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PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

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Indication:	Major Depressive Disorder
Applicant:	Forest Laboratories, Inc.
Review Division:	Division of Psychiatry Products (HFD-130)
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1 Executive Summary

1.1 Introduction

This NDA is being submitted by Forest Laboratories, Inc. under section 505(b)(1) of the Federal Food, Drug, and Cosmetic Act for Levomilnacipran for the treatment of major depressive disorder (MDD). Levomilnacipran (1S, 2R enantiomer) is the more active of the two enantiomers present in the racemate milnacipran, which has been approved outside of the United States for the treatment of major depressive disorder and in the United States for the treatment of fibromyalgia (Savella™).

1.2 Brief Discussion of Nonclinical Findings

Levomilnacipran was adequately assessed in nonclinical studies to support its approval for the treatment of major depressive disorder. The Sponsor has studied and characterized its pharmacologic profile, including the mechanism of action and efficacy, and safety using appropriate *in vitro* and *in vivo* models/assays. Pharmacokinetic studies demonstrated systemic exposure to levomilnacipran and in addition, provided the absorption, distribution, metabolism, and excretion profiles. The general toxicology profile of levomilnacipran was evaluated in mice, rats, and non-human primates following acute and chronic administration via oral route, which is the intended clinical route. Genotoxic potential of levomilnacipran was assessed using standard *in vitro* and *in vivo* assays. The carcinogenic potential of levomilnacipran was assessed in a standard two-year study in male and female Sprague-Dawley rats and in a short-term study in Tg.rasH_2 mice. The potential adverse effects of levomilnacipran on fertility, fetal-embryonic development and peri-/post-natal development were evaluated in rats and rabbits.

<u>Pharmacology</u>: Levomilnacipran is a potent and selective serotonin- and norepinephrine reuptake inhibitor (SNRI) with no appreciable binding affinity or uptake inhibition potential at the dopamine transporter. Levomilnacipran has no apparent affinity (>10 μM) for adrenergic, muscarinic, cholinergic, histaminergic receptors and ion channels including, sodium, potassium, calcium and chloride channels. Levomilnacipran was shown to have moderate efficacy in some mouse and rat models of depression and anxiety.

<u>Major human metabolite</u>: Following oral administration of levomilnacipran to humans, one major human metabolite [(*N*-desethyl levomilnacipran (F17400)] was produced at greater than 10% of the total levomilnacipran exposure. The ratio of C_{max} and AUC between *N*-desethyl levomilnacipran and the parent were in the range of 8.5%-14.1% and 11.3%-17.1%, respectively. The *N*-desethyl levomilnacipran metabolite was characterized as pharmacologically inactive and was the major metabolite identified in all three preclinical species as well. The plasma concentration of *N*-desethyl levomilnacipran in all three nonclinical species was significantly higher than in human plasma (at 60 mg oral dose). The safety of this major metabolite has been adequately assessed in nonclinical studies.

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General toxicity: In general toxicity studies in rats (up to 6 month duration) and cynomolgus monkeys (up to 1 year), levomilnacipran appeared to be devoid of significant toxicity up to one half of the dose that produced convulsions and/or death. Exposures that resulted in convulsions and/or deaths in animals were well above those in humans at therapeutic doses.

Genotoxicity: Levomilnacipran was not genotoxic when tested in the standard *in vitro* and *in vivo* genotoxicity assays.

Carcinogenicity: Levomilnacipran was not carcinogenic in rats and Tg.rasH2 mice, in standard assays.

Reproductive toxicity: Levomilnacipran had no adverse effects on fertility in rats up to a dose of 100 mg/kg/d, a dose that is ~8 times the MRHD, on a mg/m^2 basis. Similarly, levomilnacipran was not teratogenic in rats or rabbits up to a dose that is 8 times and 16 times, respectively the MRHD, on a mg/m^2 basis. Levomilnacipran caused significant decrease in maternal boy weight and increase in early post-natal mortality and a decrease in pup body weight at a dose that is ~5 times the MRHD. The no-adverse effect dose for maternal and offspring toxicity was 7 mg/kg/d, a dose that is 0.6 times the MRHD, on a mg/m^2 basis.

In summary, the overall nonclinical pharmacology and toxicology evaluations in rodents, rabbits and monkeys indicate that levomilnacipran has a safety profile similar to other drugs in this class and with adequate margins of exposure relative to the intended use in patients. From a nonclinical perspective, there are no issues that would prevent the approval of this NDA.

1.3 Recommendations

1.3.1 Approvability

Approvable

1.3.2 Additional Non Clinical Recommendations

None at this time

Labeling recommendations

The following are this Reviewer's recommendations for sections 8.1, 12.1, 12.2, and 13 of labeling.

Section 8: Use in specific populations

8.1 Pregnancy

Pregnancy Category C

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Risk Summary

Levomilnacipran was not teratogenic in rats and rabbits during the period of organogenesis. However, an increase in early post natal rat pup mortality was seen at a dose equivalent to 5 times the maximum recommended human daily dose of 120 mg on a mg/m2 basis.

Animal data

No teratogenic effects were observed when levomilnacipran was administered to pregnant rats or rabbits during the period of organogenesis at doses up to 100 mg/kg/day. This dose is 8 and 16 times (in rats and rabbits, respectively) the maximum recommended human daily dose (MRHD) of 120 mg on a mg/m^2 basis. Fetal body weights were reduced in rats, and skeletal ossification was delayed in both rats and rabbits at this dose; these effects were not observed at doses up to 2.4 times the MRHD in rats or 5 times the MRHD in rabbits.

When levomilnacipran was administered to pregnant rats at an oral dose of 5 times the MRHD during organogenesis and throughout pregnancy and lactation, there was an increase in early postnatal pup mortality and among the surviving pups, pre- and post-weaning pup weight gain was reduced up to at least 8 weeks of age; however, physical and functional developments including reproductive performance of the progeny were not affected. These effects were not seen at 0.6 times the MRHD.

Section 12: Clinical Pharmacology

12.1 Mechanism of action

The exact mechanism of the antidepressant action of levomilnacipran is unknown, but is thought to be related to the potentiation of serotonin and norepinephrine in the central nervous system, through inhibition of their reuptake. Non-clinical studies have shown that levomilnacipran is a potent and selective serotonin and norepinephrine reuptake inhibitor (SNRI).

12.2 Pharmacodynamics

Levomilnacipran lacked significant affinity for numerous receptors and ion channels, including serotonergic ($5HT_{1-7}$), α- and β- adrenergic, muscarinic, or histaminergic receptors and Ca^{2+}, Na^+, K^+ or Cl^- channels *in vitro*. Levomilnacipran also lacked monoamine oxidase (MAO) inhibitor activity.

Section 13: Nonclinical Toxicology

Carcinogenesis

Levomilnacipran administered by oral gavage to rats for 2 years and Tg.rasH2 mice for 6 months did not increase the incidence of tumors in either study.

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Rats received levomilnacipran at dosages up to 90/70 mg/kg/day (dosage lowered in males after 45 weeks of dosing). The 90 mg/kg/day dose is 7 times the recommended human daily dose of 120 mg on a mg/m2 basis.

Tg.rasH2 mice received levomilnacipran at dosages up to 150 mg/kg/day. This dose is 6 times the recommended human daily dose of 120 mg on a mg/m2 basis.

Mutagenesis

Levomilnacipran was not mutagenic in the *in vitro* bacterial mutation assay (Ames test) and was not clastogenic in an *in vivo* micronucleus assay in rats. Additionally, levomilnacipran was not genotoxic in the *in vitro* mouse lymphoma (L5178Y $TK^{+/-}$) cell forward mutation assay.

Impairment of fertility

When levomilnacipran was administered orally to male and female rats before mating, through mating and up to Day 7 of gestation at doses up to 100 mg/kg/day, no effects were observed on fertility. This dose is 8 times the recommended human daily dose of 120 mg on a mg/m2 basis.

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2 Drug Information

2.1 Drug:

Levomilnacipran hydrochloride (FETZIMA™)

CAS Registry Number: 96847-54-0 (levomilnacipran base); 175131-60-9 (levomilnacipran hydrochloride)

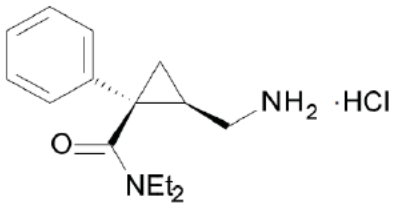
Generic Name: Levomilnacipran

Code Name: F2695

Chemical Name: (1S, 2R)-2-(aminomethyl)-*N,N*-diethyl-1-phenylcyclopropanecarboxamide hydrochloride

Molecular Formula/Molecular Weight: $C_{15}H_{22}N_2O$/246.35 g/mol

Structure or Biochemical Description:



Pharmacologic Class: Selective serotonin and norepinephrine reuptake inhibitor (SNRI)

2.2 Relevant INDs, NDAs, BLAs and DMFs:

IND 104,483

2.3 Drug Formulation:

Capsule (Extended Release) of 20, 40, 80, and 120 mg strengths

2.4 Comments on Novel Excipients:

There are no novel excipients; Levomilnacipran capsules contain the inactive ingredients, sugar spheres, povidone (b) (4), talc, ethylcellulose (b) (4), and triethyl citrate

2.5 Comments on Impurities/Degradants of Concern:

Earlier during development, drug substance starting material (b) (4) were identified by CMC as plausible structural alerts for genotoxicity. The Sponsor was advised to develop analytical methods capable of measuring/controlling the levels of these impurities in all drug substance batches (CMC communication dated 5-20-2009; IND-

104,483). Accordingly, the Sponsor agreed to control the levels of [redacted] (b) (4) and [redacted] (b) (4) to no more than [redacted] (b) (4) per day by the time of NDA submission. The Sponsor also agreed to develop analytical methods to control and detect the levels of [redacted] (b) (4) and [redacted] (b) (4) levels in all drug batches at least below [redacted] (b) (4) levels. At the time of NDA submission, the levels of these impurities in various drug substance batches were controlled to levels that were specified by CMC team and there are no longer concerns regarding these impurities at this time.

2.6 Proposed Clinical Population and Dosing Regimen

Levomilnacipran is indicated for the chronic treatment of adult patients with major depressive disorder (MDD). The initial and target dose of levomilnacipran is 40 to 120 mg once daily.

2.7 Regulatory Background

Levomilnacipran has been studied for the treatment of MDD under an Investigational New Drug application (IND 104,483) submitted to the Food and Drug Administration by the current Sponsor (Forest Laboratories, Inc.) on February 6, 2009. The Sponsor met with the FDA to discuss the MDD program at a Type B End-of-Phase-2 meeting on 18 May, 2009. Safety and efficacy of levomilnacipran was discussed and agreed upon at the Type B Pre-NDA Meeting held on 4 May, 2012. Levomilnacipran (1S, 2R enantiomer) is the more active of the two enantiomers present in the racemate milnacipran, which has been approved outside of the United States for the treatment of major depressive disorder and in the United States for the treatment of fibromyalgia (Savella™).

3 Studies Submitted

3.1 Studies Reviewed

All submitted studies, including pharmacology, pharmacokinetics and pivotal toxicology studies in various species were reviewed.

3.2 Studies Not Reviewed

Previously submitted (IND 104,483) pharmacology and acute toxicology studies that were resubmitted with this application were not reviewed. In addition, dose range-finding studies for the chronic toxicity studies and reproductive toxicity studies in various species were not reviewed in detail.

3.3 Previous Reviews Referenced

IND 104,483; short-term chronic toxicity studies in rats and monkeys up to 13 weeks duration were previously reviewed by this Reviewer and are summarized and/or reproduced in the relevant sections of this review.

4 Pharmacology

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Levomilnacipran (F2695) is a selective serotonin (5-HT)-norepinephrine (NE) reuptake inhibitor (SNRI), with negligible affinity for the dopamine transporter. *In vitro* studies have shown high binding affinity for human (recombinant) norepinephrine transporters (hNET expressed in MDCK cells: K_i = 71-90 nM) and human (recombinant) serotonin transporters (hSERT expressed in HEK-293 cells: K_i = 11 nM), but not for human (recombinant) dopamine transporter (hDAT expressed in CHO cells: K_i = not calculable; but > 10 μ). In functional studies, levomilnacipran inhibited the uptake of both NE and 5-HT with equal potency (IC_{50} = 11 nM and 16-19 nM, respectively at hNET and hSERT stably expressed in CHO cells); in contrast, levomilnacipran did not block dopamine (DA) uptake at dopamine transporter (DAT) for concentrations up to 10 μM. Levomilnacipran had no significant binding affinity at 81 other receptors, transporters, and ion channels, including DA, 5-HT, adrenergic, muscarinic, and histamine receptors, and the DA transporter. Levomilnacipran exhibited low binding affinity (K_i = 1.7 μM) at the phencyclidine (PCP) binding site and, weakly inhibited NMDA-evoked NR1/NR2A and NR1/NR2B glutamate receptor-mediated currents (IC_{50} = 28 and 24 μM, respectively).

4.1 Primary Pharmacology

Mechanism of action: The pathophysiology of major depressive disorder (MDD) as well as its treatment is believed to involve alterations in noradrenergic and serotonergic function in the CNS. Some recent studies suggest that treatment of MDD with antidepressants that simultaneously enhance serotonin and norepinephrine neurotransmission may be more effective than the current treatment strategies.

Levomilnacipran, being developed by Forest Laboratories, was shown to exhibit selective NE and 5-HT reuptake inhibition. Results from four *in vitro* studies show that levomilnacipran exhibits very high binding affinity for human and rat NE and 5-HT transporters (summarized in Table 1, below).

Table 1 *In vitro* binding affinity of levomilnacipran at the NET and SERT

(Excerpted directly from the Pharmacology Written Summary section of this NDA, pp 7)

Test System (Study Number, year)	*Binding Affinity K_i (nM)*	
	NET	*SERT*
Rat brain homogenate (Study 1236/1, 2011)	11	7
Rat cortical transporters (Study F2695-19855, 2004)	na	2.4
Human recombinant transporters (Study 1236/1, 2011)	91	11
Human recombinant transporters (Study F2695-19857, 2004)	71	na

na = not applicable; NET = norepinephrine transporter; SERT = serotonin transporter

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Table 2 *In vitro* uptake inhibition by levomilnacipran at the NET and SERT transporters

(Excerpted directly from the Pharmacology Written Summary section of this NDA, pp 7)

Test System (Study Number, year)	*Uptake Inhibition IC_{50} (nM)*		*NE Selectivity Ratio (SERT/NET)*
	NET	*SERT*	
Rat brain homogenate (Study P158, 1994)	31.5	103	3.2
Rat brain synaptosomes* (Study 1240/1, 2011)	62	68	1.1
Rat brain synaptosomes** (Study 165bis.CM.md, 1989)	15	46	3.1
Human recombinant transporters (Study 1033056, 2008)	11	16	1.5
Human recombinant transporters (Study 1237/1, 2011)	11	19	1.7

NET = norepinephrine transporter; SERT = serotonin transporter

* NE reuptake inhibition was measured in synaptosomes from the frontal cortex and 5-HT uptake inhibition was measured in synaptosomes from the cerebral cortex

** NE and 5-HT reuptake inhibition were measured in synaptosome preparations from rat hippocampus

The affinities of levomilnacipran were evaluated using ligand binding on membrane preparations from cells expressing (recombinant) human SERT, NET, and DAT (expressed in HEK-293, MDCK and CHO, respectively) as well as at native rat SERT and NET from cortical membrane preparations. Levomilnacipran showed similar affinities at rSERT (K_i = 7 nM) and rNET (K_i = 11 nM). However, levomilnacipran had higher affinity at hSERT (K_i = 11 nM) than at hNET (K_i = 91 nM). Levomilnacipran did not bind to recombinant human DAT (K_i below detection levels).

Functional effects of levomilnacipran on NE, 5-HT and DA uptake were tested using recombinant human transporters expressed CHO cells; results (shown in Table 2, above),showed that levomilnacipran inhibited 5-HT and NE uptake in a concentration-dependent manner (IC_{50} = 11 nM and 19 nM, respectively). In contrast, levomilnacipran did not block DA uptake at the dopamine transporter (IC_{50} >100 μM). In a second functional study using recombinant hNET (expressed in MDCK cells) and hSERT (expressed in HEK-293 cells), levomilnacipran block of NE and 5-HT uptake was identical (IC_{50} = 11 nM and 16 nM, respectively). In a third functional assay, norepinephrine, serotonin and dopamine uptake inhibition properties of levomilnacipran were examined using rat brain synaptosomes; levomilnacipran potently inhibited NE and 5-HT uptake (IC_{50} = 62 nM and 68 nM, respectively). In contrast, levomilnacipran was over 100-fold less potent at inhibiting DA uptake (IC_{50} >10 μM). In a fourth study using rat brain homogenates, levomilnacipran exhibited ~3-fold greater potency at NET versus 5-HT transporters (IC_{50} = 31.5 nM and 103 nM, respectively); levomilnacipran have no effect on the uptake of DA in the rat striatum.

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Noradrenergic activity of levomilnacipran was evaluated in isolated rat seminal vesicles; levomilnacipran (100 nM to 30 µM) produced a concentration-dependent potentiation of NE (10 nM to 100 µM)-induced contraction of the seminal vesicles (there was a left-ward and parallel shift to the norepinephrine dose-response curve, significantly reducing the norepinephrine EC_{50} values at each of the concentrations of levomilnacipran tested). The effect of levomilnacipran on contractile response is most likely due to inhibition of NE uptake by the nerve endings.

Effects of levomilnacipran on the extracellular levels of NE and 5-HT:

Levomilnacipran (administered via a microdialysis probe in the prefrontal cortex of freely-moving Sprague-Dawley rats) produced a concentration-dependent increase in the extracellular levels of NE, 5-HT, and DA; the effects on 5-HT and NA were similar in terms of magnitude (maximal increases of 387% and 331%, respectively, above baseline at a concentration of 1 mM) and larger than the increase evoked in DA (83%) at the same concentration. The minimum effective concentrations of (levomilnacipran) 1 µM and 10 µM for NE and 5-HT, respectively, were interpreted by the Sponsor as being consistent with the *in vitro* profile of levomilnacipran showing greater potency at NET than SERT. As per the Sponsor's narrative the increase in extracellular DA levels are also consistent with previous reports that drugs selectively blocking NE uptake increase both NE and DA in the prefrontal cortex[1,2]. The Sponsor also cited a published repot in which it was found that DA transporters are expressed at low levels in the prefrontal cortex and NE transporters in the prefrontal cortex uptake both NE and DA[3]; therefore, increases in DA concentration may be due to inhibition of the NE transporter. In an *in vivo* study, single oral administration of levomilnacipran (20 mg/kg) increased NE, 5-HT, and DA levels 2-3 fold in Sprague-Dawley rat medial prefrontal cortex relative to saline control (measured by intracerebral microdialysis); increases in NE levels persisted for a longer duration (up to 8 h, post-dose) than increases in 5-HT (0-160 min, post-dose) and DA (40-240 min, post-dose) and the increases in NE levels were numerically higher than increases in 5-HT or DA levels (86% for NE versus 36% and 67%, respectively for 5-HT and DA, above the levels in saline controls).

In another *in vivo* dose-response study, single oral (gavage) administration of levomilnacipran (5, and 20 mg/kg) resulted in increased NE levels (40 and 50%, respectively compared to saline treatment; monitored by intracerebral microdialysis) in the rat prefrontal cortex; the 20 mg/kg dose also increased DA levels (55%, relative to saline control). However, the 5-HT levels were not affected by either dose.

In a dual-probe microdialysis study (probes placed in prefrontal cortex and striatum), sub-chronic (12 days) oral (gavage) administration of levomilnacipran (40 mg/kg/d) in Sprague-Dawley rats had no effect on basal levels of NE, 5-HT or DA in the prefrontal cortex. Whereas, prefrontal cortical NE levels were significantly increased on day 12,

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[1] Rowley et al., 1998; *Neuropharmacology* 37:937-944
[2] Rowley et al., 2000; *Naunyn-Schmeif Arch Pharmacol*., 361:187-192
[3] Carboni and Silvagni 2004; *Crit Rev Neurobiol*., 16: 121-128

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post-dose (42%, $p < 0.01$); 5-HT and DA levels were unchanged. In the striatum, levomilnacipran did not change basal or post-dose 5-HT and DA levels. Duloxetine (10 mg/kg/d), another SNRI, had very similar results in the levels of NE, 5-HT, and DA from both regions of the brain.

Levomilnacipran activity related to proposed indication:

Antidepressant-like and anxiolytic-like efficacy of levomilnacipran was demonstrated in multiple rodent models.

Levomilnacipran was tested in several different **behavioral models of depression** to demonstrate the antidepressant-like effects. In one study (study #P170) in male NMRI mice, antidepressant-like effect of levomilnacipran was evaluated using the forced swim test model (behavioral despair). Levomilnacipran (16, 32, and 64 mg/kg, p. o.) caused significant reduction in the duration of immobility 60 min after dosing at all three doses tested (185 sec in vehicle control versus 109, 71, and 75 sec, respectively at 16, 32, and 64 mg/kg; $p < 0.01$ to 0.001; maximal effect was seen at 32 mg/kg); the positive control imipramine also caused significant reduction in the duration of immobility (32 sec, $p < 0.001$), predictive of its antidepressant-like effect.

In a second study (behavioral despair- Porsolt) using forced swim test (study #P092) in Swiss mice, levomilnacipran (1, 3, and 10 mg/kg, i. p.) produced a dose-dependent reduction in the duration of immobility 1 hr after drug treatment (185 sec in control versus 148, 101, and 65 sec, respectively; the effective dose was 3 mg/kg; $p < 0.001$). In a third study (study #1238/1), also using forced swim test in male OF1 mice, levomilnacipran (5, 10, 20, and 40 mg/kg, i. p.) significantly reduced the duration of immobility 30 min after drug administration (169 sec in vehicle control versus 155, 123, 76 and 53 sec, respectively at 5, 10, 20 and 40 mg/kg; statistically significant only at 20 and 40 mg/kg, $p < 0.01$; minimum effective dose was 20 mg/kg).

In the tail suspension test, another behavioral model of depression, levomilnacipran (16, 32, and 64 mg/kg, p.o.) in male NMRI mice (study # P170) produced significant reduction in the duration of immobility at 64 mg/kg dose, indicative of anti-depressant-like activity (80 sec control versus 22 sec at 64 mg/kg; p 0.05); the positive control imipramine also caused significant reduction in the duration (36 sec, $p < 0.05$) of immobility. In a second study using tail suspension test in NMRI male mice (study #1239/1), levomilnacipran (0.63, 2.5, 10, 20, and 40 mg/kg, i. p.) significantly reduced immobility at doses 10 and 20 mg/kg 30 min after drug administration (144 sec in control versus 81 and 109 sec, respectively; $p < 0.01$).

However, in the learned helplessness model of depression in male Wistar rats, sub-chronic administration of levomilnacipran (10, 20 and 40 mg/kg/day; oral; study #0PF29) failed to produce improvements in the escape performance in the active avoidance task (during which 30 stimulus-shock treatment was given) on all 3 days tested, demonstrating the lack of antidepressant-like effects. On the other hand, the reference compound imipramine (64 mg/kg; oral) significantly improved escape performance in

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the active avoidance task on all 3 days of testing demonstrating the reversal of behavioral impairment caused by an experience of uncontrollable aversive events.

The **anxiolytic-like effect** of levomilnacipran was evaluated in male Sprague-Dawley rats in two studies (study #s1065/1 and 1235/1) by testing the ability of the drug to attenuate conditioned stress-induced ultrasonic vocalizations (USVs). Animals were trained to associate a conditioned stimulus with inescapable mild electric shock. In both studies, i. p. administration (0.63, 2.5, 5 and 10 mg/kg) of levomilnacipran 30 min before testing resulted in a dose-dependent reduction in USV duration in response to the conditioned stimulus, indicating anxiolytic-like effect of the drug; the minimal effective dose (both studies) was 5 mg/kg ($p < 0.01$) and the ED_{50} dose range was 3.95-4.2 mg/kg.

The efficacy of levomilnacipran was also evaluated for its anxiolytic-like effects in several other animal models such as Vogel Conflict test (study # 05.154/3; p.o. and 05.313/4/B; s.c.), and Elevated Plus-Maze test (study # 05.155/3; p.o. and 05.313/4/A; s.c.) in male Han Wistar rats and Four Plates test (study # 05.153/3 and 05.311/4/B; s.c) and Marble burying test (study # 05.156/3; p.o. and 05.311/4/A; s.c.) in male NMRI mice. The results of single-dose (3, 10 and 30 mg/kg) and chronic doses (0.1, 0.3, 1 mg/kg/day) studies showed no anxiolytic-like effects by levomilnacipran in any of the animal models at any of the dose levels tested. Clobazam (32-64 mg/kg) was used as positive control and produced significant anxiolytic-like effects in all the four animal models, thus validating the test. The effect of venlafaxine, an SNRI, was also evaluated in the above studies for its anxiolytic-like effects for comparison purpose; neither acute nor chronic doses of venlafaxine produced anxiolytic-like effects in the Elevated Plus-Maze, Vogel Conflict test, or Four-Plates test (study #s 05.153/3; 05.154/3; 05.155/3; 05.311/4/D; 05.313/4/C; 05.313/4/D). However, acute dose of venlafaxine (30 mg/kg; p.o.) produced anxiolytic-like activity in the Marble burying test (study # 05.156/3), but lacked anxiolytic-like activity upon chronic (0.1 – 1.0 mg/kg) administration (study # 05.311/4/C).

Catecholaminergic activity of levomilnacipran was evaluated by assessing antagonism of tetrabenazine (TBZ)-induced ptosis, potentiation of yohimbine toxicity and induction of mydriasis (study # P092). Oral administration of levomilnacipran (0.1 to 30 mg/kg) to male Swiss mice produced dose-dependent decrease (-15% to -95%; ED_{50} = 0.8 mg/kg) in TBZ-induced ptosis. Oral administration of levomilnacipran (0.3 to 30 mg/kg) prior to sub lethal dose of yohimbine (25 mg/kg s.c.) also increased the number of deaths (20% to 90% deaths; ED_{50} = 1.7 mg/kg) in male Swiss mice. Following direct (*in situ*) application of a solution of levomilnacipran (0.5 and 1%) to the eye of male Swiss mice, statistically significant increase in pupil diameter was observed, thus demonstrating the induction of mydriasis. Induction of mydriasis by levomilnacipran (1-50 mg/kg; i.p.) was also demonstrated *in vivo* in male Swiss mice in a separate experiment.

Catecholaminergic activity of levomilnacipran was further evaluated by assessing its effects on reserpine-induced behaviors (hypothermia, ptosis and akinesia) in male

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NMRI mice (study # P132). Levomilnacipran at lower concentrations (2-16 mg/kg) attenuated reserpine-induced hypothermia; however, at higher dose (64 mg/kg) it enhanced reserpine-induced hypothermia. In separate experiments, levomilnacipran (2-32 mg/kg) was also shown to antagonize reserpine-induced ptosis and attenuated reserpine-induced akinesia in NMRI mice. Desipramine (positive control) produced moderate to robust antagonism of reserpine-induced behaviors, thus validating the test.

4.2 Secondary Pharmacology

In a series of *in vitro* binding studies (study #s 1077/2; F2695-19855; F2695-19856; F2695-19857) that included 81 receptors, ion channels, or transporter targets, levomilnacipran was found to have high binding affinity at the NE and 5-HT transporters (as already shown in section 4.1); however, did not show significant binding affinity/selectivity (IC_{50} > 10 μM) at other targets, including serotonin (5-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_3, 5-HT_{5A}, 5-HT_6, and 5-HT_7), adrenergic (α1, α2, β1, and β2), dopamine (D1, D2, D3, D4, and D5), muscarinic (M1-5) and histamine (H1, H2) receptors and the dopamine transporter (DAT). *In vitro* binding affinity of levomilnacipran at specific 5-HT receptor subtypes (human: 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}) were also evaluated and showed no significant binding affinity at any of the drug concentrations tested (0.01 nM - 10μM).

In the *in vitro* receptor binding assays, levomilnacipran was found to have low binding affinity for the PCP binding site (K_i = 1.7 μM); however, its functional significance is unclear. Nevertheless, in functional studies using patch-clamp techniques, levomilnacipran dose-dependently inhibited NMDA-evoked human NR1/NR2A and NR1/NR2B glutamate receptor-mediated whole-cell currents (in HEK-293 cells) with an IC_{50} of 28 and 24 μM, respectively (study #080432.DCC). Likewise, in a separate study (study # 1147/2) using two-electrode voltage-clamp, levomilnacipran dose-dependently inhibited glutamate-evoked whole-cell currents in *Xenopus* oocytes expressing recombinant human NR1/NR2B receptors (IC_{50} = 4.7 μM).

Ever since John Olney and colleagues demonstrated neuronal vacuolation and degeneration in developing rat brain following ketamine (a non-competitive NMDAR antagonist) administration[4] (the so-called 'Olney lesion'), there is a general concern in some quarters that NMDAR antagonists in general are a suspect candidate for 'Olney lesion'. As mentioned above, levomilnacipran was found to have a low affinity binding to the PCP site and in order to understand the functional significance of the observed binding (it is thought that the PCP binding site lies very deep within the NMDAR channel and by implication, antagoinists acting at the PCP site may act like an open channel blocker), the Sponsor has conducted a functional study using recombinant human NMDARs (NR1/NR2A and NR1/NR2B) expressed in HEK-293 cells and measured the effects of levomilnacipran on NMDA-evoked whole-cell currents using patch-clamp techniques (study #080432.DCC). Levomilnacipran blocked the NMDA-induced whole-

[4] Ikonomidou C., et al, 1999; Science, 283: 70-74

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cell currents in a concentration-dependent manner with an IC_{50} of 28 and 24 μM, respectively for the two combinations. These half maximal inhibitory concentrations of levomilnacipran at the NMDARs are ~1368 and 2363 times lower than the levomilnacipran-induced reuptake inhibition (IC_{50}) of NE and 5-HT at the hNET and hSERT, respectively. Thus, levomilnacipran is not only a weak antagonist at the NMDARs (however, the specific mechanism of antagonism is unclear from the limited information obtained from the above functional studies) but also has a large margin as far as its blocking affinity for NE and 5-HT reuptake inhibition. It is worth noting that levomilnacipran block of NMDARs in this study is significantly weaker than dizocilpine (MK-801), the prototypical non-competitive NMDAR antagonist or PCP (MK-801 IC_{50} = 45-82 nM, and PCP IC_{50} = 182-240 nM for rat NR1/NR2A or NR1/NR2B combinations[5]).

Moreover, the C_{max} at steady-state in healthy human volunteers following oral daily dosing of 120 mg of levomilnacipran was 340 ng/mL (1.4 μM), a concentration at least 20 times lower than the half maximal inhibitory concentration of levomilnacipran obtained in the above *in vitro* study. Assuming that the concentration of levomilnacipran in the brain is lower than in the plasma, there is even a wider margin for antagonism of NMDARs in the brain. In conclusion, this Reviewer is not overly concerned about the (possible) levomilnacipran-induced block of NMDA subtype of the glutamate receptors and consequent neurotoxicity.

Anticholinergic activity of levomilnacipran was examined by assessing its effects on oxotremorine-induced behaviors (central and peripheral) in NMRI mice (study # P132). Oral administration of F2695 at doses of 2-16 mg/kg significantly attenuated oxotremorine-induced hypothermia (central cholinergic effects); however, at the highest dose of 64 mg/kg, levomilnacipran enhanced oxotremorine-induced hypothermia. While levomilnacipran had no significant effects on oxotremorine-induced tremors, it showed only a modest effect (reduction) on oxotremorine-induced akinesia (both central cholinergic effects). Similarly, levomilnacipran showed only slight attenuation of oxotremorine-induced peripheral cholinergic signs such as lacrimation and salivation. The positive control atropine (8 mg/kg, p.o.) produced profound antagonism of oxotremorine-induced behaviors.

4.3 Safety Pharmacology

Safety pharmacology assessments included *in vitro* and *in vivo* effects of levomilnacipran on central nervous system, cardiovascular system and respiratory system in multiple non-clinical species.

Effects on CNS

The neurobehavioral effects of a single oral dose of levomilnacipran (10, 30, 60, 90, 110, 140, 170 and 200 mg/kg) were assessed in male Sprague-Dawley rats using

[5] Avenet P., et al, 1997; Neuroscience Lett., 223 :133-36

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standard observational battery (study # F2695-22628). General physical condition/behavior, motor activity, and body temperature were assessed 1, 2, 3, and 24 h, post-dose. Levomilnacipran caused decrease in motor activity 1-3 hr post-dose at the doses ≥110 mg/kg; however, significant increase in motor activity was noted 24 hr post-dose, especially at doses ≥ 140 mg/kg. Likewise, levomilnacipran significantly decreased arousal within 1 to 3 hr of dosing at the doses ≥ 90 mg/kg. At dose ≥ 110 mg/kg, there was a significant alteration in posture and induced palpebral ptosis. At the highest dose of 200 mg/kg, there was a statistically significant modification of the gait (not specified) in all animals. Furthermore, levomilnacipran produced a dose-dependent and statistically significant decrease in body temperature (↓ 0.6 °C to 3.0 °C; $p < 0.05$-0.01), 1 2 and 3 hr post-dose. With the exception of a decrease of arousal and a modification of posture in some animals, all neurobehavioral changes were comparable to control, 24 hr post-dose.

In a second study, the neurobehavioral effects of a single oral dose of levomilnacipran (16, 32, 64, 128, 256 and 512/kg) were assessed in male NMRI mice using the Irwin test (study # P132). Behavioral changes were recorded at 15, 30, 60, 120, and 180 min and 24 hr after dosing. Convulsions and death occurred at the highest dose of 512 mg/kg (3/3) within 20 to 60 min after dosing. Mortality also occurred at the dose level of 256 mg/kg (1/3); in addition, significant hypothermia (-4.5 °C), mydriasis, and sedation were seen at 256 mg/kg dose. At the dose level of 128 mg/kg the effects included slight sedation, loss of traction, decreased reactivity to touch, decreased muscle tone, significant hypothermia (-3.4 °C) and mydriasis in all animals, within 30 to 60 min after dosing and lasted for up to 180 min. At the 64 mg/kg dose, only mydriasis and slight hypothermia (-2 °C) were seen and at 32 mg/kg, only mydriasis was seen.

Effect of levomilnacipran on motor coordination was assessed in the rotarod test following a single oral dose (32, 64 and 128 mg/kg; study # P132). Levomilnacipran did not produce significant effect on the drop-off time at any of the dose levels. Even though the drop-off time at all 3 dose levels showed a decreasing trend (-18%, -54% and -45%, respectively compared to vehicle control), the changes were not statistically significant. The positive control diazepam (4 mg/kg), markedly decreased the drop-off time (-78%; $p < 0.01$).

Using the delayed non-matching to position (DMNTP) task, the effects of levomilnacipran (acute; study #1224/1 and sub-chronic- five days; study # 1225/1) on working memory (short-term retention of continuously updated information), executive function (ability to do the operant task at short retention interval) and processing speed (latency times to do the operant task at short retention interval or “sampling phase” and long retention interval or “choice phase”) were assessed in middle-aged rats (17-18 months old). Acute administration of levomilnacipran (0.63 – 20 mg/kg; i.p.) did not impact working memory (the levels of accuracy in terms of percentage of correct responses) in comparison to vehicle-treated rats. Similarly, acute administration of levomilnacipran had no impact on the processing speed (latency times to the SP or CP) or executive function (no change in performance at the 1, 20 or 50 sec delay), when compared to vehicle control group. Sub-chronic administration of levomilnacipran (10

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mg/kg/d; i.p.) slightly but statistically significantly improved working memory (increased the percentage of correct response at 50 sec) in comparison to the control group. Levomilnacipran had no adverse effect on executive function; however, at the high dose of 20 mg/kg/d, the processing speed was slightly but statistically significantly slower than control (mean latency was 0.88 sec higher than the control group, 1.16 sec, control versus 2.04 sec at 20 mg/kg/d).

Effects on Cardiovascular System:

Effects of levomilnacipran on human ether-a-go-go related (hERG) K^+ -currents: The effects of levomilnacipran on hERG K^+ -currents were assessed *in vitro* in CHO-K1 cells stably expressing the hERG K^+ channels using patch-clamp techniques in the whole-cell configuration (study # F2695-19457). Levomilnacipran (0.001, 0.01, 0.1, 1, and 10 μM) inhibited the hERG K^+ channel maximum tail current amplitude in a concentration-dependent manner (14 to 31% inhibition range); however, the actual IC_{50} could not be determined because the maximum inhibition was only 31% at the highest concentration tested and therefore, the estimated IC_{50} value is greater than 10 μM. The positive control, cisapride (3 μM), produced 85% inhibition of the hERG K^+ channel tail current amplitude, thus validating the study.

Effect of levomilnacipran on cardiac action potential in isolated canine Purkinje fibers: The effect of levomilnacipran (0.01, 0.1, 1, and 10 μM) on cardiac action potential was assessed in isolated Purkinje fibers from 3 male Beagle dogs (study # F2695-19458). Action potential amplitude, resting potential, maximal rate of depolarization, and action potential duration (APD_{50}, APD_{70}, and APD_{90}) were measured. Levomilnacipran at concentrations of 0.01 through 10 μM had no significant effect on the action potential parameters when measured at the normal stimulation rate of 1 Hz. However, when measured at the slower stimulation rate of 0.33 Hz, levomilnacipran produced a statistically significant increase in APD_{90} (↑ 19 ms) at 1 μM and in both APD_{70} (↑22 ms) and APD_{90} (↑ 37 ms) at 10 μM, suggesting a slight reverse use-dependency. Cisapride (positive control; 0.3 μM) produced statistically significant increase in the duration of action potential at both 1 and 0.33 Hz stimulation rates (↑ 9-93 ms and ↑ 38-445 ms, respectively), thus validating the method.

In vitro study of the chronotropic effect of levomilnacipran on the guinea pig isolated right atrium: Effect of cumulative increasing concentrations of levomilnacipran (0.1 nM – 10 μM) on the spontaneous contractions of the guinea pig atrium was evaluated *in vitro* (study # P164). Levomilnacipran did not exert significant chronotropic effects on the guinea pig isolated right atrium.

Effects of levomilnacipran on cardiovascular parameters investigated by telemetry in conscious dogs: The effect of a 5-day repeated oral treatment with levomilnacipran (10 mg/kg/d) on blood pressure, heart rate, ECG parameters, and clinical signs were assessed in freely moving female Beagle dogs equipped with telemetry devices (n = 6; 10 mg/kg/d; (study # F2207-16383). Levomilnacipran produced a statistically significant increase in heart rate (125 beats/min, compared to 110 beats/min in the control)

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following single or 5 -day administration and the maximum effect occurred between 0.5 to 1.5 hr. In addition, there was a statistically significant increase in diastolic blood pressure following single or 5-day treatment with levomilnacipran (72-89 mm Hg in control versus 81 to 110 mm Hg at 10 mg/kg). Moreover, there was **prolongation of QTc** (Bazzett) interval following single or 5-day treatment with levomilnacipran (↑ 21 ms or 9% on both Day 1 and Day 5). Moderate mydriasis was noted in 5/6 dogs and in some dogs it persisted for more than 24 hr after drug administration. Minimal decrease in the mean body weight gain (-6% compared to vehicle control) and a slight reduction in food consumption were also noticed in dogs treated with levomilnacipran for 5 days.

Effects of levomilnacipran on arterial blood pressure, heart rate and electrocardiogram following oral administration in the conscious cynomolgus monkey monitored by telemetry (study # AA 30935): The effect of single oral doses of levomilnacipran (0, 5, 15, 45 mg/kg) on cardiovascular function (arterial blood pressure, heart rate and ECG parameters) were assessed in cynomolgus monkeys (3/sex/dose; nasogastric intubation; Latin crossover design, with a minimum washout period of 2 days). Treatment-related clinical signs included sporadic and delayed vomiting episodes. Statistically significant (not dose-related) increases in mean arterial blood pressure, systolic blood pressure, and diastolic blood pressure at all three doses, with the maximum increase at 0.5 hr post-dose and returning to normal levels by 4 hr after drug administration. In addition, there was a very pronounced and statistically significant **increase in QT and QTc intervals at the HD of 45 mg/kg** (↑ 16% and 18%, respectively using Bazzett and Fredericia formulas), compared to the pre-test values and occurred between 2 and 6 hr post-dose (average plasma concentration of levomilnacipran between 2 and 8 hr post-dose was 4448 ng/ml). At the high dose, there was a moderate, though statistically significant increase in QRS duration and occurred between 3 and 6 hr post-dose. There was a slight, however statistically significant (< 1 °C, from pre-test) **decrease in body temperature** at the HD and occurred after 4 hr post-dose. PK analysis showed that the C_{max} values at the three oral doses (5, 15, and 45 mg/kg) of levomilnacipran were 617 ng/mL, 1595 ng/mL and 7234 ng/mL, respectively.

Cardiac and systemic hemodynamic effects of levomilnacipran in anesthetized open chest dogs: Cardiac and systemic hemodynamic effects of levomilnacipran (0.01, 0.03, 0.1, 0.3, 1, 3, and 10 mg/kg; ascending, i.v. bolus at 20 min intervals) were evaluated in the anesthetized open chest male Beagle dogs (n = 4-6; study # P171). Levomilnacipran caused increase in blood pressure at the lowest doses and significant hypotension at the highest dose. The lowest 2 doses of levomilnacipran also caused increase in heart rate; however, at higher doses the heart rate was significantly decreased. Aortic blood flow was progressively depressed as the concentration of levomilnacipran was increased. There were no significant increases in the PR and QTc intervals.

Effects on Respiratory System

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Respiratory effects after intravenous injection of increasing doses of levomilnacipran in the conscious rat: The effect of levomilnacipran (ascending doses: 1, 2, 4, 8, 16, 32, 64 and 128 µmol/kg in glucose) on blood gases were assessed in conscious male Sprague Dawley rats surgically implanted with arterial and venous catheters and sub-dermal platinum needle electrodes (study # P162). Following the highest dose (128 µmol/kg), there was a marginal, increase in pCO_2 (5.8 kPa versus 5.2 kPa in control; $p < 0.05$) together with a decrease in pH (7.48 versus 7.43 in control; $p < 0.05$), indicating a decreased respiration. The racemate (6.3 kPa versus 5.2 kPa in control; $p < 0.05$) and F2696 (5.7 kPa versus 5.2 kPa in control; $p < 0.05$) at the highest dose level produced marginal, though statistically significant increase in pCO_2 compared to placebo indicating a slight decrease in respiration (however, there was no decrease in pO_2).

Evaluation of effects of levomilnacipran on respiration in the unrestrained conscious rat following oral administration: The effect of single oral administration of levomilnacipran (20, 63, and 200 mg/kg) on various respiratory parameters (respiratory rate, tidal volume, minute volume, airway resistance index, peak inspiratory and peak expiratory flows and inspiration and expiration times) was evaluated by the whole body plethysmography method in conscious male Wistar rats (study # CEPC 08-443). At the low and mid doses, there were no treatment-related effects on any of the respiratory parameters. At the high dose of 200 mg/kg, there was a statistically significant increase in respiratory rate (+75%, compared to pre-dose and lasted from 30 to 240 min post-dose) and statistically significant decreases in tidal volume (-27% to -64%, compared to pre-dose and lasted from 60 to 240 min post-dose) and minute volume (-15% to -53%, compared to pre-dose, lasting from 90 to 240 min post-dose). In addition, there were decreases in peak inspiratory flow (-5% to -57%; $p < 0.01$, compared to pre-dose) and inspiration time (-18% to -25%; $p < 0.01$, compared to pre-dose). Similarly, there were decreases in expiration time (-21% to -55%; $p < 0.01$, compared to pre-dose) and peak expiratory flow (-8% to -43%, compared to pre-dose- but not statistically significant). Finally, there was a statistically significant increase in airway resistance index, lasting from 60 to 240 min post-dose (pre-dose airway resistance expressed as PenH units of 0.56 versus 0.23 to 0.48 –effects between t = 30 min to t = 240 min expressed as variation calculated in relation to pre-dose values; $p < 0.01$). Carbamylcholine chloride (30 mg/kg; p.o.) was used as positive control and it induced typical bronchoconstrictor effects (characterized by pronounced increase in airway resistance index; pre-dose airway resistance index expressed as PenH units of 0.60 versus 0.41 at t = 180 min post-dose; $p < 0.01$). In conclusion, the top dose of levomilnacipran induced respiratory depressive and bronchoconstrictor effects. Therefore, the NOAEL of levomilnacipran on respiratory parameters in conscious rats is 63 mg/kg, when administered through the oral route. The mean exposure values from the satellite rats are as follows:

At the low dose of 20 mg/kg, the mean exposure = 844 ng/mL
At the mid dose of 63 mg/kg, the mean exposure = 4193 ng/mL
At the high dose of 200 mg/kg, the mean exposure = 8429 ng/mL

Note: At the top dose, 3 rats out of 13 (replacement inclusive) died after dosing- one immediately after dosing; a second one died 70 min after dosing; the third one about 85

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min after dosing. All rats displayed similar changes in the respiratory parameters as the other top dose rats.

Renal System: No studies were provided.

Gastrointestinal System: No studies were provided.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Pharmacokinetic properties of levomilnacipran (F2695) were evaluated in mice, rats, rabbits, and monkeys following intravenous and oral administration and were compared to humans. The dosing solutions were made up in 0.9% NaCl for intravenous administration and in ultra pure water for oral administration.

Methods of Analysis: Measured [^{14}C]-F2695 and F2695 dose levels administered in each animal were used for all pharmacokinetic calculations. Radiochemical purity of intravenous and oral dosing solutions was determined using HPLC method with on-line radioactivity detection. Levomilnacipran concentrations of the intravenous and oral dosing solutions were determined using a HPLC method with UV detection. LC-MS/MS and/or LC/ESI-MS/MS methods were used for determination of the concentrations of levomilnacipran and the metabolites. Total radioactivity level (plasma, urine and cage wash, blood and feces) was determined by liquid scintillation counting. Metabolite profiling was done using HPLC method with on-line radioactivity detection and chemical structures of main metabolites were identified or characterized using LC-MS^n method.

Absorption

Single-dose studies: Absorption was rapid in mice, rats and monkeys following oral administration of [^{14}C]-F2695 (single dose of 5 mg/kg) with mean T_{max} of 0.17, 0.25 and 2 hr, respectively. The exposure in terms of AUC was dose-proportional in mice; however, the exposures in rats and monkeys were greater than dose-proportional in the dose range of 5-50 mg/kg (in rats) and 5-30 mg/kg (in monkeys); however, there was no significant gender difference in C_{max} and AUC values in any of the species. The absolute oral bioavailability was greater than 71% in male rats and male monkeys in the dose ranges examined (5-50 mg/kg in rats and 5-30 mg/kg in monkeys). Following intravenous administration of levomilnacipran, the apparent total clearance (CL) and apparent volume of distribution (V_d) were 4.6 L/hr/kg and 5.5 L/kg in rats and 0.2 L/hr/kg and 0.9 L/kg in monkeys, respectively. In mice CL and V_d (estimated following oral dosing) were 4.9 L/hr/kg and 8.7 L/kg, respectively. Following single oral administration of [^{14}C] F2695, ~64% of the radioactivity was recovered (urine and feces) from mice in 0-48 hr, ~93% of the radioactivity was recovered from rats in 0-72 hr, and ~73% of the radioactivity was recovered from monkeys in 0-168 hr. The terminal elimination half life

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($T_{1/2}$) of F2695 was 1.2 hr in mice, 0.8 hr in rats and 3.3 hr in monkeys (study #s CEPC 09-0025, CEPC 09-0026, CEPC 09-0027 and M108).

Levomilnacipran produced detectable plasma exposure of *N*-desethyl levomilnacipran (F17400), a pharmacologically inactive metabolite of levomilnacipran in rats and monkeys (11% to 29% and 30% to 47% of levomilnacipran ACU, respectively). In mice (at 5 mg/kg), the exposure of the metabolite was relatively low (1% to 3% of levomilnacipran ACU). The T_{max} of F17400 was slightly later than that of levomilnacipran in both rats and monkeys and $T_{1/2}$ was longer than levomilnacipran (1.8 hr in rats and 5.9 hr in monkeys; see Table 3 & Table 4, below).

Repeat-dose studies: In mice, following 28-day and 26 week repeated administration, plasma exposures of levomilnacipran were comparable to those on Day 1, indicating no accumulation of levomilnacipran in mice with repeated dosing (study № AC32RU.2G3R.BTL and AC32RU.7G8R.BTL). In rats, plasma exposures following repeated administration were higher than on Day 1, indicating accumulation (study № F2695-22629; M105; 1712-001; 1712-005); however, in female rats, the exposures of levomilnacipran were similar between GD6 and GD17 (study № AA78122). In cynomolgus monkeys, repeated dosing for 90 days or 1 year (study № F2695-19529 and № 1712-006) showed similar C_{max} and AUC values and did not indicate any evidence of accumulation. Exposure of *N*-desethyl levomilnacipran (F17400) was detected in repeat dose studies in rats and monkeys, but was significantly less than levomilnacipran (study № 1712-005 and study № 1712-006). In female rabbits, exposures of levomilnacipran were greater than dose proportional at all doses and exposures on GD19 were higher than on GD6 (study № AA78128).

Table 3 PK parameters of levomilnacipran following single dose administration of [^{14}C]-levomilnacipran

(Excerpted directly from the Pharmacokinetic Written Summary section of this NDA, pp 24)

Species	*Sex*	*Route of Dosing*	*Dose*	*CL or CL/F*	*V or V/F*	*C_{max}/Dose*	*T_{max}*	*AUC_{0-t}/Dose*	*$T_{½}$*	*Renal Clearance*	*F[a]*	*Reference*
			mg/kg	*L/h/kg*	*L/kg*	*ng/mL/mg*	*h*	*h·ng/mL/mg*	*h*	*L/h/kg*	*%*	
Mouse	Male	PO	5	4.9	8.7	137	0.17	201	1.2	1.43	NA	M108
	Male	PO	50	NA	NA	122	0.25	169	15.0	NA	NA	CEPC 09-0026
	Female	PO	50	NA	NA	162	0.25	221	18.0	NA	NA	CEPC 09-0026
Rat	Male	IV	5	4.6	5.5	NA	NA	222	0.8	NA	NA	CEPC 09-0025
	Male	PO	5	NA	NA	162	0.25	159	0.9	NA	71.4	CEPC 09-0025
	Male	PO	50	NA	NA	161	0.5	333	2.3	NA	100	CEPC 09-0025
	Female	PO	50	NA	NA	179	0.25	372	3.4	NA	100[b]	CEPC 09-0025
Monkey	Male	IV	5	0.2	0.9	NA	NA	885	3.3	NA	NA	CEPC 09-0027
	Male	PO	30	0.1	1.1	167	4	1335	6.2	NA	100	CEPC 09-0027
	Female	PO	30	0.3	2.1	176	3	1224	5.9	NA	100[b]	CEPC 09-0027

a no value exceeding 100% is shown.

b referenced to male IV data

AUC = area under the plasma concentration versus time curve; AUC_{0-t} = area under the plasma concentration versus time curve from time zero to time t; C_{max} = maximum plasma drug concentration; F = bioavailability fraction.

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Table 4 PK parameters of F17400 (metabolite) following single dose administration of [^{14}C]-levomilnacipran

(Excerpted directly from the Pharmacokinetic Written Summary section of this NDA, pp 25)

Species	*Sex*	*Route of Dosing*	*Dose*[a]	*C_{max}/Dose*	*T_{max}*	*AUC_{0-t}/Dose*	*$T_{½}$*	*AUC Ratio (F17400/Levomilnacipran)*	*Reference*
			mg/kg	*ng/mL/mg*	*h*	*h·ng/mL/mg*	*h*	*%*	
Mouse	Male	PO	5	NA	NA	NA	NA	NA	M108
	Male	PO	50	1	0.5	2	NA	1	CEPC 09-0026
	Female	PO	50	2	0.5	7	19.5	3	CEPC 09-0026
Rat	Male	IV	5	11	1.0	32	1.8	14	CEPC 09-0025
	Male	PO	5	19	1.0	46	1.5	29	CEPC 09-0025
	Male	PO	50	8	1.0	44	2.7	13	CEPC 09-0025
	Female	PO	50	7	1.0	41	4.5	11	CEPC 09-0025
Monkey	Male	IV	5	38	3	407	5.9	47	CEPC 09-0027
	Male	PO	30	29	6	421	7.9	32	CEPC 09-0027
	Female	PO	30	27	6	362	7.0	30	CEPC 09-0027

a Levomilnacipran dose

AUC = area under the plasma concentration versus time curve; AUC_{0-t} = area under the plasma concentration versus time curve from time zero to time t; C_{max} = maximum plasma drug concentration.

Distribution

Tissue distribution: Tissue distribution of levomilnacipran was studies in male and female Sprague-Dawley (albino) and male Lister Hooded (pigmented) rats by quantitative whole body phosphor imaging following oral administration of a 5 mg/kg dose of [^{14}C]-levomilnacipran HCl (study № CEPC 09-0028). Following oral administration, the distribution of radioactivity was rapid and showed extensive distribution in all anatomical and biological structures. The general distribution patterns and concentrations of total radioactivity in the male and female albino rats and the male pigmented rats were similar. Distribution and uptake of radioactivity was good in the majority of tissues with tissue/blood ratios greater than 1. Tissues associated with biotransformation and elimination (gastrointestinal tract, liver, and kidneys) tended to have highest maximum concentrations of radioactivity. In addition, the highest radioactivity in the brain was observed in the thalamus. Elimination of radioactivity from tissues was rapid, with decreased tissue levels observed at 24 hr post-dose. In male and female albino and male pigmented rats, elimination of radioactivity from tissues was complete in the majority of tissues by 72 h post-dose. Binding to the melanin of the pigmented tissues was evident with levels in the eye choroid layer and pigmented fur still present at 168 h post-dose.

Plasma protein binding: *In vitro* binding of [^{14}C]-levomilnacipran to human RBC, human plasma proteins and isolated human plasma proteins (human serum albumin, α1- acid glycoprotein, γ- globulin) showed low binding to human plasma proteins (22%), human serum albumin (11%), γ- globulin (4%) and $α_1$-acid glycoprotein (4%) across a concentration range of 1 to 1000 ng/ml (study № CEPC 05-0164).

Metabolism

In vivo metabolism

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Metabolism of levomilnacipran was investigated *in vivo* in mice (study № CEPC 09-0026), rats (study № CEPC 09-0025; study № CEPC 11-0426) and monkeys (study № CEPC 09-0159; study № CEPC 09-0267; study № CEPC 11-0267) following oral administration of [^{14}C]-levomilnacipran and measuring the metabolites in plasma, urine and feces. In general, following the oral administration of [^{14}C]-levomilnacipran to animals, ~70% of the radioactivity was excreted in urine, indicating high oral absorption of levomilnacipran. For metabolite profiling, urine and feces samples were collected at different periods ([0-24] hr, [24-48] hr and 48-72] hr) after dosing and plasma samples were collected at 0.25, 0.5, 1, 2, 4, 6, and 10 hr after dosing. Following oral administration of [^{14}C]-levomilnacipran, the dose was excreted in urine mainly as unchanged levomilnacipran by mice (73% males and 64% females), rats (50% males and 67% females), and monkeys (36% males and 46% females); the remaining levomilnacipran was transformed to its metabolites and excreted.

Following <u>oral administration of levomilnacipran to humans</u>, one major human metabolite [(*N*-desethyl levomilnacipran (F17400)] was present at greater than 10% of the total levomilnacipran exposure. This metabolite was characterized as a pharmacologically inactive metabolite and was the major metabolite identified in all three preclinical species as well. In humans, the ratio of C_{max} and AUC between the major metabolite, *N*-desethyl levomilnacipran and the parent were in the range of 8.5%-14.1% and 11.3%-17.1%, respectively.

Following oral administration of levomilnacipran (50 mg/kg) to male and female mice, levomilnacipran was the major circulating compound accounting for ~75% of the circulating total radioactivity in plasma. The major circulating metabolite was *N*-desethyl levomilnacipran (F17400) and accounted for ~5% of the levomilnacipran plasma exposure (males and females).

Following oral administration of levomilnacipran (30 mg/kg) to male and female monkeys, levomilnacipran was the major circulating compound in plasma up to 10 hr after dosing. The major circulating metabolite was *N*-desethyl levomilnacipran (F17400) and the corresponding plasma exposure accounted for 27.5% (in males) and 33% (in females) of the plasma exposure of the parent drug. Plasma exposure of other metabolites were <3% of levomilnacipran.

Following oral administration of levomilnacipran (50 mg/kg) to male and female rats, levomilnacipran was the major circulating compound (~60%) of the total radioactivity. The major circulating metabolite was *N*-desethyl levomilnacipran (F17400) and the minor metabolite was *p*-hydroxy levomilnacipran glucuronide accounting for 13.4% and 3%, respectively (Table 9 and Table 10 below).

The measured plasma concentrations and excretion of *N*-desethyl levomilnacipran metabolite in mice, rats, and monkeys and are summarized along with the results of a study in humans in Table 5 & Table 6, below. The *N*-desethyl levomilnacipran (F17400) and *p*-hydroxy levomilnacipran metabolites were produced by all three preclinical species. In mice, there was only minimum metabolism of levomilnacipran and only

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small amounts of metabolites were excreted. The *N*-desethyl levomilnacipran (F17400) and *p*-hydroxyl levomilnacipran metabolites were the metabolites excreted by rats, and *N*-desethyl levomilnacipran (F17400) was the major metabolite excreted by monkeys. In rats and monkeys, a small amount of levomilnacipran dose was found in the plasma as levomilnacipran *N*-carbamoyl glucuronide metabolite (1.3% and 4.1% of the dose, respectively) and excreted (Table 13 and Table 14, below). Likewise, a small amount of the *N*-desethyl levomilnacipran metabolite was excreted as the *N*-desethyl levomilnacipran *N*-carbamoyl glucuronide metabolite by rats [(0.6% of the dose as amide linkage) and monkeys (2.3% of the dose as amine and amide linkages); Table 7 and Table 8, below)]. In rats, a small amount of the *p*-hydroxy levomilnacipran metabolite was excreted as the *p*-hydroxy levomilnacipran glucuronide metabolite (~4% of the dose; Table 11 and Table 12, below).

Table 5 Mean plasma concentrations of *N*-Desethyl levomilnacipran metabolite following single oral dose of [^{14}C]-levomilnacipran to mice, rats, monkeys, and humans

(Excerpted directly from the Pharmacokinetic Written Summary section of this NDA, pp 33)

	Plasma Concentrations of N-Desethyl Levomilnacipran Metabolite (ng Equivalent of Levomilnacipran/mL)									
Species	*Mouse*		*Rat*				*Monkey*			*Human*
Study	**CEPC 09-0026***		**CEPC 09-0025***			**CEPC 11-0426**	**CEPC 09-0027***		**CEPC 11-0267**	**CEPC 09-0471**
Dose (mg/kg)	***50***		***5***	***50***		***50***	***30***		***30***	***60 mg***
Sex	*Male*	*Female*	*Male*	*Male*	*Female*	*Male*	*Male*	*Female*	*Male*	*Male*
Time (h)										
0.25	25.5	77.4	62.6	188.2	242.0	NS	NS	NS	NS	NS
0.5	39.1	123.6	87.1	308.8	317.2	NS	NS	NS	NS	NS
1	34.2	86.8	105.1	445.6	409.2	375	252.8	274.2	310	6.6
2	17.1	50.3	52.9	400.4	400.3	408	530.4	530.4	462	10.1
3	NS	NS	NS	NS	NS	NS	NS	NS	NS	16.3
4	8.3	23.6	24.4	361.1	213.6	247	874.6	841.9	707	16.4
6	3.5	9.7	5.4	183.3	165.9	140	966.0	919.7	815	17.1
8	NS	NS	NS	NS	NS	NS	NS	NS	NS	15.9
10	2.4	4.9	1.4	35.1	47.9	16.8	748.2	646.6	623	NS
12	BLQ	NS	BLQ	NS	NS	NS	NS	NS	NS	12.4
24	BLQ	6.6	BLQ	1.9	3.3	NS	163.6	120.8	NS	NC
32	BLQ	0.6	BLQ	BLQ	1.8	NS	NS	NS	NS	NS
48	BLQ	2.0	BLQ	BLQ	BLQ	NS	16.7	8.4	NS	BLQ
72	BLQ	BLQ	BLQ	BLQ	BLQ	NS	2.7	2.1	NS	BLQ

*The concentrations reflect the sum of N-desethyl levomilnacipran metabolite and its glucuronide.
NS = no sample was collected: NC = not calculated; BLQ = below limit of quantification

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Table 6 Excretion of *N*-Desethyl levomilnacipran metabolite following single oral dose of [^{14}C]-levomilnacipran to mice, rats, monkeys, and humans

(Excerpted directly from the Pharmacokinetic Written Summary section of this NDA, pp 34)

	Excretion of N-Desethyl Levomilnacipran Metabolite (% of Dose)								
Species	*Mouse*		*Rat*			*Monkey*			*Human*
Study	**CEPC 09-0026**		**CEPC 09-0025**		**CEPC 11-0426**	**CEPC 09-0027**		**CEPC 11-0267**	**CEPC 09-0471**
Dose (mg/kg)	***50***		***50***		***50***	***30***		***30***	***60 mg***
Sex	*Male*	*Female*	*Male*	*Female*	*Male*	*Male*	*Female*	*Male*	*Male*
Excretion Route									
Urine	0.1	1.9	12.2	9.1	7.9	18.2	23.2	12.4	18.2
Feces	0.3	0.8	0.6	0.3	ND	1.0	0.2	ND	ND
Total	0.4	2.7	12.7	9.4	7.9	19.2	23.4	12.4	18.2

ND = not determined

As in non-clinical species, following oral administration of [^{14}C]-levomilnacipran to humans, the dose was excreted in urine mainly as unchanged levomilnacipran (58.4% of the dose). The *N*-desethyl levomilnacipran (F17400) was the major metabolite excreted by humans (18.2%; Table 6, above). A small amount of the *N*-desethyl levomilnacipran metabolite was excreted as the *N*-desethyl levomilnacipran *N*-carbamoyl glucuronide metabolite (3.2% of the dose as amide linkage). Likewise, a small amount of the levomilnacipran was excreted as the levomilnacipran *N*-carbamoyl glucuronide metabolite (3.8% of dose as amine linkage). Small amounts of *p*-hydroxy levomilnacipran metabolite (0.9% of dose) and its glucuronide (1.2% of dose) were also excreted by humans (Table 7, Table 8, Table 10, Table 12, below). The proposed biotransformation pathways of levomilnacipran in mouse, rat and monkey are shown in Figure 1, below.

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Table 7 Mean plasma concentrations of *N*-desethyl levomilnacipran *N*-carbamoyl glucuronide metabolite following single oral dose of [^{14}C]-levomilnacipran to rats, monkeys and humans

(Excerpted directly from the Pharmacokinetic Written Summary of this NDA, pp 36)

	Plasma Concentrations of N-Desethyl Levomilnacipran N-Carbamoyl Glucuronide Metabolite (ng Equivalent of Levomilnacipran/mL)		
Species	*Rat*	*Monkey*	*Human*
Study	**CEPC 11-0426**	**CEPC 11-0267**	**CEPC 09-0471**
Dose (mg/kg)	***50***	***30***	***60 mg***
Sex	*Male*	*Male*	*Male*
Time (h)			
1	23.7	92.8	7.3
2	26.7	203	13.4
3	NS	NS	23.1
4	25.7	314	28.0
6	9.50	357	27.7
8	NS	NS	25.3
10	2.25	280	NS
12	NS	NS	17.1

NS = no sample was collected

Table 8 Excretion *N*-Desethyl levomilnacipran *N*-carbamoyl glucuronide metabolite following single oral dose of [^{14}C]-levomilnacipran to rats, monkeys, and humans

(Excerpted directly from the Pharmacokinetic Written Summary section of this NDA, pp 36)

	Excretion of N-Desethyl Levomilnacipran N-Carbamoyl Glucuronide Metabolites (% of Dose)		
Species	*Rat*	*Monkey*	*Human*
Study	**CEPC 11-0426**	**CEPC 11-0267**	**CEPC 09-0471**
Dose (mg/kg)	***50***	***30***	***60 mg***
Sex	*Male*	*Male*	*Male*
Excretion Route			
Urine	0.6	2.3	3.2
Feces	ND	ND	ND
Total	0.6	2.3	3.2

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Table 9 Mean plasma concentrations of *p*-hydroxy levomilnacipran metabolite following single oral dose of [^{14}C] levomilnacipran to rats, monkeys and humans

(Excerpted directly from the Pharmacokinetic Written Summary of this NDA, pp 37)

	Plasma Concentrations of p-Hydroxy Levomilnacipran Metabolite (ng Equivalent of Levomilnacipran/mL)				
Species	*Rat*		*Monkey*		*Human*
Study	**CEPC 09-0025**		**CEPC 09-0027**		**CEPC 09-0471**
Dose (mg/kg)	***50***		***30***		***60 mg***
Sex	*Male*	*Female*	*Male*	*Female*	*Male*
Time (h)					
1	NO	NO	NO	NO	5.4
2	NO	NO	83.3	NO	4.7
3	NS	NS	NS	NS	7.8
4	NO	26.6	68.6	NO	6.6
6	12.4	NO	74.0	NO	5.4
10	10.3	NO	NO	84.4	NS

NO = not observed; NS = no sample was collected

Table 10 Excretion of *p*-hydroxy levomilnacipran metabolite following single oral dose of [^{14}C]-levomilnacipran to mice, rats, monkeys, and humans

(Excerpted directly from the Pharmacokinetic Written Summary section of this NDA, pp 38)

	Excretion of p-Hydroxy Levomilnacipran Metabolite (% of Dose)						
Species	*Mouse*		*Rat*		*Monkey*		*Human*
Study	**CEPC 09-0026**		**CEPC 09-0025**		**CEPC 09-0027**		**CEPC 09-0471**
Dose (mg/kg)	***50***		***50***		***30***		***60 mg***
Sex	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*
Excretion Route							
Urine	NO	NO	0.06	NO	1.7	1.4	0.9
Feces	0.01	0.2	15.7	9.2	0.2	0.10	ND
Total	0.01	0.2	15.76	9.2	1.9	1.5	0.9

NO = not observed; ND = not determined

Table 11 Mean plasma concentrations of *p*-hydroxy levomilnacipran glucuronide metabolite following single oral dose of [^{14}C]-levomilnacipran to rats

(Excerpted directly from the Pharmacokinetic Written Summary of this NDA, pp 39)

	Plasma Concentrations of p-Hydroxy Levomilnacipran Glucuronide Metabolite (ng Equivalent of Levomilnacipran /mL)	
Species	*Rat*	
Study	**CEPC 09-0025**	
Dose (mg/kg)	***50***	
Sex	*Male*	*Female*
Time (h)		
0.25	NO	182.2
0.5	311.2	467.9
1	352.6	544.4
2	344.3	547.1
4	342.4	330.3
6	116.0	211.1
10	86.7	133.6

NO = not observed;

Table 12 Excretion of *p*-hydroxy levomilnacipran glucuronide metabolite following single oral dose of [^{14}C]-levomilnacipran by rats and humans

(Excerpted directly from the Pharmacokinetic Written Summary section of this NDA, pp 39)

	Excretion of p-Hydroxy Levomilnacipran Glucuronide Metabolite (% of Dose)		
Species	*Rat*		*Human*
Study	**CEPC 09-0025**		**CEPC 09-0471**
Dose (mg/kg)	***50***		***60 mg***
Sex	*Male*	*Female*	*Male*
Excretion Route			
Urine	3.7	4.3	1.2
Feces	NO	NO	ND
Total	3.7	4.3	1.2

NO = not observed; ND = not determined

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Table 13 Plasma concentrations of levomilnacipran *N*-carbamoyl glucuronide metabolite following single oral dose of [^{14}C] levomilnacipran in rats, monkeys and humans

(Excerpted directly from the Pharmacokinetic Written Summary of this NDA, pp 41)

	Plasma Concentrations of Levomilnacipran N-Carbamoyl Glucuronide Metabolite (ng Equivalent of Levomilnacipran/mL)		
Species	*Rat*	*Monkey*	*Human*
Study	**CEPC 11-0426**	**CEPC 11-0267**	**CEPC 09-0471**
Dose	***50 mg/kg***	***30 mg/kg***	***60 mg***
Sex	*Male*	*Male*	*Male*
Time (h)			
1	46.6	163	10.7
2	49.6	152	14.2
3	NS	NS	17.9
4	16.2	194	15.5
6	1.96	225	13.9
8	NS	NS	9.4
10	0.7	66	NS
12	NS	NS	3.6

NS = no sample was collected

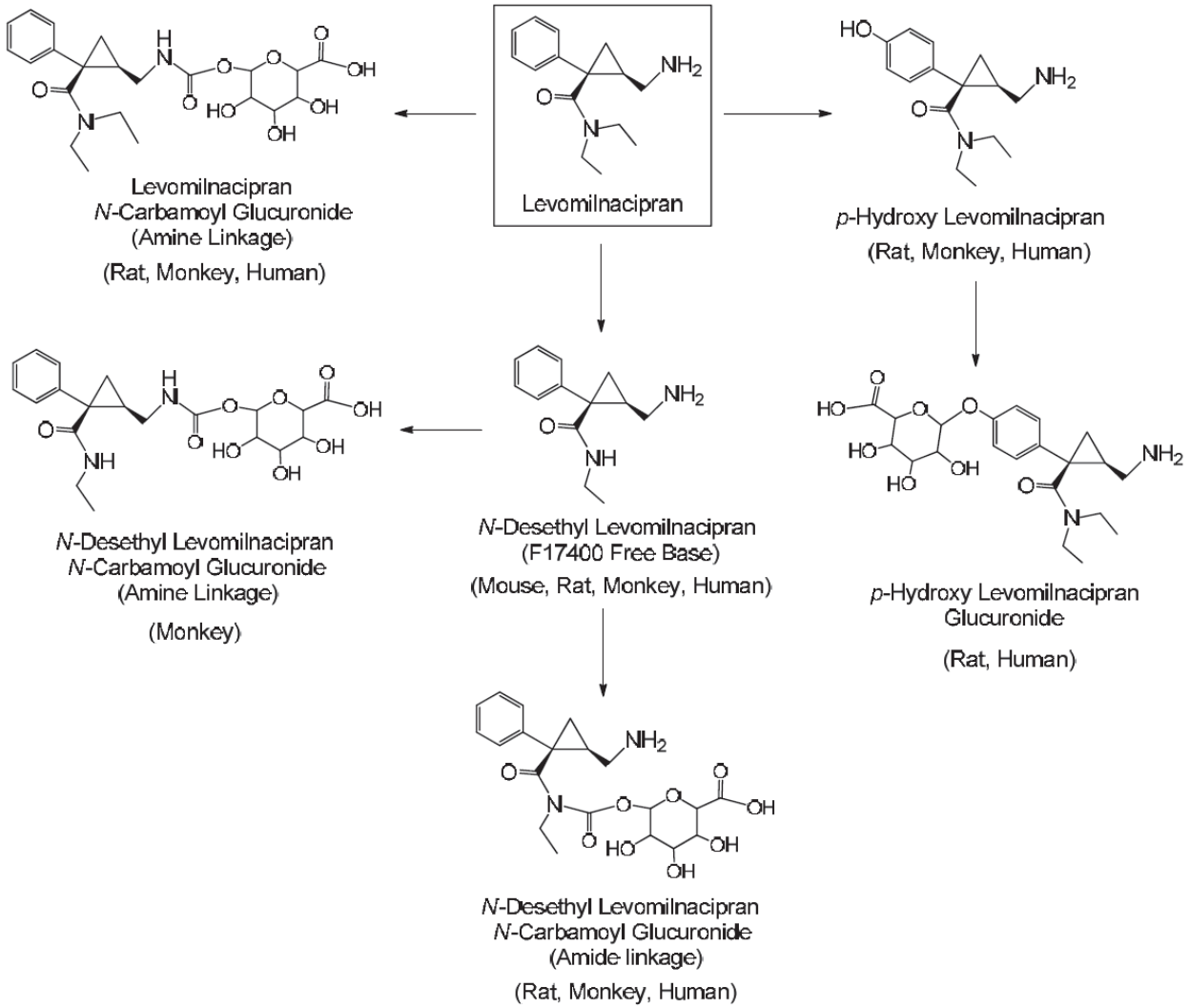
Table 14 Excretion of levomilnacipran *N*-carbamoyl glucuronide metabolite following single oral dose of [^{14}C] levomilnacipran in rats, monkeys and humans

(Excerpted directly from the Pharmacokinetic Written Summary of this NDA, pp 41)

	Excretion of Levomilnacipran N-Carbomyl Glucuronide Metabolite (% of Dose)		
Species	*Rat*	*Monkey*	*Human*
Study	**CEPC 11-0426**	**CEPC 11-0267**	**CEPC 09-0471**
Dose	***50 mg/kg***	***30 mg/kg***	***60 mg***
Sex	*Male*	*Male*	*Male*
Excretion Route			
Urine	1.3	4.1	3.8
Feces	ND	ND	ND
Total	1.3	4.1	3.8

ND = not determined

Figure 1 Proposed biotransformation pathways of levomilnacipran in mouse, rat, and monkey (Excerpted directly from the Pharmacokinetic Written Summary section of this NDA, pp 42)



In summary, following oral administration of levomilnacipran to mice, rats and monkeys, all four levomilnacipran metabolites present in human plasma were observed in the rat and monkey plasma; however, only the *N*-desethyl levomilnacipran metabolite (F17400) was the only metabolite observed in the mouse plasma. Following the oral administration of levomilnacipran to mice (50 mg/kg), rats (50 mg/kg), and monkeys (30 mg/kg), the plasma concentration of the major metabolite, *N*-desethyl levomilnacipran (F17400) was ~7-fold higher in mice, ~26-fold higher in rat, and ~56-fold higher in monkeys than found in human plasma at 60 mg oral dose. In rats, plasma concentrations of levomilnacipran *N*-carbamoyl glucuronide and *N*-desethyl levomilnacipran *N*-carbamoyl glucuronide metabolites were similar to those of human plasma (at 60 mg oral dose); however, in monkeys the plasma concentrations of these two metabolites were approximately 12-fold higher than those of the human plasma.

In humans, following single-dose or multiple dose administration of levomilnacipran, the ratios of C_{max} and AUC between the major metabolite (*N*-desethyl levomilnacipran) and

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the parent compound were in the range of 8.5%-14% and 11%-17%, respectively. Thus, the interspecies comparison of metabolism indicates that the exposure of all metabolites in humans is supported by animal safety studies.

In vitro metabolism

Metabolism of levomilnacipran by human liver microsomes, human hepatocytes, and recombinant human CYP enzymes was slow and limited. Following the incubation of levomilnacipran with liver microsomes for 1.5 hr, less than 2% of levomilnacipran substrate was metabolized. Similarly, low metabolism was observed with human hepatocyte cultures for 2 days. Moreover, biotransformation was low following long incubation period (120 min) with cDNA-expressed human CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2 E1, and 3A4 enzymes (study № PK07MXH1).

Daily treatment (for three consecutive days) of freshly isolated human hepatocytes with 1 to 10 μM of levomilnacipran did not induce the enzyme activities of CYP1A2, 2C9, 3A4/5, and UGT1A6. Likewise, there was no significant inhibition on CYP1A2, 2A6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4/5 (midazolam, testosterone, and nifedipine as substrates) enzymes in human liver microsomes when evaluated by pre-incubation of levomilnacipran with and without NADPH; however, (at 10 μM) there was a slight (20%) inhibition of CYP3A4/5 with pre-incubation with NADPH only when nifedipine was the substrate (Study № CEPC 05-0174).

The inhibition potential of the major metabolite, *N*-desethyl levomilnacipran (F17400; at 0.1 to 100 μM) on CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4/5 (midazolam and testosterone as substrates) enzymes was also evaluated in a pool of human liver microsomes; no significant inhibition was observed, except a slight (~23%) inhibition CYP3A4/5 at a high concentration of 100 μM (Study № XT105102).

Excretion

The elimination of [^{14}C]-levomilnacipran was rapid in both male mice and male monkeys following single oral administration of 5 mg/kg of [^{14}C]-levomilnacipran. The radioactivity of the administered dose was mainly recovered in urine and the terminal half life was 1.2 hr in mice and 3.3 hr in monkeys. In mice, ~ 34% of the administered dose was excreted in urine as unchanged levomilnacipran and ~14% of the dose was excreted in feces. Similarly, in monkeys the administered dose was mainly excreted in urine, however, only ~8% was excreted as unchanged levomilnacipran (study № M108; study № M106).

Following administration of [^{14}C]-levomilnacipran at higher doses in mice (study № CEPC 09-0026), rats (№ CEPC 09-0025), and monkeys (№ CEPC 09-0027), majority of the radioactivity was excreted rapidly (within 24 hr post-dose) in urine in all three species (80% in monkeys and 90% in mice and rats); the rests of the radioactivity of the dose was recovered in feces and cage. The excretion of the radioactive materials by

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these three preclinical species is comparable to the results obtained in humans (Table 15, below).

Excretion in milk

The transfer of levomilnacipran and its metabolites to milk was evaluated after administering a single oral dose (5 mg/k) of [^{14}C]-levomilnacipran to lactating female rats (study № CEPC 10-0002). A small portion of the dose was secreted into milk; the highest concentration of radioactivity was observed at 0.5 hr post-dose (2674 ng eq/mL) and decreased rapidly to 44 ng eq/mL at 24 hr post-dose. Small amount of *N*-desethyl levomilnacipran metabolite (F17400) was also observed in the milk (331 ng eq/mL) at 0.5 hr post-dose.

Table 15 Excretion of radioactive materials following single oral dose of [^{14}C]-levomilnacipran to mice, rats, monkeys and humans

(Excerpted directly from the Pharmacokinetic Written Summary section of this NDA, pp 46)

Species	*Dose (mg/kg)*	*Sex*	*Excretion (% of Dose)*			
			Urine	*Feces*	*Cage* [a]	*Total*
Mouse	50	Male	71.2	14.7	7.3	93.1
	50	Female	65.0	17.6	12.6	95.2
Rat	50	Male	66.6	27.5	4.1	98.2
	50	Female	79.3	16.3	3.3	98.8
Monkey	30	Male	64.5	7.8	20.7	93.0
	30	Female	67.7	3.8	13.8	85.3
Human	60 mg	Male	93.6	3.8	NA	97.4

a The radioactivity recovered in the cage represents the sum of cage wash, cage rinse, and cage wipe
NA = not applicable

Pharmacokinetic drug-drug interaction

No *in vivo* drug-drug interaction studies were performed. The following *in vitro* drug-drug interaction study results are obtained from the Sponsor's PK Written Summary section (individual study reports were not provided in this NDA submission; however, individual studies were referenced in the PK Written Summary).
In the *in vitro* drug-drug interaction studies, Caco-2 cell model was used to evaluate the potential of levomilnacipran being a P-glycoprotein (P-gp) substrate. The bi-directional ratio at the levomilnacipran concentrations of 1.6 µM and 17.8 µM was ~4 and this ratio decreased to 2.3 at the higher concentration of 160 µM, indicating that levomilnacipran is a weak P-gp substrate. Using Caco-2 cell model, the inhibitory effects of levomilnacipran (concentration range of 10-480 µg/mL or 40 to 1950 µM) on P-gp were evaluated; the IC50 for levomilnacipran was ~104 µg/mL (423 µM), showing that levomilnacipran is a poor inhibitor of P-glycoprotein. The potential of levomilnacipran being a BCRP (breast cancer resistant protein) substrate was evaluated using Caco-2 cell model ([3H]-E3S, a known BCRP substrate was used as a probe to determine the activity of BCRP). The efflux ratio at levomilnacipran concentration of 1.4 µM was 3.9 (a value identical to the P-gp substrate activity, and therefore attributable to P-gp substrate

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activity), indicating that levomilnacipran was not a BCRP substrate. The same Caco-2 cell model was used to evaluate the inhibitory potential of levomilnacipran at 100 and 200 µM. In the absence of levomilnacipran, the E3S efflux ratio was 16.5 and this did not change significantly at 100 and 200 µM of levomilnacipran, indicating that levomilnacipran is not an inhibitor of BCRP.

The potential of levomilnacipran to be a substrate of the human organic anion transporters 1 (OAT1) and 3 (OAT3), human organic anion transporting polypeptide 1B1 and 1B3 (OATP1B1 and OATP1B3), and organic cation transporter 2 (OCT2) was evaluated using MDCK-II cells expressing the particular transporter or a control vector. The net transporter-mediated uptake of levomilnacipran was not changed at levomilnacipran concentrations of 0.56, 1.4 and 14 µM, indicating that levomilnacipran is not a substrate of OATP1B1, OATP1B3, OAT1, OAT3, and OCT2. The above model system was used to evaluate the inhibitory potential of levomilnacipran on OAT1, OAT3, OATP1B1, OATP1B3, and OTC2; levomilnacipran at a concentration of 140 µM did not significantly inhibit the activity of OATP1B1, OATP1B3, OAT1, and OAT3; however, the activity of OCT2 was reduced by ~77%.

The *N*-desethyl levomilnacipran (F1400), the major metabolite of levomilnacipran was evaluated for being a substrate or inhibitor of P-gp (at concentrations of 1.8, 20.1, and 183.2 µM in Caco-2 cells), OAT1, OAT3, and OCT2 (at concentrations of 0.7, 7, and 70 µM in MDCK-II cells). The *N*-desethyl levomilnacipran (F1400) was neither a substrate nor inhibitor for P-gp, OAT1, OAT3, and OCT2.

5.2 Toxicokinetics

Toxicokinetic properties of levomilnacipran are included in general toxicology studies.

6 General Toxicology

6.1 Single-Dose Toxicity

The toxicological potential of levomilnacipran following single oral dose was evaluated in rats and mice. Clinical signs of acute toxicity were tremors and convulsions. The approximate lethal dose was 140 mg/kg in mice and 215 mg/kg in rats. The calculated LD_{50} dose was 270 mg/kg in mice and 238 mg/kg in rats (see Table 16 and Table 17, below).

Table 16 Acute toxicity and LD_{50} dose for levomilnacipran in Sprague-Dawley rats (Values compiled by the Reviewer from study № T065)

Species/strain	ROA	LD_{50} values (M and F combined), mg/kg	Findings
			≥140 mg/kg; convulsions, 140 mg/kg; death (20% mortality)

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Species/strain	ROA	LD_{50} values (M and F combined), mg/kg	Findings
Mouse/Swiss	Oral gavage	270	≥236 mg/kg; prostration; 236 mg/kg; death (40% mortality) ≥308 mg/kg; hypomotricity 308 mg/kg; death (80% mortality) ≥400 mg/kg; death (100% mortality)

Table 17 Acute toxicity and LD_{50} dose for levomilnacipran in Swiss mice (Values compiled by the Reviewer from study № T090)

Species/strain	ROA	LD_{50} values (M and F combined), mg/kg	Findings
Rat/Sprague-Dawley	Oral gavage	238	215 mg/kg; ↓ BW (F, 24%); death (30% mortality) ≥215 mg/kg; subdued behavior, prostration; ≥261 mg/kg; tremors; 261 mg/kg; death (70% mortality) 316 mg/kg; tremors; death (90% mortality) ≥ 464 mg/kg; clonic convulsions; death (100% mortality). -congested lungs and intestine (deceased).

6.2 Repeat-Dose Toxicity

Summary of short-term repeat dose studies (1-week, 4-weeks and 13-weeks) in rats:

Rat sub-chronic studies of 1, 4, and 13 week duration were reviewed by this Reviewer under IND 104,483 (SDN-002 and SDN-004) and are summarized below.

F2695- One week oral (gavage) dose range-finding study in the rat (study № 314/518; Sponsor's code: T068):

The objective of this non-GLP study was to evaluate the general tolerance of F2695 in the Sprague-Dawley rats following daily oral (gavage) administration for 8 consecutive days and to select dose levels for a subsequent 4 week toxicity study. F2695 was administered by oral gavage to Sprague-Dawley rats (5/sex/dose) for 8 consecutive days at the dose levels of 75, 100 and 150 mg/kg/d (F2695, batch № PL 01 221). Morbidity/mortality, clinical signs, body weight, food consumption and gross pathology parameters evaluated.

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There were no deaths in this study. Treatment-related clinical signs included hypersalivation in all animals in both sexes at all three doses. There were no treatment-related effects on body weights and food consumption. There were no treatment-related macroscopic findings. The dose levels of up to 150 mg/kg/d was well tolerated and could be chosen as the high dose for the 4 week toxicity study.

F2695- 4 week oral (gavage) toxicity study in the rat (study № 314/516; Sponsor's code: T070):

The objective of this GLP study was to determine the toxicity of F2695 following daily oral administration to rats for 4 consecutive weeks. F2695 was administered by oral gavage to Sprague-Dawley rats (10/sex/dose) for 4 weeks at the dose levels of 10, 35 and 120 mg/kg/d (F2695, batch № PL 01-221). Morbidity/mortality, clinical signs, body weight, food consumption, ophthalmology, clinical pathology, gross pathology and histopathology parameters were evaluated.

There were no deaths in this study. Hypersalivation was observed (lasting for several minutes, post-dose) in all high dose animals during the entire study period and in majority of mid dose females during weeks 3 and 4 of the study. Slight, but statistically significant decrease in body weight gain in mid dose (↓5-7%) and high dose (↓5-9%) males (only) correlated with decreased food consumption (10 to 16% in MDM and 10 to 17% in HDM, respectively). There were no treatment-related effects on ophthalmology, hematology and clinical chemistry parameters. Minimal decreases (↓10 to 30%, relative to body weight) in organ weights (adrenal, liver, spleen and heart) were noted in MDM and HDM; however, without microscopic correlates. There were no macroscopic findings at necropsy. Treatment-related microscopic findings included <u>centrilobular hypertrophy (slight) of the liver in 8/10 HDM and 4/10 HDF and minimal centrilobular hypertrophy and vacuolation of the liver in 1/10 MDM</u>.

13-week toxicity study by the oral route (gavage) in rats followed by a 4-week recovery period (study № 932; Sponsor's code: F2695-22629):

The purpose of this GLP study was to investigate the toxicity of F2695 when given daily to rats for 13 consecutive weeks and followed by a 4-wek recovery period. F2695 was administered to four groups of 15 M and 15 F Sprague-Dawley rats daily at 0, 10, 35 and 120 mg/kg/d (F2695, batch № LP 110) over a 13-week period. At the end of the treatment period, 5 animals/sex/group were kept for a 4-week recovery period. Morbidity/mortality, clinical signs, body weight, food/water consumption, ophthalmology, clinical pathology, gross pathology and histopathology parameters were evaluated. Plasma kinetic parameters were also determined at the end of the treatment period.

There were two unexplained deaths- one MDM (35 mg/kg/d) on Day 32 and one HDF (120 mg/kg/d) on Day 52, both of which may be attributable to treatment-related causes. Treatment-related clinical findings were salivation in all HD animals and in some mid dose animals. Other treatment-related effects were decreased body weight gain and food consumption (~10%), slightly elevated ALT and ALP levels, and centrilobular

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hypertrophy of the liver (reversible) in HDF. In males, treatment-related effects were centrilobular hypertrophy of the liver (reversible) and increased urinary sodium and chloride outputs [although there was reduction in body weight gain (~9% in one study and 13% in a second study) in HDM in the 4-week studies, this was not replicated in the 13-week study].

TK analysis indicated that all treated animals were exposed to F2695. In males, the T_{max} occurred at 0.33 h on Day 1 and Week 13; in females, the T_{max} occurred at 0.33-1 hr on Day 1 and Week 13. The systemic exposure (AUC) was greater than dose proportional in both males and females on Day 1 and Week 13; the exposure was slightly higher in females than males at all dose levels on Day 1 and Week 13. The exposure after repeated administration was slightly higher during Week 13 compared to Day 1, indicating some accumulation.

Chronic Toxicity Study in Rats

Study title: F2695: A 6-month oral toxicity study in rats with a 4-week recovery

Study no.:	1712-005
Study report location:	eCTD
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 7, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Levomilnacipran HCl, lot #L0003039; 100.1 (% m/m) purity

Key Study Findings

Levomilnacipran (F2695) was administered orally to male and female rats at 0, 10, 30, and 120/100 (M/F) mg/kg/d for 6 months. Treatment-related findings were:

- Death of one HDM (on study day 50)
- Clinical signs at the high dose such as decreased activity, salivation, hunched posture, and hair discolored yellow
- Significant reduction in body weight/body weight gain in HDM
- Increased liver weight in HDM with histopathological correlates
- Minimal to mild centrilobular hepatocyte hypertrophy (9/11) in HDM
- The NOAEL 30 mg/kg/d (males), based on minimal to mild centrilobular vacuolation of hepatocytes at 120 mg/kg/d and 100 mg/kg/d (females), based on no adverse toxicity up to the highest dose.
- The doses used are considered adequate, based on treatment-related decrease in body weights/body weight gains in HDM (~13%, compared to control; in addition, the high dose of 120 mg/kg/d represents approximately one-half the calculated acute median lethal dose (LD_{50}) of 238 mg/kg .

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Methods

Doses:	0, 10, 30, and 120 (males)/100 (females) mg/kg/day (see Table 18, below)
Frequency of dosing:	Once a day
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	Solution/distilled water
Species/Strain:	Rats/CD® [Crl:CD® (SD)]
Number/Sex/Group:	15/sex/group
Age:	~6 weeks old
Weight:	Males: 266 to 352g; females: 177 to 237g
Satellite groups:	9/sex/group (treatment) and 3/sex (control)
Unique study design:	None
Deviation from study protocol:	No significant deviations that would affect the study outcome

Table 18 Chronic toxicity study in rats; study design

(Excerpted directly from the study report 1712-005; pp 17)

Group Assignments			
Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male	Female
Main Study			
1	0	15[c]	15[d]
2	10	15[c]	15[c]
3	30	15[c]	15[c]
4	120/100[a]	15[d]	15[c]
Toxicokinetic			
5	0	3 + 3[b]	3 + 3[b]
6	10	9 + 2[b]	9 + 2[b]
7	30	9 + 2[b]	9 + 2[b]
8	120/100[a]	9 + 2[b]	9 + 2[b]

[a]Male animals were administered 120 mg/kg/day; females were administered 100 mg/kg/day.
[b]Additional animals included as replacement animals.
[c]Five animals were evaluated during the 4 week recovery period.
[d]Due to two animals that died on study (one male on Day 50 and one female on Day 41), only four animals were evaluated during the 4 week recovery period.

Observations and Results

Mortality- Observed for mortality/morbidity at least twice daily.

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Results: One HDM was found dead on Day 50 (#5051); the Sponsor could not determine the cause of death after microscopic examination (Reviewer note: no adverse clinical signs or body weight changes were observed in this particular animal). One control female (#5113) was found dead on Day 41; according to the Sponsor, the cause of death was accidental injury, most likely due to gavage trauma. One HDM in the TK group was replaced on Day 4; however, the Sponsor did not state the reason for doing so.

Clinical Signs- Detailed clinical examination performed once weekly. On occasion, clinical observations were recorded at unscheduled intervals.
Results: Treatment-related clinical signs were observed mainly in HDM and HDF and included decreased activity, salivation, hunched posture (only in 3/15 females), and discolored yellow (ventral surface); notable clinical signs are listed in Table 19, below.

Table 19 Detailed clinical findings during treatment with F2695

(Excerpted directly from the study report 1712-005; pp 29)

Notable Detailed Clinical Observations[a] Treatment Period											
		F2695 Dose (mg/kg/day)									
Clinical Observation	Study Weeks	0		10		30		100		120	
		M	F	M	F	M	F	--	F	M	--
Salivation	1, 2, 4, 8-12, 18, 21-26	0	0	0	0	0	0	--	17/3	11/4	--
Hair discolored, yellow	7-26	0	0	0	0	0	5/1	--	31/6	13/1	--
Hunched Posture	3, 7-13	0	0	0	0	0	0	--	9/3	0	--
Decreased Activity	1	0	0	0	0	0	0	--	15/15	15/15	--

[a]Number of times observed/Number of animals affected
M - Male
F - Female

During the recovery period, only discolored yellow (ventral surface) was noted, although less frequently in MDF and HDF.

Body Weights- recorded weekly; weekly body weight change was calculated relative to previous weight; total body weight change at Week-26 was calculated relative to the Week- 1 body weight.

Results: Significant **reduction in BW and BWG of HDM**; overall, the mean body weights were statistically significantly lower (-6 to 13%) than control from weeks 2 to 26 as illustrated in Figure 2, below. However, the mean body weights of HDF were comparable to control (see Figure 3, below).

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During the **recovery period, BW gain in HDM was significantly higher** than control group (56 g in HDM versus 4.4 g in control); however, at the end of the recovery period, the mean BW in HDM were lower by 7.2% (not statistically significant) compared to the control group (see Figure 4, below).

Figure 2 Sponsor's figure (with minor formatting modifications by the Reviewer) showing decrease in body weight gain in HDM rats

(Star, solid line) compared with control (square, solid line); study report 1712-005, pp 44

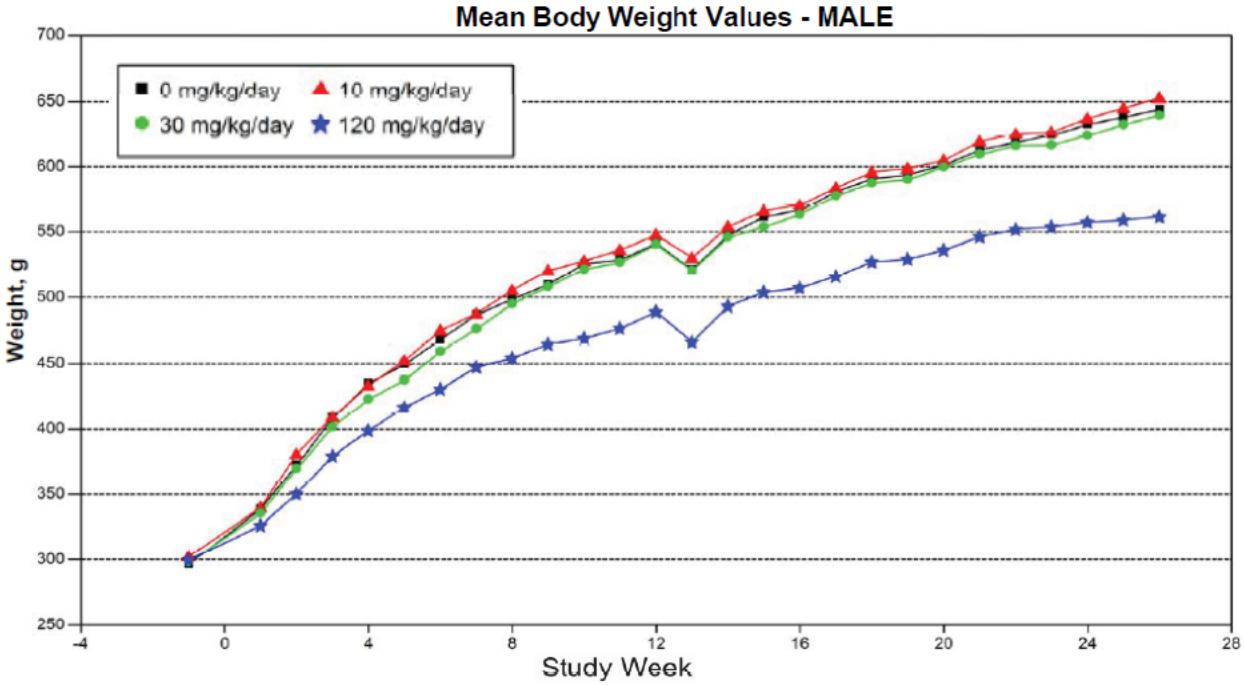


Figure 3 Sponsor's figure (with minor formatting modifications by the Reviewer) showing no significant changes in body weight gain in HDF rats

(Star, solid line) compared with control (square, solid line); study report 1712-005, pp 45

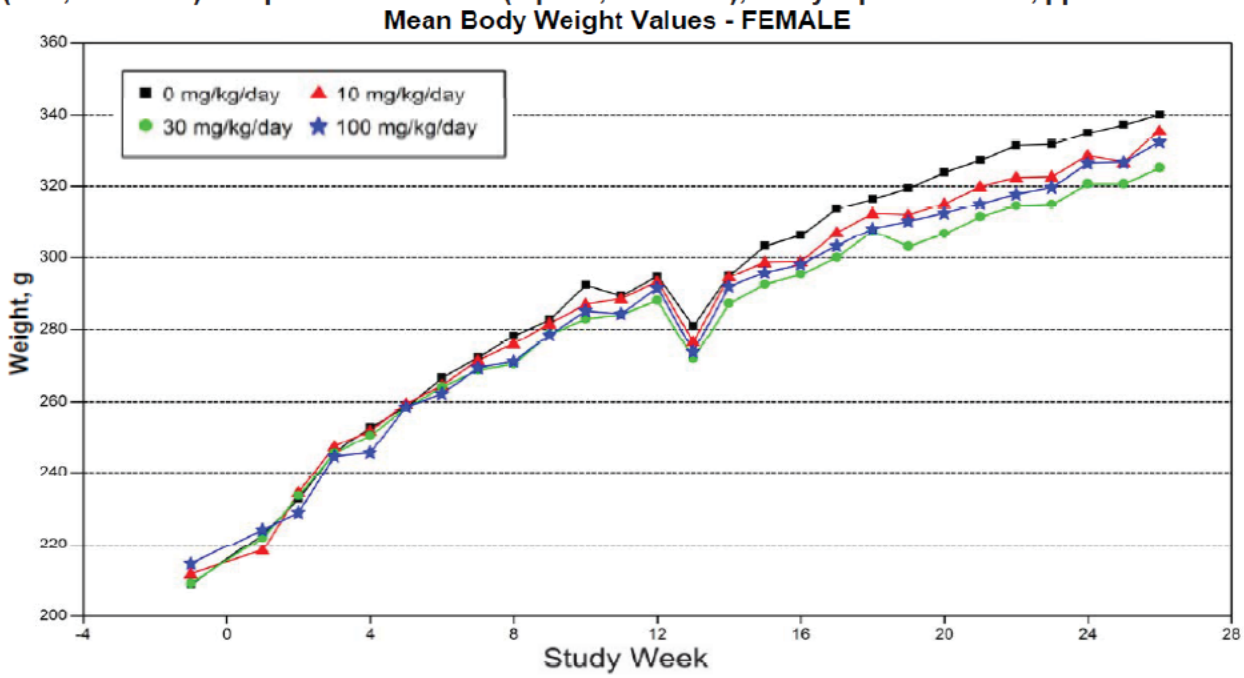
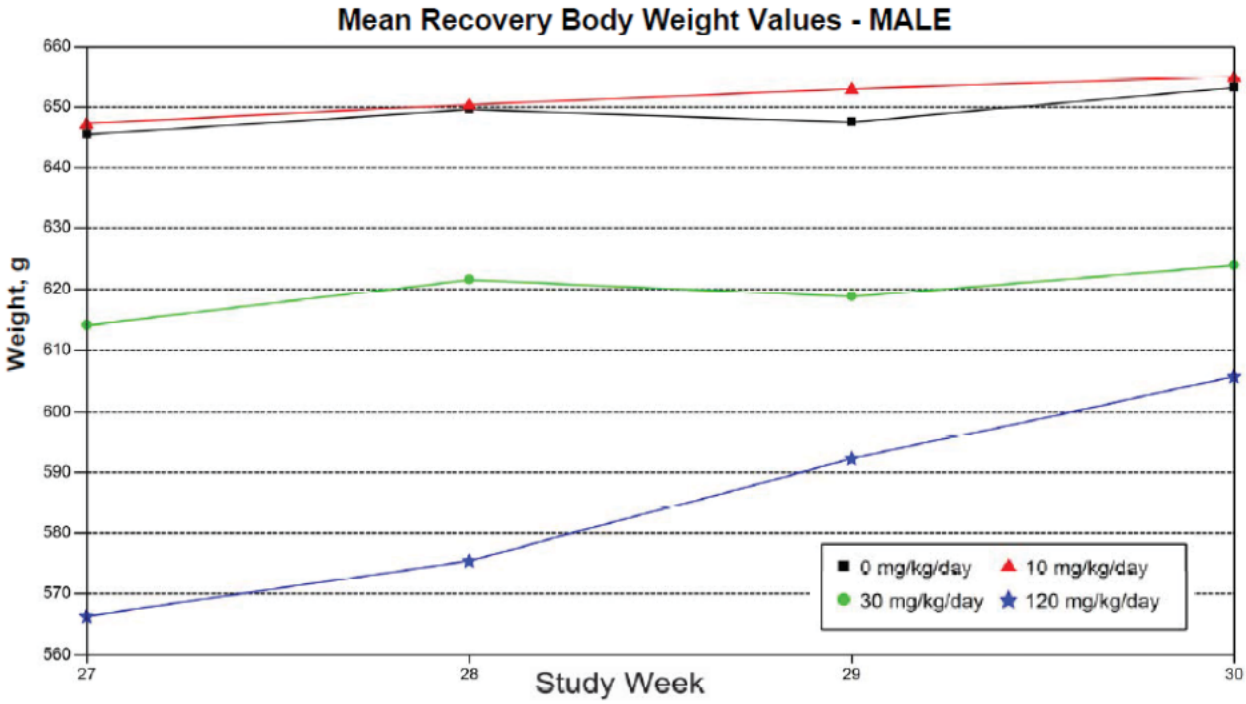


Figure 4 Sponsor's figure (with minor formatting modifications by the Reviewer) showing recovery of body weight gain in HDM rats

(Star, solid line) compared with control (square, solid line); study report 1712-005, pp 46



Feed Consumption- measured weekly; due to group housing, food consumption was measured as a weekly estimated mean value for each animal.

Results: Lower mean food consumption in HDM throughout the study (~5-11%; see Figure 5, below) which correlated with decrease in BW/BW gain. However, food consumption in HDF was only slightly lower than control throughout the study except at Weeks 1, 3, 8, 10, and 22, when the decreases were significant and statistically meaningful (~7-10%, compared to control; see Figure 6, below).

During the recovery period, mean food consumption was higher in HDM than control group (~5-18%), although the increases were not statistically significant, except at Week 27 (see Figure 7, below). On the other hand, mean food consumption in HDF was similar to control throughout the recovery period.

Figure 5 Sponsor's figure (with minor formatting modifications by the Reviewer) showing decrease in food consumption in HDM rats

(Star, solid line) compared with control (square, solid line); study report 1712-005, pp 51

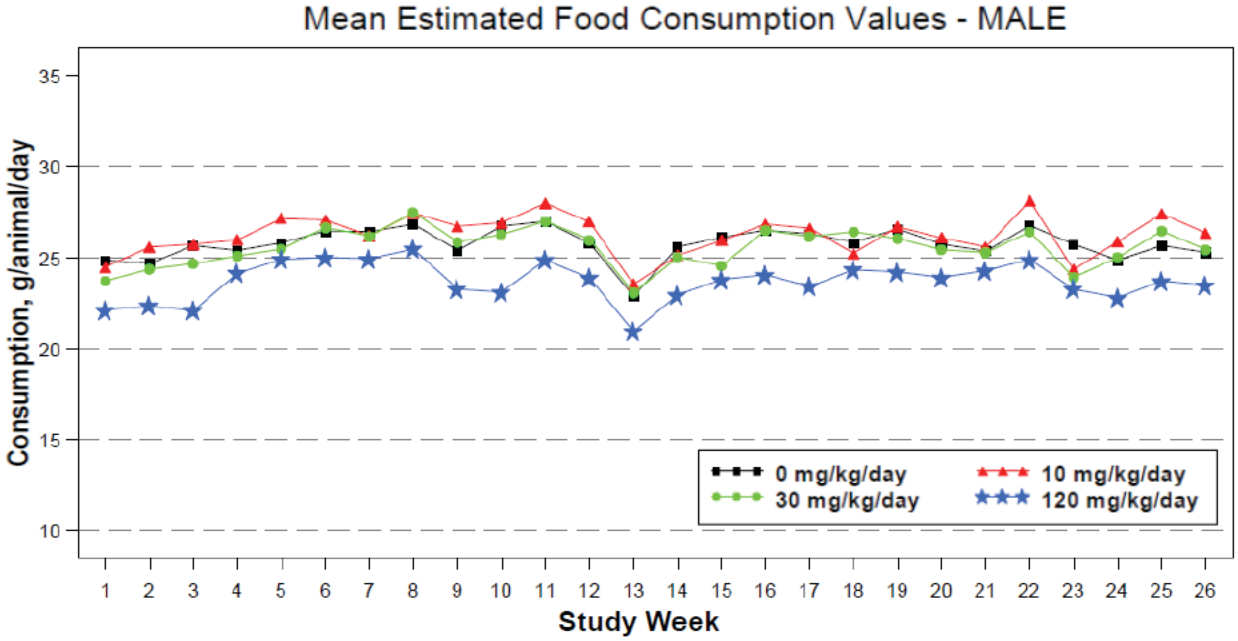


Figure 6 Sponsor's figure (with minor formatting modifications by the Reviewer) showing no apparent changes in food consumption in HDF rats

(Star, solid line) compared with control (square, solid line); study report 1712-005, pp 52

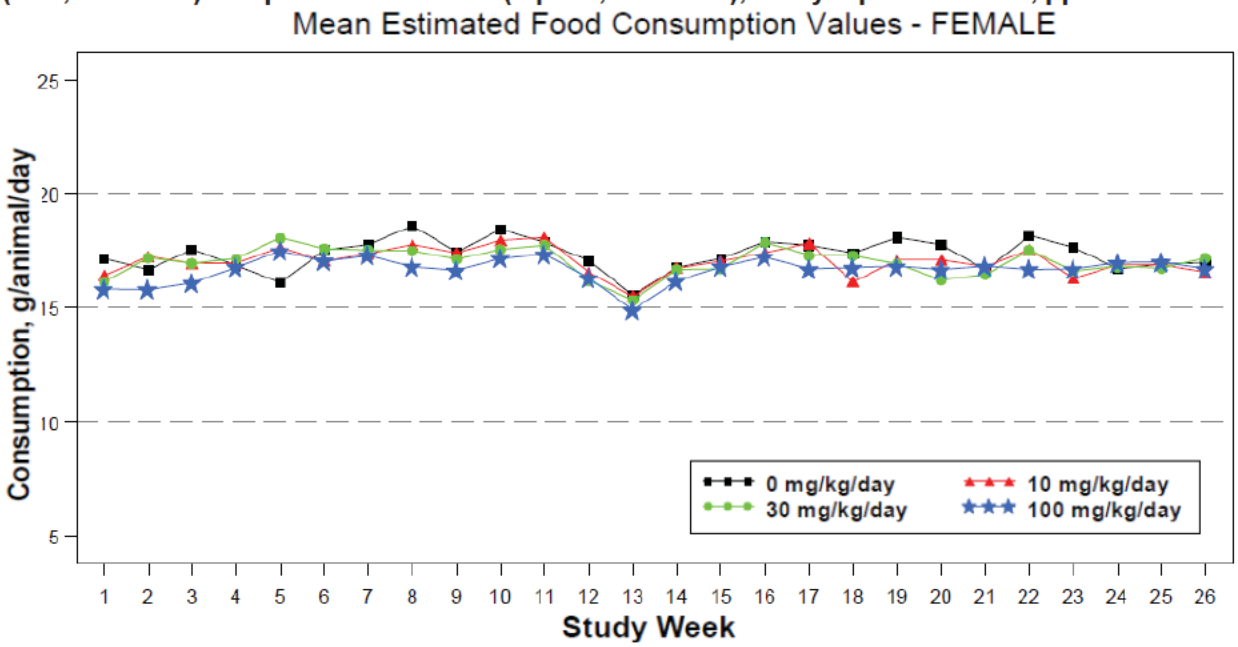
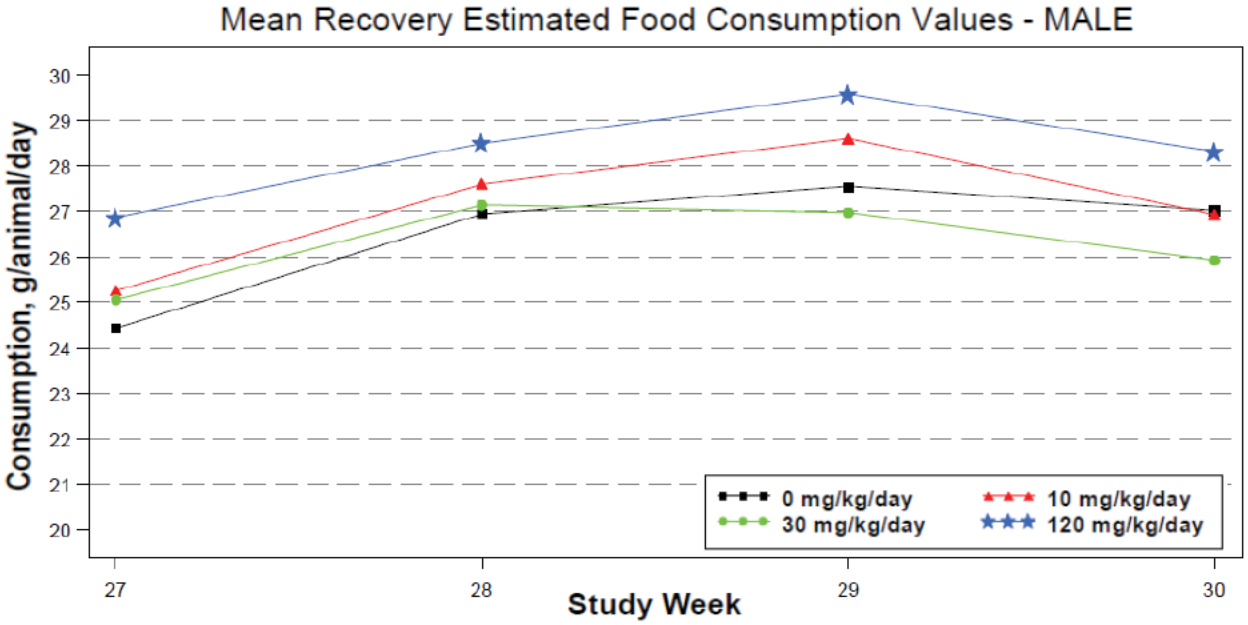


Figure 7 Sponsor's figure (with minor formatting modifications by the Reviewer) showing increased food consumption in HDM rats during recovery

(Star, solid line) compared with control (square, solid line); study report 1712-005, pp 53



Ophthalmoscopy- The examinations were conducted before the start of treatment and at Week 26 (main study group) and Week 30 (recovery group); 15/sex/group (main) and 5/sex/group (recovery)

Results: No treatment-related changes were observed

Clinical Pathology:

Clinical pathology evaluations conducted on all surviving main study animals at Week 13 and prior to scheduled terminal (Day 183) or recovery (Day 211) necropsies. Week 13 blood samples (3.0 mL) were collected via the orbital sinus, and terminal and recovery blood samples (4.0 mL) were collected from the abdominal vena cava.

Hematology: Blood collected into tubes containing K3EDTA (for hematology) and sodium citrate (for coagulation) and the following parameters were evaluated at Weeks 13 (15/sex/group), 26 (10/sex/group) and 30 (5/sex/group): WBC (leucocytes), RBC (erythrocytes), Hb, HCT, MCV, MCH, MCHC, PLT, ARC, NE, band NE, LYM, MN, EO, Bas and other cells.

> Results: No treatment-related changes in hematology and coagulation parameters were observed.

Clinical Chemistry: Blood collected into tubes containing serum separator and the following parameters were evaluated at Weeks 13 (15/sex/group), 26 (10/sex/group) and 30 (5/sex/group): Na, K, Cl, P, AP, TBIL, GGT, AST, ALT, SODH, BUN, Creat, TP, ALB, Glb, ALB/Glb ratio, Chol and GLUC.

> Results: No treatment-related changes in clinical chemistry parameters were observed.

Urinalysis: Animals were housed in stainless steel metabolism cages and urine was collected for at least 12 hr; the following parameters were evaluated at Weeks 13, 26 and 30: volume, specific gravity and pH.

> Results: Increase in urine volume was observed in HDM and HDF at Week 13 (↑47% and 113%, respectively) and Week 26 (↑173% and 94%, respectively; the Sponsor was uncertain if the effects were treatment-related. Since the urine volume returned to control levels at the end of recovery period, the volume changes may be related to treatment.

Gross Pathology- At the end of the study (terminal and recovery[6]), rats were necropsied and examined for macroscopic pathological findings.

Results: There were no treatment-related macroscopic findings

[6] Due to early deaths of one female in group 1 and one male in group 4, the number of animals included in the recovery phase was reduced to maintain the 10/sex/group in the main study necropsy.

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Organ Weights- Organ weights (see Table 20, below) were recorded for all main study animals at the scheduled necropsies and organ weight ratios were calculated (relative to body and brain weights).

Table 20 List of full complement of organs and tissues from the 6-month rat study (Excerpted directly from the study report 1712-005, pp 669)

- Adrenal (2)*
- Aorta
- Bone with marrow [femur]
- Bone with marrow [sternum]
- Bone marrow smear [2 collected][a]
- Brain [cerebrum, midbrain, cerebellum, medulla/pons]*
- Epididymis (2)
- Eye including optic nerve (2)
- Gastrointestinal tract:
  - esophagus
  - stomach [glandular and nonglandular]
  - duodenum
  - jejunum
  - ileum
  - cecum
  - colon
  - rectum
- Gonads:
  - ovary (2)* with oviduct (2)
  - testis (2)*
- Gross lesions
- Heart*
- Joint, tibiofemoral
- Kidney (2)*
- Lacrimal gland, exorbital (2)
- Larynx
- Liver [3 sections collected; 2 examined]*
- Lung with bronchi [collected whole; 2 sections examined]*
- Lymph nodes: mandibular and mesenteric
- Mammary gland [process females only]
- Pancreas
- Peyer's patch
- Pituitary*
- Prostate* and seminal vesicle (2)*
- Salivary gland, mandibular/sublingual [2 collected; 1 examined]*[b]
- Salivary gland, parotid [2 collected; 1 examined]
- Sciatic nerve
- Skeletal muscle, biceps femoris
- Skin
- Spinal cord [cervical, thoracic, and lumbar]
- Spleen*
- Thymus*
- Thyroid/parathyroid (2)*
- Tongue
- Trachea
- Ureters (2)
- Urinary bladder
- Uterus [both horns]/Cervix*
- Vagina

[a]Bone marrow smears were collected at the scheduled necropsy and held.
[b]The combined weight of the right mandibular/sublingual salivary gland was obtained.
*Organ weighed
(2) Paired organ

Results: Mean liver weight (relative to body weight) was statistically significantly increased (+12%) in terminal HDM, compared to control (no change in absolute liver weight) and the increased relative liver weight was correlated with the microscopic findings of centrilobular hypertrophy and vacuolation (see below in the histopathology section). Mean spleen weights were statistically significantly decreased in terminal HDM (absolute and relative to brain weight; -23% and -22%, respectively) and in terminal HDF (absolute and relative to both body and brain weights; -21%, -18%, and -21%, respectively), compared to control. Mean adrenal gland weights were statistically

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significantly decreased in terminal MDF (absolute and relative to both body and brain weights; -20% and -19%, respectively) and terminal HDF (absolute; -18%), compared to control. Mean prostate weights were statistically significantly decreased in terminal HDM (absolute and relative to brain weight; -23% and -22%, respectively) and mean seminal vesicle weights (absolute and relative to both body and brain weights) were statistically significantly decreased in terminal males at all three doses (-16 to -29%). There were no microscopic correlates for the weight changes in spleen, adrenal, prostate and seminal vesicles. There were no treatment-related organ weight changes in recovery animals. Even though the magnitude of the organ weight changes appears to be small, the toxicological significance can not be undermined.

Histopathology- Microscopic examination of tissues (listed in the histopathology inventory, see Table 15, above) from control and HD rats (including tissues from two main study animals found dead; #s 5051 and 5113) were performed.
Note: The Sponsor has added Protocol Amendments: Amendment # 5 to clarify that the organs or tissues will be examined from the Recovery animals and Amendment #6 to clarify that liver from males will be microscopically examined from Main study groups 2 and 3 and Recovery groups 1 to 4 (the liver has been identified as a target organ by the study pathologist and hence the need for Amendment #6).

Adequate Battery: yes

Peer Review: Yes; conducted by two pathologists (one in-house pathologist and a reviewing pathologist from (b) (4) reached similar conclusions on histopathological findings.

Histological Findings: Treatment-related microscopic findings were present in the liver of terminal and recovery male rats. Minimal to mild centrilobular hepatocyte hypertrophy was present in 9/11 terminal HDM rats. In addition, centrilobular vacuolation of hepatocytes was present in 3/10 terminal MDM (minimal severity) and 7/11 terminal HDM (minimal severity in 6 rats and mild severity in 1 rat). In recovery males, minimal centrilobular vacuolation of hepatocytes was present in one 1/4 HDM.

Special Evaluation

None

Toxicokinetics- Blood samples were obtained from cohorts of 3 animals/sex/dose level at 1 hr after the dose (Group 5, control) or at pre-dose and at 0.5, 1, 4, 12 and 24 hr after the dose on Days 1, 90 and 180 [Groups 6-8, 10, 30, and 120/100 (M/F) g/kg/d]. The TK parameters were determined for F2695 and its main metabolite.

Results: The TK parameters for F2695 are summarized in Table 21, below; plasma concentrations of F2695 were similar in males and females after 10 or 30 mg/kg/d on all study days. $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}24}$ and C_{max} for F2695 were slightly higher in females than in males after 10 and 30 mg/kg/d on Days 1 and 90, but were similar between males and females on Day 180. $AUC0\text{-}_{tlast}$ and $AUC_{0\text{-}24}$ increased slightly more than dose

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proportional on Days 1, 90 and 180; there was slight accumulation of F2695 (~1.5-fold) after repeated administration.

Table 21 Toxicokinetic parameters for F2695 after single and repeated oral administration in male and female rats for 6 months

(Excerpted directly from the study report 1712-005, pp 34)

Day	Dose mg/kg/day	Sex	$AUC_{0\text{-}tlast}$ (hr•ng/mL)	$AUC_{0\text{-}24}$ (hr•ng/mL)	C_{max} (ng/mL)	T_{max} (hr)
1	10	Male	1360	1410	493	0.500
		Female	2200	2260	1070	0.500
		Mean	1780	1830	783	0.500
	30	Male	6340	6670	2140	0.500
		Female	9590	10800	2630	1.00
		Mean	7970	8730	2380	0.750
	120	Male	47000	47000	7180	0.500
	100	Female	51300	51300	7340	1.00
		Mean	NC	NC	NC	NC
90	10	Male	2080	2190	694	0.500
		Female	2710	2880	989	0.500
		Mean	2390	2540	842	0.500
	30	Male	9670	9670	2010	1.00
		Female	12400	12400	2590	0.500
		Mean	11000	11000	2300	0.750
	120	Male	58900	58900	5720	1.00
	100	Female	57100	57100	6720	1.00
		Mean	NC	NC	NC	NC
180	10	Male	3020	3090	954	1.00
		Female	3280	3440	1080	0.500
		Mean	3150	3270	1020	0.750
	30	Male	11700	11700	2760	1.00
		Female	15000	15000	2990	1.00
		Mean	13300	13300	2870	1.00
	120	Male	77600	77600	10300	1.00
	100	Female	72000	72000	9950	1.00
		Mean	NC	NC	NC	NC

NC – Not Calculated; males and female received different doses

The TK parameters for the main metabolite, F17400 are summarized in Table 22, below; plasma concentrations of F17400 were similar in males and females following the administration of F2695 at 10 and 30 mg/kg/d on all study days. $AUC_{0\text{-}tlast}$ and $AUC_{0\text{-}24}$ increased in proportion to F2695 dose on all study days. There was no evidence for accumulation of F17400 on repeated administration of F2695. Exposure to F17400 as estimated by metabolite to parent ratios of $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}24}$ and C_{max} was less than that to F2695 (ratios ranged from 0.0421 to 0.521).

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Table 22 Toxicokinetic parameters for the F17400 (major metabolite) after single and repeated oral administration in male and female rats for 6 months

(Excerpted directly from the study report 1712-005, pp 35)

Day	Dose mg/kg/day	Sex	$AUC_{0\text{-tlast}}$ (hr•ng/mL)	$AUC_{0\text{-}24}$ (hr•ng/mL)	C_{max} (ng/mL)	T_{max} (hr)
1	10	Male	683	733	131	1.00
		Female	658	658	152	0.500
		Mean	670	696	142	0.750
	30	Male	2180	2180	308	1.00
		Female	2250	2250	338	1.00
		Mean	2210	2210	323	1.00
	120	Male	7930	7930	596	4.00
	100	Female	6500	6500	511	4.00
		Mean	NC	NC	NC	NC
90	10	Male	825	825	135	1.00
		Female	750	750	115	1.00
		Mean	788	788	125	1.00
	30	Male	2480	2480	264	1.00
		Female	1990	1990	212	1.00
		Mean	2240	2240	238	1.00
	120	Male	7070	7070	406	4.00
	100	Female	4860	4860	359	4.00
		Mean	NC	NC	NC	NC
180	10	Male	899	899	196	1.00
		Female	783	783	115	1.00
		Mean	841	841	155	1.00
	30	Male	2660	2660	328	1.00
		Female	2090	2090	255	1.00
		Mean	2370	2370	292	1.00
	120	Male	7300	7300	536	4.00
	100	Female	5470	5470	419	4.00
		Mean	NC	NC	NC	NC

NC – Not Calculated; males and female received different doses

Dosing Formulation Analysis

Dosing formulations were prepared weekly and stored at 2 to 8 °C. The nominal concentrations of 2, 6, 20, and 24 mg/mL were used for preparations. Analysis of dosing formulations for homogeneity indicated that the low (2 mg/mL) and high (24 mg/mL) concentrations used in this study were homogeneous based on percent recovery of ±10% of the nominal concentrations for samples collected from top, middle, and bottom strata. The dosing formulations demonstrated stability up to 14 days at 2 to 8 °C. The concentrations of all doses of F2695 were determined at Weeks 1, 14, and 26; the average percent recovery of F2695 from each dose concentration was within ±10% of the nominal mg/mL concentrations and therefore, dosing was considered acceptable.

Summary: This study was conducted in CD® rats to evaluate the toxicity of F2695 (levomilnacipran), after daily oral administration (10, 30, and 120/100 mg/kg/d) for 26 weeks, to determine the toxicokinetics after 1, 90, and 180 days, and to evaluate reversibility of any observed changes following a 4 week post-dose observation period. One HDM was found dead on Day 50; the Sponsor could not determine the cause of death after microscopic examination. In a previous 13 week oral toxicity/TK study in rats (study #932, under IND 104,483) administered F2695 at the dose levels of 0, 10, 35 and 120 mg/kg/d, one mid dose male and one high dose female were found dead without clear relationship to treatment; nonetheless, treatment-related causes were not ruled out by the Sponsor. In addition, the high dose of 120 mg/kg/d represents approximately one-half the calculated acute median lethal dose (LD_{50}) of 238 mg/kg (study #T065 1990, under IND 104,483). Therefore, this Reviewer considers the HDM death as related to treatment-related causes.

Notable treatment-related clinical findings included decreased activity, salivation, hunched posture (females), and hair discolored yellow in HDM and HDF. There were treatment-related effects on body weights/body weight gains in HDM during the treatment period; body weights in HDM were lower by ~13%, compared to control. Although body weight gain during the recovery period indicated a trend towards reversibility, the mean body weights in HDM at the end of recovery were 7.2% less than control. Throughout the treatment period, the mean food consumption was consistently lower in HDM compared to control; however, food consumption increased during the recovery. Even though urine volumes were slightly higher and accompanied by lower specific gravity in HDM and HDF, it is not clear if these changes were treatment-related; nevertheless, these changes were reversed at recovery. There were no treatment-related effects on other clinical pathology parameters.

Treatment-related changes in organ weights included increased mean liver weight (relative to body weight) in terminal HDM (+12%, compared to control); this is despite a 13% decrease in mean body weight in the HDM. The increased liver weight was correlated with the microscopic findings of centrilobular hypertrophy and vacuolation.

Mean spleen weights were statistically significantly decreased in terminal HDM and terminal. Mean adrenal gland weights were statistically significantly decreased in terminal MDF and HDF. Mean prostate weights were statistically significantly decreased in terminal HDM and mean seminal vesicle weights were statistically significantly decreased in terminal males at all three doses. There were no microscopic correlates for the weight changes in spleen, adrenal, prostate and seminal vesicles. There were no treatment-related organ weight changes in recovery animals.

Treatment-related microscopic findings were present in the liver of terminal and recovery males; microscopic findings included minimal to mild centrilobular hepatocyte hypertrophy in HDM (9/11) and minimal to mild centrilobular vacuolation of hepatocytes in MDM (3/10) and HDM (7/11). In the recovery males, minimal centrilobular vacuolation of hepatocytes was present in 1/4 HDM; an indication that the microscopic changes in the liver may be reversible.

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The NOAEL = 30 mg/kg/d in males (minimal to mild centrilobular vacuolation of hepatocytes at 30 mg/kg/d, however, without correlating effects on clinical pathology or organ weights and without similar effects in females) and 100 mg/kg/d in females (no adverse toxicity up to the highest dose).

Summary of short-term repeat dose studies in cynomolgus monkeys:

F2695: A dose range-finding oral toxicity study in naïve cynomolgus monkeys (study № 1712-007):

The purpose of this study was to determine the maximum tolerated dose (MTD) after single doses and to evaluate and characterize the oral toxicity of the estimated MTD after 14 consecutive days of dosing with F2695 in monkeys.

Experimentally naïve cynomolgus monkeys (12 males and 12 females), 2 years and 8 months old and weighing 2.4 to 2.8 kg were assigned using three sample randomization procedures (one per phase) to control and treatment groups as shown in Table 23, below; the vehicle and F2695 were administered via oral gavage (dose levels and frequency of dosing is as shown in Table 24, below.

Table 23 Three sample randomization of cynomolgus monkeys

(Excerpted directly from the study report 1712-007, pp 12-13)

Group Assignments – Phase A			
Group Number	Dose Level (mg/kg)	Number of Animals	
		Male[a]	Female[b]
1	60/90/140	1	1
2	70/120/200	1	1

[a]Weight for males at randomization – 2.80 kg
[b]Weight range for females at randomization – 2.40 to 2.45 kg

Group Assignments – Phase B			
Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male[a]	Female[b]
3	0	2	2
4	140	2	2
5	200	2	2

[a]Weight range for males at randomization – 2.75 to 3.04 kg
[b]Weight range for females at randomization – 2.04 to 2.77 kg

Group Assignments – Phase C			
Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male[a]	Female[b]
3[c]	0	2	2
6	80	2	2
7	100	2	2

[a]Weight range for males at randomization – 2.35 to 3.08 kg
[b]Weight range for females at randomization – 2.04 to 3.20 kg
[c]The same animals in Phase B were used for dosing in Phase C

The Phase A dose levels were selected by the Sponsor based on previous studies with similar analog. Phase B dose levels were selected based on the results in Phase A and Phase C doses were selected based on the results of Phase B.

Table 24 Dose levels and frequency of dosing with F2695 in the various Phases

(Excerpted directly from the study report 1712-007, pp 15)

Phase A		
Animal Number[a]	Dose Level	Number of Days Dosed
101 and 102	60/90/140 mg/kg	3
103 and 104	70/120/200 mg/kg	3
[a]Each animal dosed once at each dose level with a 2 to 4 day washout period between doses.		

Phase B[c]		
Animal Number[a,b]	Dose Level	Number of Days Dosed
105 through 108	0 mg/kg/day	1
109 through 112	140 mg/kg/day	1
113 through 116	200 mg/kg/day	1
[a]Animal 113 died after blood collection. [b]Animals 110 and 111 were euthanized *in extremis* on Day 1. [c]Due to mortality, Phase B terminated after 1 day of dosing.		

Phase C		
Animal Number	Dose Level	Number of Days Dosed
105 through 108	0 mg/kg/day	14
117 through 120[a]	80 mg/kg/day	14
121 through 124	100 mg/kg/day	14
[a]Animal 120 was dosed twice and euthanized *in extremis* on Day 2.		

Morbidity, mortality, clinical signs and body weights were recorded daily during all 3 phases. Clinical pathology evaluations were conducted on all surviving Phase C animals (clinical pathology parameters were also conducted on two females that were euthanized- female #111 at 140 mg/kg in Phase B and female #120 at 80 mg/kg in Phase C). Plasma concentrations of F2695 were determined for Phase B animals (pre dose and on Day 1, post-dose) and Phase C animals (on Days 1 and 14).

Results: All animals survived Phase A escalated dosing at 60, 90, 140 (group 1) and 70, 120, 200 (group 2) mg/kg/d. In Phase B, two animals dosed at 140 mg/kg/d (male #110 and female # 111) were euthanized *in extremis* on Days 1 and 2, respectively and one male (#113) dosed at 200 mg/kg/d died after blood draw on Day 1. All three animals exhibited decreased activity, clonic convulsions and bilateral dilated pupils; due to mortality and clinical signs, the Sponsor discontinued dosing for Phase B after 2 days. In Phase C, one female (#120) dosed at 80 mg mg/kg/d was euthanized on Day

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2 due to severe clinical signs; notable clinical signs prior to euthanasia were decreased activity, ataxia, tremors, partially or completely closed eyelids, inappetance, hunched posture and dilated pupils.

There were no major treatment-related clinical signs in Phase A; clinical signs in Phase A were limited to vomitus, lacerations (digit or tail), and soft or watery feces.

In Phase B, treatment-related clinical signs included decreased activity (1 M and 2 F at 140 mg/kg and 2 M and 2 F at 200 mg/kg), clonic convulsions (1 M and 1 F at 140 mg/kg and 1 M at 200 mg/kg), pupils dilated (all animals), ataxia (1 M at 200 mg/kg) and partially/completely closed eyelids (1 M at 200 mg/kg).

In Phase C, treatment-related clinical findings included partially/completely closed eyelids (all animals at both doses), dilated pupils (all animals at both doses), distended abdomen (males at both doses), inappetance (all animals at both doses), hunched posture (all animals at 80 mg/kg and males at 100 mg/kg), tremors (2 M and 1F at 80 mg/kg and 2 M and 1 F at 100 mg/kg), ataxia (1 F each at 80 and 100 mg/kg), decreased activity (all animals at both doses), and emesis (males and females at 100 mg/kg).

There were no treatment-related changes in body weights in any phase of this study. Likewise, there were no treatment-related changes in hematology and coagulation parameters. There were minimal, dose-dependent increases in ALT, compared to controls, in both males and females dosed at 80 (+29% and +9%, respectively) and 100 mg/kg/d (47% and 53%, respectively); although minimal, the increases in ALT may be an indication of hepatocellular injury.

Clinical pathology parameters were also determined on two female monkeys that were euthanized (animal #111 at 140 mg/kg in Phase B and animal #120 at 80 mg/kg in Phase C). Compared to control, animal #120 had a mild increase in neutrophils (↑171%). Similarly, compared to control, animal # 111 had mild increases in GGT (↑97%), AST (↑477%), and ALT (↑477%).

The TK parameters for F2695 were similar in males and females in Phase B (Table 25, below); generally the C_{max} and AUC_{0-24} increased in approximate proportion to dose. The T_{max} was similar at 140 and 200 mg/kg. In Phase C, the TK parameters for F2695 were also similar in males and females (Table 26, below); there were no significant differences between the TK parameters for Days 1 and 14. Generally, the C_{max} and AUC_{0-24} increased in proportion to dose. The T_{max} was similar at 80 and 100 mg/kg/d.

In conclusion, a single oral dose of 60 and 70 mg/kg of F2695 to naïve monkeys did not produce clinical signs of toxicity. Based on the dose escalation in Phase A from 60 to 90 mg/kg/d without toxicity, the dose of 90 mg/kg/d is considered to be the MTD.

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Table 25 Day 1 toxicokinetic parameters for F2695 in male and female monkeys given an oral dose in Phase B

(Excerpted directly from the study report 1712-007, pp 25)

Dose (mg/kg/day)	Sex	Animal Number	C_{max} (ng/mL)	AUC_{0-24} (hr•ng/mL)	T_{max} (hr)
140	Male	109	19100	287000	4.00
	Female	112	12600	196000	4.00
	Combined		15900	241000	4.00
200	Male	114	21800	380000	8.00
	Female	115	17400	153000	4.00
	Female	116	9670	82700	2.00
	Mean		13500	118000	3.00
	Combined		16300±6140	205000±155000	4.67±3.06

Table 26 Day 1 and 14 toxicokinetic parameters for F2695 in male and female monkeys given daily oral dose in Phase C

(Excerpted directly from the study report 1712-007, pp 26)

Day	Dose (mg/kg/day)	Sex	Animal Number	C_{max} (ng/mL)	AUC_{0-24} (hr•ng/mL)	T_{max} (hr)
1	80	Male	117	12700	179000	
			118	14300	159000	8.00
			Mean	13500	169000	4.00
		Female	119	12900	165000	6.00
			120	14100	144000	4.00
			Mean	13500	154000	4.00
			Combined	13500±816	162000±14600	4.00
	100	Male	121	15800	217000	5.00±2.00
			122	13400	196000	8.00
			Mean	14600	207000	8.00
		Female	123	21100	195000	8.00
			124	15200	199000	2.00
			Mean	18200	197000	8.00
			Combined	16400±3310	202000±10300	5.00
14	80	Male	117	17900	149000	6.50±3.00
			118	11900	134000	2.00
			Mean	14900	141000	4.00
		Female	119	14500	176000	3.00
			Combined	14800±3010	153000±21200	2.00
	100	Male	121	10700	153000	2.67±1.15
			122	12400	132000	4.00
			Mean	11600	143000	4.00
		Female	123	20300	212000	4.00
			124	15000	202000	4.00
			Mean	17700	207000	8.00
			Combined	14600±4190	175000±38200	6.00

13-week toxicity study by oral route (gavage) in cynomolgus monkeys followed by a 4-week treatment-free period (study № 26215 TCP; Sponsor's code: F2695-19529):

This sub-chronic study of 13 weeks duration in Cynomolgus monkeys was reviewed by this Reviewer under IND 104,483 (SDN-002) and the results are summarized below.

The purpose of this GLP study was to investigate the toxicity of F2695 when given daily to cynomolgus monkeys for 13 consecutive weeks and followed by a 4-wek recovery period. F2695 (batch № LP 110) at 0, 5, 15, and 45 mg/kg/d) was administered orally (gavage) to cynomolgus monkeys (6/sex/dose in control and HD; 4/sex/dose in LD and MD; mean BW of 2.5 to 2.7 kg; 24 months old). Animals were checked daily for morbidity/mortality and clinical signs. BW were recorded once before allocation of animals to groups, on Day 1 of treatment and then weekly until the end of the study. Food consumption was estimated daily throughout the study. Ophthalmoscopy, ECG and blood pressure measurements were performed before commencement of treatment and in weeks 4 and 12/13. Clinical pathology parameters were measured before the commencement of treatment, and in weeks 4 and 13. Gross pathology and histopathology parameters were evaluated on completion of the treatment and/or recovery period.

Results: There were no deaths and there were no clinical signs related to treatment with F2695. There was treatment-related decrease in body weight gain in HDF (~10% less than control; no weight gain throughout the treatment period); this effect was no longer observed during the recovery period. There was reduced food consumption (↓25-75% of the quantity given) by MDM, HDM and HDF on some occasions throughout the study; however, this effect was no longer observed during the recovery period. Although there were no treatment-related effects on ECG parameters, in weeks 4 and 13, lower systolic pressure was observed in MDM and HDM and in week 13 in HDF; this effect seems to persist during and up to the end of the recovery period.

There were no treatment-related effects on ophthalmology, hematology and blood chemistry or urinalysis parameters. No treatment-related alterations were seen at necropsy. No significant differences in organ weights and there were no treatment-related microscopic findings.

The plasma concentrations measured on days 1 and 90 showed an increase in exposure higher than the dose increase in males and females. The T_{max} ranged from 1.75 to 4 hr. At the high dose of 45 mg/kg/d, the exposure was higher in males than in females.

The NOAEL was the mid dose of 15 mg/kg/d (decreased body weight gain in HDF).

Chronic Toxicity Study in Monkeys

Reference ID: 3326668

Study title: F2695: A 1-year oral toxicity study in monkeys with a 4-week recovery

Study no.:	1712-006
Study report location:	eCTD
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 23, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Levomilnacipran HCl, L0003039; 100.1 (% m/m) purity

Key Study Findings

Levomilnacipran was administered orally to male and female cynomolgus monkeys at 0, 0, 5/10, 15/30, and 45/70/90 mg/kg/day for 1 year. Treatment-related findings were:

- Clinical signs, notably at the high dose, such as decreased activity, emesis, salivation, hunched posture, pupil dilated and self mutilation
- Decreased body weight gain in high dose males and females
- Increased liver weight in mid dose and high dose males and females and persisted during the recovery period (only in high dose groups)
- Minimal panlobular hepatocyte hypertrophy in males and females of the high dose groups. Minimal to mild midzonal vacuolation of hepatocytes in males and females of the high dose groups
- The NOAEL is considered to be 30 mg/kg/d

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Methods

Doses:	0, 5/10, 15/30, and 45/70/90 mg/kg/day [dose escalation was done as explained in the footnote of Table 27, below]. Initial dose selection was based on previous 13-week oral study (study № 26215 TCP) and the dose range-finding study in monkeys (study № 1712-007)
Frequency of dosing:	Once a day
Route of administration:	Oral gavage (nasogastric dosing[7])
Dose volume:	5 mL/kg
Formulation/Vehicle:	Solution/distilled water
Species/Strain:	Cynomolgus monkey (*Macaca fascicularis*)
Number/Sex/Group:	6/sex/group (4/sex/group for LD and MD (Table 10, below)
Age:	~2 years and 7 months to 4 years 1 month
Weight:	Males: 2.25 to 3.25 kg; females: 2.15 to 2.75 kg
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	No significant deviations that would affect the study outcome

Table 27 Chronic toxicity in monkeys; study design

(Excerpted directly from the study report 1712-006, pp 17)

Group Assignments				
Group Number	Dose Level (mg/kg/day)	Concentration (mg/mL)	Number of Animals	
			Male	Female
1[a]	0	0	6	6
2[b]	5/10	1/2	4	4
3[b]	15/30	3/6	4	4
4[a,c]	45/70/90	9/14/18	6	6

[a]Two animals/sex in the control and high dose groups were retained for a 4 week recovery
[b]Dose levels were increased from 5 and 15 mg/kg/day to 10 and 30 mg/kg/day, respectively beginning on Day 8. The concentration levels were increased appropriately.
[c]Beginning on Day 6 the 45 mg/kg/day dose level was increased to 70 mg/kg/day. On Day 11, the dose level was increased to 90 mg/kg/day.

[7] On Day 245, due to high incidence of emesis, the route of administration was changed from oral gavage to nasogastric for three HDM. On Day 269, the route of administration for all remaining animals in the high dose groups was changed from oral gavage to nasogastric. In response to the death of two animals following nasogastric administration, beginning on Day 294, the route of administration for all animals in the high dose group was returned to oral gavage.

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Observations and Results

Mortality- Observed for mortality/morbidity, injury, and the availability of food and water at least twice daily.
There were no treatment-related deaths. Two animals in the 45/70/90 mg/kg/d group (female #120, found dead on Day 289 and male #137, died on Day 293, shortly after nasogastric dosing) were found dead; macroscopic (moderate to severe red discoloration of the lungs) and microscopic (edema and congestion in multiple sections of the lungs) findings were consistent with intubation error as the cause of deaths.

Clinical Signs- Detailed clinical examination performed weekly, beginning on Day 7. On occasion, clinical observations were recorded at unscheduled intervals.

Results: Treatment-related clinical findings were mainly seen in mid dose and high dose animals and are summarized in Table 28, below; notable clinical findings were decreased activity, eyelids partially or completely closed pupils dilated and hunched posture. There were one to two incidences of tremor in HDM and HDF (the Sponsor did not consider this as treatment-related; however, this Reviewer does not agree with their conclusion because in the range-finding study, there were incidences of tremors at doses as low as 80 mg/kg/d and convulsion at slightly higher doses). Emesis occurred in males and females in a dose-dependent manner (however, the Sponsor treated this effect as being caused by bad taste or sensation rather than a systemic effect of the drug substance). Increased incidence of abrasions and scabbed areas was observed mainly in mid and high dose animals; even though the incidence was lower during the recovery period, the Sponsor was unsure if the effects were treatment-related. Similarly, it is unclear if the self mutilation behavior, mainly observed in mid and high dose animals, is treatment-related; the Sponsor had cited published literature to indicate that self mutilation behavior has been reported in primates.

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Table 28 Levomilnacipran treatment-related clinical signs in monkeys

(Excerpted directly from the study report 1712-006; pp 29)

Notable Detailed Clinical Observations for Weeks 1 to 53								
	0 mg/kg/day		5/10 mg/kg/day		15/30 mg/kg/day		45/70/90 mg/kg/day	
Observation	M	F	M	F	M	F	M	F
Abrasion	2/2	10/2	5/1	5/1	24/3	52/4	51/5	18/6
Activity Decreased	0/0	0/0	0/0	0/0	0/0	1/1	24/5	12/5
Emesis	0/0	4/1	2/1	2/1	1/1	3/3	20/6	32/3
Emesis after dosing	20/2	40/3	58/3	137/3	69/4	55/4	364/6	55/3
Emesis during dosing	5/2	5/1	1/1	3/1	11/3	1/1	20/5	6/2
Eyelids partially/ completely closed	0/0	0/0	0/0	0/0	10/2	6/3	1674/6	1695/6
Posture hunched	0/0	0/0	0/0	0/0	0/0	17/3	47/4	22/4
Pupil dilated	0/0	0/0	0/0	0/0	24/3	26/3	221/6	74/4
Salivation after dosing	0/0	0/0	0/0	0/0	16/2	1/1	59/6	12/3
Scabbed area	1/1	17/4	12/3	7/2	99/3	83/3	68/5	89/6
Self mutilation	0/0	2/1	0/0	0/0	83/1	74/1	13/2	0/0
Tremors	0/0	0/0	0/0	0/0	0/0	0/0	2/1	1/1
Number of times observed/Total number of animals affected								

Body weights- Recorded weekly

Results: Body weight data are illustrated in Figure 8 & Figure 9, below; during the course of the study, mean body weight gain was lower by ~30% in HDM and 45% in HDF, compared to the respective control groups. In HDM, the differences did not reach statistical significance at any point of measurement interval; however, in HDF, the differences occasionally reached statistical significance. During the 4-week recovery period, body weight gain in both males and females were comparable to control groups. There were no treatment-related effects on body weights in the low and mid dose groups.

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Figure 8 Sponsor's figure (with minor formatting modifications by the Reviewer) showing decrease in body weight gain in HDM monkeys

(Star, solid line) compared with control (square, solid line); study #1712-006, pp 95

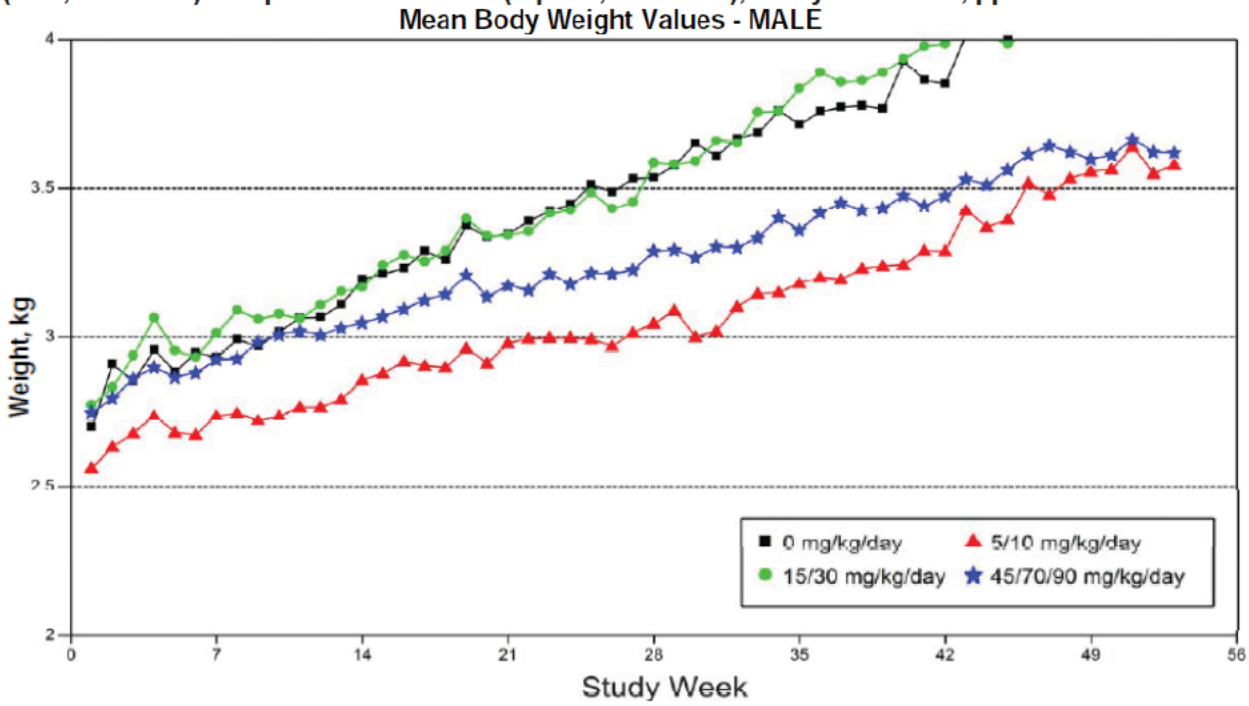
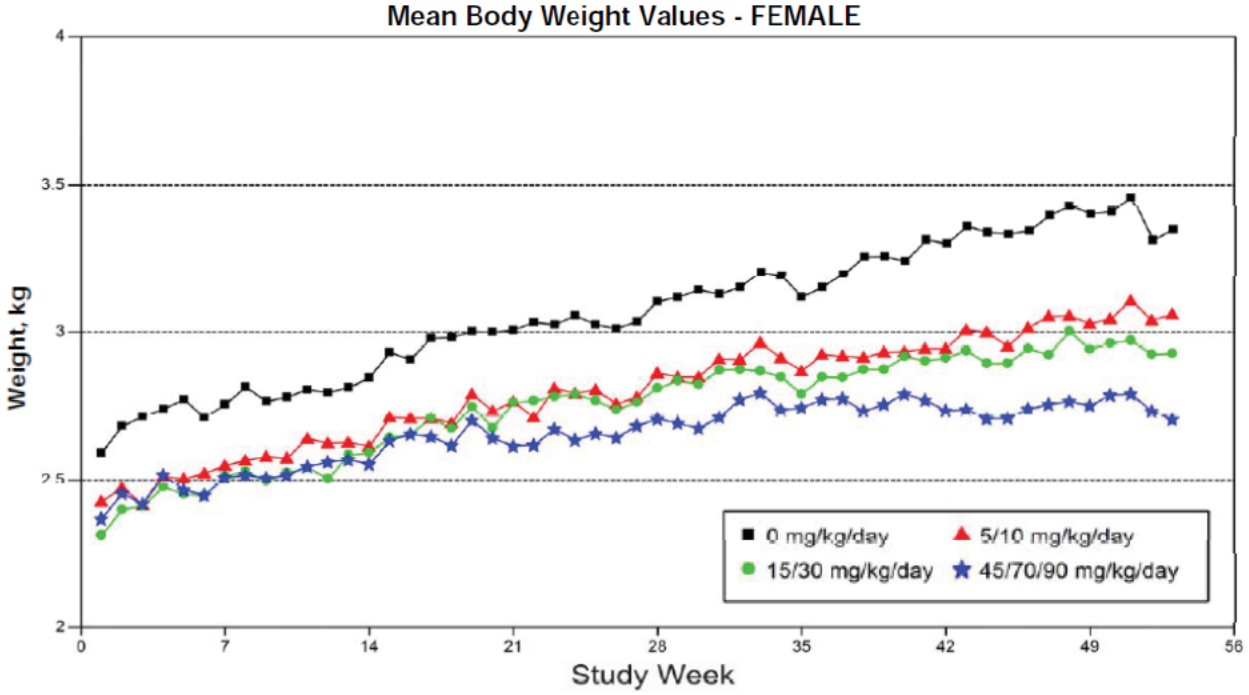


Figure 9 Sponsor's figure (with minor formatting modifications by the Reviewer) showing decrease in body weight gain in HDF monkeys

(Star, solid line) compared with control (square, solid line); study #1712-006, pp 96



Ophthalmoscopy- Ophthalmoscopic examinations were conducted on all animals pretest (Day -7 or -1) and again prior to scheduled terminal or recovery necropsy.

Results: No treatment-related changes in ophthalmic parameters were observed.

Electrocardiography- All animals in all groups received ECG examination pre-test, Day 1(~1 to 2 hr, post-dose ± 15 min), during the last week of dosing (Day 363) and during the last week of recovery (Day 391).

Results: There were no treatment-related effects on ECG parameters (RR, PR, QT, and QT_c intervals, and QRS duration). All animals were either in sinus rhythm or sinus arrhythmia, both of which are reported to be normal in monkeys[8]. Sinus tachycardia (defined as an average heart rate greater than 270 bpm for a single interval) was observed in 14 animals; however, it did not occur in a dose dependent manner and the incidence was higher in the control group (according to the veterinary cardiologist's report, sinus tachycardia is often a normal variant in cynomolgus monkeys).

Clinical Pathology:

Clinical pathology evaluations conducted on all animals pretest, Week 13, and all surviving animals prior to terminal or recovery necropsy. Blood samples (~4.8 to 5.8 mL) were collected from the femoral artery from animals that were fasted overnight (but had access to drinking water). Urine samples were collected using steel pans placed under the cages for at least 16 hours.

Hematology- Blood collected into tubes containing K3EDTA (for hematology) and sodium citrate (for coagulation) and the following parameters were assessed: leukocytes, erythrocytes, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets, absolute reticulocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, other cells, activated partial thromboplastin time (APTT) and prothrombin time.

Results: There were no consistent treatment-related changes in hematology parameters at any dose. There was a minor increase in leukocytes in HDF at week 52 (+1.57-fold, compared to the pretest and Week 13 values); the increased leukocytes were mainly neutrophils and could be attributed to two high dose females (#s139 and 140) whose neutrophil values were ~1.9-fold higher than their pretest values.

Clinical chemistry- Blood collected into tubes containing serum separator (no anticoagulant) and the following parameters were evaluated: Na, K, Cl, Ca, P, alkaline phosphatase, total bilirubin, γ-glutamyltransferase, AST, ALT, sorbitol dehydrogenase, creatine kinase, urea nitrogen, creatinine, total protein, albumin, globulin, albumin/globulin ratio, cholesterol and glucose.

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[8] Macallum, GE and Houston, BJ. Characterization of cardiac alterations in nonsedated cynomolgus monkeys. J Am Vet Res 1993; 54(2): 327-332.

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Results: Findings were limited to minor changes in clinical chemistry parameters that could be attributed to treatment with F2695. There was a slight decrease in total bilirubin in males and females of all three doses at termination; ~60% lower than pretest values. There was a slight increase in AST and ALT at termination in HDM (13% and 49%, respectively), compared to pretest values. At the high dose, albumin was decreased 18% and 16% in males and females, respectively at Week 13, and 20% and 19%, respectively at termination. The albumin to globulin ratios were decreased at Week 13 and at termination in males (25%) and females (~17%), compared with pre-test values. In one HDM (#116) there was a slight increase of γ-glutamyltransferase at Week 13 and at termination (↑40%, compared to pretest value) and this remained elevated at recovery.

Urinalysis: Urine volume, specific gravity and pH were evaluated.

Results: There were no consistent treatment-related effects on urinalysis parameters; one HDM (#116) had increased urine volume and low specific gravity with mild glucosuria at termination.

Gross Pathology- Necropsy examination performed on animals found dead and all surviving animals at scheduled terminal (Day 366) and recovery (Day 394) necropsies. A full complement of tissues and organs (see Table 29, below) was collected from all animals.

Results: There were no toxicologically significant treatment-related macroscopic findings.

Organ Weights- Organs specifically marked in Table 29 were weighed at the schedules terminal or recovery necropsy and organ weight ratios were calculated relative to body and brain weights.

Results: Treatment-related changes in liver weights were seen in terminal and recovery males and females (see Table 30, below). Statistically significant increases in liver weights were seen in HDM and HDF (+32% and +41%, respectively, compared to control) with histopathological correlates (see below). Liver weights relative to body and brain weighs were also increased in HDM and HDF. At recovery, liver weights were slightly increased (see Table 31, below); however, there were no microscopic correlates.

Histopathology- A list of organs and tissues examined are presented in the histopathology inventory table (Table 29, below).

Adequate Battery: Yes

Peer Review: Yes, by a pathologist other than the study pathologist

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Results: Treatment-related microscopic findings in terminal monkeys included minimal panlobular hepatocyte hypertrophy in all three terminal (3/3) HDM and two terminal (2/3) HDF monkeys. In addition, minimal to mild midzonal vacuolation of hepatocytes was present in one of the terminal HDM monkeys that had panlobular hepatocyte hypertrophy and one HDF (that did not show panlobular hypertrophy). The above observations were not present in recovery monkeys.

Table 29 List of organs and tissues (1 year monkey study) collected and preserved for examination

(Excerpted directly from the study report 1712-006; pp 966)

- Adrenal (2)*
- Aorta
- Bone with marrow [femur]
- Bone with marrow [rib]
- Bone with marrow [sternum]
- Bone marrow smear [2 collected][a]
- Brain [cerebrum, midbrain, cerebellum, medulla/pons]*
- Epididymis (2)
- Eye including optic nerve (2)
- Gallbladder
- Gastrointestinal tract:
  - esophagus
  - stomach [cardia, fundus, and pylorus]
  - duodenum
  - jejunum
  - ileum
  - cecum
  - colon
  - rectum
- Gonads:
  - ovary (2)* with oviduct (2)
  - testis (2)*
- Gross lesions
- Heart*
- Joint, tibiofemoral
- Kidney (2)*
- Larynx
- Liver [3 sections collected; 2 examined]*
- Lung with bronchi [collected whole; 2 sections examined]*
- Lymph nodes: mandibular and mesenteric
- Mammary gland [process females only]
- Pancreas
- Peyer's patch
- Pituitary*
- Prostate* and seminal vesicle (2)
- Salivary gland, mandibular [2 collected; 1 examined]*[b]
- Salivary gland, parotid [2 collected; 1 examined]
- Salivary gland, sublingual [2 collected; 1 examined]
- Sciatic nerve
- Skeletal muscle, rectus femoris
- Skin
- Spinal cord [cervical, thoracic, and lumbar]
- Spleen*
- Tail tip[c]
- Thymus*
- Thyroid/parathyroid (2)*
- Tongue
- Trachea
- Ureter (2)
- Urinary bladder
- Uterus/Cervix*
- Vagina

[a]Bone marrow smears were collected at the scheduled necropsy and held.
[b]Only the right mandibular salivary gland weight was obtained.
[c]Tail tips were only collected for animals that had tail amputations performed.
(2) Paired organ
*Weighed organ

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Table 30 Treatment-related organ weight changes seen in terminal male and female cynomolgus monkeys

(Excerpted directly from the study report 1712-006, pp 959)

Test Article-related Organ Weight Changes - Terminal Male and Female (Percent change relative to control)								
Dose level: mg/kg/day	0		5/10		15/30		45/70/90	
Sex	M	F	M	F	M	F	M	F
Number Examined	4	4	4	4	4	4	3	3
Liver (g)	72	55	–6	+12	+11	+16	+32[a]	+41[b]
Liver/BWt%	2	2	+20	+13	+23	+21[a]	+50[b]	+56[b]
Liver/BrWt ratio	1	1	–7	+11	+9	+8	+31	+40[b]

[a]Significantly different from control; ($p<0.05$) +- Increased
[b]Significantly different from control; ($p<0.01$) – - Decreased
BWt - Body Weight M – Male
BrWt - Brain Weight F - Female

Table 31 Treatment-related organ weight changes seen in recovery male and female cynomolgus monkeys

(Excerpted directly from the study report 1712-006, pp 959)

Test Article-related Organ Weight Changes - Recovery Male and Female (Percent change relative to control)				
Dose level: mg/kg/day	0		45/70/90	
Sex	M	F	M	F
Number Examined	2	2	2	2
Liver (g)	60	64	+17	–10
Liver/BWt%	2	2	+22	+18
Liver/BrWt ratio	1	1	+16	+5

[a]Significantly different from control; ($p<0.05$) +- Increased
[b]Significantly different from control; ($p<0.01$) – - Decreased
BWt - Body Weight M – Male
BrWt - Brain Weight F - Female

Toxicokinetics- Blood samples (~1 mL) were collected from all surviving animals via the femoral artery for determination of F2695 and the metabolite F17400. Samples were collected prior to dosing and at 1, 2, 4, 8, and 24 hours post-dose on Days 1, 11, 90, 180, and 365.

Results: The TK parameters for Levomilnacipran (F2695) were similar in males and females on Days 1, 11, 90, 180 and 365. The combined mean values are shown in Table 32, below. Systemic exposure increased slightly more than in proportion to dose

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between 5/10 and 15/30 mg/kg/d and in approximate proportion to dose between 15/30 and 45/70/90 mg/kg/d.

Table 32 Toxicokinetic parameters for F2695 in male and female monkeys given daily oral doses

(Compiled by the Reviewer from the study report 1712-006, pp 904-908)

Dose (mg/kg/d)	Day	$AUC_{(0-24)}$ (ng*h/mL)	C_{max} (ng/mL)	T_{max} (h)	$t_{½}$ (h)
5/10*	1	2700	307	3.3	4.7
	11	7300	855	4.0	3.9
	90	6760	787	3.1	4.3
	180	6240	705	3.0	3.3
	365	7210	848	3.3	4.4
15/30*	1	16200	1710	4.0	4.1
	11	42300	5460	3.1	3.5
	90	36900	4550	3.3	3.5
	180	31200	3940	3.0	3.7
	365	41400	4870	2.9	3.9
45/70/90**	1	61200	6150	4.0	3.7
	11	137000	14000	3.8	3.3
	90	151000	14200	4.2	3.2
	180	94700	8400	4.0	3.7
	365	161000	14600	4.2	4.3

*Dose was raised on Day 8

**Dose was raised on Day 6 and again on Day 11

Table 33 Toxicokinetic parameters for the metabolite, F14700, in male and female monkeys given daily oral doses of F2695

(Compiled by the Reviewer from the study report 1712-006, pp 910-915)

Dose (mg/kg/d)	Day	$AUC_{(0-24)}$ (ng*h/mL)	C_{max} (ng/mL)	T_{max} (h)	$t_{½}$ (h)
5/10*	1	2270	186	4.0	7.0
	11	3980	305	4.0	7.3
	90	3300	267	4.0	7.2
	180	3290	246	4.0	7.7
	365	3670	278	4.5	7.2
15/30*	1	7540	567	4.5	7.4
	11	10600	827	4.0	7.4
	90	11100	829	4.0	6.9
	180	8490	572	4.0	8.2
	365	10700	743	5.0	7.2
45/70/90**	1	16300	1120	6.3	6.5
	11	17000	1120	5.7	8.2
	90	22800	1490	6.3	7.2
	180	15900	998	6.3	7.9
	365	22600	1370	6.0	12.9

*Dose was raised on Day 8

**Dose was raised on Day 6 and again on Day 11

There was slight accumulation of F2695 following repeated dosing. The T_{max} and $t_{1/2}$ did not vary with dose or duration of treatment with F2695.

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The toxicokinetic parameters for the metabolite, F14700 were also similar in males and females and the combined mean values are shown in Table 33, above. Exposure to F14700 was significantly less than that of F2695. Systemic exposure increased in proportion to dose between 5/10 and 15/30 mg/mg/d and less than proportion to dose between 15/30 and 45/70/90 mg/kg/d. There was no accumulation of F14700 following repeated dosing with F2695.

The ratio of exposure to metabolite versus parent drug decreased with increasing dose, suggesting that the metabolizing enzyme(s) was saturated.

Dosing Formulation Analysis-

Dosing formulations were prepared weekly and stored at 2 to 8 °C. The nominal concentrations used for Week 1 dosing were 1, 3, and 14 mg/mL; beginning Week 2, the nominal concentrations of 2, 6, or 18 mg/mL were used for preparations. Analysis of dosing formulations for homogeneity indicated that the low (1 mg/mL) and high (18 mg/mL) concentrations used in this study were homogeneous based on percent recovery of ±8% of the nominal concentrations for samples collected from top, middle, and bottom strata. Under a validation study by the study facility ((b) (4) study #1712-002), F2695 in water was found to be stable for at least 25 h under ambient conditions (protected from light) and for at least 8 days under refrigerated conditions and therefore, stability analysis was not conducted as part of this study. The concentrations of all doses of F2695 were determined at Week 1 (5, 15, 45, and 70 mg/kg), and Weeks 2, 13, 27, 40, and 52 (10, 30, and 90 mg/kg); the average percent recovery of F2695 from each dose concentration was within ±8% of the nominal mg/mL concentrations and therefore, dosing was considered acceptable.

Summary: This study was conducted in cynomolgus monkeys to evaluate the toxicity of F2695 (levomilnacipran), after daily oral gavage or nasogastric administration (0, 10, 30, and 90 mg/kg/d) for 1 year, to determine the toxicokinetics after 1, 11, 90, 180, and 365 days, and to evaluate reversibility of any observed changes following a 4 week post-dose observation period.

There were no treatment-related deaths during this study. Treatment-related notable clinical findings were decreased activity, eyelids partially or completely closed, pupils dilated, emesis and hunched posture. There were one to two incidences of tremor in HDM and HDF.

There were treatment-related effects on body weights/body weight gains in HDM and HDF; during the course of the study, mean body weight gain was lower by ~30% in HDM (not statistically significant) and 45% in HDF (occasionally reached statistical significance), compared to the respective control groups.

There were minor treatment-related changes in clinical pathology parameters (slight increase in AST and ALT in HDM), liver weights (increases in absolute and relative to body/brain weights in HDM and HDF) with histopathological correlates (minimal

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panlobular hepatocyte hypertrophy in HDM and HDF, minimal to mild midzonal vacuolation of hepatocytes in HDM and HDF; both reversible after cessation treatment). The high dose of 45/70/90 mg/kg/d was associated with high plasma exposure as indicated by C_{max} and AUC_{0-24} of 14,600 ng/mL and 161,000 ng*hr/mL, respectively for F2695 and 1370 ng/mL and 22,600 ng*hr/mL, respectively, for the metabolite F17400 on Day 365. Based on the histopathology and clinical pathology findings for liver, the NOAEL is 30 mg/kg/d.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Mutagenicity test on bacteria (*Salmonella typhimurium* his- and *Escherichia coli* trp-) using B. N. Ames's technique

Study no.:	(b) (4) 041009/F2695 (b) (4)
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 3, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	F2695, Batch # OP110, 100.6%

Key Study Findings

Levomilnacipran (F2695) was **negative** for mutagenicity in a valid Ames assay.

Methods

Strains:	Salmonella typhimurium- TA1535, TA1537, TA98, and TA100 Escherichia coli- WP2 pKM101 and WP2 *uvrA* pKM101
Concentrations in definitive study:	50, 150, 500, 1500, and 5000 µg/plate
Basis of concentration selection:	F2695 was tested in a preliminary cytotoxicity test using all the test strains in the absence and presence of S9 at concentrations ranging from 50 to 5000 µg/plate
Negative control:	Solvent, distilled water
Positive control:	Without S9: sodium azide (TA1535 and TA100), 9-aminoacridine (TA1537), 2-nitroflurene (TA98), mitomycin C (WP2pKM101), potassium dichromate (WP2 uvrA pKM101); with S9: 2-anthramine (TA1535, TA1537, TA98, TA100), benzo[a]pyrine (WP2 pKM101, WP2 uvr pKM101)
Formulation/Vehicle:	Solution/distilled water
Incubation & sampling time:	Bacterial strains were exposed (plate incorporation method) to F2695 up to 48 hours

Study Validity: The assays were considered valid based on the following criteria: appropriate bacterial strains were used; appropriate positive controls were used; maximum concentration of the test article was used (5000 µg/plate); the positive controls resulted in significant increase in the number of revertant colonies (mean increases of > 4 times), the study was conducted in the presence and absence of metabolic activation and the assays were done in triplicate.

Results

Except a slight toxicity in the preliminary mutagenicity assay without metabolic activation in the strain TA1537, there were no indications of cytotoxicity at any of the concentrations of F2695 in the absence or presence of metabolic activation. Similarly, there were no indications of precipitation at any of the concentrations of F2695. The results indicated that F2695 did not increase the number of revertant colonies with any of the 6 bacterial strains either in the presence or absence of S9 metabolic activation system. Thus, F2695 was considered **negative** for mutagenicity in the bacterial reverse mutation tests (Ames assay).

Reference ID: 3326668

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Mutation assay at the TK locus in L5178Y mouse lymphoma cells using a microtiter cloning technique (trifluorothymidine resistance)

Study no.:	(b) (4) 050109/F2695 (b) (4)
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 23, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	F2695, Batch # OP110, 100.6% (87.1% active fraction)

Key Study Findings

Levomilnacipran (F2695) was **negative** for inducing forward mutation at the thymidine kinase (tk) locus in mouse lymphoma (L5178Y $TK^{+/-}$) cells in the presence and absence of metabolic activation system.

Methods

Cell line:	L5178Y $TK^{+/-}$ mouse lymphoma cells
Concentrations in definitive study:	65.54, 81.92, 102.4, 128, 160, 200 µg/mL (-S9, 3 hr); 49.15, 61.44, 76.8, 96 and 120 µg/mL (-S9, 24 hr); 81.92, 102.4, 128, 160 and 200 µg/mL (+S9, 3 hr); 81.92, 102.4, 128, 160, 200, and 250 µg/ml (+S9, 24 hr)
Basis of concentration selection:	Cytotoxicity assessment was performed in the absence and presence of S9 at concentrations ranging from 75 to 5000 µg/ml. Concentrations of F2695 to determine the mutagenicity after 3 and 24 hr exposures (+/- S9) were determined from this cytotoxicity assay
Negative control:	Solvent, distilled water
Positive control:	Methyl methane sulfonate (-S9, 2 µg/mL for 24 hr and 10 µg/mL for 3 hr); cyclophosphamide (+S9, 2 µg/mL).
Formulation/Vehicle:	Solution/distilled water
Incubation & sampling time:	Treatment/incubation for 3 hr (+/- S9) and 24 hr (+/-S9). Expression time: 2 days after treatment

Study Validity:

Reference ID: 3326668

The study was considered **valid** based on the following criteria: (i) L5178Y $TK^{+/-}$ cell cultures were treated with at least 4 concentrations of the test substance, (ii) the test substance was assessed in duplicate and the highest concentration of the test substance reduced the cell survival at least by 80%, (iii) the mean solvent control cloning efficiency was within the specified range (65% and 120%), (iv) the mean solvent control mutant frequency (MF) value was within the specified range (50-170 x 10^{-6}), and (v) positive controls (MMS and CP) gave clear increase in MF above the concurrent solvent control (at least 300 x 10^{-6}).

Results

The results of the study showed that there was significant increase in toxicity at high concentrations as reflected in the relative total growth (RTG): RTG was decreased to 67-24% at the highest 3 concentrations compared to solvent control following 3-hr exposure in the presence of S9; however, RTG following 3 hr exposure in the absence of S9 was decreased to 40-7% at the highest 3 concentrations and following 24-hr exposure in the absence of S9, RTG was decreased to 32-3% at the highest 3 concentrations compared to solvent control. Although there was a slight but statistically significant increase (mean induction ratio of 1.6) in MF of small colonies at the highest concentration (200 µg/ml) of F2695 in the first assay without S9, there was no increase in MF in the second assay or both assays with S9. The solvent control and positive control mutant frequencies were within the acceptable range, demonstrating the mutability of the cell line. Therefore, it is concluded that F-2695 is not mutagenic. The study met all the validity criteria and F2695 was considered **negative** in its mutagenic effects.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Study of genotoxic activity using the micronucleus test in rat (2 treatments, 1 sampling time)

Study no:	(b) (4) 041010/F2695/ (b) (4)
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 23, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	F2695, Batch # OP110, 100.6% (87.1% active fraction)

Key Study Findings

Levomilnacipran (F2695) was **not clastogenic** in the *in vivo* micronucleus test in male and female Sprague-Dawley rats.

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Methods

Doses in definitive study:	20, 40, 80 mg/kg/d x 2 (males) and 40, 80, 200 mg/kg/d x 2 (females)
Frequency of dosing:	Once a day
Route of administration:	Oral
Dose volume:	10 mL/kg
Formulation/Vehicle:	Solution/distilled water
Species/Strain:	Rat/Sprague-Dawley
Number/Sex/Group:	5 (7 for HD)/sex/group
Satellite groups:	None
Basis of dose selection:	Based on preliminary toxicity assay; MTD was equal to 200 mg/kg (x2) in males and 320 mg/kg (x2) in females (in a previous acute toxicity study, LD_{50} was 226 and 256 mg/kg in males and females, respectively). Two females died between 2 and 4 hr after the first dose of 320 mg/kg. One male died between 2 and 4 hr after the second dose of 320 mg/kg.
Negative control:	Distilled water
Positive control:	Cyclophosphamide, 25 mg/kg, i.p.

Study Validity

The study was considered **valid** based on the finding that the mean micronuclei/1000 PCE in the vehicle control fell within the historical control data and the CP (positive control)-treated rats showed statistically significant increase in the mean micronuclei/1000 PCE compared to the vehicle control group. In addition, there was statistically significant decrease of PCE/NCE ratio in males and females treated with CP.

Results

The results showed that F2695 did not produce increase in the frequency of micronuclei up to the maximum tolerated dose of 80 mg/kg (x 2) in males and 200 mg/kg (x 2) in females. In addition, there was no difference in PCE/NCE ratio in F2695 treated animals at any dose. Plasma concentration of F2695 was determined to demonstrate exposure. The high doses used for females [200 mg/kg (x 2)] and for males [80 mg/kg (x 2)] were determined to be an MTD in a preliminary study, where one male and two female rats died at the dose level of 320/mg (x 2) and 1 male rat died at the dose level of 200 mg/kg (x 2). Adverse clinical signs included slight to strong decrease in motor activity, partially closed eyes and decrease in body temperature in both sexes at their respective high doses.

7.4 Other Genetic Toxicity Studies

None

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8 Carcinogenicity

8.1 Rat carcinogenicity study

Study title: F2695: A 104-week oral carcinogenicity study in rats

Study no.:	1712-001
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 20, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	L0003039 (Batch: OP210), 100.1 (% m/m)
CAC concurrence:	Yes

Key Study Findings

- Oral (gavage, in distilled water) administration of F2695 to Sprague-Dawley rats at 0, 0 (2 identical vehicle control groups), 10, 30, and 90/70 mg/kg/d (HD in males was lowered in Week 45) for 104 weeks (dosing in HD female group was discontinued in Week 87) did not result in increased number of any tumor types or neoplastic lesions.
- The MTD was demonstrated based on slightly increased mortality in HD females and decreased body weights at HD, mainly in males (≥10%).
- Treatment-related non-neoplastic microscopic findings were limited to centrilobular hepatocellular hypertrophy, increased eosinophilic foci of cellular alteration, increased clear foci of cellular alteration, and vacuolation of centrilobular hepatocytes.

Adequacy of Carcinogenicity Study

This study was conducted to assess the carcinogenic potential of F2695 (levomilnacipran HCl) and to establish the toxicokinetic profile of the test article following repeated oral administration (gavage) for 104 weeks in to male and female Sprague-Dawley rats. The doses used in this study were approved by the E-CAC (see recommendations below), based on MTD in short-term studies. Based on the Sponsor's request (and approved by the Division), the high dose in males was lowered (from 90 to 70 mg/kg/d) beginning in Week 45 due to significant decrease in mean body weight (14%) in that group. Beginning in Week 87 (Day 605), dosing was discontinued in HD female group (based on increased mortality and the Sponsor's request and subsequent approval by the Division), and all surviving HD females were sacrificed in Week 93 (Day 646; based on the Division's recommendation to prematurely terminate

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when the number of survivors decreases to 15). The doses were considered adequate based on the statistically significant decrease (≥10%) in body weight in HDM, compared to the combined control groups 1 and 2 (as well as to control groups 1 and 2 individually). In addition, the survival of HD females was statistically significantly lower when compared to the combined control groups 1 and 2 (as well as to control groups 1 and 2 individually); nevertheless, enough rats were exposed for a sustained amount of time to the risk of late developing tumors. Notable treatment-related findings included decreased mean body weights at ≥30 mg/kg/d for males and 90 mg/kg/d for females, compared to controls. Treatment-related toxicological findings included (non-neoplastic) centrilobular hepatocellular hypertrophy, increased eosinophilic foci of cellular alteration, increased clear foci of cellular alteration and vacuolation of centrilobular hepatocytes in HD males and HD females. Toxicokinetic analysis during this study showed that exposure to F2695 was similar in male and female rats at the low dose and slightly higher in females than males at the mid and high doses. At the high dose, the exposure ($AUC_{(0-24)}$) was ~12 to 14-fold (males and females) of the human exposure at the MRHD of 120 mg/d. In conclusion, the levomilnacipran carcinogenicity study in rats is considered adequate.

Appropriateness of Test Models

Sprague-Dawley rats were selected for the long-term carcinogenicity study based on the recommendations (ICH Guidance S1B); moreover rat is the usual rodent model used for evaluating the toxicity of various classes of chemicals and for which there is large historical database. Oral (gavage) administration was chosen because that is the intended clinical route and a sufficient number of animals were used (60/sex/group) in the study.

Evaluation of Tumor Findings

Based on the Sponsor's analyses, repeated daily administration of F2695 to male and female rats for two years did not show statistically significant positive dose response relationship in any of the tumor types, or higher tumor incidence in the treated groups in any of the tumor types compared to the controls in either sex. In males, when both identical control groups were combined and compared to the treated groups, benign pheochromocytoma of the adrenal glands was statistically decreased based on the Fisher Exact Test at all dose levels and there was a statistically significant trend with both Cochran-Armitage Trend Test and the Peto Test (28/120, 4/60, 4/60 and 5/60, at combined control, LD, MD, and HD, respectively)[9]. In females, when both identical control groups were combined, there was a statistically significant decrease in the incidence of adenoma of the pars distalis of the pituitary gland by the Fisher's Exact

[9] The Sponsor noted that there were no statistically significant differences for any tumor type in treated groups when compared to control group 1 (males); however, when control group 2 was compared to the treated groups, benign pheochromocytoma of the adrenal glands was statistically decreased.

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Test (96/120 and 35/60 for controls and HD, respectively) and a statistically significant trend with the Cochran-Armitage Trend Test[10].

Study results were independently reviewed and evaluated by the CDER statistical reviewer (Mohammad Atiar Rahman, Ph.D.) of the Division of Biometrics (see statistical review). Briefly, he found a statistically significant dose-response relationship in mortality across treatment groups in female rats. The pair-wise comparison in females showed statistically significant increased mortality in HD females compared to the combined control. However, none of the tested tumor types showed a statistically significant dose-response relationship in either sex. Similarly, pair-wise comparisons did not show statistically significant increase in the incidence of any tumor type in any of the treated groups compared to the combined control.

[10] The Sponsor noted that there were no statistically significant differences for any tumor type (females) when control group 2 was compared to treated groups (females); however, when control group 1 was compared to all other treated groups, the incidence of adenoma pars distalis of the pituitary was statistically decreased at the HD group; similarly, there was a statistically significant trend for decreased incidence of granular cell tumor of the uterus.

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Methods

Doses:	0, 0, 10, 30, 90 mg/kg/d (HD lowered from 90 to 70 mg/kg/d in males beginning in Week 45)
Frequency of dosing:	Once a day
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Solution/distilled water
Basis of dose selection:	13-week oral toxicity study (study № 932; Sponsor's code: F2695-22629; reviewed under IND 104,483 and summarized in section 6.2, above) at 0, 10, 35 and 120 mg/kg/d. Mortality (one male at 35 and one female at 120) without clear relationship to treatment. Reduced body weight gain (-10%) and food consumption (-12%) in HD females. Centrilobular hypertrophy of the liver with amorphous eosinophilic cytoplasm in hepatocytes (one MD male and most HD animals); NOAEL was 10 mg/kg/d. The Sponsor's proposed high dose levels of 120 mg/kg/d in males and 100 mg/kg/d in females were amended to 90 mg/kg/d by the E-CAC prior to study initiation.
Species/Strain:	Rat, Sprague-Dawley; CD® [Crl:CD® (SD)]
Number/Sex/Group:	60/sex/group (see Table 30, below for group assignment)
Age:	~8 weeks old at the start of dosing
Animal housing:	Two to three per cage in polyboxes with non-aromatic bedding; in the event of an animal death, the remaining animal(s) were housed singly or with the remaining cagemate.
Paradigm for dietary restriction:	Food and water *ad libitum*
Dual control employed:	Yes, identical vehicle groups.
Interim sacrifice:	No
Satellite groups:	11/sex/group (controls 6/sex/group; see Table 34, below)
Deviation from study protocol:	The study was designed for 104 weeks of dosing; however, due to increased mortality in HD female group, dosing was discontinued on Day 605 (~ week 86). In addition, there were some other minor deviations; however, according to the study director these deviations did not affect the quality or integrity of the study.

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Table 34 Group assignments in the 2-year rat carcinogenicity study

(Excerpted directly from this study report 1712-001; pp 16)

Group Assignments			
Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male	Female
Main Study			
1	0 (Control 1)	60	60
2	0 (Control 2)	60	60
3	10	60	60
4	30	60	60
5 (female)	90	NA	60[a]
5 (male)	90/70[b]	60	NA
Toxicokinetic (TK)			
6	0 (Control 1)	3+3[c]	3+3[c]
7	0 (Control 2)	3+3[c]	3+3[c]
8	10	9+2[c]	9+2[c]
9	30	9+2[c]	9+2[c]
10 (female)	90	9+2[c]	9+2[c]
10 (male)	90/70[b]	9+2[c]	9+2[c]
Sentinel[d]			
89	NA	20	20

NA – Not Applicable
[a] Dosing for Group 5 females was discontinued beginning on Day 605.
[b] Beginning in Week 45, the dose level for Groups 5 and 10 males was reduced to 70 mg/kg/day.
[c] Additional animals included as possible replacements.
[d] Untreated animals utilized for serological health screens.

Observations and Results

Mortality

Animals were observed for morbidity, mortality, injury, and the availability of food and water twice daily; beginning Week 53, a third mortality check was also conducted.

Table 35 Mortality rates at the end of treatment

(Compiled by the Reviewer from Sponsor's Table 1 of the study report 1712-001; pp 98-110)

Group (mg/kg/d)	Control 1 (0)		Control 2 (0)		LD (10)		MD (30)		HD (90)*	
	M	F	M	F	M	F	M	F	M	F
Number of animals	60	60	60	60	60	60	60	60	60	60
Number of deaths	40	38	39	37	42	39	46	44	34	44
Percentage	67	63	65	62	70	65	77	73	57	73

***beginning in Week 45, the dose level for HDM was reduced to 70 mg/kg/d**

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Figure 10 Sponsor's figure (with minor formatting modifications by the Reviewer) showing the mean survival estimates in male rats in the 2-year carcinogenicity study

Study report 1712-001, pp 84

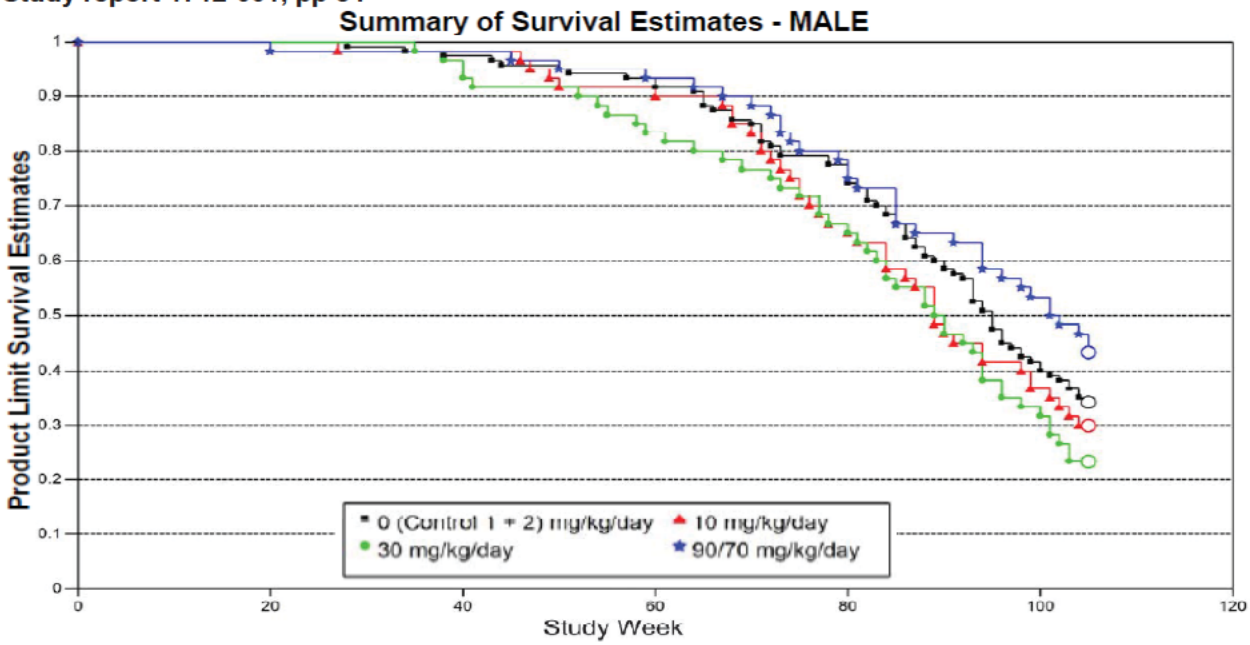
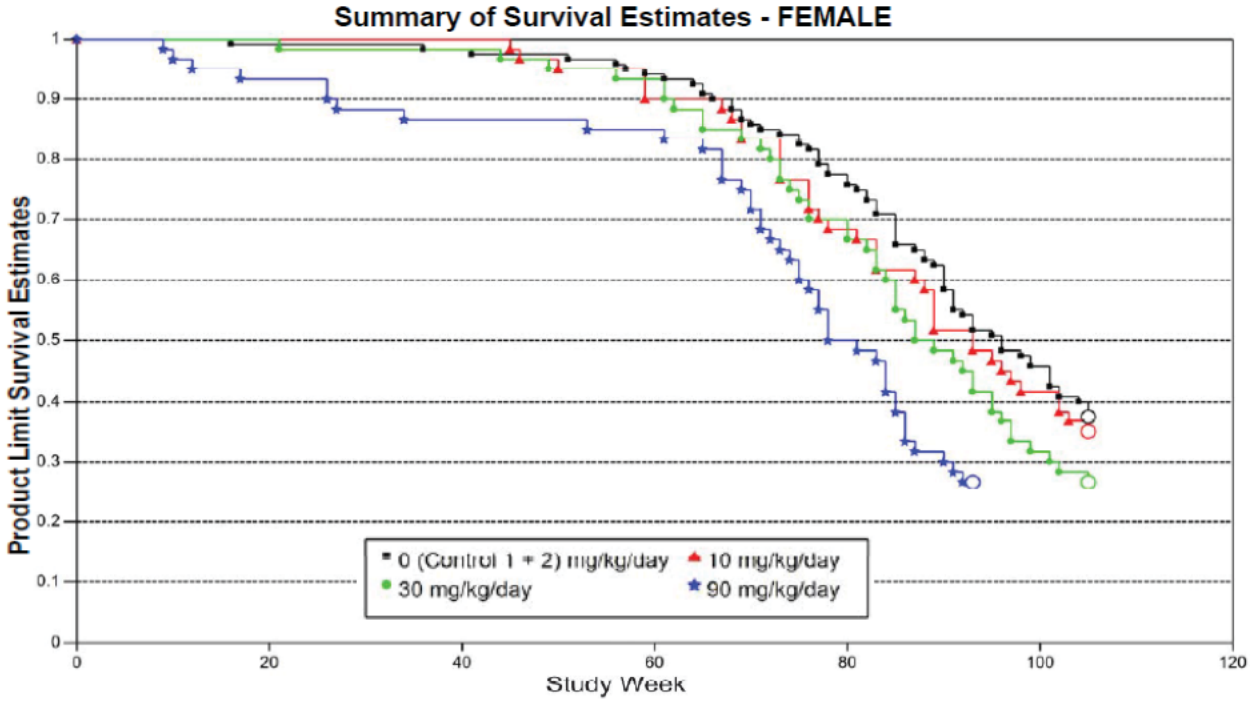


Figure 11 Sponsor's figure (with minor formatting modifications by the Reviewer) showing the mean survival estimates in female rats in the 2-year carcinogenicity study

Study report 1712-001, pp 87



Mortality rates at the end of the treatment period are shown in Table 35, above and the survival estimates are illustrated in Figure 10 and Figure 11 (males and females, respectively), above.

A total of 201 males and 202 females in the main study died or were euthanized. There were no treatment-related effects on the cause of death in either sex. According to the Sponsor's narrative, all causes of death/moribundity were of the type commonly seen in this type of study in rats. The most common cause of death was pituitary tumor and undetermined in both sexes and mammary tumors in females (see Table 36, below). The overall incidence of survival at the termination was similar to that of controls for males at all doses (30, 23, and 43%, respectively at 10, 30 and 90/70 mg/kg/d versus 30 and 35%, respectively in control groups 1 and 2) and for females at dose levels of 10 and 30 mg/kg/d (35 and 27%, respectively versus 37 and 38%, respectively in control groups 1 and 2), but was statistically significantly decreased for females at 90 mg/kg/d when compared to control 1, control 2, and the combined control groups 1 and 2 (HD females were terminated early on Day 646 and the corresponding survival percentage was 27%; the estimated survival percentage at the end of the 2-year study for HDF was 0%). At the end of 52 weeks, percentage of survival in high dose males and females were 95% and 87%, respectively. There was more than 50% survival in high dose males at the end of Week 91 (38/60); however, in the high dose female group, the survival was only 28% (17/60) at the end of Week 91.

Table 36 Factors contributing to mortality in various treatment groups

(Tabulated by the Reviewer from the study report 1712-001; Appendix C, pp 212-382

Sex		Males					Females				
Dose (mg/kg/d)		0	0	10	30	90/70	0	0	10	30	90
Number of animals		60	60	60	60	60	60	60	60	60	60
Factors contributing to death	Number of decedents @ (SNC)*	40 (20)	39 (21)	42 (18)	46 (14)	34 (26)	38 (22)	37 (23)	39 (21)	44 (16)	44 (16)
Pituitary gland adenoma, pars distalis, benign, primary		26 (16)	27 (14)	30 (12)	27 (9)	18 (18)	31 (20)	29 (16)	32 (15)	30 (11)	27 (8)
Mammary gland											
Adenocarcinoma, malignant, primary							18 (4)	13 (1)	9 (3)	14 (5)	11 (4)
Adenoma, benign, primary							0 (0)	3 (1)	2 (1)	3 (2)	1 (0)
Carcinosarcoma, malignant, primary							0 (0)	0 (0)	0 (0)	1 (0)	0 (0)
Fibroadenoma, benign, primary							15 (12)	15 (15)	18 (9)	15 (9)	13 (4)

@Died or euthanized; *SNC- scheduled necropsy

Clinical Signs

Detailed clinical examination performed prior to randomization; observations of main study animals performed weekly during the study (on occasion, clinical observations

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recorded at unscheduled intervals). Palpation of tissue masses conducted monthly for the first six months and twice monthly thereafter.

Results: Treatment-related clinical findings included increased incidences of teeth cut and salivation in HDM and salivation in HDF, compared to controls (Table 37, below). In addition, dose-related increased incidences of yellow discolored hair were observed in MDF and HDF. Several other clinical findings appeared to be unrelated to treatment since they occurred in a non-dose-dependent manner and were also observed in control groups.

Table 37 Sponsor's tabulated summary of clinical findings

(Excerpted directly from the Sponsor's study report 1712-001, pp 1410)

Dose (mg/kg/day)	0 (Control 1)		0 (Control 2)		10		30		90/70	
Sex	M	F	M	F	M	F	M	F	M	F
Number of Animals										
At Start of Study	60	60 60		60	60 60		60	60 60		60
Died or Sacrificed Moribund	40	38 39		37	42 39		46	44 34		44
Terminal Sacrifice	20	22 21		23	18 21		14	16 26		16[e]
Survival of Carcinogenicity Animals (%)	33	37 35		38	30 35		23	27 43		27
Clinical Observations[f]										
Teeth cut	6/3	66/5	0/0	4/3	38/6	5/2	0/0	0/0	81/11	9/6
Salivation	0/0	1/1	0/0	7/2	2/2	0/0	5/3	9/5	154/27	206/17
Hair discolored, yellow	50/2	78/5	17/5	82/6	4/3	21/3	23/4	196/16	7/2	683/35
Mass Findings	–	– –		–	– –		–	– –		–
Serological Health Screen	–	– –		–	– –		–	– –		–
Hematology	–	– –		–	– –		–	– –		–

– = No noteworthy findings

e - For females administered 90/mg/kg/day, 16 animals survived to the early termination on Day 646. This equates to a 27% survival for this group.

f - Weeks 1 to104. Reported as Number of Times Observed/Total Number of Animals Affected.

There were no treatment-related effects/trend in the incidence of palpable mass; the following incidences of mass findings were observed: 8, 8, 7, 12, and 10% in males at 0, 0, 10, 30, and 90/70 mg/kg/d, respectively and 53, 43, 48, 47, and 40% in females at 0, 0, 10, 30, and 90 mg/kg/d, respectively.

Body Weights

Main study and TK animals were weighed weekly during the study for the first 13 weeks, then once every 4 weeks thereafter.

Results: Body weight data are shown in Table 38 and Figure 12 and Figure 13, below; treatment-related decreased mean body weights were observed at ≥30 mg/kg/d in males and ≥90 mg/kg/d in females during the study when compared with controls. The decreases in HD males were in the range of 7 to 11%, consistently reaching statistical significance throughout the treatment period, when compared to both control groups and the combined control 1& 2. The decreases in MD males were minimal (3 to 6%), however, reaching statistical significance during the initial stage of treatment when compared to both control groups and the combined control 1 & 2. The decreases in HD females were in the range of 6 to 8%; however, reaching statistical significance only at certain intervals during the initial treatment period.

Table 38 Sponsor's tabulated summary of mean body weight changes (%) during the 2 year

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carcinogenicity study in rats

(Excerpted directly from the Sponsor's study report 1712-001; pp 4011)

Dose (mg/kg/day)	0 (Control 1)		0 (Control 2)		10		30		90/70	
Sex	M	F	M	F	M	F	M	F	M	F
Body Weight (g) (%)[g]										
Groups 2, 3, 4, 5 vs. 1										
Week 1	303.0	211.0	+2	-2 +2		+1	-1	-1	-2	-1
Week 10	529.7	288.4	+0	+0	-0	+2	-2	+1	-9**	-3
Week 21	622.4	325.2	+2	-1 +2		+3	-1	+1	-10**	-3
Week 37	722.8	371.4	+2	-2 +0		+3	-4	+2	-14**	-5
Week 61	796.1	435.3	+4	-2	+1	+2	-4	+1	-12**	-6
Week 69	814.2	450.8	+3	+1	+1	+3	-3	+4	-12**	-6
Week 73	816.9	466.3	+3	+0	+0	+4	-3	+3	-12**	-7
Week 89	824.3	495.1	+6	+3	+2	+7	-3	-0	-11**	+3
Week 101	750.0	508.2	+5	+1	+8	+1	+3	-5	-4**	NA
Groups 3, 4, 5 vs. 2										
Week 1	NA	NA	308.5	207.1	-0	+3	-3	+1	-4** +1	
Week 10	NA	NA	532.3	289.0	-1	+2	-2	+1	-9**	-3
Week 21	NA	NA	634.8	322.3	-0	+4*	-3	+2	-11**	-2
Week 37	NA	NA	733.8	363.5	-1	+5*	-5*	+4	-15**	-3
Week 61	NA	NA	825.7	425.4	-2	+5	-7**	+4	-15**	-3
Week 69	NA	NA	837.4	454.1	-2	+3	-6*	+3	-14**	-7
Week 73	NA	NA	844.6	466.9	-3	+4	-6*	+3	-15**	-8
Week 89	NA	NA	876.8	509.9	-4	+4	-9*	-3	-16**	+0
Week 101	NA	NA	785.8	511.3	+3	+1	-2	-6 -8		NA

Dose (mg/kg/day)	0 (Control 1 + Control 2)		10		30		90/70	
Sex	M	F	M	F	M	F	M	F
Groups 3, 4, 5 vs. 1+2								
Week 1	305.8	209.0	+1	+2	-2	+0	-3**	+0
Week 10	531.0	288.7	-1	+2	-2	+1	-9**	-3
Week 21	628.6	323.7	+1	+4*	-2	+2	-10**	-2
Week 37	728.4	367.4	-0	+4	-4* +3		-14**	-4
Week 61	810.9	430.4	-0	+4	-5**	+3	-14**	-5
Week 69	826.1	452.4	-1	+3	-4	+3	-13**	-6
Week 73	831.3	466.6	-2	+4	-5	+3	-13**	-8*
Week 89	851.3	502.5	-2	+5	-6	-2	-13**	+2
Week 101	768.3	509.7	+5	+1 +0		-5	-6	NA

NA – Not applicable; * – $p < 0.05$; ** – $p < 0.01$

g - For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences). All percent difference values are rounded to the nearest whole number.

Figure 12 Sponsor's figure (with minor formatting modifications by the Reviewer) illustrating the changes in mean body weights in male rats during the 2-year carcinogenicity study

Study Report 1712-001, pp 90

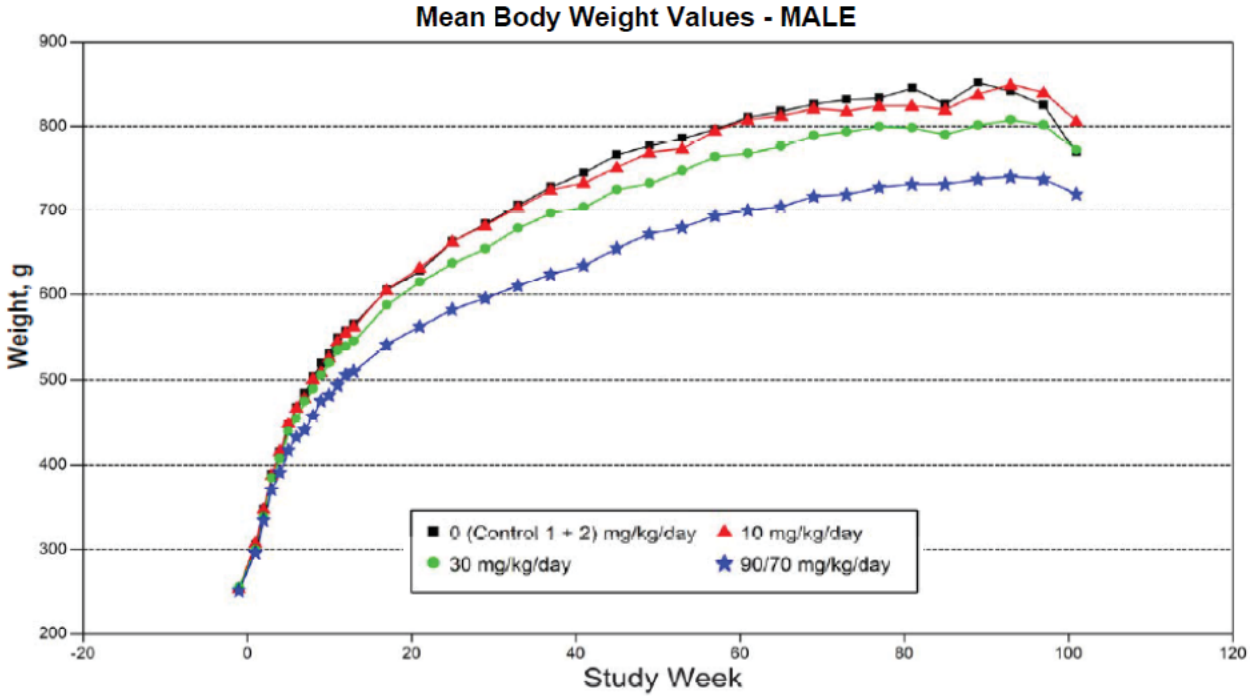
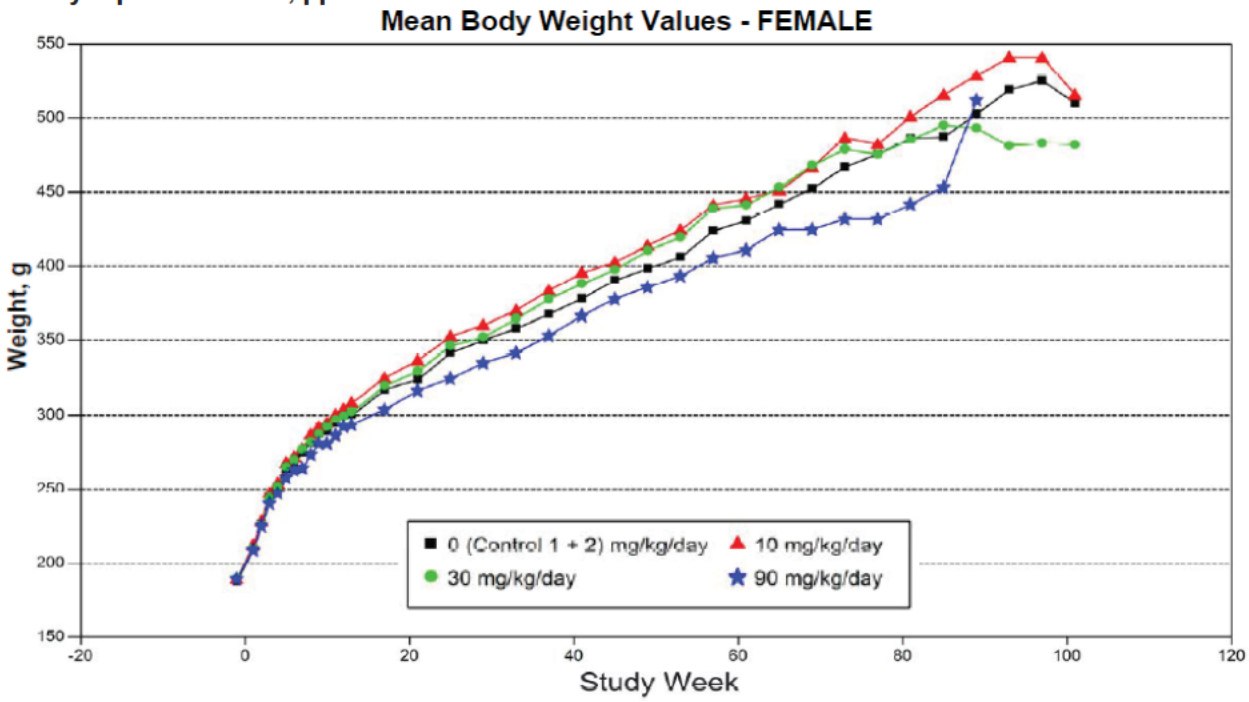


Figure 13 Sponsor's figure (with minor formatting modifications by the Reviewer) illustrating the changes in mean body weights in female rats in the 2-year carcinogenicity

Study report 1712-001, pp 92



Feed Consumption

Food consumption for main study animals was determined weekly during the first 13 weeks, then once every 4 weeks thereafter.

Results: There were minimal treatment-related effects on food consumption during the initial stages of treatment; statistically significant decreases in mean food consumption were noted in males at ≥30 mg/kg/d, and to a lesser extent in females at 90 mg/kg/d, somewhat correlating with the decreases in body weights (see Table 39, below). However, mean food consumption values were comparable to controls by Week 49 in MDM, by Week 69 in HDM, and by Week 65 in HDF.

Table 39 Sponsor's tabulated summary of changes in mean food consumption (%) during the 2 year carcinogenicity study in rats

(Excerpted directly from the Sponsor's study report 1712-001; pp 4012)

Dose (mg/kg/day)	0 (Control 1)		0 (Control 2)		10		30		90/70	
Sex	M	F	M	F	M	F	M	F	M	F
Food Consumption (g/animal/day) (%)[g]										
Groups 2, 3, 4, 5 vs. 1										
Week 1	22.68	16.11	+4	-0	+2	+0	-9**	-7**	-8**	-9**
Week 10	25.77	16.59	+4*	+6*	+2	+8**	-1	+5	-5**	+2
Week 21	25.41	17.10	+3	-5	+2	-1	-1	+5	-9**	-5
Week 37	24.91	17.27	+5**	-3	+0	-2	-4*	-2	-8**	-9**
Week 61	26.69	18.60	+1	-2	-2 -3		-1	-3	-7**	-7*
Week 69	25.21	16.80	-0	+2	-2	-7	+5	+4	-2	+8
Week 73	24.26	17.55	+4	+2	-2 +1		+8*	+6	+3	+4
Week 89	25.76	17.01	+2	+9	-4 +6		-8	-1	-6	+51**
Week 101	21.22	19.49	+4	-5	+12	-11	+0	-12 +9		NA
Groups 3, 4, 5 vs. 2										
Week 1	NA	NA	23.56	16.08	-2	+0	-12**	-6**	-12**	-8**
Week 10	NA	NA	26.85	17.52	-2	+3	-5**	-0	-9**	-3
Week 21	NA	NA	26.27	16.17	-1	+4**	-4**	+11**	-12**	+0
Week 37	NA	NA	26.27	16.76	-5**	+1	-9**	+1	-13**	-7**
Week 61	NA	NA	27.09	18.23	-3	-1	-3 -1		-8**	-5
Week 69	NA	NA	25.18	17.21	-2	-9	+5	+1	-1	+5
Week 73	NA	NA	25.27	17.86	-6	-1	+4	+5	-1	+2
Week 89	NA	NA	26.18	18.55	-6	-3	-9	-10 -8		+38**
Week 101	NA	NA	22.04	18.49	+8	-7	-3 -7		+5	NA

Dose (mg/kg/day)	0 (Control 1 + Control 2)		10		30		90/70	
Sex	M	F	M	F	M	F	M	F
Groups 3, 4, 5 vs. 1+2								
Week 1	23.12	16.09	-0	+0	-11**	-6**	-10**	-8**
Week 10	26.31	17.06	-0	+6*	-3	+2	-7**	-1
Week 21	25.84	16.63	+1	+2	-3*	+8**	-11**	-3
Week 37	25.60	17.01	-3*	-0	-6**	-0	-10**	-8**
Week 61	26.89	18.41	-2	-2	-2	-2	-8**	-6*
Week 69	25.19	17.00	-2	-8	+5	+3	-2	+7
Week 73	24.78	17.70	-4	+0	+6	+5	+1	+3
Week 89	25.97	17.78	-5	+2	-9	-6	-7	+44**
Week 101	21.63	19.00	+10	-9	-2	-10	+7	NA

NA = Not applicable; * = $p < 0.05$; ** = $p < 0.01$

g - For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences). All percent difference values are rounded to the nearest whole number.

Clinical Pathology

Blood samples for hematology parameters (WBC, RBC, NEU, Band NEU, Lymph, MONO, EOS, BASO and other cells) were collected from all main study animals at the scheduled necropsy and for main study animals euthanized *in extremis*. The animals had access to water and food prior to sample collection.

Results: There were no treatment-related effects on hematology parameters; erythrocytes were slightly (though statistically significantly) increased in MDM and HDM (~5 to 10%, relative to control 1 and control 2 and the combined controls 1&2).

Gross Pathology

Detailed necropsy examinations were performed on animals euthanized *in extremis*, animals found dead and all surviving main study animals at the scheduled necropsy.

Results: There were no treatment-related macroscopic findings in either sex; treatment with levomilnacipran did not result in increase in tumor incidence and/or increase in any tumor type. Even though there was a statistically significant increased mortality in the high dose female group, compared to the combined control group, there was no statistically significant positive dose response relationship in any of the observed tumor types or tumor incidence. All macroscopic observations were of the type commonly found in rats of this strain and age at the testing facility.

Histopathology

Microscopic examination was performed on protocol-designated sections of tissues (see Histopathology Inventory, Table 40, below) from all main study animals. The slides were examined by a board-certified veterinary pathologist.

Peer Review: Yes, re-examination of all tissues from 10% of the control groups (groups 1 & 2) and 10% of the high dose group animals selected randomly, all hyperplasias and neoplasias diagnosed by the study pathologist and target tissue (liver) from all animals in all groups were done by the peer review pathologist.

Neoplastic

There were no treatment-related increases in tumor incidence in either sex. The Sponsor's datasets were subjected to independent statistical analyses by Dr. Mohammad Atiar Rahman of the Division of Biometrics (see statistical review) for dose response relationships and pair-wise comparisons of control groups with each of the treated groups. Based on his analyses, none of the tested tumor types was considered to have statistically significant positive dose response relationship in either sex. Similarly, pair-wise comparisons did not show statistically significant increase in the incidence of any tumor type in any of the treated groups compared to the combined control.

Sponsor's analysis (using survival unadjusted and survival adjusted) showed statistically significant negative dose response relationship for the incidence of benign pheochromocytoma of the adrenal glands in males and the pair-wise comparisons showed statistically significant decreased incidence of benign pheochromocytoma of the adrenal glands when control group 2 and combined control groups were compared to the treated groups (18/60, 28/120, 4/60, 4/60, and 5/60 for control 2, combined control, LDM, MDM, and HDM, respectively).

Table 40 List of tissues and organs examined microscopically (2 year rat carcinogenicity in rats)

(Excerpted directly from the study report; Appendix L- Pathology Report, study 1712-001; pp 1114)

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- Adrenal (2)
- Aorta
- Bone with marrow [femur]
- Bone with marrow [sternum]
- Bone marrow smear [2 collected][a]
- Brain [cerebrum, midbrain, cerebellum, medulla/pons]
- Clitoral gland (2)
- Coagulating gland (2)
- Epididymis (2)
- Eye including optic nerve (2)
- Gastrointestinal tract:
  - esophagus
  - stomach [glandular and nonglandular]
  - duodenum
  - jejunum
  - ileum
  - cecum
  - colon
  - rectum
- Gonads:
  - ovary (2) with oviduct (2)
  - testis (2)
- Gross lesions
- Harderian gland (2)
- Heart
- Joint, tibiofemoral
- Kidney (2)
- Lacrimal gland, exorbital (2)
- Larynx
- Liver [3 sections collected; 2 examined]
- Lung [collected whole; 2 sections examined]
- Lymph nodes: mandibular, mesenteric and regional where applicable
- Mammary gland [process females only]
- Nasal tissue [Levels A, B, C, and D]
- Pancreas
- Peyers patch
- Pharynx
- Pituitary
- Preputial gland (2)
- Prostate and seminal vesicle (2)
- Salivary gland, mandibular/sublingual [2 collected; 1 examined]
- Salivary gland, parotid [2 collected; 1 examined]
- Sciatic nerve
- Skeletal muscle, biceps femoris
- Skin
- Spinal cord [cervical, thoracic, and lumbar]
- Spleen
- Thymus
- Thyroid/parathyroid (2)
- Tissue masses
- Tongue
- Trachea
- Ureter (2)
- Urinary bladder
- Uterus [both horns]/Cervix
- Vagina
- Zymbal's gland [2]

[a]Bone marrow smears were collected at the scheduled necropsy and held.

(2) Paired organ

Sponsor's analysis in female rats (using survival unadjusted and survival adjusted) showed statistically significant negative dose response relationship for the incidence of granular cell tumor of vagina and uterus. The survival unadjusted test also showed statistically significant negative dose response relationship for the incidence of c-cell adenoma of the thyroid and adenoma of the pars distalis of the pituitary gland. In pair-wise comparison with control 1, there was a statistically significant decreased incidence of c-cell adenoma of the thyroid in mid dose females (12/60 and 2/60 for control 1 and MDF, respectively) and adenoma of the pars distalis of the pituitary gland at the high dose (51/60 and 35/60 for control 1 and HDF, respectively). In addition, in pair-wise comparison using the combined control groups, the incidence of adenoma of the pars distalis of the pituitary gland was statistically decreased at the high dose (96/120 and 35/60 for combined controls and HDF, respectively).

A comparison between the Sponsor's findings and the findings of the independent statistical analyses was attempted by this Reviewer; Tables 41-43, are excerpted from the statistical review of Dr. Rahman and according to his analysis, there was a statistically significant negative dose response relationship for the incidence of benign

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pheochromocytoma of the adrenal glands in males and showed statistically significant decreased incidence of benign pheochromocytoma of the adrenal glands when combined control groups were compared to the treated groups (Table 41, highlighted), thus in agreement with the Sponsor's analysis. However, in his analysis the negative dose response relationship for the decreased incidence of adenoma of the pars distalis of the pituitary gland in female groups and the decreased incidence of adenoma of the pars distalis of the pituitary gland in HD females were not statistically significant (Table 42, highlighted). Similarly, the decreased incidence of c-cell adenoma of the thyroid in mid dose females were not statistically significant, although the negative dose response relationship for the incidence of c-cell adenoma of the thyroid in female groups was statistically significant (Table 43, highlighted). The Sponsor's finding of the statistically significant negative dose response relationship for the incidence of granular cell tumor of vagina and uterus in female groups was corroborated by Dr. Rahman's independent analysis (Table 43, highlighted).

Table 41 Part of Table 3A (highlighted by the Reviewer) of the statistical review

Excerpted directly from the independent statistical review; pp 14

Table 3A: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons
Male Rats

Organ Name	Tumor Name	0 mg Com C N=120	10 mg Low N=60	30 mg Med N=60	70 mg High N=60	P_Value Dose Resp	P_Value Com C vs. L	P_Value Com C vs. M	P_Value Com C vs. H
adipose tissue,	HIBERNOMA	0	0	1	0	0.4030	.	0.3109	.
adrenal glands	ADENOMA, CORTICAL	4	2	1	0	0.9334	0.6320	0.4963	0.8253
	CARCINOMA, C-CELL	1	0	0	0	0.5871	0.3140	0.3025	0.3465
	CARCINOMA, CORTICAL	1	2	1	0	0.7214	0.2353	0.5189	0.3492
	LYMPHOMA	1	0	0	0	0.5871	0.3140	0.3025	0.3465
	PHEOCHROMOCYTOMA	28	4	4	5	0.9963	0.9917	0.9899	0.9936

Com C: Combined Control

Table 42 Part of Table 3B (highlighted by the Reviewer) of statistical review

Excerpted directly from the independent statistical review; pp 21-22

Table 3B: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons
Female Rats

Organ Name	Tumor Name	0 mg Com C N=120	10 mg Low N=60	30 mg Med N=60	70 mg High N=60	P_Value Dose Resp	P_Value Com C vs. L	P_Value Com C vs. M	P_Value Com C vs. H
pituitary gland	ADENOMA, PARS DISTAL	96	47	41	35	0.9208	0.6346	0.8665	0.8644
pituitary gland	ADENOMA, PARS INTERM	1	0	0	0	0.5664	0.3200	0.3033	0.2741
	CARCINOMA, PARS DIST	5	2	2	1	0.6846	0.3919	0.3660	0.5377
	PITUICYTOMA, PARS NE	0	0	1	0	0.3628	.	0.3089	.
	SARCOMA, HISTIOCYTIC	1	0	0	0	0.5639	0.3175	0.3008	0.2721

Com C: Combined Control

Table 43 Part of Table 3B (highlighted by the Reviewer) of statistical review

Excerpted directly from the independent statistical review; pp22

Table 3B: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons
Female Rats

Organ Name	Tumor Name	0 mg Com C N=120	10 mg Low N=60	30 mg Med N=60	70 mg High N=60	P_Value Dose Resp	P_Value Com C vs. L	P_Value Com C vs. M	P_Value Com C vs. H
thyroid gland	ADENOMA, C-CELL	17	5	2	3	0.9554	0.7619	0.9647	0.8629
	ADENOMA, FOLLICULAR	0	2	2	0	0.4905	0.1006	0.0972	.
	CARCINOMA, C-CELL	3	0	0	0	0.9199	0.6892	0.6655	0.6207
	CARCINOMA, FOLLICULA	1	0	1	0	0.4216	0.3200	0.5163	0.2741
tongue	CARCINOMA, SQUAMOUS	1	0	0	0	0.5664	0.3200	0.3033	0.2741
urinary bladder	PAPILLOMA, TRANSITIO	0	0	0	1	0.1637	.	.	0.2741
uterus with cer	GRANULAR CELL TUMOR	7	1	1	0	0.9762	0.7866	0.7663	0.9001
	LEIOMYOMA	1	0	0	0	0.5664	0.3200	0.3033	0.2741
	LEIOMYOSARCOMA	1	0	1	0	0.4216	0.3200	0.5163	0.2741
	POLYP, STROMAL	7	3	1	2	0.7021	0.4110	0.7554	0.4837
	SARCOMA, HISTIOCYTIC	1	0	0	0	0.5639	0.3175	0.3008	0.2721
	SARCOMA, STROMAL	1	0	0	1	0.3074	0.3200	0.3033	0.4822
vagina	GRANULAR CELL TUMOR	8	3	2	0	0.9753	0.4914	0.6493	0.9290
	LEIOMYOSARCOMA	1	0	0	0	0.5664	0.3200	0.3033	0.2741

Com C: Combined Control

Non Neoplastic

Treatment-related centrilobular hepatocellular hypertrophy (minimal to mild severity) was observed in the liver of male rats at 90/70 mg/kg/d and female rats at 90 mg/kg/d (see Table 44, below). According to the Sponsor's narrative, hypertrophy was

characterized by enlargement of hepatocytes surrounding central veins and the cytoplasm of the affected hepatocytes was increased and stained slightly more eosinophilic and homogeneously than in unaffected animals. Additionally, eosinophilic foci of cellular alteration were increased in the liver of both HDM and HDF; however, clear foci of cellular alteration were only increased in HDM. Vacuolation of centrilobular hepatocytes was increased in HDM.

Table 44 Sponsor's table showing treatment-related microscopic findings in the liver (Excerpted directly from the study report 1712-001; pp 31)

Test Article-related Microscopic Findings Liver										
Dose level: mg/kg/day	Control 1		Control 2		10		30		90/70	90
Sex	M	F	M	F	M	F	M	F	M	F
Number Examined										
Liver										
Focus of cellular alteration, eosinophilic	3	7	5	8	6	5	4	6	12	15
-minimal	2	5	5	4	6	5	4	5	10	13
-mild	1	2	0	3	0	0	0	1	2	2
-moderate	0	0	0	1	0	0	0	0	0	0
Hypertrophy, hepatocyte, centrilobular	0	0	0	0	0	0	0	0	50	31
-minimal	0	0	0	0	0	0	0	0	34	28
-mild	0	0	0	0	0	0	0	0	16	3
Vacuolation, centrilobular	3	2	1	0	1	1	6	1	32	3
-minimal	3	0	1	0	1	1	5	1	27	2
-mild	0	2	0	0	0	0	1	0	5	1

M - Male
F - Female

Toxicokinetics

Blood samples were collected from cohorts of 3 TK animals/group on Days 1, 90, 180, and 365 at 0.5, 1, 4, 12, and 24 hr, post-dose (control animals at 1 hr, post-dose).

Results: Systemic exposure to F2695 in terms of AUC_{0-24} and C_{max} was similar in male and female rats on Days 1, 90, 180 and 365 following 10/mg/d and slightly higher in females than in males after 30 and 90/70 mg/kg/d (Table 45, below). On Day 1, AUC_{0-24} and C_{max} increased more than in proportionally to dose between 10 and 90 mg/kg/d. Following repeated dosing for 365 days, there was evidence of slight accumulation; accumulation ratios ranged from 0.722 to 2.72 for AUC_{0-24} and C_{max} and were similar across doses. For each dose, accumulation ratio tended to increase between Day 90 and Day 365. T_{max} and $T_{1/2}$ were similar across dose level and days of dosing.

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Table 45 Sponsor's table (excerpted and reconstructed by the Reviewer) showing the toxicokinetic parameters for F2695 in male and female rats in the 2-year carcinogenicity study

Report 1712-001; pp 1061-1067

Day	Dose (mg/kg/day)	Sex	AUC_{0-24} (hr•ng/mL)	C_{max} (ng/mL)	$t_{1/2}$ (hr)	T_{max} (hr)
1	10	M	1320	431	1.46	0.500
		F	2380	742	1.47	0.500
		Combined	1850	586	1.47	0.500
	30	M	7120	2410	2.01	0.500
		F	8490	2410	2.16	0.500
		Combined	7800	2410	2.09	0.500
	90	M	35000	4550	2.70	0.500
		F	27800	5720	2.76	0.500
		Combined	31400	5130	2.73	0.500
90	10	M	2190	523	2.76	0.500
		F	3240	982	1.89	0.500
		Combined	2710	753	2.33	0.500
	30	M	8570	1740	3.21	1.00
		F	12400	4420	2.30	0.500
		Combined	10500	3080	2.75	0.750
	90	M	39100	4280	3.66	1.00
		F	62700	8670	3.25	1.00
		Combined	50900	6470	3.46	1.00
180	10	M	2900	755	2.62	0.500
		F	4020	1090	2.43	0.500
		Combined	3460	925	2.53	0.500
	30	M	11000	3490	2.54	0.500
		F	15100	2610	2.62	0.500
		Combined	13100	3050	2.58	0.500
	90	M	60900	6350	3.23	1.00
		F	74800	8710	3.43	1.00
		Combined	67900	7530	3.33	1.00
365	10	M	3640	945	2.13	0.500
		F	3860	1260	2.32	0.500
		Combined	3750	1100	2.22	0.500
	30	M	10900	2110	2.79	1.00
		F	15300	2830	3.60	1.00
		Combined	13100	2470	3.19	1.00
	90/70*	M	33500	4510	2.96	1.00
		F	75500	9220	2.51	1.00

*Dose was lowered for male rats beginning Week 45.

Dosing Solution Analysis

Dosing formulation analyses for stability, concentration, and homogeneity were performed. Results of the homogeneity analysis (duplicate samples from the top, middle, and bottom of the low and high concentrations) showed that the samples were within the acceptance criteria (100 ± 10%). Stability analysis following 7, 14, and 94 days of refrigerated (2-8 °C) storage of duplicate samples from the middle stratum at the low and high concentrations showed that the average recovery was within the acceptance criteria of 100 ± 10%. Concentration verification for Week 1 and every three months thereafter until Week 104 [0 (vehicle control), 2, 6, and 18 mg/mL concentrations) showed an average recovery of 100 ± 10%; Test article was not detected in any of the vehicle control samples.

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Summary

The carcinogenic potential of F2695 (levomilnacipran, HCl) was assessed in male and female Sprague-Dawley rats following repeated oral administration (gavage) for 104 weeks. The doses of the test article [0, 0 (2 identical vehicle control groups), 10, 30, and 90 mg/kg/d] were approved by the E-CAC based on MTD in short-term studies. The high dose in males was lowered (from 90 to 70 mg/kg/d) beginning in Week 45 due to significant decrease in mean body weight in that group. Beginning in Week 87 (Day 605), dosing was discontinued in HD female group due to increased mortality in that group.

Treatment-related decrease in mean body weights were noted at ≥30 mg/kg/d for males and at 90 mg/kg/d for females, when compared to controls. The overall survival in males of all treatment groups was comparable to control groups; however, the overall survival was statistically significantly decreased for females at 30 and 90 mg/kg/d, compared to control groups. There were no treatment-related increases in any of the observed tumor types in either sex. Pair wise comparisons also did not show statistically significant increase in the incidence of any tumor type in any of the treated groups, when compared to the combined control groups. Treatment-related non-neoplastic microscopic findings included centrilobular hepatocellular hypertrophy, increased eosinophilic foci of cellular alteration, increased clear foci of cellular alteration, and/or vacuolation of centrilobular hepatocytes. Based on treatment-related decrease in mean body weights at doses ≥30 mg/kg/d for males and ≥90 mg/kg/d for females, it is concluded that MTD was achieved in the 2-year rat carcinogenicity study.

The human exposure margin (at the maximum recommended human dose of 120 mg/d) based on the systemic exposure at the highest dose of 90 mg/kg/d is ~13-fold (see Table 46, below)

Table 46 Rat-Human multiples of exposure for levomilnacipran, HCl

[Human exposure margin calculated by the Reviewer using the TK parameters for the 2-year carcinogenicity study in Sprague-Dawley rats (Table 12, above) and human PK parameters from Table 11.2.2-1 of the Clinical Study Report LVM-PK-15, pp 46]

Rat 2-year carcinogenicity study	Dosage (mg/kg/d)	Rat TK Parameters#		Human Exposure Margin (MRHD = 120 mg/d)	
		C_{max} (ng/mL)	AUC_{0-24} (ng*h/mL)	C_{max} (ng/mL)	AUC_{0-t} (ng*h/mL)
		M + F	M + F	M + F	M + F
F2695	10	925	3460	4	1.5
	30	3050	13100	13	2.5
	90	7530	67900	33	13

TK measurements of Day 180

8.2 Mouse carcinogenicity study

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Study title: F2695: 26-week repeated dose oral carcinogenicity study in Tg.rasH2 mice

Study no.:	AC32RU.7G8R.BTL
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 31, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	F2695, lot #L0003039 (Sample 0001), L0003838 (Sample 0002); 100.1% m/m for Sample 001 and 99.7% m/m for Sample 002
CAC concurrence:	Yes

Key Study Findings

- Male and female Tg.rasH2 mice were dosed orally (by gavage, in distilled water) at 0, 15, 50 and 150 mg/kg/d for 26 weeks; the doses, including the positive control (1000 mg/kg/d, urethane, i.p.), had been approved by the Executive-CAC.
- MTD was not demonstrated.
- Statistically significant increase in the incidence of splenic hemangiosarcomas in HD males only, when compared to vehicle control. However, the numerical increase in the incidence of hemangiosarcomas was only slightly higher than the historical control range by one animal and therefore, the biological significance of the finding is not clear.
- In high dose males and females, there was a numerical increase in the combined incidence of hemangiosarcomas in multiple organs; however, the incidence was within the historical control range.

Adequacy of Carcinogenicity Study

The purpose of this study was to assess the carcinogenic potential of F2695 (levomilnacipran HCl) and to establish the toxicokinetic profile of the test article following repeated oral administration (gavage) for 26 weeks in hemizygous Tg.rasH2 mice. The Tg.rasH2 mouse model is considered to be a suitable alternative model to the conventional 2-year mouse carcinogenicity bioassay. Oral (gavage) administration was chosen because that is the intended clinical route and a sufficient number of animals were used (25/sex/group) in the study. In addition, an appropriate positive control group (urethane; i.p.) was used to demonstrate the validity of the test system's susceptibility to carcinogen. The doses for this assay (0, 15, 50 and 150 mg/kg/d) were selected based on the Executive CAC recommendations (meeting minutes sent to the Sponsor on August 12, 2010). The Sponsor's (proposed) choice of doses for this study

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(25, 75 and 150 mg/kg/d) was based on a 5-day range-finding study in CByB6H1 wild type littermate mice (doses: 25, 50, 100, 200, and 300 mg/kg/d) in which one hundred percent mortality was observed at 300 mg/kg/d and some deaths at 200 mg/kg/d. The high dose of 150 mg/kg/d in this study is approximately one-half the dose at which all animals in both sexes died in the 5-day range-finding study in CByB6H1 wild type littermate mice; it is worth noting that there were virtually no treatment-related signs of toxicity up to and including the high dose of 150 mg/kg/d in a separate 28-day repeat dose study in Tg.rasH2 transgenic mice.

Treatment with F2695 did not increase mortality at the doses used in the study. There were no severe clinical signs, no changes in body weights, and no non-neoplastic lesions that could be attributed to treatment with F2695. Thus, it appears that the doses used in this study are below the MTD. There was a statistically significant increase in the incidence of hemangiosarcomas in the HD male group only; however, the numerical increase in the incidence of hemangiosarcomas is only slightly higher than the historical control established at the test facility (b) (4). Toxicokinetic analysis showed that exposure to F2695 was similar in males and females on Days 1 and 178. Increased mortality, clinical signs of toxicity and changes in body weights were observed in the positive control (urethane) group, as expected based on the toxicity of urethane. In addition, an increase in pulmonary and splenic lesions was observed in the positive control group, thus validating the test assay.

Appropriateness of Test Models

The Tg.rasH2 mouse model is considered to be a suitable alternative model to the conventional 2-year mouse carcinogenicity bioassay. Oral (gavage) administration was chosen because that is the intended clinical route and a sufficient number of animals were used (25/sex/group) in the study. In addition, an appropriate positive control group (urethane; i.p.) was used to demonstrate the validity of the test system's susceptibility to carcinogen.

Evaluation of Tumor Findings

Repeated daily administration of F2695 to male and female rasH2 transgenic mice for six months resulted in a statistically significant increase in the incidence of hemangiosarcomas in the spleen of high dose males, but not in females. There was a numerical increase in the incidence of hemangiomas and hemangiosarcomas in multiple organs in HDM and HDF; however, the increases did not reach statistical significance. The Sponsor noted that those numerical increases in splenic hemangiosarcomas were only slightly higher than the historical range established at the testing facility (b) (4). There were no other treatment-related lesions (neoplastic or non-neoplastic) in this study. In the positive control animals (both male and female groups), there was a statistically significant increase in the incidence of pulmonary tumors (multiple adenomas and carcinomas) as well as statistically significant increase in the incidence of splenic hemangiosarcomas when compared to controls, thus validating the assay.

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Study results were independently reviewed and evaluated by the CDER statistical reviewer (Mohammad Atiar Rahman, Ph.D.) of the Division of Biometrics (see statistical review). According to his review, there was a statistically significant dose response relationship for the incidence of hemangiosarcoma in the spleen (only) of both male and female mice (see Table 47, below). However, the pair-wise comparisons did not show statistically significant increase in splenic hemangiosarcoma or any other tumor types in any of the treated groups, when compared to the vehicle control. The pair-wise comparisons showed statistically significant increase in the incidence of lung and spleen tumors in the positive control (male and female) groups, when compared to the respective vehicle controls.

Table 47 Statistical Reviewer's table (highlighted by this Reviewer)

Excerpted directly from the independent statistical review; pp 8

Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pairwise Comparisons in Mice

							P-Value			
Sex	Organ Name	Tumor Name	Veh C* N=25	Low N=25	Med N=25	High N=25	Dose Resp	Veh C vs. L	Veh C vs. M	Veh C vs. H
Male	spleen	hemangiosarcoma	1	0	1	5	0.0058*	0.5000	0.2551	0.0941
Female	spleen	hemangiosarcoma	0	1	0	3	0.0326*	0.5000	.	0.1173

* Veh C: Vehicle Control

Methods

Doses:	0, 15, 50, and 150 mg/kg/d and 1000 mg/kg/d (positive control),
Frequency of dosing:	Once daily, for 26 consecutive weeks (3 doses of positive control on study days 1, 3, and 5)
Dose volume:	10 mL/kg/d
Route of administration:	Oral gavage (positive control, i.p. injection)
Formulation/Vehicle:	Solution/sterile water (positive control: solution/ saline)
Basis of dose selection:	Based on the results of a previous range-finding study
Species/Strain:	Tg.rasH2 transgenic mice (main study) and CByB6F1 wild type littermate mice for TK
Number/Sex/Group:	25/sex/group (15/sex/positive control)
Age:	8 weeks (21-26 g males and 16-21 g females, at the beginning of the study)
Animal housing:	Environmentally controlled room; one per cage during dosing and two per cage during acclimation
Paradigm for dietary restriction:	*ad libitum*
Dual control employed:	No (positive control employed)
Interim sacrifice:	No
Satellite groups:	TK, 38/sex/group (8/sex/group for control)- see Table 48, below for study design
Deviation from study protocol:	No

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Table 48 Tg.rasH2 carcinogenicity; study design

(Excerpted directly from this study report AC32RU.7G8R.BTL, pp 14)

Group	Dose levels (mg/kg/day)	Dose Concentration (mg/mL)	Number of Animals			
			Main Study (Tg.rasH2)		TK Study (wild type littermates)**	
			Male	Female	Male	Female
Group 1 (Vehicle)	0	0	25	25	8	8
Group 2 (Positive Control)	1000 (urethane)*	100	15	15	---	---
Group 3	15	1.5	25	25	38	38
Group 4	50	5	25	25	38	38
Group 5	150	15	25	25	38	38
Total	---	---	115	115	122	122

*The Positive Control animals were administered a total of 3 intraperitoneal (i.p.) injections on Study Days (SD) 1, 3 and 5.

**Extra TK animals (2/sex) were used to try to ensure adequate animals for TK bleeding

Observations and Results

Mortality

Observed twice daily, at least 6 hr apart

Results: There were no test article-related deaths; however, mortality was observed in the main study (see Table 49, below) and was limited to 1 male and 1 female of the high dose group (Day 40 and 141, respectively), 1 male in the low dose group (Day 3) and 2 males in the vehicle control group (Day 148 and 176, respectively).

Table 49 Summary of mortality in Tg.rasH2 mice in the main study

(Excerpted directly from this study report AC32RU.7G8R.BTL; pp 367)

Group	Sex	Mode of Death	Animal Number	Day of Removal	Cause of Death
1	Male	Natural Death	5504	176	Undetermined
1	Male	Moribund Sacrifice	5508	148	Multicentric Sarcoma
3	Male	Natural Death	5543	3	Undetermined
5	Male	Natural Death	5592	40	Undetermined
5	Female	Natural Death	5717	141	Splenic Hemangiosarcoma

Group 1 (male) – 0 mg/kg/day
Group 3 (male) – 15 mg/kg/day
Group 5 (male and female) – 150 mg/kg/day

The cause of death for one of the control males was multicentric sarcoma and the cause of death for the second control male was undetermined. The cause of death for

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the low dose male and the high dose male was undetermined. The cause of death for the high dose female was splenic hemangiosarcoma.

In the positive control groups, 7 males and 8 females did not survive to scheduled sacrifice; deaths were attributed to urethane toxicity (lungs with bronchiolar adenoma/carcinoma and splenic hemangiosarcoma).

According to the Sponsor's narrative, 3 high dose males and 2 high dose females in the TK group (CByB6F1 wild type littermate) mice were found dead during the study period. No necropsy data for the dead TK animals were provided by the Sponsor; however, the death is likely to be treatment-related considering the fact that the lowest lethal dose in the 5-day range-finding study in CByB6F1 mice was 200 mg/kg/d.

Clinical Signs

Observed twice daily, within 2 hr after dosing; in addition, detailed hands-on examinations were performed for main study animals on Day 1 and weekly thereafter (for the positive control animals, the cage side observations were performed on Days 1, 3 and 5 within 2 hr after dosing).

Results: During cage side observations, the most common treatment-related clinical signs were hunched posture and rapid and shallow breathing; these findings were more frequent in females than in males at dose levels ≥50 mg/kg/d. During hand-on observation, the most common treatment-related findings were hunched posture, thinness and rapid and shallow breathing at doses ≥15 mg/kg/d; however, in males, these signs were mostly seen at the high dose and mainly after study Day 99. In females, the incidence of hunched posture was dose dependently increased and reached statistical significance at the high dose. The incidence of rapid/shallow breathing was higher in mid and high dose females and mainly seen after study Day 99.

In positive control animals, urethane treatment-related clinical signs included ataxia, decreased motor activity, impaired gait, prostrate/hunched posture and rapid/shallow breathing.

Body Weights

Body weights for the main study animals and TK animals measured on Day 1, weekly through week 13, and biweekly thereafter.

Results: There were no treatment-related changes in the group mean body weights between the treated and control groups. Some sporadic, statistically significant differences in the group mean body weight changes between treated and control groups were observed during the treatment period; however, the absolute body weight gain (Day 1 to Day183) was similar in control and treatment groups. In the positive control groups (male and female), the mean body weights were higher (at the time of termination) than the mean body weight of the control groups (23 and 27%, respectively in male and female groups).

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Feed Consumption

Measured (main study animals only) weekly

Results: There were no meaningful differences in the group mean food consumption between the treated and control groups that could be attributed to treatment with F2695; however, sporadic statistically significant differences were noted at some measurement intervals. In the positive control groups (male and female), the mean food consumption was higher than controls by an average of 22% during Day 57 through Day 113, most of the increases were statistically significant.

Gross Pathology

Necropsy on all surviving main study animals on Day 183 or 184 (surviving positive control animals during week 18). The following tissues/organs (Table 50, below) for each main study animal were collected and fixed in 10% NBF.

Table 50 List of organs/tissues collected for macroscopic and microscopic evaluations (6-month Tg.rasH2 carcinogenicity study)

(Excerpted directly from this study report AC32RU.7G8R.BTL; pp 366)

Adrenal glands	Pancreas
Aorta	Parathyroid glands
Bone (femur and sternum)	Pituitary gland
Bone marrow (femur and sternum)	Prostate gland
Brain	Salivary gland
Epididymides	Sciatic nerve
Esophagus	Seminal vesicles
Eyes	Skeletal muscle (thigh)
Gall bladder	Skin, mammary area
Gross lesions	Small intestine (duodenum, jejunum, and ileum)
Harderian gland	Spinal cord (cervical, thoracic, and lumbar)
Heart	Spleen
Kidneys	Stomach
Large intestine (cecum, colon, rectum)	Testes
Liver	Thymus
Lungs and bronchi	Thyroid glands
Lymph nodes (mesenteric, mediastinal, and mandibular)	Trachea
Mammary gland (Females only)	Urinary bladder
Nasal cavity	Uterus
Ovaries	Vagina

Results: Increase in spleen nodules in high dose males (5/25) was the only treatment-related macroscopic finding (with microscopic correlates); although there were spleen nodules in 2 high dose females (2/25), this was not considered to be treatment-related

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because it fell within historical control range (see Table 51, below). In the urethane treated positive control groups, treatment-related increase in lung nodules (22 and 25 nodules in males and females, respectively) and spleen nodules (17 nodules each in males and females) were observed, thus validating the study.

Organ Weights

At termination (main study animals except for the positive control group and for animals found dead/moribund); adrenal gland, brain, heart, kidneys, liver, spleen, and testes/ovaries were weighed.

Results: Statistically significant differences in organ weights in high dose males and females, compared to control (see Table 52, below); however, there were no lesions to account for the decrease in absolute splenic weight and the increase in relative liver weight. Since the differences are small, the Sponsor did not consider these changes to be of biologically significant.

Table 51 Historical control data for the 6 month carcinogenicity study in Tg.rasH2 mice (Excerpted directly from the study report AC32RU.7G8RU.BTL; pp 616-617)

Tissues/Organs	Male (n=525)		
	Total (# of animals)	%	Range (# of animals)
Lungs			
Lungs, adenoma; single	55	10.5	0-6
Lungs, adenoma; multiple	8	1.5	0-1
Lungs, carcinoma	4	0.8	0-2
Lungs, adenoma and carcinoma	-	-	0-7
Spleen			
Spleen, hemangiosarcoma	19	3.6	0-4
Hemangiosarcoma, Other Tissues			
Testes	4	0.8	0-1
Liver	1	0.2	0-1
Lungs	1	0.2	0-1
Seminal vesicles	1	0.2	0-1
Nasal cavity	1	0.2	0-1
Subcutis	1	0.2	0-1
Epididymides	1	0.2	0-1
Hemangiosarcoma, All Tissues	-	-	0-4
Other Tumors			
Spleen, lymphoma	1	0.2	0-1
Skin, papilloma	2	0.4	0-1
Harderian glands, adenoma	7	1.3	0-2
Stomach, nonglandular, papilloma	2	0.4	0-1
Stomach, nonglandular, squamous cell carcinoma	2	0.4	0-1
Skin, sarcoma	1	0.2	0-1
Prostate, transitional cell carcinoma	1	0.2	0-1
Multiple organ, mesothelioma	2	0.4	0-1
Jaw. Sarcoma	1	0.2	0-1
Liver, adenoma	1	0.2	0-1
Nasal Cavity, adenoma	3	0.6	0-2

Tissues/Organs	Female (n=525)		
	Total (# of animals)	%	Range (# of animals)
Lungs			
Lungs, adenoma; single	36	6.9	0-6
Lungs, adenoma; multiple	4	0.8	0-1
Lungs, carcinoma	4	0.8	0-1
Lungs, adenoma and carcinoma	-	-	0-6
Spleen			
Spleen, hemangiosarcoma	20	3.8	0-4
Hemangiosarcoma, Other Tissues			
Mammary glands	1	0.2	0-1
Skin (multiple sites)	3	0.6	0-1
Uterus	9	1.7	0-2
Lungs	1	0.2	0-1
Kidney	1	0.2	0-1
Bone	1	0.2	0-1
Ovary	2	0.4	0-1
Subcutis	1	0.2	0-1
Vagina	1	0.2	0-1
Hemangiosarcoma, All Tissues	-	-	0-5
Other Tumors			
Spleen, lymphoma	4	0.8	0-2
Spleen, leukemia	2	0.4	0-1
Liver, leukemia	1	0.2	0-1
Harderian glands, adenoma	13	2.5	0-4
Harderian glands, carcinoma	2	0.4	0-2
Stomach, nonglandular, papilloma	3	0.6	0-1
Stomach, nonglandular, squamos cell carcinoma	1	0.2	0-1
Lymphoma, multiple organ	3	0.6	0-1
Skin; squamos cell carinoma	1	0.2	0-1
Nasal cavity, adenocarcinoma	4	0.8	0-1
Ovaries; teratoma	1	0.2	0-1
Ovaries; leiomyosarcoma	1	0.2	0-1
Multiple organ, mesothelioma	2	0.4	0-1
Thymus, thymoma	1	0.2	0-1
Stomach; adenocarcinoma	1	0.2	0-1

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Table 52 Sponsor's table summarizing the statistically significant differences in organ weights

Excerpted directly from this study report AC32RU.7G8R.BTL; pp 23

Parameter	Males		Females	
	Dose Group	Significant difference (%)	Dose Group	Significant difference (%)
Spleen Wt.	5	↓ (12.2)	5	↓ (23.7)
Relative Spleen Wt.	-	-	5	↓ (24.5)
Relative Kidney Wt.	-	-	3	↓ (3.9)
Relative Liver Wt.	5	↑ (5.7)	-	-

↑ = Statistically significantly ($p < 0.05$) increased compared to Group 1.
↓ = Statistically significantly ($p < 0.05$) decreased compared to Group 1.
- = Difference was not exhibited in this cohort.

Gross lesions

Treatment-related increase in spleen nodules in HD males (5/25) related to the increase in hemangiosarcomas (discussed below under neoplastic lesions). In the positive control group (group 2 males and females), there was increased incidence of pulmonary and splenic lesions and all males and females in the group were affected.

Histopathology

At termination, routine exam of tissues listed in Table 50, above in all groups (except the positive control group in which only lung and spleen were examined) were conducted.

Peer Review: Yes; a microscopic peer review was performed on all proliferative lesions from all animals, including from the positive control group and all tissues from 20% of the animals. Differences of opinion between the study and reviewing pathologists were resolved through consensus.

Neoplastic

Neoplastic lesions in lungs: Statistically significant increased incidence of pulmonary tumors (single adenomas, multiple adenomas and carcinomas) were observed in the positive control (male and female) group in comparison with vehicle control, demonstrating the validity of the test (Group 2 in Table 53, below).

Table 53 Sponsor's table showing the incidence of neoplastic lesions in the lungs

Excerpted directly from this study report AC32RU.7G8R.BTL, pp 369; Note: group 2 is the positive control.

MALE						
	Group 1	Group 2	Group 3	Group 4	Group 5	HCR
Adenoma, single	3	0	1	3	1	0-6
Adenoma, multiple	0	15[1]	0	0	0	0-1
Carcinoma	0	8[1]	0	0	0	0-2
All Lung Tumor	3	15[1,2]	1	3	1	0-7
FEMALE						
	Group 1	Group 2	Group 3	Group 4	Group 5	HCR
Adenoma, single	0	0	1	1	0	0-6
Adenoma, multiple	0	15[1]	0	0	0	0-1
Carcinoma	0	11[1]	0	0	0	0-1
All Lung Tumor	0	15[1,2]	1	1	0	0-6

25 mice examined in each of the vehicle and test article treated dose groups of both sexes. 15 mice examined in each of the positive control dose groups.
Group 1 (male and female) – 0 mg/kg/day
Group 2 (male and female) – Positive Control (urethane, 1000 mg/kg/day)
Group 3 (male and female) – 15 mg/kg/day
Group 4 (male and female) – 50 mg/kg/day
Group 5 (male and female) – 150 mg/kg/day
HCR: Historical Control Range (See Appendix B).
[1] Statistically significant compared to control Group 1 (see Appendix A)
[2]: Multiple adenomas and/or carcinomas were present in the same animal in some of the urethane treated mice

The incidence of single or multiple adenomas, carcinomas and the combined incidence of all pulmonary tumors in the levomilnacipran treated groups were comparable to the vehicle control group (see Table 53, above); there were no statistically significant differences either for incidence or for trend.

Neoplastic lesions in spleen: Statistically significant increased incidence of splenic hemangiosarcomas were observed in the positive control (male and female) group in comparison with vehicle control, once again demonstrating the validity of the test (Group 2 in Table 54, below). The incidence of splenic hemangiosarcomas is comparable between the vehicle control and levomilnacipran treated low and mid dose groups of both sexes and falls within the historical control range; however, in the high dose groups of both sexes there is a numerical increase in the number of hemangiosarcomas when compared to the vehicle control groups (see Table 54, below). This numerical increase in the high dose female group was not statistically significant, compared to vehicle control and the incidence fell within the historical control range. However, in the high dose male group, the numerical increase was statistically significant, compared to vehicle control and the incidence exceeded the historical control by one animal (Table 54, below).

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Table 54 Sponsor's table showing the incidence of neoplastic lesions in the spleen

Excerpted directly from this study report AC32RU.7G8R.BTL, pp 370; Note: group 2 is the positive control.

MALE						
	Group 1	Group 2	Group 3	Group 4	Group 5	HCR
Hemangiosarcoma	1	14[1]	0	1	5[1]	0-4
FEMALE						
	Group 1	Group 2	Group 3	Group 4	Group 5	HCR
Hemangiosarcoma	0	15[1]	1	0	3	0-4

25 mice examined in each of the vehicle and test article treated dose groups of both sexes. 15 mice examined in each of the positive control dose groups.
Group 1 (male and female) – 0 mg/kg/day
Group 2 (male and female) – Positive Control (urethane, 1000 mg/kg/day)
Group 3 (male and female) – 15 mg/kg/day
Group 4 (male and female) – 50 mg/kg/day
Group 5 (male and female) – 150 mg/kg/day
HCR: Historical Control Range (See Appendix B)
[1] Statistically significant compared to control Group 1 (see Appendix A)

Table 55 Sponsor's table showing the incidence of hemangiomas and hemangiosarcomas in multiple organs including spleen

Excerpted directly from this study report AC32RU.7G8R.BTL, pp 371

MALE					
	Group 1	Group 3	Group 4	Group 5	HCR
Hemangiosarcomas					
Spleen	1	0	1	5[1]	0-4
Combined incidence	1	0	1	5[1]	0-4
FEMALE					
	Group 1	Group 3	Group 4	Group 5	HCR
Hemangiosarcomas					
Spleen	0	1	0	3	0-4
Multicentric	1	0	0	0	NR
Nasal Cavity	0	1	0	0	0-1[2]
Salivary gland[3]	0	0	1	0	NR
Perineum	0	0	0	1	0-1[4]
Combined incidence	1	2	1	4	0-5

25 mice examined in each of the vehicle and test article treated dose groups of both sexes.
Group 1 (male and female) – 0 mg/kg/day
Group 3 (male and female) – 15 mg/kg/day
Group 4 (male and female) – 50 mg/kg/day
Group 5 (male and female) – 150 mg/kg/day
[1] Statistically significant compared to control Group 1 (see Appendix A)
[2]Only reported in males
[3]A hemangioma was diagnosed in this organ. Hemangiosarcomas diagnosed in all other organs.
[4]The incidence of skin hemangiosarcomas
HCR: Historical Control Range (See Appendix B)
NR: Not previously reported

Hemangiomas and hemangiosarcomas in multiple organs including spleen: Comparison of incidence of hemangiomas and hemangiosarcomas in multiple organs between the vehicle control and levomilnacipran-treated groups of males and females are shown in Table 55, above. The combined incidence of all hemangiosarcomas and

hemangiomas in multiple organs in the low and mid dose males and all three doses in female mice is comparable with the incidence in vehicle control group and fell within the historical range. In high dose males and females, there is a numerical increase in the combined incidence of hemangiosarcomas in multiple organs; according to the Sponsor, this numerical increase is a reflection of either low incidence of hemangiosarcomas in the vehicle control males or lack of splenic hemangiosarcomas in vehicle control female mice. Moreover, in females, these tumors in multiple organs (other than spleen) did not show a particular trend for involvement of a tissue or an organ system. The increase in the combined incidence of hemangiosarcomas in high dose males was greater than the historical range and the Sponsor's analysis showed that the increase was also statistically significant when compared to the vehicle control. However, as already mentioned above (see Table 47, above), according to our CDER statistician, even though there was a statistically significant dose response relationship for the incidence of hemangiosarcoma in the spleen (only) of both males and females, the pair-wise comparisons did not show statistically significant increase in splenic hemangiosarcoma or any other tumor types in any of the treated groups, when compared to the vehicle control. On the other hand, the pair-wise comparisons showed statistically significant increase in the incidence of lung and spleen tumors in the positive control (male and female) groups, when compared to the respective vehicle controls.

Other tumors in multiple organs: The incidence of all other tumors (non vascular and non pulmonary) shown in Table 56, below involves isolated organs and did not show levomilnacipran treatment-related increase and fell within the historical range.

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Table 56 Sponsor's table comparing the incidence of other tumors in multiple organs

Excerpted directly from this study report AC32RU.7G8R.BTL, pp 372

Male					
	Group 1	Group 3	Group 4	Group 5	HCR
Sarcoma, multicentric	1	0	0	0	0-1
Nasal Cavity, adenocarcinoma	0	0	0	1	0-2[1]
Harderian gland, adenoma	1	1	0	0	0-2
Perineum, papilloma	1	0	0	0	0-1[2]
Female					
	Group 1	Group 3	Group 4	Group 5	HCR
Harderian gland, adenoma	2	1	0	1	0-4
Harderian gland, carcinoma	1	0	0	0	0-2
Salivary gland, adenocarcinoma	0	0	1	0	NR
Thymoma	3	1	2	1	0-1
Perineum, papilloma	0	1	1	0	NR
Uterus, deciduoma	0	0	0	1	NR
Uterus, sarcoma	0	0	1	0	NR

25 mice examined in each of the vehicle and test article treated dose groups of both sexes.
Group 1 (male and female) – 0 mg/kg/day
Group 3 (male and female) – 15 mg/kg/day
Group 4 (male and female) – 50 mg/kg/day
Group 5 (male and female) – 150 mg/kg/day
[1]Adenoma reported in HCR
[2]The incidence of skin papilloma
HCR: Historical Control Range (See Appendix B)
NR: Not previously reported
There were no statistically significant differences in the incidence of these tumors in Groups 3-5, compared to Group 1

Non Neoplastic

There were no treatment-related non-neoplastic lesions in any dose group in either sex. Although a number of non-neoplastic lesions were observed in multiple organs across the dose groups, all those lesions were considered to be background/spontaneous in nature due to their comparable occurrence across the dose groups.

Toxicokinetics

Days 1 and 178; pre-dose, 0.25, 0.5, 2, 8 and 24 hrs

Results: The TK parameters for levomilnacipran (F2695) were similar between male and female mice on Days 1 and 178 (Table 57, below). Levomilnacipran was rapidly absorbed with T_{max} ranging from 0.25 to 0.5 hr at all doses between Days 1 and 178. Systemic exposure in terms of AUC_{last}, AUC_{0-24}, and C_{max} increased in proportion to dose on both days. Combined $t_{1/2}$ ranged from 1.81 to 2.57 hr. There was no evidence for accumulation of levomilnacipran on repeated dosing.

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Table 57 Sponsor's table showing toxicokinetic parameters for F2695 in Tg.rasH2 mice (Excerpted directly from this study report AC32RU.7G8R.BTL; pp 24)

Day	Dose (mg/kg/day)	Sex	AUC_{0-24} (hr•ng/mL)	$AUC_{0-tlast}$ (hr•ng/mL)	C_{max} (ng/mL)	$t_{1/2}$ (hr)	T_{max} (hr)
1	15	Male	4110	4000	2030	NC	0.500
		Female	4800	4690	2000	NC	0.500
		Combined	4450	4340	2020	NC	0.500
	50	Male	14200	14200	5840	2.37	0.250
		Female	14000	13400	5940	1.24	0.250
		Combined	14100	13800	5890	1.81	0.250
	150	Male	46900	46900	11800	2.60	0.500
		Female	54500	54500	10200	2.31	0.250
		Combined	50700	50700	11000	2.45	0.375
178	15	Male	3970	3730	1950	1.37	0.250
		Female	4930	4730	2170	NC	0.500
		Combined	4450	4230	2060	NC	0.375
	50	Male	13600	13600	6030	2.25	0.250
		Female	17900	16300	5720	1.63	0.250
		Combined	15800	15000	5870	1.94	0.250
	150	Male	48300	48300	13100	2.54	0.250
		Female	63900	63900	10600	2.61	0.250
		Combined	56100	56100	11900	2.57	0.250

Dosing Solution Analysis

Dosing formulations were analyzed to check the concentration of levomilnacipran (F2695) 4 times during the study period.

Results: Stability of the formulated levomilnacipran in water was determined by the Sponsor; at concentrations of 1 mg/mL and 24 mg/mL, the formulated test article was stable for 10 days under refrigeration. All three sets of dose formulations analyzed (1.5, 5, and 15 mg/mL) met the acceptance criteria of 90-110% of target concentration and ≤ 5% RSD. No test article was detected in the vehicle control samples.

Summary: The doses of F2695 (levomilnacipran HCl) for this 6-month carcinogenicity assay (15, 50 and 150 mg/kg/d; by oral gavage in distilled water) were selected based on the Executive CAC recommendations. The recommended high dose of 150 mg/kg/d was approximately one-half of the lethal dose (300 mg/kg/d) in the 5-day range-finding study in CByB6H1 wild type littermates (it should be noted that there were virtually no treatment-related signs of toxicity up to and including the high dose of 150 mg/kg/d in a separate 28-day repeat dose study in Tg.rasH2 transgenic mice).
Daily administration of F2695 (levomilnacipran HCl) to Tg.rasH2 mice and CByB6F1 wild type mice did not result in treatment-related mortality. There were also no treatment-related severe clinical signs, no changes in body weights and no non-neoplastic lesions. A statistically significant increase in the incidence of splenic

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hemangiosarcomas was observed in males of high dose group only, when compared to vehicle control group. However, the numerical increase in the incidence of hemangiosarcomas was only slightly higher than the historical control value (5 in the HDM versus 0-4 in the historical control) and therefore, the biological significance of this finding is unclear. Moreover, analysis by our CDER statistician did not show statistically significant increase in splenic hemangiosarcomas or any other tested tumor types in any of the levomilnacipran-treated groups, compared to vehicle control (see Table 58 and Table 59, below).

Table 58 CDER statistical Reviewer's pair-wise comparison showing the lack of statistically significant increases in the incidence of tumors in male mice

(Excerpted directly from the Statistical Review, pp 24)

Table 6A: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons
Male Mice

Organ Name	Tumor Name	0 mg Veh C# N=25	15 mg Low N=25	50 mg Med N=25	150 mg High N=25	P_Value Dos Resp	P_Value Veh C vs. L	P_Value Veh C vs. M	P_Value Veh C vs. H
harderian gland	adenoma	1	1	0	0	0.8170	0.7553	0.5102	0.5000
lungs with bron	alveolar-bronchiolar	3	1	3	1	0.7363	0.6957	0.3535	0.6957
perineum	papilloma	1	0	0	0	0.7526	0.5000	0.5102	0.5000
spleen	hemangiosarcoma	1	0	1	5	0.0058*	0.5000	0.2551	0.0941

#Veh C: Vehicle Control

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Table 59 CDER statistical Reviewer's pair-wise comparison showing the lack of statistically significant increases in the incidence of tumors in female mice

(Excerpted directly from the Statistical Review, pp 25)

Table 6B: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons Female Mice

Organ Name	Tumor Name	0 mg Veh C# N=25	15 mg Low N=25	50 mg Med N=25	150 mg High N=25	P_Value Dos Resp	P_Value Veh C vs. L	P_Value Veh C vs. M	P_Value Veh C vs. H
cavity, nasal	hemangiosarcoma	0	1	0	0	0.4949	0.5000	.	.
harderian gland	adenoma	2	1	0	1	0.6180	0.5000	0.7551	0.4844
	carcinoma	1	0	0	0	0.7475	0.5000	0.5000	0.4898
lungs with bron	alveolar-bronchiolar	0	1	1	0	0.4898	0.5000	0.5000	.
perineum	hemangiosarcoma	0	0	0	1	0.2424	.	.	0.4898
	papilloma	0	1	1	0	0.4898	0.5000	0.5000	.
salivary glands	adenocarcinoma	0	0	1	0	0.2424	.	0.5000	.
	hemangioma	0	0	1	0	0.2424	.	0.5000	.
spleen	hemangiosarcoma	0	1	0	3	0.0326*	0.5000	.	0.1173
thymus	thymoma	3	1	2	1	0.7256	0.6954	0.5000	0.6798
uterus	deciduoma	0	0	0	1	0.2424	.	.	0.4898
	sarcoma	0	0	1	0	0.2424	.	0.5000	.

#Veh C: Vehicle Control

The human exposure margin (at the maximum recommended human dose of 120 mg/d) based on the systemic exposure at the highest dose of 150 mg/kg/d is ~11-fold (see Table 60, below)

Table 60 Mouse-Human multiples of exposure for levomilnacipran, HCl

Human exposure margin calculated by the Reviewer using the TK parameters for the 26-week carcinogenicity study in Tg.rasH2 mice (Table 11, above) and human PK parameters from Table 11.2.2-1 of the Clinical Study Report LVM-PK-15, pp 46]

Tg.rasH2, 6-month carcinogenicity study	Dosage (mg/kg/d)	TK Parameters#, @		Human Exposure Margin (MRHD = 120 mg/d)	
		C_{max} (ng/mL)	AUC_{0-24} (ng*h/mL)	C_{max} (ng/mL)	AUC_{0-t} (ng*h/mL)
		M + F	M + F	M + F	M + F
F2695	15	2060	4450	8.9	0.9
	50	5870	15800	25	3
	150	11900	56100	51	11

TK measurements of Day 178
@ TK measurements in CByB6F1 wild type littermates

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: F2695- Fertility toxicity study by the oral route (gavage) in the male and female Wistar rat (Segment I)

Study no.:	AA78124
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 10, 2009
GLP compliance:	Yes (OECD and in (b) (4)
QA statement:	Yes
Drug, lot #, and % purity:	F2695 (also known as F2695A1), batch #OP210, 100.1 (% m/m)

Key Study Findings

- Effects of levomilnacipran (F2695) on male and female Wistar rats dosed orally at 0, 10, 30 and 100 mg/kg/d before mating, through mating, and up to Day 7 of gestation were investigated.
- Dose-dependent reduction in the mean body weight gain in males and females (statistically significant only at the high dose) and food consumption (only at the high dose.
- No treatment-related effects on reproductive performance or fertility, including an assessment of sperm count and motility and the reproductive indices, and no adverse macroscopic or weight changes associated with the reproductive organs.
- The NOAEL was 100 mg/kg/d for gonadal function, mating behavior, reproductive performance and early gestation in male and female rats.

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Methods

Doses:	0, 10, 30, and 100 mg/kg/d (High dose was selected based on a previous dose range-finding study on embryo-fetal development in rats where no marked maternal toxicity was noted at 80 mg/kg/d (report #0314/118; referenced in Seg II study review, below). In addition, in a separate dose range-finding oral pre- and post-natal study where effects on the dams (low body weight gain and low food consumption as well as low pup viability were observed at 100 mg/kg/d (report # AA80683, referenced in Seg III review, below).
Frequency of dosing:	Once daily
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Solution/purified water for injection
Species/Strain:	Rats/Wistar: Cr:WI (Gix/BRL/Han) IGS BR
Number/Sex/Group:	20 M and 20 F
Satellite groups:	None
Study design:	F2695 or vehicle was administered once daily to males 28 days before mating, during mating and until the day before necropsy (maximum nine weeks of treatment). F2695 or vehicle was administered to females for 14 days before mating, throughout mating and until Day 7 of gestation inclusive (maximum six weeks of treatment). Animals were paired on the basis of one male and one female from the same group (see Table 61, below) for a maximum of 21 days. Vaginal smears were taken daily from the females during cohabitation and the day of mating was confirmed by the presence sperm in the smear and was taken as Day 0 of gestation (G0). The dosages were selected based on the results of a previous range-finding study.
Deviation from study protocol:	No significant deviations that would affect the study outcome

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Table 61 Sponsor's table showing the fertility toxicity study design in rats

(Excerpted directly from this study report AA78124; pp 21)

Group number	Number of animals		Animal numbers (cage numbers are indicated in brackets)	
	Males	Females	Males	Females
1	20	20	1 to 20 (1 to 4)	81 to 100 (17 to 20)
2	20	20	21 to 40 (5 to 8)	101 to 120 (21 to 24)
3	20	20	41 to 60 (9 to 12)	121 to 140 (25 to 28)
4	20	20	61 to 80 (13 to 16)	141 to 160 (29 to 32)

Observations and Results

Mortality

All animals were observed twice daily

Results: There were no treatment-related deaths in any group

Clinical Signs

All animals were observed prior to dosing and at least one hour after dosing for clinical signs, throughout the treatment period.

Results: The only treatment-related clinical sign was hypersalivation after dosing in mid and high dose animals in both sexes. There was a dose-related increase in the number of animals affected and/or the frequency of occurrence; all MDM on three to eight occasions and 9/20 MDF on one or two occasions, versus all HDM on eight to nine occasions and 19/20 HDF on two to four occasions (the occurrence was not confined to initial days of dosing; rather, it was spread out through out the dosing period). In some low dose males (5/20), hypersalivation was noted on few occasions.

Body Weight

Individual body weights were recorded twice weekly (males) and twice weekly during pre-mating and mating periods for females and on days 0, 4, 8 and 13 of gestation.

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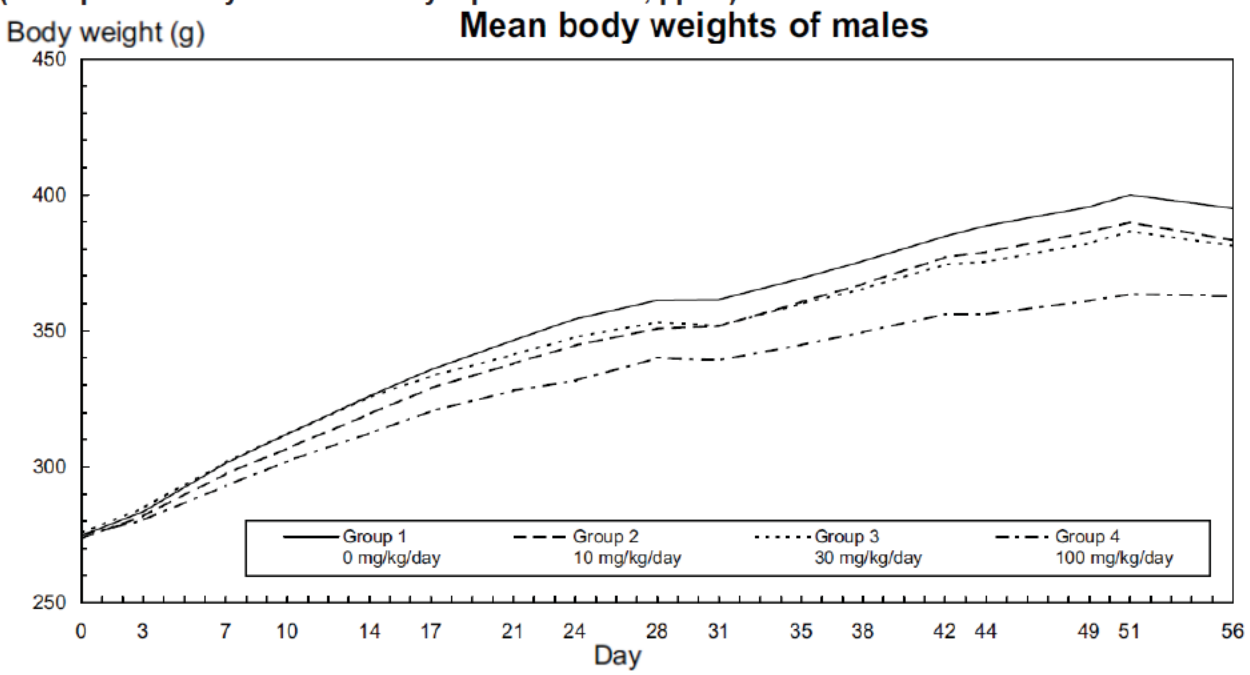
Figure 14 Sponsor's figure showing the mean body weights of male rats during treatment with F2695

(Excerpted directly from this study report AA78124; pp 41)

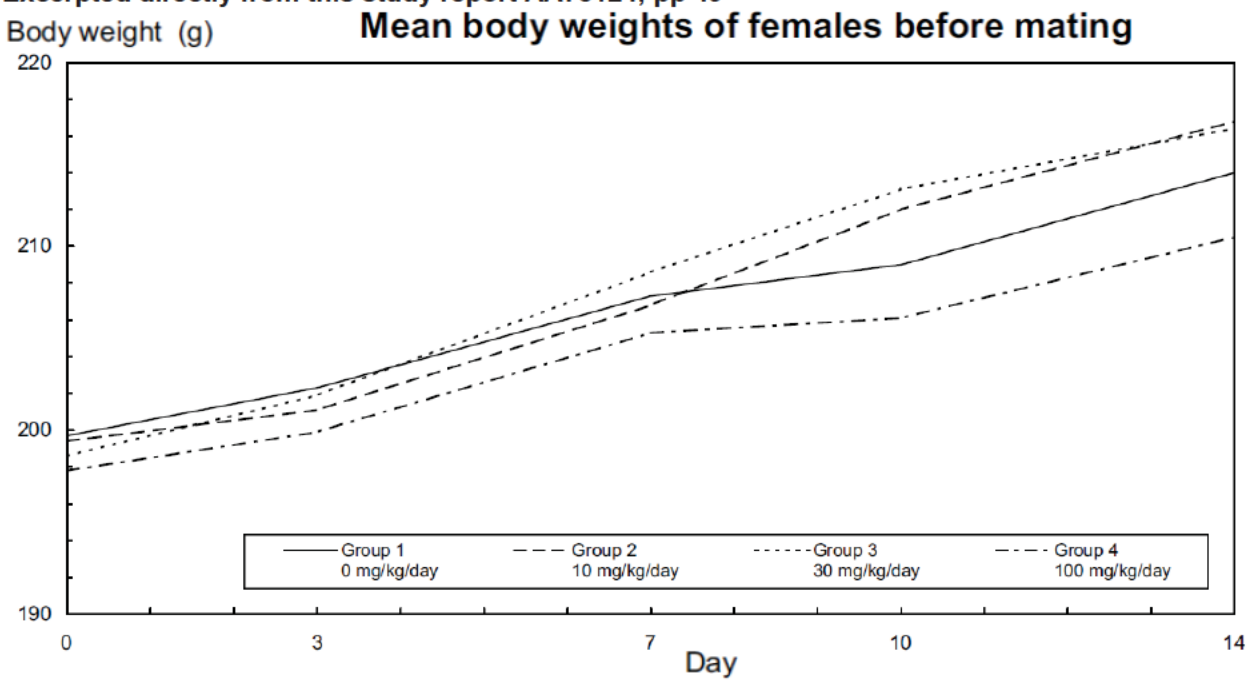


Results

Males: Treatment-related lower mean body weight gains during the dosing period (Days 0 to 56; Figure 14, above) in all drug treated groups, when compared to control; although the lower body weight gains appear to be dose-related, the decrease was statistically significant only for the high dose group (-9%, -12%, and -27%, at LD, MD, and HD, respectively). This decrease in the HDM was mainly due to a significantly low mean body weight gain during the first three weeks of dosing and as a result the absolute mean body weight was statistically significantly lower than control from Day 24 to termination. The absolute mean body weights for the LD and MD groups at the end of dosing period were comparable to control.

Figure 15 Sponsor's figure showing the mean body weights of female rats before mating period (rats treated for 14 days with F2695 before mating period)

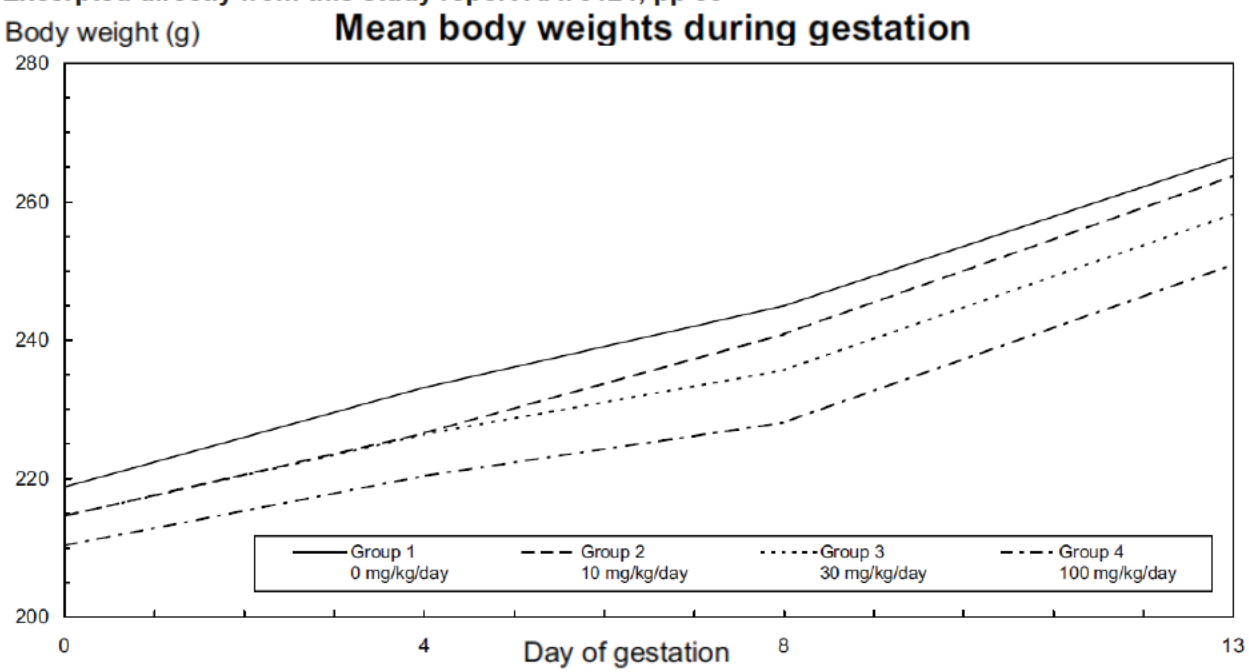
Excerpted directly from this study report AA78124; pp 49



Females: In females, the overall mean body weight gain in the treated groups during the pre-mating period (Days 0 to 14) was comparable to control (see Figure 15, above). However, the mean body weight gain during days 0 to 8 of gestation (treatment period) was statistically significantly lower (-32%) in the high dose group than in the control (see Figure 16, below) and consequently, the absolute mean body weight was statistically significantly lower (-6 or -7%) than the control from GD 8 through to termination. Similarly, the mean body weight gain was slightly lower for the mid dose group, compared to control (-19%) during GD 0-8; however the decrease was not statistically significant and since the terminal mean body weight was not affected, the lower mean body weight gain may not be of toxicological significance. The mean body weight gain in the low dose group was comparable to control throughout gestation.

Figure 16 Sponsor's figure showing the mean body weights of female rats during gestation (rats treated with F2695 for 14 days before mating, throughout mating and until day 7 of gestation)

Excerpted directly from this study report AA78124; pp 55



Feed Consumption

Food consumption of the males and females was measured weekly during pre-mating period and food consumption of pregnant females was measured for days 0 to 8 and 8 to 13 of gestation.

Results:

Males: Mean food consumption in HDM was slightly lower (-5 to -8%) during the first 3 weeks of dosing, when compared to control (see Figure 17, below); however, the decrease was not statistically significant [note: there was a statistically significant decrease in mean body weight gain (-27%) in HDM]. Mean food consumption in MDM and LDM was comparable to control group.

Females: Mean food consumption was statistically significantly lower in HDF, compared to control group (-10 to -12%; see Figure 18 and Table 62, below) during the pre-mating period (contrast with the no significant change in mean body weight gain in females during the pre-mating period; Figure 15, above). However, mean food consumption was statistically significantly lower in the mid and high dose females (-9 and -17%, respectively; see Table 63, below) during days 0 to 8 of gestation (treatment period). During post- treatment period (days 8 to 13 of gestation), mean food consumption was similar between control and treated groups (Table 63, below).

Figure 17 Sponsor's graph showing the mean food consumption in males

(Excerpted directly from the study report AA78124, pp 61)

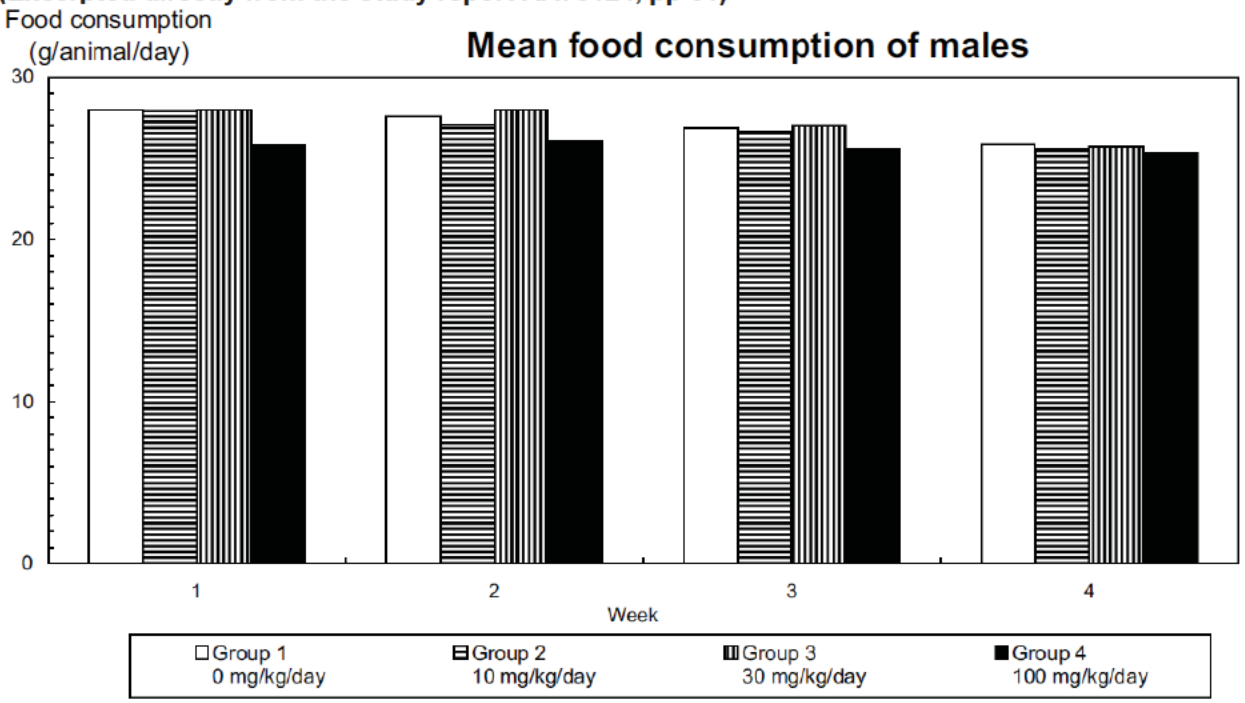


Figure 18 Sponsor's graph showing the mean food consumption in females during the pre-mating period

(Excerpted directly from the study report AA78124, pp 65)

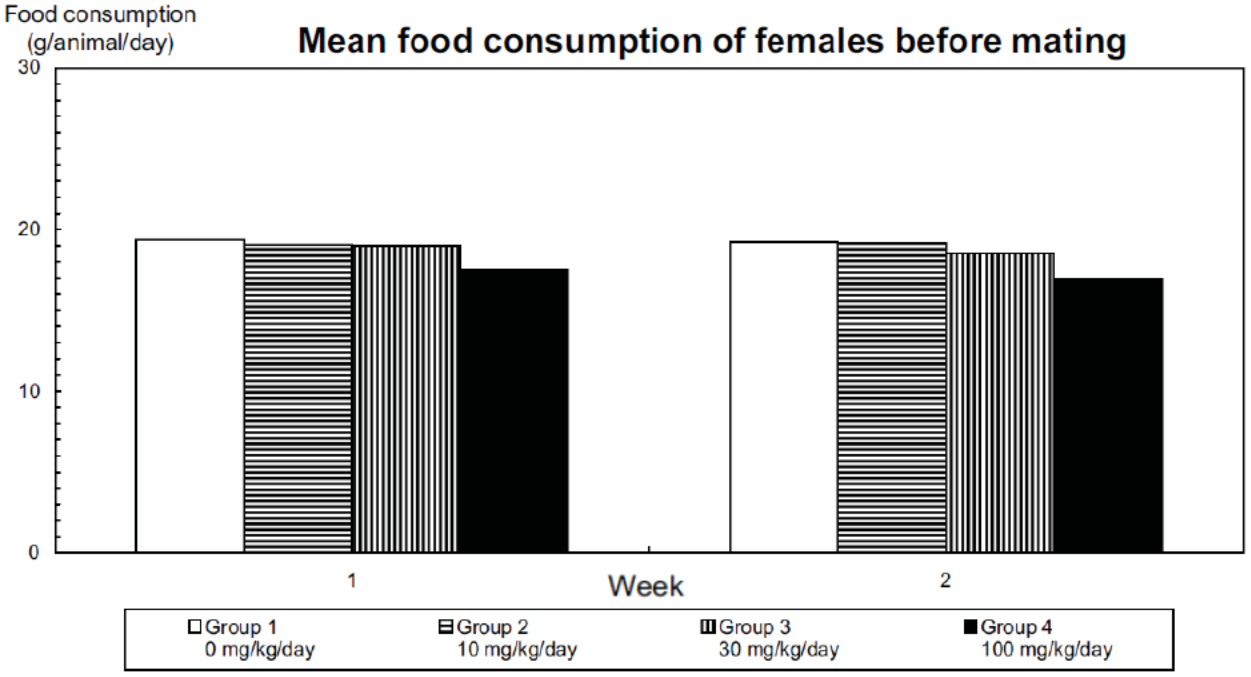


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Table 62 Sponsor's table showing the intergroup food consumption by cage

(Excerpted directly from the study report AA78124, pp 63)

Day numbers relative to Start Date

Group	Sex	From: / To:	0 / 7	7 / 14
1	f	Mean	19.35	19.24
		S.D.	0.91	0.82
		N	4	4
2	f	Mean	19.01	19.18
		S.D.	1.02	0.94
		N	4	4
3	f	Mean	18.97	18.54
		S.D.	1.07	0.63
		N	4	4
4	f	Mean	17.50*	16.91**
		S.D.	0.78	0.69
		N	4	4

Statistical analysis performed - Arithmetic mean values presented.
Statistical significances arise from automatic comparisons with the principal control.
Principal Control Group: 1

Food consumption units are g/animal/day

Group 1 - 0 mg/kg/day Group 2 - 10 mg/kg/day Group 3 - 30 mg/kg/day Group 4 - 100 mg/kg/day

Table 63 Sponsor's table showing the mean food consumption in females during gestation

(Excerpted directly from this study report AA78124; pp 67)

SUMMARY OF FOOD CONSUMPTION DURING GESTATION (GRAMS/ANIMAL/DAY)

		Group 1 Control 0 mg/kg/day	Group 2 Low dose 10 mg/kg/day	Group 3 Intermed. dose 30 mg/kg/day	Group 4 High dose 100 mg/kg/day
DAYS 0 TO 8	MEAN	19.2 k	18.4	17.4**	15.9**
	S.D.	1.1	2.5	1.3	1.3
	N	19	19	18	18
DAYS 8 TO 13	MEAN	21.6 d	22.2	20.9	20.7
	S.D.	1.5	2.6	1.8	1.9
	N	19	19	18	18

Statistical key: d-Anova/Dunnett test k-Kruskal-Wallis/Dunn test ** - p<0.01

Toxicokinetics

Not performed

Dosing Solution Analysis

Levomilnacipran (F2695) was prepared weekly; dosing analysis performed on day 0 and the last week of dosing; concentrations of levomilnacipran aqueous solutions

(formulated at 2, 6, and 20 mg/mL) were within the nominal concentrations since the deviations from the nominal concentrations ranged from 0.3% to 1.7%. No test article was detected in the vehicle control samples.

Necropsy

All animals were necropsied according to the following schedule:
-Females were submitted to a caesarean section on day 13 of gestation.
-Males were necropsied after the end of the caesarean sections of the females.
-One unmated female was necropsied after completion of the mating period.

There were no treatment-related gross pathological findings; the seminal vesicles were small in one MDM (# 58) and the prostate was small in one HDM (# 62); however, the mating performance and the fertility in these males were similar to other males of the same group and therefore, these gross findings may be unrelated to treatment.

Organ weights

Ovaries from all females were weighed paired. The testes and epididymides of all males were weighed separately.

Males: In HD males, the relative testis weights were statistically significantly higher (11%) compared to control; this is due to the lower body weights because there was no significant difference in absolute testis weights (final mean body weight of HDM was ~9% lower than control). Absolute epididymis weights were statistically significantly lower in HDM and MDM, compared to control (11% and 9.6%, respectively); however, the Sponsor did not consider these differences to be toxicologically relevant.

Females: In HD females, the absolute ovary weight was lower when compared to control (↓~6%); however, the difference was not statistically significant.

Sperm analysis: There were no treatment-related differences in mean sperm count; a slightly lower mean sperm count was noted in the LD group (↓~9%); however, in the absence of similar finding in the higher dose groups, this slightly lower sperm count may be incidental. Moreover, there were no treatment-related differences in mean percentage of motile sperm or motility parameters in any group. There were no remarkable findings for the males that failed to induce pregnancy (#21- LDM; #s 56 and 58- MDM; #s 66 and 74- HDM).

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

Animals were paired on the basis of one male and one female from the same group for a maximum of 21 days. Vaginal smears were taken daily from the females during co-habitation. The day of mating was confirmed by the vaginal plug/presence of sperm in vaginal smear and was taken as Day 0 of gestation (G0). The following reproductive

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indices were calculated: pre-coital interval (in days), copulation index (in %), fertility index (in %), pre-implantation loss (in %), post-implantation loss (in %).

Table 64 Sponsor's table showing summary of mating performance and fertility

(Excerpted directly from the study report AA78124; pp 71)

Group Treatment	1 0 mg/kg/day	2 10 mg/kg/day	3 30 mg/kg/day	4 100 mg/kg/day
Number of females:				
Paired	20	20	20	20
Inseminated	19	20	20	20
Pregnant	19	19	18	18
Pre-coital interval - Days				
Mean	3.26	2.35	2.80	2.95
S.D.	2.84	1.27	2.80	2.82
N	19	20	20	20
Copulation index (%)	95	100	100	100
Fertility index (%)	100	95	90	90

Results: There were no treatment-related adverse effects on the mating performance in any group (see Table 64, above); all animals mated, except one control female (#85) which failed to mate with male #5 (sperm analysis for this particular male did not show any abnormal values) and majority of the animals in each group (18 in control, 19 each in LD, MD, and HD groups) showed evidence of insemination within the first 4 days of pairing (considered to be the approximate duration of a normal estrous cycle). There were no significant differences in the mean pre-coital interval between control and treated groups (2.35, 2.8 and 2.95 days in LD, MD and HD respectively versus 3.26 days in the control). Likewise, there were no treatment-related effects on fertility; however, one female in the LD and 2 each in the MD and HD groups did not become pregnant and this incidence is consistent with the historical control range provided by the Sponsor. The fertility and copulation indices were comparable between control and treated groups (see Table 64, above).

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Table 65 Sponsor's table showing the summary of caesarian section data

(Excerpted directly from the study report AA78124, pp 76)

	Group 1 Control 0 mg/kg/day	Group 2 Low dose 10 mg/kg/day	Group 3 Intermed. dose 30 mg/kg/day	Group 4 High dose 100 mg/kg/day
Number of dams	19	19	18	18
Corpora Lutea				
per group	280	272	244	246
per dam: mean	15 d	14	14	14
st. dev.	3	1	2	2
Implantations				
per group	262 f	262	228	231
% of Corpora lutea	93.6	96.3	93.4	93.9
per dam: mean	14 d	14	13	13
st. dev.	3	1	2	2
Viable Embryos				
per group	249	241	221	219
per dam: mean	13 d	13	12	12
st. dev.	3	3	3	2
% of Implantations				
per group	95.0 f	92.0	96.9	94.8
per dam: mean	95 k	92	96	95
st. dev.	7	18	6	8
Postimplantation Loss				
total per group	13 f	21	7	12
% of implantations	5.0	8.0	3.1	5.2
per dam: mean	1 k	1	0	1
st. dev.	1	2	1	1
No. of dams affected	7	9	6	7
Preimplantation Loss				
per group	18 f	10	16	15
% of Corpora lutea	6.4	3.7	6.6	6.1
per dam: mean	1 k	1	1	1
st. dev.	1	1	2	2
No. of dams affected	12	8	9	6

Statistical key: d=Anova/Dunnett test f=Chi2/Fisher Exact test k=Kruskal-Wallis/Dunn test

There were no treatment-related adverse effects on mean number of corpora lutea, implantation sites, viable embryos, percentage pre- and post-implantation loss in any group (see Table 65, above).

9.2 Embryonic Fetal Development in rats

Key Study Findings

- Mated female Wistar rats, dosed orally (gavage) at 0, 10, 30, and 100 mg/kg/d on GD 6-17.
- Treatment-related clinical signs included hypersalivation and piloerection.
- Treatment-related reduction in body weight gain and slight reduction in food consumption at the high dose throughout the treatment period. Absolute body weight was lower than control (in high dose group) from GD 12 through to termination.

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- Lower mean fetal body weights and reduced ossification and bipartite ossification of the sternebrae at the high dose.
- No evidence of teratogenicity; maternal NOEL was 30 mg/kg/d based on reduced food consumption and decreased body weight/body weight gain at 100 mg/kg/d. The NOEL for embryo-fetal development was 30 mg/kg/d, based on decreased body weights of the fetus and delayed ossification at 100 mg/kg/d.

Observations and Results

Maternal Parameters:

Mortality

Maternal mortality/morbidity (monitored twice daily):

Results: There were no treatment-related deaths in any group.

Clinical Signs

Maternal clinical signs (recorded once daily).

Results: Treatment-related clinical signs were piloerection, in the high dose group (8/25) from GD16 onwards, and hypersalivation in the mid and high dose rats (7/25 in the mid and all 25 in the high dose groups, respectively). Other clinical signs such as hair loss, scabs, erethema and vaginal discharge (in 1 high dose female only) were noticed in few animals in all treatment groups, including 1-2 control animals; the Sponsor classified these findings as incidental.

Body Weight

Maternal body weight (weighed on days 0, 6, 9, 12, 15, 18, and 20 of gestation).

Results: Statistically significant reduction of body weight gains in the high dose rats during treatment days (GD 6 to GD18) compared to control (-29%); the difference was greater during the initial stages of dosing (see Table 66, below). Consequently absolute mean body weight was statistically significantly lower than control animals from GD12 through termination (↓~6 to 8%, compared to control; see Figure 19, below).

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Table 66 Body weight gains (in grams) and difference (%) when compared to control during treatment period

(Excerpted directly from the study report AA78122, pp 32)

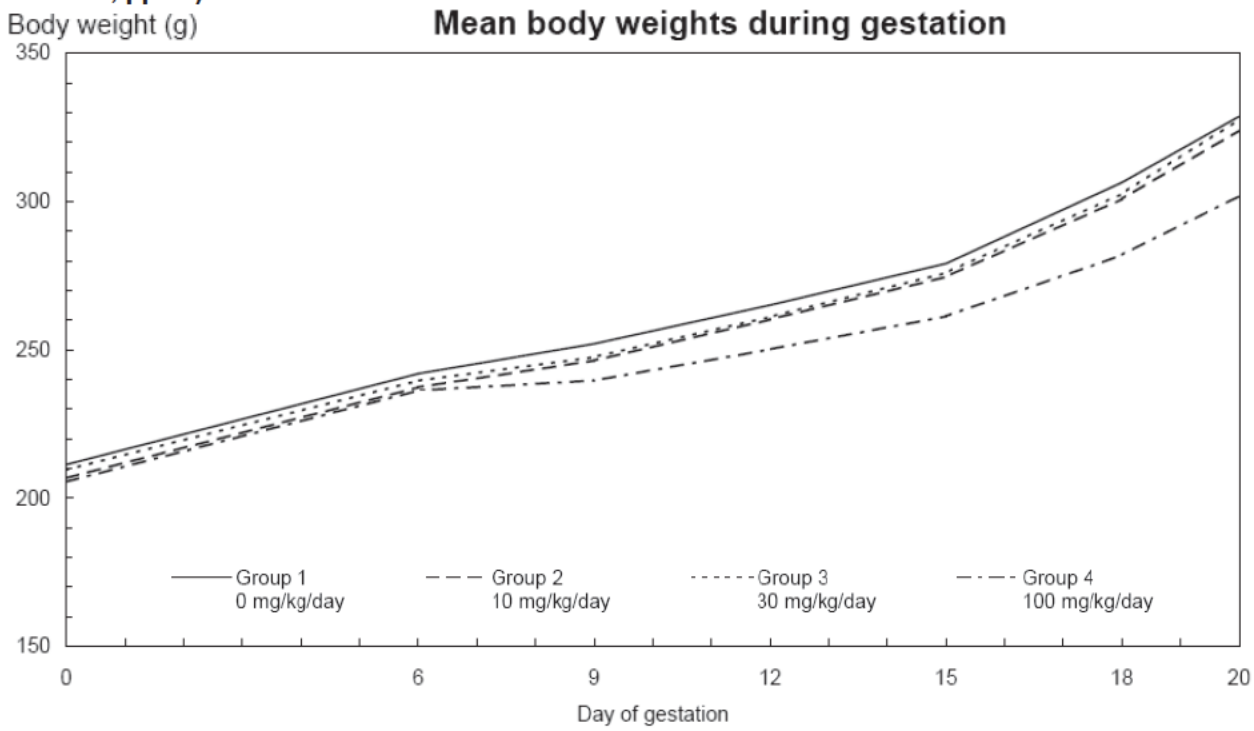
Group	1	2	3	4
GD 6-9	10.0	9.0	7.9	3.2**(-68%)
GD 9-12	13.2	13.7	13.6	10.5**(-20%)
GD 12-15	13.9	14.5	14.8	11.2 (-19%)
GD 15-18	27.4	26.2	26.7	20.6**(-25%)
GD 6-18	64.4	63.4	63.1	45.6 **(-29%)

** : $p < 0.01$

Mean body weight gains in the low and mid dose groups were comparable to control.

Figure 19 Mean body weights of female rats treated with levomilnacipran during GD 6-17

(Excerpted directly from the study report AA78122 with minor formatting modifications by the reviewer, pp 43)



Feed Consumption

Maternal food consumption (measured for the days of 0 to 6, 6 to 9, 9 to 12, 12 to 15, 15 to 18 and 18 to 20 during gestation).

Results: Slight, though statistically significant reduction in mean food consumption in the high dose group for each interval throughout the treatment period; the decrease in food consumption by the high dose group was -12 to -13%, compared to control, at each time period and overall from GD6 through GD18. There were no treatment-related effects on mean food consumption in the low and mid dose groups.

Toxicokinetics

Blood samples (~1.3 mL) were collected from all TK animals from all groups via the retro-orbital sinus for determination of levomilnacipran. Samples were collected at 0.5, 1, 2, 4, 8, and 24 hours post-dose on GD 6 and GD 17.

Results: The TK parameters for Levomilnacipran (F2695) are shown in Table 67, below. Levomilnacipran exposure was demonstrated in all treated groups on days 6 and 17 of gestation, with a dose related increase in plasma exposure; the increase in systemic exposure was linear on both days.
Note: The Sponsor noted that F2695 was found in 2 control female rat samples; trace levels (BLQ) in one female and 1.67 ng/mL in the second female on GD 6 at the 24 h time-point. An investigation at the testing facility could not identify the origin of this contamination, but led to the conclusion that no control animal could have been treated with the test item.

Table 67 Toxicokinetic parameters of levomilnacipran (F2695) in female rats during gestation (Excerpted directly from the study report AA78122, pp 320)

Group	Dose	Day	C_{max}	T_{max}	AUC_{last}	C_{max} / dose	AUC_{last} / dose	Day 17 vs Day 6	
	$mg.kg^{-1}.day^{-1}$		$ng.mL^{-1}$	h	$ng.mL^{-1}.h$			C_{max}	AUC_{last}
2	10	Day 6	1103	0.5	1999	110	200	0.7	1.3
		Day 17	777	0.5	2593	77.7	259		
3	30	Day 6	3123	0.5	8355	104	279	0.7	1.2
		Day 17	2182	0.5	10061	72.7	335		
4	100	Day 6	7970	0.5	37736	79.7	377	0.7	1.2
		Day 17	5701	0.5	45738	57.0	457		

Dosing Solution Analysis

Dosing formulations were prepared weekly and refrigerated at ~4 °C. The aqueous solution (concentrations from 1 mg/mL to 24 mg/mL) was stable for 5 hours at room temperature and 10 days under refrigeration (stability study reference F2695REC-32RM102). The concentrations of all doses of F2695 were determined on the first and last days of dosing (nominal concentrations 2, 6, and 20 mg/mL); the deviations from the nominal concentrations ranged from -5.5% to +1.2% (the testing facility requirement being ± 15%). No test article was detected in the vehicle control sample.

Necropsy

All females were killed by CO_2 inhalation followed by exsanguination on GD 20; dissected and examined for macroscopic pathological changes. The ovaries and uterus of each female were removed and examined.

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Maternal macroscopic findings: There were no treatment-related macroscopic findings in any group.

Litter data

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Pregnancy incidence:

There were 23, 24, 25, and 24 pregnant females (out of 25/group), respectively in groups 1 to 4 and all had viable fetuses (Table 68, below).

Pre-implantation data: The numbers of corpora lutea and implantations, and the percentage pre-implantation loss were comparable in all groups.

Post-implantation data: The percentage post-implantation loss in the high dose group was higher (↑57%), when compared to control; this was true when compared on per animal basis as well (↑55%). This was perhaps accounted by the slightly higher incidences of early as well as late resorptions, when compared to the control group. It is worth noting that the percentages of early and late resorptions per animal remained close to the historical control range of values (0.8 to 1.4, for early resorptions and 0 to 0.2, for late resorptions) and therefore, may not be of any toxicological significance as pointed out by the Sponsor.

Table 68 Maternal variables for segment II study of 0, 10, 30, and 100 mg/kg/d oral (gavage) doses of F2695 in Wistar rats

(Compiled by the Reviewer from the study report AA78122, pp 104-107)

PARAMETER	DOSE, MG/KG/D (GESTATIONAL D6-17)			
	0	10	30	100
TOTAL PREGNANCY RATE	23/25	24/25	25/25	24/25
MORTALITIES	0	0	0	0
PREGNANT FEMALES AT TERMINATION	23	24	25	24
MEAN IMPLANTATION SITES	11.3	10.6	11.8	10.8
MEAN CORPORA LUTEA	12.0	11.5	12.7	11.9
PRE-IMPLANTATION LOSS %, PER DAM	6.1%	7.6%	6.4%	11.1%
MEAN LIVE FETUSES	10.3	9.7	11.2	9.4
% M	49.3%	47.9%	49.2%	52.6%
% F	50.7%	52.1%	50.8%	47.4%
POST-IMPLANTATION LOSS (TOTAL)	21	22	16	33
MEAN PER ANIMAL	0.9	0.9	0.6	1.4
% IMPLANTS/ANIMAL	7.7%	10.0%	4.9%	12.6%
MEAN DEAD FETUSES	0	0	0	0
EARLY RESORPTIONS (TOTAL)	20	21	13	27
MEAN PER ANIMAL	0.9	0.9	0.5	1.1
% IMPLANTS/ANIMAL	7.4%	9.3%	4.1%	10.5%
LATE RESORPTIONS (TOTAL)	1	1	3	6

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PARAMETER	DOSE, MG/KG/D (GESTATIONAL D6-17)			
	0	10	30	100
MEAN PER ANIMAL % IMPLANTS/ANIMAL	0 0.3%	0 0.7%	0.1 0.8%	0.3 2.0%
TOTAL RESORPTIONS, %, PER LITTER	3.9 %	3.7%	2.4%	5.8 %

Fetal data:

Offspring (Malformations, Variations, etc.)

Compared to control group, there was a statistically significantly lower mean fetal weight (↓11%) in the high dose group (equally in both male and female fetuses; Table 69, below). There was a slightly lower mean fetal weight (↓6%; not statistically significant) in the mid dose group as well; however, it appears to be due individual variations in fetal weight.

Table 69 Mean fetal body weight in Segment II study (Wistar rat)

(Values compiled by the Reviewer from Table 4 of the study report AA78122; pp 53)

	GROUP 1 CONTROL 0 MG/KG/D	GROUP 2 LOW DOSE 10 MG/KG/D	GROUP 3 MID DOSE 30 MG/KG/D	GROUP 4 HIGH DOSE 100 MG/KG/D
MEAN FETAL BODY WEIGHT (G)	3.5 D	3.4	3.3	3.1 **
MALES	3.6 D	3.5	3.4	3.2 **
FEMALES	3.4 D	3.4	3.2	3.0 **

Statistical key: d = Anova/Dunnet test ** = $p < 0.01$

Fetal examinations:

Table 70, below shows the numbers of fetuses (litters) that were examined for the presence of external, internal and skeletal malformations/variations following treatment with levomilnacipran during the period organogenesis (GD6 to GD17).

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Table 70 Number of fetuses (litters) submitted to external, internal or skeletal examinations in the Segment II study in Wistar rats treated with levomilnacipran

(Excerpted directly from the study report AA78122; pp 35)

Group	1	2	3	4
External examination	238 (23)	233 (24)	280 (25)	226 (24)
Internal examination	111 (23)	112 (23)	135 (25)	107 (24)
Skeletal examination	127 (23)	121 (24)	145 (25)	119 (24)

External examinations: No treatment-related external abnormalities were observed at any dose. The occurrence of anasarca and a malrotated hind limb in one control fetus (female #14) may be considered as incidental.

Visceral observations: There were no fetal visceral abnormalities in any group.

Skeletal observations: Skeletal malformations were limited to 3 pups, 1 from the control group and 2 from different litters in the HD group, as shown in Table 71, below. There were two fetuses from two different litters (female #s 84 and 91) in the high dose group, one (female #84) with malformed hindlimbs (short long bones) and the second one (female #91) with a small mandible and malformed skull. In the control group, there was one fetus (female # 14) with malformed cervical and thoracic vertebrae. According to the Sponsor's narrative, although there were no such findings in the historical control data, these findings were considered incidental since each finding was only in one fetus.

At the high dose of 100 mg/kg/d, the fetal incidence of skeletal changes (Table 72, below) were higher than the concurrent control and historic control values; however, only the incomplete ossification of $2^{nd}/4^{th}$ sternebrae were statistically significantly higher, compared to control. The above mentioned fetal anomalies and variations of skeletal changes at the mid dose also showed a tendency to a slightly higher incidence, consistent with a minor delay in ossification.

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Table 71 Malformation and incidence in the segment II study in Wistar rats treated with F2695 (Excerpted directly from the study report AA78122, pp 36)

Group	1	2	3	4
Malformed hindlimbs	0	0	0	1 [1]
Small mandible and malformed skull	0	0	0	1 [1]
Cervical and thoracic vertebrae malformed	1 [1]	0	0	0
Total number of malformed fetuses	**1 [1]**	**0**	**0**	**2 [2]**

Number of fetuses affected [number of litters affected]

There was a slightly higher incidence of anomalies and variations of the sternebrae, such as reduced ossification, bipartite ossification and asymmetry in the high dose group (see Table 68, below).

Table 72 Rat fetal incidence of skeletal changes in sternebrae (Excerpted directly from the study report AA78122; pp 37)

Findings with % of affected fetuses	Historical control data (2006-2008)	Group 1	Group 2	Group 3	Group 4
Bipartite ossification (2nd, 3rd or 5th)	0.35	1.6	0.8	3.4	5.0
Asymmetry	0.94	0.8	0.0	0.7	4.2
$1^{st}/3^{rd}$: incomplete ossification	8.93	3.9	3.3	4.8	11.8
6th: unossified	2.35	2.4	0.0	5.5	7.6
$2^{nd}/4^{th}$: incomplete ossification	19.04	6.3	14.9	25.5	37.8
5th: unossified	4.11	3.1	4.1	6.2	12.6

In conclusion, oral (gavage) treatment of pregnant female Wistar rats with levomilnacipran (F2695) at 10, 30, and 100 mg/kg during organogenesis was associated with maternal clinical findings and reductions in mean body weight gain and food consumption in the high dose group only. There were no treatment-related maternal findings in the mid dose and low dose groups and therefore, the mid dose of 30 mg/kg/d was considered NOEL for maternal toxicity. Treatment-related embryo-fetal findings included statistically significant lower mean fetal weight and an increase in the incidence of anomalies and variations of the sternebrae such as reduced ossification,

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bipartite ossification and asymmetry in the high dose group, probably indicative of a delay in ossification. Based on low fetal weight at 100 mg/kg/d, the NOEL for embryo-fetal development was 30 mg/kg/d. However, since the fetal skeletal changes were most likely due to a delay in ossification and probably of no toxicological significance, it is safe to conclude that there was no treatment-related adverse effect on fetal morphological development at doses up to 100 mg/kg/d.

9.3 Additional embryonic fetal development study in rabbits

Key Study Findings

- Pregnant New-Zealand White rabbits were treated with levomilnacipran (F2695) throughout the period of organogenesis (day 6 to day 19 of gestation) at oral (gavage) doses of 10, 30, and 100 mg/kg/d.
- Maternal mortality at the high dose, possibly treatment-related
- Initial body weight loss and decreased body weight gain, reduced food consumption (a reduction in fecal output for several days, consistent with reduced food intake) at the high dose.
- Minor delay in ossification of a few digit bones (mainly phalanges) in the high dose group fetuses.
- No evidence of teratogenicity; the NOAEL for maternal toxicity was 30 mg/kg/d, based on maternal toxicity at the high dose of 100 mg/kg/d (mortality, initial body weight loss and reduced food consumption); the NOAEL for embryo-fetal development was 100 mg/kg/d, since it appears that the minor delays in ossification may have no physiological significance.

Observations and Results

Maternal parameters:

Mortality

All females were observed at least twice daily; aborting or dead females were necropsied.

Three females in the high dose group (#s 3207, 3223 and 3225) were found dead on day GD18 or GD19. According to the Sponsor's narrative, there were no remarkable clinical changes prior to death and there were no findings at necropsy that could explain the cause of death (and this Reviewer concurs); however, the Sponsor did not rule out a possible association with treatment due to the occurrence in the high dose group only and at the end of the treatment period in all three cases.

One control female (#3142) was sacrificed after aborting on day GD18.

Clinical Signs

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All females were observed daily for clinical signs.

At the high dose, a reduction in fecal output for several females (15 out of 22 females) was observed between GD6 and GD12 and this was consistent with significant reduction in food consumption and lower body weight gain/body weight loss at the high dose during the same period; therefore, should be considered as treatment-related. Moreover, there was slight reduction in fecal output for some females (5-7 out of 22 females) in the mid dose, (associated with a slight, though statistically significant reduction in food consumption). No other treatment-related clinical signs were observed at any dose level.

Body Weight

All females were weighed on days 0, 6, 9, 13, 16, 20, 24 and 29 of gestation.

There was a treatment-related significant reduction in body weight gains in the HD females, when compared to control during GD6 to GD20. This reduction was initially due to significant mean body weight loss during GD6 and GD9 involving almost all high dose females (20/22 females). There were no differences in mean body weight gain in the low and mid dose females when compared to control during the treatment period (see Table 73 and Figure 20, below).

Table 73 Body weight change (in grams) in NZW rabbits during the treatment period (Excerpted directly from the study report AA78128; pp 32)

Group	1	2	3	4
GD 6-9	31	37	42	-61 **
GD 6-20	249	288	279	149 **

** : $p \leq 0.01$

GD: day of gestation

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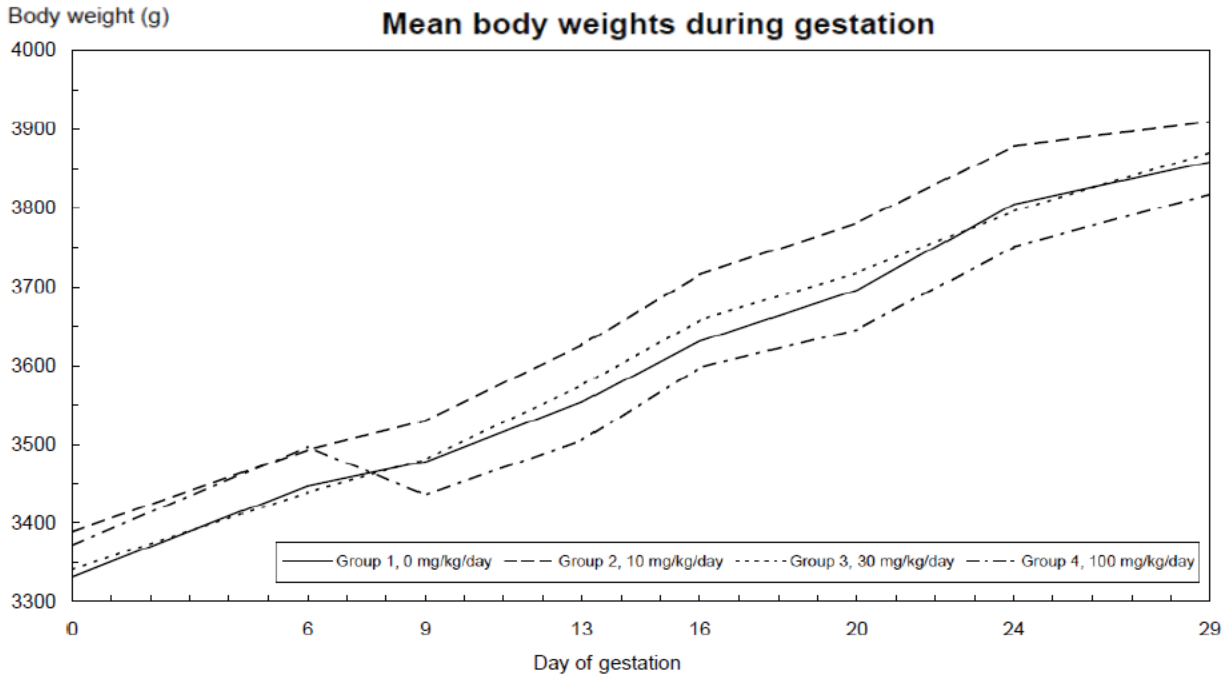
Figure 20 Mean body weights in NZW rabbits during gestation

(Excerpted directly from the study report AA78128 with minor formatting modifications by the Reviewer, pp 45



Feed Consumption

Food consumption of each female was measured daily from the day of arrival to day 29 of gestation.

When compared to control, there was a significant reduction (-25%) of food consumption in the HD females (Table 74, below) during GD6 to GD20. Similarly, in the mid dose group, mean food consumption was reduced during the first 3 days of treatment period by -13% compared to control. The mean food consumption was similar between the control and low dose group. After the treatment period, mean food consumption in all treatment groups was slightly higher than the control group.

Table 74 Difference in mean food consumption (%) during the treatment period, between control and treated groups

(Excerpted directly from the study report AA78128, pp 33)

Group	2	3	4
GD 6-9	-	- 13 % *	- 37 % **
GD 9-13	-	-	-33 % **
GD 13-16	-	-	-13 %
GD 16-20	-	-	- 18 % **
GD 6-20	-	-	- 25 % **

* : $p \leq 0.05$, ** : $p \leq 0.01$
GD: day of gestation

Toxicokinetics

Blood samples were collected from six females of the test article treated groups and three females from the vehicle control group via an ear artery (1 mL on GD 6 and 1.3 mL on GD 19). Samples were collected at 0.5, 1, 2, 4, 8, and 24 hours post-dose on GD 6 and GD 17.

The TK parameters for Levomilnacipran (F2695) are shown in Table 75, below. Levomilnacipran exposure was demonstrated in all test article treated groups on days 6 and 19 of gestation. The maximum plasma concentrations (C_{max}) were reached at ~0.5 h, post-dose for all treated groups on days 6 and 19. Systemic exposure in terms of C_{max} and AUC_{last} increased with dose on both days; however, the increase was non-linear on both days.

Table 75 Toxicokinetic parameters of levomilnacipran in female rabbits during gestation

(Excerpted directly from the study report AA78128, pp 337)

Group	Dose	Day	C_{max}	T_{max}	AUC_{last}	C_{max} / dose	AUC_{last} / dose	Day 19 vs Day 6	
	$mg.kg^{-1}.day^{-1}$		$ng.mL^{-1}$	h	$ng.mL^{-1}.h$			C_{max}	AUC_{last}
2	10	Day 6	31.8	0.5	70.0	3.18	7.0	2.2	1.7
		Day 19	70.2	0.5	121	7.02	12.1		
3	30	Day 6	187	0.5	469	6.23	15.6	3.1	2.3
		Day 19	583	0.5	1101	19.4	36.7		
4	100	Day 6	1566	1	4427	15.7	44.3	4.5	4.9
		Day 19	6994	0.5	21715	69.9	217		

Dosing Solution Analysis

Dosing formulations were prepared weekly and refrigerated at ~4 °C (Group 4 formulation prepared daily, just before dosing). The aqueous solution (concentrations from 1 mg/mL to 24 mg/mL) was stable for 5 hours at room temperature and 10 days under refrigeration (stability study reference F2695REC-32RM102). The concentrations

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of all doses of F2695 were determined on the first and last days of dosing (nominal concentrations 5, 15, and 50 mg/mL); the deviations from the nominal concentrations ranged from -1.6% to 3.0% (the testing facility requirement being ± 15%). No test article was detected in the vehicle control sample.

Necropsy

All surviving females were sacrificed on GD 29 by sodium pentobarbitone (i.v.) followed by exsanguination and examined for macroscopic pathological changes. The ovaries and uterus of all females were removed and examined.

There were no treatment-related maternal macroscopic findings in any group.

Litter data

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Pregnancy incidences: There were 20/22, 21/22, 21/22 and 19/22 pregnant female rabbits in groups 1 to 4, respectively at the time of terminal caesarian examination, all of which had live fetuses (Table 76, below). One control female (#3142) aborted early in the study (GD 18); according to the Sponsor, this was perhaps a normal physiological response to the presence of a single implant in that animal.

Pre-implantation data: The mean pre-implantation loss appeared to be higher in all treatment groups than the control group (Table 76, below); however, this may be due to a significantly lower concurrent control value than the historical control value (the mean pre-implantation loss in the historical control group obtained from 3 different studies was 15.9%). Moreover, the number of implantation sites at all three doses was comparable to control. Therefore, the higher incidence of pre-implantation loss in the treated groups when compared to control may not be treatment-related.

Table 76 Maternal variables for segment II study in NZ White rabbits

(Compiled by the Reviewer from the study report AA78128, pp 53-55)

PARAMETER	DOSE, mg/kg/d (gestational D6-19)			
	0	**10**	**30**	**100**
Total pregnancy rate	20/22	21/22	21/22	19/22
Drug-related mortalities	0	0	0	3
Gravid rabbits completing study	21	22	22	19
Mean implantation sites	9.1	8.2	8.5	8.9
Mean corpora lutea	9.6	9.2	9.8	10.2
Pre-implantation loss %, per dam	5.4	11.6	13.3	13.6
Mean live fetuses (%) %M %F	9.0(100%) 46.2 53.8	7.5 (100%) 44.1 55.9	8.2 (100%) 51.5 48.5	7.8 (100%) 50.7 49.3
Mean dead fetuses	0.0	0.0	0.0	0.0
Post-implantation loss, total	3.0	15.0	5.0	20.0

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PARAMETER	DOSE, mg/kg/d (gestational D6-19)			
	0	10	30	100
Mean per animal	0.2	0.7	0.2	1.1
% implants per animal	1.5	9.4	3.5	9.1
Early resorptions, total	2.0	9.0	3.0	16.0
Mean per animal	0.1	0.4	0.1	0.8
% implants per animal	1.1	6.5	2.4	7.3
Late resorptions, total	1	6	2	4
Mean per animal	0.1	0.3	0.1	0.2
% implants per animal	0.4	2.8	1.1	1.8

Post-implantation data: There was a slightly higher post-implantation loss in the low dose and the high dose group; however, this increased post-implantation loss was not dose-related and was mainly due to one female (#3210) in the high dose group with 7 early resorptions that influenced the group mean value and an unusually low percentage post-implantation loss in the concurrent control group compared to the historical control values (the mean post-implantation loss in the historical control group obtained from 3 different studies ranged between 3.8 and 5.7% versus 1.5%, in concurrent control). The mean live litter size was slightly lower in all three dose groups than control group, perhaps as a consequence of the differences in both pre- and post-implantation loss (Table 76, above).

Fetal data

Offspring (Malformations, Variations, etc.)

Fetal sex ratio: No treatment-related effects on sex ratio of fetuses; proportion of male fetuses was 46.2%, 44.1%, 51.5%, and 50.7%, respectively at 0, 10, 30, and 100 mg/kg/d.

Fetal body weights: No treatment-related effects on fetal body weights; mean fetal body weights (male and female combined) on a litter basis were 34.9g, 37.6g, 36.1g and 34.6g, respectively at 0, 10, 30 and 100 mg/kg/d.

Fetal examinations: The numbers of fetuses (litters) submitted to the different examinations were as shown in Table 77, below:

Table 77 Embryo/fetal variables for segment II study in NZ White rabbits

(Compiled by the Reviewer from study report AA78128; pp 53-55 and 129-241; Appendices 9-11)

PARAMETER	DOSE, MG/KG/D (GESTATIONAL D6-19)			
	0	10	30	100
TOTAL LITTERS WITH LIVE FETUSES	20	21	21	19
MEAN LIVE FETUSES/LITTER	9.0	7.5	8.2	7.8
MALES (%)	4.1 (45.6%)	3.3 (44.1%)	4.2 (51.5%)	3.9 (50.7%)
FEMALES (%)	4.9 (54.4%)	4.2 (55.9%)	4.0 (48.5%)	3.8 (49.3%)

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PARAMETER	DOSE, MG/KG/D (GESTATIONAL D6-19)			
	0	10	30	100
MEAN FETUS WEIGHTS, G	34.9	37.6	36.1	34.6
TOTAL FETUSES EXAMINED FOR EXTERNAL ABNORMALITIES	180	158	173	149
TOTAL FETUSES EXAMINED FOR VISCERAL ABNORMALITIES	180	158	173	149
TOTAL FETUSES EXAMINED FOR SKELETAL ABNORMALITIES	180	158	173	149
TOTAL FETUSES WITH MALFORMATIONS (LITTERS)	1 (1)	5 (4)	0 (0)	4 (3)
MALFORMATION INCIDENCES:				
VISCERAL, FETUSES (LITTERS)	0 (0)	1 (1)	0 (0)	0 (0)
SKELETAL, FETUSES (LITTERS)	1 (1)	4 (3)	0 (0)	4 (3)
EXTERNAL, FETUSES (LITTERS)	0 (0)	1 (1)	0 (0)	2 (2)
FETUSES/LITTER WITH MALFORMATIONS (%)	1 (0.6)	6 (3.8)	0 (0)	6 (4.0)
LITTERS WITH MALFORMATIONS (%)	1 (5)	5 (23.8)	0 (0)	5 (26.3)
FETUSES/LITTER WITH VARIATIONS (%)	5.6 (52.7%)	5.2 (56.5%)	4.9 (50.5 %)	4.4 (47%)
LITTERS WITH VARIATIONS (%)	19 (95%)	20 (95.2%)	19 (90.5%)	16 (84%)

External observations:

In the low dose group, one fetus (# 1) from one litter (# 3163) had ectrodactyly of the first digit. Similarly, in the high dose group, two fetuses (# 5 from litter 3212 and # 3 from litter 3214) had ectrodactyly; fetus # 5 from litter 3212 had ectrodactyly for several digits and fetus # 3 from litter 3214 had ectrodactyly at the first digit (see Table 78, below).

One fetus (# 9) from one litter (3172) at the low dose was acaudate.

Table 78 Incidence of ectrodactyly at external examination

(Excerpted directly from the study report AA78128, pp 36)

Group	1	2	3	4
Ectrodactyly (first digit)	0	1 [1]	0	1 [1]
Ectrodactyly (several digits including first digit of forepaws)	0	0	0	1 [1]
Total fetuses [litters] affected	0	1 [1]	0	2 [2]

Number of fetuses affected [number of litters affected]

Other external anomalies were a localized edema in one fetus (# 2) from one litter (3184) at the low dose and hyperflexion of the limb in one fetus (# 3) from one litter (3148) in the control group; both these findings were considered by the Sponsor as incidental (see Table 79, below).

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Table 79 Incidence of anomalies at external examination

(Excerpted directly from the study report AA78128, pp 36)

Group	1	2	3	4
Limb : hyperflexion	1[1]	0	0	0
Oedema	0	1[1]	0	0
Total fetuses [litters] affected	1[1]	1[1]	0	0

Number of fetuses affected [number of litters affected]

Soft tissue observations: Malformations were limited to one fetus (female #3184) in the low dose group that had hydrocephaly; no such malformations were seen at any other dose level and therefore, it appears to be spontaneous in origin and not related to treatment.

Other soft tissue anomalies or variations did not appear to be treatment-related: absence of azygos lobe in the lung (1 fetus in the control group; 3 fetuses- 3 different females- in low dose group; 7 fetuses- 3 different females- in mid dose group and 1 fetus in the high dose group), small size of the lung/fused lung (1 fetus in low dose group), dilated third cerebral ventricle (1 fetus in low dose; same female #3184 that had a fetus with hydrocephaly) and vacuole in the midbrain (1 fetus in mid dose group).

Skeletal observations: Skeletal malformations were limited to paw/digit malformations (see Table 80, below) in the low dose (2 fetuses) and high dose groups (3 fetuses):

Table 80 Paw/digit malformations in rabbit fetuses

(Excerpted directly from the study report AA78128, pp 37)

Group	1	2	3	4
Ectrodactyly/brachydactyly (first digit)	0	2 [1]	0	2 [1]
Malformed paws	0	0	0	1 [1]

Number of fetuses affected [number of litters affected]

At the low and high doses, there were 2 fetuses each (low dose female #3163 and high dose female #3214) with ectrodactyly/brachydactyly (limited to first digit). In addition, at the high dose, one fetus (female #3212) had multiple digit defects; due to their low incidence as well as lack of any fetus with less severe cases of absent bones/claws of the first digit, the Sponsor considered these malformations as spontaneous in origin and not elated to treatment.

Higher incidence of incomplete ossification of the phalanges of forepaw and hindpaw was seen in high dose group fetuses compared to concurrent control and historical control data (41 fetuses/9 litters in the HD group versus 13 fetuses/7 litters in the concurrent control group and 22/399 fetuses in the background control). Similarly,

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incidence of incomplete ossification of the 1st metacarpal was seen in the high dose group fetuses compared to concurrent control and historical control data (11 fetuses/5 litters in the HD group versus 2 fetuses/2 litters in the concurrent control group). Even though the number and location of the affected phalanges were not different between the treatment groups (less than 4 phalanges of the forepaws and/or hindpaws), the severity was slightly higher in the high dose group (as shown by the number of fetuses with unossified phalanges) and therefore, the above findings may be treatment-related.

Other skeletal malformations included one control fetus with sternoschisis (female # 3151; fetus #7), one fetus in each of low dose (female # 3266; fetus # 6) and high dose (female # 3217; fetus # 7) with malformed cervical and thoracic vertebrae causing scoliosis.

Reduced or absent interparietal bone was also noted in all treatment groups as shown in Table 81, below. However, there was no dose-related increase in the incidences and as per the Sponsor's narrative there was no statistical significance in any group; moreover, these findings were not associated with other malformations of head bones or in the cervical region. In the historical control data provided by the Sponsor, spontaneous occurrence of absent interparietal bone was ~ 2% (97 fetuses affected out of 4610); therefore, it appears to be unrelated to treatment with F2695. In addition, the Sponsor has cited a report by Guittin et al[11] in which a similar incidence of absent or reduced interparietal bone (overall incidence of up to 16%) was reported to argue that these findings reflect biological variations rather than drug-related effects.

Table 81 Incidence of absent or reduced interparietal bone

(Excerpted directly from the study report AA78128, pp 39)

Group	1	2	3	4
Absent interparietal				
Fetal incidence: absolute value	1	6	10	2
Fetal incidence: %	1.1	7.1	10.8	2.5
Reduced interparietal				
Fetal incidence: absolute value	6	10	12	7
Fetal incidence: %	6.3	11.8	12.9	8.9
Overall incidence (%)	7.3	18.8	23.7	11.4

[11] Guittin et al; In Birth Defects Res Part A Clin Mol Teratol, 2004 May, 70 (5): 303 (Absent interparietal bone as a variation in rabbit strain fetuses).

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In conclusion, treatment of female New Zealand White rabbits with levomilnacipran (10, 30, and 100 mg/kg/d) during organogenesis resulted in maternal toxicity which consisted of mortality, initial body weight loss, and reductions in mean body weight gain and food consumption at the high dose of 100 mg/kg/d. In the mid dose rabbits, treatment-related changes were limited to reduced food consumption (transient; only at the beginning of the treatment period). The mid dose of 30 mg/kg/d was therefore considered as NOAEL for maternal toxicity.

There were no treatment-related changes in the caesarean section data, including embryo-fetal survival and fetal weight in any dose group. Fetal data showed a slight delay in ossification of digit bones (mainly phalanges) in the high dose group compared to control. According to the Sponsor, this delay was most likely related to the significant decrease in maternal body weight gain during gestation at the high dose and hence, may be of no physiological or toxicological significance and the NOAEL for embryo-fetal development was considered to be 100 mg/kg/d (however, considering the large number of fetuses in the high dose group with delayed ossification, this Reviewer thinks that it may be treatment-related, especially because this happened in the absence of treatment-related effects on fetal body weight).

9.4 Prenatal and Postnatal Development

Study title: F2695- Pre- and post-natal development study by the oral route (gavage) in the rat (Segment III)

Study no.:	AA78125
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 31, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	F2695 (also referred as F2695A1); batch #OP210; 100.1 m/m (potentiometric assay)

Key Study Findings

- Effects of oral administration of levomilnacipran (F2695) at doses of 0, 7, 20, and 60 mg/kg/d on pregnant Wistar rats during the period of organogenesis and through parturition/lactation (GD6 through post-natal day 21) were evaluated.
- Maternal (F_0) findings:
  - Decreased body weight gains at mid dose and high dose during gestation (-8% and -16%, respectively) and lactation (-39% and -46%, respectively).

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Reduction in food consumption at mid dose and high dose during gestation (-7% for both groups) and lactation (-7% and -18%, respectively)

- No treatment-related effects on gestation index, delivery index, live birth index, and gestation length.

- Increased pup mortality at HD (only) on the first day *post partum* (14 HD pups from 7 litters versus one pup in control); consequently the mean HD live litter size before culling was reduced.

- The NOAEL for maternal toxicity is 7 mg/kg/d, based on reduced body weight gains and food consumption at the mid dose of 20 mg/kg/d.

- Pup (F_1) findings:
  - Dose-dependent decrease in pup weight at birth; thereafter, pup weight gains was reduced for the entire period of lactation to weaning (LD4 to 21) and consequently, mean pup weight was lower at weaning for all dose groups; during the initial 4 to 5 weeks of post-weaning period, the mean body weight gains in F1 males and females of all three treatment groups were slightly lower than control. Mean body weight gains of F1 females during gestation were comparable in all groups.

  - Slight delay in physical development based on delayed incisor eruption and pinna unfolding and earlier eye opening in the HD and MD pups.

  - Maternal (F_0) treatment with levomilnacipran did not affect the F_1 adult behavioral parameters (swimming ability, learning, memory, motor activity or exploratory behavior).

  - Maternal (F_0) treatment with levomilnacipran did not affect the fertility and reproductive performance in F_1 adults.

  - Maternal (F_0) treatment with levomilnacipran did not affect the caesarian-section data of adult F_1 females (corpora lutea counts, pre- and post-implantation losses and live litter sizes).

  - The NOAEL for toxicity in the offspring is 7 mg/kg/d, based on reduced body weight gain at the mid dose of 20 mg/kg/d….

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Methods

Doses: 0, 7, 20, and 60 mg/kg/d. Doses were selected based on a previous dose range-finding study (MDS study № AA80683) where the test item was given by oral gavage to females (7-8/group) from Day 6 of gestation up to Day 7 of lactation at doses of 0, 30, 60 and 100 mg/kg/d. During the first week of treatment, there was a slightly low maternal body weight gain at all three doses. At the high dose of 100 mg/kg/d, a high mortality rate of pups was noted at birth and during the first day of lactation. In addition, the body weights of the pups were significantly lower at the high dose. At the mid dose of 60 mg/kg/d, the number of live pups and body weights at birth were statistically lower than control group. At the low dose of 30 mg/kg/d, the number of live pups and body weights at birth were lower than control; however, the differences were not statistically significant; therefore, 60 mg/kg/d was chosen as the top dose for the pivotal study.

Frequency of dosing: Once daily from GD 6 to day 20 of lactation (inclusive) of the F1 pups.

Dose volume: 5 mL/kg/d

Route of administration: Oral gavage

Formulation/Vehicle: Solution/purified water

Species/Strain: Rats/Wistar rats: Cr;:WI (Gix/BRL/Han) IGS BR

Number/Sex/Group: 25/females/group

Satellite groups: Yes; 6/females/group (3 females in control)

Study design: Levomilnacipran (F2695) oral doses of 0, 7, 20 and 60 mg/kg/d were administered to pregnant Wistar rats from implantation through the end of lactation (GD 6 through postnatal Day 21). The F_0 dams were allowed to deliver and nurse their pups (F_1); pup viability/litter size, clinical signs, weight, presence of milk in pup stomach, physical and functional development (pinna detachment, incisor eruption, righting reflex) were assessed weekly. On day 4 post-partum, the size of each main study litter was adjusted (culling) to yield 4 males and 4 females per litter. After weaning of the F_1 pups (day 21 post-partum), at least one male and one female pup per litter were selected to yield 25 males and 25 females in each dose group for evaluation of growth, sexual maturation (balano-preputial separation in males or vaginal opening in females), mating behavior and neurobehavioral development (learning and memory, motor activity or exploratory behavior). Caesarean sections of F_1 females were performed on GD 13 for implantation data (F_2).

Deviation from study protocol: No significant deviations that would affect the study outcome.

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Observations and Results

Maternal (F_0) parameters: mortality (checked twice daily), clinical signs (observed daily), body weight (individual body weights measured on days 0, 6, 9, 12, 15, 18 and 20 of gestation and days 1, 4, 7, 10, 14, 17 and 21 of lactation), food consumption (individual consumption measured on days 0-6, 6-9, 9-12, 12-15, 15-18 and 18-20 of gestation and days 1-4, 4-7, 7-10, 10-14, and 14-21 of lactation), pregnancy and parturition and litter data were recorded. For exposure determination in F0 dams, the satellite females from each group were sampled on days 6 and 17 of gestation. For exposure determination in pups (F_1), all surviving pups from satellite females in all groups were sampled on day 4 *post partum* within 2 hr after dosing of the mother.

F_1 offspring parameters: F1 generation parameters included the following: number of pups born (live and dead), number of pups with milk in the stomach versus total number of pup on day 1 *post-partum*, external abnormalities, number, weight and sex of live pups (on days 1, 4, 7, 14 and 21 *post-partum*), physical development (as assessed by the intra-litter onset and duration of pinna unfolding, incisor eruption and eye opening), behavioral and functional tests (surface righting reflex on day 8 *post-partum*, gripping reflex on day 17 *post-partum* and pupil reflex and auditory reflex on day 21 *post-partum*), external and necropsy findings of dead pups.

After weaning of the F_1 pups (day 21 post-partum), at least one male and one female pup per litter were selected to yield 25 males and 25 females in each group for post-weaning behavioral test and mating (~8 weeks after weaning- 11 weeks of age). Females were examined from day 28 *post-partum* to detect the day of vaginal opening; males were examined from day 38 *post-partum* to detect the day of balano preputial skinfold cleavage; post-weaning behavioral tests were initiated at 5-7 weeks of age (water maze at 5 to 6 weeks of age and open field test at 7 weeks of age); mating of F_1 animals (one male and one female from the same group were paired for up to 21 days) to produce F_2 generation was initiated at 11 weeks of age. Successful mating was confirmed by the presence of sperm in the vaginal smear or a vaginal plug and was taken as Day 0 of gestation. Pre-coital time was noted for each female. Pregnant F_1 females were killed by CO_2 inhalation and necropsied on gestation day 13 or 14 to determine the pregnancy status, number of corpora lutea, number and distribution of intrauterine implantation (viable embryos and intrauterine deaths-resorptions). The following reproductive indices were calculated:

F_1 Pre-coital interval (in days) = Number of days until successful insemination/number of inseminated animals.
F_1 Copulation (mating) index (in %) = Number of animals copulated (or inseminated)/number of paired animals x 100.
F_1 Fertility index (in %) = Number of animals inducing pregnancy (or pregnant)/number of animals copulated (or inseminated) x 100.
F_1 Pre-implantation loss (in %) = number of corpora lutea – number of implantations/number of corpora lutea x 100.
F_1 Post-implantation loss (in %) = Number of implantations – number of live embryos/number of implantations x 100.
Sex ratio (proportion of male pups) (in %) = number of males/number of pups x 100.

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Results:

F_0 Dams

Survival:

One HD female (#78) was found dead on day 7 *post partum*; no abnormal clinical signs prior to death except hair loss on a forelimb. Necropsy showed dark lungs (all lobes); stomach/intestine full of 'material' and the animal was stiff. Cause of death undetermined; the Sponsor considered the death as incidental.

Clinical signs:

Treatment-related hypersalivation immediately after dosing on multiple occasions in all HD females, ~half of MD females and three LD females. Raised hair was observed on few occasions in the drug treated groups; however, the significance of this finding is unclear.

Body weight:

During gestation: At the high dose, the mean BW on GD20 was slightly lower than control (-4%; not statistically significant). Overall, the mean BWG from GD6 to GD20 was reduced (-16%; $p < 0.01$) compared to control; this was mainly due to reduced BWG from GD6 to GD9 (-41%, $p < 0.01$) and from GD15 to GD18 (-15%, < p 0.05), compared to control (see Figure 21, below). At the mid dose, the mean BW was slightly lower than control (-2.4%, not statistically significant). The BWG from GD6 to GD20 slightly lower than control (-7.5%, $p < 0.05$). The mean BW/BWG at the low dose was comparable to control.
After birth, all mean BWs were comparable between the treated and control groups.

During lactation: At the high dose, the maternal BW at each measurement interval from LD7 to LD 21 was slightly lower than control (between -4 and -6%; $p < 0.01$); the maternal BWG was lower than control between LD1 and LD21 (-46% to -60%; $p < 0.01$); see Figure 22, below. At the mid dose, the mean BW was slightly lower than control at various measurement intervals (on LD4 = -2%, on LD14 = -3.5%, $p < 0.05$; and on LD17 = -5%, $p < 0.01$ and terminal LD21 = -3.9%, $p < 0.05$). Although the mean BWG at the mid dose was lower than control at some measurement intervals, the differences were statistically significant only at LD1 to 4 (-33%, p < 0.05) and the overall decrease at the end of lactation period (LD1 to LD21) was statistically significant (-39%, $p < 0.01$). At the low dose, the BW/BWG was comparable to control.

Feed consumption:

During gestation: Occasional, slightly lower food consumption in HD (-8%) and MD (-7%) rats, when compared to control.
During lactation: Food consumption was lower in HD (-18%, p = 0.01; at all intervals

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from LD1 to LD21) and MD (-6%, p = 0.05; from LD10 to LD21); LD food consumption was comparable to controls (see Figure 23, below).

Uterine content:

No treatment-related effects on gestation index, delivery index, live birth index, and gestation length (see Table 82, below). All pregnant dams in all groups gave birth to live pups except for one HD dam (#92) that was found to have only three uterine implantation sites (total litter resorption); this isolated incident was not considered to be treatment-related by the Sponsor. The incidence of stillborn pups was low and comparable in the treated and control groups. The mean uterine implantation sites were lower in HD group, compared to control (10.9 compared to 11.3; not statistically significant); however, unlikely to be treatment-related according to the Sponsor because the implantation is normally complete by GD 6. The mean number of pups born was slightly lower in the HD group than control (9.3 compared with 10.5; not statistically significant), partly due to the slightly lower number of implantation sites in HD group. The pup mortality on the first day *post partum* was higher in the HD group compared to control (14 HD pups from 7 litters versus one pup in control); consequently the mean HD live litter size before culling was reduced (p = 0.05 on days 1 and 4). The percentage of male pups was slightly lower in the LD and HD groups; however, in the absence of a dose-relationship, this may be of no toxicological consequence.

Necropsy observation:

No treatment-related gross pathological findings in F_0 maternal rats on days 21/22 *post partum*.

Toxicokinetics:

All dams from the treated groups were exposed to levomilnacipran on GD6 and GD17 and the plasma concentrations increased in relation with the dose on days 6 and 17. Similarly, all pups (male and female) born of the treated mothers were exposed to levomilnacipran on day 4 *post partum* and the plasma concentrations for each litter increased with the dose (Table 83, below).

Dosing Solution Analysis:

The test item was prepared (weekly) as a solution in the vehicle (purified water) at concentrations of 1.4, 4 and 12 mg/mL. Aqueous solution from 1 mg/mL to 24 mg/mL was stable for 5 hr at RT and 10 days under refrigeration. Chemical analyses of the dosing formulations indicated that the achieved concentration mean were within the intended values (97.6 to 102.7%of the nominal concentration).

Other: None

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Figure 21 Sponsor's figure (with minor formatting modification by the Reviewer) showing the mean maternal (F_0) body weights during gestation

(Excerpted directly from the study report AA78125, pp 63)

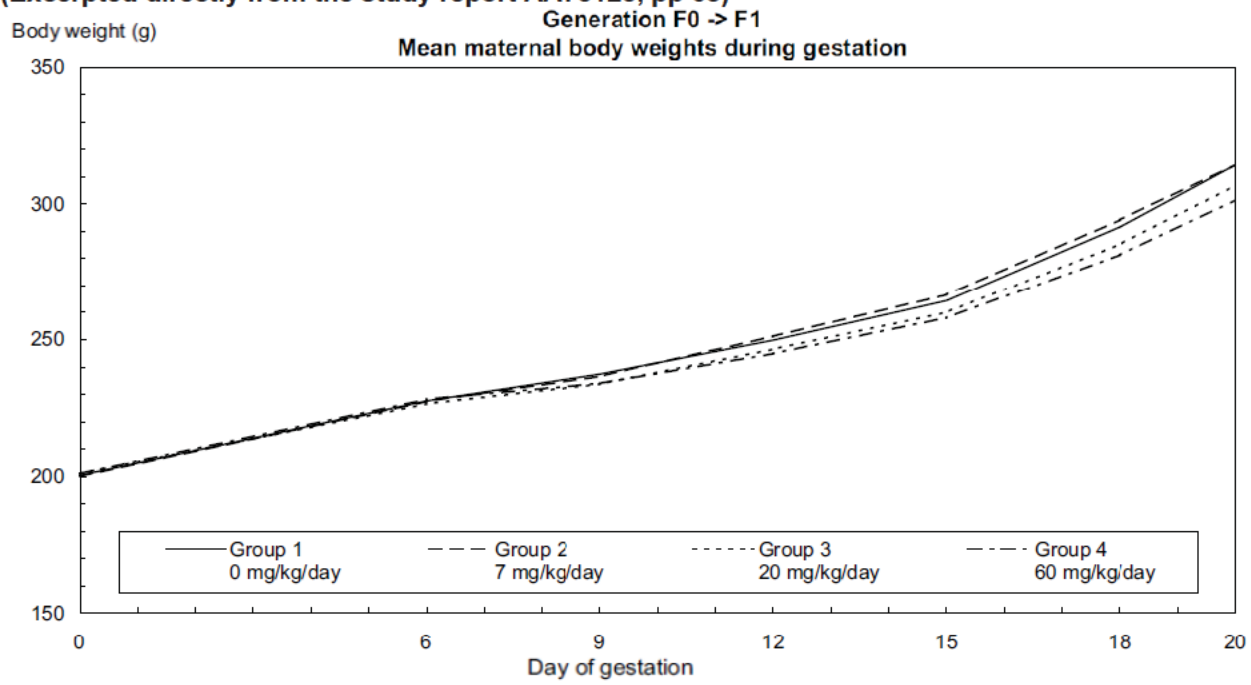

Figure 22 Sponsor's figure (with minor formatting modification by the Reviewer) showing the mean maternal (F_0) body weights during lactation

(Excerpted directly from the study report AA78125, pp 69)

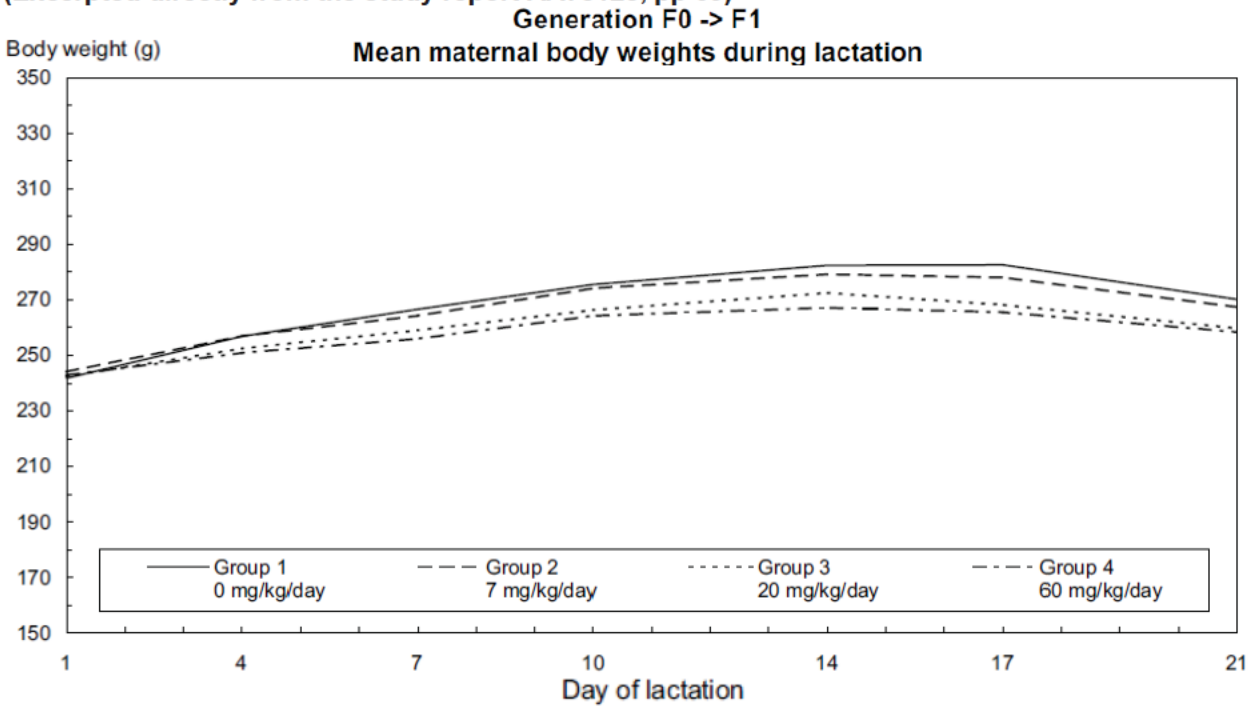

Figure 23 Sponsor's figure (with minor formatting modifications by the Reviewer) showing the mean maternal (F_0) food consumption during lactation phase

Excerpted from the study report AA78125pp 79

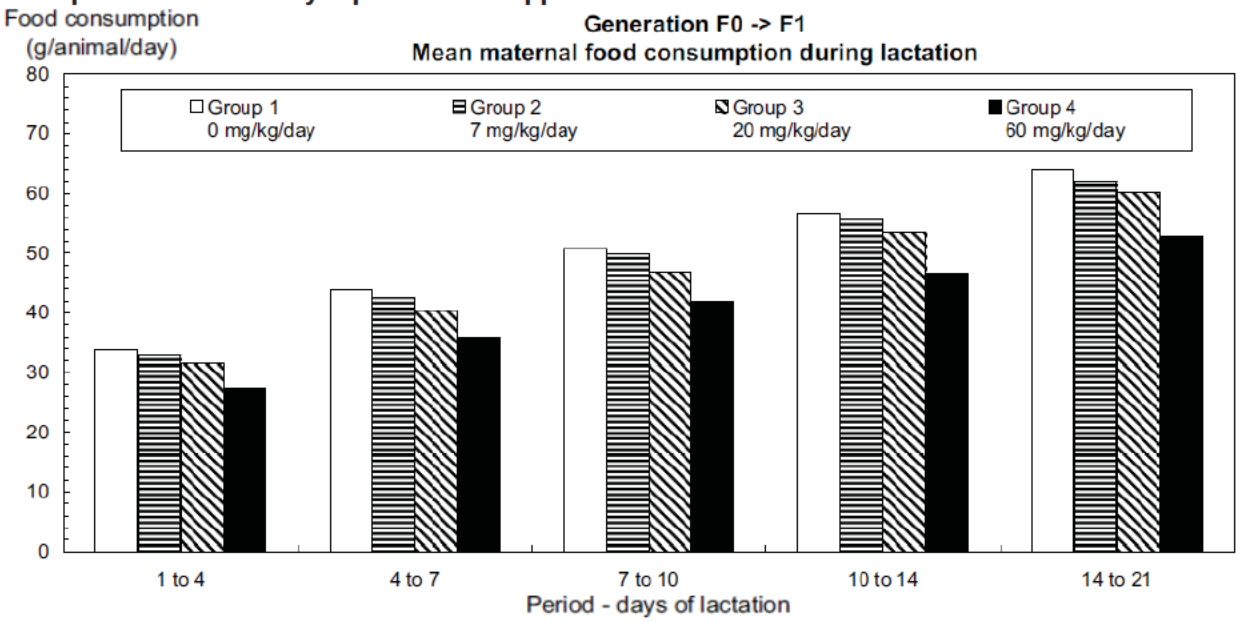

Table 82 Summary of reproductive findings in maternal (F_0) rats

(Values compiled by the Reviewer from the study report AA78125, pp 81-83)

		Group 1 0 mg/kg/d	Group 2 7 mg/kg/d	Group 3 20 mg/kg/d	Group 4 60 mg/kg/d
Females Mated	N	25 f	25	25	25
	%	100.0	100.0	100.0	100.0
Females Pregnant	N	24 f	25	24	25
Fertility Index	%	96.0	100.0	96.0	100.0
Females with live born	N	24 f	25	24	23
Gestation Index	%	100.0	100.0	100.0	92
Females completing delivery	N	24 f	25	24	23
	%	96.0	100.0	96.0	92.0
With stillborn pups	N	4 f	5	5	6
	%	16.7	20.0	20.8	26.1
With all stillborn	%	0.0	0.0	0.0	0.0
Duration of gestation (mean)		22.1 d	22.1	22.2	22.2
Pups delivered	N	251 f	269	247	214
	(mean)	10.5 d	10.8	10.3	9.3
Live born	N	246 f	263	240	207
Live birth Index	%	98.0	97.8	97.2	96.7
Stillborn	N	5 f	6	7	7
	%	2.0	2.2	2.8	3.3
Pups dying, missing and/or Cannibalized:					
Day 0	N	0 f	0	0	0
Days 1-4	N	1 f	0	4	15**
	%	0.4	0.0	1.7	7.2
Days 5-21	N	1 f	0	0	0
Pups surviving 4 days:	N	245 f	263	236	192**
Viability Index	%	99.6	100.0	98.3	92.8
Pups surviving 21 days:	N	186 f	200	190	159
Lactation Index	%	99.5	100.0	100.0	100.00
Implantation sites Per litter (mean)		11.3 d	11.7	11.5	10.9
Live pups/litter (mean)					
Day 1		10.2 d	10.5	9.8	8.4*
Day 4 (preculling)		10.2 d	10.5	9.8	8.3*
Day 4 (postculling)		7.8 k	8.0	7.9	6.9
Day 7		7.8	8.0	7.9	6.9
Day 14		7.8	8.0	7.9	6.9
Day 21		7.8	8.0	7.9	6.9
Sex Ratio- Male pups: total pups					
Day 0	%	52.7	46.8	50.6	45.6
Day 21	%	52.7	45.5	52.1	45.9

Statistical key: d = Anova/Dunnett test; f = Chi^2/Fisher Exact test; k = Kruskal/Wallis/Dunn test; * p<0.05; ** p<0.01

F_0 Gestation index (%) = (number of females with live pups/number of pregnant animals) x 100
Delivery index (%) = (number of pups delivered/number of implantations) x 100
F_0 Live birth index (%) = (number of pups born alive/number of pups born) x 100
F_0 Viability index (%) = (number of pups alive on day 4/number of pups alive at birth) x 100
F_0 Lactation index (%) = (number of pups alive on day 21/number of pups alive on day 4 after adjustment of litter size) x 100.

Table 83 Mean plasma concentrations of F2695 (ng/mL) in the female rat (F_0 generation) and pups (F_1 generation)

Excerpted directly from the study report AA78125; pp 43

Study Day	Time after dosing (hour)	7 mg/kg/day group	20 mg/kg/day group	60 mg/kg/day group
Females (F0 generation)				
G6	0.5	671	2221	4693
	6	30	139	889
G17	0.5	565	2481	3745
	6	37	395	1395
Pups (F1 generation)				
L4 Male pups	2	94.6	140	1196
L4 Female pups	2	36.4	175	993

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F_1 Generation

Survival:

The pup mortality on the first day *post partum* was higher in the HD group compared to control (14 HD pups from 7 litters versus one pup in control); consequently the mean HD live litter size before culling (day 4) was reduced (p = 0.05 on days 1 and 4). One more HD pup died on day 2, but thereafter pup survival was similar in all treated and control groups. As a result of the early deaths in the high dose group, the viability index was slightly reduced in the high dose group (99.6% control versus 92.8% HD; p = 0.001). The percentage of male pups was slightly lower in the LD and HD groups on post-natal day 21; however, in the absence of a dose-relationship, this may be of no toxicological consequence (see Table 82, above).

There were no deaths amongst the selected F_1 animals.

Clinical signs:

No abnormal clinical signs in F_1 pups related to treatment of the dams.

Body weight:

Reduction in pup weight (male and female combined) on LD1 (statistically significant at HD and MD and not at LD); the mean differences were -18%, -8% and -4% in HD, MD and LD, respectively compared to control (see Table 84 and Figures 25 & 26, below). The rate of pup growth from birth to weaning was reduced in all three dose groups compared to control. The BWG (male and female combined) from LD4 (post culling) to LD21 was statistically significantly lower in HD, MD and LD groups, compared to control; at weaning on day 21, the difference in group mean pup weight was -6% (p < 0.05), -8% (p < 0.01) and -17% (p < 0.01) respectively at LD, MD and HD compared to control.

At the time of selection/culling of F_1 males and females, the body weights were statistically significantly lower than control as shown in Table 84 and Figure 24 & Figure 25; these differences persisted up to the time of mating at 11 weeks of age and beyond as shown in Table 85, below. In fact, during the first 4 weeks after weaning the mean BWGs were statistically significantly lower in HDM (-13%, p < 0.05), MDM (-7%, < p 0.05), HDF (-8%, < p 0.05) and MDF (-9%, p < 0.01). After words, the mean BWGs in the mid and high dose groups were comparable with the control; however, the BWs of HDM and MDM were slightly, though statistically significantly lower than control. During gestation (of F_1 females), the maternal BW of the high dose group was slightly lower (-6.5%; p < 0.05; see Table 86, below) than control even though the maternal BWG was comparable in the treated and control groups.

Feed consumption:

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Food consumption of F_1 males up to the time of mating was slightly (~10%) though statistically significantly lower in all three treated groups than control. However, in females, food consumption was comparable between treated and control groups.

Physical development:

A summary of the physical development parameters in F_1 generation pups are shown in Table 87, below. Compared to control, there was a slight delay in the incisor eruption and pinna unfolding in HD pups. The incisor eruption was slightly delayed in MD pups as well. Some pups in the HD and MD groups showed slightly earlier than usual eye opening and according to the Sponsor, this is a frequent occurrence in small pups (which this Reviewer was not aware of in the context of retarded growth).

Neurological assessment:

There were no treatment-related effects on the reflex parameters (surface righting, gripping, pupillary and auditory responses; Table 87, below).

Reproduction:

There were no treatment-related effects on the age of secondary sexual development (balano-preputial separation in males or vaginal opening in females), even though there was a treatment-related decrease in body weights of MDF, HDF and HDM (see Table 88, below). Similarly, there were no treatment-related effects on the mating performance and fertility in the F_1 generation animals (Table 89, below); all animals from the treated groups mated and except one LDF (#350), all females in each group were inseminated within the first 4 days of pairing.

Other:

Presence of milk in the stomach was confirmed for most of the live main study pups in all groups on LD1 and most of the satellite pups in all groups on LD1 and LD4.

One stillborn pup from a LD dam (#35) had multiple malformations, including anasarca, short limbs and some defects of the abdominal organs (liver- pale; stomach- absent; gall bladder- absent; urinary track- absent; kidney- absent and guts, gonads- absent); since this appears to be an isolated incident, it may be unrelated to treatment with the test article.

In the post-weaning behavioral test (water maze), there were no treatment-related effects on the swimming ability, learning or memory in males and females (water maze). Similarly, there were no treatment-related effects on motor activity or exploratory behavior in the open field test in males and females.

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Table 84 Mean pup weights (F_1 male & female combined)

Excerpted directly from the study report AA78125; pp 48

Pup weight and gain (g)	Control	7 mg/kg/day	20 mg/kg/day	60 mg/kg/day
Day 1	6.5	6.2	6.0*	5.3**
Day 1 to Day 4	3.7	3.5	3.6	3.2**
Day 4 (before culling)	10.2	9.7	9.5	8.4**
Day 4 (after culling)	10.2	9.7	9.5	8.5**
Day 4 to Day 7	5.7	5.6	5.4	4.9**
Day 7	15.9	15.3	15.0	13.3**
Day 7 to Day 14	15.3	14.5	13.8**	12.9**
Day 14	31.1	29.9	28.8**	26.3**
Day 14 to Day 21	18.1	16.6*	16.3**	14.5**
Day 21	49.2	46.4*	45.1**	40.8**
Day 4 to Day 21	39.0	36.7*	35.6**	32.3**

* = $p < 0.05$; ** = $p < 0.01$

Table 85 Mean F_1 generation body weights from weaning to mating

(Excerpted directly from the study report AA78125, pp 52)

Body weight (BW) and Body weight gain (BWG)	Control	7 mg/kg/day	20 mg/kg/day	60 mg/kg/day
Day 0 BW				
-males	50.3	46.8*	45.9**	41.5**
-females	48.3	45.4*	44.6**	40.7**
Days 0 to 28 BWG				
-males	175.2	166.3	162.9*	153*
-females	118.2	111.1	108.0**	108.4*
Day 28 BW				
-males	225.5	213.0*	208.8**	194.5**
-females	166.4	156.5*	152.5*	149.1**
Days 28 to 56 BWG				
-males	118.0	118.7	116.4	113.2
-females	46.3	50.9	53.1*	51.7
Day 56 BW				
-males	343.5	331.7	325.2*	307.7**
-females	212.7	207.4	205.7	200.8

* = $p < 0.05$; ** = $p < 0.01$

Day 0: day of weaning.

Table 86 Summary of gestation (F_1) body weights in grams

(Excerpted directly from the study report AA78125; pp 128)

		Group 1 Control 0 mg/kg/day	Group 2 Low dose 7 mg/kg/day	Group 3 Intermed. dose 20 mg/kg/day	Group 4 High dose 60 mg/kg/day
DAY 0	MEAN	217.7 d	213.7	210.7	204.9
	S.D.	20.3	18.7	16.6	13.5
	N	23	21	25	23
DAY 4	MEAN	231.4 d	226.9	222.8	216.2*
	S.D.	20.2	18.7	18.5	14.5
	N	23	21	25	23
DAY 8	MEAN	241.9 d	236.0	232.7	225.7*
	S.D.	21.5	19.0	19.3	15.9
	N	23	21	25	23
DAY 13	MEAN	262.9 d	258.4	253.7	245.8*
	S.D.	22.1	20.6	20.8	17.7
	N	23	21	25	23

Statistical key: d=Anova/Dunnett test * = p<0.05

Figure 24 Mean pup (F_1 male) body weights during lactation

(Excerpted directly from the study report AA78125 with minor formatting modifications by the Reviewer; pp 91)

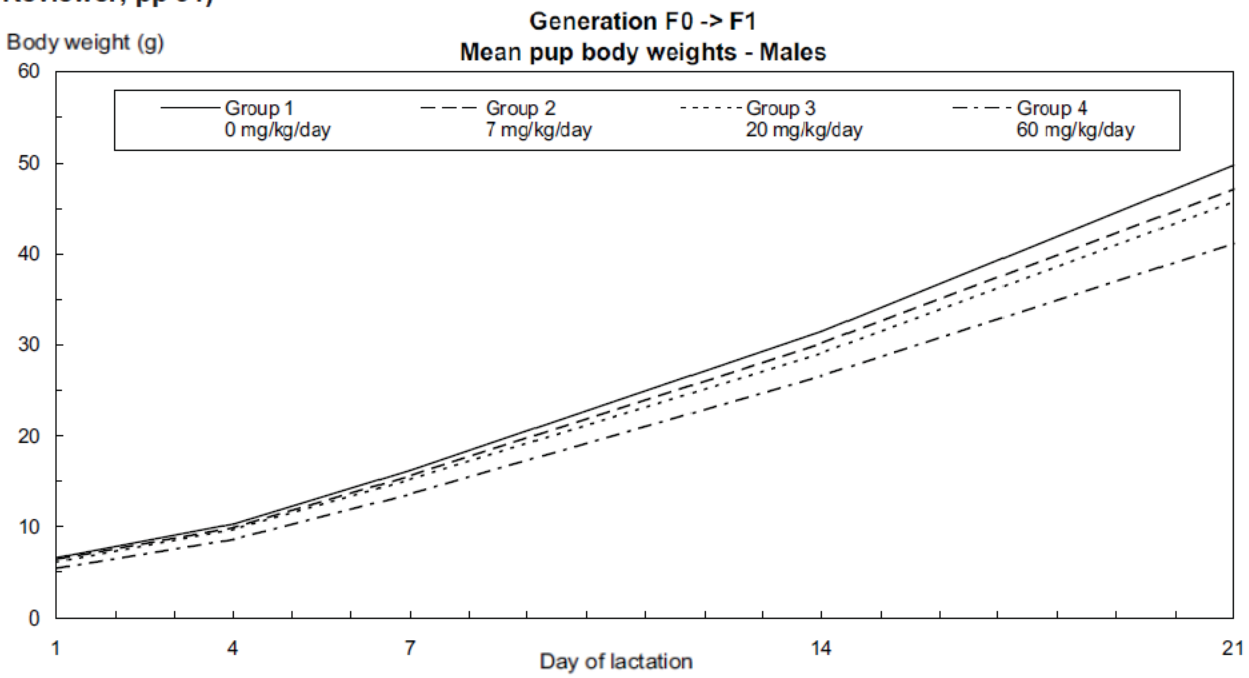

Figure 25 Mean pup (F_1 female) body weights during lactation

(Excerpted directly from the study report AA78125 with minor formatting modifications by the Reviewer; pp 92)

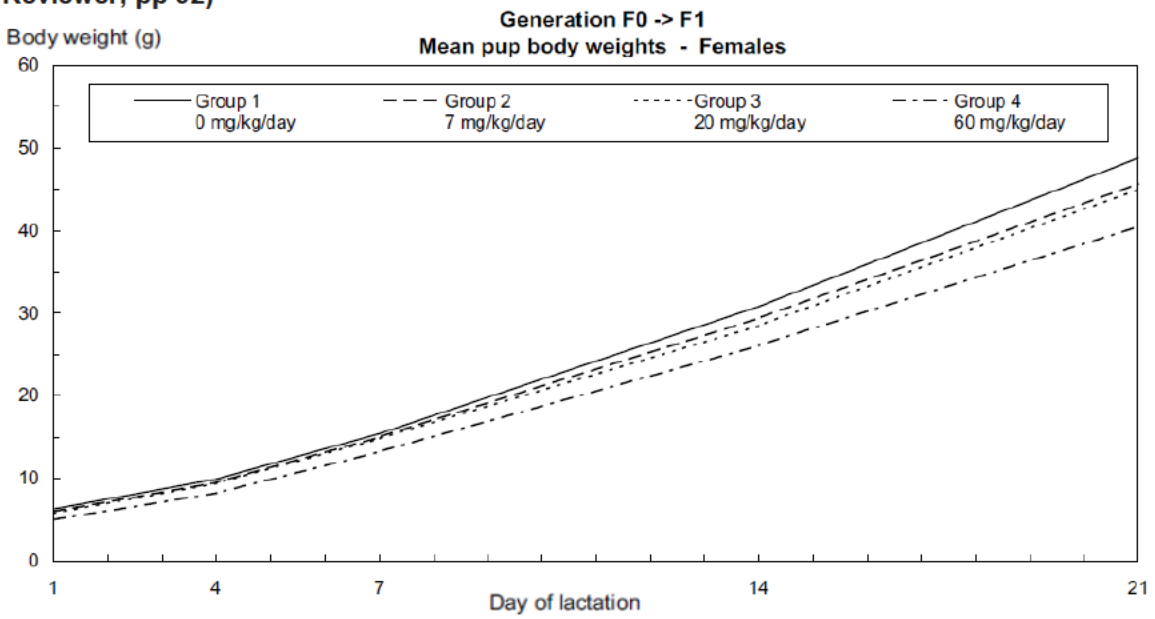

Table 87 Summary of reflex and physical development (F_1 generation)

(Excerpted directly from the study report AA78125 with minor formatting modification by the Reviewer; pp 94)

Group / Dose level	1 0 mg/kg/day	2 7 mg/kg/day	3 20 mg/kg/day	4 60 mg/kg/day
PINNA UNFOLDING - % of pups positive:				
day 1 *post-partum*	0	1	4**	0
day 2 *post-partum*	31	30	29	20*
day 3 *post-partum*	93	97	91	76**
day 4 *post-partum*	100	100	100	100
INCISOR ERUPTION - % of pups positive:				
day 7 *post-partum*	0	0	0	0
day 8 *post-partum*	0	4*	0	1
day 9 *post-partum*	11	12	4*	4
day 10 *post-partum*	49	44	26**	19**
day 11 *post-partum*	84	88	72*	57**
day 12 *post-partum*	97	100	94	92
day 13 *post-partum*	100		100	95
day 14 *post-partum*				95 [1]

Group / Dose level	1 0 mg/kg/day	2 7 mg/kg/day	3 20 mg/kg/day	4 60 mg/kg/day
EYE OPENING - % of pups positive:				
day 12 *post-partum*	0	1	0	12**
day 13 *post-partum*	7	11	23**	45**
day 14 *post-partum*	78	79	78	87
day 15 *post-partum*	99	100	100	100
day 16 *post-partum*	99			
day 17 *post-partum*	100			
SURFACE RIGHTING REFLEX - day 8 *post-partum* - % of pups positive	100	100	100	100
GRIPPING REFLEX - day 17 *post-partum* - % of pups positive	100	100	100	100
PUPILLARY REFLEX - day 21 *post-partum* - % of pups positive	99	100	100	100
AUDITORY REFLEX - day 21 *post-partum* - % of pups positive	100	100	100	100

[1] less than 100 % due to death of pups * P (probability) ≤ 0.05 ** P ≤ 0.01

Table 88 Sponsor's table (with minor formatting modifications by the Reviewer) showing the summary of sexual maturation in F_1 generation

Study report AA78125, pp 97

Summary of sexual maturation
Generation F1=>F2

	Vaginal opening		Balano preputial skinfold cleavage	
	Post-partum day of occurrence	Body weight (g)	*Post-partum* day of occurrence	Body weight (g)
Group 1: 0 mg/kg/day				
MEAN	33	107	47	208
S.D.	2	12	2	21
N	25	25	25	25
Group 2: 7 mg/kg/day				
MEAN	34	102	47	195
S.D.	2	12	3	24
N	25	25	25	25
Group 3: 20 mg/kg/day				
MEAN	33	97**	47	197
S.D.	2	13	3	25
N	25	25	25	25
Group 4: 60 mg/kg/day				
MEAN	34	96**	47	185**
S.D.	2	10	3	24
N	25	25	25	25

* P (probability) ≤ 0.05 ** $P \leq 0.01$

Table 89 Summary of mating performance and fertility in F_1 generation

(Excerpted directly from the study report AA78125; pp 138

GROUP TREATMENT	1 0 mg/kg/day	2 7 mg/kg/day	3 20 mg/kg/day	4 60 mg/kg/day
NUMBER OF MALES:				
Paired	25	25	25	25
Copulated	24	25	25	25
Induced pregnancy	23	23	25	23
NUMBER OF FEMALES:				
Paired	25	25	25	25
Inseminated	24	25	25	25
Pregnant	23	23	25	23
Not pregnant	1	2	0	2
No viable fetuses	0	1	0	0
PRE - COITAL INTERVAL - DAYS				
MEAN	2.25	3.04	2.32	2.72
S.D.	1.22	2.54	1.11	1.10
N	24	24 [1]	25	25
COPULATION INDEX (%)	96	100	100	100
FERTILITY INDEX (%)	96	92	100	92

[1] mating not detected for one pair of rats

F_2 Generation:

Pregnant F_1 females were killed and necropsied on gestation day 13 or 14 to determine the pregnancy status, number of corpora lutea, number and distribution of intrauterine implantation (viable embryos and intrauterine deaths). Caesarian section data (see Table 90, below) showed no treatment-related effects on the pre-implantation data (mean numbers of corpora lutea, uterine implantations and percentage of pre-implantation loss) and the post-implantation data (incidence of embryonic resorption and the mean live litter size).

Table 90 Summary of caesarean section data (F_2 generation)

(Excerpted directly from the study report AA78125, pp 140)

	Group 1 Control 0 mg/kg/day	Group 2 Low dose 7 mg/kg/day	Group 3 Intermed. dose 20 mg/kg/day	Group 4 High dose 60 mg/kg/day
Number of dams	23	23	25	23
Corpora Lutea				
per group	306	299	310	281
per dam: mean	13 d	13	12	12
st. dev.	3	2	2	1
Implantations				
per group	282 f	276	296	260
% of Corpora lutea	92.2	92.3	95.5	92.5
per dam: mean	12 d	12	12	11
st. dev.	2	3	2	2
Viable Embryos				
per group	263	262	276	248
per dam: mean	11 d	11	11	11
st. dev.	3	3	2	2
% of Implantations				
per group	93.3 f	95.6	93.2	95.4
per dam: mean	93 k	92	93	95
st. dev.	8	21	9	7
Postimplantation Loss				
total per group	19 f	14	20	12
% of implantations	6.7	5.1	6.8	4.6
per dam: mean	1 k	1	1	1
st. dev.	1	1	1	1
No. of dams affected	13	9	13	9
Preimplantation Loss				
per group	24 f	23	14	21
% of Corpora lutea	7.8	7.7	4.5	7.5
per dam: mean	1 k	1	1	1
st. dev.	1	2	1	1
No. of dams affected	17	9	9	13

Statistical key: d=Anova/Dunnett test f=Chi2/Fisher Exact test k=Kruskal-Wallis/Dunn test

Summary: In this pre- and post-natal developmental toxicity study, oral (gavage) administration of levomilnacipran (F2695) to Wistar rats at 7, 20 or 60 mg/kg/d from implantation (GD6) through to weaning of the F_1 offspring (post-natal day 21) was associated with the following findings: dose-related reduction in maternal (F_0) body weight gain and food consumption during gestation and lactation at 20 and 60 mg/kg/d; increased pup loss during the first day *post-partum* at 60 mg/kg/d; dose-related reduction in pup birth weight at all three doses (statistically significant at mid and high doses). The NOAEL for maternal toxicity was the low dose of 7 mg/kg/d, based on reduced body weight gains and food consumption at the next high dose of 20 mg/kg/d.

In the F_1 generation pups, there was a dose-related reduction in pre- and post-weaning pup weight gain at all three doses (statistically significant only at 20 and 60 mg/kg/d) and consequently, the mean body weights were reduced up to at last 8 weeks of age.

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There were no other treatment-related effects on physical and functional development of the F_1 offspring. There were no treatment-related effects on the caesarian section data of pregnant F_1 females. The NOAEL for development of F_1 offspring was 7 mg/kg/d, based on reduced body weight gain at the mid dose of 20 mg/kg/d (even though after weaning, the mean body weight gains of the low dose F_1 offspring remained lower than controls by ~6% during four weeks, it was not considered adverse due to the small magnitude).

10 Special Toxicology Studies

None

11 Integrated Summary and Safety Evaluation

Pharmacology

Levomilnacipran is a selective serotonin-norepinephrine reuptake inhibitor (SNRI). Levomilnacipran binds with high affinity to recombinant human norepinephrine and serotonin transporters (hNET and hSERT, respectively) expressed in mammalian cell lines (K_i = 71-90 nM and 11 nM, respectively for hNET and hSERT) as well as to native rat serotonin and norepinephrine transporters from cortical membrane preparations (K_i = 7 nM and 11 nM, respectively for NET and SERT). However, levomilnacipran did not bind to recombinant human dopamine transporter (K_i below detection levels, but > 10 μM).

Four functional studies (*in vitro*) were conducted to demonstrate levomilnacipran-induced inhibition of NE and 5-HT uptake by their respective transporters. In an uptake inhibition study using recombinant human NET, SERT and DAT stably expressed in CHO cells, levomilnacipran inhibited 5-HT and NE uptake in a concentration-dependent manner (IC_{50} = 11 nM and 19 nM, respectively), but did not inhibit DA uptake at the dopamine transporter (IC_{50} >100 μM). In another expression study using recombinant human NET and human SERT (expressed in MDCK and HEK-293 cells, respectively), the extent of levomilnacipran block of NE and 5-HT uptake was identical (IC_{50} = 11 nM and 16 nM, respectively). Similarly, in rat brain synaptosomes, levomilnacipran was shown to potently inhibit NE and 5-HT uptake (IC_{50} = 62 nM and 68 nM, respectively); however, levomilnacipran was over 100-fold less potent at inhibiting DA uptake (IC_{50} >10 μM). In rat brain homogenates, levomilnacipran exhibited ~3-fold greater potency at NET than at SERT (IC_{50} = 31.5 nM and 103 nM, respectively); however, levomilnacipran had no effect on the uptake of DA in rat striatum.
Levomilnacipran has no significant binding affinity at 81 other receptors, transporters, and ion channels, including dopamine (DA), 5-HT, adrenergic, muscarinic, and histamine receptors, the DA transporter (DAT) and Ca^{2+}, Na^+, K^+ and Cl^- channels. Levomilnacipran showed a low binding affinity (K_i = 1.7 μM) at the phencyclidine (PCP) binding site; however, its functional significance is unclear.

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In rats, the pharmacological action of levomilnacipran through noradrenergic and serotonergic mechanisms was further demonstrated by some indirect functional assays. Levomilnacipran produced a concentration-dependent potentiation of NE-induced contraction of the rat seminal vesicles, an effect most likely due to inhibition of NE uptake by the nerve endings. Similarly, levomilnacipran administration to prefrontal cortex of freely moving Sprague-Dawley rats resulted in a concentration-dependent increase in the extracellular levels of NE and 5-HT in the prefrontal cortex; the minimum effective concentrations of levomilnacipran were 1 μM and 10 μM for NE and 5-HT, respectively. Likewise, single oral administration of levomilnacipran (20 mg/kg) increased NE and 5-HT levels 2-3 fold in Sprague-Dawley rat medial prefrontal cortex. In a dual-probe microdialysis study, oral administration of levomilnacipran in Sprague-Dawley rats for 11 days had no significant effect on the basal levels of NE, 5-HT or DA in the prefrontal cortex or stratum; however, following dosing on Day 12, prefrontal cortical NE levels were significantly increased (42%, $p < 0.01$); however, the levels of 5-HT and DA were unchanged. Duloxetine (10 mg/kg/d), another SNRI, had very similar effects on the levels of NE, 5-HT, and DA from both regions of the brain.

Levomilnacipran appears to have varying degrees of antidepressant-like efficacy in some animal models of depression such as forced swim test and tail suspension test in mice (with a minimal effective dose of 10-20 mg/g); however, levomilnacipran failed to produce antidepressant-like activity in the learned helplessness model of depression in male Wistar rats. Levomilnacipran also showed anxiolytic-like efficacy in a rat model of conditioned stress-induced ultrasonic vocalizations (USVs); however, in several other anxiolytic models, such as Vogel Conflict test, Elevated Plus-Maze, Four Plates test and Marble burying test, levomilnacipran failed to show anxiolytic-like effects.

Catecholaminergic activity of levomilnacipran was demonstrated in four different studies; for example, oral administration of levomilnacipran to male Swiss mice produced a dose-dependent antagonism of tetrabenazine (TBZ)-induced ptosis and potentiated yohimbine-induced deaths. Levomilnacipran (in solution) produced mydriasis in male Swiss mice. Induction of mydriasis was also demonstrated *in vivo* in male Swiss mice when levomilnacipran was administered intraperitoneally. Catecholaminergic activity of levomilnacipran was further demonstrated by its effects on reserpine-induced behaviors such as hypothermia, ptosis and akinesia; levomilnacipran attenuated reserpine-induced hypothermia and akinesia, and antagonized reserpine-induced ptosis in male NMRI mice.

Levomilnacipran has only modest anticholinergic (both central and peripheral) activity as demonstrated by its minimal (attenuating) effects on oxotremorine-induced behaviors (hypothermia, tremor, akinesia, lacrimation, salivation and defecation) in NMRI mice.

<u>Safety Pharmacology</u>: With regard to safety, levomilnacipran was assessed *in vitro* and *in vivo* for effects on central nervous system (CNS), cardiovascular system (CV) and respiratory system (RS) in multiple non-clinical species.

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The neurobehavioral effects of levomilnacipran were consistent with its mechanism of action and consisted of mydriasis, sedation, loss of traction, decreased muscle tone, decreased motor activity, decreased arousal and a dose-dependent decrease in body temperature at low to moderate doses and convulsions and death at higher doses (mice).

With regard to cardiovascular safety, levomilnacipran produced *in vitro* cardiac effects such as increase in APD_{90} (↑ 19 ms) at 1 μM and in both APD_{70} (↑22 ms) and APD_{90} (↑ 37 ms) at 10 μM, suggesting a slight reverse use-dependency in isolated Purkinje fibers (dogs). Levomilnacipran also inhibited the human ether-a-go-go related (hERG) K^+ channel maximum tail current amplitude in a concentration-dependent manner, with an (estimated) IC_{50} value of > 10 μM.

In vivo cardiac effects of levomilnacipran were observed in freely moving Beagle dogs; levomilnacipran (single or 5-day treatment at 10 mg/kg) produced a statistically significant increase in heart rate and QTc interval (↑ 21 ms or 9% on both Day 1 and Day 5). Similarly, in conscious cynomolgus monkeys, levomilnacipran (0, 5, 15, 45 mg/kg) produced statistically significant increases in mean arterial blood pressure, systolic blood pressure, and diastolic blood pressure at all three doses; in addition, there was a pronounced increase in QT and QTc intervals at the HD of 45 mg/kg (↑ 16% and 18%, respectively (the corresponding C_{max} was 7234 ng/m, which is ~21-fold higher than the C_{max} of 341 ng/mL or 1.4 μM associated with the highest clinical dose of 120 mg/d). The Sponsor had conducted a thorough QT study as part of this NDA.

With regard to respiratory safety, levomilnacipran had no significant effects on respiratory parameters, except at the high dose of 200 mg/kg (the Cmax associated with this dose was 8429 ng/mL, which is ~25-fold higher than the C_{max} of 341 ng/mL associated with the highest clinical dose of 120 mg/d).

Results from nonclinical pharmacology studies suggest that levomilnacipran is a potent and selective SNRI with antidepressant-like activity and an acceptable safety pharmacology profile.

Pharmacokinetics

Pharmacokinetic properties of levomilnacipran were evaluated in mice, rats, rabbits, and monkeys following intravenous and oral administration and were compared to humans. In humans, one major metabolite was identified; *N*-desethyl levomilnacipran (F17400), a pharmacologically inactive metabolite of levomilnacipran. The ratio of C_{max} and AUC between the metabolite and the parent were in the range of 8.5%-14.1% and 11.3%-17.1%, respectively. The *N*-desethyl levomilnacipran was also produced in rats and monkeys in substantial quantities and in mice <5% of the parent.

Exposure to levomilnacipran (both C_{max} and AUC) was dose proportional in mice and greater than dose-proportional in rats and monkeys. Levomilnacipran was absorbed rapidly in mice, rats and monkeys following oral administration with mean T_{max} ranging

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from 0.17 to 2 hr. The terminal elimination half life ($T_{1/2}$) ranged from 1.2 in mice to 3.3 hr in monkeys (in humans, a median T_{max} of levomilnacipran is 6 to 8 hr after oral dosing and declines with a $T_{1/2}$ of ~12 hr). Levomilnacipran showed rapid and extensive distribution in all anatomical and biological structures in albino rats and male pigmented rats following oral administration. Gastrointestinal tract, liver, and kidneys showed the highest concentrations of radioactivity. The highest distribution in the brain was observed in the thalamus.

Levomilnacipran exhibited low binding (*in vitro*) to human plasma proteins (22%), human serum albumin (11%), γ- globulin (4%) and α_1-acid glycoprotein (4%).

Following oral administration of levomilnacipran to mice, rats and monkeys, majority of the administered dose was excreted in urine primarily as unchanged levomilnacipran. The remaining levomilnacipran was metabolized and excreted. The *N*-desethyl levomilnacipran (F17400) was the major metabolite present in the plasma of all three preclinical species. The minor metabolites present in plasma (≤5% of the parent) are *p*-hydroxy levomilnacipran, levomilnacipran *N*-carbamoyl glucuronide metabolite, *N*-desethyl levomilnacipran *N*-carbamoyl glucuronide metabolite and *p*-hydroxy levomilnacipran glucuronide metabolite. The *N*-desethyl levomilnacipran and *p*-hydroxy levomilnacipran metabolites were the metabolites excreted by rats (11% and 12%, respectively). The *N*-desethyl levomilnacipran metabolite was the major metabolite excreted by monkeys (~18%). In addition, a small amount (≤ 4%) of levomilnacipran was excreted as the levomilnacipran *N*-carbamoyl glucuronide metabolite by rats and monkeys. Similarly, a small amount (≤ 2%) of the *N*-desethyl levomilnacipran metabolite was excreted as the *N*-desethyl levomilnacipran *N*-carbamoyl glucuronide metabolite by rats and monkeys. A small amount (~4%) of the *p*-hydroxy levomilnacipran metabolite was excreted as the *p*-hydroxy levomilnacipran glucuronide metabolite by rats.

The elimination of levomilnacipran was rapid in mice, rats and monkeys following single oral administration of levomilnacipran; the administered dose was mainly recovered in urine in all three species (80% in monkeys and 90% in mice and rats). The excretion of the radioactive materials by these three preclinical species was comparable to humans.

When administered orally to lactating rats, a small portion of the levomilnacipran dose was secreted into milk; the highest concentration of radioactivity was observed at 0.5 hr post-dose (2674 ng eq/mL) and decreased rapidly to 44 ng eq/mL at 24 hr post-dose. Small amount of *N*-desethyl levomilnacipran metabolite was also observed in the milk (331 ng eq/mL) at 0.5 hr post-dose.

Levomilnacipran had no significant inhibition potential on CYP enzymes (human liver microsomes), except a mild inhibition (20% to 30%) of CYP2C9 and 3A4/5 activities. Similarly, the major inactive metabolite *N*-desethyl levomilnacipran (F17400) did not show inhibitory activity of major CYP isozymes. Levomilnacipran showed no significant induction of CYP enzymes *in vitro* (human liver hepatocytes).

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No *in vivo* drug-drug interaction studies were performed. In the *in vitro* drug-drug interaction studies (Caco-2 cell model), levomilnacipran was found to be a weak substrate (bi-directional ratio = 2 – 4) and a poor inhibitor (IC_{50} = 423 μM) of P-gp. Levomilnacipran was neither a substrate nor an inhibitor of BCRP in the Caco-2 cell model. Similarly, levomilnacipran was neither a substrate nor an inhibitor of human transporters expressed in MDCK-II cells (OAT1, OAT3, OATP1B1 and OATP1B3); however, the activity of OCT2 was moderately (77%) reduced. The *N*-desethyl levomilnacipran (F1400), the major metabolite of levomilnacipran was neither a substrate nor inhibitor for P-gp, OAT1, OAT3, and OCT2.

As in non-clinical species, following oral administration of levomilnacipran to humans, the majority of the administered dose was excreted in urine mainly as unchanged levomilnacipran (58.4% of the dose). The *N*-desethyl levomilnacipran metabolite was the major metabolite excreted by humans (18%). Other metabolites (<4% of the dose) of levomilnacipran in humans were the levomilnacipran *N*-carbamoyl glucuronide metabolite (amine linkage), *p*-hydroxy levomilnacipran metabolite, and *N*-desethyl levomilnacipran *N*-carbamoyl glucuronide metabolite (amide linkage). Apart from these four levomilnacipran metabolites, no additional metabolites were observed in humans.

General toxicology

Levomilnacipran was adequately tested following acute and chronic oral administration in OF1 mice, Sprague-Dawley rats and cynomolgus monkeys.

Clinical signs of acute toxicity were tremors and convulsions in mice and rats, and decreased activity, clonic convulsions, ataxia, hunched posture and bilateral dilated pupils in monkeys. The approximate lethal dose was 140 mg/kg in mice, 215 mg/kg in rats and 140 to 200 mg/kg in monkeys; even though acute dose of 80 mg/kg in monkeys produced severe clinical signs that resulted in euthanasia, acute doses <80 mg/kg did not produce any clinical signs of toxicity, except vomitus. The calculated LD_{50} dose was 270 mg/kg in mice and 238 mg/kg in rats. In monkeys, an acute dose of 80 mg/kg was considered to be the MTD, based on clinical signs (such as tremors and ataxia) and minimal, increases in ALT in both males and females.

Levomilnacipran-related toxicity following chronic dosing was limited to decreased body weight/weight gains and minimal to mild centrilobular hypertrophy/vacuolation of the liver in both rats and monkeys mainly seen at the high doses. In rats the NOAEL was 30 mg/kg/d (males) and 100 mg/kg/d (females), with a corresponding exposure (AUC) of 11700 ng*hr/mL and 72000 ng*hr/mL, respectively. In monkeys, the NOAEL was 30 mg/kg/d, with a corresponding exposure of 41400 ng*hr/mL. These doses represent an-animal-to human exposure multiples of 2-fold (male rats), 14-fold (female rats) and 8-fold (monkeys), respectively relative to the human exposure (AUC 5200 ng*hr/mL) obtained at the MRHD of 120 mg/d.

Following sub-chronic dosing in rats (4 weeks and 13 weeks; 10, 35, 120 mg/kg/d) and in cynomolgus monkeys (13 weeks; 5, 15, and 45mg/kg/d), the only significant

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treatment-related findings were decreased body weight gain in both species (↓~9% to 10% in rats and 13% in monkeys) at the high doses and centrilobular hypertrophy (mild) of the liver only in rats at the high dose of 120 mg/kg/d (without significant changes in ALT or ALP levels).

In the pivotal six-month study in rats (10, 30, and 120/100 mg/kg/d), apart from an unexplained death of a high dose male, the noteworthy dose-limiting toxicity was decreased body weights/body weight gains at the high dose of 120 mg/kg/d in male rats (↓13%), without full recovery after dosing cessation. In addition, the high dose also was associated with minimal to mild centrilobular hepatocyte hypertrophy and minimal centrilobular vacuolation of hepatocytes, which were reversible after dosing cessation. Regarding the 'unexplained death' of a high dose male rat, this Reviewer considers it is likely to be due to treatment-related causes because the high dose of 120 mg/kg/d represents approximately one-half the calculated acute median lethal dose (LD_{50}) of 238 mg/kg.

In the pivotal 1 year study in cynomolgus monkeys (10, 30, and 90 mg/kg/d), the most notable treatment-related findings were decreased body weight gain at the high dose in both males and females (~30 to 45%). In addition, there was an increase in liver weights (absolute) with histopathological correlates (minimal panlobular hepatocyte hypertrophy and minimal to mild midzonal vacuolation of hepatocytes) at the high dose.

Genotoxicity

Levomilnacipran was adequately tested in *vitro* and *in vivo* and did not demonstrate potential for genotoxicity. It was not mutagenic in *Salmonella typhimurium* or *Escherichia coli* in the absence or presence of metabolic activation (Ames test) or in the *in vitro* (L5178Y $TK^{+/-}$) forward mutation assay in mouse lymphoma cells, in the presence or absence of metabolic activation system. Likewise, levomilnacipran was not clastogenic in the *in vivo* micronucleus assay in rats at oral doses (two consecutive doses) up to 200 mg/kg/d.

Carcinogenicity

The carcinogenic potential of levomilnacipran was assessed in a standard two-year study in Sprague-Dawley rats and in a short-term (6-month) study in Tg.rasH_2 mice; the study protocols were approved by E-CAC prior to study initiation. Levomilnacipran was not carcinogenic in either study.

In the rat study, following daily oral administration of levomilnacipran (10, 30, 90 mg/kd/d; these doses were 0.8, 2.4, and 7 times the MRHD, respectively on a mg/m^2 basis) for 2 years, the overall survival was statistically significantly decreased for females at 30 and 90 mg/kg/d, compared to two identical vehicle control groups. There were no treatment-related, biologically relevant increases in the incidence of neoplasms in either sex at doses up to the high dose of 90 mg/kg/d. The maximum tolerated dose (MTD) was demonstrated based on slightly increased mortality in females at 30 and 90

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mg/kg/d and decreased body weights at the high dose, mainly in male rats (≥10%). Based on the systemic exposure at the highest dose of 90 mg/kg/d, the human exposure margin (at the MRHD of 120 mg/d) is ~13-fold.

In Tg.rasH_2 mice, daily oral administration of levomilnacipran (15, 50, and 150 mg/kg/d; these doses were 0.6, 2, and 6 times the MRHD, respectively on a mg/m^2 basis) for six months was not considered carcinogenic. There was a statistically significant increase in the incidence of splenic hemangiosarcomas in high dose males, when compared to vehicle control group. However, the numerical increase was only slightly higher than the historical control value and there were such findings in females. Therefore, it was not considered as a significant finding. Based on the systemic exposure at the highest dose of 150 mg/kg/d, the human exposure margin (at the MRHD of 120 mg/d) is ~11-fold.

Reproductive toxicity

The potential adverse effects of levomilnacipran on fertility, fetal-embryonic development and peri-/post-natal development were evaluated in rats and/or rabbits.

In the fertility and early embryonic study (Segment I) in male and female rats treated orally with levomilnacipran (10, 30, and 100 mg/kg/d; males treated 4 weeks prior to pairing and females treated 14 days before mating), there were no treatment-related effects on reproductive performance or fertility, including sperm count and motility, and mating behavior at doses up to 100 mg/kg/d. There was a reduction in mean body weight gain in males and females at the high dose of 100 mg/kg/d (-27% in males and -32% in females). The NOAEL was 100 mg/kg/d for gonadal function, mating behavior, reproductive performance and early gestation in male and female rats; this dose that is 8 times a human dose of 120 mg/day (on a mg/m^2 basis).

In the embryo-fetal development studies (Segment II) in pregnant rats and rabbits, treatment with levomilnacipran (10, 30, and 100 mg/kg/d in both species) during the period of organogenesis (GD-6 through GD-17 in rats and GD-6 through GD-18 in rabbits) was not teratogenic up to the high dose of 100 mg/kg/d in both species. There was maternal toxicity at the high dose in rats based on reduced body weight gains (-29%) and food consumption (-12%, compared to control) during the treatment period. Similarly, there was maternal toxicity in rabbits, based on initial weight loss and reduced weight gains (-144%, compared to control), and reduced food consumption (-25% to -37%, compared to control) during the treatment period. At these maternally toxic doses there was also some embryo-fetal toxicity. In the high dose rats, the mean fetal weight was statistically significantly lower (-11%, compared to control) and there were some variations of the sternebrae such as reduced ossification, bipartite ossification and asymmetry, most likely an indication of delayed ossification. Likewise, in the high dose rabbit fetuses, there was a slight delay in ossification, but only of digit bones and fetal weights were unaffected; these findings were also probably related to the reduced maternal body weight gain/weight loss. There were no fetal malformations in rats or rabbits at any dose up to the high dose of 100 mg/kg/d, a dose that is 8 and

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16 times, respectively the MRHD of 120 mg/day (on a mg/m^2 basis). On the basis of exposure, this dose represents animal-to-human exposure multiples of 9-fold (rat AUC = 45738 ng*hr/mL) and 4-fold (rabbit AUC = 21715 ng*hr/mL) compared to the human exposure from an oral dose of 120 mg/d of levomilnacipran (AUC = 5200 ng*/hr/mL). The NOEL doses for embryo-fetal development in rats and rabbits are the mid dose of 30 mg/kg/d, based on lower fetal weight and delayed ossification of sternebrae in rats and delayed ossification of digit bones in rabbits at the high dose of 100 mg/kg/d, a dose 2.4 and 5 times, respectively a human dose of 120 mg/day (on a mg/m^2 basis).

In the pre- and post- natal study (Segment III), when levomilnacipran (7, 20, and 60 mg/kg/d) was orally administered to pregnant rats during the period of organogenesis and through parturition/lactation to weaning, the maternal toxicity was limited to decreased body weight gains (as a result of decreased food consumption) at the mid and high doses (-8 and -16%, respectively). There was increased pup mortality on the first day *post partum* at the high dose of 60 mg/kg, a dose that is 5 times a human dose of 120 mg/day (on a mg/m^2 basis). Additionally, pup birth weight was decreased at the mid and high doses (that continued during the initial 4-5 weeks of post-weaning period), perhaps a direct consequence of significantly decreased maternal body weight gain at the high dose and to a lesser extent at the mid dose. There was a slight delay in pinna unfolding and incisor eruption, in the mid and high dose F_1 pups. However, there were no treatment-related effects on behavioral parameters, fertility and reproductive performance of F_1 generation adults. There were no treatment-related effects on the caesarian section data of pregnant F1 females. The no-adverse effect dose for maternal and offspring toxicity was 7 mg/kg/d; this dose is 0.6 times the MRHD of 120 mg/day (on a mg/m^2 basis).

Safety Evaluation

Levomilnacipran is an SNRI with high binding affinity for human (recombinant) as well as rat NE and 5-HT transporters *in vitro*. In addition, in functional studies, levomilnacipran inhibited the uptake of both NE and 5-HT with more or less equal potency. Levomilnacipran had no appreciable binding affinity or uptake inhibition potential at the dopamine transporter. In an *in vivo* study in freely moving rats, levomilnacipran was shown to increase both NE and 5-HT levels in the prefrontal cortex. Levomilnacipran was shown to have efficacy in some mouse and rat models of depression and anxiety.

In nonclinical safety pharmacology studies, levomilnacipran was found to have some minor cardiovascular effects such as increase in blood pressure and QTc interval in monkeys at a dose that is ~7 times the MRHD of 120 mg/d, on a mg/m^2 basis or ~21 times the C_{max} (341 ng/mL or 1.4 μM) associated with the clinical dose of 120 mg/d (the Sponsor had subsequently conducted a thorough QT study in healthy human subjects). Levomilnacipran also had significant respiratory effects such as decreased tidal volume and minute volume, and increase in airway resistance index at a dose that is ~16-fold higher than the MRHD dose (on a mg/m^2 basis) or ~25 times the C_{max} of 341 ng/mL associated with the highest clinical dose of 120 mg/d).

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With regard to the pharmacokinetic parameters, levomilnacipran is rapidly absorbed in all nonclinical species (T_{max} 1 to 3 hr) and extensively distributed in all anatomical and biological structures. Levomilnacipran has low level of binding to plasma proteins in humans (*in vitro*; 22%) and animals. Exposure to levomilnacipran (both AUC and C_{max}) was dose-proportional in mice and greater than dose-proportional in rats and monkeys. Metabolism of levomilnacipran was similar across all species including humans. Following oral administration of levomilnacipran to mice, rats, monkeys and humans, the majority of the dose is excreted in urine primarily as unchanged form; and the remaining dose is metabolized and excreted. *N*-desethyl levomilnacipran (F17400) was the major metabolite circulating in human plasma (≥10% of the parent) and was present at significant levels in all three preclinical species. Three minor metabolites (≤5% of the parent) are *p*-hydroxy levomilnacipran, levomilnacipran *N*-carbamoyl glucuronide metabolite and *p*-hydroxy levomilnacipran glucuronide.

Following oral administration of levomilnacipran to mice, rats and monkeys, all four levomilnacipran metabolites present in human plasma were observed in the rat and monkey plasma; however, *N*-desethyl levomilnacipran (F17400) was the only metabolite observed in the mouse plasma. The plasma concentration of *N*-desethyl levomilnacipran (F17400) was ~7-fold higher in mice, ~26-fold higher in rat, and ~56-fold higher in monkeys than found in human plasma at 60 mg oral dose. In humans, following single-dose or multiple dose administration of levomilnacipran, the ratios of C_{max} and AUC between the major metabolite (*N*-desethyl levomilnacipran) and the parent compound were in the range of 8.5%-14% and 11%-17%, respectively. Therefore, it is concluded that the safety of the major human metabolite has been adequately covered in nonclinical safety studies.

Levomilnacipran was adequately tested following acute and chronic oral administration in mice, Sprague-Dawley rats and cynomolgus monkeys. CNS signs, including convulsions, were seen in all species, but only at doses/exposures 6-23-fold higher (on a mg/m^2 basis) than the MRHD. Following chronic administration of levomilnacipran to rats and monkeys, drug-related findings were minor and limited to decreased body weight/weight gains and minimal to mild centrilobular hypertrophy/vacuolation of the liver in both rats and monkeys mainly seen at the high doses and was shown to be reversible in both species. In rats the NOAEL was 30 mg/kg/d (males) and 100 mg/kg/d (females), and in monkeys, the NOAEL was 30 mg/kg/d. These doses represent an-animal-to human exposure multiples of 2-fold (male rats), 14-fold (female rats) and 8-fold (monkeys), respectively relative to the human exposure (AUC 5200 ng*hr/mL) obtained at the MRHD of 120 mg/d.

Levomilnacipran was adequately tested *in vitro* and *in vivo* for potential genotoxicity and it was not genotoxic. In the carcinogenicity studies in rats and transgenic mice, levomilnacipran was not considered to be carcinogenic.

Levomilnacipran had no adverse effects on fertility in rats (NOAEL was 100 mg/kg/d in male and female rats) and it was not teratogenic in rats or rabbits; however, there was some developmental toxicity, namely delayed ossification, in both species and lower

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body weights in rat fetuses at higher doses (rat NOEL was 30 mg/kg/d; rabbit NOAEL 100 mg/kg/d). In the peri- and post-natal development study in rats, levomilnacipran administration resulted in increased pup mortality immediately following birth and a decrease in pup body weight at birth that continued through the initial 4-5 weeks of post-weaning period. However, there were no treatment-related effects on behavioral parameters, fertility and reproductive performance of F_1 generation adults; and there were no treatment-related effects on the caesarian section data of pregnant F_1 females. The no-adverse effect dose for maternal and offspring toxicity was 7 mg/kg/d; this dose is 0.6 times the MRHD of 120 mg/day (on a mg/m^2 basis).

Overall nonclinical pharmacology and toxicology evaluations in rodents, rabbits and monkeys indicate that levomilnacipran has a safety profile similar to other drugs in this class and with adequate margins of exposure relative to the intended use in patients (see Table 91, below).

Table 91 Safety margins calculated by the Reviewer based on exposure data

Toxicity	Species	NOAEL (mg/kg) M/F	Safety Margin Based on AUC*
General	Rat (6 months)	30/100	2 (M); 14 (F)
	Monkey (1 year)	30/30	8
Carcinogenicity	Rat	90/90	12 (M); 14 (14)
	Tg.rasH2 mice	150/150	9 (M); 12 (F)
Reproductive and Development			
Fertility	Rat	100/100	8x**
Embryo/fetal development	Rat	30	2
	Rabbit	100	4
Pre- and post- natal development	Rat	7	0.1

*AUC in human: 5200 ng.hr/ml at 120 mg/day.
**On a mg/m^2 basis, in the absence of TK data

12 Appendix/Attachments

None

Reference ID: 3326668

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/s/

ARIPPA RAVINDRAN
06/17/2013

LINDA H FOSSOM
06/17/2013
I agree with the conclusions and recommendations in Dr. Ravindran's very thorough review.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 204168 **Applicant: Forest Laboratories** **Stamp Date: 25-Sept-2012**

Drug Name: Levomilnacipran HCl **NDA Type: 505(b)(1)**

On **initial** overview of the NDA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	√		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	√		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	√		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	√		I have contacted the Stats reviewer about the carcinogenicity data sets. I have contacted the Biopharm reviewer about major human metabolites (>10% of total circulating drug species). The submitted clinical studies are for MDD in adults; juvenile animal studies are not required at this stage
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	√		The route of administration of the formulation to be marketed is not different from the formulation used in the toxicology studies
6	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 applicant submitted a rationale to justify the alternative route?	√		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	√		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	√		No special studies were requested at the Pre-NDA meeting.
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?		√	The proposed labeling does not include human safety margins, but doses and NOAELs are included, so margins can be calculated.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	√		Several potential process impurities (b) (4) have been dealt with under the IND. I am not aware of any other impurities, degradants or novel excipients that would need qualification; however, I will co-ordinate with CMC during the review cycle.
11	Has the applicant addressed any abuse potential issues in the submission?		√	Levomilnacipran is an SNRI, a class not considered to pose a concern for abuse.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?	--	--	Not applicable

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ___Yes_____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Arippa Ravindran, Ph.D. — October 31, 2012
Reviewing Pharmacologist — Date

Linda Fossom, Ph.D. — November 6, 2012
Team Leader/Supervisor — Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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/s/

ARIPPA RAVINDRAN
11/06/2012

LINDA H FOSSOM
11/06/2012