CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

204447Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 204447
Submission date: October 2, 2012
Drug: vortioxetine
Applicant: Takeda Pharmaceuticals USA, Inc and Takeda Global Research Development Center, Inc.
Indication: Major Depressive Disorder (MDD)

Reviewing Division: Division of Psychiatry Products

Discussion:
The pharmacology/toxicology reviewer initially recommended that vortioxetine could be approved from the pharmacology/toxicology perspective for the indication of MDD if a genotoxic impurity, (b) (4) was limited so that the maximum daily intake did not exceed (b) (4). The applicant subsequently submitted an amendment to the relevant DMF updating the specification for the starting material. This amendment was reviewed by the quality reviewer. A limit of (b) (4) will apply for the potential impurity (b) (4). This ensures that the content in the API of (b) (4) will be below the (b) (4) limit (calculated at the highest daily dose (20 mg) of vortioxetine).

Vortioxetine was tested in 2 year carcinogenicity studies in rats and mice using oral gavage administration. Both studies were found to be acceptable by the executive carcinogenicity assessment committee. No drug-related neoplasms were noted in mice. The Executive Carcinogenicity Assessment Committee concluded that polypoid adenomas of the rectum in high dose (40 mg/kg/bid) female rats were drug-related.

Vortioxetine caused some developmental toxicity in rats and rabbits. This included decreased fetal and pup body weight, delayed ossification and decreased pup survival in rats. Decreased fetal body weight and delayed ossification were also seen in rabbits. A few CNS malformations were observed in vortioxetine treated rabbits although the relationship to drug-treatment is not clear.

Conclusions:
The pharmacology/toxicology reviewer and supervisor conducted a thorough evaluation of the nonclinical information submitted in support of this NDA. I agree that this NDA may be approved for the above indication. A pregnancy category of C appears appropriate. I have provided comments on labeling to the Division separately.

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/s/

PAUL C BROWN
09/24/2013

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	204447
Supporting document/s:	SDN-1/SN0000
Applicant's letter date:	October 1, 2012
CDER stamp date:	October 2, 2012
Product:	Brintellix (vortioxetine)
Indication:	Major Depressive Disorder
Applicant:	Takeda Pharmaceuticals USA, Inc and Takeda Global Research Development Center, Inc.
Review Division:	Division of Psychiatry Products
Reviewer:	Antonia Dow, PhD
Supervisor/Team Leader:	Linda Fossom, PhD
Division Director:	Mitch Mathis, MD (Acting Director)
Project Manager:	Hiren Patel, PharmD

Disclaimer

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1 Executive Summary

1.1 Introduction

This application is a 505(b)(1) NDA for Brintellix (vortioxetine). The proposed indication is major depressive disorder (MDD) and the application is based on clinical trials in adults. Vortioxetine is a new molecular entity that acts as a potent and selective inhibitor of the serotonin transporter, with additional activities at some serotonin receptors (antagonist at 5-HT3, 5-HT7, and 5-HT1D; partial agonist at 5-HT1B; agonist at 5-HT1A). Vortioxetine has not been approved outside the U.S.

1.2 Brief Discussion of Nonclinical Findings

Vortioxetine was shown to be a potent and selective inhibitor of the serotonin transporter, without significant activity at the norepinephrine and dopamine transporters. In addition, vortioxetine is a potent antagonist at 5-HT3 receptor, a moderate antagonist at 5-HT1D receptor, a moderate agonist at 5-HT1A receptor, a moderate to weak partial agonist at 5-HT1B, and a weak antagonist at 5-HT7 receptor. Vortioxetine had mixed results demonstrating antidepressant-like activity in behavioral animal models.

The four major-human metabolites M3 (Lu AA39835 glucuronide), Lu AA34443, M4(b) (Lu AA34443 glucuronide), and M12 are considered qualified and no additional studies are needed.

General toxicology studies in two species (rat and dog) were conducted to support chronic use of vortioxetine. General toxicities seen in rat or dog that might have clinical relevance are convulsions, kidney and liver pathology, and pupillary dilation. Convulsions were noted after acute dosing in rats and dogs at 195 and 16 times, respectively, the maximum recommended human dose (MRHD) of 20 mg on a mg/m2 basis; however, no convulsions were seen in chronic rat and dog studies at doses 39 and12 times, respectively, the MRHD. In rat, kidney and liver pathology related to the presence of vortioxetine metabolite crystals was noted at doses 38 times the MRHD, but not at 7 times the MRHD. In dog, pupillary dilation occurred at doses 8 times the MRHD, but not at 6 times the MRHD.

Vortioxetine was not genotoxic. Vortioxetine treatment resulted in a slight, but statistically significant, increase in the incidence of polypoid adenomas of the rectum of female rats at 39 times the MRHD, but not at 15 times the MRHD. No drug-related tumors were seen in male rats or in male or female mice at up to 20, 12, and 24 times, respectively, the MRHD.

Vortioxetine was not teratogenic in rats or rabbits at doses up to 78 and 58 times, respectively, the MRHD. However, some developmental toxicities were seen in rat and rabbit at 15 and 10 times, respectively, the MRHD, but not at 5 and 2 times, respectively, the MRHD. No effect on fertility was seen in rats. A pre- and postnatal development study in rat showed a decrease in the number of live-born pups, an

increase in early postnatal pup mortality, and delayed development (based on eye opening only) at doses up to 20 times the MRHD, but not at 5 times the MRHD.

The only Pharmacology/Toxicology issue that could prevent approval of this NDA is a genotoxic impurity, (b) (4). This impurity was identified (b) (4) for drug substance and needs to be limited so that the maximum daily clinical dose does not exceed (b) (4).

1.3 Recommendations

1.3.1 Approvability

Vortioxetine can be approved from the Pharmacology/Toxicology perspective for the indication of MDD if the genotoxic impurity, (b) (4) is limited during this review cycle so that the maximum daily clinical dose does not exceed (b) (4).

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Below are my recommendations for sections 8.1, 12.1, 12.2, and 13 of labeling. Prior to sending the draft labeling to the Sponsor, I will be working with the Maternal Health Team to write section 8.1. In addition, labeling will be negotiated with Sponsor after submission of this review and this may not be the final wording for labeling.

Section 8: Use in specific populations

8.1 Pregnancy

Teratogenic Effects

Pregnancy Category C

Vortioxetine caused some developmental toxicity in rats and rabbits, but there were no findings of teratogenicity that were clearly vortioxetine related. There are no adequate and well controlled studies of BRINTELLIX in pregnant women. When treating pregnant women with BRINTELLIX, carefully consider whether the potential benefits outweigh the potential risks of treatment.

No teratogenic effects that were clearly vortioxetine related were seen when vortioxetine was given to pregnant rats and rabbits during the period of organogenesis at oral doses up to 160 and 60 mg/kg/day, respectively. These doses are 77 and 58 times, in rats and rabbits, respectively, the maximum recommended human dose (MRHD) of 20 mg on a mg/m2 basis. Decreased fetal body weight and delayed ossification occurred in rat and rabbit at 15 and 10 times, respectively, the MRHD; these effects were not observed at doses up to 5 times the MRHD in rats and 2 times the MRHD in rabbits.

When vortioxetine was administered to pregnant rats at oral doses up to 20 times the MRHD during organogenesis and throughout pregnancy and lactation, the number of live-born pups was decreased and early postnatal pup mortality was increased at the high dose. Additionally, pup weights were decreased at birth to weaning and

development (specifically eye opening) was slightly delayed. These effects were not seen at 5 times the MRHD.

Section 12: Clinical Pharmacology

12.1 Mechanism of Action

The mechanism of the antidepressant effect of vortioxetine is not fully understood, but is thought to be related to its enhancement of serotonergic activity in the CNS through selective inhibition of serotonin reuptake. Vortioxetine is also an antagonist at serotonergic 5-HT3 receptors; however, the net result of this action on serotonergic transmission and its role in vortioxetine's antidepressant effect are unknown.

12.2 Pharmacodynamics

Vortioxetine binds with high affinity to the serotonin transporter (Ki=1.6 nM), but not to the norepinephrine (Ki=113 nM) and dopamine (Ki>1000 nM) transporters. Vortioxetine potently and selectively inhibits reuptake of serotonin (IC50=5.4 nM). Vortioxetine also binds to the 5-HT3 (Ki=3.7 nM), 5-HT1D (Ki=54 nM), 5-HT1B (Ki=33 nM), and 5-HT1A (Ki=15 nM) receptors and is a 5-HT3 and 5-HT1D receptor antagonist, 5-HT1B receptor partial agonist, and 5-HT1A receptor agonist.

Section 13: Nonclinical Toxicology

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

Carcinogenicity studies were conducted in which CD-1 mice and Wistar rats were given oral doses of vortioxetine up to 50 and 100 mg/kg/day for male and female mice, respectively, and 40 and 80 mg/kg/day for male and female rats, respectively, for 2 years. The doses are approximately 12, 24, 20, and 39 times, respectively, the maximum recommended human dose (MRHD) of 20 mg on a mg/m^2 basis.

In rats, the incidence of polypoid adenomas of the rectum was statistically significantly increased in females at doses 39 times the MRHD, but not at 15 times the MRHD. This finding was not observed in male rats at 20 times the MRHD.

In mice, vortioxetine was not carcinogenic in males or females at doses up to 12 and 24 times, respectively, the MRHD.

Mutagenicity

Vortioxetine was not genotoxic in the *in vitro* bacterial reverse mutation assay (Ames test), an *in vitro* chromosome aberration assay in cultured human lymphocytes, and an *in vivo* rat bone marrow micronucleus assay.

Impairment of Fertility
Treatment of rats with vortioxetine at doses up to 120 mg/kg/day had no effect on male or female fertility, which is 58 times the maximum recommended human dose (MRHD) of 20 mg on a mg/m2 basis.

2 Drug Information

2.1 Drug

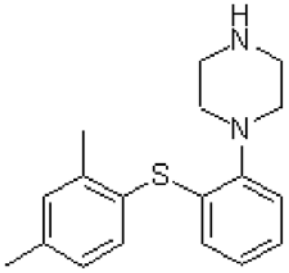
Generic Name: vortioxetine

Code Name: Lu AA21004

Chemical Name: 1-[2-(2,4-Dimethylphenylsulfanyl)phenyl]piperazine

Molecular Formula/Molecular Weight: $C_{18}H_{22}N_2S$/298.4

Structure:



Pharmacologic Class: serotonin reuptake inhibitor with additional serotonin receptor activity (antagonist at 5-HT3, 5-HT7, and 5-HT1D; partial agonist at 5-HT1B; agonist at 5-HT1A)

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 76307: For the treatment of depression; Sponsored by Takeda
IND 112581: For the treatment of cognitive dysfunction in adult patients with major depressive disorder; Sponsored by Lundbeck
DMF (b) (4): For manufacturing of vortioxetine; held by Lundbeck

2.3 Drug Formulation

Immediate release, film-coated tablets in 5, 10, 15, and 20 mg strength

2.4 Comments on Novel Excipients

The drug product does not contain any novel excipients. Excipients for tablet core are mannitol, microcrystalline cellulose, hydroxpropyl cellulose, sodium starch glycolate, and magnesium stearate and for film coat are hypromellose (b) (4), titanium dioxide, and polyethylene glycol 400.

2.5 Comments on Impurities/Degradants of Concern

CDER ONDQA reviewer, Dr. Wendy Wilson-Lee, Ph.D., identified structural alerts for two potentially genotoxic impurities in the drug substance (b) (4) and for a degradant in the drug product. The CDER Computational Toxicology Group conducted quantitative structure-activity relationship (QSAR) modeling for these three

impurities/degradants using Derek Nexus 3.01 (DX), Leadscope Model Applier 1.5.0-4 (LMA), and MC4PC. The following is a summary of the findings:

(b) (4)

(b) (4) is used (b) (4) in drug substance synthesis. The Sponsor's proposed limit is NMT (b) (4) which would be (b) (4) for MRHD of 20 mg/day. The limit of (b) (4) is below the (b) (4) limit for oral exposure (b) (4) in the EMA Guidance (b) (4) (January 2007). Therefore, the limit of (b) (4) is acceptable from the pharmacology/toxicology perspective.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed indication is for the treatment of major depressive disorder (MDD). The proposed starting dose in adults is 10 mg once daily with a proposed dose range between 5 mg and 20 mg.

[1] Thompson, C.Z, Hill, L.E., Epp, J.K., Probst, G.S., 1983. The induction of bacterial mutation and hepatocyte unscheduled DNA synthesis by monosubstituted anilines. Environ. Mutagen. 5, 803-811

[2] Zimmer, D., Mazurek, J., Petzold, G., Bhuyan, B.K., 1980. Bacterial mutagenicity and mammalian cell DNA damage by several substituted anilines. Mutat. Res. 77, 317-326

2.7 Regulatory Background

H. Lundbeck A/S was the initial developer or vortioxetine. In 2008, Lundbeck transferred sponsorship of this IND to Takeda Global Research & Development Center, Inc, but continued to co-develop vortioxetine. Takeda Global Research & Development Center, Inc submitted the current NDA on behalf of Takeda Pharmaceuticals USA, Inc.

3 Studies Submitted

3.1 Studies Reviewed

All submitted pivotal studies, in various species were reviewed in detail, except the juvenile animal study.

3.2 Studies Not Reviewed

Preliminary dose-range finding studies and non-pivotal studies for deciding nonclinical safety of vortioxetine were not reviewed in detail. In addition, because the approval for MDD will be based on clinical trials in adults, a juvenile animal study was not reviewed.

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

In Vitro Binding and Activity

Vortioxetine: Vortioxetine inhibits the reuptake of serotonin at the human serotonin reuptake transporter (5-HTT) *in vitro*, but has lower affinity for the human norepinephrine and dopamine transporters (NET (70-fold) and DAT (>625-fold), respectively) (Table 1). Vortioxetine also inhibits serotonin reuptake in rat synaptosomes (cIC50 = 5.3 nM).

Table 1: *In vitro* binding (Ki) and uptake (cIC50) data for human 5-HTT, NET, and DAT

Reuptake Site	Ki (nM)	cIC50 (nM)*
h5-HTT	1.6	5.4
hNET	113	107
hDAT	>1000	>1000

*cIC50 = corrected IC50 (Cheng-Prusoff correction factor was applied)

[Data from NDA 204447 submission; Report No. 929-900 035]

In addition to binding to SERT, vortioxetine has in vitro binding activity at five serotonin receptors (human and rat): 5-HT3, 5-HT7, 5-HTD1, 5-HT1B, and 5-HT1A (Table 2). Although, vortioxetine's affinity for the human serotonin receptors (except 5-HT3) is lower than it's affinity for human SERT (11-, 34-, 21-, and 9-fold for 5-HT7, 5-HTD1, 5-HT1B, and 5-HT1A, respectively). Compared to human, vortioxetine has a similar affinity for rat 5-HT1B receptors, a higher affinity for rat 5-HT3 and 5-HT1D receptors (3- and 15-fold, respectively), and a lower affinity for rat 5-HT7 and 5-HT1A receptors (11- and 15-fold, respectively).

Functional activity at the 5-HT3, 5-HT7, 5-HTD1, 5-HT1B, and 5-HT1A receptors were examined in recombinant cell lines expressing human or rat receptors (Table 2). At human and rat ***5-HT3 receptor***, vortioxetine acted as an antagonist on 5-HT3 receptors expressed in oocytes (human: IC50 = 12 nM; rat: IC50 = 0.18 nM) and HEK cells (human: IC50 = 10 nM). At human and rat ***5-HT7 receptor***, vortioxetine had weak antagonist activity in a FLIPR assay (human: IC50 = 1271 nM; cIC50 (Kb) = 450 nM) and cAMP assay (rat: IC50 = 11,000 nM; cIC50 (Kb) = 2080 nM), respectively. At human and rat ***5-HT1D receptor***, vortioxetine had moderate antagonist activity in a cellular dielectric spectroscopy based assay (human: IC50 = 369 nM; cIC50 (Kb) = 25 nM; rat: IC50 = 260 nM; cIC50 (Kb) = 43 nM). At human ***5-HT1B receptor***, vortioxetine had weak to moderate partial agonist activity at the high-affinity site in a membrane-based GTPγS assay (EC50 = 120 nM; intrinsic activity 55%) and a whole cell-based cAMP assay (EC50 = 460 nM; intrinsic activity 22%). At human ***5-HT1A receptor***, vortioxetine had moderate agonist activity in a GTPγS assay (EC50 = 199 nM; intrinsic activity 96%).

Table 2: *In vitro* binding and functional activity data for vortioxetine at human and rat 5-HTT and serotonin receptors 5-HT3, 5-HT7, 5-HT1D, 5-HT1B, and 5-HT1A

	Human			Rat		
	Binding affinity	Functionality		Binding affinity	Functionality	
Target	Ki (nmol/L)(a)	IC50/EC50 (nmol/L)	Kb (b) (nmol/L)	Ki (nmol/L)	IC50 (nmol/L)	Kb (b) (nmol/L)
5-HT3 receptor antagonism	3.7 (c)	12 (d) 10 (e)	3.5 (d)	1.1 (c)	0.18 (d)	ND
5-HT7 receptor antagonism	18 (c)	1271 (e)	450 (e)	200 (e)	11,000 (e)	2080 (e)
5-HT1D receptor antagonism	54.2 (c)	369(c)	25	3.7 (c)	260 (c)	43 (c)
5-HT1B partial agonism	33 (f)	120, IA=55% (g) 460, IA=22% (h)	NR	16 (i)	ND	NR
5-HT1A agonism	15 (c)	199, IA=96% (c)	NR	232 (i)	ND	NR
5-HTT inhibition	1.6 (j)	5.9 (c)	5.4 (c) (k)	ND	5.3 (l)	ND

EC50=50% effective concentration, IA=intrinsic activity (relative efficacy as compared to the full agonist), ND=not determined, NR=not relevant.
(a)Y nmol/L Lu AA21004 = 0.29845 x Y ng/mL Lu AA21004.
(b) Kb=cIC50=IC50/(1 + ([A]/(EC50 of agonist)) where EC50 of agonist in this particular experiment and [A] is the concentration of agonist.
(c) Chinese hamster ovary (CHO) cells; (d) Xenopus oocytes (XO) cells; (e) HEK 293 cell; (f) HELA cells;
(g) GTPγS assay in HELA cells; (h) cAMP assay in HELA cells; (i) Cerebral cortex; (j) Peak Rapid 293 cells;
(k) cIC50 = IC50/(1+S/Km), where S is the radio-substrate concentration and Kn is the Michaelis-Menten rate constant for radio-substrate to the transporter
(l) synaptosomes.

[Table from NDA 204447 submission; Nonclinical Overview, page 10]

At human ***5-HT2B receptor***, vortioxetine did not have agonist activity up to 1 µM and had weak antagonist activity (IC50 >1 µM) in a functional agonist and antagonism assay using 5-HT as the agonist and measuring intracellular Ca^{2+} by fluorimetry in CHO cells. Vortioxetine had *in vitro* binding affinity for human ß1 adrenoceptor (Ki = 46.2 nM), but only had weak antagonistic activity in HEK 293 cells (IC50 = 840 nM). Vortioxetine was not found to have binding affinities of <100 nM for other receptors examined, including human ß2 adrenoceptor (Ki = 385 nM), dopamine receptor D1 (Ki = 1100 nM; IC50 = 2800), delta opioid receptor (Ki = 520 nm; IC50 880), mu opioid receptor (Ki = 210 nM; IC50 = 410 nm), and GABA receptor (< 10% inhibition at 1 µM in a high throughput screen).

Metabolites of Vortioxetine: In a CEREP screening panel, a major human metabolite, ***Lu AA34443***, at 1 µM inhibited [^{3}H]BRL 43694, [^{3}H](-)CGP 12177, [^{3}H]8-OH-DPAT, [^{3}H]LSD binding to human 5-HT3, ß1 adrenergic, 5-HT1A, and 5-HT7 receptors, respectively, by 99, 77, 69, and 68%, respectively. The Sponsor did not perform follow-up studies to determine Ki or activity at these receptors and IC50s were not determined.

The reactive intermediate to Lu AA34443, ***Lu AA34994***, inhibits the reuptake of serotonin at human 5-HTT (cIC50 = 7.9 nM) with similar potency as vortioxetine. Lu AA34994 also had weak inhibitory activity at human delta 2 (Ki = 150 nM; cIC50 = 250 nM), kappa (Ki = 250 nM; cIC50 = 250), and mu (Ki = 160 nM; cIC50 = 380 nM) opioid receptors. A non-major human metabolite, ***Lu AA39835***, also inhibits the reuptake of serotonin at human 5-HTT (cIC50 = 15.5 nM).

Ex Vivo and In Vivo Occupancy Studies in Rat Brain

Ex vivo and *in vivo* occupancy studies in rat brain were conducted for 5-HTT, 5-HT3 receptor, 5-HT1B receptor, and 5-HT1A receptor.

5-HTT: 5-HTT occupancy was determined ex vivo by the prevention of binding of [^{3}H]DASB in brain slices taken 0.5 or 1 hour after SC injection or oral administration, respectively, by vortioxetine (0.08, 0.19, 0.39, 0.79, 3.90 or 7.90 mg/kg SC; 2.5, 5, 10 and 20 mg/kg oral) to male Sprague-Dawley rats (Report No. 929 900 2007 034). Vortioxetine produced a dose-dependent increase in 5-HTT occupancy (SC: 20 – 95%; ED50 = 0.6 mg/kg; Oral: 5 – 75%; ED50 = 12 mg/kg). The Sponsor calculated the EC50 for plasma and brain exposure of vortioxetine to be 63 ng/mL and 553 ng/g, respectively, for SC administration and 40 ng/mL and 1007 ng/g, respectively, for oral administration.

5-HTT occupancy was determined *in vivo* in male Sprague-Dawley rats by competition of [^{3}H]MADAM (a selective SERT ligand) with vortioxetine (5 mg/kg/day for 3 days, SC by osmotic minipump) in ventral hippocampus (Report No. 007-308-2007). 5-HTT occupancy was 41% and the plasma concentration of vortioxetine was 87 ng/mL.

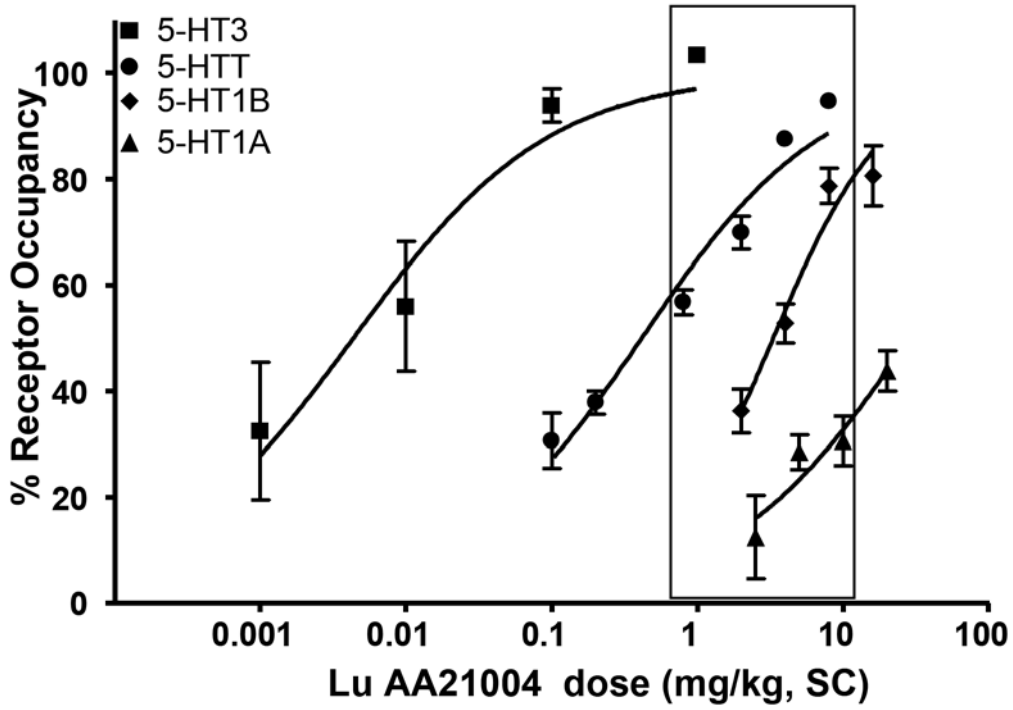
5-HT3: 5-HT3 receptor occupancy was determined ex vivo by the prevention of binding of [^{3}H]LY278584 (a 5-HT3 receptor antagonist) in brain slices taken 1 hour after subcutaneous (SC) injection of vortioxetine (0.001, 0.01, 0.01 and 1.0 mg/kg) to male Sprague-Dawley rats (Report No. 929-300 2012 059). Vortioxetine produced a dose-dependent increase in 5-HT3 receptor occupancy (30 – 100%; ED50 = 0.004 mg/kg), while ondansetron (positive control) produced occupancy ranging from 8 – 40% over the same dose range (an ED50 was not calculated for ondansetron). The Sponsor calculated the EC50 for plasma and brain exposure of vortioxetine to be 0.6 ng/mL and 8 ng/g, respectively.

5-HT1B: 5-HT1B receptor occupancy was determined *ex vivo* by the prevention of binding of [^{3}H]GR125743 (selective 5-HT1B receptor antagonist) in brain slices taken 1 hour after SC injection of vortioxetine (2, 4, 8, and 16 mg/kg) to male Sprague-Dawley rats (Report No. 929-300 2011 041). Vortioxetine produced a dose-dependent increase in 5-HT1B receptor occupancy (36 – 81%; ED50 = 3.3 mg/kg), while SB216641 (positive control) produced 30% occupancy at 7.5 mg/kg. The Sponsor calculated the EC50 for plasma and brain exposure of vortioxetine to be 150 ng/mL and 1600 ng/g, respectively.

5-HT1A: 5-HT1A receptor occupancy was determined in male Sprague-Dawley rats by in vivo cold competition binding of vortioxetine (2.5, 5, 10, and 20 mg/kg, SC) with WAY-100635 (5-HT1A receptor antagonist) using liquid chromatography and mass spectrometry (Report No. 929-300 2011 042). Vortioxetine produced a dose-dependent increase in 5-HT1A receptor occupancy (7 – 44%), while pindolol (positive control) produced 73% occupancy at 3 mg/kg. Vortioxetine plasma and brain exposures ranged from 166 – 898 ng/mL and 1849 – 19,759 ng/g, respectively.

The brain receptor occupancy data following SC vortioxetine dosing in rats for 5-HTT and 5-HT3, 5-HT1B, and 5-HT1A receptors are summarized in Sponsor's Figure 1. The box indicates the rat dose range the Sponsor considers to be equivalent to the clinical dose range.

Figure 1: Brain Receptor Occupancies from *Ex Vivo* Studies Conducted in Rats



[Figure from NDA 204447 submission; Nonclinical Overview, page 12]

In Vivo Mechanistic Studies in Rodents

Serotonin syndrome, Bezold-Jarisch reflex assay, *in vivo* microdialysis, and serotonin neuronal firing in the rat dorsal raphe nucleus were *in vivo* mechanistic studies conducted in rat to further characterize vortioxetine's functional activity at 5-HTT and 5-HT3 receptor and vortioxetine's effect on neurotransmitter release and serotonin neuronal firing.

5-HT Syndrome: The effect of vortioxetine and a non-major human metabolite, Lu AA39835, on 5-HT syndrome was determined in CD-1 mice (Report No. 929-900 2007 026). 5-hydroxytryptophan (5-HTP)-potentiated behavioral syndrome (5-HT or serotonin

syndrome) is a number of behavioral changes (including flat body posture, head twitches, tremor, Straub tail) that occur when 5-HTP is administered with compounds that block 5-HTT. Vortioxetine at doses of 7.9 and 15.8 mg/kg given SC produced 5-HT syndrome in mice, while Lu AA39835 at doses up to 12.3 mg/kg did not.

In Vivo Antagonist Activity at 5-HT3 Receptor: The *in vivo* antagonist activity of vortioxetine was quantified using the Bezold-Jarisch reflex assay (5-HT3 receptor agonist-induced blood pressure reflex) in Wistar rats (Study No. 12475). Vortioxetine (0.01 – 3 mg/kg) dose-dependently suppressed the Bezold-Jarish reflex with an ED50 of 0.11 mg/kg, while ondansetron (a positive control; 0.003 – 0.3 mg/kg) suppressed the Bezold-Jarish reflex with an ED50 of 0.021 mg/kg.

In Vivo Microdialysis: *In vivo* microdialysis studies were conducted in freely moving male Wistar or Sprague-Dawley rats to measure the effects of a single SC dose or 3 SC doses of vortioxetine on neurotransmitter release in brain (ventral hippocampus (VH), medial prefrontal cortex (mPFC), and nucleus accumbens (NAcc)).

A single dose of vortioxetine at 2.5, 5, or 10 mg/kg increased dose-dependently 5-HT, norepinephrine, dopamine (although, not statistically significant in mPFC), and histamine levels in the mPFC and VH (histamine not examined in VH) over 180 minutes (5-HT: minimally effective dose (MED) = 2.5 mg/kg mPFC and VH; NE and DA: MED = 5 mg/kg mPFC and 10 mg/kg VH; Histamine: MED = 5 mg/kg VH). In the NAcc, 5-HT was increased (MED = 5 mg/kg), but not NE or DA. Acetylcholine (ACh) levels were not affected by vortioxetine treatment in the mPFC, VH, and NAcc in one study, but were increased in the mPFC and VH in a second study when a cholinesterase inhibitor was used with a MED = 5 mg/kg. Glutamate levels in the mPFC, VH, and NAcc and GABA levels in the mPFC were not affected by vortioxetine treatment. (Report Nos. 929-900 2007 032, 929-900 2007 033, 005-308-2007, and 929-900 2010 110).

Vortioxetine at 5 mg/kg/day (41% 5-HTT occupancy) for 3 days increased extracellular 5-HT in the VH. Vortioxetine at 5, 10, 19, or 28 mg/kg/day for 3 days increased extracellular 5-HT, NE, and DA in the mPFC and VH (not DA) compared to controls (5-HT: mPFC – ↑ at 10 – 28 mg/kg/day, ~57 – 98% occupancy at 5-HTT, VH - ↑ at 19 – 28 mg/kg/day, ~98% occupancy at 5-HTT ; NE: mPFC and VH - ↑ at 28 mg/kg/day, 98% occupancy at 5-HTT; DA: mPFC - ↑ at 28 mg/kg/day, 98% occupancy at 5-HTT). Vortioxetine had no effect on ACh levels in these studies; although a cholinesterase inhibitor was not used. (Report Nos. 007-308-2007, 929-900 2010 095, 929-900 2010 111)

Serotonin Neuronal Firing in the Rat DRN: The effects of vortioxetine on 5-HT neuronal firing in the dorsal raphe nucleus (DRN) were examined in two studies in anesthetized male Sprague-Dawley rats using *in vivo* electrophysiological recordings. (Report Nos. 929-900 2008 065 and 929-300 2011 044)

A single dose of vortioxetine at 0.25 – 1.25 mg/kg and 0.25 – 1 mg/kg (IV) suppressed DRN 5-HT neuronal firing dose-dependently (ED50 = 0.548 and 0.44 mg/kg,

respectively). This effect was reversed by IV administration of the 5-HT1A receptor antagonist WAY-100635 (0.05 mg/kg); suggesting that the effect of vortioxetine in the rat DRN involves presynaptic 5-HT1A receptors (in the DRN 5-HT1A receptors exert an inhibitory influence on 5-HT cell firing and 5-HT release). In addition, this effect was blocked dose-dependently by pre-administering a 5-HT3 receptor agonist SR57227 (0.005 mg/kg; IV). Fluoxetine at 1 – 7 mg/kg, IV, suppressed DRN 5-HT neuronal firing (ED50 = 2.73 mg/kg). This effect was reversed by WAY-100635 administration, but was not blocked by pre-administration of SR57225.

Vortioxetine at doses of 5 mg/kg/day (minipump) for 5 hours and 10 hours resulted in a decrease in spontaneous DRN 5-HT neuronal firing by 42 – 58% compared to controls. However, 5 mg/kg/day vortioxetine for 1 – 14 days had no affect on spontaneous firing. Fluoxetine (10 mg/kg/day; SC) administered for 7 days reduced firing activity by 55%, but had no effect when administered for 14 – 21 days (earlier time points were not measured based on previous published studies). Vortioxetine reduced the desensitization of 5-HT1A somatodendritic receptors caused by administration of a 5-HT1A receptor agonist flesinoxan (0.1 – 700 mg/kg, IV). Co-administration of vortioxetine (5 mg/kg/day, SC) and SR57227 (1 mg/kg/day, SC; 5-HT3 receptor antagonist) for 3 days decreased firing activity by 37%.

Animal Models of Antidepressant and Anxiolytic Activity

Vortioxetine had an antidepressant-like effect in the forced swim test in mice, but not in the chronic mild stress model of depression and in the olfactory bulbectomized model in rats. Although, the antidepressant desipramine did not have an effect in the olfactory bulbectomized model in rats as well and this is most likely a failed study. Vortioxetine had an anxiolytic/antidepressant-like effect in the novelty-induced suppression of feeding paradigm and increased cell proliferation and maturation of immature neurons in the dentate gyrus of the hippocampus consistent with the antidepressant fluoxetine. Vortioxetine had an anxiolytic-like effect in the mouse marble burying test, the rat social interaction test, and in conditioned fear-induced vocalization model in rats.

4.2 Secondary Pharmacology

Learning and Memory

Vortioxetine enhanced contextual memory in fear conditioning, increased exploration of novel objects in the novel object recognition test, and dose-dependently reversed the effects of serotonin depletion in the novel object recognition test and Y-maze spontaneous alternation test in rats.

Analgesia

Vortioxetine had an analgesia-like effect in the mouse formalin model of pain and on thermal hyperalgesia, but not mechanical allodynia, in the rat chronic constriction nerve injury model of neuropathic pain. Vortioxetine did not have analgesic-like effects on mechanical allodynia and thermal hyperalgesia in the rat carrageenan paw inflammation model of pain.

Rat Sexual Behavior
At doses of vortioxetine and fluoxetine (10 mg/kg/bid for up to 24 days) that produced 5-HTT occupancy of 70 and 95%, respectively, vortioxetine did not have statistically significant effects on rat sexual behavior while fluoxetine did.

4.3 Safety Pharmacology

Safety pharmacology studies were performed to examine vortioxetine's effect on the central nervous, cardiovascular, respiratory, renal, and gastrointestinal systems. Only key findings for the safety pharmacology studies are discussed in this review.

Central Nervous System
Sprague-Dawley rats acutely dosed with up to 40 mg/kg (oral) vortioxetine had no vortioxetine-related behavioral or physiological changes for 4 hours postdose in an Irwin test and had no vortioxetine-related effects on locomotor activity, grip strength, and performance on a rotarod for 24 hours postdose. Beagle dogs dosed with 0.75 – 6 mg/kg (intravenous) vortioxetine in two cardiovascular and respiratory studies showed a dose-dependent increase in mild or moderate sedation at all doses and had whimpering/whining/barking and mild to moderate abdominal pain at doses ≥1.5 mg/kg over the three hour examination period.

Cardiovascular and Respiratory Systems
In vitro, vortioxetine is a weak reversible inhibitor of hERG channels with an IC50 of 3.3 μM and an inhibitor of human cardiac SCN5A sodium channels (associated with cardiac arrhythmias in humans) with an IC50 of 930 nM.

Ex vivo, vortioxetine concentrations up to 3 μM did not affect basal heart rate, but did modify isoprenaline's dose response—low concentrations (<300 nM) of vortioxetine had a positive chronotropic effect and high concentrations (1 – 3 μM) of vortioxetine had a negative chronotropic effect—in isolated right atrium from male Sprague-Dawley rats.

In anesthetized, ventilated New Zealand White rabbits, vortioxetine given by intravenous infusion at doses up to 10 mg/kg had no effect on ECG morphology and did not produce pro-arrhythmic activity; however, vortioxetine did dose-dependently increase heart rate and left ventricular pressure X heart rate with a NOEL of 6 mg/kg.

In two conscious Beagle dogs receiving 6 mg/kg vortioxetine IV infusion in a pilot study, blood pressure increased >25 mmHg. In addition, heart rate (HR) was increased during vortioxetine infusion but not after infusion was terminated. No vortioxetine-related changes were seen in blood pressure or HR for dogs receiving 1 and 3 mg/kg in this study or in a second pivotal study where 3 mg/kg was the highest dose tested. In the pilot study, no differences in PR, QRS, and QT intervals were seen, but minor changes in T-wave morphology was noted at 3 and 6 mg/kg. In the pivotal study, the only ECG change seen was a small (4 – 5 msec) increase in PR interval post dose. In the pilot study, all vortioxetine dosed dogs (1 – 6 mg/kg) had a decrease in arterial blood pH, pO2, blood hemoglobin saturation, and an increase in arterial blood pCO2 compared to predose levels. In the pivotal study, a dose-dependent decrease in pH was also noted

at 3 mg/kg, but all other changes were observed in vehicle dosed dogs; therefore, were not vortioxetine-related.

In anesthetized Beagle dogs that received increasing doses of vortioxetine IV, arterial blood pressure was decreased by ≤ 7 mmHg dose-dependently and a dose-dependent increase in PR interval was see at all doses.

In anesthetized, ventilated guinea pigs, 10 and 20 mg/kg vortioxetine IV decreased heart rate for the 120 minutes measured. There were no significant effects of vortioxetine on airway resistance, dynamic lung compliance, and mean arterial blood pressure, suggesting that vortioxetine is not acting as a ß2-adrenoceptor agonist.

In conscious, freely-moving male rats, oral vortioxetine at 40 mg/kg reduced peak expiratory flow (PEF) when measured by whole body plethysmography with a NOEL of 20 mg/kg. No other vortioxetine-related changes to respiration were seen.

Renal System

Wistar rats (8/sex/group) dosed with 20 or 40 mg/kg/bid (oral gavage) for 5 days were tested for renal function after saline loading over a 24 hour period in a metabolism cage. For males and females dosed with 40 mg/kg/bid at 0 – 2 hours from last dose, mean urine osmolarity (↑40%) and albumin levels (↑69%) were increased for males and mean urine chloride was increased (↑88%) for females. For males dosed with 40 mg/kg/bid at 2 – 6 hours from last dose, mean urinary volume was decreased (↓60%), mean urine sodium was increased (↑43%), and mean urine chloride was decreased (↓62%). No changes were seen for rats dosed with 20 mg/kg/bid or at other time points.

Gastrointestinal System

Wistar rats (6 – 8/group) acutely dosed with 40 and 100 mg/kg vortioxetine, but not 20 mg/kg, had increased stomach weights and increased stomach contents (primarily bedding material when bedding present at time of test). Charcoal transit time was slightly decreased for all vortioxetine dosed groups; although, not statistically significantly. This data suggests that vortioxetine may decrease gastric emptying in rats.

5 Pharmacokinetics/ADME

5.1 PK/ADME

Absorption, distribution, metabolism, and excretion (ADME) were examined in CD-1 mice, Han Wistar rats, Sprague-Dawley rats, Lister Hooded rats, and Beagle dogs. All species used in toxicology studies, except New Zealand White rabbits, were used in PK studies. The formulations used in the PK studies were vortioxetine in 5% hydroxypropyl-beta-cyclodextrin (HP-ß-CD) and 10% HP-ß-CD.

Absorption

Vortioxetine was absorbed after oral administration in nonclinical species. Peak plasma concentrations generally were 1 – 2 hours in rat and 4 – 6 hours in dog after a single dose and 1 – 2 hours after the first or second dose of the day in rat and 4 hours in dog after repeat oral dosing. Oral bioavailability was low in rat (~10%) and dog (48%), but higher in human (75%). PK after oral single dose, oral repeat dose, and IV administrations in rat and dog are shown in Table 3, Table 4, and Table 5. PK/toxicokinetic (TK) data after repeat oral dosing is reviewed in more detail in the general toxicology, carcinogenicity, and reproductive toxicology sections.

Table 3: Mean PK parameter estimates of vortioxetine following single dose oral administration

Species/Strain	Sex	Dose (mg/kg)	AUC(0-inf) (ng·hr/mL)	Cmax (ng/mL)	Tmax (hr)	T1/2 (hr)	BA(a) (%)	Study Identifier [Reference]
Rat/Wistar	M	2	22.4	3.85	2	3.0	-	[10088]
	M	6	116	20.7	1	3.8	6.7	
	M	20	564	83.9	2	3.9	-	
	F	2	28.0	5.34	2	2.9	-	
	F	6	155	42.1	2	2.5	10	
	F	20	1151	161	2	3.8	-	
Dog/Beagle	M+F	0.25	20.4	1.45	4.3	6.1	-	[10087]
		0.75	209	10.8	6.0	7.3	48	
		2.5	425	26.4	4.5	7.9	-	

(a) IV data used for calculation of the bioavailability are shown in Table 3.c.

[Table from NDA 204447 submission; Pharmacokinetics Written Summary, page 10]

Table 4: Mean PK parameter estimates of vortioxetine following repeat dose oral administration

Species/Strain	Sex	Dose (mg/kg)	AUC(0-inf) (ng·hr/mL)	Cmax (ng/mL)	Tmax (hr)	T1/2 (hr)	Study Identifier [Reference]
Rat/Wistar	M	2	25.7(a)	4.33	1	2.9	[10088]
	M	6	87.7(a)	13.4	1	2.9	
	M	20	757(a)	111	1.5	10	
	F	2	28.2(a)	5.76	1.5	2.9	
	F	6	173(a)	29.2	4	3.5	
	F	20	1419(a)	186	2	5.7	
Dog/Beagle	M+F	0.25	32.5	3.01	4.4	5.4	[10087]
		0.75	197	18.2	4.5	9.4	
		2.5	1290	68.0	4.1	24	

(a) Value corrected for residual area attributed to remaining Lu AA21004 from previous dose.

[Table from NDA 204447 submission; Pharmacokinetics Written Summary, page 10]

Table 5: Mean PK Parameter Estimates of vortioxetine following single dose IV administrations

Species/Strain	Sex	Dose (mg/kg)	AUC(0-inf) (ng·hr/mL)	Vd (L/kg)	CL (L/hr/kg)	T1/2 (hr)	Study Identifier [Reference]
Rat/Wistar	M	0.5	144	10.2	3.46	2.1	[10088]
	M	1.5	411	12.7	3.65	2.4	
	M	5	1015	16.2	4.93	2.3	
	F	0.5	124	10.0	4.05	1.7	
	F	1.5	435	12.7	3.44	2.6	
	F	5	1220	12.4	4.10	2.1	
Dog/Beagle	M+F	0.125	74.6	15.8	2.06	5.6	[10087]
	M+F	0.375	174	23.7	2.53	6.7	
	M+F	1.25	742	21.2	1.82	8.1	

[Table from NDA 204447 submission; Pharmacokinetics Written Summary, page 11]

Distribution

The tissue distribution of vortioxetine after a single dose was examined in male Lister Hooded and Han Wistar rats using radiolabeled vortioxetine ([^{14}C]Lu AA21004) at 20 mg/kg. Vortioxetine and metabolites were extensively distributed to all tissues examined. Maximum plasma concentrations were at 2 hours post dose and there was more drug-related material in tissues than blood at 2 and 8 hours post dose. By 24 hours, liver and eye were the only tissues to have greater concentrations than blood and kidney had similar concentrations to blood. The distribution of drug-related material were similar for Lister Hooded and Han Wistar rats, but concentrations of drug-related material were higher in melanin containing tissues of pigmented rats (Lister Hooded) compared to albino rats (Han Wistar); therefore, drug-related material most likely binds to melanin. However, the Sponsor states that the UV absorption maxima for vortioxetine are between 199 and 225 nM, which is below the UV absorption of concern for phototoxicity of ≥290 nM. The tissue distribution in Listar Hood rats is shown in Table 6.

Table 6: Tissue distribution of radioactivity in the tissue of male Listar Hooded rats following a single 20 mg/kg oral dose of [^{14}C]-vortioxetine

	Concentration (μg equivalent of [^{14}C]Lu AA21004 free base/g or mL)				
Tissue	**2 hr**	**8 hr**	**24 hr**	**72 hr**	**168 hr**
Blood	1.01	0.635	0.701	0.173	<LLOQ
Bone marrow	3.48	2.17	0.137	<LLOQ	<LLOQ
Brain	2.77	1.39	0.136	0.157	<LLOQ
Eye	1.88	3.97	2.11	1.66	0.436
Kidney	10.3	4.18	0.652	0.305	0.103
Liver	22.3	10.4	1.90	0.433	0.138
Lung	15.5	7.81	0.223	0.173	<LLOQ
Pituitary gland	5.12	5.50	<LLOQ	<LLOQ	<LLOQ
Thymus	2.12	1.79	0.142	<LLOQ	<LLOQ
Thyroid gland	3.16	2.07	0.239	<LLOQ	<LLOQ

LLOQ=lower limit of quantification.
Study Identifier: [10349].

[Table from NDA 204447 submission; Pharmacokinetics Written Summary, page 14]

Administration of [^{14}C]-vortioxetine to pregnant and lactating rats showed drug-related material was distributed throughout the maternal and fetal tissue and was in milk secretion. However, concentrations of drug-related material were lower in the fetus than maternal tissue (~ 10-fold for tissues with the greatest amount of radioactivity in the fetus). Milk to plasma ratio was 1, 1.2, and 0.5 at 2, 6, and 24 – 72 hours post-dose.

In vitro plasma protein binding of [^{14}C]-vortioxetine was >98.8% for human, mice, rats, rabbits, and dogs. Binding to plasma protein was independent of concentrations. Plasma protein binding to the major-human metabolite, Lu AA34443, was 62.9 – 74.5% for all species tested and was independent of concentration. There was no preferential partitioning of ^{14}C-voritoxetine into red blood cells in mice, rats, and dogs.

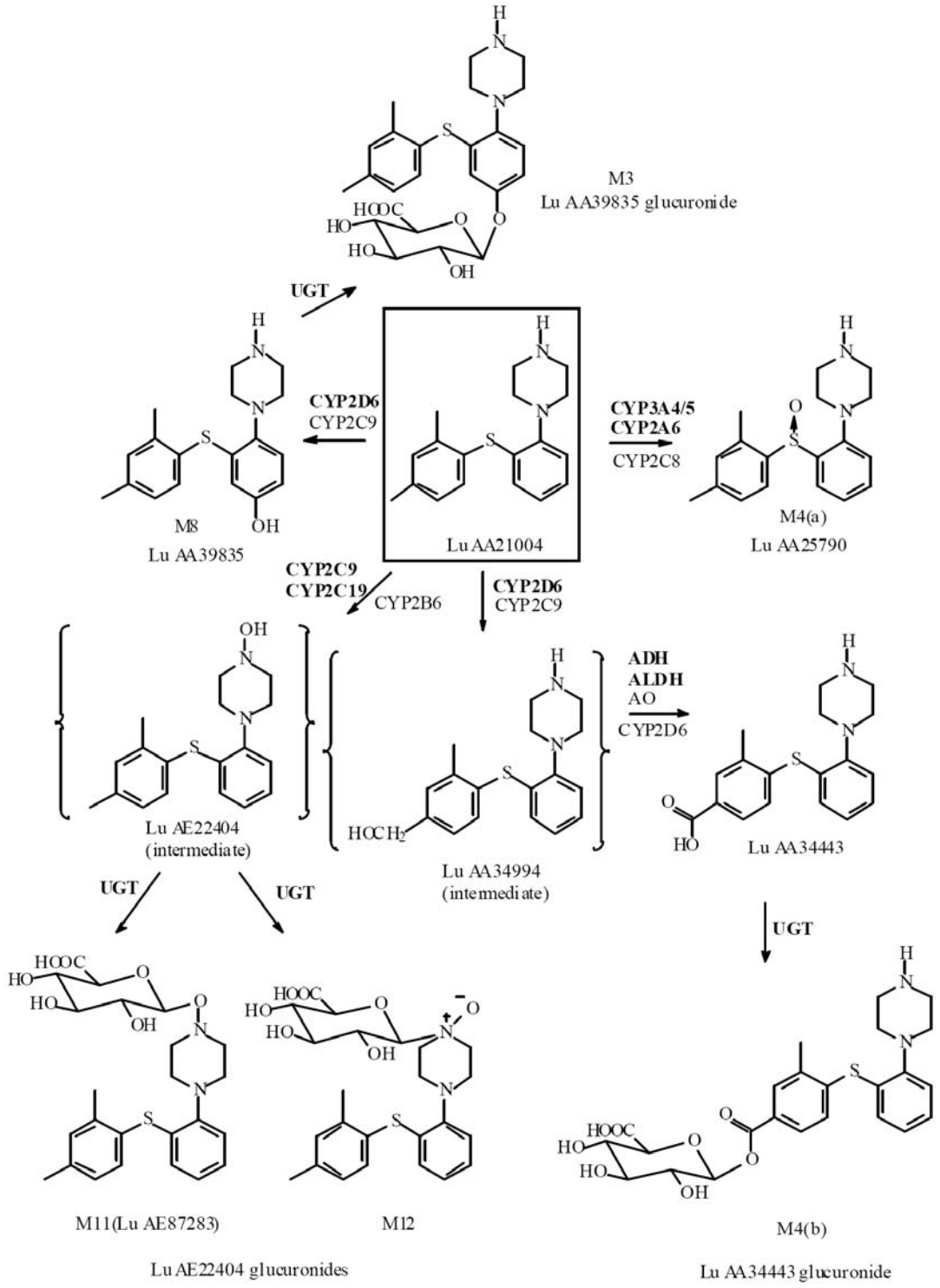
Metabolism

Humans, mice, rats, and dog extensively metabolize vortioxetine *in vivo*.

Human: The human biotransformation scheme is shown in Figure 2. In a single oral dose mass balance study, M3 (Lu AA39835 glucuronide), M4(b) (Lu AA34443 glucuronide), Lu AA34443, and M12 were each identified as being present at more than 10% of total circulating radioactivity and are considered major-human metabolites (Table 7). Lu AA34443 was the major metabolite excreted in urine and feces (>80%), followed by its glucuronide conjugate M4(b). Only a small amount (1.6%) of vortioxetine was present in feces.

CYP2D6, CYP2C9, CYP3A4/5, CYP2A6, CYP2C8, CYP2C19, and CYP2B6 are the cytochrome P450 isozymes responsible for most of vortioxetine metabolism (Figure 2). The formation of Lu A34443 from Lu AA34994 is primarily catalyzed through alcohol dehydrogenase and aldehyde dehydrogenase (Figure 2).

Figure 2: Human biotransformation scheme showing the enzymes involved in the metabolism of vortioxetine



The isoenzymes written in **bold** are expected to be the primary enzymes involved.
The intermediates (shown in parenthesis) were not detected in vivo, but were seen in vitro [10431].

[Table from NDA 204447 submission; Pharmacokinetics Written Summary, page 22]

Table 7: Metabolite % of total radioactivity for [14C]-vortioxetine (Lu AA21004) and its radiolabeled metabolites in plasma from healthy male subjects following a single oral administration of 50 mg free base (1.85 MBq) of [14C]-vortioxetine

	Metabolite % of total radioactivity			
Metabolite (a)	4 hours (n=6)	12 hours (n=6)	24 hours (n=6) (b)	72 hours (n=1) (c)
M3 (glucuronide)	10 ± 7	12 ± 5	11 ± 2	8
M4(b) (glucuronide)	16 ± 12	14 ± 10	11 ± 4	19
Lu AA34443	28 ± 22	20 ± 12	14 ± 6	14
Lu AA39835	4 ± 3	4 ± 2	4 ± 2	4
M12 (glucuronide)	22 ± 11	33 ± 34	20 ± 8	36
Lu AA21004	8 ± 5	7 ± 2	10 ± 9	13
M11 (glucuronide)	11 ± 5	8 ± 4	8 ± 3	8
Total	99 ± 58	99 ± 65	77 ± 30	102

Source: Study No. 10882, Table 8 and Study No. 10477 CSR amendment 1, Table 9.
(a) Refer to Figure 2.a in Module 2.7.2 for biotransformation scheme.
(b) No other metabolites were observed, thus the lower recovery at this time point was ascribed to experimental error.
(c) Only one sample obtained from subject R0104 at 72 hours post-dose was analyzed in study No. 10882.

[Table from NDA 204447 submission; Response to FDA Information Request Dated 10/19/12, page 2]

Mouse: The metabolites Lu AA34443, M3, Lu AA25790, M4(b), and M12 were detected in plasma of CD-1 mice. Non-human metabolites were also detected in plasma. Vortioxetine is highly metabolized in mice, only 2% of the administered dose was excreted as parent in feces and parent was not detected in urine of males or females. Lu AA34443 was the major metabolite in urine (65%) and feces (43%).

Rat: In plasma of rat, the metabolites Lu AA34443, M3, M4(b), and Lu AA39835 were detected. Non-human metabolites were also detected in plasma. Vortioxetine was found at 2.7% of administered dose in feces and was not detected in urine. Lu AA34443 was the major metabolite excreted in rat (22% in urine and 26% in feces). Lu AA39835 and M4 (Lu AA34443 glucuronide and Lu AA25790) accounted for 19 and 8%, respectively. Both were predominantly excreted in feces. The other metabolites accounted for less than 2% of the administered dose. Sex differences were seen; females formed less Lu AA34443 (38 vs. 58%) and more Lu AA39835 (30 vs. 8%) than males. The Sponsor measured Lu AA34443 in rat general toxicology, carcinogenicity, and reproductive and development studies.

Dog: In plasma of dog, the metabolites Lu AA34443, M3, M4(a), M4(b), M11, and M12 were detected. Lu AA34443 was the major metabolite excreted accounting for 43% of the administered dose and was predominantly excreted in feces. Vortioxetine accounted for 6.1 – 9.5% and was primarily excreted in feces. The Sponsor measured Lu AA34443 in dog general toxicology studies.

Metabolism Comparison Across Species: The major-human metabolites, each accounting for greater than 10% of total circulating drug-related species, are M3 (Lu AA39835 glucuronide), M4(b) (Lu AA34443 glucuronide), Lu AA34443, and M12. Lu AA34443 is present in plasma at >3-fold (and as much as 71-fold) of human in mouse, rat, dog and rabbit (as measured in the toxicology animal studies); therefore, is adequately covered in nonclinical studies. Because M4(b) is a glucuronide of Lu AA34443 it is also not a concern. Lu AA39835 is detected in rat, dog, and rabbit in general toxicology studies; therefore, the glucuronide of Lu AA39835, M3, is not a concern.

However, M12 is not detected in rat and was not assessed in rabbit. M12 is detected in mouse and dog at 0.1- and 0.7-fold to human at the MRHD of 20 mg. M12 is an N-glucuronide of the reactive intermediate Lu AE22404. Lu AE22404 has only been detected in human hepatocytes *in vitro* and was not detected *in vivo* in humans. Although, M12 is not present in rat and was not covered in toxicology studies using rat, it is likely to be pharmacologically inactive and water soluble (and readily excreted) because it is a Phase II glucuronide conjugate.

CYP Induction and Inhibition: *In vitro*, vortioxetine and the metabolite Lu AA34443 did not induce synthesis of mRNA encoding CYP1A2, 2A6, 2B6, 2C8, 2C19, 3A4/5. In addition, vortioxetine and its metabolites; Lu AA34443, Lu AA34994, Lu AA39835, and Lu AA25790; did not inhibit CYP1A2, 2A6, 2B6, 2D6, 2E1, 3A4 (IC50 >34 μM). However, vortioxetine and Lu AA38835 inhibited CYP2C9 (Ki = ~15 – 30 and 8 μM, respectively), Lu AA39835 inhibited CYP2C19 (Ki <1 μM), and Lu AA25790 and Lu AA34443 inhibited CYP3A4/5 mediated midazolam metabolism (Ki = ~9 μM). Vortioxetine and Lu AA34443 inhibited CYP2C8 (Ki = 9.34 and 4.24 μM, respectively). The Sponsor conducted *in vivo* drug-drug interaction studies which they state did not demonstrate a "…clinically meaningful inhibitory effect of Lu AA21004 and/or its metabolites on these enzymes." See the Clinical Pharmacology Review for a review of the *in vivo* drug-drug interaction studies.

P-glycoprotein Substrate and Inhibition: In vitro, vortioxetine is not a good substrate for P-gp and only showed moderate inhibitory potential towards P-gp (IC50 = 4.4 μM for vortioxetine; IC50 = 0.717 and 0.567 μM for cyclosporine A and ketoconazole, respectively).

Excretion

The main route of excretion in humans of radiolabeled vortioxetine and drug-related material is via the urine (59 and 26% recovery of administered dose in urine and feces respectively). In contrast, the main route of excretion in mouse, rat, and dog is feces (mouse: 84% feces vs. 14% urine; rat: 69 vs. 33%, dog: 59 – 65 vs. 23 – 33% of administered dose). In rats, biliary excretion was 46% of total radioactivity. Recovery of radioactivity was 100% in rats and mice by 168 hours; however, recovery was only 90% after 168 hours and 85% after 360 hours in dogs and humans, respectively. In dogs, continued collection for an extra 24 hours showed excretion of radioactivity was still ongoing.

6 General Toxicology

6.1 Single-Dose Toxicity

Rat

In Wistar (HsdBrlHan: WIST) rats (2 – 5/sex/group), two doses by oral gavage of ≥200 mg base/kg given an hour apart resulted in convulsions and/or death in one or more rats and severe clinical signs including hypoactivity, tremors, and piloerection. Single doses by oral gavage of 400 and 500 mg base/kg produced less severe clinical signs of marked sensitivity to disturbance, rapid breathing, and fur staining and did not result in death. The acute MTD was considered to be a single dose of 500 mg base/kg.

In Wistar (HsdBrlHan: WIST) rats (2 – 5/sex/group) given vortioxetine as an intravenous (IV) injection, doses ≥30 mg base/kg resulted in convulsions and/or death. A convulsion followed by euthanasia occurred in 1 male dosed with 40 mg base/kg. Two rats, 1 female dosed with 40 mg base/kg and 1 male dosed with 30 mg base/kg, were found dead 5 - 10 min postdose. The remaining males and all females dosed with 30 mg base/kg had irregular breathing, hypoactivity, and unsteady gait immediately postdose. All rats dosed with 20 mg base/kg had hypoactivity reported 5 min postdose. All surviving rats gained weight after the single IV injection and no gross findings were seen at necropsy. The acute MTD was considered to be 20 mg base/kg.

Mouse

In CD-1 mice (2 – 5/sex/group), two doses by oral gavage of ≥300 mg/kg given an hour apart resulted in severe clinical signs, including roughened coat, cold body surface temperature, incoordination, leaning to one side, loose gelatinous feces, hypoactivity (2 x 400 mg/kg only), and suspected or confirmed convulsions. Tremors, unsteady gait, eyes partly closed, and convulsion (1 female) were noted in mice dosed with two doses of 200 mg/kg given an hour apart. Single doses of 400 and 500 mg/kg produced severe clinical signs of marked sensitivity to touch, rapid and/or labored breathing, incoordination, unsteady gait, tremors, salivation, and eyes partly closed. A single dose of 200 and 300 mg/kg produced less severe clinical signs of mild tremors, marked sensitivity to touch, eyes partly closed, and hypoactivity. The Sponsor considered the acute MTD to be a single dose of 300 mg/kg.

In CD-1 mice (2 – 5/sex/group), a single intravenous (IV) injection of 30 mg base/kg resulted in tremors and a convulsion in male mice. A single IV injection of 20 mg base/kg did not affect clinical signs and all mice gained weight. At necropsy, no gross findings clearly related to vortioxetine were noted. The acute MTD was considered 20 mg base/kg.

Dog

Single-dose studies were not conducted in dog.

6.2 Repeat-Dose Toxicity

6.2.1 Rat Repeat-Dose Toxicity

***Summary of studies shorter than 6 months in duration in rat*:**

8 – 10-Day Oral MTD Study in Wistar Rats (Study No. 10110; non-GLP)
Han Wistar rats (5/sex/group; except for HD with 2/sex) were dosed by oral gavage with 0, 20/150 (dose increased to 150 on Day 5), 40, 80, or 100 mg/kg/bid for 10 or 8 (100 mg/gk/bid only) days in 10% hydroxypropyl-beta-cyclodextrin (HP-β-CD) and 4.4% glucose. One 100 mg/kg/bid female rat was found dead on Day 9 and one 20/150 mg/kg/bid male rat was found dead on Day 10; cause of death was not determined. Clinical signs were slight to moderate sedation in males and females at 80 mg/kg/day, muscle tremors, slight salivation, soft or liquid feces, ungroomed and bristly coat at all doses, and red discoloration of the urine on Day 10 and 11 at 150 mg/kg/bid for 2 male rats. Body weight loss and decreased feed consumption (males only) were seen for males receiving ≥80 mg/kg/bid and females receiving ≥100 mg/kg/bid. The kidney was identified as a target organ for toxicity in male rats based on increased organ weight and macroscopic and microscopic findings associated with increased plasma creatinine and urea at ≥80 mg/kg/bid. There were no kidney changes noted for females dosed up to 150 mg/kg/bid. The MTD was considered below 80 mg/kg/bid for males and females.

4-Week Oral Toxicity Study in Wistar Rats (Study No. 10194; GLP-compliant)
Han Wistar rats (10/sex/group) were dosed by oral gavage (in 10% HP-ß-CD containing 4.4% glucose monohydrate) with 0, 10, 20, or 40 mg/kg/bid for 4 weeks. Small increases in prothrombin time (≤8%) were noted for HD males and females and decreases in activated partial thromboplastin time (≤25%) were noted for MD and HD males. Increases were noted for plasma cholesterol (≤30%) and triglycerides (≤63%) in MD and HD females, bilirubin (≤100%) in MD and HD males and HDF, and alkaline phosphatase (≤36%) in all male dose groups. Decreases were noted for plasma aspartate aminotransferase (≤15%) in HDM and sodium (≤4%) in all female dose groups. Urinary volume was increased and specific gravity decreased for MD and HD females. Increased adrenal weights (body weight adjusted) and minimal diffuse cortical hypertrophy were noted for HDM. Liver weights were slightly increased for HDF without a corresponding histopathological change. Exposures (AUC and Cmax) generally increased more than dose-proportional, females had higher exposures than males, and exposures were higher at Day 29 than Day 1.

13-Week Oral Toxicity Study in Wistar Rats Followed by a 4-Week Recovery Period (Study No. 10304; GLP-compliant)
Wistar rats (10/sex/group) were dosed by oral gavage (in 10% HP-ß-CD) containing 4.4% glucose monohydrate) with 0, 10, 20, or 40 mg/kg/bid for 13 weeks. No vortioxetine-related deaths occurred and clinical signs were limited to isolated incidence of salivation in rats at all dose groups. MD and HD females gained more body weight compared to controls over the 13 weeks with no corresponding difference in food consumption.

A small increase in prothrombin time (≤8%) was noted for all male dose groups in Week 6 and a small decreases in activated partial thromboplastin time (≤18%) was noted for all dose groups in Week 13. Decreases were noted for platelet count (12%) for HDM in Week 13. Increases were noted for lymphocyte count (38%) and total white blood cell count (30%) for HDF in Week 6 and neutrophils (≤60%) and total white blood cell count (≤32%) for MD and HD females in Week 13.

Increases in alkaline phosphatase activity (≤58%)was noted for LD (W6 only), MD, and HD males and MD and HD females in Weeks 6 and 13. Increases were noted for plasma glucose (≤34%) for MD and HD males and females in Week 6, triglycerides (33%) for HDF in Week 6, and electrolytes for MD and HD males and females Weeks 6 and 13. Decreases were noted for plasma urea (≤14%) for MD and HD females in Week 6, and creatinine (6%) for HDF in Week 6.

Urinary volume was decreased for HDF with corresponding decreases in protein in Week 6 and sodium and potassium in Week 13. Urinary volume was slightly increased with corresponding increase in sodium, potassium, and chloride, and decreased specific gravity for MD and HD males in Week 13. Crystals were present in the urine of 2 MD and all HD males and 7 HD females in Week 13. There were no differences noted after the recovery period.

Liver and kidney weights were increased in Weeks 13 for HD males and females with corresponding histopathology findings. Centrilobular hepatocyte hypertrophy in all male dose groups and in HDF and midzonal hepatocyte vacuolation in HDM were seen. Glomerulonephritis in the kidney was noted in 3 HDM. Most of the changes were not seen after the recovery period.

Exposures (Cmax and AUC) to vortioxetine increased more than dose-proportionally, were similar for males and females, and increased from Day 1 to Week 13 (Table 8). Exposures at Week 13 to a major-human metabolite, Lu AA34443, increased slightly less than dose-proportional for Cmax and slightly more than dose-proportional for AUC and exposures for males were approximately 2-fold higher than for females (Table 9).

Table 8: Summary of group mean TK parameters for vortioxetine in rat

Lu AA21004		10 mg/kg b.i.d.		20 mg/kg b.i.d.		40 mg/kg b.i.d.	
		males	females	males	females	males	females
C_{max} (nmol/L)[a]	Day 1, single dose	163	157	352	572	1217	1310
	Week 13, multiple dose	488	506	1312	1610	3043	2856
t_{max} (h)	Day 1, single dose	1.0	1.0	1.0	1.0	1.0	1.0
	Week 13, multiple dose	1.0	1.0	12[c]	1.0	14[d]	12[c]
AUC_{0-10h} (h x nmol/L)[a]	Day 1, single dose	479	485	1235	1672	3508	4551
	Week 13, multiple dose	1388	1613	4778	5029	17860	14165
AUC_{0-24h} (h x nmol/L)[a]	Week 13, multiple dose	2810	3556	10426	10238	38319	32957
CL/F (L/h/kg)	Week 13, multiple dose	24	19	13	13	7.0	8.1
AI[b]	Week 13, multiple dose	2.9	3.3	3.9	3.0	5.1	3.1

The calculation of toxicokinetic parameters were based on data from 3 rats / sex / time point / dose group where possible.
[a]: Conversion factor for Lu AA21004: 1 nmol/L = 0.2984 mg/mL.
[b]: Accumulation Index: $AUC_{0-10,\ week\ 13}/AUC_{0-10,\ Day\ 1}$.
[c]: equal to 2 hours after administration of the second daily dose.
[d]: equal to 4 hours after administration of the second daily dose.

[Table from NDA 204447 submission; Study No. 10304, page 37]

Table 9: Summary of mean TK parameters for Lu AA34443 in rat

Lu AA34443		10 mg/kg b.i.d.		20 mg/kg b.i.d.		40 mg/kg b.i.d.	
		males	females	males	females	males	females
C_{max} (nmol/L)[a]	Week 13, multiple dose	4254	2681	6756	4723	10607	6140
t_{max} (h)	Week 13, multiple dose	1.0	1.0	12[b]	1.0	12[b]	12[b]
AUC_{0-10h} (h x nmol/L)[a]	Week 13, multiple dose	15106	8378	28993	16335	60543	33048
AUC_{0-24h} (h x nmol/L)[a]	Week 13, multiple dose	26638	13929	58171	33490	134048	60688[c]
MR	Week 13, multiple dose	9.5	3.9	5.6	3.3	3.5	n.a.

The calculation of toxicokinetic parameters were based on data from 3 rats / sex / time point / dose group where possible.
[a]: Conversion factor for Lu AA3443: 1 nmol/L = 0.3284 mg/mL.
[b]: Equal to 2 hours after administration of the second daily dose.
[c]: AUC_{0-16h}.

[Table from NDA 204447 submission; Study No. 10304, page 38]

Study title: Lu AA21004 Toxicity Study by Twice Daily Oral Gavage Administration to Wistar Rats for 26 Weeks Followed by a 12 Week Recovery Period Amended Final Report Number 3

Study no.:	LBK 150; Reference no. 11013
Study report location:	(b) (4)
Conducting laboratory and location:	
Date of study initiation:	February 1, 2005
GLP compliance:	Yes, except for TK data
QA statement:	Yes
Drug, lot no., and % purity:	Lu AA21004, 2018324, 100%

Key Study Findings

- Male and female Wistar rats were dosed orally (by gavage, in 10% hydroxypropyl-beta-cyclodextrin) at 0, 10, 20 or 40 mg/kg/bid for 26 weeks followed by a 12-week recovery period.
- Death of one high dose male at Week 15 possibly due to renal pathology.
- Small increase in the incidence of unilateral linear superficial corneal opacities in high dose males and females.
- Crystals were seen in the urine of mid and high dose males and females in Weeks 13 and 26 and erythrocytes were seen in the urine of high dose males in Week 26. Crystals and erythrocytes were not seen in the urine after the recovery period.
- Increased urinary volume, decreases in urinary electrolytes, and increases in urinary electrolytes; enlarged, pale, granular kidneys with pelvic dilation; corresponding increased weight; and histopathological findings (including tubular basophilia/regenerative hyperplasia, dilatation of collecting ducts, tubular dilatation, papillary necrosis/foreshortened papilla, hyperplasia of papillary and/or pelvic epithelium, and papillary and/or tubular inflammation) possibly related to the presence of crystals in kidney were seen in high dose males.
- Increased liver weight was seen in mid and high dose females. Increased incidence and severity of liver histopathological findings; including increased plasma ALP activity, bile duct hyperplasia, centrilobular hepatocyte hypertrophy, low incidence of crystalline material in the bile ducts, pericholangitis, and focal hepatocyte necrosis; in mid and high dose males and females were seen, with greater incidence in males than females.
- Luminal dilatation, fibrosis, crystalline material in lumen, and epithelial hyperplasia of the extrahepatic bile duct was noted in a small number of high dose males and females.

- The NOAEL for liver and kidney pathology is 10 mg/kg/bid, which is 10 times the MRHD of 20 mg on a mg/m2 basis.

Methods

Doses:	0, 10 (LD), 20 (MD), or 40 (HD) mg/kg/bid
Frequency of dosing:	Twice a day for 26 weeks
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	Solution/10% HP-ß-CD and 4.4% glucose monohydrate
Species/Strain:	Rat/Wistar (Crl:WI BR)
Number/Sex/Group:	12/sex/group main; 6/sex/control and HD recovery
Age:	39 – 45 days at start of dosing
Weight:	Males: 117 – 163 g; Females: 102 – 136 g
Satellite groups:	3/sex/controls and 18/sex/dose group for TK
Unique study design:	None
Deviation from study protocol:	Study protocol and SOP deviations were found for the TK evaluation performed at (b) (4) which resulted in the inability to claim GLP compliance for the TK data.

Observations and Results

Mortality

One MDF, two HDM, and two HDF died prematurely. One HDF (No. 120) was euthanized during Week 12 of the recovery period because of trauma to the eye after blood sampling for clinical chemistry; therefore, this death is not considered vortioxetine related. One HDM (No. 164) and one HDF (No. 219) died during Weeks 4 and 21, respectively, most likely from dosing errors and their deaths are most likely not vortioxetine related. One MDF (No. 73) was euthanized during Week 24 because of poor clinical condition. Clinical chemistry analysis suggested the cause of death to be widespread inflammatory changes (low erythrocyte count; high neutrophil, eosinophil, and monocyte counts; high plasma ALP activity; high plasma glucose and triglyceride concentrations; and low plasma albumin concentration) and the relationship to vortioxetine is unclear. One HDM (No. 39) was euthanized during Week 15 because of poor clinical condition. Upon examination the cause of death was determined to be urinary tract lesions (pelvic, tubular, and collecting duct dilation; moderate tubular basophilia/regenerative hyperplasia; and dilation of the ureters) which is consistent with findings in other HDM; therefore, the cause being vortioxetine related cannot be ruled out. However, crystalline material was not seen in the glomeruli/tubules/papilla and papillary necrosis and interstitial fibrosis was not seen.

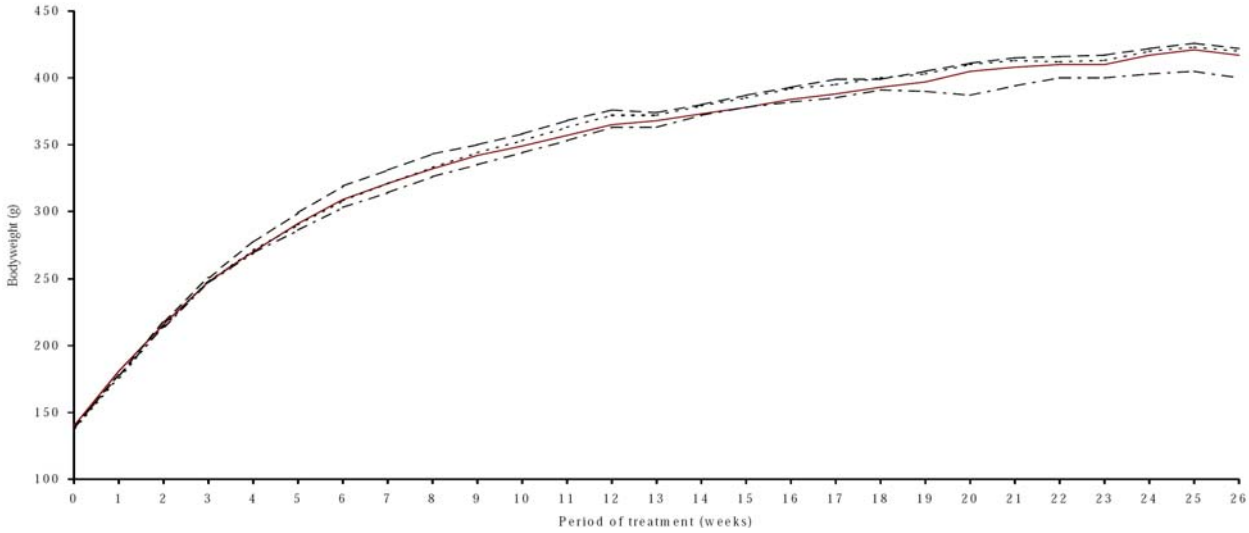
Clinical Signs

Isolated incidences of salivation and associated staining were seen starting Day 4 and continuing throughout dosing in all dose groups. Salivation was not observed during the recovery period.

Body Weights

HDM gained slightly less weight than controls (2.3 vs. 6%) from Week 18 through the end of dosing and weighed 4% less than controls at the end of dosing (Figure 3). MDF and HDF gained 32 – 36% more weight during Week 1 compared to controls (Figure 4). Body weight gain remained similar for MDF and HDF compared to controls for the remaining dosing period; however, MDF and HDF weighed 7 and 3%, respectively, more than controls at the end of dosing because of the initial higher body weight gain (Figure 4).

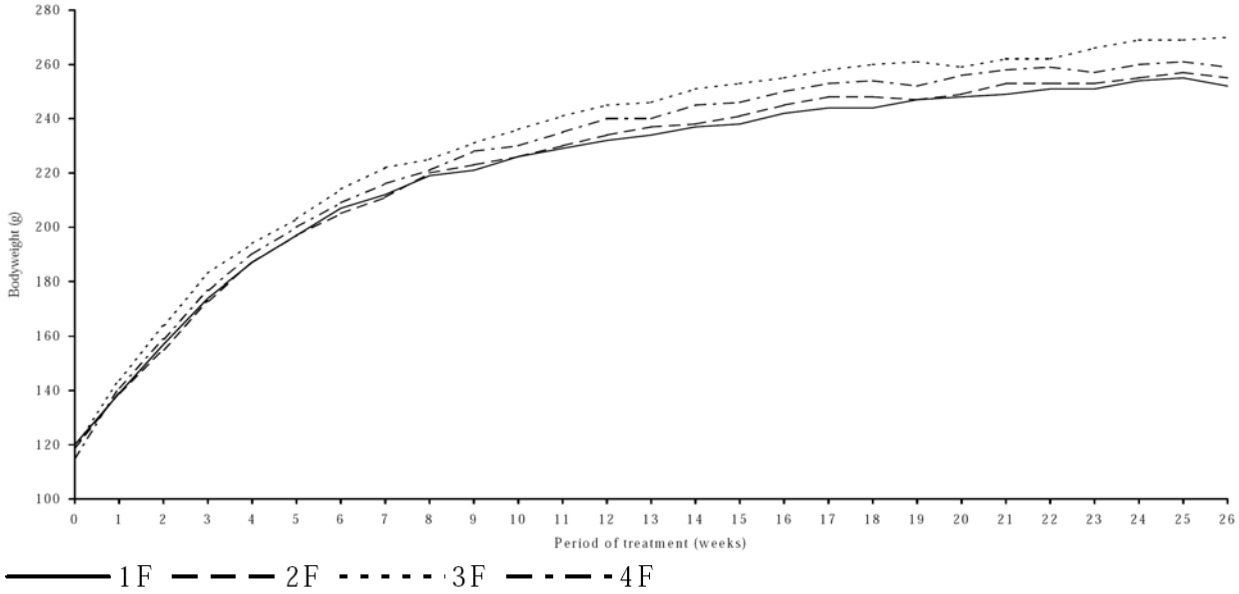
Figure 3: Group mean male body weights over the 6-month dosing period in rat



1M = control, 2M = 10 mg/kg/bid, 3M = 20 mg/kg/bid, 4M = 40 mg/kg/bid

[Figure from NDA 204447 submission; Study No. LBK 150, page 56]

Figure 4: Group mean female body weights over the 6-month dosing period in rat



1F = control, 2F = 10 mg/kg/bid, 3F = 20 mg/kg/bid, 4F = 40 mg/kg/bid

[Figure from NDA 204447 submission; Study No. LBK 150, page 57]

Feed Consumption

There was no vortioxetine-related effect on feed consumption.

Ophthalmoscopy

There was a small increase in the incidence of unilateral linear superficial corneal opacities in HD males and females compared to controls in Week 26 with no effect seen with LD and MD rats (0/18, 1/12, 1/12, and 4/17 for control, LD, MD, and HD males; 2/18, 2/2/12, 3/11, and 6/18 for control, LD, MD, and HD females). No difference was seen at the end of the recovery period (1/6, 1/6, 2/6, and 2/6 for control males, HDM, control females, and HDF, respectively).

Hematology

Increases in white blood cells (↑33 and 27% for HDM and HDF, respectively), due to increases in neutrophils (↑112%) and eosinophils (↑ 55%) in males and lymphocytes (↑30%) in females, was noted in HD males and females compared to controls in Week 26. A slight increase in prothrombin time was noted for MD and HD females (↑8%) in Week 13 and MD and HD males (7 and 14%, respectively) in Week 26 compared to controls. A slight decrease in activated partial thromboplastin time was noted for HD males in Weeks 13 and 26 (↓11 and 9%, respectively) and in HDF (↓9%) in Week 26 compared to controls. Hematocrit was increased for HDF (↑36%) in Week 26 compared to controls. No significant effects were seen at the end of the recovery period.

Clinical Chemistry

Alkaline phosphatase activities were increased for MD and HD males and females compared to controls in Weeks 13 and 26 (W13: ↑25, 29, 30 and 51%; W26: ↑20, 43, 48, and 76% for MDM, HDM, MDF, HDF, respectively). Alanine aminotransferase and

aspartate aminotransferase activities were not changed with treatment. Plasma urea concentrations were decreased 12% for HDM in Week 13 and HDF in Week 26. Plasma sodium, potassium, chloride, calcium, and phosphorous were slightly, but in most instances, statistically significantly increased for MD and HD males in Weeks 13 and 26 (Table 10). Plasma potassium and chloride were also slightly, but statistically significantly increased for MD and HD (Cl only) females in Weeks 13 and 26 (Table 10). Total protein was slightly, but statistically significantly increased for MD and HD males compared to controls in Weeks 13 and 26 most likely due to increased Alpha 1 globulin in Week 13 and albumin in Week 26 (W13: Total Protein- 3%, A1- 15%; W26: Total Protein- 6 – 7%, Alb-8%). None of these changes were seen after the recovery period.

Table 10: Percent mean increase in electrolytes over control in rats at Weeks 13 and 26

Electrolyte	Week	Dose (mg/kg/bid)			
		Male		Female	
		20	40	20	40
Na	13	0.7%*	2%**		
	26	0.7%**	2%**		
K	13	6%**	8%**	10%**	10%**
	26	2%	2%	9%*	6%**
Cl	13	1%	1%*		2%**
	26	1%	2%*		2%*
Ca	13	4%**	4%**		
	26	3%**	8%**		2%*
Phos	13	11%**	8%**		
	26	5%	16%*		

*p<0.05; **p<0.01

[Data from NDA 204447 submission; Study No. LBK 150, pages 94 – 108]

Urinalysis

In Week 13 and 26, HDM had increased urinary volume (↑23 and 102%, respectively) and slightly decreased specific gravity (↓0.9%) compared to controls. Males of all doses had decreased urinary sodium and potassium (MD and HD only) during Week 13 (except potassium) and 26 compared to controls (Na: W13-↓20, 29, and 36% for LD, MD, and HD; W26-↓27, 42, and 32% for LD, MD, and HD; K: W26-↓23 and 21% for MD and HD). No significant effects were seen in females during Weeks 13 and 26 or in males after the recovery period.

Microscopic examination indicated a presence of crystals in the urine of MD and HD males and females (only 1/12 MDF in W13) during Weeks 13 and 26. No crystals were seen in the urine of HD males and females after the recovery period. Erythrocytes in the urine were seen at an increased incidence in HDM during Week 26, but not after the recovery period. The incidence of crystals and erythrocytes in the urine of rats at Week 13 and 26 are shown in Table 11.

Table 11: Incidence of erythrocytes and crystals in urine of rats after 13 and 26 weeks of dosing and 12 weeks of recovery

Group and sex		1M	2M	3M	4M	1F	2F	3F	4F
Dosage (mg/kg b.i.d.)	Grade	0	10	20	40	0	10	20	40
Erythrocytes									
Week 13	0	15	11	12	18	18	12	12	18
	1	3	1	0	0	0	0	0	0
	N	18	12	12	18	18	12	12	18
Week 26	0	17	12	12	8	18	12	11	18
	1	1	0	0	4	0	0	0	0
	2	0	0	0	5	0	0	0	0
	N	18	12	12	17	18	12	11	18
Week R12	0	6	-	-	5	5	-	-	4
	1	0	-	-	1	1	-	-	2
	N	6	0	0	6	6	0	0	6
Crystals									
Week 13	0	18	12	4	0	18	12	11	2
	1	0	0	6	6	0	0	1	11
	2	0	0	2	12	0	0	0	5
	N	18	12	12	18	18	12	12	18
Week 26	0	18	12	7	7	17	12	11	4
	1	0	0	5	7	1	0	0	9
	2	0	0	0	3	0	0	0	5
	N	18	12	12	17	18	12	11	18
Week R12	0	6	-	-	6	6	-	-	6
	N	6	0	0	6	6	0	0	6

0 Not detected
1 A few observed in some fields examined
2 A few observed in all fields examined
N Number of animals examined

[Table from NDA 204447 submission; Study No. LBK 150, page 46]

Gross Pathology

There was an increased incidence of enlarged, pale, granular kidneys with pelvic dilation in HDM compared to controls. There was an increased incidence of pale areas on the lungs in HDF compared to controls. There was an increased incidence of distended and thickened bile ducts with abnormal contents in HD males and females compared to controls. After the recovery period, there was an increased incidence of pale areas on the lungs of HD males and females. No effect was seen in the kidney and bile duct after the recovery period.

Organ Weights

The organs that were weighed are included in the histopathology inventory table in the Appendix (Table 91, page 152).

Increased kidney weight (absolute and relative to body weight) was noted for HDM (↑45%) and to a smaller extent for HDF (↑7%) compared to controls. After the recovery period, HDM had a small increase (↑13%) in relative kidney weight; although, there was no effect on absolute weight for males and absolute or relative for females. Increased liver weight (absolute and relative) was noted for MD and HD females (↑8 -15 and 13%, respectively) compared to controls. The increased liver weight relative to body weight was dose related. There was no effect on liver weight after the recovery period.

Decreased salivary gland and seminal vesicle weights (absolute and relative) were noted for HDM (↓20% for both) compared to controls. Increased spleen weight (relative) was noted for HDM (↑10%) compared to controls. There was no effect on salivary glands, seminal vesicle, or spleen weights after the recovery period.

Histopathology

Adequate Battery: Yes. The organs and tissues examined microscopically in controls and HD rats are listed in the histopathology inventory table in the Appendix (Table 91, page 152).

Peer Review: Yes

Histological Findings: Vortioxetine-related histopathological findings were seen in liver, kidney, and extrahepatic bile duct. Incidental findings were noted in the lungs.

Liver

Histopathological findings in the liver after 26 weeks of dosing are summarized in Table 12. Increased incidence and severity of bile duct hyperplasia was seen in MD and HD males and MD and HD females compared to controls; although, the incidence is much smaller in females than males. An increased incidence of centrilobular hepatocyte hypertrophy was noted in HDM and to a lesser extent in HDF. A low incidence of crystalline material in the bile ducts, pericholangitis, focal hepatocyte necrosis, and pigment in macrophages was noted primarily in HD males and females with no occurrence in the control group.

After the recovery period, the only reported finding was bile duct hyperplasia in 5/6 HDM and 1/5 HDF with no occurrence in controls. The severity was minimal for all but 1 HDM which was slight.

Table 12: Histopathological changes in the liver of rat after 26 weeks of dosing

Group and sex		1M	2M	3M	4M	1F	2F	3F	4F
Dosage (mg/kg b.i.d.)		0	10	20	40	0	10	20	40
Bile duct hyperplasia	Total	1	1	4	9	0	0	2	4
	Minimal	1	1	3	4	0	0	1	2
	Slight	0	0	1	4	0	0	1	2
	Moderate	0	0	0	1	0	0	0	0
Crystalline material in bile ducts	Total	0	0	0	2	0	0	0	1
	Present	0	0	0	2	0	0	0	1
Pericholangitis	Total	0	0	0	1	0	0	0	3
	Minimal	0	0	0	1	0	0	0	0
	Slight	0	0	0	0	0	0	0	3
Centrilobular hepatocyte hypertrophy	Total	5	2	3	10	1	2	2	4
	Minimal	5	2	3	10	1	2	2	4
Focal hepatocyte necrosis	Total	0	0	1	2	0	0	1	1
	Minimal	0	0	0	1	0	0	1	1
	Slight	0	0	1	1	0	0	0	0
Pigment in macrophages	Total	0	0	0	3	0	0	0	3
	Minimal	0	0	0	3	0	0	0	2
	Slight	0	0	0	0	0	0	0	1
Number of animals examined		12	12	12	11	12	12	11	12

[Table from NDA 204447 submission; Study No. LBK 150, page 48]

Kidney

Histopathological findings in the kidney of males after 26 weeks of dosing are summarized in Table 13. The presence of crystalline material in the tubules, papilla, and glomeruli was noted in 54% of HDM, with no findings of crystalline material in controls or other dose groups including HDF. The presence of the crystalline material may be the cause of the other reported findings which occurred in HDM but not controls or other dose groups, including tubular basophilia/regenerative hyperplasia, dilatation of collecting ducts, tubular dilatation, papillary necrosis/foreshortened papilla, hyperplasia of papillary and/or pelvic epithelium, and papillary and/or tubular inflammation. Interstitial fibrosis and pelvic dilatation occurred at increased incidence and severity in HDM compared to controls and LDM.

After the recovery period, similar histopathological findings seen after 26 weeks of dosing were noted in 1/6 HDM.

Table 13: Histopathological changes in the kidney of rat after 26 weeks of dosing

Group and sex Dosage (mg/kg b.i.d.)		1M 0	2M 10	3M 20	4M 40
Crystalline material in tubules/papilla/glomeruli	Total	0	0	0	6
Tubular basophilia/regenerative hyperplasia	Total	0	0	0	7
	Slight	0	0	0	1
	Moderate	0	0	0	5
	Marked	0	0	0	1
Dilatation of collecting ducts	Total	0	0	0	6
	Minimal	0	0	0	2
	Slight	0	0	0	2
	Moderate	0	0	0	1
	Marked	0	0	0	1
Tubular dilatation	Total	0	0	0	7
	Minimal	0	0	0	1
	Slight	0	0	0	5
	Moderate	0	0	0	1
Papillary necrosis/foreshortened papilla	Total	0	0	0	5
	Minimal	0	0	0	2
	Slight	0	0	0	3
Hyperplasia of papillary/pelvic epithelium	Total	0	0	0	6
	Minimal	0	0	0	1
	Slight	0	0	0	4
	Moderate	0	0	0	1
Interstitial fibrosis	Total	0	1	0	6
	Minimal	0	1	0	2
	Slight	0	0	0	4
Papillary/tubular inflammation	Total	0	0	0	7
	Minimal	0	0	0	2
	Slight	0	0	0	5
Pelvic dilatation	Total	2	4	0	6
	Minimal	1	2	0	1
	Slight	1	1	0	5
	Moderate	0	1	0	0
Number of animals examined		12	12	12	11

[Table from NDA 204447 submission; Study No. LBK 150, page 49]

Extrahepatic Bile Duct

Luminal dilatation, fibrosis, crystalline material in lumen, and epithelial hyperplasia were noted in 1 – 4 rats in the HD for males and females (except crystalline material was not noted in males), but were not seen in controls or lower dose groups. No findings were reported in the extrahepatic bile duct after the recovery period.

Table 14: Histopathological changes in the extrahepatic bile duct of rat after 26 weeks of dosing

	Males				Females			
Dose (mg/kg/bid)	0	10	20	40	0	10	20	40
Luminal dilatation	0	0	0	4	0	0	0	3
Fibrosis	0	0	0	2	0	0	0	3
Crystalline material in lumen	0	0	0	0	0	0	0	1
Epithelial hyperplasia	0	0	0	3	0	0	0	1
Number of animals examined	12	12	12	11	12	12	11	12

[Data from NDA 204447 submission; Study No. LBK 150, page 144]

Lungs

A small increase in incidence of foamy alveolar macrophages in the lungs of dosed females after 26 weeks of dosing and of HD males and females after the recovery period compared to controls was noted (Table 15). The foamy alveolar macrophages may correlate with the finding of pale areas of the lung upon gross examination. However, the incidence of foamy alveolar macrophages in the control male group after 26 weeks of dosing is similar to the dosed females; therefore, this is most likely an incidental finding.

Table 15: Incidence of foamy alveolar macrophages in the lungs of rats after 26 weeks of dosing and after 12 weeks of recovery

	Males				Females			
Dose (mg/kg/bid)	0	10	20	40	0	10	20	40
after 26 weeks of dosing	4	1	3	4	2	6	5	6
after 12 weeks of recovery	1	--	--	3	1	--	--	2

[Data from NDA 204447 submission; Study No. LBK 150, pages 136 and 145]

Toxicokinetics

Plasma exposures for vortioxetine and a major human metabolite (Lu AA34443) were determined on Day 1 and in Weeks 13 and 26. Because of sparse sampling in Week 13 (only at 1, 3, and 10 hours), estimation of TK parameters was only performed for Day 1 and Week 26. Due to misconduct at (b) (4) the TK data were not found to be GLP compliant. Based on remedial action, all dose groups for males on Day 1, the control (Lu AA34443 only), and the 40 mg/kg/bid dose groups in Week 26 were considered unreliable and were excluded from the TK evaluation. The plasma concentrations of Lu AA34443 for rats dosed with 10 and 20 mg/kg/bid in Week 26 changed as a result of reprocessing and were updated to reflect the reprocessed data. The Sponsor considered the TK data for the 10 and 20 mg/kg/bid dose groups reliable and adequate to establish a human safety ratio.

Vortioxetine

In general, plasma exposures (Cmax and AUC) were generally greater than dose proportional for vortioxetine. For females, exposures increased from Day 1 to Week 26 (for 10 and 20 mg/kg/bid). In Week 26, exposures were slightly higher for males than

females. The TK parameters are summarized in Table 16. The rat-to-human exposure margins for vortioxetine at the NOAEL for liver and kidney pathology are summarized in Table 17.

Table 16: Summary of mean TK parameters for vortioxetine

Occasion	Sex	Dosage mg/kg b.i.d.	C_{max} nmol/L	t_{max} h	AUC_{0-10h} h*nmol/L	AUC_{10-24h} h*nmol/L	AUC_{0-24h} h*nmol/L	CL/F L/hr/kg	AI
Day 1	M	10	nc*	nc*	nc*	nc*	nc*	nc*	na
		20	nc*	nc*	nc*	nc*	nc*	nc*	na
		40	nc*	nc*	nc*	nc*	nc*	nc*	na
Day 1	F	10	163	1	589	na	634	52.8	na
		20	706	1	2300	na	2542	26.3	na
		40	1090	1	4633	na	5651	23.5	na
Week 26	M	10	642	3	2799	2128	4928	13.6	nc*
		20	1925	1	7542	8019	15561	8.61	nc*
		40	nc*	nc*	nc*	nc*	nc*	nc*	nc*
Week 26	F	10	579	1	1682	1755	3437	nc*	2.86
		20	1809	12~	5687	8120	13806	9.71	2.47
		40	nc*	nc*	nc*	nc*	nc*	nc*	nc*

na = not applicable
~ = t_{max} value attained at 2 h post-second daily dose
nc* = not calculated; insufficient number of plasma concentrations available for toxicokinetic analysis

[Table from NDA 204447 submission; Study No. LBK 150, page 40]

Table 17: Rat-to-human exposure margin for vortioxetine at the NOAEL of 10 mg/kg/bid for liver and kidney pathology

Sex	AUC_{0-24h} (ng.h/mL)*	Exposure Margin at MRHD#
M	1471	2
F	1026	1.6

*Rat AUC_{0-24h} from Table 16 (Week 26) converted from nmol.h/L to ng.h/mL using the conversion factor 0.29845 (From Sponsor's Pharmacokinetics Written Summary, page 5). #Human repeat dose AUC_{0-24h} at 20 mg = 646 ng.hr/mL (From Sponsor's Summary of Clinical Pharmacology, Table 5.2, page 166).

Lu AA34443 (A Major Human Metabolite)

In general, exposures at Cmax were less than dose proportional and AUC exposures were greater than dose proportional for Lu AA34443. For females, exposures (AUC) increased from Day 1 to Week 26 (for 10 and 20 mg/kg/bid). In Week 26, exposures were higher for males than females. The TK parameters are summarized in Table 18. The rat-to-human exposure margin for Lu AA34443 at 20 mg/kg/bid vortioxetine (because the TK data from 40 mg/kg/bid is compromised) to the MRHD are summarized in Table 18.

Table 18: Summary of mean TK parameters for Lu AA34443

Occasion	Sex	Dosage mg/kg b.i.d.	C_{max} nmol/L	t_{max} h	AUC_{0-10h} h*nmol/L	AUC_{10-24h} h*nmol/L	AUC_{0-24h} h*nmol/L	AI	MR
Day 1	**M**	**10**	nc*	nc*	nc*	na	nc*	na	nc*
		20	nc*	nc*	nc*	na	nc*	na	nc*
		40	nc*	nc*	nc*	na	nc*	na	nc*
Day 1	**F**	**10**	2678	1	6612	na	7291	na	11.2
		20	6254	1	18487	na	20496	na	8.04
		40	9636	1	34830	na	42956	na	7.52
Week 26	**M**	**10**	5242	1	16462	13352	29814	nc*	5.88
		20	7970	1	39603	35265	74868	nc*	5.25
		40	nc*	nc*	nc*	nc*	nc*	nc*	nc*
Week 26	**F**	**10**	3135	1	9260	6760	16020	1.40	5.50
		20	5413	1	20757	23794	44551	1.12	3.65
		40	nc*	nc*	nc*	nc*	nc*	nc*	nc*

na = not applicable
nc* = not calculated; insufficient number of plasma concentrations available for toxicokinetic analysis

[Table from NDA 204447 submission; Study No. LBK 150, page 41]

Table 19: Rat-to-human exposure margin for major human metabolite Lu AA34443 at 20 mg/kg/bid vortioxetine

Sex	AUC_{0-24h} (ng.h/mL)*	Exposure Margin at MRHD#
M	24,589	44
F	14,632	26

*Rat AUC_{0-24h} from Table 18 (Week 26) converted from nmol.h/L to ng.h/mL using the conversion factor 0.32843 (From Sponsor's Pharmacokinetics Written Summary, page 5). #Human repeat dose AUC_{0-24h} at 20 mg = 563 ng.hr/mL (From Sponsor's Summary of Clinical Pharmacology, Table 5.2, page 166).

Dosing Solution Analysis

All formulations were prepared fresh each week and were refrigerated and protected from light until use. Formulation samples taken in Weeks 1, 13, and 26 were determined to be within 100.2 – 107.8% of the nominal concentrations.

6.2.2 Dog Repeat-Dose Toxicity

Summary of studies shorter than 52 weeks in duration in dog:

1-2 Week Oral MTD Finding Study in Dogs (Study No. 10243; non-GLP)
Beagle dogs (2/group) were dosed by oral gavage (in 5% hydroxypropyl-beta-cyclodextrin (HP-ß-CD)) with 25 and 10 mg/kg/day for one week and 15 mg/kg/day for 2 weeks. Clinical signs were pupil dilation after dosing in all doses; abdominal muscle contraction, salivation, slight to moderate vomiting, stiff body, whining, slight tremor, slight sedation at 15 and 25 mg/kg; and subdued behavior at 25 mg/kg. Body weight and food consumption were decreased in dogs receiving 25 mg/kg and 15 mg/kg (only slight body weight decrease and no decrease in food consumption). The MTD was considered to be 25 mg/kg.

4-Week Oral Toxicity Study in Dogs (Study No. 10071; GLP-compliant)
Beagle dogs (3/sex/group) were dosed by oral gavage (in 5% HP-ß-CD) with 0, 3.75, 7.5, and 15 mg/kg/day. Clinical signs were pupil dilation after dosing in all dose groups and slight to marked salivation at 15 mg/kg. Small increases in QT interval (7 – 14 ms) were seen for MD and HD females in Week 1 and HDM in Week 4 compared to controls; however, heart rate was highly variable for all groups and QT interval was not corrected. Small increases were noted for plasma creatinine in MDM and MD and HD females (≤13%), total protein in HD males and females (≤5%), and albumin for MD and HD males and HDF (≤12%). Small decreases were noted for plasma cholesterol in HD males and females (≤9%) and plasma triglycerides for MD and HD males and females (≤30%). However, there was high variability for all these parameters predose.

Decreased absolute and relative brain weight (in HDM and HDF), pituitary weight (in HDM and all dose female), pancreas weight (in MD and HD males and MD females), and lung weights (in all male dose groups and MDF) and increased absolute and relative heart weight (MD and HD males), adrenal weight (HD males), and thymus weight (all dose groups) were noted compared to controls without any histopathological correlation. Mild cytoplasmic vacuolation of midzonal hepatocytes were noted in 3/3 HDM and 1/3 HDF. Staining for triglycerides and glycogen were negative; although, the tissue was inadequately fixed and preservation of glycogen is uncertain.

Exposures (AUC and Cmax) generally increased more than dose-proportional, females had higher exposures than males, and exposures were higher at Day 28 than Day 1; although, there was high individual dog variability in the groups.

13-Week Oral Toxicity Study in Dogs Followed by a 4-Weeks Recovery Period (Study No. LBK 132; Reference No. 10300; GLP-compliant)
Beagle dogs (3/sex/group) were dosed by oral gavage (in 5% HP-ß-CD) with 0, 3.75 (LD), 5 (MD), 7.5 (HD), and 15 mg/kg/day for 13 weeks. Due to severe clinical signs and a premature death at the 15 mg/kg, dosing was stopped after 2 doses for 4 weeks and resumed at 10 mg/kg/day. However, because of clinical signs and a second premature death after one dose, 3 males and 3 females were re-allocated to the 7.5 mg/kg/day

recovery phase group (dosing at 7.5 mg/kg/day for 13 weeks followed by a 4-week recovery period).

Two doses of 15 mg/kg/day produced clinical signs of pupil dilation, inappetance, over activity, and unsteady or abnormal gait in males and females and prostration, excessive urination, and vocalization in males. In addition, one 15 mg/kg/day dosed female had two convulsions at 3 and 4 hours postdosing on Day 2 and was prematurely euthanized. At 10 mg/kg/day (1 dose), clinical signs were similar to those seen at 15 mg/kg/day and a second female had a convulsion after dosing and was prematurely euthanized.

A dose-related increase in severity and persistence of pupil dilation was seen at all doses in males and females throughout the dosing period. Excessive urination and occasional tremors and unsteady gait in MD and HD males were noted in the first two weeks of dosing. Abnormal gait, tremors, aggression with vocalization, and piloerection in HDM was noted in Weeks 1 and 7. Ophthalmic examination confirmed that there was incomplete pupil constriction in the light at all doses with severity increasing with dose. After the 4-week recovery period, there was no difference in pupillary response in HD compared to controls.

There was no clear vortioxetine-related effect on ECG parameters and heart rate.

Small increases were noted for plasma glucose in HDM in Weeks 6 and 13 (≤23%) and for total plasma protein in MD and HD males and HD females in Week 6 and HDM in Week 13 (≤7%). Plasma glucose and total protein were still increased 10% for HDM compared to controls after the 4-week recovery period.

There were no vortioxetine-related effects on organ weight or gross pathology. Minimal to slight involution/atrophy in the thymus was seen in 2/3 MDM, 1/3 MDF, 1/3 female controls, 2/3 MDF, and 1/3 HDF in Week 13. After the 4-week recovery period, minimal to moderate involution/atrophy was seen in 2/2 male controls, 3/3 HDM, and 3/3 HDF. Due to a lack of a dose response and to some findings in the control groups, a relationship to vortioxetine is unlikely. Hepatocellular vacuolation was not seen in this study.

Exposures (Cmax and AUC) to vortioxetine general increased dose-proportionally, males had higher exposures than females, and exposures were higher at Day 91 than Day 1 (Table 20); although, there was high individual dog variability in the groups.

Table 20: Summary of group mean (±SD) TK parameters for vortioxetine in dog

Sex	Dosage (mg/kg/day)	Group	Day	T_{max} (h)	C_{max} (nmol /L)	AUC_{inf} $AUC_{0\text{-}24h}$ (nmol·h/L)	$T_{1/2}$ (h)
Male	3.75	2	1	3.3±1.2	360±144	3454±1432	5.5±1.4
Female	3.75	2	1	3.3±1.2	228±113	2845(n=2)	5.1(n=2)
Male	5	3	1	4.0±0	502±102	5273±913	5.5±0.61
Female	5	3	1	2.7±1.2	447±90	3696±1136	4.8±0.32
Male	7.5	4	1	2.7±1.2	937±375	9328±3253	6.3±0.83
Female	7.5	4	1	2.7±1.2	603±139	5039±531	5.5±0.88
Male	10	5	1	2.0±0	1049±123	10629±2388	6.9±1.8
Female	10	5	1	2.0±0	892±227	9189±1221	5.1±0.73
Male	15	5	1	2.8±1.1	1791±284	19097±4180	6.3±1.1
Female	15	5	1	2.4±0.89	1220±638	13033±11211	5.9±1.5
Male	3.75	2	91	3.3±1.2	491±196	4334±1964	6.3(n=2)
Female	3.75	2	91	2.7±1.2	465±256	3541±2167	5.0±0.55
Male	5	3	91	2.0±1.0	853±197	6793±1500	5.4±0.44
Female	5	3	91	2.0±1.0	642±129	5073±161	5.8±0.53
Male	7.5	4	91	2.7±1.2	1018±200	9918±1948	6.1±0.69
Female	7.5	4	91	2.0±0	822±107	6432±452	4.8±0.17
Male	7.5	5A	91	2.7±1.2	1167±353	11263±4382	5.9±0.28
Female	7.5	5A	91	3.3±1.2	1001±132	9775±880	6.6±1.1

Conversion factor for Lu AA21004: 1nmol/L=0.2984 mg/mL.
*AUC_{INF} for Day 1 and $AUC_{0.24h}$ for Day 91
Exposure levels (Day 91) for Groups 4 and 5A combined:
$AUC_{0\text{-}24h}$ (nmol·h/L) : 10591±3121 (males) and 8104±1935 (females)
C_{max} (nmol/L) : 1093±269 (males) and 912±145 (females)

[Table from NDA 204447 submission; Study No. LBK 132, page 43]

Plasma exposures (Cmax and AUC) on Day 91 to the major human metabolite Lu AA34443, the reactive intermediate to Lu AA34443, Lu AA34994, a non-human metabolite Lu AA25790, and a non-major human metabolite, Lu AA39835, for this 13-week study were determined in Study No. 10715 (Table 21). The highest exposures were for the Lu AA34443 and the lowest for Lu AA39835. Exposures for Lu AA34443 were greater than for parent. Exposures generally increased dose-proportionally for Lu AA34443, Lu AA34994, and Lu AA25790 and less than dose proportional for Lu AA39835. Exposures were similar for males and females for all metabolites.

Table 21: Summary of group mean TK parameters for metabolites

Sex	Dose	Group	C_{max} (nmol/L) Lu AA34443	AUC_{0-24h} (nmol·h/L) Lu AA34443	C_{max} (nmol/L) Lu AA34994	AUC_{0-24h} (nmol·h/L) Lu AA34994
m	3.75	2	1467 ± 92	16638 ± 4942	9.9 ± 2.5	114 (n=2)
m	5	3	1823 ± 320	17380 ± 1801	18 ± 5.0	140 ± 33
m	7.5	4	2738 ± 519	29221 ± 8270	21 ± 1.7	246 ± 14
m	7.5	5A	3077 ± 327	31518 ± 6513	31 ± 7.3	283 (n=2)
f	3.75	2	1655 ± 95	16605 ± 1550	14 ± 5.1	114 (n=2)
f	5	3	2260 ± 308	22855 ± 834	17 ± 4.9	na
f	7.5	4	3110 ± 308	28584 ± 4395	16 ± 6.2	186 ± 52
f	7.5	5A	2570 ± 573	31560 ± 4831	23 ± 4.8	252 ± 26

Sex	Dose	Group	C_{max} (nmol/L) Lu AA25790	AUC_{0-24h} (nmol·h/L) Lu AA25790	C_{max} (nmol/L) Lu AA39835	AUC_{0-24h} (nmol·h/L) Lu AA39835
m	3.75	2	17 ± 1.9	158 (n=2)	5.6 ± 4.1	na
m	5	3	36 ± 11	245 ± 104	5.1 ± 1.2	62 ± 20
m	7.5	4	26 ± 6.2	184 (n=2)	7.5 ± 2.1	99 (n=2)
m	7.5	5A	44 ± 10	322 ± 129	7.9 ± 3.8	na
f	3.75	2	14 ± 5.2	98 ± 57	6.1 ± 3.2	92 (n=2)
f	5	3	25 ± 6.9	167 ± 20	5.1 ± 3.1	72 ± 50
f	7.5	4	18 ± 4.4	na	5.7 ± 3.0	51 ± 32
f	7.5	5A	30 ± 2.6	311 ± 54	8.0 ± 1.9	115 ± 356

[Data from NDA 204447 submission; Study No. 10715, page 5]

Plasma exposures (Cmax and AUC) of vortioxetine and the metabolites Lu AA34443 and Lu AA34994 were additionally analyzed for dogs dosed with 10 mg/kg/day from this 13-week study in Study No. 10459. The exposures (Cmax) for the female dog who had a convulsion (No. 148) were compared to the group means (Table 22). Compared to females, the female dog that had a convulsion had a higher Cmax for vortioxetine and the metabolites compared to the group mean Cmax and all individual Cmaxs. Compared to males, the female dog had higher exposures for group mean Cmax, but there were 1-2 male dogs that had similar exposures for vortioxetine and the two metabolites. Individual dog exposures are not shown here, except for No. 148.

Table 22: Cmax (nM) from Day 1 10 mg/kg dose group for vortioxetine, Lu AA34443, and Lu AA34994 from dog that had a convulsion, female group mean (contains No. 148), and male group mean

Anylate	No. 148	Female Mean	Male Mean
Vortioxetine	1180	892	1050
Lu AA34443	4010	2630	3520
Lu AA34994	5.71	3.38	4.10

[Data from NDA 204447 submission; Study No. 10459]

The NOAEL was 7.5 mg/kg/day based on convulsions in one female at 15 mg/kg/day and in one female at 10 mg/kg/day.

Study title: Toxicity Study by Oral Gavage Administration to Beagle Dogs for 52 Weeks Followed by a 12 Week Recovery Period Amended Final Report

Study no.:	LBK 146/053960; Reference no. 10892
Study report location:	Archives of Lundbeck
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 18, 2004
GLP compliance:	Yes, except for TK data
QA statement:	Yes
Drug, lot no., and % purity:	Lu AA21004 HBr, 2018324, 100%

Key Study Findings

- Beagle dogs were dosed orally (by gavage, in 5% hydroxypropyl-beta-cyclodextrin) at 0, 3.75, 5, or 7.5 mg/kg/day for 52 weeks followed by a 12-week recovery period.
- There were no premature mortalities.
- Clinical signs were limited to pupillary dilation after dosing in mid and high dose males and high dose females.
- There were small increases in plasma glucose for all dosed males and high dose females throughout the dosing period and decreases in plasma triglycerides for high dose males and females in Week 52.
- There was a dose-dependent increase in urine volume in males in Week 52.
- Spleen and thymus weights were slightly decreased in mid and high dose males and ovary, pituitary, and uterus weights were slightly decreased in high dose females. There were no histopathological correlates.
- The NOAEL is 6- and 8-times the MRHD of 20 mg on a mg/m2 basis, based on pupillary dilation males and females, respectively.

Methods

Doses:	0, 3.75 (LD), 5 (MD), or 7.5 (HD) mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	Solution/5% HP-ß-CD
Species/Strain:	Dog/Beagle
Number/Sex/Group:	4/sex/group main; 2/sex/control and HD recovery
Age:	20 – 23 weeks at start of dosing
Weight:	Males: 7.8 – 10.5 kg; Females: 6.7 – 9.4 kg
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Study protocol and SOP deviations were found for the TK evaluation performed at (b) (4) which resulted in the inability to claim GLP compliance for the TK data.

Observations and Results

Mortality

There were no premature mortalities.

Clinical Signs

Slight to moderate (isolated occasions marked) pupillary dilation was seen in all HD dogs 0.5 – 2 hours after dosing with variable frequency (isolated to frequent incidents). Three MD males had isolated incidents of pupillary dilation.

Body Weights

MD and HD males and all female dose groups gained slightly more body weight (non-dose dependent) over the 52 weeks than controls (↑11, 4, 7.3, 14, and 11% for MDM, HDM, LDF, MDF, and HDF, respectively).

Feed Consumption

MD and HD males and females non-dose dependently consumed 7 – 16% less feed than controls during Week 1. Males continued to consume slightly less feed than controls to Week 4, while females consumed similar amounts to controls at Week 2. Feed consumption was similar for the remainder of the dosing period.

Ophthalmoscopy

There were no vortioxetine-related ophthalmology findings.

ECG

There were no consistent findings suggesting a vortioxetine-related effect on heart rate and ECG parameters. QT and QTcV for individual dogs (1 HDM and 1LDF at W13, 1 LDM and 1HDF at W26, 1 control male and LDM at W26 and W52) were occasionally above the 95% maximum confidence limit for the background data from this laboratory—the Sponsor considered these dogs to have prolonged QT/QTcV. For one of the LDMs, it was the same dog in W26 and W52 and this male had a high QT and QTcV interval prior to the start of dosing. In addition, one control dog was above the 95% maximum confidence limit in Weeks 26 and 52, there was no dose-response, and there was no difference for the mean values of QT and QTcV for dosed groups compared to controls.

Two hours after dosing, there was a non-dose-dependent, 17 – 26% and 15 – 39% increase in systolic, diastolic, and mean arterial pressure in all male dose groups in Week 26 and LD and MD males in Week 52, respectively. Only the HD was statistical significant it Week 26 and there were no statistically significant findings in Week 52. There was no difference in any blood pressure measurements at 24 hours after dosing in Weeks 26 and 52.

Hematology

The pattern of variation did not indicate any vortioxetine-related effect on hematology parameters.

Clinical Chemistry

Plasma glucose concentrations were slightly increased for HDF (↑7 – 10%) and non-dose-dependently increased for all male dose groups (↑10– 19%) in Weeks 13 (HDF only), 26, and 52 compared to controls. Plasma glucose concentrations for HD males and females were similar to controls after the 12-week recovery period. Plasma cholesterol was slightly decreased for HDM (↓16%) in Weeks 13 and 26 and HDF (↓20%) in Week 52 compared to controls. However, plasma cholesterol was decreased 9 – 13% for HD males and females compared to controls prior to dosing. Plasma triglycerides were decreased 21% for HDM and 50% for HDF compared to controls in Week 52. Plasma cholesterol and triglycerides were not analyzed after the 12-week recovery period.

Urinalysis

There was a dose-dependent (significant at HD) increase in urine volume in dosed males compared to controls in Week 52 (↑34, 53, and 141% for LD, MD, and HD, respectively). However, increase urine volume was not seen at Weeks 13 and 26 for males or all time points for females.

Gross Pathology

There were no vortioxetine-related macroscopic findings.

Organ Weights

The organs that were weighed are included in the histopathology inventory table in the Appendix (Table 91, page 152). For males, spleen weight was decreased 23 – 33% (unadjusted and relative to body weight) for MD and HD males and thymus was non-dose-dependently decreased 21 – 39% unadjusted and 33 – 43% relative to body weight compared to controls. For females, ovary weight, pituitary weight, and uterus weight were decreased 25 – 28% (unadjusted and relative to body weight), 20 – 29% (unadjusted and relative to body weight), and 83% unadjusted and 48% relative to body weight, respectively for HDF compared to controls. Thymus weight was non-dose-dependently increased 50 – 79% (unadjusted and relative to body weight) for all female dose groups compared to controls. In all cases, individual animal weights within a group were highly variable and there were no histopathological correlates.

Histopathology

Adequate Battery: Yes. The organs and tissues examined microscopically are listed in the histopathology inventory table in the Appendix (Table 91, page 152).

Peer Review: Yes, by a reviewing pathologist.

Histological Findings: There were no vortioxetine-related histopathological findings.

Toxicokinetics

Plasma exposures for vortioxetine and a major human metabolite (Lu AA34443) were determined predose and 1, 2, 4, 8, 10, and 24 hours after dosing on Day 1 and in Weeks 26 and 52 and predose and 4 hours after dosing in Weeks 13 and 39. Due to

misconduct at (b) (4) the TK data were not found to be GLP compliant. Based on remedial action, the Sponsor determined that the LD male and female vortioxetine data from Week 52 and all data for Lu AA34443, except Day 1, were impacted.

Vortioxetine

In general, plasma exposures (Cmax and AUC) of vortioxetine were generally dose proportional or greater than dose proportional. AUC exposures tended to increase over time for males and females. Exposures were slightly higher for males than females at the LD, but similar for the MD and HD at all time points. The TK parameters are summarized in Table 23. The dog-to-human exposure margins for vortioxetine at the NOAEL for pupillary dilation are summarized in Table 24.

Table 23: Summary of mean TK parameters for vortioxetine in dog

Occasion	Sex	Dose (mg/kg)	C_{max} (nmol/L)	AUC_{0-24h} (h*nmol/L)
Day 1	Male	3.75	501	3033
		5	582	4163
		7.5	1111	7205
	Female	3.75	191	1450
		5	682	4597
		7.5	923	7179
Week 26	Male	3.75	445	3577
		5	552	5374
		7.5	1206	10632
	Female	3.75	242	1837
		5	755	5951
		7.5	1238	11692
Week 52	Male	3.75	444	4254
		5	686	7543
		7.5	1034	10226
	Female	3.75	298	2276
		5	755	6629
		7.5	968	10556

[Table from NDA 204447 submission; Study No. LBK 146, page 36]

Table 24: Dog-to-human exposure margin for vortioxetine at the NOAEL for pupillary dilation

Sex	Dose (mg/kg/day)	AUC_{0-24h} (ng.h/mL)*	Exposure Margin at MRHD#
M	3.75	1270	2
F	5	1978	3

*Rat AUC_{0-24h} from Table 23 (Week 52) converted from nmol.h/L to ng.h/mL using the conversion factor 0.29845 (From Sponsor's Pharmacokinetics Written Summary, page 5). #Human repeat dose AUC_{0-24h} at 20 mg = 646 ng.hr/mL (From Sponsor's Summary of Clinical Pharmacology, Table 5.2, page 166).

Lu AA34443 (A Major Human Metabolite)
In general, exposures (Cmax and AUC) were dose-proportional or greater than dose-proportional for Lu AA34443 on Day 1. Exposures were similar for males and females on Day 1. AUC exposures for Lu AA34443 were 2.5 – 4.7-fold the exposures to parent, vortioxetine, on Day 1. The TK parameters are summarized in Table 25.

Table 25: Summary of mean TK parameters for Lu AA34443 in dog

Occasion	Sex	Dose (mg/kg)	C_{max} (nmol/L)	AUC_{0-24h} (h*nmol/L)
Day 1	Male	3.75	978	7830
		5	1343	11641
		7.5	2193	18022
	Female	3.75	1007	6841
		5	1766	12957
		7.5	1986	18080
Week 26	Male	3.75	1705	16303
		5	NC	NC
		7.5	NC	NC
	Female	3.75	1796	16375
		5	NC	NC
		7.5	NC	NC
Week 52	Male	3.75	NC	NC
		5	NC	NC
		7.5	2933	29069
	Female	3.75	NC	NC
		5	NC	NC
		7.5	NC	NC

[Table from NDA 204447 submission; Study No. LBK 146, page 37]

Dosing Solution Analysis

All formulations were prepared fresh each week. Formulation samples taken in Weeks 1, 13, 26, 39, and 52 were determined to be within 100 – 109.5% of nominal concentrations.

7 Genetic Toxicology

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

Study title: Lu AA21004: Ames Test in Four Strains of Salmonella typhimurium and Two Strains of Escherichia coli

Study no.:	113-855; Reference no. 10073
Study report location:	Regulatory Central Archive, H. Lundbeck A/S, DK-2500 Valby, Copenhagen Denmark
Conducting laboratory and location:	H. Lundbeck A/S, Ottiliavej 9, DK-2500 Valby, Copenhagen Denmark
Date of study initiation:	February 17, 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot no., and % purity:	Lu AA21004, 1001258, 100%

Key Study Findings

- Vortioxetine was negative for mutagenicity in bacterial cells in a valid Ames test.

Methods

Strains:	Salmonella typhimurium – TA98, TA100, TA1535, and TA1537; Escherichia coli – WP2 pKM101 and WP2 uvrA pKM101
Concentrations in definitive study:	Experiments 1 and 1a – 2.344, 4.688, 9.375, 18.75, 37.5, 75, 150, and 300 µg/plate Experiments 2 – (+S9) 9.375, 18.75, 37.5, 75, 150, 300; (-S9) 2.344, 4.688, 9.375, 18.75, 37.5, 75 µg/plate
Basis of concentration selection:	The concentrations for Experiments 1 and 1a were based on the cytotoxicity seen (≥150 µg/plate +S9; ≥25 µg/plate –S9) in preliminary studies (Study Nos. 99875 and 99934). The concentrations for Experiment 2 were based on the cytotoxicity seen in Experiments 1 and 1a.
Negative control:	Dimethyl sulphoxide (DMSO)
Positive control:	Without S9 – 9-Aminoacridine (TA1537), 4-nitroquinoline-N-oxide (WP2 pKM101, WP2 uvrA pKM101), Daunomycin (T98), Sodium azide (TA100, TA1535); With S9 – 2-aminoanthracene (TA100, TA1537, TA1535, WP2 uvrA pkM101, WP2 pkM101), Benzo(a)pyrene (TA98)
Formulation/Vehicle:	Solution/DMSO

Incubation & sampling time:	The plate incorporation assay was used for Experiment 1 and 1a and the pre-incubation assay was used for Experiment 2 (strains were incubated with test article for 1 hour prior to addition to agar plates). Plates incubated for 2 – 3 days at 37^{o}C.

Study Validity

The selection of the bacterial tester strains was adequate. Dose selection for the plate incorporation assay and the pre-incubation assay was adequate based upon cytotoxicity that was seen in all test strains with and without S9 at least one dose for each test performed. A minimum of 3 non-cytotoxic doses were tested for each strain with and without S9 except for strain TA98 in the pre-incubation assay in which only 2 non-cytotoxic doses were tested. However, an adequate number of non-cytotoxic doses (4) were tested in the plate incorporation assay and the results were consistent for both assays with TA98 strain.

The positive controls yielded the expected mutation frequencies that were significantly greater than the negative controls. The negative control mutation frequencies were within the historical control range except in Experiment 1 where strain WP2 uvrA pKM101 values were significantly above the historical range with and without S9. Therefore, the data from strain WP2 uvrA pKM101 in Experiment 1 were not considered valid and Experiment 1a was performed to retest strain WP2 uvrA pKM101.

Results

No precipitation of vortioxetine was observed. Cytotoxicity (defined as a reduction or lack of bacterial lawn or a statistically significant decrease in the mean number of revertants) was observed at concentrations ≥37.5 µg/plate without S9 and ≥150 µg/plate with S9 for plate incorporation assay and ≥9.375 µg/plate without S9 and ≥75 µg/plate with S9 for pre-incubation assay.

Vortioxetine showed no increase in the number of reverse mutations of any strain with and without S9 in the plate incorporation and pre-incubation assays.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Lu AA21004 (Batch No. 1001258): Chromosome Aberration Assay in Cultured Human Peripheral Blood Lymphocytes

Study no.:	10204; Report No. 112-855
Study report location:	Regulatory Central Archive, H. Lundbeck A/S, DK-2500 Valby, Copenhagen Denmark
Conducting laboratory and location:	Non-Clinical Safety Research, H. Lundbeck A/S, Ottiliavej 9,DK-2500 Valby, Copenhagen Denmark
Date of study initiation:	March 26, 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot no., and % purity:	Lu AA21004, 1001258, 100%

Key Study Findings

- Vortioxetine was negative for clastogenicity in human peripheral blood lymphocytes in a valid *in vitro* chromosome aberration assay.

Methods

Cell line:	Human peripheral blood lymphocytes
Concentrations in definitive study:	-S9 for 20 hrs – 1.63, 2.07, 2.63, 3.34, 4.24, 5.38, 6.83, 8.68, 11 µg/mL; ±S9 for 3 hrs - 1.63, 2.07, 2.63, 3.34, 4.24, 5.38, 6.83, 8.68, 11, 14, 17.8, 22.6 µg/mL
Basis of concentration selection:	The concentrations were based on cytotoxicity seen in preliminary studies. Test article precipitated at concentrations >597 µg/mL. The test solution did not change the pH of the cell culture media.
Negative control:	Dimethyl sulphoxide (DMS0)
Positive control:	-S9 – methyl methanesulphonate; +S9 – Cyclophosphamide
Formulation/Vehicle:	Solution/DMSO
Incubation & sampling time:	In Experiment 1, cells with or without S9 were incubated with test article for 3 hours followed by a 17-hour recovery. In Experiment 2, cells without S9 were incubated with test article for 20 hours and cells with S9 were incubated for 3 hours followed by a 17-hour recovery. Colchicine was added 1.5 hours prior to harvest.

Study Validity

The appropriate number of cells was evaluated and two replicates of each concentration were tested. Only cells with 46 chromosomes were scored and a minimum of 200 cells per negative control and vortioxetine treated cells and 50 cells per positive control treated cells were analyzed. Dose selection for analysis based on mitotic index was adequate. The number of structural aberrations for the negative controls was within the historical vehicle control range. The number of structural aberrations for the positive controls was significantly greater than the negative controls.

Results

Vortioxetine decreased the mitotic activity (43.3 – 65.2%) of human peripheral blood lymphocytes at ≥6.63 µg/mL without S9 and ≥6.63 µg/mL with S9. No evaluable metaphases were present at higher concentrations.

Vortioxetine showed no increase in the number of aberrant metaphases or in polyploidy at any evaluable concentration with or without S9 in both experiments performed.

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

Study title: Lu AA21004: Induction of micronuclei in the bone marrow of treated rats

Study no:	356/240; Reference no. 11468
Study report location:	(b) (4)
Conducting laboratory and location:	
Date of study initiation:	March 14, 2006
GLP compliance:	Yes, except for TK
QA statement:	Yes
Drug, lot no., and % purity:	Lu AA21004, 2018324, 100%

Key Study Findings

- Vortioxetine is not clastogenic in a valid *in vivo* micronucleus assay at oral doses up to 305 mg/kg.

Methods

Doses in definitive study:	125, 250, and 305 mg/kg/day
Frequency of dosing:	Once daily for two days ~24 hours apart
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	Solution/15% hydroxypropyl-ß-cyclodextrin (HP-β-CD)
Species/Strain:	Rat/Wistar Crl:WI
Number/Sex/Group:	6 males/group
Satellite groups:	3 males dosed with 305 mg/kg/day for PK

Basis of dose selection:	Dose range finding in 3/sex/group (200 and 500 mg/kg/day) in this study. 500 mg/kg was chosen as the high dose to be tested in the dose range finding study because of solubility issues. The intended high dose for the definitive study was 500 mg/kg; however, base upon analysis of the dosing solution, the actual dose was 305 mg/kg. Because no differences in toxicity were seen between males and females, the definitive study was conducted in males only.
Negative control:	15% HP-β-CD
Positive control:	Cyclophosphamide at 20 mg/kg

Study Validity

PK assessment showed systemic exposure and dosing was adequate based on solubility limitations. The incidence of micronucleated polychromatic erythrocytes (PCE) in the negative control group was consistent with the historical vehicle control data from this laboratory. The incidence of micronucleated PCE in the positive control group was statistically significantly increased compared to the negative control group.

Results

MD and HD rats did not gain weight over the dosing period while the vehicle control rats gained an average of 13g over the dosing period. No statistically significant increase in the number of micronucleated PCE was seen in any of the vortioxetine dosed groups.

8 Carcinogenicity

8.1.1 Rat Carcinogenicity

Study title: Lu AA21004: Carcinogenicity study by twice daily oral gavage administration to Wistar Han rats for 104 weeks and Amendment 1

Study no.:	LBK0201; Reference no. 11689
Study report location:	(b) (4)
Conducting laboratory and location:	
Date of study initiation:	March 7, 2007
GLP compliance:	Yes, except TK
QA statement:	Yes
Drug, lot no., and % purity:	Lu AA21004 (hydrobromide salt), 2108448, 99.1%
CAC concurrence:	Yes

Key Study Findings

- Male and female Wistar rats were dosed orally (by gavage, in 10% hydroxypropyl-beta-cyclodextrin (HP-ß-CD)) at 0 (water), 0 (vehicle), 2, 7, or 20 mg/kg/bid and 0 (water), 0 (vehicle), 5, 15, or 40 mg/kg/bid, respectively, for 104 weeks.
- The doses had been approved by the Executive CAC with the high dose in males based on MTD (renal pathology) and the high dose in females based on a 25-fold AUC ratio to human exposure, provided that the maximum clinical dose does not increase such that the rat-to-human AUC ratio falls below 25.
- The MTD was demonstrated for females based on increased mortality due to blockage of the common bile duct by crystals. The MTD was not demonstrated for males; although, the high dose is adequate based on the MTD (renal pathology) in the 26-week general toxicology study.
- There was a statistically significant increase in polypoid adenomas of the rectum in high dose females (40 mg/kg/bid) with a safety ratio of 15-fold at the NOAEL to the MRHD of 20 mg on mg/mg2 bases.
- There were numerical increases in the incidences of hepatocellular adenomas and combined hepatocellular adenomas and carcinomas in high dose males and females (20 and 40 mg/kg/bid, respectively), hemangiomas in the mesenteric lymph node in mid and high dose males (7 and 20 mg/kg/bid, respectively), and histiocytic sarcomas in high dose males (20 mg/kg/bid). However, statistical significance was not reached. Non-neoplastic findings of hepatotoxicity and crystalline material in the hepatic bile ducts were seen in high dose males and mid and high dose females.

Adequacy of Carcinogenicity Study

The study design (including route of administration, number of rats, length of dosing, and two control groups) of this 104-week rat carcinogenicity study is considered adequate. The HD for males and females (20 and 40 mg/kg/bid, respectively) was selected with concurrence from the Executive CAC based on the MTD (renal pathology) for males and on AUC for females in the 26-week rat general toxicology study (Meeting Minutes dated January 17, 2007).

In males, dosing with vortioxetine up to 20 mg/kg/bid did not increase mortality, cause significant clinical signs, or adversely affect body weight and is below an MTD. However, the HD was determined based on renal pathology in the 26-week general toxicology study; and therefore, is considered adequate. In females, dosing with vortioxetine up to 40 mg/kg/bid did increase premature mortality due to partial or total blockage of the common bile duct by crystals; therefore, the HD is at or above the MTD and is considered adequate. Although premature mortality was higher for the HD females compared to both control groups, survival was adequate (36% survived to termination) and sufficient numbers of rats were exposed for a sustained amount of time to adequately asses the risk of late developing tumors. At the HD in females, vortioxetine did not cause significant clinical signs or adversely affect body weight.

Appropriateness of Test Models

The 104-week carcinogenicity study in Wistar rats is an appropriate model. The use of two controls (water and HP-ß-CD) was appropriate because oral HP-ß-CD is known to cause pancreatic neoplasms in rat.

Evaluation of Tumor Findings

The liver, mesenteric lymph node, hematopoietic tissue, and rectum were target organs of neoplastic changes.

The incidence of hepatocellular adenomas was numerically increased for males and females at the HD (20 and 40 mg/kg/bid, respectively) and the incidence is slightly higher than the historical control range for this laboratory (b) (4). However, the increase did not reach statistical significance for a common tumor; although, there was a significant trend for females. Also, the increase in the combination of hepatocellular adenomas and carcinomas for males and females did not reach statistical significance for a common tumor. Non-neoplastic findings of hepatotoxicity and crystalline material in the hepatic bile ducts were seen in HD males and MD and HD females. In general, the incidence and severity of non-neoplastic findings in the liver was less in males than females most likely due to the lower doses in males, except for the finding of centrilobular hepatocyte hypertrophy which was only seen in males.

The incidence of hemangiomas in the mesenteric lymph node was numerically increased for MD and HD males and the incidence is slightly higher than the historical control range for this laboratory. The increase was significant for trend test; however, did not reach statistical significance for the pairwise comparison or for the combination of hemangiomas and hemangiosarcomas at all sites. No hemangiosarcomas were

found in the mesenteric lymph node in males. It should be noted that the incidence of hemangiomas in the mesenteric lymph node for the vehicle control was in the middle of the historical control range and the historical control range does not contain rats that have received the vehicle, HP-ß-CD.

The incidence of histiocytic sarcomas was slightly increased for HD males compared to vehicle controls and the incidence was slightly higher than the historical control range for this laboratory. However, the increase did not reach statistical significance and the male water control group had one rat reported to have a histiocytic sarcoma, which is in the high end of the historical control range.

There was a statistically significant increase in polypoid adenomas of the rectum in HD females. No polypoid adenomas were seen in either control group for males or females or in the historical control data for this laboratory. The vehicle, HP-ß-CD, has been shown previously to increase neoplasms of the large intestine of rat at 5000 mg/kg (4/50 males and 2/50 females), with no neoplasms seen at 500 mg/kg[3]. Although in the current carcinogenicity study all groups that received vehicle (control and vortioxetine) had increased epithelial hyperplasia and mucosal inflammation in the large intestine (cecum, colon, and rectum) compared to water controls, a clear dose response was seen for polypoid adenomas while no dose response was seen for the increased hyperplasia and inflammation. Because all groups received the same volume of vehicle (5 mL/kg; 500 mg/kg/bid) and there is still a dose response for polypoid adenomas in females, a drug effect cannot be discounted. Therefore, I consider the finding of polypoid adenomas in the rectum vortioxetine related.

Study results were independently reviewed and evaluated by the CDER statistical reviewer, Matthew Jackson, Ph.D. (see Statistical Review and Evaluation). Dr. Jackson's statistical analysis confirmed that the increased incidence of hepatocellular adenomas and the combination of hepatocellular adenomas and carcinomas in males and females did not meet the standard for statistical significance for common tumors (trend $p<0.005$, pairwise $p<0.01$; Table 26 and Table 27). In addition, Dr. Jackson's statistical analysis confirmed that statistical significance for both trend test and pairwise comparisons were not met for hemangiomas in the mesenteric lymph node and histiocytic sarcomas for HD males (Table 26). Dr. Jackson's statistical analysis confirmed that the increased incidence of polypoid adenomas in the rectum of HD females did meet statistical significance for both trend test and pairwise comparison for a rare tumor (trend $p<0.025$, pairwise $p<0.05$; Table 27).

[3] Pharmacology/Toxicology review of NDA 20-966 for itraconazole dated 3/19/1999

Table 26: Statistical results for neoplasms in male rat compared to vehicle (HP-ß-CD) control

Organ/Tissue	Tumor	Dose (mg/kg/bid) n= 55/group					Trend test p-value	Pairwise Test (V vs. HD) p-value
		W	V	2	7	20		
Liver	adenoma	1	0	2	2	4	0.0364	0.0539
	carcinoma	0	0	1	0	1	0.3046	0.4898
	adenoma + carcinoma	1	0	3	2	5	0.0277	0.0252
Mesenteric lymph node	Hemangioma	3	6	6	13	15	0.004	0.0163
	Hemangiosarcoma	0	0	0	0	0	--	--
	All sites hemangioma + hemangiosarcoma	4	7	9	13	15	0.0180	0.0313
Hematopoietic tissue	Histiocytic sarcoma	1	0	0	1	3	0.0173	0.1137

W = Water Control; V = Vehicle Control
[Data from Dr. Jackson's statistical analysis]

Table 27: Statistical results for neoplasms in female rat compared to vehicle (HP-ß-CD) control

Organ	Tumor	Dose (mg/kg/bid) n=55/group					Trend test p-value	Pairwise Test (V vs. HD) p-value
		W	V	5	15	40		
Liver	adenoma	1	0	1	0	4	0.0072	0.0474
	carcinoma	0	0	0	1	0	0.4709	--
	adenoma + carcinoma	1	0	1	1	4	0.0103	0.0474
Rectum	polypoid adenoma	0	0	0	1	4	0.0034	0.0448

W = Water Control; V = Vehicle Control
[Data from Dr. Jackson's statistical analysis]

Methods

Doses: Male: 0 (water), 0 (vehicle), 2 (LD), 7 (MD), or 20 (HD) mg/kg/bid; Female: 0 (water), 0 (vehicle), 5 (LD), 15 (MD), or 40 (HD) mg/kg/bid
Frequency of dosing: Twice daily (10 hours apart)
Dose volume: 5 mL/kg
Route of administration: Oral gavage
Formulation/Vehicle: Solution/10% HP-ß-CD and 4.4% glucose monohydrate

Basis of dose selection:	Dose selection is based on the 13- and 26-week rat toxicology studies (Study Nos. 10304 and LBK/0150) with dosing at 10, 20, and 40 mg/kg/bid. For males, the HD of 20 mg/kg/bid was based on the MTD for renal pathology. For females, the HD of 40 mg/kg/bid was based on a 25-fold AUC ratio to human exposure, provided that the rat-to-human AUC ratio falls below 25 (Executive CAC concurrence January 17, 2007).
Species/Strain:	Rat/Wistar Han (Crl: WI (Han))
Number/Sex/Group:	55/sex/group
Age:	30 – 40 days
Animal housing:	5 of the same sex/cage
Paradigm for dietary restriction:	Free access to feed and water
Dual control employed:	Yes, water and vehicle
Interim sacrifice:	No
Satellite groups:	15/sex/group for TK
Deviation from study protocol:	Study protocol and SOP deviations were found for the TK evaluation performed at (b) (4) which resulted in the inability to claim GLP compliance for the TK data.

Observations and Results

Mortality

The distribution of mortality rate by group and sex is listed in Table 28. There were 189 premature deaths (69 males and 120 females). HDF had higher early mortality compared with the water and vehicle control groups (36% survival vs. 67 and 55% survival, respectively). Two HDF were euthanized early due to the presence of a polypoid adenoma in the rectum. The cause of premature death for the remaining HDF was considered due to partial or total blockage of the common bile duct by crystals.

Table 28: Mortality rate at end of dosing

Group/sex	1M	2M	3M	4M	5M	1F	2F	6F	7F	8F
Dose (mg/kg b.i.d.)	0	0*	2	7	20	0	0*	5	15	40
Total number of deaths	16	10	14	11	18	18	25	16	26	35
Total number surviving	39	45	41	44	37	37	30	39	29	20
Percentage survival	71	82	75	80	67	67	55	71	53	36

* Vehicle (hydroxypropyl-β-cyclodextrin) control

[Table from NDA 204447 submission; Study No. LBK0201, page 47]

The statistical analyses performed by Dr. Jackson showed a dose related effect on survival in female rats (significant trend test, p=0.0003; but pairwise comparison of HDF with vehicle control not significant, p=0.0587), but not male rats.

Clinical Signs

Piloerection was observed in HDF throughout dosing, in one MDF in Week 48, in one HDM in Week 28, and in all HDM in Week 48. Chin rubbing and salivation occurred from Day 3 and throughout dosing in MD and HD males and all dosed females (except salivation was not observed in LDF).

There was no effect of vortioxetine on the incidence and multiplicity (number/rat) of palpable masses. The time of first onset of palpable masses was earlier for HDF compared to the other female groups (86, 86, 88, 79, and 72 weeks for water, vehicle, LD, MD, and HD females, respectively); however, there was no effect for males.

Body Weights

Vortioxetine had no adverse effect on body weight. MD and HD males gained more weight compared to vehicle for most of the dosing period except starting in Week 88 when HDM stopped gaining weight. At the end of dosing HDM only weighed 2% more than vehicle, while MDM weighed 8% more than vehicle. MD and HD females gained more weight for most of the dosing period and LD females gained more weight starting in Week 80 compared to vehicle. At the end of dosing, LD, MD, and HD females weighed 6, 10, and 6% more than vehicle.

In general, males and females given HP-ß-CD vehicle (control and vortioxetine dosed, except MD males and females) gained less weight than the water controls during the dosing period (starting Week 12 for control and LD males, Week 28 for control and LD females, Week 60 for HDM, and Week 69 for HDF). Vehicle, LD, and HD males weighed on average 8 – 10% less than water controls at the end of dosing (Week 104). Vehicle, LD, and HD females weighed on average 4 – 10% less than water controls at the end of dosing, with the vehicle group weighing the lowest.

Feed Consumption

Males given vehicle (control and vortioxetine dosed, except MDM) consumed 4 – 5% less feed compared to water controls. Vortioxetine dosed males and females generally consumed similar amounts of feed to vehicle controls.

Ophthalmology

There were no vortioxetine related ophthalmology findings.

Hematology

A small decrease in red blood cells (3%) was noted in Weeks 52 (significant) and 104 for HDM. In Week 104, there was a small, but significant increase in mean cell hemoglobin (3%) and mean cell hemoglobin concentrations (2%) for MDM (MCHC only) and HDM. In contrast, a small significant increase in red blood cells (4%) and in mean cell hemoglobin concentrations (2%) was noted in Week 52 only for MDF (MCHC only) and HDF. A small significant decrease in mean cell volume (4%) was noted in HDF in Week 52.

Increases in white blood cells (≤40%), neutraphils (≤60%), and monocytes (≤63%) were noted in HD males and females in Weeks 52 and 104. Neutraphils (≤24%) and monocytes (≤45%) were also increased for MD males in Week 52.

Clinical Chemistry

At the end of the dosing period, alkaline phosphatase and amino transferase activities were increased for HD males, MD females, and HD females compared to vehicle (ALP: 11, 48, and 72%, respectively; ALT: 61, 49, and 40%, respectively). Plasma urea concentrations were decreased for HD males, MD females, and HD females (13, 15, and 26%). Plasma phosphorous was increased for HD males, MD females, and HD females (11, 9, and 17%, respectively). Plasma potassium and calcium were increased for MD and HD females (K: 10 and 18%, respectively; Ca: 1.9 and 4%, respectively). Plasma triglycerides were increased 33% for HD females.

Urinalysis

Male and female rats dosed with HP-ß-CD (control and vortioxetine) had decreased urinary volume (≤35%) compared to water controls in Week 103. However, compared to HP-ß-CD vehicle controls, male rats had a dose-related increase in urinary volume (18, 23, and 26% for LD, MD, and HD, respectively). Urine protein concentrations were increased dose dependently in males (69, 165, and 230% for LD, MD, and HD, respectively) and in HDF (242%).

Microscopic examination of urine indicated a high presence of crystals in the urine of HDF (Table 29). Urine samples from these HDF were LC-MS/MS analyzed to determine the chemical composition of the crystals in Study No. 13185. The crystals were determined to consist of the major-human metabolite, Lu AA34443.

Table 29: Incidence of crystals in the urine

Group/sex	1M	2M	3M	4M	5M	1F	2F	6F	7F	8F
Dose (mg/kg b.i.d.)	0	0*	2	7	20	0	0*	5	15	40
Animals with crystals	8	12	8	6	9	4	3	2	4	17
Mean number of crystals	3	12	4	2	2	0	1	0	0	16
0-10	17	12	18	18	19	20	19	20	20	10
11-20	2	3	1	2	1	0	1	0	0	4
21-30	1	3	0	0	0	0	0	0	0	1
31-40	0	1	0	0	0	0	0	0	0	2
>41	0	1	1	0	0	0	0	0	0	3
No. of animals	20	20	20	20	20	20	20	20	20	20

* Vehicle (hydroxypropyl-β-cyclodextrin) control

[Table from NDA 204447 submission; Study No. LBK0201, page 51]

Gross Pathology

The list of organs and tissues evaluated histopathologically is included in the histopathology inventory in the Appendix of this review (Table 91, page 152).

Vortioxetine-related changes were seen in the liver, rectum, extrahepatic bile duct, mesenteric lymph node, and kidneys. There were no differences in the incidences of macroscopic changes between the water or vehicle controls.

Liver
Masses and pale areas were seen at increased incidence in HD males and females. Increase incidence of pale areas was also noted for LD and MD males. Dark liver was seen at increased incidence in HDF. Macroscopic findings in the liver are summarized in Table 30. The masses and pale areas correlate with the neoplastic and non-neoplastic findings seen in the liver.

Table 30: Macroscopic findings in the liver

Group/sex	1M	2M	3M	4M	5M	1F	2F	6F	7F	8F
Dose (mg/kg b.i.d.)	0	0	2	7	20	0	0	5	15	40
Mass(es)	2	2	3	3	8	2	3	2	4	12
Pale area(s)	8	7	18	24	32	11	21	15	26	35
Dark	0	0	1	0	2	1	2	1	4	8
N° of animals examined	55	55	55	55	55	55	55	55	55	55

Group 1M/F = water control; Group 2M/F = vehicle control

[Table from NDA 204447 submission; Study No. LBK0201, pg 51]

Rectum
Masses were seen in 1/55 MDM, 2/55 MDF, and 2/55 HDF. Intussusception was seen in 1 MDM, 1 HDM, and 3 HDF. Masses and intussusceptions were not seen in control rats (water or vehicle). The masses and intussusceptions correlate with the neoplastic findings seen in the rectum.

Extrahepatic Bile Duct
Distension, the presence of abnormal contents, and thickening were noted at increased incidence in the extrahepatic bile ducts of HDM, MDF, and HDF (Table 31). Masses were noted in 1 HDM and 1 MDF. The presence of abnormal contents is likely the crystalline material seen microscopically in the lumen of the bile duct.

Table 31: Macroscopic findings in the extrahepatic bile duct

Group/sex	1M	2M	3M	4M	5M	1F	2F	6F	7F	8F
Dose (mg/kg b.i.d.)	0	0	2	7	20	0	0	5	15	40
Distended	11	20	14	14	36	8	11	10	15	41
Abnormal contents	0	3	0	0	8	1	1	0	5	27
Thickened	1	0	0	0	12	0	2	1	4	23
Mass(es)	0	0	0	0	1	0	0	0	1	0
N° of animals examined	55	55	55	55	55	55	55	55	55	55

Group 1M/F = water control; Group 2M/F = vehicle control

[Table from NDA 204447 submission; Study No. LBK0201, page 52]

Mesenteric Lymph Node

Enlargement and dark appearance of the mesenteric lymph nodes were seen at increased incidence in MD and HD males compared to water and vehicle controls (enlargement: 11% vs. 1.8 – 3.6%; Dark appearance: 22 – 35% vs. 3.6%). Enlargement of the mesenteric lymph nodes was only seen in 1.8 and 3.6% of MD and HD females, respectively; however enlargement was not seen in any of the control females. The incidence of darkened appearance was similar for HD females and vehicle controls.

Kidneys

Pale areas on the surface of the kidney was seen at increased incidence in all male dose groups and in MD and HD females (Males: 7, 9, 16, 16, and 27% for water, vehicle, LD, MD, and HD, respectively; Females: 5, 3.6, 5, 7, and 18% for water, vehicle, LD, MD, and HD, respectively). Dark appearance to the kidney was seen at increased incidence in HD males and MD and HD females (M: 9% vs. 0 – 3.6%; F: 22 – 36% vs. 5 – 7%).

Histopathology

Peer Review: Yes, by a consultant pathologist. A peer review occurred for all tumors for which increased incidence was considered vortioxetine related and when the Sponsor considered necessary for organs/tissues where increased tumor incidence was seen in vortioxetine or vehicle dosed groups. In addition to peer review, the Sponsor requested a Scientific Advisory Group (SAG), chaired by the peer review pathologist, to evaluate the increased incidence of hepatocellular tumors, hemangiomas, histiocytic sarcomas, and rectal polypoid adenomas in this study. The Sponsor used the opinion of the SAG panel in their interpretation of the study.

Findings: The liver, mesenteric lymph node, hematopoetic tissue, and rectum were target organs of neoplastic changes. Vehicle-related neoplastic findings were seen in the pancreas. Incidental neoplastic findings were seen in the cecum, mammary gland, thyroid, and uterus.

The liver, kidney, stomach, bile duct, mesenteric lymph node, thyroid, and cervix were target organs for non-neoplastic changes. Vehicle-related non-neoplastic findings were seen in pancreas and large intestine. Incidental non-neoplastic findings were seen in the thyroid and spleen.

Liver

Neoplastic: The incidence of hepatocellular adenomas was increased in HD males and females compared to water and vehicle controls (7.3% vs. 1.8 and 0%, respectively). The incidence for HDM and HDF of 7.3% is higher than the historical control range for this laboratory of 0 – 6.7% for males (n=757) and 0 – 5.5% for females (n=757). Neoplastic findings in the liver are summarized in Table 32.

Table 32: Neoplastic findings in the liver

Group/sex	1M	2M	3M	4M	5M	1F	2F	6F	7F	8F
Dose (mg/kg b.i.d.)	0	0	2	7	20	0	0	5	15	40
Hepatocellular adenoma	1	0	2	2	4*†	1	0	1	0	4††
Hepatocellular carcinoma	0	0	1	0	1	0	0	0	1	0
Hepatocellular adenoma and carcinoma combined	1	0	3	2	5*†	1	0	1	1	4†
N° of animals examined	55	55	55	55	55	55	55	55	55	55

One-tailed test for a trend using nominal dose levels, with the vehicle control group. † $p < 0.05$ †† $p < 0.01$
One-tailed pairwise comparison test against the vehicle control group. * $p < 0.05$

Group 1M/F = water control; Group 2M/F = vehicle control

[Table from NDA 204447 submission; Study No. LBK0201, page 54]

According to the Sponsor, statistical analysis of the data showed a significant trend test for hepatocellular adenomas (p=0.025 for males and p=0.008 for females) and for the combination of hepatocellular adenomas and carcinomas (p=0.013 for males and p=0.010 for females). Only the pairwise comparison for HDM was significant (p=0.038 for adenomas and p=0.019 for adenomas and carcinomas). Hepatocellular carcinomas alone were not significant.

Non Neoplastic: Non-neoplastic findings in the liver are summarized in Table 33. Most of the findings are associated with the portal area of the liver and are related to the presence of crystals in the bile ducts. Crystalline material in bile ducts and increased incidence and severity of bile duct hyperplasia and dilatation (HDF only) and cholangitis/pericholangitis were seen in HDM and MD and HD females. An increased incidence and severity of multilocular biliary cyst, peribiliary fibrosis, oval cell proliferation, and periportal mastocytosis was seen in HDF. An increased incidence and severity of hepatocyte necrosis was seen in MD and HD females. An increased incidence and severity of centrilobular hepatocyte hypertrophy with vacuolation was seen in MD and HD males. An increased incidence of pigmented macrophages and hepatocytes was seen in the HDF, most likely secondary to the crystals in the bile duct.

Table 33: Non-neoplastic findings in the liver

Group/sex	1M	2M	3M	4M	5M	1F	2F	6F	7F	8F
Dose (mg/kg b.i.d.)	0	0	2	7	20	0	0	5	15	40
Bile duct hyperplasia										
Minimal	6	1	1	0	6	8	1	2	6	10
Slight	0	0	0	0	2	0	1	0	4	13
Moderate	0	0	0	0	1	0	0	0	1	7
Marked	0	0	0	0	2	0	0	0	0	5
Severe	0	0	0	0	0	0	0	0	0	1
Total	6	1	1	0	11	8	2	2	11	36
Bile ducts – dilatation										
Minimal	2	1	0	0	2	2	1	0	0	9
Slight	0	0	0	0	1	0	0	0	1	5
Moderate	0	0	0	0	0	0	0	0	0	3
Total	2	1	0	0	3	2	1	0	1	17
Biliary cyst, multilocular										
Minimal	1	2	1	1	1	2	1	2	0	2
Slight	0	0	0	1	1	0	2	1	2	7
Moderate	0	0	0	1	0	1	0	1	1	2
Total	1	2	1	3	2	3	3	4	3	11
Peribiliary fibrosis										
Minimal	4	10	1	2	2	5	2	1	2	11
Slight	2	1	0	0	1	0	0	0	1	10
Moderate	0	0	0	0	0	0	0	0	0	1
Total	6	11	1	2	3	5	2	1	3	22

Text table 25 Summary of non-neoplastic findings in the liver for all rats

Group/sex	1M	2M	3M	4M	5M	1F	2F	6F	7F	8F
Dose (mg/kg b.i.d.)	0	0	2	7	20	0	0	5	15	40
Oval cell proliferation										
Minimal	0	0	0	0	0	0	0	0	1	3
Slight	0	0	0	0	2	0	0	0	0	5
Total	0	0	0	0	2	0	0	0	1	8
Periportal mastocytosis										
Minimal	0	0	0	0	0	0	0	0	0	3
Slight	0	0	0	0	0	0	0	0	0	3
Total	0	0	0	0	0	0	0	0	0	6
Cholangitis / pericholangitis										
Minimal	1	0	1	0	3	1	0	1	3	12
Slight	0	0	0	0	1	0	0	0	2	8
Moderate	0	0	0	0	1	0	0	0	0	5
Total	1	0	1	0	5	1	0	1	5	25
Crystalline material in bile ducts										
Minimal	0	0	0	0	7	0	0	0	6	16
Slight	0	0	0	0	1	0	0	0	1	11
Moderate	0	0	0	0	0	0	0	0	0	1
Total	0	0	0	0	8	0	0	0	7	28
Hepatocyte vacuolation, centrilobular										
Minimal	2	1	4	10	4	0	0	0	1	2
Slight	1	0	0	3	14	1	1	1	2	1
Moderate	3	0	0	0	6	1	0	0	0	0
Marked	0	0	0	0	1	0	0	0	0	0
Total	6	1	4	13	25	2	1	1	3	3
Hepatocyte hypertrophy, centrilobular										
Minimal	0	1	3	10	19	0	5	4	4	3
Slight	0	0	0	1	8	1	0	0	1	0
Moderate	0	0	0	0	2	0	0	0	0	0
Total	0	1	3	11	29	1	5	4	5	3
Bacterial presence										
Slight	0	0	0	0	0	0	0	0	1	3
Moderate	0	0	0	0	0	0	0	0	0	1
Total	0	0	0	0	0	0	0	0	1	4
Inflammatory cell infiltration (non portal)										
Minimal	2	1	2	3	3	4	5	5	4	6
Slight	1	1	0	0	0	1	2	1	2	6
Moderate	0	0	0	0	0	0	0	0	0	2
Total	3	2	2	3	3	5	7	6	6	14

Group/sex	1M	2M	3M	4M	5M	1F	2F	6F	7F	8F
Dose (mg/kg b.i.d.)	0	0	2	7	20	0	0	5	15	40
Hepatocyte necrosis, focal										
Minimal	4	1	3	2	1	5	3	0	2	6
Slight	2	1	1	0	2	0	0	0	3	5
Moderate	1	1	0	1	2	1	0	0	3	5
Marked	0	0	0	0	1	0	0	0	0	3
Total	7	3	4	3	6	6	3	0	8	19
Abscessation										
Presence	0	0	0	0	0	0	0	0	0	2
Total	0	0	0	0	0	0	0	0	0	2
N° of animals examined	55	55	55	55	55	55	55	55	55	55

Group/sex	1M	2M	3M	4M	5M	1F	2F	6F	7F	8F
Dose (mg/kg b.i.d.)	0	0	2	7	20	0	0	5	15	40
Pigmented macrophages										
Minimal	0	0	0	0	4	4	2	4	3	13
Slight	0	0	2	1	1	2	1	1	2	5
Moderate	2	1	1	0	0	1	2	0	2	0
Marked	0	0	0	0	0	2	1	0	0	0
Severe	0	0	0	0	0	0	0	0	0	1
Total	2	1	3	1	5	9	6	5	7	19
Pigments in hepatocytes										
Minimal	0	0	0	0	1	0	7	9	8	13
Slight	2	0	0	0	1	0	1	2	1	4
Moderate	0	1	1	0	0	3	2	0	1	1
Marked	0	0	0	0	0	1	0	0	0	0
Total	2	1	1	0	2	4	10	11	10	18
N° of animals examined	55	55	55	55	55	55	55	55	55	55

Group 1M/F = water control; Group 2M/F = vehicle control

[Table from NDA 204447 submission; Study No. LBK0201, pages 59 – 61, 66]

The SAG considers the increased incidence of hepatocellular adenomas in HD males and females not to pose a risk to humans[4]. Specifically, the SAG report states that

[4] Gopinath C., Maronpot H.P., Greaves P., (2010) – Scientific Advisory Group Report on the increased incidence of some tumours in rodent carcinogenicity studies with Lu AA21004.

The increased incidence of hepatocellular adenomas mediated by non genotoxic mechanism of persistent hepatotoxicity at these high dose levels poses no carcinogenic risk to humans at therapeutic doses.

Mesenteric Lymph Node

Neoplastic: The incidence of hemangeomas in the mesenteric lymph node was increased in MD and HD males compared to water and vehicle controls (24 and 27% vs. 5 and 11%, respectively). The incidence for MD and HD males of 24 and 27%, respectively, is higher than the historical control range of 4 – 20% for males (n=756) for this laboratory. There were no findings of hemangiosarcomas in the mesenteric lymph node in any males and in only 1 vehicle control and 1 HD female. Neoplastic findings in the mesenteric lymph node are summarized in Table 34. There was no effect on the incidence of hemangiomas and hemangiosarcomas at all sites (Table 35).

Table 34: Neoplastic findings in the mesenteric lymph node

Group/sex	1M	2M	3M	4M	5M	1F	2F	6F	7F	8F
Dose (mg/kg b.i.d.)	0	0	2	7	20	0	0	5	15	40
Haemangioma	3	6	6	13†	15*††	1	3	2	1	4
Haemangiosarcoma	0	0	0	0	0	0	1	0	0	1
Angiomatous hyperplasia (1)	4	7	6	3	12	2	1	1	4	3
N° of animals examined	55	55	55	55	55	55	55	55	55	54

One-tailed test for a trend using nominal dose levels, with the vehicle control group. † $p < 0.05$ †† $p < 0.01$
One-tailed pairwise comparison test against the vehicle control group. * $p < 0.05$
(1) Angiomatous hyperplasia is a proliferative non-neoplastic lesion, it was included here to help in the interpretation of the proliferative lesions

Group 1M/F = water control; Group 2M/F = vehicle control

[Table from NDA 204447 submission; Study No. LBK0201, page 55]

Table 35: Hemangiomas and hemangiosarcomas at all sites in males

Site	Tumor	Dose (mg/kg/bid)				
		W	V	2	7	20
Mesenteric LN	Hemangioma	3	6	6	13	15
Mandibular LN	Hemangioma	0	0	0	0	1
Renal LN	Hemangioma	1	0	0	0	0
Spleen	Hemangioma	0	1	0	0	0
	Hemangiosarcoma	0	0	1	0	0
	Combined	0	1	1	0	0
Skeletal Muscle	Hemangiosarcoma	0	1	1	0	0
Skin	Hemangiosarcoma	0	0	0	0	1
All sites*	Hemangioma + Hemangiosarcoma	4	7	9	13	15

W = Water Control, V = Vehicle Control; *Each rat is counted once, even if there are multiple tumors

[Data from NDA 204447 submission; Study No. LBK0201]

According to the Sponsor, statistical analysis of the data showed a significant trend test for hemangiomas (p=0.003) and the pairwise comparison was significant for HDM (p=0.012).

Non Neoplastic: An increased incidence of macrophage aggregates and sinus histiocytosis was seen in HDF and increased incidence of angiomatous hyperplasia was seen in HDM (Table 36). An increased incidence and severity of sinus erythrocytosis/erythrophagocytosis was seen in HDM compared to controls. This finding may be secondary to the increased presence of hemangiomas in HDM.

Table 36: Non-neoplastic findings in the mesenteric lymph node

Group/sex	1M	2M	3M	4M	5M	1F	2F	6F	7F	8F
Dose (mg/kg b.i.d.)	0	0	2	7	20	0	0	5	15	40
Macrophage aggregates										
Minimal	13	15	26	20	18	28	29	27	29	13
Slight	23	19	17	15	19	9	8	13	12	22
Moderate	5	4	3	0	2	2	6	2	1	14
Total	41	38	46	35	39	39	43	42	42	49
Sinus histiocytosis										
Minimal	1	0	3	4	3	1	6	8	12	16
Slight	0	1	0	0	1	2	3	2	2	11
Moderate	0	0	0	0	0	0	1	0	0	2
Total	1	1	3	4	4	3	10	10	14	29
Angiomatous hyperplasia										
Minimal	1	2	0	1	3	2	1	1	2	0
Slight	3	5	4	2	8	0	0	0	2	3
Moderate	0	0	2	0	1	0	0	0	0	0
Total	4	7	6	3	12	2	1	1	4	3
N° of animals examined	55	55	55	55	55	55	55	55	55	54

Group/sex	1M	2M	3M	4M	5M	1F	2F	6F	7F	8F
Dose (mg/kg b.i.d.)	0	0	2	7	20	0	0	5	15	40
Sinus erythrocytosis/erythrophagocytosis										
Minimal	3	4	6	9	3	6	6	8	5	6
Slight	2	5	2	6	11	4	1	1	1	5
Moderate	1	3	4	7	12	1	3	1	2	3
Marked	1	1	0	0	0	0	3	1	0	0
Severe	0	0	0	0	1	0	1	0	0	0
Total	7	13	12	22	27	11	14	11	8	14
N° of animals examined	55	55	55	55	55	55	55	55	55	54

Group 1M/F = water control; Group 2M/F = vehicle control

[Tables from NDA 204447 submission; Study No. LBK0201, pages 64 and 69]

The SAG considers the increased incidence of hemangeomas in the mesenteric lymph node in MD and HD males not to be relevant to humans[5]. Specifically, the SAG report states that

[5] Gopinath C., Maronpot H.P., Greaves P., (2010) – Scientific Advisory Group Report on

An increase in incidence of haemangioma of mesenteric lymph node was seen in male rats, which was associated with increased incidence of angiomatous hyperplasia. This benign response is considered to be rat specific, due to exaggerated susceptibility to angioproliferative stimuli in this species.

Hematopoietic Tissue

Neoplastic: The incidence of histiocytic sarcoma was increased in HDM compared to water and vehicle controls (5.5% (3/55) vs. 1.8 (1/55) and 0%, respectively). The incidence of 5.5% is above the historical control range of 0 – 1.8% (n=757). According to the Sponsor, statistical analysis of the data showed a significant trend test (p=0.016); but none of the pairwise comparisons were significant. There is no evidence of an increase incidence of related non-neoplastic findings.

The SAG considers the increased incidence of histocytic sarcoma in HD males not to be treatment related[1]. Specifically, the SAG report states that

The minor numerical differences seen in the incidence of histiocytic sarcomas in male rats from the high dose group was due to fortuitous distribution and not treatment related.

Large Intestine (Cecum, Colon, and Rectum)

Neoplastic: Polypoid adenomas of the rectum were seen in 1/55 (1.8%) MDM, 1/55 (1.8%) MDF and 4/55 (7.3%) HDF. No polypoid adenomas were seen in any other groups including the water and vehicle controls. In addition, no polypoid adenomas of the rectum have been seen in the historical control data for this laboratory (n=696 and 697 for males and females, respectively) and is considered a rare tumor. The Sponsor's statistical analysis of the data for females showed a significant trend test for polypoid adenomas (p=0.002) with a significant pairwise comparison of HDF to vehicle controls (p=0.032).

A sarcoma-NOS (not otherwise specified) was seen in the cecum of 1/55 HDM. This finding was not statistically significant and is most likely an incidental finding.

Non Neoplastic: Epithelial hyperplasia and mucosal inflammation was seen at an increased incidence in the large intestine (cecum, colon, and rectum) of all HP-ß-CD vehicle (control and vortioxetine) dosed groups compared to water controls. There was no difference in incidence or severity for the hyperplasia and inflammation seen in the vehicle and vortioxetine dosed groups. Therefore, hyperplasia and inflammation of the large intestine is considered vehicle-related and not vortioxetine-related. Table 37,

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the increased incidence of some tumours in rodent carcinogenicity studies with Lu AA21004.

Table 38, and Table 39 summarize the non-neoplastic findings in the cecum, colon, and rectum, respectively.

Table 37: Non-neoplastic findings in the cecum

Group/sex	1M	2M	3M	4M	5M	1F	2F	6F	7F	8F
Dose (mg/kg b.i.d.)	0	0	2	7	20	0	0	5	15	40
Epithelial hyperplasia										
Minimal	1	11	14	13	15	3	22	28	23	21
Slight	0	10	13	4	10	1	16	14	7	10
Moderate	0	0	1	0	1	0	2	0	2	1
Total	1	21	28	17	26	4	40	42	32	32
Mucosal inflammation										
Minimal	1	13	6	6	4	6	21	10	5	6
Slight	0	1	1	3	2	1	0	1	5	0
Moderate	0	0	1	0	0	0	0	0	0	0
Total	1	14	8	9	6	7	21	11	10	6
N° of animals examined	54	55	54	55	54	55	51	54	53	50

Group 1M/F = water control; Group 2M/F = vehicle control

[Table from NDA 204447 submission; Study No. LBK0201, page 69]

Table 38: Non-neoplastic findings in the colon

Group/sex	1M	2M	3M	4M	5M	1F	2F	6F	7F	8F
Dose (mg/kg b.i.d.)	0	0	2	7	20	0	0	5	15	40
Epithelial hyperplasia										
Minimal	0	18	13	13	9	1	21	7	9	5
Slight	0	1	7	6	0	0	3	10	6	7
Moderate	0	0	0	0	1	0	0	0	0	0
Total	0	19	20	19	10	1	24	17	15	12
Mucosal inflammation										
Minimal	0	8	5	4	4	0	7	3	7	5
Slight	0	0	0	1	0	1	0	0	1	1
Total	0	8	5	5	4	1	7	3	8	6
N° of animals examined	55	55	55	55	55	54	54	55	55	53

Group 1M/F = water control; Group 2M/F = vehicle control

[Table from NDA 204447 submission; Study No. LBK0201, page 70]

Table 39: Non-neoplastic findings in the rectum

Group/sex	1M	2M	3M	4M	5M	1F	2F	6F	7F	8F
Dose (mg/kg b.i.d.)	0	0	2	7	20	0	0	5	15	40
Epithelial hyperplasia										
Minimal	1	16	4	7	10	1	24	16	17	18
Slight	2	7	5	4	7	0	10	6	7	7
Moderate	0	0	3	0	1	0	3	1	1	1
Total	3	23	12	11	18	1	37	23	25	26
Mucosal inflammation										
Minimal	0	4	1	1	3	0	7	3	6	4
Slight	0	0	0	1	1	0	0	0	0	0
Total	0	4	1	2	4	0	7	3	6	4
N° of animals examined	54	55	55	55	55	55	55	55	55	52

Group 1M/F = water control; Group 2M/F = vehicle control

[Table from NDA 204447 submission; Study No. LBK0201, page 70]

The SAG considered the findings of polypoid adenomas in the rectum to be vehicle mediated and not relevant to humans[6]. Specifically the SAG report states that

> A few rectal polypoid adenomas reported especially in the female rats from the high dose group was related to the vehicle driven hyperplasia and inflammation of the large intestine. This is a known response to HP-beta-cyclodextrin. This does not pose any carcinogenic risk for human use as this vehicle is not a component of the clinical formulation.

Although it is true that there is vehicle mediated epithelial hyperplasia and mucosal inflammation of the cecum, colon, and rectum, it is less clear that the finding of polypoid adenomas in the rectum are also vehicle mediated. There was no difference in incidence or severity for the hyperplasia and inflammation seen in the vehicle control and vortioxetine dosed groups. However, there is a clear dose response for the polypoid adenoma in the rectum of female rats.

The SAG report cites the Pharmacology/Toxicology Review of itraconazole (NDA 020966) for the finding that polypoid adenomas of the rectum are HP-ß-CD mediated. Because the clinical formulation of itraconazole used HP-ß-CD as the vehicle, HP-ß-CD was tested alone in an oral rat carcinogenicity study (0, 500, 2000, and 5000 mg/kg HP-ß-CD for 25 months). In this study a slight increase in neoplasms of the large intestine

[6] Gopinath C., Maronpot H.P., Greaves P., (2010) – Scientific Advisory Group Report on the increased incidence of some tumours in rodent carcinogenicity studies with Lu AA21004.

at 5000 mg/kg was seen (Table 40). However, there was a clear dose response and no neoplasms were seen at the low dose of 500 mg/kg. In the case of the current study, the vehicle is 10% HP-ß-CD with a dose volume of 5 mL/kg for all groups. Therefore, all vehicle dosed groups (control and vortioxetine) received the same amount of HP-ß-CD (500 mg/kg/bid). In addition, the amount of HP-ß-CD given to the rats in the current study is below the 2000 and 5000 mg/kg dose where neoplasms were seen. Therefore, I do not consider the finding of polypoid adenoma in the rectum to be HP-ß-CD mediated, but to be vortioxetine related.

Table 40: Incidence of neoplastic and non-neoplastic changes in the large intestine of rat after oral dosing with HP-ß-CD

Male rats	0 mg/kg	500 mg/kg	2000 mg/kg	5000 mg/kg	p [d]
non-neoplastic changes					
cecum hypertrophy	3/49	10/49	36/49[c]	41/48[c]	
colon hypertrophy	1/49	0/50	20/48[c]	40/49[c]	
rectum hypertrophy	0/49	0/50	19/50[c]	40/49[c]	
neoplastic changes					
adenocarcinoma	0/50	0/50	1/50	4/50	0.0050
Female rats	**0 mg/kg**	**500 mg/kg**	**2000 mg/kg**	**5000 mg/kg**	**p [d]**
non-neoplastic changes					
cecum hypertrophy	0/50	0/49	16/50[c]	45/49[c]	
colon hypertrophy	0/50	0/49	4/50	41/49[c]	
		0/49	1/50	38/49[c]	
neoplastic changes					
- adenocarcinoma	0/50	0/50	050	1/50	0.2569
- adenoma	0/50	0/50	0/50	1/50	0.2830

Chi-square (one-tailed): [a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$ [d]One-sided p-value for trend. Test for positive trend, Peto monograph, WHO, IARC, Lyon 1980, pp.311-426.

[Table from Pharmacology/Toxicology review of NDA 020966 for itraconazole dated 3/19/1999, page 15]

Pancreas

Neoplastic: The incidence of acinar cell adenoma in the pancreas was increased in vehicle and LD males compared to water controls (3.6 and 7.3% vs. 1.8%, respectively). The incidence for vehicle and LD males of 3.6 and 7.3%, respectively, is higher than the historical control range for this laboratory of 0 – 1.7% for males (n=756). The incidence of focal acinar cell hyperplasia was also increased in all vehicle dosed males (control, LD, MD, and HD) compared to water controls (see below). Oral HP-ß-CD is known to

cause acinar pancreatic neoplasms in rats[7] with no NOAEL identified; therefore, the increase in acinar cell adenoma and focal acinar cell hyperplasia are related to the HP-ß-CD vehicle. Because HP-ß-CD is not used in the clinical formulation, there is no carcinogenic risk for humans.

Non Neoplastic: Acinar cell hyperplasia was seen in all vehicle treated (control and dosed) male groups (incidence of 0, 5 (9%), 4 (7.3%), 6 (11%), and 4 (7.3%) for water, vehicle, LD, MD, and HD males, respectively); therefore, this finding is vehicle related.

Kidney

Non Neoplastic: Non-neoplastic findings in the kidney are summarized in Table 41. Crystalline material in the kidney and increased incidence and severity of cortical tubular dilation was seen in HDM and MD and HD females. An increased incidence of chronic progressive nephropathy and interstitial inflammation was seen in HD females. Cellular tubular casts were seen occasionally in MD and HD males and all dosed females with the highest incidence in HDF.

An increased incidence and/or severity of cortical tubular pigments and vacuolation was seen in all vehicle treated groups (control and vortioxetine). Therefore, this finding is related to vehicle and not vortioxetine.

[7] Pharmacology/Toxicology review of NDA 020966 for itraconazole dated 3/19/1999

Table 41: Non-neoplastic findings in the kidney

Group/sex	1M	2M	3M	4M	5M	1F	2F	6F	7F	8F
Dose (mg/kg b.i.d.)	0	0	2	7	20	0	0	5	15	40
Crystalline material										
Minimal	0	0	0	0	2	0	0	0	1	5
Slight	0	0	0	0	1	0	0	0	2	2
Moderate	0	0	0	0	1	0	0	0	0	1
Marked	0	0	0	0	0	0	0	0	0	2
Total	0	0	0	0	4	0	0	0	3	10
Chronic progressive nephropathy										
Minimal	29	26	18	29	27	17	13	15	19	21
Slight	6	3	5	10	8	3	2	1	1	10
Moderate	2	2	5	3	5	1	1	0	0	2
Marked	0	0	2	1	1	0	0	0	1	0
Severe	0	0	0	1	0	0	0	0	0	0
Total	37	31	30	44	41	21	16	16	21	33
Cortical tubular dilatation										
Minimal	1	0	1	0	4	3	1	1	3	3
Slight	0	1	0	0	1	1	2	0	4	5
Moderate	0	1	0	1	1	0	0	0	0	2
Total	1	2	1	1	6	4	3	1	7	10
Tubular cast (cellular)										
Minimal	0	0	0	1	1	0	0	1	2	5
Slight	0	0	0	1	1	0	0	0	1	0
Moderate	0	0	0	0	0	0	0	0	0	2
Total	0	0	0	2	2	0	0	1	3	7
Interstitial inflammation										
Minimal	16	11	7	16	15	1	3	1	2	3
Slight	3	2	7	7	8	0	1	0	1	4
Moderate	0	1	1	2	1	1	0	0	0	3
Total	19	14	15	25	24	2	4	1	3	10
N° of animals examined	55	55	55	55	55	55	53	54	52	52

Group/sex	1M	2M	3M	4M	5M	1F	2F	6F	7F	8F
Dose (mg/kg b.i.d.)	0	0	2	7	20	0	0	5	15	40
Cortical tubular pigments										
Minimal	1	34	37	38	31	29	6	16	12	21
Slight	0	13	12	9	6	7	31	31	29	22
Moderate	0	0	0	1	1	0	12	7	9	6
Marked	0	0	0	0	0	0	1	0	1	2
Total	1	47	49	48	38	36	50	54	51	51
Cortical tubular vacuolation										
Minimal	0	17	21	23	25	0	7	3	9	9
Slight	0	2	5	7	6	0	2	1	3	7
Moderate	0	3	5	2	3	0	1	0	2	2
Marked	0	0	0	0	1	0	0	0	0	0
Total	0	22	31	32	35	0	10	4	14	18
N° of animals examined	55	55	55	55	55	55	53	54	52	52

Group 1M/F = water control; Group 2M/F = vehicle control

[Table from NDA 204447 submission; Study No. LBK0201, page 62]

Extrahepatic Bile duct

Non Neoplastic: Crystals were seen in the bile duct of HDM and MD and HD females (Table 42). An increased incidence and severity of luminal dilatation, epithelial hyperplasia, fibrosis, and inflammatory cell infiltration was also seen in HDM and MD and HD females.

Table 42: Non-neoplastic findings in the extrahepatic bile duct

Group/sex	1M	2M	3M	4M	5M	1F	2F	6F	7F	8F
Dose (mg/kg b.i.d.)	0	0	2	7	20	0	0	5	15	40
Luminal dilatation										
Minimal	9	12	10	2	8	4	7	9	6	8
Slight	1	4	4	2	14	3	1	2	5	9
Moderate	2	3	0	0	5	1	2	0	3	14
Marked	0	1	0	1	0	0	1	0	2	5
Severe	0	0	0	0	0	0	0	0	0	3
Total	12	20	14	5	27	8	11	11	16	39
Epithelial hyperplasia										
Minimal	5	2	1	3	10	1	4	1	7	6
Slight	0	1	1	0	12	1	0	0	3	12
Moderate	0	2	0	0	3	0	0	0	2	8
Marked	0	1	0	0	0	0	0	0	0	5
Total	5	6	2	3	25	2	4	1	12	31
Crystalline material In lumen										
Minimal	0	0	0	0	2	0	0	0	0	6
Slight	0	0	0	0	4	0	0	0	0	6
Moderate	0	0	0	0	1	0	0	0	1	3
Marked	0	0	0	0	0	0	0	0	0	1
Severe	0	0	0	0	0	0	0	0	0	1
Total	0	0	0	0	7	0	0	0	1	17
Inflammatory cell infiltration										
Minimal	0	0	0	0	2	0	0	0	1	2
Slight	0	0	0	0	0	0	0	0	0	2
Moderate	0	0	0	0	0	0	1	0	0	1
Total	0	0	0	0	2	0	1	0	1	5
Fibrosis										
Minimal	1	4	1	0	7	1	1	0	1	9
Slight	1	1	0	0	1	0	0	0	1	12
Moderate	0	0	0	0	0	0	1	0	1	2
Total	2	5	1	0	8	1	2	0	3	23
N° of animals examined	39	48	43	45	47	36	31	40	33	44

Group 1M/F = water control; Group 2M/F = vehicle control

[Table from NDA 204447 submission; Study No. LBK0201, page 63]

Stomach

Non Neoplastic: An increased incidence and severity (females only) of dilated glands in the glandular region of the stomach was seen at the HD in males and females (Table

43). An increased incidence of hyperkeratosis of the nonglandular region and epithelial hyperplasia of the limiting ridge was seen in HDF compared to controls (Table 43). The Sponsor reports that these changes are seen in early decedents and therefore, the correlates with the increased incidence of premature mortality seen in HDF.

Table 43: Non-neoplastic findings in the stomach

Group/sex	1M	2M	3M	4M	5M	1F	2F	6F	7F	8F
Dose (mg/kg b.i.d.)	0	0	2	7	20	0	0	5	15	40
Dilated glands – glandular region										
Minimal	9	6	5	8	21	6	13	9	16	20
Slight	2	0	0	0	1	0	1	0	0	16
Moderate	0	0	0	0	0	0	0	0	0	5
Total	11	6	5	8	22	6	14	9	16	41
N° of animals examined	55	55	55	52	55	55	55	55	55	55

Group/sex	1M	2M	3M	4M	5M	1F	2F	6F	7F	8F
Dose (mg/kg b.i.d.)	0	0	2	7	20	0	0	5	15	40
Hyperkeratosis – nonglandular region										
Minimal	3	1	1	1	2	3	2	1	2	7
Slight	2	2	0	0	0	1	2	2	2	4
Total	5	3	1	1	2	4	4	3	4	11
Epithelial hyperplasia – limiting ridge										
Minimal	6	5	5	4	5	5	4	3	5	8
Slight	1	2	2	0	3	1	0	2	2	3
Moderate	0	0	0	2	0	0	0	0	1	0
Total	7	7	7	6	8	6	4	5	8	11
N° of animals examined	55	55	55	52	55	55	55	55	55	55

Group 1M/F = water control; Group 2M/F = vehicle control

[Table from NDA 204447 submission; Study No. LBK0201, pages 61 and 68]

Thyroid

Non Neoplastic: An increased incidence of cystic follicular hyperplasia was seen in HDM (16%) compared to controls (7% for water and vehicle).

Uterine Cervix

Non Neoplastic: An increased incidence of epithelial hyperplasia was seen in the uterine cervix of HDF (20%) compared to controls (1.8 and 5.5% for water and vehicle, respectively).

Spleen

Non neoplastic: Increased incidence and severity of extramedullary hemopoiesis was seen in HDF compared to controls (Table 44). Most likely, this is secondary to changes seen in the liver.

Table 44: Non-neoplastic findings in the spleen

Group/sex	1M	2M	3M	4M	5M	1F	2F	6F	7F	8F
Dose (mg/kg b.i.d.)	0	0	2	7	20	0	0	5	15	40
Extramedullary haemopoiesis										
Minimal	1	0	0	0	1	3	3	1	5	2
Slight	0	0	1	1	2	3	2	3	1	6
Moderate	0	2	1	0	1	1	3	1	0	1
Marked	1	0	1	0	1	0	0	0	0	2
Total	2	2	3	1	5	7	8	5	6	11
N° of animals examined	55	55	55	55	55	55	55	55	55	55

Group 1M/F = water control; Group 2M/F = vehicle control

[Table from NDA 204447 submission; Study No. LBK0201, page 67]

Toxicokinetics

Plasma exposures of vortioxetine, a major human metabolite (Lu AA34443), and a non-major human metabolite (Lu AA39835) were determined in Weeks 1, 13, 26, and 105 at only 2 and 24 (except Week 105) hours post dosing. Due to misconduct at (b) (4), the TK data from Weeks 1, 13, and 26 were not found to be GLP compliant. However, the TK data from Week 105 was GLP compliant. Based on remedial action, the Sponsor considers the TK data from Weeks 1, 13, and 26 not compromised.

At 2 hours postdose, all tested rats were exposed to vortioxetine and Lu AA34443. Exposure was generally greater than dose proportional for vortioxetine and more than or dose proportional for Lu AA34443. For Lu AA39835, exposures were not detected at 2 hours for LD males and in general were low for all other groups. Exposure for Lu AA39835 was generally greater than dose proportional for males and less than or dose proportional for females. For vortioxetine, Lu AA34443, and Lu AA39835 accumulation was seen over the 105 week dosing period.

Vortioxetine

Because of the sparse sampling, estimation of TK parameters was not performed for the carcinogenicity study. As a result, human exposure margins for vortioxetine need to be calculated from the 4-, 13-, and/or 26-week rat general toxicology studies (Table 45, Table 46, and Table 47). Due to misconduct, the 26-week TK data was found not to be

GLP compliant and the Sponsor considers the data from the 40 mg/kg/bid dose groups in Week 26 to be unreliable and excluded this data from the TK evaluation. The graph in Figure 5 compares exposures (AUC) for vortioxetine from the 4-, 13-, and 26-week studies. In general, exposures for males was higher than for females and tended to increase with length of dosing suggesting that there is accumulation.

The rat-to-human exposure for vortioxetine at the NOAEL for polypoid adenomas in the rectum based on rat exposures at Week 4, 13, or 26 from the general toxicology studies is shown in Table 48. The dose at the NOAEL in females of 15 mg/kg/bid was not used in the 4-, 13-, or 26-week study and therefore were interpolated from Figure 5. The exposure margins are 3 – 7-fold for males and 3 – 4-fold for females.

Table 45: Summary of mean Cmax, Tmax, and AUC at Day 29 for vortioxetine in the 4-week rat general toxicology study

Parameter	Males (mg/kg/bid)			Females (mg/kg/bid)		
	10	20	40	10	20	40
Cmax (nmol/L)	300	1150	2990	462	1590	2610
Tmax (h)	2*	2*	2*	2*	2*	2
$AUC_{0\text{-}24h}$ (nmol.h/L)	2270	6832	24532	3096	10385	31464

* Time after second daily dose

[Data from NDA 204447 submission; Study No. LBK 126, page 35]

Table 46: Summary of mean Cmax, Tmax, and AUC at Week 13 for vortioxetine in the 13-week rat general toxicology study

Parameter	Males (mg/kg/bid)			Females (mg/kg/bid)		
	10	20	40	10	20	40
Cmax (nmol/L)	488	1312	3043	506	1610	2856
Tmax (h)	1	12*	14*	1	1	12*
$AUC_{0\text{-}24h}$ (nmol.h/L)	2810	10426	38319	3556	10238	32957

* Tmax equal to 2 – 4 hours post dose of the second daily dose

[Data from NDA 204447 submission; Study No. LBK 129, page 37]

Table 47: Summary of mean Cmax, Tmax, and AUC at Week 26 for vortioxetine in the 26-week rat general toxicology study

Parameter	Males (mg/kg/bid)			Females (mg/kg/bid)		
	10	20	40	10	20	40
Cmax (nmol/L)	642	1925	NC*	579	1809	NC*
Tmax (h)	3	1	NC*	1	12	NC*
$AUC_{0\text{-}24h}$ (nmol.h/L)	4928	15561	NC*	3437	13806	NC*

*NC = not calculated; insufficient number of plasma concentrations available for TK analysis

[Data from NDA 204447 submission; Study No. LBK 150, page 40]

Figure 5: Comparison of exposures (AUC_{0-24h}) for vortioxetine at the end of 4-week, 13-week, and 26-week rat general toxicology studies

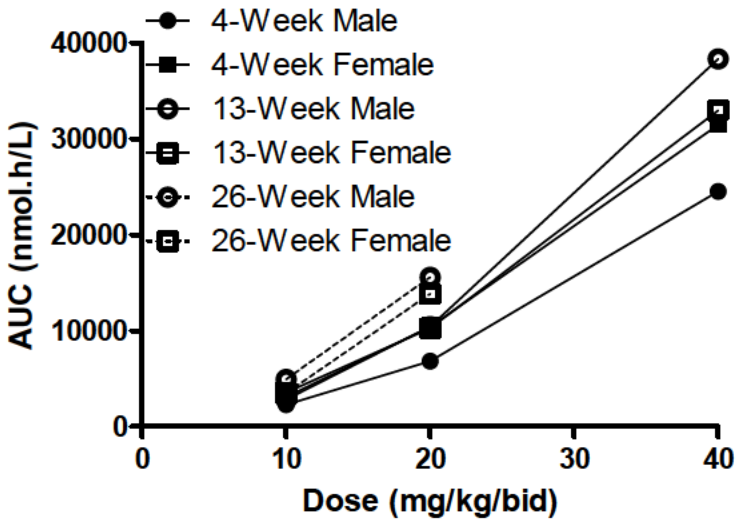


Table 48: Rat-to-human AUC ratio for vortioxetine at the NOAEL for polypoid adenomas of the rectum, based on rat exposures at Week 4, 13, or 26

Study	Sex	Dose (mg/kg/bid)	AUC_{0-24h} (ng.h/mL)*	Exposure Margin at MRHD#
4-Week	M	20	2039	3
	F	15	2012^	3
13-Week	M	20	3112	5
	F	15	2058^	3
26-Week	M	20	4644	7
	F	15	2573^	4

*Rat AUC_{0-24h} from Table 45 (Day 28), Table 46 (Week 13), and Table 47 (Week 26) converted from nmol.h/L to ng.h/mL using the conversion factor 0.29845 (From Sponsor's Pharmacokinetics Written Summary, page 5). #Human repeat dose AUC_{0-24h} at 20 mg = 646 ng.hr/mL (From Sponsor's Summary of Clinical Pharmacology, Table 5.2, page 166). ^ AUC_{0-24h} was interpolated from the graph in Figure 5.

Lu AA34443 (A Major Human Metabolite)

Estimation of TK parameters of Lu AA34443 was performed from exposure data in the 13- and 26-week, but not the 4-week, study (Table 51). Because the exposure data at 40 mg/kg/bid is compromised for the 26-week study, only the 13-week study was used for females. The exposure margin for the HD in males and females to the MRHD is 35 – 44.

Table 49: Summary of mean Cmax, Tmax, and AUC at Week 13 for Lu AA34443 in the 13-week rat general toxicology study

Parameter	Males (mg/kg/bid)			Females (mg/kg/bid)		
	10	20	40	10	20	40
Cmax (nmol/L)	4254	6756	10607	2681	4723	6140
Tmax (h)	1	12*	12*	1	1	12*
$AUC_{0\text{-}24h}$ (nmol.h/L)	26638	58171	134048	13929	33490	60688

* Tmax equal to 2 hours post dose of the second daily dose

[Data from NDA 204447 submission; Study No. LBK 129, page 38]

Table 50: Summary of mean Cmax, Tmax, and AUC at Week 26 for Lu AA34443 in the 26-week rat general toxicology study

Parameter	Males (mg/kg/bid)			Females (mg/kg/bid)		
	10	20	40	10	20	40
Cmax (nmol/L)	5242	7970	NC*	3135	5413	NC*
Tmax (h)	1	1	NC*	1	1	NC*
$AUC_{0\text{-}24h}$ (nmol.h/L)	29814	74868	NC*	16020	44551	NC*

*NC = not calculated; insufficient number of plasma concentrations available for TK analysis

[Data from NDA 204447 submission; Study No. LBK 150, page 41]

Table 51: Rat-to-human AUC ratio for major human metabolite Lu AA34443

Study	Sex	Dose (mg/kg/bid)	$AUC_{0\text{-}24h}$ (ng.h/mL)*	Exposure Margin at MRHD#
13-Week	M	20	19,105	34
	F	40	19,932	35
26-Week	M	20	24,589	44

*Rat $AUC_{0\text{-}24h}$ from Table 49 (Week 13) and Table 50 (Week 26) converted from nmol.h/L to ng.h/mL using the conversion factor 0.32843 (From Sponsor's Pharmacokinetics Written Summary, page 5).
#Human repeat dose $AUC_{0\text{-}24h}$ at 20 mg = 563 ng.hr/mL (From Sponsor's Summary of Clinical Pharmacology, Table 5.2, page 166).

Dosing Solution Analysis

All formulations were prepared fresh each week and were refrigerated and protected from light until use. Formulation samples taken in Weeks 1, 13, 26, 39, 52, 65, 78, 91, and 103 were determined to be within 93.5 – 107.2% of the nominal concentrations.

8.1.2 Mouse Carcinogenicity

Study title: Lu AA21004: Carcinogenicity study by oral gavage administration to CD-1 mice for 103/104 weeks

Study no.:	LBK0202; Reference 11688
Study report location:	(b) (4)
Conducting laboratory and location:	
Date of study initiation:	March 20, 2007
GLP compliance:	Yes, except TK
QA statement:	Yes
Drug, lot no., and % purity:	Lu AA21004, 2108448, 99.1%
CAC concurrence:	Yes

Key Study Findings

- Male and female CD-1 mice were dosed orally (by gavage, in 15% hydroxypropyl-beta-cyclodextrin (HP-ß-CD)) at 0, 5, 15, or 50 mg/kg/day and 0, 10, 30, or 100 mg/kg/day, respectively, for at least 102 weeks.
- The doses had been approved by the Executive CAC with high doses based on a 25-fold AUC ratio to human exposure, provided that the maximum clinical dose did not increase such that the mouse-to-human AUC ratio falls below 25.
- MTD was not demonstrated and exposure margins are not quite 25-fold.
- There was a numerical increase in the incidence of hepatocellular adenomas and combined hepatocellular adenomas and carcinomas in high dose males (50 mg/kg/day) which correlated with increased hepatotoxicity in high dose males. The incidence of hepatocellular adenomas was higher than the historical control range; but did not reach statistical significance for a common tumor.

Adequacy of Carcinogenicity Study

The study design (including route of administration, number of mice, and length of dosing) of this 103/104-week mouse carcinogenicity study is considered adequate. The HD for males and females (50 and 100 mg/kg, respectively) was selected based on AUC from the 13-week mouse general toxicology study with concurrence from the Executive CAC, provided that the maximum clinical dose did not increase such that the mouse-to-human AUC ratio falls below 25 (Meeting Minutes dated January 17, 2007). Although the maximum clinical dose of 20 mg did not increase, the human PK data used to determine the AUC ratio was preliminary (early in development) and the current exposure multiples based on final human PK are below 25. The AUC ratio is ~ 20 for males and ~ 13 – 22 for females.

Dosing with vortioxetine up to 50 and 100 mg/kg in male and female mice, respectively, did not increase mortality, cause any clinical signs, or adversely affect body weight.

Non-neoplastic findings were most likely related to the presence of crystalline material in the bile duct of the liver, the gall bladder, and the external bile duct in HD males and to a much lesser extent in HD females. Therefore, the doses used in this study are below an MTD. There was a numerical increase in the incidence of hepatocellular adenomas in HD males compared to controls that most likely is related to the hepatotoxicity.

The HD for males is considered adequate. The male mouse-to-human AUC ratio is 20 which is reasonably close to the 25 limit. In addition, HD males had an increased incidence of hepatocellular adenomas and non-neoplastic findings related to the presence of crystalline material.

The adequacy of the HD for females is more questionable. Because of the lack of female exposure data at 100 mg/kg in mouse general toxicology studies, exposure multiples are estimated based on the linearity of the exposure-to-dose graph of the 4- and 13-week data (Figure 6, page 93) and the exposure at 100 mg/kg for males. The most conservative estimate for the female mouse-to-human AUC ratio is 13 and the least is 22. The AUC ratio of 22 is reasonably close to the 25 limit; although, the AUC ratio of 13 is not. In addition, there were no vortioxetine-related neoplastic findings in females and minimal non-neoplastic findings. However, considering that the maximum clinical dose did not increase, the top range of the mouse-to-human AUC ratio is reasonably close to the 25 limit, and at least minimal non-neoplastic findings were seen at the HD, I consider the HD for females adequate.

Appropriateness of Test Models

The 103/104-week carcinogenicity study in CD-1 mice is an appropriate model.

Evaluation of Tumor Findings

Hepatocellular adenomas and carcinomas were present in all male groups, including controls, except males dosed with 15 mg/kg (MD) which had no findings of hepatocellular carcinomas. The incidence of adenomas was numerically increased for males at the HD (50 mg/kg) and the incidence is slightly higher than the historical control range for this laboratory (b) (4). However, the increase did not reach statistical significance for a common tumor and the control incidence was in the high end of the historical control range. There was a trend for an increase in the combination of hepatocellular adenomas and carcinomas; although, not statistically significant for a common tumor.

The increased incidence of hepatocellular adenomas correlates with non-neoplastic findings of hepatotoxicity in HD males—increased incidence of peribiliary fibrosis and/or inflammation, crystalline material in bile ducts, bile duct hyperplasia, and eosinophilic inclusions in bile duct epithelium. Findings of hepatotoxicity were also seen in HD females (100 mg/kg); although, at decreased incidence and severity.

Study results were independently reviewed and evaluated by the CDER statistical reviewer, Matthew Jackson, Ph.D. (see Statistical Review and Evaluation). Dr. Jackson's statistical analysis confirmed that the increased incidence of hepatocellular

adenomas and the combination of hepatocellular adenomas and carcinomas did not meet the standard for statistical significance for a common tumor (Table 52).

Table 52: Statistical results for hepatocellular adenoma and carcinoma in male mice

Tumor	Control n=60	5 mg/kg n=60	15 mg/kg n=60	50 mg/kg n=60	Trend test p-value	Pairwise Test (C vs. HD) p-value
Hepatocellular adenoma	11	13	9	18	0.0283	0.0964
Hepatocellular carcinoma	4	3	0	5	0.1754	0.5000
Hepatocellular adenoma + carcinoma	14	15	9	22	0.0099	0.0611

[Data from Dr. Jackson's statistical analysis]

Methods

Doses:	Male : 0, 5 (LD), 15 (MD), or 50 (HD)mg/kg/day Female: 0, 10 (LD), 30 (MD), or 100 (HD) mg/kg/day
Frequency of dosing:	Once a day
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Solution/15% HP-ß-CD
Basis of dose selection:	Dose selection is based on the 4- and 13-week mouse toxicology studies (Study Nos. LBK 145 and LBK143). Doses for the 4-week study were 50, 100, and 150 mg/kg/day in male and 50, 150, and 200 mg/kg/day in females. Doses for the 13-week study were 12.5, 25, and 50 mg/kg/day for males and 50, 150, and 200 mg/kg/day in females. The HD of 50 (males) and 100 (females) mg/kg/day was based on a 25-fold AUC ratio to human exposure, provided that the maximum clinical dose did not increase such that the mouse-to-human AUC ratio falls below 25 (Executive CAC concurrence January 17, 2007).
Species/Strain:	Mice/Crl:CD-1™(ICR) BR
Number/Sex/Group:	60/sex/group
Age:	40 – 43 days
Animal housing:	3 of same sex/cage
Paradigm for dietary restriction:	Free access to feed and water
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	21/sex/group (TK)

Deviation from study protocol:	Due to a high rate of mortality in the control and HD males, dosing was terminated at Week 102 for all male groups. Study protocol and SOP deviations were found for the TK evaluation performed at (b) (4) which resulted in the inability to claim GLP compliance for the TK data.

Observations and Results

Mortality

There was no vortioxetine-related effect on mortality. There were a total of 263 premature deaths (129 males and 134 females). Mortality was high in control and HD males, which resulted in early termination of dosing at Week 102 for males. The distribution of mortality rate by group and sex is listed in Table 53.

Table 53: Group distribution of mortality

Group/sex Dose (mg/kg/day)	1M 0	2M 5	3M 15	4M 50	1F 0	5F 10	6F 30	7F 100
Total number of deaths	38	28	26	37	31	34	35	34
Total number surviving	22	32	34	23	29	26	25	26
Percentage survival	36.7	53.3	56.7	38.3	48.3	43.3	41.7	43.3

[Table from NDA 204447 submission; Study No. LBK0202, page 39]

Clinical Signs

There were no effects of vortioxetine on clinical signs or palpable masses.

Body Weights

In general, vortioxetine had no adverse effects on body weight throughout the dosing period. HDM and MDF gained more weight than controls and had increased mean body weights (5 and 6%, respectively) at the end of dosing.

Feed Consumption

LD and MD males had decreased food consumption compared to controls (5 – 18%) from Weeks 24 through the end of dosing.

Hematology

In Week 52, MD and HD females had decreased white blood cells (≤34%), lymphocytes (≤35%), basophiles (50%), monocytes (≤40%), and large unstained cells (≤64%). The decrease seen in HDF was statistically significant. There were no vortioxetine-related effects on hematology parameters in Week 104.

Gross Pathology

The list of organs and tissues evaluated histopathologically is included in the histopathology inventory in the Appendix of this review (Table 91, page 152).

Vortioxetine-related changes were seen in the liver, gall bladder, and bile duct of HD males and females.

Liver

Masses and pale areas were seen at increased incidence in HDM and pale areas were seen at increased incidence in HDF (Table 54). The masses and pale areas may correlate with the neoplastic and non-neoplastic findings seen in the liver.

Table 54: Vortioxetine-related macroscopic findings in the liver

Group/sex	1M	2M	3M	4M	1F	5F	6F	7F
Dose (mg/kg/day)	0	5	15	50	0	10	30	100
Mass(es)	22	22	24	30	10	3	4	9
Pale area(s)	8	10	5	22	5	4	4	12
No. of animals examined	60	60	60	60	60	60	60	60

[Table from NDA 204447 submission; Study No. LBK0202, page 40]

Gall Bladder

Abnormal contents were seen in the gall bladder at increased incidence in HD males and females (41 HDM vs. 0 controls and 40 HDF vs. 3 controls). Distension of the gall bladder was seen at increased incidence in HD males (16 HDM vs. 9 controls).

Bile Duct

Distension of the bile duct was seen in 10% of HDM compared to no controls.

Histopathology

Peer Review: Yes, by a consultant pathologist. A peer review occurred for all tumors for which increased incidence was considered vortioxetine related and when necessary other lesions, organs, or tissues. In addition to peer review, the Sponsor requested a Scientific Advisory Group (SAG), chaired by the peer review pathologist, to evaluate the increased incidence of hepatocellular adenomas in the study. The Sponsor used the opinion of the SAG panel in their interpretation of the study.

Findings: The liver, gall bladder, and bile duct were the main target organs of neoplastic and/or non-neoplastic changes in both sexes with a higher incidence and severity in males.

Liver

Neoplastic: The liver was the main target organ of neoplastic changes in males (Table 55). Hepatocellular adenomas and carcinomas were present in all male groups,

including controls, except MDM which had no findings of hepatocellular carcinomas. The incidence of adenomas was increased in HDM compared to controls (30% vs. 18%, respectively). The incidence for HDM of 30% is higher than the historical control range of 6.1 – 24.1% for this laboratory.

Table 55: Neoplastic findings in the liver

Group/sex Dose (mg/kg/day)	1M 0	2M 5	3M 15	4M 50	1F 0	5F 10	6F 30	7F 100
Hepatocellular adenoma	11	13	9	18*	2	0	0	2
Hepatocellular carcinoma	4	3	0	5	0	0	0	0
Hepatocellular adenoma and carcinoma combined	14	15	9	22††	2	0	0	2
Number of animals examined	60	60	60	60	60	60	60	60

One-tailed trend test. †† $p < 0.01$ * $p < 0.05$

[Table from NDA 204447 submission; Study No. LBK0202, page 42]

According to the Sponsor, statistical analysis of the data showed a significant trend test for hepatocellular adenomas in males (p=0.026) and for the combination of hepatocellular adenomas and carcinomas in males (p=0.010); but, none of the pairwise comparisons were significant. Hepatocellular carcinomas alone were not significant.

Non Neoplastic: Peribiliary fibrosis and/or inflammation, crystalline material in bile ducts, bile duct hyperplasia, and eosinophilic inclusions in bile duct epithelium were seen in HD males and females (Table 56).

Table 56: Summary of non-neoplastic findings in the liver

Group/sex	1M	2M	3M	4M	1F	5F	6F	7F
Dose (mg/kg/day)	0	5	15	50	0	10	30	100
Peribiliary fibrosis/inflammation								
Minimal	0	0	0	10	0	0	0	3
Slight	0	0	0	13	0	0	0	3
Moderate	0	0	0	2	0	0	0	0
Total	0	0	0	25	0	0	0	6
Crystalline material in bile ducts								
Minimal	0	0	0	5	0	0	0	2
Slight	0	0	0	10	0	0	0	3
Moderate	0	0	0	2	0	0	0	0
Total	0	0	0	17	0	0	0	5
Bile duct hyperplasia								
Minimal	0	0	0	13	0	0	0	3
Slight	0	0	0	12	0	0	0	1
Total	0	0	0	25	0	0	0	4
Eosinophilic inclusions in bile duct epithelium								
Minimal	0	0	0	8	0	0	0	3
Slight	0	0	0	1	0	0	0	1
Moderate	0	0	0	1	0	0	0	0
Total	0	0	0	10	0	0	0	4
Number of animals examined	60	60	60	60	60	60	60	60

[Table from NDA 204447 submission; Study No. LBK0202, page 43]

The SAG considers the increased incidence of hepatocellular adenomas in HD males not to be relevant to humans[8]. Specifically, the SAG report states that

The minimal increase noted in the incidence of hepatocellular adenomas in the male mice from the high dose group is a rodent specific response mediated by the nongenotoxic mechanism of persistent hepatotoxicity at this dose level. This is not an indicator of carcinogenic risk to humans.

[8] Gopinath C., Maronpot H.P., Greaves P., (2010) – Scientific Advisory Group Report on the increased incidence of some tumours in rodent carcinogenicity studies with Lu AA21004.

Gall Bladder

Non Neoplastic: Epithelial hyperplasia was seen in 2 HDM and 1 HDF. Crystalline material was seen in the lumen of the gall bladder of HD males and females. Luminal dilation was slightly increased in HD males and females. Eosinophilic epithelial inclusions were seen in one MDM and 4 HDF. The crystalline material in the lumen of the gall bladder may correlate with the abnormal contents seen macroscopically. The non-neoplastic findings in the gall bladder are summarized in Table 57.

Table 57: Summary of non-neoplastic findings in the gall bladder

Group/sex	1M	2M	3M	4M	1F	5F	6F	7F
Dose (mg/kg/day)	0	5	15	50	0	10	30	100
Epithelial hyperplasia								
Minimal	0	0	0	2	0	0	0	1
Total	0	0	0	2	0	0	0	1
Crystalline material in lumen								
Minimal	0	0	0	12	0	0	0	3
Slight	0	0	0	20	0	0	0	19
Moderate	0	0	0	13	0	0	0	9
Marked	0	0	0	1	0	0	0	5
Total	0	0	0	46	0	0	0	36
Luminal dilatation								
Present	7	11	8	23	17	21	21	36
Total	7	11	8	23	17	21	21	36
Eosinophilic epithelial inclusions								
Minimal	0	0	1	0	0	0	0	2
Slight	0	0	0	0	0	0	0	2
Total	0	0	1	0	0	0	0	4
Number of animals examined	52	52	57	58	59	56	53	56

[Table from NDA 204447 submission; Study No. LBK0202, page 44]

Bile Duct (External)

Non Neoplastic: Crystalline material in the lumen of the bile duct was seen in 2/23 HDM and 1/25 HDF. Subepithelial fibrosis, inflammation, and edema was seen in 2/23 HDM. Epithelial hyperplasia was seen in 2/23 HDM.

Toxicokinetics

Plasma exposures of vortioxetine, a major human metabolite (Lu AA34443), and a non-major human metabolite (Lu AA39835) were determined in Weeks 1, 13, 26, 104 (M), and 105 (F) at only 2 and 24 (except at Week 104/105) hours post dosing. Due to misconduct at (b) (4), the TK data from Weeks 1, 13, and 26 were not found to be GLP

compliant. However, the TK data from Weeks 104 and 105 were GLP compliant. Based on remedial action, the Sponsor considers the TK data from Weeks 1, 13, and 26 not compromised.

At 2 hours postdose, all tested mice were exposed to vortioxetine and its metabolites, Lu AA34443 and Lu AA39835. Exposure was generally less than dose proportional for vortioxetine and more than dose proportional for Lu AA34443 and Lu AA39835. Because of the sparse sampling, estimation of TK parameters was not performed. Instead, the Sponsor compared the plasma concentrations from this study to TK data obtained in the 13-week mouse general toxicology study. In general, the mean plasma concentrations for vortioxetine were similar for the 13-week and carcinogenicity studies.

Vortioxetine

Because the estimation of TK parameters was not performed for the carcinogenicity study, human exposure margins for vortioxetine need to be calculated from the 4- and/or 13-week mouse general toxicology studies (Table 47 and Table 59). The graph in Figure 6 compares vortioxetine exposures for the 4- and 13-week studies. In general, the increase in dose results in a linear increase in exposure (except 4-week males). In addition, the exposure at 50 mg/kg in males is similar for the two studies. Therefore, the mouse-to-human exposure for the high dose in males to the MRHD of 20 mg is straight forward and is ~20-fold (Table 60).

However, the mouse-to-human vortioxetine exposure for the high dose in females is not straight forward. The carcinogenicity study high dose of 100 mg/kg was not used in either the 4- or 13-week study. Because the increase in dose results in a linear increase in exposure, the exposures at 100 mg/kg can be interpolated from Figure 6. In addition, males were dosed with 100 mg/kg in the 4-week study and the exposures are only 0.9 – 1.5-fold the interpolated exposures for females; therefore, the exposures of males at 100 mg/kg might be used to calculate exposure multiples. Based on the three data points, the mouse-to-human exposure for the high dose in males to the MRHD of 20 mg is 13 – 22-fold (Table 60).

Table 58: Summary of mean Cmax, Tmax, and AUC for vortioxetine at Day 28 in the 4-week mouse general toxicology study

Parameter	Males			Females		
	50 mg/kg	100 mg/kg	150 mg/kg	50 mg/kg	150 mg/kg	200 mg/kg
Cmax (nmol/L)	4556	5868	12,121	2701	5239	6899
Tmax (h)	1	1	2	4	8	4
$AUC_{0\text{-}24h}$ (nmol.h/L)	36,498	47,429	136,559	23,040	58,114	77,093

[Data from NDA 204447 submission; Study No. LBK 145, page 30]

Table 59: Summary of mean Cmax, Tmax, and AUC for vortioxetine in 13-week mouse general toxicology study

Parameter	Time	Males			Females		
		12.5 mg/kg	25 mg/kg	50 mg/kg	50 mg/kg	150 mg/kg	200 mg/kg
Cmax (nmol/L)	D1	2029	3686	4237	3485	3747	4746
	W13	2732	3348	4863	2554	3589	3969
Tmax (h)	D1	1	2	1	1	8	4
	W13	1	2	8	4	1	2
AUC_{0-24h} (nmol.h/L)	D1	10020	16860	28114	24242	54208	82996
	W13	12140	21354	42536	21347	36089	43923

[Data from NDA 204447 submission; Study No. LBK143; Text-Table 1, page 33]

Figure 6: Comparison of exposures (AUC_{0-24h}) at the end of 4-week and 13-week mouse general toxicology studies

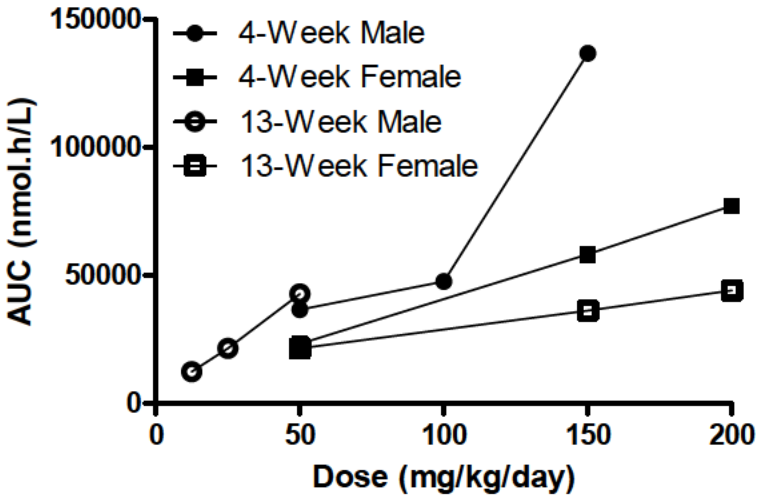


Table 60: Mouse-to-human AUC ratio for vortioxetine based on mouse values at Week 4 or 13

Study	Sex	Dose (mg/kg/d)	AUC_{0-24h} (ng.h/mL)*	Exposure Margin at MRHD#
4-Week	M	50	10,893	17
		100	14,155	22
	F	100	12,172^	19
13-Week	M	50	12,695	20
	F	100	8591^	13

*Mouse AUC_{0-24h} from Table 58 (Day 28) and Table 59 (Week 13) converted from nmol.h/L to ng.h/mL using the conversion factor 0.29845 (From Sponsor's Pharmacokinetics Written Summary, page 5).
#Human repeat dose AUC_{0-24h} at 20 mg = 646 ng.hr/mL (From Sponsor's Summary of Clinical

Pharmacology, Table 5.2, page 166). ^AUC_{0-24h} at 100 mg/kg/day for females in 4- and 13-week studies was interpolated from the graph in Figure 6.

Lu AA34443 (A Major Human Metabolite)

Estimation of TK parameters for Lu AA34443 was performed from exposure data in the 13-week, but not the 4-week, study (Table 61). Using the exposure (AUC) at 50 mg/kg in the males and interpolation of the exposure at 100 mg/kg in females, the exposure margin to the MRHD is 3 and 5.7, respectively (Table 62).

Table 61: Summary of mean Cmax, Tmax, and AUC for a major human metabolite Lu AA34443 in 13-week mouse general toxicology study

Parameter	Time	Males			Females		
		12.5 mg/kg	25 mg/kg	50 mg/kg	50 mg/kg	150 mg/kg	200 mg/kg
Cmax (nmol/L)	D1	239	2744	2840	4085	3269	3667
	W13	230	494	3322	2025	2895	4309
Tmax (h)	D1	1	2	1	1	1	1
	W13	1	1	1	1	1	1
AUC_{0-8h} (nmol.h/L)	D1	607	4773	6143	8316	10596	18304
	W13	575	1007	5079	4681	15051	19336

[Data from NDA 204447 submission; Study No. LBK143; Text-Table 2, page 34]

Table 62: Mouse-to-human AUC ratio for a major human metabolite Lu AA34443

Study	Sex	Dose (mg/kg/d)	AUC_{0-24h} (ng.h/mL)*	Exposure Margin at MRHD#
13-Week	M	50	1668	3
	F	100	3198^	5.7

*Mouse AUC_{0-24h} from Table 61 (Week 13) converted from nmol.h/L to ng.h/mL using the conversion factor 0.32843 (From Sponsor's Pharmacokinetics Written Summary, page 5). #Human repeat dose AUC_{0-24h} at 20 mg = 563 ng.hr/mL (From Sponsor's Summary of Clinical Pharmacology, Table 5.2, page 166). ^AUC_{0-24h} at 100 mg/kg/day for females in 13-week study was extrapolated from the data in Table 61.

Dosing Solution Analysis

All formulations were prepared fresh each week and were refrigerated and protected from light until use. Formulations samples taken in Weeks 1, 13, 26, 39, 52, 65, 78, 91, and 103 were determined to be within 94.6 – 104.2% of the nominal concentrations. Vortioxetine was not found in any vehicle samples.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

A preliminary dose range finding study (Study No. LBK0239) in sexually mature male and female Sprague-Dawley rats was conducted at oral doses of 5, 15, and 40 mg/kg/bid from 2 weeks prior to mating through postnatal day (PND) 6. There was no vortioxetine-related effect on body weight and food consumption for males or females. There was no vortioxetine-related effect of on mating, fertility parameters, implantation sites, mean duration of gestation, mean number of pups born, pup survival, pup clinical signs, and necropsy findings in adults and pups. Female pup body weight was slightly decreased in a dose-related manner for female pups on PND1, but not on PND7.

Due to no effects on bodyweight, feed consumption, lack of clinical signs, and no effect on mating or fertility in Study No. LBK0239, a higher dose of 60 mg/kg/bid was examined (Study No. LBK0262). Male and female Sprague-Dawley rats (5/sex) were dosed orally with 60 mg/kg/bid vortioxetine for 14 days. There were no vortioxetine-related premature mortalities, clinical signs, effects on body weight or feed consumption, or macroscopic necropsy findings.

Based on results of this study, 60 mg/kg/bid was selected as the high dose for the main fertility and early embryonic development study reviewed in detail in this section.

Study title: Lu AA21004: Oral (Gavage) Twice Daily Dosing Fertility and Early Embryonic Development Study in the Rat and Amendments 1 and 2

Study no.:	LBK0240; Reference no. 11682
Study report location:	(b) (4)
Conducting laboratory and location:	
Date of study initiation:	February 15, 2007
GLP compliance:	Yes, except TK data
QA statement:	Yes
Drug, lot no., and % purity:	Lu AA21004, 2018324, 98.8%

Key Study Findings

- Male and female Sprague-Dawley rats were dosed orally (by gavage, in 10% hydroxypropyl-beta-cyclodextrin (HP-ß-CD) and 4.4% glucose monohydrate) at 0, 20, 40, or 60 mg/kg/bid starting 29 days (males) or 15 days (females) and prior to and continuing through mating.
- An MTD was demonstrated in males based on premature mortality due to kidney pathology, decreased body weight and feed consumption, renal pelvis dilatation, and presence of white and/or yellow calculi in kidneys and ureters at 60 mg/kg/bid. The NOAEL is 40 mg/kg/bid.
- In females, an MTD was not demonstrated.

- Vortioxetine had no effect on male and female fertility and early embryonic development in rats at doses up to 58X the MRHD of 20 mg on a mg/m2 basis.

Methods

Doses:	0, 20 (LD), 40 (MD), or 60 (HD) mg/kg/bid
Frequency of dosing:	Twice daily
Dose volume:	6 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Solution/10% HP-ß-CD and 4.4% glucose monohydrate (isotonic)
Species/Strain:	Rats/Sprague-Dawley (Crl:CD (SD) IGS BR VAF PLUS)
Number/Sex/Group:	20/sex/group
Satellite groups:	None
Study design:	Males were dosed for 29 days prior to mating (pre-pairing period), during mating, and until necropsy. Females were dosed for 15 days prior to mating (pre-pairing period), during mating, and until Gestation Day (GD) 6. Females were necropsied on GD13. After the pre-pairing period, each female was paired with a male from the same dose group.
Deviation from study protocol:	Study protocol and SOP deviations were found for the TK evaluation performed at (b) (4) which resulted in the inability to claim GLP compliance for the TK data.

Observations and Results

Mortality

There were four premature mortalities. Due to dosing trauma, one MDM (No. 42) was found dead on Day 12 and one MDF (No. 132) was sacrificed for humane reasons on Day 26 (GD2). Due to clinical signs, two HDM (Nos. 73 and 76) were killed on Days 18 and 52, respectively. Clinical observations for HDM No. 73 were cold body surface, abnormal gait, decreased activity, hunched body posture, head tilting, and piloerection and for HDM No. 76 were red discharge from urethral opening and brown staining of perinasal and peribuccal areas. Findings at necropsy for Nos. 73 and 76 included distended stomach with food material, thickened duodenal mucosa, distended ureters with ureter containing white material, severe pelvic dilation of kidneys, presence of white calculi in kidneys, large abnormal size kidneys with white calculi, and/or dark and pale areas in kidney cortex.

Clinical Signs

Except for the clinical signs seen in HDM Nos. 73 and 76 discussed above, there were no vortioxetine-related clinical signs.

Body Weight

Mean body weight gain was lower at several time points throughout the dosing period for HDM compared to controls. At the end of dosing, HDM had gained an average of

14% less body weight and weighed an average 6% less than controls. There was no vortioxetine-related difference in body weight or body weight gain in females during the pre-pairing or gestation periods.

Feed Consumption

HDM consumed 5 and 9% less feed during Days 43 – 50 and 50 – 57, respectively, compared to controls

During the pre-pairing period, all female dose groups consumed 6 – 9% less feed than controls on Days 1 – 8. There was no significant change in feed consumption on Days 8 – 15. Feed consumption for MD and HD females was decreased 4 and 8%, respectively, compared to controls on GD0 – 7 and was increased 3 and 7%, respectively, compared to controls on GD7 – 13. During gestation, only the feed consumption for HD females on GD0 – 7 was below the laboratory background range of 25 – 29 g (HDF: 24g).

Toxicokinetics

Plasma exposures of vortioxetine, a major human metabolite (Lu AA34443) and a non-major human metabolite (Lu AA39835) were determined on Day 29 for males and Day 15 for females predose and at only 2 and 10 hours postdose. Due to misconduct at (b) (4), GLP compliance is not claimed for the TK data. Based on remedial action, the Sponsor considers the bioanalytical data reliable.

Exposure was generally dose proportional or more than dose proportional for vortioxetine (except for females from 40 to 60 mg/kg/bid at 2 hours where there was a decrease in exposure), Lu AA34443, and Lu AA39835 (except for females from 40 to 60 mg/kg/bid at 10 hours where there was a decrease in exposure). In general, no sex-related difference in exposure to vortioxetine was seen. Exposure to Lu AA34443 and Lu AA39835 was approximately 2X greater in males than females at 20 and 40 mg/kg/bid for Lu AA34443 and at 40 and 60 mg/kg/bid for Lu AA39835.

Because of the sparse sampling, estimation of TK parameters was not performed. Instead, the Sponsor compared the plasma concentrations of vortioxetine and Lu AA34443 from this study to TK data obtained at the 10 hour time point from Week 13 (the only time point the Sponsor can directly compare) of the 26-week general toxicity study in Wistar rat. The mean plasma concentrations for vortioxetine and Lu AA34443 were similar for males and females at the 20 mg/kg/bid dose for the 26-week and fertility studies. However, mean plasma concentrations for vortioxetine and Lu AA34443 for males and females at 40 mg/kg/bid were approximately double for the 26-week study compared to the fertility study.

Dosing Solution Analysis

All formulations were prepared fresh each week. Formulation samples taken in Weeks 1, 4, and 8 were determined to be within 7% of the nominal concentrations.

Necropsy

Renal pelvis dilatation of one or both kidneys was noted in 7/20 HDM and 2/ 20 MDM. Four of the seven HDM with renal pelvis dilation also were noted to have white and/or yellow calculi in the kidneys and two of these four also had distended ureters with white material. Three HDM that did not have renal pelvis dilatation had white and/or yellow calculi in their kidneys. No MDM were noted to have the white and/or yellow calculi in their kidneys. The presence of the white and/or yellow calculi is consistent with findings from the chronic rat study and is most likely vortioxetine-related.

Abnormal sized right and/or left submandibular lymph nodes were noted in 4/20 HDM and 2/20 MDM.

Large abnormal sized right or left testis was noted in 1/20 HDM (No. 68) and 1/20 MDM (No. 47) at necropsy. However, there was no vortioxetine-related effect on mean absolute or body weight-related testes weight (Mean absolute: 3.61, 3.58, 3.78, and 3.55 g for 0, 20, 40, and 60 mg/kg/bid, respectively; body weight related: 0.76, 0.76, 0.78, 0.79% for 0, 20, 40, and 60 mg/kg/bid, respectively; No. 47 5.71 g/1.28%; No. 68 5.81g/1.23%).

One LDF had moderate right kidney pelvic dilation. There were no other macroscopic necropsy findings for females.

Fertility Parameters

There was no vortioxetine-related effect on mean female estrus cycle (2, 2, 1.9, and 1.9 complete estrous cycles for 0, 20, 40, and 60 mg/kg/bid, respectively) and time to mating (2.7, 2.5, 2.7, and 2.2 days for 0, 20, 40, and 60 mg/kg/bid, respectively). The mean number of copulation plugs was lower at the HD compared to controls (3.4 vs. 4.7); however, the HD value of 3.4 was within the background range of 2.7 – 5.4. There was no vortioxetine-related effect on male and female fertility and mating (Table 63 and Table 64). All pairs mated and had successful pregnancies except one MD pair—the female (No. 132) was sacrificed on GD2 for humane reasons due to dosing error.

The mean number of headless sperm was statistically higher for HDM compared to controls (Table 63). However the mean value of 2.33 is in the historical control background range of 1.00 – 5.84. There were no other vortioxetine-related findings for sperm morphology or semen analysis (motility, concentration, and actual path or straight line velocities).

There was no significant vortioxetine-related effect for females dosed till GD6 on the mean number of corpora lutea, implantations, live embryos, or pre- and post-implantation loss (Table 64).

Table 63: Mean fertility parameters in male rat

Parameter	Dose (mg/kg/bid)			
	0	20	40	60
Mating Index	100%	100%	100%	100%
Fertility Index	100%	100%	94.7%	100%
Sperm Morphology				
normal	193	197	197	195
headless	1.05	1.40	1.16	2.33*
no tails	0.65	0.2	0.26	0.78
Mis. abnormalities	0.9	0.45	0.84	1.11
Semen Analysis				
Motility	98.4%	98.1%	95.9%	97.6%
Concentration (M/mL)	13.1	12.8	13.6	14.5
Velocity-actual path (µm/s)	62.2	63.2	62.2	63.7
Velocity-straight line (µm/s)	34.5	36.6	34.5	37.4

* $p<0.01$ (trend test)

[Data from NDA 204447 submission; Study No. LBK0240]

Table 64: Mean fertility parameters in female rat

Parameter	Dose (mg/kg/bid)			
	0	20	40	60
Mating Index	100%	100%	100%	100%
Fertility Index	100%	100%	95%	100%
Corpora lutea/rat	16.8	15.8	16.7	15.9
Implantations/rat	16.2	14.3	15.5	15.0
Preimplantation loss	3.7%	9.3%	6.9%	4.9%
Early resorptions	14	21	11	15
Dead embryos	0	0	0	0
Post implantation loss	4.2%	6.9%	3.7%	5.3%
Live embryos/litter	15.5	13.3	14.9	14.3

[Data from NDA 204447 submission; Study No. LBK0240]

9.2 Embryonic Fetal Development

9.2.1 Rat Embryonic Fetal Development

A preliminary dose range finding study (Study No. 10145) in pregnant female Sprague-Dawley rats was conducted at oral doses of 10, 20, 30, and 40 mg/kg/bid from GD6 - 17. Decreased feed consumption was noted for vortioxetine-dose rats throughout dosing. There was no impairment of reproductive parameters and no external fetal abnormalities were noted. Based on the results of this study, doses of vortioxetine at 5, 15, and 40 mg/kg/bid were selected for the main study reviewed in detail in this section.

Two pivotal studies were conducted in pregnant rats: initially at doses up to 40 mg/kg/bid, then a second study at doses of 60 and 80 mg/kg/bid.

Study title: Lu AA21004: Oral (Gavage) Developmental Toxicity Study in the Rat

Study no.:	LBK0156; Reference no. 10143
Study report location:	Regulatory Central Archive at H. Lundbeck A/S
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 4, 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot no., and % purity:	Lu AA21004, 1001258, 99.8%

Key Study Findings

- Pregnant Sprague-Dawley rats were dosed orally (by gavage, in 10% hydroxypropyl-beta-cyclodextrin (HP-ß-CD) and 4.4% isotonic glucose monohydrate) at 0, 5, 15, or 40 mg/kg/bid during the period of organogenesis (gestational day 6 – 17).
- Maternal toxicity was not seen.
- Delayed ossification of the fetal skeletal system without a corresponding decrease in fetal weight occurred at 15 and 40 mg/kg/bid.
- The NOAEL for maternal toxicity is ≥40 mg/kg/bid and for fetal developmental toxicity is 5 mg/kg/bid based on delayed ossification, which is 39 and 5X, respectively, the MRHD of 20 mg on a mg/m2 basis.
- There was no evidence of teratogenicity in rats at doses up to 39X the MRHD of 20 mg on a mg/m2 basis.

Methods

Doses:	0, 5 (LD), 15 (MD), or 40 (HD) mg/kg/bid
Frequency of dosing:	Twice daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage

Formulation/Vehicle:	Solution/10% HP-β-CD and 4.4% glucose monohydrate
Species/Strain:	Rat/Hsd:Sprague Dawley SD
Number/Sex/Group:	24 females/group
Satellite groups:	None
Study design:	Pregnant rats were dosed from gestational day (GD) 6 – 17. The fetuses were delivered by c-section on GD20 and examined.
Deviation from study protocol:	No significant deviations

Observations and Results

Mortality

No rats died or were euthanized.

Clinical Signs

No vortioxetine-related clinical signs were noted.

Body Weight

There were no differences in group mean body weights for dosed rats compared to controls, except for MD dams that gained slightly more body weight during the dosing period and weighed 4% more than controls at the end of dosing.

Feed Consumption

Dosed dams consumed significantly less feed (5 – 24%) than controls from GD 6 – 9 with HD dams consuming the least amount of feed. HD dams continued to consume significantly less feed than controls (7 – 13%) for the rest of the dosing period. After dosing stopped, MD and HD dams consumed 6% more than controls.

Toxicokinetics

Mean plasma exposures for vortioxetine were determined on GD6 and 17 predose (GD17 only) and at only 2 and 10 hours postdose. Exposure was greater than dose proportional at GD6 and 17. Exposures at 15 and 40 mg/kg/bid on GD17 were approximately 1.5 – 3 times the exposures on GD6, while exposures at 5 mg/kg/bid were similar on both days.

Because of sparse sampling, estimation of TK parameters was not performed. Instead, the Sponsor compared the plasma concentrations at 40 mg/kg/bid from this study to TK data obtained at 2 and 10 hours on Days 1 and 29 in the 4-week general toxicology study in Wistar rats. Mean plasma concentrations at 40 mg/kg/bid were approximately double for the 4-week study in Wistar rats compared to this embryo-fetal development study in Sprague-Dawley rats, except at 2 hours on Day 17 which was similar to the 2 hour time point on Day 29 of the general toxicology study.

Dosing Solution Analysis

All formulations were prepared twice and were protected from light. Formulation samples taken on both days of preparation were determined to be within 102.2 – 108.1% of the nominal concentrations.

Necropsy

There were no vortioxetine-related necropsy findings.

Cesarean Section Data

There were no vortioxetine-related effects on the mean number of corpora lutea or implantations. There was a non-significant, non-dose dependent increase in the percent of postimplantation loss for the dosed groups; although, there was no difference in the mean number of live fetuses per dam. There was an increase in early resorptions per dam for LD and MD compared to controls; although, there was no difference for HD compared to controls.

Offspring (Malformations, Variations, etc.)

The offspring data is summarized in Table 65. There was no effect on fetal weight and sex rate. There was a small, but significant increase in placental weight at the MD and HD.

Table 65: Summary of offspring data in rat

Parameter	Dose (mg/kg/bid)			
	0	5	15	40
Mean live fetuses/litter	11.8	11.4	11.9	12.4
Mean sex rate (% males)	47.7%	49.2%	51.5%	51.5%
Mean fetus weight (g)	3.92	4.01	3.98	3.91
Mean placental weight (g)	0.61	0.62	0.66**	0.63*
Total number of litters examined	23	22	24	23
Number of fetuses examined	272	250	285	273
Fetuses with malformations (litters)	5 (3)	4 (4)	1(1)	1 (1)
Fetuses with minor abnormalities (mean % of fetuses examined)	39 (14.2%)	32 (13%)	44 (15.3%)	43 (16.2%)
Fetuses with variations (mean % of fetuses examined)	136 (50.5%)	150* (60.3%)	151 (53.2%)	144 (50.5%)

*p<0.05; **p<0.01

[Data from NDA 204447 submission; Study No. LBK0156]

Malformations occurred in 5(3), 4(4), 1(1), and 1(1) control, LD, MD, and HD fetuses (litters), respectively. One control, LD, and MD fetus had an interrupted aortic arch. The MD fetus also had scoliosis of the cervical, thoracic, lumbar, sacral or caudal vertebra and was absent one or more neural arches of the lumbar vertebra. One control had a malrotated hindlimb. Three control fetuses (from the same litter) had micrognathia of jaw; cleft palate and palantine; microglossia of tongue (1 fetus); anasarca of the body; short mandible and rib; malformed scapula; short humerus, radius, and ulna of forelimb;

and short femur, tibia, and fibula of hindlimb. Two LD fetuses from separate litters had undescended testis and one LD fetus had meningocele of the brain. The HD fetus had malformed scapula and clavicle of pectoral girdle; malformed humerus, radius, and ulna of forelimb; and malformed femur, tibia, and fibula of hindlimb.

There was a statistically significant increase in the number of fetuses with variations following maternal dosing of LDF compared to controls (Table 65). The variation that occurred at significantly higher rates in fetuses of LDF compared to controls was dilated ureter (Table 66). Although not significant, the incidence of dilated ureter was also increased in fetuses of MDF and HDF compared to controls. However, this increase is not dose-dependent and the values for the fetuses from MDF and HDF were within the historical control range and the values for the fetuses from the LDF were only slightly higher than the historical control range.

Minor abnormalities and variations that occurred at significantly higher rates in fetuses of MDF and HDF compared to controls were related to delayed ossification of the fetal skeleton in the skull and the centra of the cervical and thoracic vertebra; although, the incidences for these minor abnormalities and variations were mostly withing the background range (Table 66). There was a nonsignificant increase in the incidence of irregular ridging of the palate in fetuses of HDF compared to control (Table 66). However, the incidence was still within the background range.

Table 66: Minor abnormalities and variations that reached statistical significance in rat

Fetal Abnormality	Dose (mg/kg/bid)				Background range
	0	5	15	40	
ureter: dilated	4.6%	15.7%**	9.1%	7.5%	3.2 – 14.9%
occipital skull: incomplete ossification	2.5%	3.5%	3.1%	7.2%*	0.7 – 11.8%
centra of cervical vertebra: ossified	48.5%	59.3%	33.4%**	34%**	25.6 – 60.3%
centra of thoracic vertebra: incomplete ossification	5.1%	7%	11.3%*	10%	0 – 7.7%
centra of thoracic vertebra: bilobed ossification	7.9%	4.5%	16.9%*	15.2%*	4.8 – 22.7%
Number of thoracic vertebra: 14	0%	0.8%	3%*	2.3%	0 – 4%
Number of lumbar vertebra: 5	0%	0.8%	3%*	2.3%	0 – 4%
Rib: 14th uni or bilateral: extra	0%	0.8%	3%*	2.3%	0 – 4%
palate: irregular ridging	4.3%	5.1%	4.7%	10.2%	2.2 – 18.6%

*p<0.05; **p<0.01

[Data from NDA 204447 submission; Study No. LBK0156]

Study title: Lu AA21004: Oral (Gavage) Twice Daily Dosing Developmental Toxicity Study in the Rat and Amendment Number 1

Study no.:	LBK0280; Reference no. 12715
Study report location:	Regulatory Central Archive at H. Lundbeck A/S
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 16, 2008
GLP compliance:	Yes, except TK analysis
QA statement:	Yes
Drug, lot no., and % purity:	Lu AA21004, 1001258, 99.5%

Key Study Findings

- Pregnant Sprague-Dawley rats were dosed orally (by gavage, in 10% hydroxypropyl-beta-cyclodextrin (HP-ß-CD) and 4.4% isotonic glucose monohydrate) at 60 and 80 mg/kg/bid during the period of organogenesis (GD6 – 17).
- Maternal toxicity—characterized by decreased body weight and feed consumption—occurred at 60 and 80 mg/kg/bid.
- Fetal developmental toxicity—characterized by decreased fetal weight and delayed ossification of the fetal skeletal system—occurred at 60 and 80 mg/kg/bid.
- No NOAELs were determined for maternal or fetal developmental toxicity.
- There was no evidence of teratogenicity in rats at doses up to 78X the MRHD of 20 mg on a mg/m2 basis.

Methods

Doses:	0, 60 (LD), or 80 (HD) mg/kg/bid
Frequency of dosing:	Twice daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Solution/10% HP-β-CD and 4.4% glucose monohydrate
Species/Strain:	Rat/Sprague Dawley (Crl:CD (SD) IGS BR VAF PLUS)
Number/Sex/Group:	20 females/group
Satellite groups:	3 females/group (TK)
Study design:	Pregnant rats were dosed from GD6 – GD17. The fetuses were delivered by c-section on GD20 and examined.
Deviation from study protocol:	Study protocol and SOP deviations were found for the TK evaluation performed at (b) (4) which resulted in the inability to claim GLP compliance for the TK data.

Observations and Results

Mortality

One HDF (No. 47) was euthanized on GD14 due to clinical condition—excess salivation pre-dose, hunched posture, and noisy breathing. At necropsy, food was found in the esophagus, while there was reduced food in the rest of the GI tract. No blockage was observed upon macroscopic examination.

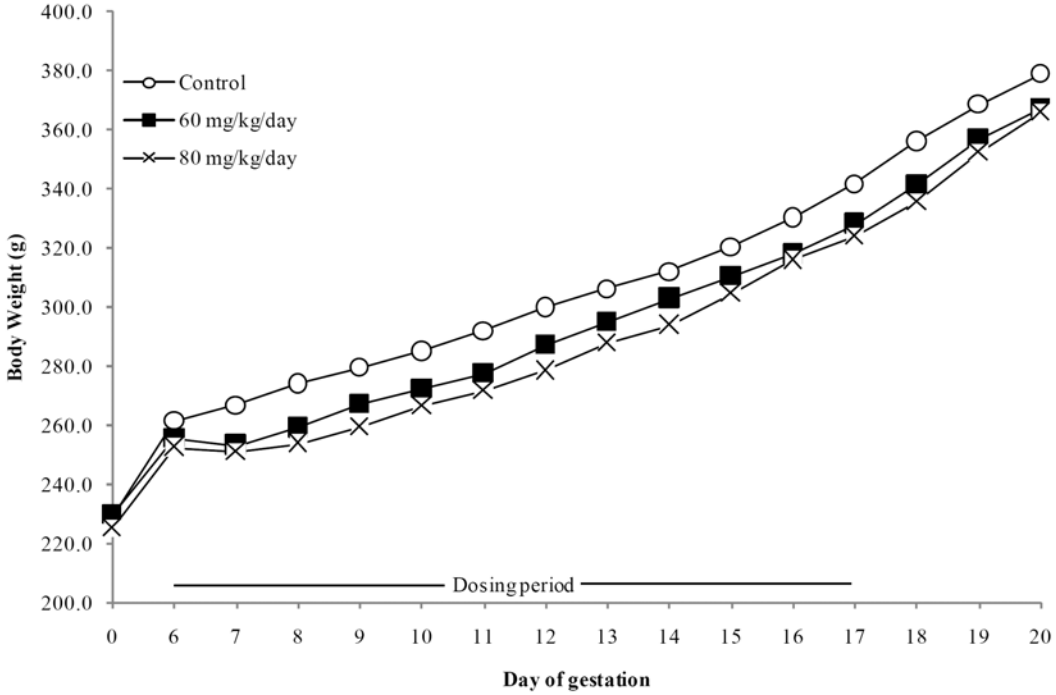
Clinical Signs

Excessive salivation was observed pre- and post-dose sporadically in 5 LDF and 5 HDF.

Body Weight

Dosed females lost on average 0.5 – 1% of their body weight after the first day of dosing and weighed on average 5 – 6% less than controls on GD7. However, dosed females gained weight at a similar rate to controls for the remainder of dosing. After the dosing period (GD18 – 20), dosed females gained 3 – 7 g more than the controls (Figure 7).

Figure 7: Group mean maternal body weight in rat



[Figure from NDA 204447 submission; Study No. LBK0280, page 37]

Feed Consumption

Dosed females consumed on average 26 – 35% less feed than controls from GD6 – 9. Dosed females consumed on average 10 – 18% less feed than controls for the remainder of the dosing period. After the dosing period (GD18 – 20), dosed females consumed 10 – 14% more feed than controls.

Toxicokinetics

Plasma exposures of vortioxetine, a major human metabolite (Lu AA34443), and a non-major human metabolite (Lu AA39835) were determined on GD17 at 1, 3, 10, 12, 16, and 24 hours after the first dose of the day. Due to misconduct at (b) (4), the TK data were not found to be GLP compliant. Based on remedial action, the Sponsor considers the TK data reliable.

Peak exposures (Cmax) were reached 1 – 12 hours postdose (1 – 3 hours after the first or second dose of the day) for vortioxetine, Lu AA34443, and Lu AA39835 on GD17. Exposures (Cmax and AUC) generally increased in a dose-proportional manner for Lu AA21004 and Lu A34443 and decreased for Lu AA39835. Exposure was greater for the metabolite Lu AA34443 compared to parent, Lu AA21004. In contrast, plasma exposure was less for Lu AA39835 compared to parent. The TK results are summarized in Table 67.

Table 67: Geometric mean TK parameters on GD17 for vortioxetine (Lu AA21004), Lu AA34443, and Lu AA39835 in rat

Analyte	Dose	C_{max}	t_{max*}	AUC_{0-10h}	AUC_{10-24h}	$t_{1/2}$
	mg/kg/dose	nmol/L	hr	hr*nmol/L	hr*nmol/L	hr
Lu AA21004	**60**	4560	12	26100	38500	8.63[2]
(n = 3)	**80**	5410	1	37300	38700	5.28[2]
Lu AA34443	**60**	10100	12	59700	83400	16.7[1]
(n = 3)	**80**	11800	3	90700	88700	6.73[2]
Lu AA39835	**60**	31.9	12	199	268	22.4[1]
(n = 3)	**80**	26.6	12	183	254	11.0[1]

[1], n = 1; [2], n = 2. *median value reported

[Table from NDA 204447 submission; Study No. LBK0280, Amendment 1, page 7]

The rat-to-human AUC ratio for vortioxetine and Lu AA34443 at 60 and 80 mg/kg/bid is shown in Table 68. The exposure margins are to the MRHD of 20 mg.

Table 68: Rat-to-human AUC ratio for vortioxetine and Lu AA34443 at 60 and 80 mg/kg/bid

Anylate	Dose (mg/kg/bid)	AUC_{0-10h} (ng.h/mL)*	Exposure Margin at MRHD[#]
Vortioxetine	60	7790	12X
	80	11,132	17X
Lu AA34443	60	19,607	35X
	80	29,789	53X

* Rat AUC_{0-10h} from Table 67 converted from nmol.h/L to ng.h/mL using the conversion factor 0.29845 for vortioxetine and 0.32843 for Lu AA34443 (From Sponsor's Pharmacokinetics Written Summary, page 5).

[#]Human repeat dose AUC_{0-24h} for vortioxetine at 20 mg = 646 ng.hr/mL and for Lu AA34443 at 20 mg = 563 ng.hr/mL (From Sponsor's Summary of Clinical Pharmacology, Table 5.2, page 166).

Dosing Solution Analysis

All formulations were prepared weekly and protected from light. Formulation samples were determined to be within 96.7 – 100.3% of the nominal concentrations.

Necropsy

There were no vortioxetine-related macroscopic findings at necropsy.

Cesarean Section Data

There were no vortioxetine-related effects on the mean number of corpora lutea, implantations, resorptions, or live fetuses.

Offspring

The offspring data is summarized in Table 69. There was a significant 7 and 9% decrease in mean fetus weight at the LD and HD, respectively, compared to controls. The fetal weight reduction is observed in both sexes. There was no vortioxetine-related effect on mean placental weight or sex rate.

Table 69: Summary of offspring data in rat

Parameter	Dose (mg/kg/BID)		
	0	60	80
Mean live fetuses/litter	12	12.2	13.4
Mean sex rate (% males)	45.4%	56.8%	54.8%
Mean fetal weight (g)	4.10	3.83**	3.74**
Mean placental weight (g)	0.55	0.59	0.54
Total number of litters examined	19	20	18
Number of fetuses examined	228	243	241
Fetuses with malformations (litters)	2 (2)	3 (2)	1 (1)
Fetuses with minor abnormalities (mean % of fetuses examined)	46 (22.3%)	68 (32.4%*)	71 (29.7%*)
Fetuses with variations (mean % of fetuses examined)	122 (54.3%)	135 (56.9%)	146 (61.4%)

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

[Data from NDA 204447 submission; Study No. LBK0280]

Malformations were rare occurring in 2(2), 3(2), and 1(1) control, LD, and HD fetuses (litters), respectively. One control and one LD fetus had an interrupted aortic arch. One control had multiple malformations—including filamentous tail, horseshoe shaped kidney, ectopic ovary, discontinuous spine, bifid cervical and thoracic neural arches, malformed neural arch, absent lumbar, sacral, and caudal vertebra, severe fused ribs, absence of one or more sternebra, and malformed pelvic girdle. One LD had cystic dilatation in the cerebral hemisphere. One LD had multiple malformations of the mouth, jaw, and head—including micrognathia of jaw, microglossia of tongue, a malformed

palate and mandible, and fused tympanic rings of the skull. The HD fetus had a constricted pulmonary arch.

There was a statistically significant increase in the percent of fetuses with minor abnormalities following maternal dosing of test article compared to controls (Table 69). The minor abnormalities and variations that occurred at significantly higher rates in fetuses of LDF and HDF compared to controls were related to delayed ossification of the fetal skeleton in skull; cervical, lumbar, sacral and caudal vertebra; sternum; forelimb; and hindlimb (Table 70). The delayed ossification of fetal skeleton correlates with the fetal weight reduction observed in the dosed groups.

Table 70: Statistically significant minor abnormalities and variations in rat

Foetal Findings	Background Data (%)	Group 1 (%)	Group 2 (%)	Group 3 (%)
Skull: interparietal: incomplete ossification variant	7.0 - 34.3	20.6	**38.1***	**35.2****
Skull: occipital: incomplete ossification variant	4.6 – 27.6	15.7	**30.2***	**36.6*****
Skull: zygomatic arch- uni- or bilateral: incomplete ossification minor	0.6 – 6.3	8.0	18.8	**15.5***
Skull: hyoid: not ossified minor	2.8 – 34.2	5.9	9.1	18.2**
Cervical vertebra: one or more neural arch: incomplete ossification minor	0.0 – 14.1	1.8	6.2	7.7*
Forelimb: one or more metacarpal 1st – 4th: incomplete ossification minor	0.0 – 7.2	2.6	3.0	**10.9****
Forelimb: 5th metacarpal- uni- or bilateral: not ossified variant	13.8 – 75.1	23.9	42.9**	53.0***
Hindlimb: one or more metatarsal: not ossified minor	0.0 – 18.4	0.0	0.0	3.8*
Sternum: 5th sternebra: not ossified variant	9.1 – 52.9	10.0	17.2*	35.9***
Sternum 6th sternebra: not ossified variant	0.0 – 56.7	4.1	7.3	25.8***
Sternum: 6th sternebra: incomplete ossification variant	0.0 – 40.8	10.7	33.5***	33.9***
Lumbar vertebra: one or more neural arch: incomplete ossification minor	0.0 – 2.6	0.0	0.6	**2.9***
Sacral vertebra: one or more neural arch incomplete ossification minor	2.3 – 43.3	12.2	22.3*	28.5**
Caudal vertebra: number of centra <=2 ossified minor	0.0 – 22.2	0.0	2.5	4.3*

Numbers in bold type are outside the background data range.
*p<0.5, ** p<0.01, *** p<0.001, statistical significance is based on the number of foetuses with the finding.

[Table from NDA 204447 submission; Study No. LBK0280, page 32]

9.2.2 Rabbit Embryonic Fetal Development

In a preliminary dose range finding study (Study No. LBK0153) in New Zealand White rabbits, single oral doses of 10 and 20 mg/kg/bid vortioxetine (in 10% hydroxypropyl-beta-cyclodextrin (HP-β-CD) and 4.4% glucose monohydrate) resulted in dose-related decrease in body weight and feed consumption compared to controls. Rabbits were next dosed with 15 mg/kg/bid for seven days. A small decrease in body weight and feed consumption compared to predose values was seen. Based on the decreased body weight and feed consumption, a high dose of 15 mg/kg/bid was selected for the rabbit developmental toxicity dose range finding study.

In a preliminary developmental toxicity dose range finding study (Study No. LBK0154), pregnant New Zealand White rabbits were orally dosed with vortioxetine (in 10% HP-β-CD and 4.4% glucose monohydrate) at 5, 10, or 15 mg/kg/bid. Decreased body weight (≤4%), feed consumption (≤83%), and feces was seen in all groups (control and vortioxetine) throughout the dosing period. There was no vortioxetine-related effect on implantations, the number of live fetuses, mean fetal and placental weight, sex rate, and external fetal abnormalities.

Based on the results of this study, doses of vortioxetine at 5, 10, and 15 mg/kg/bid were selected for the main study reviewed in detail in this section. A second pivotal study was conducted using doses of 1 and 30 mg/kg/bid, and including a water control group, as well as a HP-ß-CD vehicle control group: and also reviewed in detail in this section

Study title: Lu AA21004: Oral (Gavage) Developmental Toxicity Study in the Rabbit

Study no.:	LBK0155; Reference no. 10238
Study report location:	Regulatory Central Archive at H. Lundbeck A/S
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 4, 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot no., and % purity:	Lu AA21004, 1004239, 99.8%

Key Study Findings

- Pregnant New Zealand White rabbits were dosed orally (by gavage, in 10% HP-ß-CD and 4.4% isotonic glucose monohydrate) at 0 (water), 0, 5, 10, or 15 mg/kg/bid during the period of organogenesis (gestational day 6 – 18)
- Maternal toxicity—characterized by decreased body weight and feed consumption—occurred at 5, 10, and 15 mg/kg/bid.
- Developmental toxicity—characterized by decreased fetal weight and delayed ossification of the fetal skeletal system—occurred at 5, 10, and 15 mg/kg/bid.

- There was a small, non-dose dependent, numerical increase in CNS malformations at 5, 10, and 15 mg/kg/bid.
- No NOAEL was determined for maternal toxicity, developmental toxicity, and CNS malformations.

Methods

Doses:	0, 5 (LD), 10 (MD), or 15 (HD) mg/kg/bid
Frequency of dosing:	Twice daily
Dose volume:	2 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Solution/10% HP-β-CD and 4.4% glucose monohydrate
Species/Strain:	Rabbit/New Zealand White
Number/Sex/Group:	20 females/group
Satellite groups:	3 females/group (TK)
Study design:	Pregnant rabbits were dosed from GD6 – GD18. The fetuses were delivered by c-section on GD28 and examined.
Deviation from study protocol:	No significant deviations

Observations and Results

Mortality

There were no premature moralities.

Clinical Signs

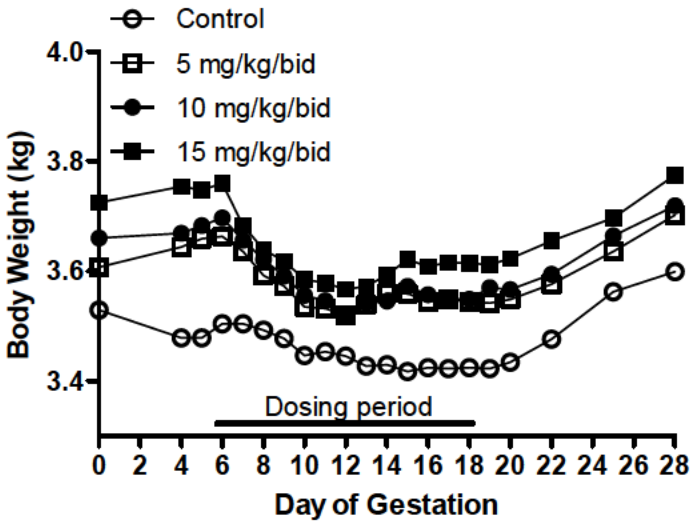
Reduced feces production was noted in females in all groups throughout the study; although, the number of rabbits and the frequency of reduced feces was greater in the dosed groups. The reduced feces corresponded with lower food intake for all groups.

Body Weight

At the start of the study, groups were not evenly distributed for body weight and group mean body weights were increased for dosed groups 2 – 6% compared to controls (Figure 8). Body weights for dosed groups remained increased 5 – 7% at the start of dosing on GD6. As a result, group mean body weights for dosed groups during the dosing period and after dosing cannot be compared to controls and are instead compared to their predose weight.

All dosed groups lost weight (0.7 – 2%) after the first day of dosing with HD females losing the most amount of weight while controls did not change weight. All groups continued to lose weight until GD12 (1.7, 4, 4.8, 5% for control, LD, MD, and HD, respectively); although, the vortioxetine dosed females lost more weight than controls. After GD12, the control and LD females maintained their weight and the MD and HD females gained weight (1.4%) through the dosing period. After the dosing period (GD19 – 28), all groups gained the same amount of weight (4 – 5%).

Figure 8: Group mean maternal body weights in rabbits



[Data from NDA 204447 submission; Study No. LBK0155, pages 35 - 36]

Feed Consumption

Feed consumption was increased for dosed groups prior to dosing by as much as 48% compared to controls. During dosing, vortioxetine dosed females consumed on average much less feed than controls from GD6 – 14 (↓27 – 49% LDF, ↓16 – 59% MDF, ↓26 – 70% compared to controls). However, all groups, including controls consumed less feed during dosing than their predosing consumption with dosed groups consuming the least amount of feed (↓≤74, 80, 88, and 87% for controls, LDF, MDF, and HDF). MDF and HDF feed consumption remained less than controls (↓≤28%) through most of the study until GD24 when feed consumption was similar.

Toxicokinetics

Plasma exposure of vortioxetine was determined on GD18 predose and at 2, 4, 7, 10, 12, 14, and 24 hours after the first dose of the day. Cmax was reached after 2 hours for all doses. Plasma exposure (AUC and Cmax) was more than dose proportional from 5 to 10 mg/kg/bid. However, due to high individual variability at the HD (7 – 9-fold difference between one rabbit and the other two), dose proportionality with the HD is not interpretable (HD individual Cmax: 164, 214, and 1526 nM; AUC: 1140, 1291, and 10,386 nM.h). The mean TK results are summarized in Table 71.

Table 71: Mean TK parameters on GD18 for vortioxetine

Dose (mg/kg/bid)	tmax (h)	Cmax (nM)	AUC_{0-24} (nM.h)
5	2	68.3	512
10	2	464	2640
15	2	635	4272

[Data from NDA 204447 submission; Study No. LBK0155, page 130]

Dosing Solution Analysis

Formulation samples were determined to be within 101.7 – 108.6% of the nominal concentrations.

Necropsy

There were no vortioxetine-related macroscopic findings at necropsy.

Cesarean Section Data

There were no vortioxetine-related effects on the mean number of corpora lutea and implantations. There was a nonsignificant increase in the number of late resorptions for all dose groups and postimplantation loss and early resorptions for the LD females. However, there was no difference in the mean number of fetuses for dosed groups compared to controls. The cesarean section data are summarized in Table 72.

Table 72: Summary of cesarean section data in rabbit

Parameter	Dose (mg/kg/bid)			
	0	5	10	15
Total pregnancy rate	19/20	18/20	17/20	19/20
Females with live fetuses	18	17	17	19
Mean corpora lutea	8.8	10.1	9.9	10.1
Mean implantation sites	7.2	9.2	9.1	8.1
Mean preimplantation loss	19.4%	8%	9.4%	19.9%
Mean postimplantation loss	18.3%	26.9%	16.4%	14.9%
Mean live fetuses	6.4	7	7.4	6.7
Mean dead fetuses	0	0	0	0
Abortions	0	0	0	0
Early resorptions (Mean)	16 (0.84)	28 (1.5)	15 (0.88)	16 (0.84)
Late resorptions (Mean)	5 (0.26)	18 (1)	14 (0.82)	11 (0.58)

[Data from NDA 204447 submission; Study No. LBK0155]

Offspring

The offspring data is summarized in Table 73. There was a significant 12, 7 and 8% decrease in mean fetus weight at the LD, MD, and HD, respectively, compared to controls. The fetal weight reduction is observed in both sexes. There was also a nonsignificant decrease in mean placental weight for all dose groups. There was no effect on the sex rate.

Table 73: Summary of offspring data in rabbit

Parameter	Dose (mg/kg/bid)			
	0	5	10	15
Mean live fetuses/litter	6.4	7	7.4	6.7
Mean sex rate (% males)	46%	49.8%	51.1%	55.7%
Mean fetal weight (g)	35.3	31.1*	32.9*	32.3*
Mean placental weight (g)	3.95	3.53	3.44	3.61
Total number of litters examined	18	17	17	19
Number of fetuses examined	115	119	126	127
Fetuses with malformations (litters)	3(3)	1(1)	3(3)	6(5)
Fetuses with minor abnormalities (mean % of fetuses examined)	54 (47%)	73* (60.8%)	85** (69%)	81** (65.9%)
Fetuses with variations (mean % of fetuses examined)	114 (99.2%)	119 (100%)	125 (99.2%)	127 (100%)

*p<0.05; **p<0.01

[Data from NDA 204447 submission; Study No. LBK0155]

Malformations occurred in 3(3), 1(1), 3(3), and 6(5) control, LD, MD, and HD fetuses (litters), respectively. Two control fetuses had pulmonary valvular atresia and one had a fused sternum. The LD fetus had spinabifida, fused neural arch of thoracic and lumbar vertebra, fused centra of lumbar vertebra, malformed neural arch of lumbar vertebra, and bifid 2nd neural arch of lumbar vertebra. The three MD fetuses had meningocele (all in different litters) and one MD fetus also had a malformed parietal skull and absent interparietal skull. Two HD fetuses from the same litter had short and malformed femur. The remaining four HD fetuses from different litters had external, visceral, and skeletal malformations: 1) gastroschisis and bifid pubis; 2) cystic dilatation of cerebellum; 3) acephaly, anencephaly, acrania, absent central and neural arch of cervical vertebra, malformed neural arch of cervical vertebra, malformed scapula of pectoral girdle, and short 2nd and 3rd digit of right hind limb; and 4) absent kidney and ureter.

Although not the same malformation, a small number of CNS malformations are seen across the dose groups in increasing severity. Spinabifida is seen in 1 LD fetus (0.8%); meningocele is seen in 3 MD fetuses from different litters (2.5%); and acephaly is seen in 1 HD fetus (0.6%) and cystic dilatation of the cerebellum is seen in 1 HD fetus (1.4%) from different litters. All individual findings are within background range for New Zealand White rabbits (Harlan data from 2/2000 to 12/2003) except the finding of meningocele which is slightly higher than the range of 0 – 1.3%. However, a background range is not given for combined CNS malformations, so it is unclear how the combined incidence compares to historical control data. The total incidence of combined CNS malformations of 0 control, 1 LD, 3 MD, and 2 HD are still small and are not dose-dependent.

There was a statistically significant increase in the number of fetuses with minor abnormalities following maternal dosing of vortioxetine compared to controls (Table 73). There was a non-dose dependent increase (nominal and/or statistically significant) in minor abnormalities and variations related to delayed ossification of the fetal skeleton in

skull, vertebra, forelimb and hindlimb in fetuses of dosed rabbits compared to controls (Table 74). In most instances, values for dosed rabbits were above the background range; although, in three instances the controls were on the high end of the background range (epiphyses and metacarpal of forelimb not ossified and incomplete ossification of phalange of hinlimb) and in three instances the incidences were within the background range (incomplete ossification of frontal skull and centra of cervical vetebra and epiphyses of hindlimb not ossified). The delayed ossification of fetal skeleton most likely correlates with the fetal weight reduction and reduced feed consumption observed in the fetuses of dosed rabbits.

There was a non-significant increase in the percent of fetuses with the common carotid artery originating from the innominate artery and the percent of fetuses with bilobed gall bladder in HD fetuses compared to controls (Table 74). The incidence for the common carotid arising from the innominate artery in HD was within the background range, while the incidence for the bilobed gall bladder in the HD was above the background range.

Table 74: Summary of minor abnormalities and variations in offspring of vortioxetine dosed rabbits (% group mean)

Fetal Abnormality	Dose (mg/kg/bid)				Background range
	0	5	10	15	
Uni- or bilateral common carotid arising from innominate artery	8.7	8.7	9	16.4	2.7 – 22.2
Bilobed gall bladder	2.2	0.8	0.8	6.8	0 – 2.7
Skull: frontal, uni- or bilateral: incomplete ossification	0	0	1.2	3.5	0 – 6.1
Skull: parietal, uni-or bilateral: incomplete ossification	2.5	**16.1***	4.4	11.8	0 – 5.3
Skull: interparietal: incomplete ossification	0	8.3	1.5	5.3	0 – 5.3
Skull: zygomatic arch, uni- or bilateral: incomplete ossification	0	0	0	3.5	0 – 1.3
Presacral vertebra: extra	14.3	14.6	**44.1*****	**36.5*****	1.9 – 17.5
Cervical vertebra: one or more centra: incomplete ossification	0	**5.3****	**5.5****	**3.8***	0 – 16.3
Forelimb: epiphyses: not ossified	41.1	**53.3***	46.1	50.0	9.6 – 42.7
Forelimb: one or more metacarpal: not ossified	11.3	**28.1****	14.3	20.8	0 – 12.4
Forelimb: one or more phalange: not ossified	2.3	**11***	7.8	10.1	0 – 7.9
Hindlimb: epiphyses: not ossified	58.1	**83.3*****	58.8	59.6	7.1 – 86.2
Hindlimb: one or more phalange: incomplete ossification	20.6	33.4	33.8	**38.9***	1.7 – 30.3

*p<0.05; **p<0.01; p<0.001

[Data from NDA 204447 submission; Study No. LBK0155, Table 8, pages 43 – 55 and Appendix 17, pages 208 – 230]

A second developmental toxicity oral dose range finding study (Study No. LBK0284) was conducted in New Zealand White rabbits with 10%HP-ß-CD control, 20 and 30 mg/kg/bid vortioxetine and a water control. Maternal toxicity—including decreased body weight (≤6%), feed consumption (≤96%), and feces compared to HP-β-CD control—was seen for vortioxetine dosed groups. Reduced body weight gain and feed consumption was also seen for the HP-β-CD control group compared to water control group. There was no vortioxetine-related effect on implantations, the number of live fetuses, and external fetal abnormalities. Lower mean fetal weight was seen at 30 mg/kg/bid when compared to HP-β-CD controls.

Based on the decreased body weight and feed consumption, 30 mg/kg/bid was determined to be the MTD for the second main study reviewed in detail in this section.

Study title: Lu AA21004: Oral (gavage) twice daily dosing developmental toxicity study in the rabbit

Study no.:	LBK0285; Reference no. 12875
Study report location:	Regulatory Central Archive at H. Lundbeck A/S, Copenhagen Denmark
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 11, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot no., and % purity:	Lu AA21004, 2018324, 99.9%

Key Study Findings

- Pregnant New Zealand White rabbits were dosed orally (by gavage, in 10% hydroxypropyl-beta-cyclodextrin) at 0 (water), 0 (vehicle), 1, or 30 mg/kg/bid during the period of organogenesis (GD6 – 18).
- Maternal toxicity—characterized by decreased body weight and feed consumption—occurred at 1 and 30 mg/kg/bid.
- Fetal developmental toxicity—characterized by decreased fetal weight, increased incidence of runts, and delayed ossification of the fetal skeletal system—occurred at 30 mg/kg/bid.
- No NOAEL was determined for maternal toxicity, but is 1 mg/kg/bid for developmental toxicity, which is 2X the MRHD of 20 mg on a mg/m2 basis.
- There was no evidence of teratogenicity in rabbits at doses up to 58X the MRHD of 20 mg on a mg/m2 basis.

Methods

Doses:	0 (water), 0 (vehicle), 1 (LD), or 30 (HD) mg/kg/bid
Frequency of dosing:	Twice daily
Dose volume:	4 mL/kg
Route of administration:	Oral gavage

Formulation/Vehicle:	Solution/10% HPβCD
Species/Strain:	Rabbit/New Zealand White (HsdlF:NZW)
Number/Sex/Group:	20 females/group
Satellite groups:	3 females/group (TK)
Study design:	Pregnant rabbits were dosed from GD6 – GD18. The fetuses were delivered by c-section on GD28 and examined.
Deviation from study protocol:	No significant deviations

Observations and Results

Mortality

There were six premature mortalities—four following abortion and two due to a decline in clinical condition most likely due to test article inhalation during dosing. One water control (No. 37), two vehicle controls (Nos. 3 and 18), and one LD (No. 60) were euthanized after spontaneous abortion. Three of these four females were noted to have low and near absent food intake; although, other females that did not abort also were reported to have low and near absent food intake. One LD (No. 42) and one HD (No. 87) were euthanized due to a decline in clinical condition, including labored breathing and decreased activity. At necropsy, the LD female had red mucosa of both the larynx and trachea proximal to the larynx and the HD female had firm lung lobes with red areas suggestive of test article inhalation.

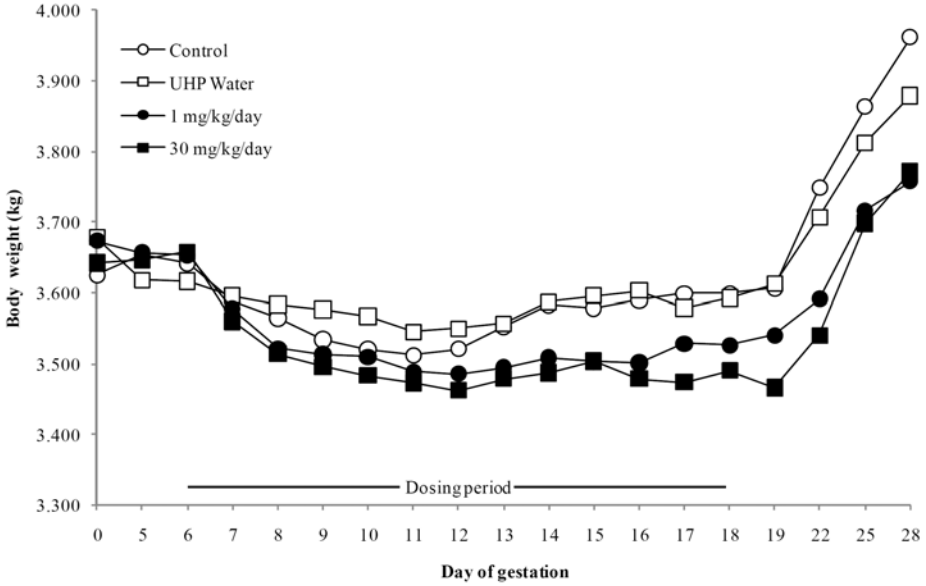
Clinical Signs

Some HD females urinated while being dosed during the end of the dosing period. Reduced feces production was noted in females in all groups; although, the duration and frequency of reduced feces was greater in HD females. For all groups the reduced feces generally corresponded with lower food intake. Loose and liquid feces were noted in 1, 4, 4, and 5 females in water control, vehicle control, LD, and HD groups, respectively. The loose and liquid feces may be due to the vehicle.

Body Weight

All groups lost weight after the first day of dosing with HD females losing the most amount of weight (0.6, 1.4, 2.1, and 2.7% for water control, vehicle control, LD, and HD, respectively). All groups continued to lose weight until GD12 (1.3, 2, 2.5, and 2.8%); although, the vehicle dosed females (control and vortioxetine) lost more weight than the water control. At GD18, LD and HD females weighed 2 and 3%, respectively, less than the vehicle controls. After the dosing period (GD19 – 28), all groups gained weight at the same rate, but due to the weight differences during the dosing period LD and HD females weighed 5% less than vehicle controls at the end of the study. Group mean maternal body weights are graphed in Figure 9.

Figure 9: Group mean maternal body weights in rabbits



[Figure from NDA 204447 submission; Study No. LBK0285, page 46]

Feed Consumption

Females given vehicle consumed on average much less feed than water controls from GD6 – 10 (↓45 – 50% for vehicle controls, ↓62 – 63% LDF, and ↓79 – 84% HDF compared to water controls). After GD10, vehicle controls consumed similar amounts of feed to water controls for the remainder of the study. Vortioxetine dosed females consumed on average much less feed than vehicle controls until GD14 for LDF (↓30 – 50%) and GD22 for HDF (↓30 – 70%).

Toxicokinetics

Plasma exposures of vortioxetine, two major human metabolites (Lu AA34443 and Lu AA34443 glucuronide (Glu)), and a non-major human metabolite (Lu AA39835) were determined on GD18 at 1, 3, 6, 10, 12, 14, 16, and 24 hours after the first dose of the day. Cmax was reached after 1 hour for vortioxetine and 4.7 – 12 hours for Lu AA34443, Lu AA39835, and Lu AA34443Glu on GD18. Plasma exposure (Cmax and AUC) generally increased dose-proportional or greater than dose-proportional for vortioxetine, Lu AA34443, and Lu AA34443Glu. However, it should be noted that for the HD, exposures were determined from only 2 rabbits and there was approximately a 5-fold difference in individual Cmax and AUC for vortioxetine between the two rabbits. Interestingly, there was less variability for the plasma exposures to the metabolites for the 2 rabbits (1.5-fold for Lu AA34443, 3.5-fold for Lu AA39835, 1-fold for Lu AA34443Glu). Plasma exposure was not detected for Lu AA39835 at the LD. Plasma exposure was greater for the metabolites Lu AA34443 and Lu AA34443Glu compared to parent, vortioxetine. In contrast, plasma exposure was less for Lu AA39835 compared to parent. The TK results are summarized in Table 75.

Table 75: Summary of mean TK parameters on GD18 for vortioxetine, Lu AA34443, Lu AA39835, Lu AA34443Glu

Anylate	Dose (mg/kg/bid)	tmax (h)	Cmax (ng/mL)	AUC_{0-24} (ng.h/mL)
Vortioxetine	1	1	2.93	10.7
	30	1	272	1900
Lu AA34443	1	4.7	150	948
	30	6.5	4500	39,700
Lu AA39835	1	ND	ND	ND
	30	6.5	0.904	9.67
Lu AA34443Glu	1	4.7	319	2130
	30	12	10,100	91600

ND = not detected

[Data from NDA 204447 submission; Study No. LBK0285, page 159]

The rabbit-to-human AUC ratio for vortioxetine and the major human metabolites, Lu AA34443 and Lu AA34443Glu, at 1 and 30 mg/kg/bid is shown in Table 76. The exposure margins for vortioxetine and Lu AA34443 are to the MRHD of 20 mg. Human exposure for Lu AA34443Glu after repeat dose was only measured at 10 mg; therefore, the exposure margins for Lu AA34443Glu are only to 10 mg and not the MRHD of 20 mg.

Table 76: Rabbit-to-human AUC ratio for vortioxetine, Lu AA34443, Lu AA34443Glu at 1 and 30 mg/kg/bid

Anylate	Dose (mg/kg/bid)	AUC_{0-24h} (ng.h/mL)	Exposure Margin at MRHD*#
Vortioxetine	1	10.7	0.02
	30	1900	3
Lu AA34443	1	948	1.7
	30	39,700	71
Lu AA34443Glu	1	2130	2.4
	30	91600	10

*Human repeat dose AUC_{0-24h} for vortioxetine at 20 mg = 646 ng.hr/mL and for Lu AA34443 at 20 mg = 563 ng.hr/mL (From Sponsor's Summary of Clinical Pharmacology, Table 5.2, page 166).
#Human repeat dose AUC_{0-24h} for Lu AA34443Glu at 10 mg = 884 ng.hr/mL (From Sponsor's Pharmacokinetics Written Summary, Table 5.c, page 20).

Dosing Solution Analysis

All formulations were prepared weekly, stored at room temperature, and were protected from light. Formulation samples were determined to be within 98 – 105% of the nominal concentrations.

Necropsy

There were no vortioxetine-related macroscopic findings at necropsy.

Cesarean Section Data

There were no vortioxetine-related effects on the mean number of corpora lutea, implantations, or resorptions (Table 77). Although, there was a significant increase in the mean number of preimplantation loss for the HD females compared to vehicle control, the increase in preimplantation loss is not vortioxetine related because the loss of embryos would have occurred before dosing began on GD6 and is also the same magnitude seen with the water control. There was a nonsignificant increase in the mean number of postimplantation loss and late resorptions for the LD females; however, this increase was not seen for the HD females. Without a dose relationship this is most likely not vortioxetine related.

Table 77: Summary of cesarean section data in rabbit

Parameter	Dose (mg/kg/bid)			
	0 (water)	0 (vehicle)	1	30
Total pregnancy rate	18/20	20/20	15/20	19/20
Females with live fetuses	16	18	12	17
Mean corpora lutea	10.9	10.8	10.7	11.3
Mean implantation sites	7.9	9.7	8.4	8.0
Mean preimplantation loss	29.3%	11%	23.1%	29%*
Mean postimplantation loss	21.2%	19.6%	27.5%	22.8%
Mean live fetuses	6.1	7.6	5.6	6.3
Mean dead fetuses	0	0	0	0
Abortions	1	2	1	0
Early resorptions (Mean)	26 (1.5)	30 (1.7)	19 (1.5)	24 (1.3)
Late resorptions (Mean)	8 (0.44)	4 (0.24)	17 (1.3)	8 (0.42)

*$p<0.05$

[Data from NDA 204447 submission; Study No. LBK0285]

Offspring

The offspring data are summarized in Table 78. There was no effect on the sex ratio. Mean fetus weight was decreased for vehicle (control and vortioxetine) dosed pregnant females compared to water controls; although, the Sponsor only performed statistical analysis for the vehicle control. Additionally, there was a nonsignificant 7% decrease in mean fetus weight at the HD compared to the vehicle control. The fetal weight reduction is observed in both sexes. Although the decreased fetal weight at the HD was not significant, there was a significant increased incidence of runts at the HD compared to vehicle control (6% vs. 1.5%).

Table 78: Summary of offspring data in rabbits

Parameter	Dose (mg/kg/bid)			
	0 (water)	0 (vehicle)	1	30
Mean live fetuses/litter	6.5	7.6	6.1	7.1
Mean sex rate (% males)	54.4%	47.4%	52%	54.8%
Mean fetal weight (g)	39	35.1†	35.4	32.5
Number of runts (litters)	1(1)	2(2)	2(2)	7(4)*
Mean placental weight (g)	4.76	4.03	4.28	3.76
Mean gravid uterus weight (g)	386.6	442.3	342.0*	359.7*
Total number of litters examined	16	18	12	17
Number of fetuses examined	104	136	73	120
Fetuses with malformations (litters)	1(1)	3(3)	5(4)	0(0)
Fetuses with minor abnormalities (mean % of fetuses examined)	51 (41.6%)	58 (41.6%)	39 (54.8%)	61 (50%)
Fetuses with variations (mean % of fetuses examined)	101 (90.4%)	133 (98.1%)	73 (100%)	119 (99.4%)

†p<0.01 compared to water control; *p<0.05 compared to vehicle control

[Data from NDA 204447 submission; Study No. LBK0285]

Malformations occurred in 1(1), 3(3), 5(4), 0(0) water control, vehicle control, LD, and HD fetuses (litters), respectively. The one water control fetus had cardiovascular malformations, pulmonary valvular atresia and severely misshapen ventricle. The three vehicle control fetuses had cardiovasicular, facial, and/or skeletal malformations: 1) severely enlarged aortic arch; 2) facial (absent left nostril, asymmetric opening of oral cavity, malformed oral cavity, microphthalmia eye) and skeletal (absent premaxilla skull, malformed and fused nasal bone, reduced orbital cavity, malformed frontal skull, zygomatic arch, and left side of palatine, malformed and fused maxilla, fused mandible, malpositioned upper incisor, and severely fused cervical vertebra); and 3) malformed rib and bowed humerus forelimb. The five LD fetuses had skeletal, cerebral, and/or cardiovascular malformations: 1) severely fused thoracic vertebra and rib; 2) severely enlarged third ventricle, cystic dilatation of cerebellum, and severely fused rib and sternebra; 3) bowed hindlimb; 4) absent and malformed cervical vertebra; and 5) aortic valvular atresia.

There was no vortioxetine-related effect on the percent of fetuses with minor abnormalities or variations. However, there was a significant increase in minor abnormalities and variations related to delayed ossification of the fetal skeleton in cervical centra, sternebra, forelimbs, and hindlimbs in HD and a cardiovascular variation to the origin of the left common carotid artery in LD and HD (Table 79) compared to vehicle controls. The delayed ossification of fetal skeleton correlates with the fetal weight reduction and reduced feed consumption observed in the HD group. Interestingly, except for the incomplete ossification of the 5th sternebra, the minor abnormalities and variations associated with delayed ossification are also increased for vehicle control and LD when compared to water control (although not statistically analyzed). This finding suggests that the weight reduction and reduced feed

consumption observed in all vehicle groups (control and vortioxetine) may have resulted in delayed ossification with an even greater effect seen with the fetuses from the HD group.

There was a statistically significant increase in the percent of fetuses with the left common carotid artery originating from the innominate artery following maternal dosing with vortioxetine compared to water and vehicle controls (Table 79). However, the group mean values for LD and HD fetuses were within the background data range.

Table 79: Statistically significant minor abnormalities and variations (% group mean)

Finding	Vehicle Control	UHP water	1 mg/kg/ b.i.d	30 mg/kg/ b.i.d	Background Data
Runted foetus	1.4	0.6	2.4	**4.0***	0.0 - 2.5
Left common carotid arising from innominate artery	**1.9**	3.3	8.5*	13.2**	2.3 - 16.6
One or more cervical centra: incomplete ossification	1.3	0.6	5.1	**7.5***	0.0 - 6.3
5th sternebra : incomplete ossification	13.3	27.1	16.3	23.8*	3.8 - 29.3
Forelimb, uni- or bilateral : epiphyses not ossified	37.7	24.0	35.6	**53.1****	9.6 - 44.6
Forelimb, one or more digit: phalanges incomplete ossification	54.7	36.5	48.2	**60.7****	5.7 - 60.6
Hindlimb, uni- or bilateral : epiphyses not ossified	58.6	49.9	**66.1**	**80.0*****	7.1 - 59.6
Hindlimb, one or more digit: phalanges incomplete ossification	15.5	8.6	25.0	28.5**	0.0 - 30.3

Numbers in bold type are outside the background data range.
Statistical analysis is carried out against the number of foetuses but shown here against the group mean percent. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.
Statistical significances relate to Group 1 versus Groups 3 and 4.

[Table from NDA 204447 submission; Study No. LBK0285, page 41]

9.3 Prenatal and Postnatal Development

Study title: Lu AA21004: Oral (Gavage) Twice Daily Dosing Pre- and Post-natal Development Toxicity Study in the Rat and Amendment 1

Study no.:	LBK0276; Reference no. 12393
Study report location:	Regulatory Central Archive at H. Lundbeck A/S
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 27, 2008
GLP compliance:	Yes, except for TK
QA statement:	Yes
Drug, lot no., and % purity:	Lu AA21004, 2018324, 99.9%

Key Study Findings

- Pregnant Sprague-Dawley rats were dosed orally (by gavage, in 10 % hydroxypropyl-beta-cyclodextrin (HP-ß-CD) and 4.4 % isotonic glucose monohydrate) at 0, 5, 20, or 60 mg/kg/bid from GD6 to PND20.
- Maternal toxicity (F_0 dams) occurred at 20 and 60 mg/kg/bid based on decreased body weight gain and decreased food consumption during gestation at 60 mg/kg/bid and decreased number of live-born pups and increased number of still born pups at 20 and 60 mg/kg/bid.
- Pup developmental toxicity (F_1 generation) occurred at 20 and 60 mg/kg/bid based on decreased survival and little to no milk in the stomach from birth to weaning, decreased body weight at birth to weaning (60 mg/kg/bid only), and delayed development based on eyes opening.
- Although body weights remained decreased for male and female F1 generation rats at 20 and 60 mg/kg/bid post-weaning; learning, auditory function, locomotor activity, sexual development, mating, and fertility were not affected by vortioxetine.
- The NOAEL for maternal toxicity and pup development is 5 mg/kg/bid and the NOAEL for post-weaning development, sexual development, mating, and fertility is 60 mg/kg/bid, which are 5 and 58X, respectively the MRHD of 20 mg on a mg/m2 basis.

Methods

Doses:	0, 5 (LD), 20 (MD), or 60 (HD) mg/kg/bid
Frequency of dosing:	Twice daily
Dose volume:	6 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Solution/10% HP-ß-CD and 4.4 % glucose monohydrate
Species/Strain:	Rat/Sprague-Dawley (Crl:CD (SD))
Number/Sex/Group:	22 females/group (F_0 dams); 20/sex/group (F_1 generation)
Satellite groups:	None

Study design:	Female rats (F_0 dams) were dosed from Gestational Day (GD) 6 to Post-natal Day (PND) 20. Maternal clinical signs, body weight, and feed consumption were recorded from GD6 up to PND21. Females gave birth and reared their offspring (F_1 generation) to weaning on PND21. Litter size and pup sex was recorded and pups were observed for development and were weighed individually during lactation. On PND4, 4 males and 4 females were selected to be reared while remaining pups and F_0 dams were necropsied on PND21 (weaning). After weaning, 20 males and 20 females from remaining F_1 generation pups from each group were randomly selected (1/sex/litter) to be allowed to mature untreated. The effects on growth, development, behavior, and reproductive performance were assessed for these F_1 generation pups. For reproductive performance, F_1 generation rats were mated for up to 7 days after reaching sexual maturity (Post-natal Week 10). All mated F_1 females were necropsied on GD13.
Deviation from study protocol:	Study protocol and SOP deviations were found for the TK evaluation performed at (b) (4) which resulted in the inability to claim GLP compliance for the TK data.

Observations and Results

F_0 Dams

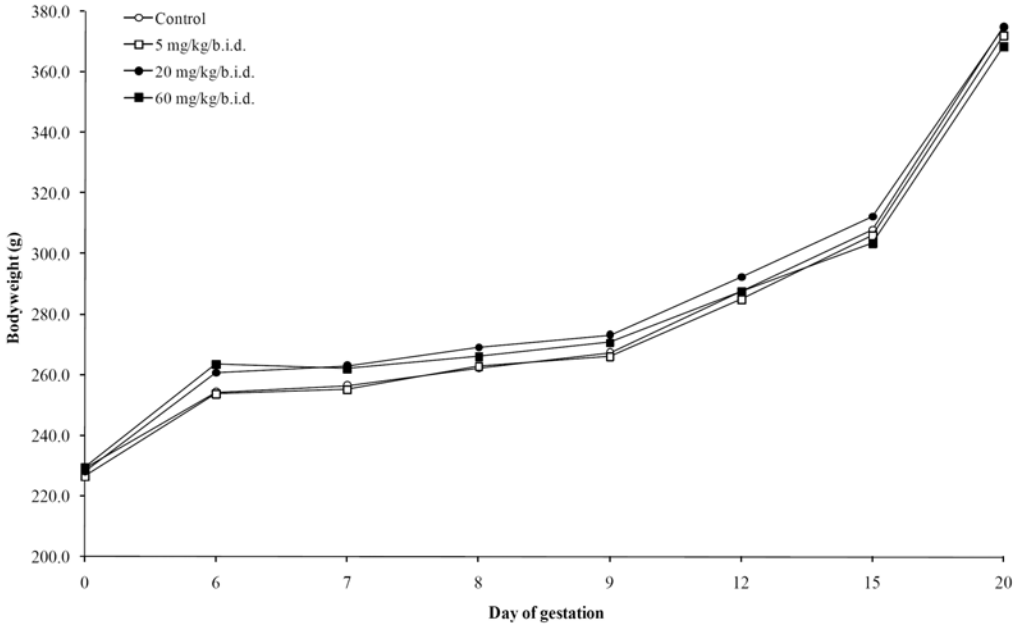
Survival: One HD female died prematurely on GD11 due to dosing error.

Clinical Signs: Pale feces were seen from GD7 through lactation for MD and HD dams. Excessive salivation was seen sporadically especially during lactation for MD and HD dams.

The length of gestation was similar for all groups. There were no clinical signs that parturition occurred at different times for any group.

Body Weight: During gestation, HD dams lost on average ~0.5% of their body weight (↓1.6 g) after the first day of dosing (GD6) while controls gained on average ~0.9% (↑2.2 g). For the remainder of gestation (GD7 – 20), HD dams gained on average slightly less weight (↓10%) than controls. Because mean body weight of HD group was 3.6% greater than control group on GD6, mean body weight for HD group cannot be directly compared to control group on GD7. Due to the body weight difference on GD6, body weight on GD20 was similar for HD group and control group, in spite of the decreased body weight gain. See Figure 10.

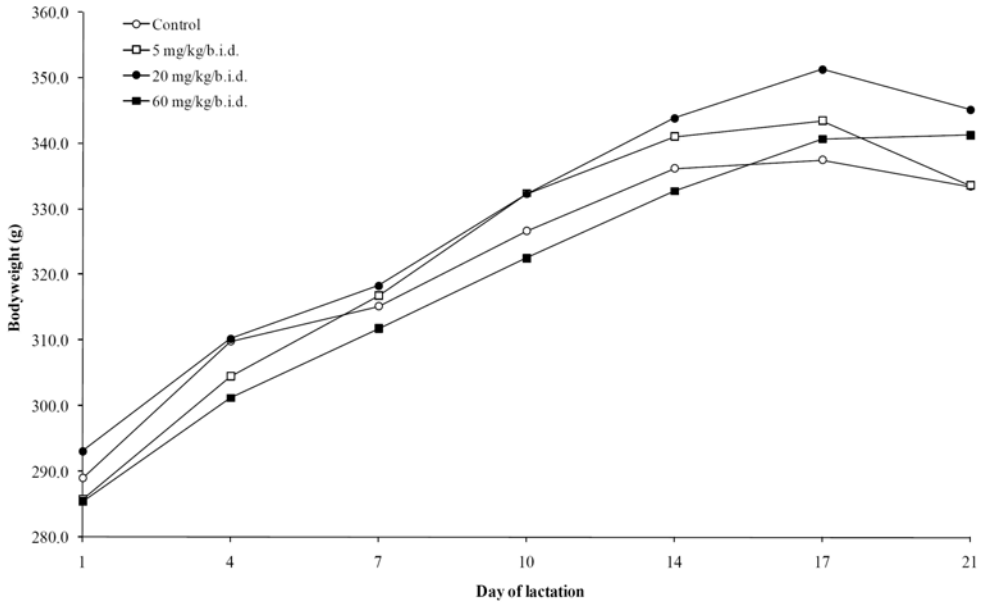
Figure 10: Group mean maternal body weight for F_0 rats during gestation



[Figure from NDA 204447 submission; Study No. LBK0276, page 52]

During lactation, MD and HD dams gained slightly less than controls from PND1 to 4 (↑7, 7, 6, and 5% for control, LD, MD, and HD, respectively). However, MD and HD dams gained more weight than controls for the rest of lactation and weighed 2 – 3.5% more than controls at the end of the lactation period (PND21). See Figure 11.

Figure 11: Group mean maternal body weight for F_0 rats during lactation



[Figure from NDA 204447 submission; Study No. LBK0276, page 53]

Feed Consumption: During gestation, MD and HD dams consumed on average 7 and 22%, respectively, less feed than controls from GD6 – 9. HD dams continued to consume less feed than controls for the remainder of the dosing period; although, to a lesser extent (↓13, 7, and 3% for GD9 – 12, 12 – 15, and 15 – 20, respectively).

During lactation, mean feed consumption was similar for controls and all dose groups throughout lactation.

Uterine Content: The mean number of implantation sites was slightly, but statistically significantly increased in the left horn of the uterus for MD and HD compared to controls; but, was slightly, non-statistically significantly decreased in the right horn with no effect on total (Left: 6, 7, 7, and 8; Right: 8, 7, 6 and 6; Total 13, 14, 14, and 14 for control, LD, MD, and HD, respectively).

There was a decrease in the number of live-born pups and an increase in the number of still births for MD and HD dams compared to controls (live born: 99.3, 99.7, 96.8, and 95.5%; still births: 2, 1, 7, and 13 for controls, LD, MD, and HD, respectively). There was no effect on sex ratio.

Necropsy Observation: There were no vortioxetine-related macroscopic findings.

Toxicokinetics: Plasma exposures of vortioxetine, a major human metabolite (Lu AA34443) and a non-major human metabolite (Lu AA39835) were determined on GD6 and PND11 predose and at only 2 and 10 hours after the first dose of the day from F_0 dams from the main study. Due to misconduct at (b) (4) GLP compliance is not claimed for the TK data. Based on remedial action, the Sponsor considers the bioanalytical data reliable.

Exposure was generally greater than dose proportional for vortioxetine (except from 20 to 60 mg/kg/bid which was less than dose proportional) and Lu AA34443 and less than dose proportional for Lu AA39835 at GD6 and PND11. Lu AA34443 exposure was 2 – 4-fold parent, vortioxetine, while Lu AA39835 exposure was 0.008 – 0.18-fold parent on GD6 and PND11.

Because of the sparse sampling, estimation of TK parameters was not performed. Instead, the Sponsor compared the plasma concentrations of vortioxetine, Lu AA34443, and Lu AA39835 from 20 and 60 mg/kg/bid groups on PND11 of this study to plasma concentrations at 2 and 10 hours postdose from Day 15 of the fertility and early embryonic development study in Sprague-Dawley rat. The Sponsor also compared plasma concentrations of vortioxetine and Lu AA34443 from 20 mg/kg/bid at 10 hours postdose from GD6 and PND11 of this study to Day 1 and Week 13 of the 26-week general toxicology in Wistar rat. Plasma exposures for Lu AA39835 were not measured in the 26-week general toxicology study. Compared to the fertility study, exposures at 2 and 10 hours postdose for vortioxetine, Lu AA34443, and Lu AA39835 were generally similar for 20 and 60 mg/kg/bid. Compared to Day 1 of the 26-week general toxicology study, exposures at 10 hours postdose on GD6 for vortioxetine and Lu AA34443 were 2.6-fold and 1.6-fold greater, respectively, for 20 mg/kg/bid. Compared to Week 13 of

the 26-week general toxicology study, exposures at 10 hours postdose on PND11 were 1.6-fold greater for vortioxetine and similar for Lu AA34443.

Dosing Solution Analysis: All formulations were prepared fresh each week. Formulation samples were determined to be within 99.1 – 103.9% of the nominal concentrations.

F_1 Generation

Survival: Pup survival to PND4 and PND21 was statistically significantly lower at the HD and at the MD and HD, respectively (Table 80). The number of pups found dead, missing (most likely cannibalized), or were euthanized prematurely was increased for MD and HD compared to controls (Dead/Missing/Euthanized: 4, 3, 19, and 30 for control, LD, MD, and HD, respectively). As a result, the total cumulative survival of pups was lower at the MD and HD compared to controls (Table 80).

Table 80: Group mean percent survival data for F1 generation litters in rat

Survival (%)	Dose (mg/kg/bid)			
	0	5	20	60
Birth (Live Birth Index)	99.3	99.7	96.8	95.5**
Post-Natal Day 4 (Viability Index)	99.1	99.2	97.2	94.7*
Post-Natal Day 21 (Lactation Index)	100	100	98.9*	97.5**
Cumulative Survival Index	98.4	98.9	92.9**	89***

*p<0.05; **p<0.01;***p<0.001

[Data from NDA 204447 submission; Study No. LBK0276, page 78]

After weaning, one MD male pup (No. 157) was euthanized on PND60 due to a decline in clinical condition—including pale body, slow breathing and piloerection. According to the Sponsor, necropsy findings included “large red lymph nodes, gelatinous mesentery and pancreas, …red fluid in the abdominal cavity…large spleen, thickened and distended bile duct, and both kidneys were green…the right lobe of the liver had an irregular shape with a cream area present and the whole of the liver appeared to be pale and had an abnormal waxy consistency.” This was the only rat to have these findings and is most likely not vortioxetine related.

Clinical Signs: MD (1) and HD (5) pups that were euthanized prematurely had clinical signs of body surface being cold, having decreased activity, the whole body was a grey or dark color, and/or slow or labored breathing. No control or LD pups were euthanized prematurely. Most of the pups found dead or prematurely euthanized had little or no milk in the stomach (0, 2, 9, and 20 for control, LD, MD, HD, respectively). There were no vortioxetine-related clinical signs seen in pups that did not die/were euthanized during lactation

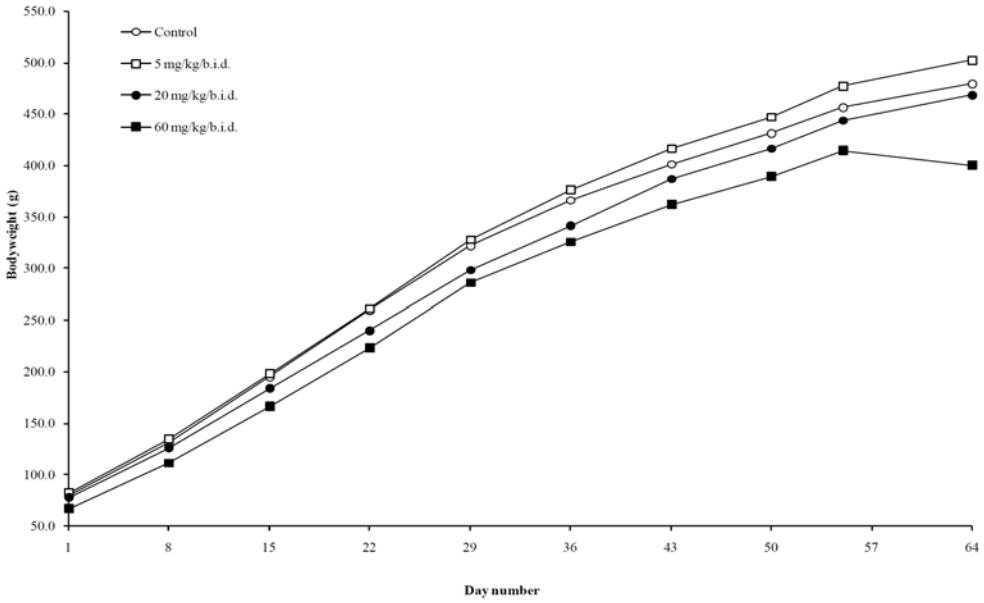
After weaning, no vortioxetine-related clinical signs were seen.

Body Weight: Mean body weight was decreased 6 and 7% at birth for male and female pups, respectively, from HD dams compared to controls. Mean body weight remained

lower throughout lactation for pups from HD dams and they weighed 10% less than controls at PND21.

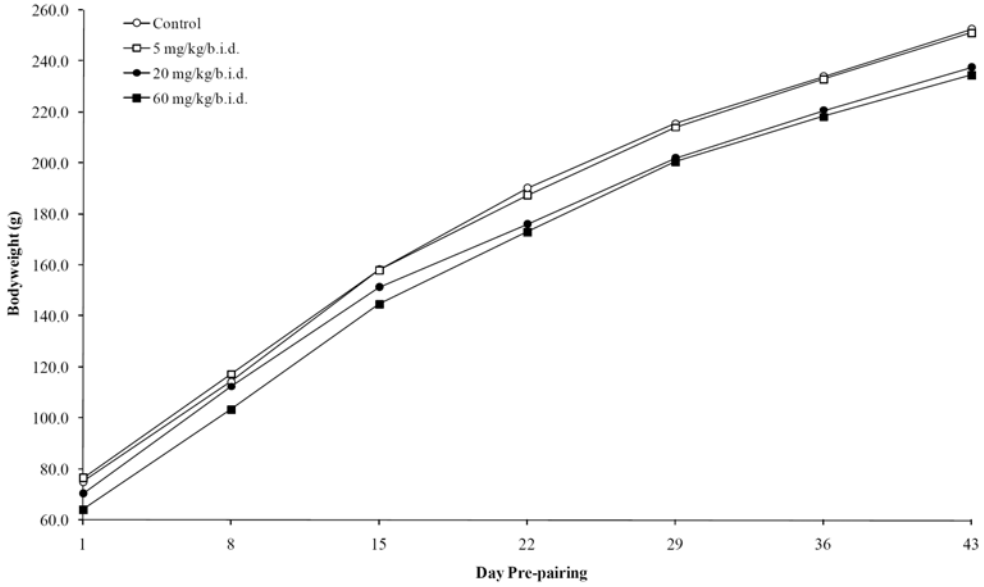
After weaning, mean body weight was decreased at PND23 for F1 males from HD dams (16%) and F1 females from MD (6%) and HD dams (15%) compared to controls. HD males continued to gain less weight than controls to PND55, after which they gained similar amounts to controls; however, body weight remained decreased by 8% at PND87. F1 males from MD dams and F1 females from MD and HD dams gained less weight than controls from PND31 to 45, after which they gained similar amounts to controls. At PND87, MD males had similar weights to controls, but MD and HD females weighed 6 – 7% less than controls. Post-weaning F1 mean body weights for males and females are graphed in Figure 12 and Figure 13, respectively.

Figure 12: Group mean body weight for F1 generation male rats post weaning



[Figure from NDA 204447 submission; Study No. LBK0276, page 56]

Figure 13: Group mean body weight for F1 generation female rats post weaning



[Figure from NDA 204447 submission; Study No. LBK0276, page 56]

At GD0, F1 females from MD and HD dams weighed 5 – 6% less than controls. However, they gained more weight than controls from GD7 – 13 and there was no significant difference in body weight for F1 females compared to controls at GD13.

Feed Consumption: Feed consumption was not recorded for F1 generation rats.

Physical Development: There was a dose-dependent decrease in the mean percent of pups with eyes open on PND15; although, only the HD group was statistically significant (64.2, 55, 51.5, and 40.1% for control, LD, MD, and HD). The Sponsor did not report the percent of eyes opened after PND15. There was a small, nonstatistically significant decrease in the mean percent of HD pups with righting reflex on PND5 compared to control (84.5 vs. 92%). There was no difference in the mean percent of dosed pups with ears open on PND3, with startle response on PND15, and with pupillary light reflex on PND21 compared to controls.

F1 generation females from dosed dams took approximately one day longer for vaginal opening compared with controls (33.7, 34.6, 34.6, and 34.9 for controls, LD, MD, and HD, respectively). However, this finding was not statistically significant or dose-dependent and the time for dosed females was within the background range for this laboratory (32.9 – 35.8, n=299). There was no difference for the mean number of days to preputial separation for males.

Neurological Assessment: There was no vortioxetine-related effect on F1 generation rats on learning in the E-maze test on PND35 and 42, auditory function using Preyer Reflex on PND 36, and locomotor coordination using a Rotarod PND28.

Reproduction: There was no group difference in the mean number of days to mate, the mean number of sperm plugs after pairing, and the copulation (no. of mated rats/no. of paired rats) and fertility (no. of fertile rats/no. of mated rats) index for males and females. All F1 females were pregnant at necropsy and there was no group difference in the mean number of corpora lutea, implantations, pre- and post-implantation loss, and live fetuses.

Necropsy: There were no vortioxetine-related macroscopic necropsy findings for F1 generation rats.

10 Special Toxicology Studies

Mechanistic Study to Examine Crystals in Bile Ducts and Renal Tract in Wistar Rats (Study No. LBK0255; GLP compliant)

This study was designed to examine the crystalline material found in the bile ducts and renal tract of Wistar rats in toxicology studies. Rats were dosed by oral gavage (in 10% hydroxypropyl-beta-cyclodextrin and 4.4% glucose monohydrate) with 0, 40, 60 or 80 mg/kg/bid for up to 26 weeks (Table 81). Clinical signs, body weight, food consumption, urinalysis, macropathology and histopathology were examined.

Table 81: Study design for mechanistic study in rat

Group	Treatment	Dose# (mg/kg b.i.d.)	Number of animals			Animals number		
			Week 4	Week 13	Week 26	Week 4	Week 13	Week 26
1	Control	0	5	5	5	1-5	6-10	11-15
2	Lu AA21004	40	0	0	10	-	-	16-25
3	Lu AA21004	60	10	10	0	26-35	36-45	-
4	Lu AA21004	80†	20	0	0	46-65	-	-

Expressed in terms of test material as supplied. A conversion factor of 1.27 was used for conversion of the dose in terms of base to that in terms of the salt.

† All animals at this dose sacrificed on Day 14 due to excessive toxicity

[Table from NDA 204447 submission; Study No. LBK0255, page 19]

Mortality and Clinical Signs

Due to severe clinical signs, rats dosed with 80 mg/kg/bid were prematurely euthanized on Day 14 instead of in Week 4. For the 80 mg/kg/bid group, clinical signs were weight loss/no weight gain, piloerect coat, dyspnea, elevated gait, hunched posture, thin build, underactivity, and consumption of bedding material. One rat dosed with 80 mg/kg/bid (no. 59) was killed on Day 13 after having three convulsions in quick succession. The last was considered marked and lasted ~ 3 minutes. Histopathological exam showed kidney pelvic dilatation due to crystalline deposits in the pelvic region and distension of the ureters.

Two rats dosed with 60 mg/kg/bid were prematurely euthanized on Days 16 (no. 34) and 73 (No. 38) due to clinical signs, including weight loss, hunched posture, a piloerect coat, underactivity, thin appearance, pallor of the whole body, and a firm area in the right ventral abdominal region (no. 38 only). Histopathological exam showed enlarged, pale, granular kidneys due to the presence of crystalline deposits. In no. 38, there was also distension of the urinary bladder and ureters.

Clinical signs for the remaining 60 mg/kg/bid and the 40 mg/kg/bid dose group were occasional piloerection, salivation, and chin rubbing.

Body Weights

There was a dose-related reduction in mean body weight gain compared to controls over the respective dosing period. Rats in the 80 mg/kg/bid dose group gained less weight compared to controls during the first two weeks of dosing with a greater effect in Week 2 (↑2 vs. 29 grams for 80 mg/kg/bid and controls, respectively), which resulted in a decrease of 14% mean body weight compared to controls at the end of dosing in Week 2. Starting in Week 3, rats in the 40 and 60 mg/kg/bid dose group gained less weight compared to controls and weighed on average 5 and 20% less than controls at the end of dosing in Weeks 26 and 13, respectively.

Feed Consumption

There was a dose-related reduction in mean food consumption compared to controls over the respective dosing period. Rats in the 60 and 80 mg/kg/bid dose groups consumed 5 and 14% less feed, respectively, than controls in Week 1. Rats in the 60 mg/kg/bid dose group continued to consume 5 – 23% less feed than controls over the 13 weeks. There was no difference in feed consumption for the 40 mg/kg/bid group compared to controls over 26 weeks.

Urinalysis

The type of crystals present in the urine of vortioxetine-treated rats was different compared to controls. The type of crystals present in the urine of controls was tri-phosphate and/or calcium oxalate. Occasionally, tri-phosphate and calcium oxalate crystals were also detected in the urine of vortioxetine-treated rats. However, the urine of vortioxetine-treated rats also contained urate and needle-shaped crystals. There was not a dose-related effect on the number of crystals in the urine or a consistent change in the number of crystals over time.

Erythrocytes were seen in the urine of two 80 mg/kg/bid rats in Week 2, five 60 mg/kg/bid rats in Week 13, and five 40 mg/kg/bid rats in Week 26.

Gross Pathology

In rats dosed with 60 mg/kg/bid for 4 (Table 82) or 13 weeks (Table 83), pale areas of the kidney, pelvic dilatation and/or renal enlargment, granular appearance of the kidney, distended ureters, thickening of the ureters, and distended and/or thickened bile ducts (W13 only) was seen. In addition, presence of abnormal contents in the kidneys, ureters, bile duct, and urinary bladder were seen.

Table 82: Macroscopic findings in rats dosed with 60 mg/kg/bid for 4 weeks

Group/sex Dose (mg/kg b.i.d.)	1M 0	3M 60
Kidneys		
Enlarged	0	2
Pelvic dilatation	0	3
Unilaterally enlarged	0	1
Granular	0	2
Pale	0	3
Pale area(s)	0	4
Bile duct		
Distended	0	1
Abnormal contents	0	1
Urinary bladder		
Abnormal contents	0	1
Ureters		
Distended	0	4
Thickened	0	2
Abnormal contents	0	1
Dark area(s)	0	1
Number of animals examined	5	9

[Table from NDA 204447 submission; Study No. LBK0255, page 37]

Table 83: Macroscopic findings in rats dosed with 60 mg/kg/bid for 13 weeks

Group/sex Dose (mg/kg b.i.d.)	1M 0	3M 60
Kidneys		
Enlarged	0	8
Pelvic dilatation	0	7
Irregular surface	0	1
Granular	0	7
Pale	0	4
Pale area(s)	0	8
Abnormal contents	0	3
Bile duct		
Distended	0	7
Abnormal contents	0	8
Thickened	0	7
Number of animals examined	5	9

Group/sex Dose (mg/kg b.i.d.)	1M 0	3M 60
Stomach		
Distended	0	4
Urinary bladder		
Abnormal contents	0	5
Ureters		
Distended	0	6
Thickened	0	8
Abnormal contents	0	1
General comments		
Animal thin	0	2
Number of animals examined	5	9

[Table from NDA 204447 submission; Study No. LBK0255, page 38 – 39]

In two rats dosed with 40 mg/kg/bid for 26 weeks, pale areas of the kidney, pelvic dilatation, granular appearance of the kidney, renal enlargement, the presence of masses in the kidney, thickened and distended ureters with abnormal contents was seen. In addition, pale areas on the liver (6 rats), distended and/or thickened bile ducts (8 rats), and abnormal contents in the bile ducts (5 rats) was seen.

Histopathology

Crystals were present in the cortex, medulla, or papilla of the kidney, but not in the liver of rats dosed with 80 mg/kg/bid for 14 days (Table 84). Crystals were present in the kidney of 2 rats dosed with 60 mg/kg/bid for 4 weeks (Table 85). In addition, 1 of these rats also had crystals present in the liver and extra-hepatic bile duct. Crystals in the kidney, liver, extra-hepatic bile ducts, and urinary bladders were most prominent in rats

dosed with 60 mg/kg/bid for 13 weeks (Table 86). Crystals were present in the kidney of 2 rats and in the liver all rats dosed with 40 mg/kg/bid for 26 weeks (Table 87).

Table 84: Histopathological findings in the kidney of rats dosed with 80 mg/kg/bid for 14 days

Group/sex		**4M**
Dose (mg/kg b.i.d.)		**80**
Kidney		
Crystal deposits – cortex		
	Minimal	4
Crystal deposits – papilla		
	Minimal	2
	Slight	1
	Total	3
Crystal deposits – pelvis		
	Slight	1
Crystal deposits – pelvic region		
	Minimal	18
	Slight	1
	Total	19
Number of animals examined		20

[Table from NDA 204447 submission; Study No. LBK0255, page 35]

Table 85: Histopathological findings in rats dosed with 60 mg/kg/bid for 4 weeks

Group/sex Dose (mg/kg b.i.d.)		1M 0	3M 60
Kidney			
Crystal deposits – cortex			
	Slight	0	1
Crystal deposits – medulla			
	Slight	0	1
Crystal deposits – papilla			
	Slight	0	1
Number of animals examined		5	9

Group/sex Dose (mg/kg b.i.d.)		1M 0	3M 60
Kidney (continued)			
Crystal deposits – pelvic region			
	Minimal	0	1
Liver			
Crystalline material – bile ducts/portal region			
	Minimal	0	1
Number of animals examined		5	9
Bile duct			
Crystal deposits – lumen			
	Minimal	-	1
Number of animals examined		0	1

- Not examined

[Table from NDA 204447 submission; Study No. LBK0255, pages 40 - 41]

Table 86: Histopathological findings in rats dosed with 60 mg/kg/bid for 13 weeks

Group/sex Dose (mg/kg b.i.d.)		1M 0	3M 60
Kidney			
Crystal deposits – cortex			
	Minimal	0	5
	Slight	0	2
	Total	0	7
Crystal deposits – medulla			
	Minimal	0	2
	Slight	0	1
	Total	0	3
Number of animals examined		5	9

Group/sex Dose (mg/kg b.i.d.)		1M 0	3M 60
Kidneys (continued)			
Crystal deposits – papilla			
	Minimal	0	2
	Slight	0	3
	Moderate	0	2
	Total	0	7
Crystal deposits – pelvis			
	Minimal	0	1
	Slight	0	3
	Total	0	4
Liver			
Crystalline material – bile ducts/portal region			
	Minimal	0	6
	Slight	0	2
	Total	0	8
Distended bile duct containing crystalline material			
	Moderate	0	1
Number of animals examined		5	9
Bile duct			
Crystal deposits – lumen			
	Minimal	-	2
	Slight	-	6
	Total	-	8
Number of animals examined		0	8
Urinary bladder			
Crystal deposits – lumen			
	Minimal	-	2
	Moderate	-	2
	Marked	-	1
	Total	-	5
Number of animals examined		0	5

[Table from NDA 204447 submission; Study No. LBK0255, pages 41 - 42]

Table 87: Histopathological findings in rats dosed with 40 mg/kg/bid for 13 weeks

Group/sex		1M	2M
Dose (mg/kg b.i.d.)		0	40
Kidney			
Crystal deposits – cortex			
	Minimal	0	2
Crystal deposits – medulla			
	Minimal	0	1
Crystal deposits – papilla			
	Minimal	0	1
	Slight	0	1
	Total	0	2
Crystal deposits – pelvis			
	Slight	0	1
Liver			
Crystalline material – bile ducts/portal region			
	Minimal	0	7
	Slight	0	1
	Total	0	8
Distended bile duct containing crystalline material			
	Minimal	0	1
	Slight	0	1
	Total	0	2
Number of animals examined		5	10
Bile duct			
Crystal deposits – lumen			
	Minimal	-	4
	Slight	-	2
	Moderate	-	1
	Total	-	7
Number of animals examined		0	8

[Table from NDA 204447 submission; Study No. LBK0255, page 43]

Kidney and liver sections from rats dosed with 40 mg/kg/bid for 26 weeks and 60 mg/kg/bid for 13 weeks were TOF-SIMS and LC-MS/MS analyzed to determine the chemical composition of the crystals in Study Nos. 11982 and 13185, respectively. These crystals consisted of a major-human metabolite, Lu AA34443, and a glucuronide of Lu AA39835, M3.

Saturated solubility of Lu AA34443 in rat, dog and human urine (Study No. 13285)
Saturated solubility measurement of Lu AA34443 was conducted in urine from rat, dog, and human. The lowest saturated solubility for human, dog, and rat urine was 133 µg/mL at pH 5.2, 199 µg/mL at pH 5.9, and 143 µg/mL at pH 5.0, respectively.

Using the saturated solubility data from Study No. 13285, the Sponsor estimated the concentration of Lu AA34443 in urine based on ADME studies (Table 88). From these estimates, the Sponsor determined that the concentration of Lu AA34443 in urine of rat exceed the solubility limit and resulted in crystal formation, while the concentration in urine of dogs was below the solubility limit and no crystals were seen. Based on the Sponsor's estimates, the concentration of Lu AA34443 in human urine will also be below the solubility limit; therefore, crystals should not form in human urine.

Table 88: Estimated mean concentrations of Lu AA34443 in urine and bile samples where no crystal formation was observed for mice, rats, and dogs compared to highest therapeutic dose in humans.

	Mouse	Rat	Dog	Human
Body weight	0.02 kg	0.25 kg	10 kg	NA
NOAEL/Therapeutic dose	50 mg/kg/day	10 mg/kg BID	7.5 mg/kg/day	20 mg
% Lu AA34443 of drug related material in urine	2% (a)	27% (a)	11% (a)	41% (b)
% Lu AA34443 of drug related material in feces	63% (a)	31% (a)	31% (a)	24% (b)
Literature values for urine volume [7-10]	0.68 - 8 mL/day	6 - 50 mL/day	200 - 1000 mL/day	602 - 2002 mL/day
Literature values for bile volume [7]	2 mL/day	23 mL/day	120 mL/day	350 mL/day
Mean concentration of Lu AA34443 in urine on a daily basis (c)(d)	29 - 3 µg/mL	225 - 27 µg/mL	41 - 8 µg/mL	14 - 4 µg/mL
lowest saturated solubility of Lu AA34443 in urine	Not determined	143 µg/mL	199 µg/mL	133 µg/mL
Mean concentration of Lu AA34443 in bile on a daily basis (c)(d)	315 µg/mL	67 µg/mL	194 µg/mL	14 µg/mL

(a) Data from study [11694], [11304] and [12284].
(b) Calculated on the basis of results from the human single dose AME study [10477] and [10882]. As an example; 59% of the administered dose was recovered in urine at 0-360 hr postdose, of these Lu AA34443 accounted for 69% of the quantified drug related material (0.69x59%=41%). 26% of the administered dose was recovered in feces at 0-360 hr post-dose, of these Lu AA34443 accounted for 92% of the quantified drug-related material (0.92x26%=24%).
(c) Concentration at the NOAEL level for the animals and the therapeutic level for humans assuming dose linearity.
(d) $C_{Lu\ AA34443\ in\ bile}$=Dose x $\%_{LuAA34443\ in\ feces}$/$Volume_{bile(0\text{-}24\ hr)}$, $C_{Lu\ AA34443\ in\ urine}$=Dose x $\%_{LuAA34443\ in\ urine}$/$Volume_{urine(0\text{-}24\ hr)}$.

[Table from NDA 204447 submission; Pharmacokinetics Written Summary, page 30]

Mechanistic Studies to Examine Convulsive Potential for Lu AA34443 in Dog (Study Nos. 10727 and 13228)

These studies was designed to determine if a major-human metabolite, Lu AA34443, has convulsive potential in dog at plasma levels similar to those measured in the 13-week general toxicology study. In the first study, two dogs (1/sex) received intravenously Lu AA34443 (2.1 mg/kg/hour in 5% HP-ß-CD containing 4.4% glucose) by infusion for 2 hours with a target Cmax value of 8000 nM. In the second study, dogs

(2/sex) received intravenously Lu AA34443 (4.2 mg/kg in 5% HP-ß-CD containing 4.4% glucose) by infusion for 1 hour.

During and after the 1 and 2-hour infusions, no convulsions were noted and there were no Lu AA34443-related clinical signs or changes in food consumption. In the first study, Cmaxs for the two dogs were 8309 and 6320 nM, which was 2- and 1.6-fold the exposures seen in the dog that convulsed in the 13-week study. In the second study, Cmaxs for the 4 dogs were 9590, 7030, 8980, and 8130 nM, which are approximately 2-fold the exposures seen in the 13-week study.

Skin Sensitization: Local Lymph Node Assay (LLNA) in Mice (Study No. 74493)
Skin sensitization potential of vortioxetine was examined in CBA/CAOlaHsd mice. Vortioxetine (30, 140, and 210 mg/ml in DMSO) was applied to the dorsal surface of each ear for 3 days and on Day 6, mice were injected with tritiated methyl thymidine and lymph nodes were removed. Lymphoproliferation was measured; a stimulation index (SI = ratio of lymphoproliferation in test groups to control groups) of ≥3 is considered a skin sensitizer.

No clinical signs or local irritation at the application site were noted during the study. The SI was ≥3 for all doses tested and increases dose-dependently (Table 89); therefore, vortioxetine has skin sensitization potential with dermal application. However, oral dosing is proposed for the indication of MDD; therefore, the potential for skin sensitization is not a concern for this indication.

Table 89: Stimulation index for vortioxetine and the positive control HCA

Group 6 (30 mg/ml Lu AA21004 in DMSO): **SI = 3.1**

Group 7 (140 mg/ml Lu AA21004 in DMSO): **SI = 7.7**

Group 8 (210 mg/ml Lu AA21004 in DMSO): **SI = 9.1**

Group 9 (25 % HCA in AOO (positive control): **SI = 6.9**

[Table from NDA 204447 submission; Study No. 74493, page 10]

11 Integrated Summary and Safety Evaluation

Pharmacology

Vortioxetine is a selective serotonin reuptake inhibitor with additional activity at five serotonin receptors: 5-HT3, 5-HT7, 5-HT1D, 5-HT1B, and 5-HT1A. It binds with high affinity to the cloned human serotonin transporter (5-HTT; Ki = 1.6 nM) and potently inhibits reuptake of serotonin (cIC50 = 5.4 nM). Vortioxetine has 70-fold lower affinity for the cloned human norepinephrine transporter (Ki = 113 nM, cIC50 = 107 nM) and >600-fold lower affinity for the dopamine transporters (Ki and cIC50 > 1000 nM). In rat brain synaptosomes, vortioxetine also potently inhibits reuptake of serotonin (cIC50 = 5.3 nM).

In addition, vortioxetine binds with high affinity to cloned human and rat 5-HT3 receptors and acts as a potent 5-HT3 receptor antagonist (H: Ki = 3.7 nM, cIC50 = 3.5 nM; R: Ki = 1.1 nM, cIC50 = 0.18 nM). Vortioxetine binds with moderate affinity for human and rat 5-HT7, 5-HT1D, 5-HT1B, and 5-HT1A receptors (H: Ki = 18, 54.2, 33, and 15 nM, respectively; R: Ki = 200, 3.7, 16, and 232 nM, respectively) and acts as a weak 5-HT7 receptor antagonist (H: cIC50 = 450 nM; R: cIC50 = 2080), moderate 5-HT1D receptor antagonist (H: cIC50 = 25 nM; R: cIC50 = 43), moderate to weak 5-HT1B receptor partial agonist (H: EC50 = 120 – 460 nM), and moderate 5-HT1A receptor agonist (H: EC50 = 199 nM).

Two metabolites of vortioxetine, Lu AA34994 and Lu AA39835, have similar affinity for human 5-HTT as vortioxetine. Although vortioxetine is only 1.4- and 3-fold more potent than Lu AA34994 and Lu AA39835, respectively, Lu AA34994 is a reactive intermediate for a major human metabolite (Lu AA34443) and is below the level of detection in a human mass balance study and Lu AA39835 is a non-major human metabolite and did not produce “serotonin syndrome” in mice suggesting that Lu AA39835 is not blocking 5-HTT in rodents *in vivo*. Lu AA34443 was found to inhibit specific binding of [^{3}H]BRL 43694 to human 5-HT3 by 99% at 1 µM in a CEREP screening panel; however, the Sponsor did not follow up and determine a Ki or IC50.

In vivo in rats, vortioxetine was shown to have occupancy at 5-HTT and rat 5-HT3, 5-HT1B, and 5-HT1A receptors. Following a single subcutaneous (SC) or oral administration of vortioxetine to rat, 50% occupancy of 5-HTT occurs at 0.6 mg/kg and 12 mg/kg respectively. Following three SC doses of vortioxetine, occupancy of 5-HTT was 41% at 5 mg/kg/day. For 5-HT3 and 5-HT1B receptors, single SC doses of vortioxetine resulted in 50% occupancy at 0.004 and 3.3 mg/kg, respectively. For 5-HT1A receptor, single SC doses of vortioxetine resulted in 44% occupancy at 20 mg/kg (highest dose tested). The acute dose of vortioxetine that produces ~ 50% occupancy (ED50) for 5-HTT and 5-HT3, 5-HT1B, and 5-HT1A receptors is consistent with vortioxetine’s binding affinity for these transporter/receptors in rat (occupancy/binding affinity: 5-HT3≥5-HTT>5-HT1B>5-HT1A). Occupancy was not examined for rat 5-HT7 and 5-HT1D receptors.

Vortioxetine's inhibition of 5-HTT and antagonist activity at 5-HT3 receptor was further demonstrated in functional (*in vitro*) studies in rodents. Vortioxetine produced "serotonin syndrome" at doses of 7.9 and 15.8 mg/kg SC in mice, suggesting inhibition of 5-HTT. In contrast, the non-major human metabolite, Lu AA39835, did not produce "serotonin syndrome" at doses up to 12.3 mg/kg. Vortioxetine dose-dependently suppressed a 5-HT3 agonist-induced blood pressure reflex (Bezold-Jarish) in rats (ED50 = 0.11 mg/kg), suggesting 5-HT3 receptor antagonist activity. In comparison, ondansetron (a known 5-HT3 receptor antagonist) suppressed this reflex with an ED50 of 0.021 mg/kg.

In vivo microdialysis and dorsal raphe nucleus (DRN) serotonin neuronal firing studies in rats also suggest functional activity of vortioxetine. After single and 3-daily doses, vortioxetine dose-dependently increased serotonin, norepinephrine, dopamine and histamine (not assessed in multiple dose study) levels in medial prefrontal cortex and ventral hippocampus (DA only shown to increase in VH after a single dose) and increased serotonin levels in the nucleus accumbens. Acetylcholine levels were also increased in the medial prefrontal cortex and ventral hippocampus, but only in the presence of a cholinesterase inhibitor. Glutamate and GABA levels were not affected after a single dose.

A single dose of vortioxetine in rat suppressed DRN serotonin neuronal firing and this effect was reversed by a 5-HT1A receptor antagonist, suggesting that the effect of vortioxetine in the rat DRN involves presynaptic 5-HT1A receptors. This effect was also blocked by pre-administering a 5-HT3 receptor agonist. Single doses of fluoxetine (a classic SSRI) suppressed DRN serotonin neuronal firing and this effect was reversed by a 5-HT1A receptor antagonist, but was not blocked by pre-administering a 5-HT3 receptor agonist. Vortioxetine (up to 5 mg/kg) given over 5 – 10 hours decreased spontaneous DRN serotonin neuronal firing by ~ 50%, but had no effect on DRN firing when given for 1 – 14 days. In contrast, fluoxetine over 7 days decreased spontaneous neuronal firing by 50%, but had no affect when given for 14 – 21 days.

Safety Pharmacology

Vortioxetine had mixed results in animal models thought to be predictive of antidepressant activity. However, it is unclear what the true predictive value of these animal models is for human antidepressant treatment and the results are not relevant at this stage of drug development, when clinical efficacy has been determined. In rat, acute oral doses up to 40 mg/kg had no CNS effects in safety pharmacology studies. Dogs dosed with ≥0.75 mg/kg IV had mild or moderate sedation, had whimpering/whining/barking, and mild to moderate abdominal pain (at doses ≥1.5 mg/kg IV).

In cardiovascular studies, vortioxetine was a weak reversible inhibitor of the hERG channel (IC50 = 3.3 µM) and an inhibitor of human cardiac SCN5A sodium channels (IC50 = 930 nM). In isolated right atrium from rat, vortioxetine did not affect basal heart rate, but did modify isoprenaline's dose response. In anesthetized rabbits and conscious dog, 10 and 6 mg/kg vortioxetine IV, respectively, increased heart rate. The NOEL for increased heart rate was 6 and 3 mg/kg in rabbit and dog respectively.

However, in anesthetized ventilated guinea pigs, IV vortioxetine at doses ≥10 decreased heart rate. In dog general toxicology studies there were no consistent findings suggesting a voritoxetine-related effect on heart rate.

Vortioxetine's effect on blood pressure is equivocal; in conscious dog, 6 mg/kg vortioxetine IV increased blood pressure by >25 mmHg and in the 52-week general toxicology study systolic, diastolic, and mean arterial pressure was increased in male dose groups, but not dose-dependently or statistically significantly. In addition, in anesthetized dogs increasing doses of vortioxetine IV, decreased arterial blood pressure by ≤ 7 mmHg. In rabbit and dog, there was no consistent finding of a vortioxetine-related effect on ECG morphology or pro-arrhythmic activity.

Vortioxetine had minimal effects on respiration. Vortioxetine may affect the gastrointestinal system by decreasing gastric emptying in rats. However, overall, ***the safety pharmacology studies did not reveal any significant areas of concern***.

Pharmacokinetics

Vortioxetine was absorbed after oral administration in nonclinical species. Oral bioavailability is low in rat and dog, but higher in human (~10, 48, and 75%, respectively). Vortioxetine was found to bind to plasma protein at >98.8% for human, mice, rats, rabbits, and dogs. Metabolite Lu AA34443 was found to bind to plasma protein at 62.9 – 74.5% for all species tested. Vortioxetine and its metabolites were extensively distributed to all tissues examined in rat, with the highest tissue concentrations seen in liver and kidney, and in eye in pigmented rats. Administration of [^{14}C]-vortioxetine to pregnant and lactating rats showed drug-related material was distributed throughout the maternal and fetal tissue and was in milk secretion. However, concentrations of drug-related material were lower in the fetus than the maternal tissue (~ 10-fold for tissues with the greatest amount of radioactivity). Milk to plasma ratio was 1, 1.2, and 0.5 at 2, 6, and 24 – 72 hours post-dose.

Vortioxetine is extensively metabolized *in vivo* in all nonclinical species and in humans. Four major-human metabolites (defined as circulating at greater than 10% of total drug-related exposure) have been identified: M3 (Lu AA39835 glucuronide), M4(b) (Lu AA34443 glucuronide), Lu AA34443, and M12. Lu A34443 is present in plasma at greater than 3-fold of human in mouse, rat, dog, and rabbit and is adequately covered in nonclinical studies. Because Lu AA34443 is covered the glucuronide of Lu AA34443, M4(b), is not a concern. Although not a major-human metabolite, Lu AA39835 is detected in rat, dog, and rabbit plasma in general toxicology studies; therefore, the glucuronide of Lu AA39835, M3, is not a concern. Lu AA34443, M4(b), and M3 are considered qualified. However, M12 is not detected in rat, the Sponsor did not look for it *in vivo* in rabbit, and it is only present at 0.1- and 0.7-fold of human exposure at the MRHD of 20 mg in mouse and dog, respectively. However, M12 is likely to be pharmacologically inactive and water soluble because it is a Phase II glucuronide conjugate. Therefore, it likely poses ***minimal safety risk for humans and no additional studies are recommended***.

The main route of excretion in humans of vortioxetine and its metabolites is via the urine (59 and 26% recovery of administered dose in urine and feces respectively). In contrast, the main route of excretion in mouse, rat, and dog is feces (mouse: 84% feces vs. 14% urine; rat: 69 vs. 33%, dog: 59 – 65 vs. 23 – 33% of administered dose). In addition, in rats, biliary excretion was 46% of total radioactivity.

General Toxicology
Vortioxetine was adequately tested in acute and chronic oral general toxicology studies in mice, rats, and dogs.

Acutely, high oral doses of vortioxetine in rats and mice (≥ two doses of 200 mg/kg given an hour apart) resulted in convulsions and/or death with a NOEL of 500 mg/kg and 300 mg/kg, respectively. No convulsions were noted in chronic rat and mouse studies with high doses of 40 mg/kg/bid and 100 mg/kg, respectively. However, in a 13-week dog study, a convulsion, which appears to be Cmax related, was seen in one dog after the second dose of 15 mg/kg and a second dog after the first dose of 10 mg/kg, making the NOEL for convulsions in dogs 7.5 mg/kg/day.

In the 26-week rat general toxicology study, kidney pathology was seen in males at the high dose of 40 mg/kg/bid and liver pathology was noted in a small number of males and females at 20 and 40 mg/kg/bid. In both the liver (bile duct) and kidney, the presence of crystals was noted and may be the cause of the histopathological findings. In addition, crystals were seen in the urine of males dosed with 40 mg/kg/bid and urinary volume was also increased. In a 2-year rat carcinogenicity study, similar findings were seen in males dosed with 20 mg/kg/day and females dosed with ≥15 mg/kg/day. The NOAEL for liver and kidney pathology is 10 mg/kg/bid in the 26-week general toxicology study and is 7 mg/kg/bid for males and 5 mg/kg/bid for females in the 2-year carcinogenicity study.

In a mechanistic study designed to examine the crystalline material in the bile ducts and renal tract of rats, crystal formation appears to be dose- and time-dependent; crystals are seen at higher doses in less time. The crystals in the liver and kidney were identified as consisting of a major-human metabolite, Lu AA34443, and a glucuronide of Lu AA39835, M3. The crystals in the urine were identified as consisting of Lu AA34443. Because there is a difference in the route of excretion for humans and rats (mainly hepatobilliary in rat and urinary in humans), ***metabolite crystals in the liver is likely a rodent specific finding***. Based on saturated solubility data in the urine and Sponsor's estimates of Lu AA34443 concentrations, the solubility limit in urine was exceeded in rat (which resulted in crystals), but not in dog or in human at the MRDH of 20 mg (no crystals observed). In doses in rat where crystals were observed, plasma Lu AA34443 was present at >26 times the plasma exposure in humans at the MRHD of 20 mg. Because the estimated amount of Lu AA34443 in human urine is below the solubility limit for humans and human plasma exposures of Lu AA34443 are at least 9 times below the NOEL for crystals in rats, ***crystals in the renal tract of rats likely poses no risk for humans***.

In the 52-week dog general toxicology study, there were no limiting toxicities observed in the 52-week dog study; however, the high dose of 7.5 mg/kg/day was the NOEL for convulsions in the 13-week general toxicology study. In the 52-week study, clinical signs were limited to pupillary dilation at ≥5 mg/kg/day in males and females with a NOAEL of 3.75 mg/kg/day for males and 5 mg/kg/day for females. Small increases in plasma glucose and decreases in triglycerides were noted in Week 52. There was also a dose-dependent increase in urine volume although no histopathological findings were seen in any organ.

Due to misconduct at (b) (4) TK data from rat and dog chronic toxicology studies were found not to be GLP compliant. In rat, remedial action determined that exposure data for vortioxetine and Lu AA34443 from males at all doses on Day 1 and males and females at 40 mg/kg/bid in Week 26 was unreliable and was excluded from the TK evaluation; however, the TK data from the 10 and 20 mg/kg/bid group were reliable at Week 26. In the dog, remedial action determined that exposure data for vortioxetine from the 3.75 mg/kg/bid dose groups in Week 52 and all exposure data for Lu AA34443, except Day 1, were unreliable. For the reliable data, plasma exposures to vortioxetine increased with repeated dosing in rat (Day 1 to Week 26) but were generally similar for dogs (Day 1 to Week 52). Vortioxetine and Lu AA34443 exposures were slightly higher for male than female rats and similar for male and female dogs. Exposure to Lu AA34443 was 3 – 6 times the exposure to vortioxetine in rat and 2.5 – 5 times the exposure to vortioxetine in dog.

Genotoxicity

Vortioxetine was adequately tested for genotoxicity. Vortioxetine was found to be negative for mutagenicity (with and without metabolic activation) in bacterial cells in an Ames assay and for clastogenicity in the *in vitro* chromosome aberration assay in cultured human lymphocytes and in an *in vivo* rat bone marrow micronucleus assay.

Carcinogenicity

Adequate two-year oral carcinogenicity studies were conducted in Wistar rats and CD-1 mice. Protocols for the carcinogenicity studies were presented to the Executive CAC on January 16, 2007 and the oral doses used in mice (5, 15, or 50 mg/kg/day for males and 10, 30, or 100 mg/kg/day for females) and female rats (5, 15, or 40 mg/kg/bid) were those recommended by the Exec CAC, with the high dose based upon AUC. The oral doses used in male rats (2, 7, or 20 mg/kg/bid) were also those recommended by the Exec CAC, with the high dose based on MTD for renal pathology in the 26-week rat general toxicology study. The vehicle was 10% HP-ß-CD and 4.4% glucose monohydrate for the rat study and 15% HP-ß-CD for the mouse study. Due to the known carcinogenicity finding of pancreatic neoplasm for oral HP-ß-CD in rats, a water control was also used in the rat study.

Due to misconduct at (b) (4) TK data from rats and mice carcinogenicity studies were found not to be GLP compliant; however, based on remedial action, the Sponsor considers the TK data reliable. Plasma exposures at 2 hours postdose indicated that both rats and mice were exposed to vortioxetine and its major human metabolite, Lu

AA34443, throughout the dosing period. Plasma exposures increased with repeated dosing in rats (Weeks 1 - 105) and mice (Weeks 1 – 104/105). Because of sparse sampling in both rat and mice studies, estimation of TK parameters was not performed and human exposure margins for vortioxetine were calculated from shorter general toxicology studies.

In rats, an MTD was demonstrated for females based on increased premature mortality due to blockage of the common bile duct by crystals. Although there was increased premature mortality of high dose female rats, the survival was adequate and sufficient numbers of female rats were exposed for a sustained amount of time for an adequate assessment of carcinogenicity. The MTD was not demonstrated for male rats; however, the high dose is considered adequate based on the MTD for renal pathology in the 26-week general toxicology study. Biologically relevant, drug-related increases in incidences of neoplasm were limited to polypoid adenomas of the rectum in females at the high dose of 40 mg/kg/bid.

The incidence of polypoid adenomas of the rectum (4 out of 55) was statistically significantly increased for female rats at 40 mg/kg/bid. No polypoid adenomas were seen in the water or HP-ß-CD vehicle control groups or in the historical control data for the laboratory (b) (4). The Sponsor wants to attribute these findings to "…vehicle driven hyperplasia and inflammation of the large intestine…" because HP-ß-CD has been shown previously to increase neoplasms of the large intestine of rat at 5000 mg/kg/day with a clear dose-response; no neoplasms were seen at 500 mg/kg/day. In the current carcinogenicity study, all male and female rat groups that received HP-ß-CD (control and vortioxetine; 500 mg/kg/bid) had increased epithelial hyperplasia and mucosal inflammation in the large intestines (cecum, colon, and rectum) when compared to the water control group. The lack of dose-response with vortioxetine for the increased hyperplasia and inflammation confirms that this was a vehicle effect. However, there was a clear dose-response for polypoid adenomas in female rats even though the same volume of vehicle (5 mL/kg; 500 mg/kg/bid) was administered to all groups. Therefore, a drug effect cannot be discounted and I, with concurrence from the Exec CAC, consider ***the finding of polypoid adenomas in the rectum of female rats vortioxetine related***.

In mice, an MTD was not demonstrated; although the high dose was considered adequate based on the clinical safety margin afforded by the AUC from the 13-week mouse general toxicology study. No biologically relevant, drug-related increases in neoplasms were seen.

Reproductive Toxicity

Vortioxetine and metabolites distributed throughout maternal and fetal tissue and were present in milk secretion. Concentrations of drug-related material were lower in the fetus than the maternal tissue, but similar in milk compared to maternal plasma up to 6 hours after dosing.

Effects of oral vortioxetine on fertility and early embryonic development were assessed in male and female Sprague-Dawley rats in the same study. Oral vortioxetine had no effect on male and female fertility and embryonic development in rats at doses up to the high dose of 60 mg/kg/bid. In males, premature mortality due to kidney pathology occurred in 2/20 males dosed at 60 mg/kg/bid. In addition, decreased body weight and feed consumption, renal pelvis dilatation, and presence of white and/or yellow calculi in kidneys and ureters were noted in males dosed with 60 mg/kg/bid, but not 40 mg/kg/bid.

In embryo-fetal development studies, there were no findings of teratogenicity in rat or rabbit that were clearly vortioxetine related at doses up to the high doses of 80 and 30 mg/kg/bid, respectively.

Pregnant Sprague-Dawley rats were dosed orally in two studies at 5, 15, and 40 mg/kg/bid or 60 and 80 mg/kg/bid during the period of organogenesis (GD6 – 17). Maternal toxicity occurred at 60 and 80 mg/kg/bid and consisted of decreased body weight and feed consumption. Fetal developmental toxicity occurred at 15, 40, 60, and 80 mg/kg/bid and consisted of decreased fetal weight (at 60 and 80 mg/kg/bid only) and delayed ossification of the fetal skeletal system. Because maternal toxicity was not seen at doses of 15 and 40 mg/kg/bid and developmental toxicity was, ***maternal toxicity alone cannot explain the delayed development; therefore, delayed skeletal development is likely a direct effect of vortioxetine***. The NOAEL for maternal toxicity is 40 mg/kg/bid and for fetal developmental toxicity is 5 mg/kg/bid. There were no vortioxetine-related malformations in rats at doses up to 80 mg/kg/bid.

Pregnant New Zealand White rabbits were dosed orally in two studies at 5, 10, and 15 mg/kg/bid or 1 and 30 mg/kg/bid during the period of organogenesis (GD6 – 18). Maternal toxicity occurred at all doses and consisted of decreased body weight and feed consumption. Of note, in the second study, a water control group was used to compare to the vehicle (HP-ß-CD) control group. Vehicle alone also resulted in decreased body weight and feed consumption; however, vortioxetine dosed groups still had greater body weight and feed consumption decreases compared to vehicle. Fetal developmental toxicity occurred in rabbits at 5, 10, 15, and 30 mg/kg/bid. Developmental toxicity was characterized by decreased fetal weight, increased incidence of runts (30 mg/kg/bid only), and delayed ossification of the fetal skeletal system. The NOAEL for maternal toxicity was not determined and is less than 1 mg/kg/bid and for fetal developmental toxicity is 1 mg/kg/bid.

Malformations were not seen in rabbit at the highest dose tested, 30 mg/kg/bid. However, in one of the rabbit studies, when CNS malformations are combined, a small, non-dose dependent increase in CNS malformations, mainly consisting of neural tube defects, is observed at 5, 10, and 15 mg/kg/bid (0, 1, 3, and 2 fetuses from different litters with CNS malformations for control, LD, MD, and HD, respectively). A CNS malformation was also noted for 1 fetus at 1 mg/kg/bid in the second rabbit study,1 rat fetus at 5 mg/kg/bid, and 1 rat fetus at 60 mg/kg/bid. However, no CNS malformations were noted in rat fetuses of the high dose groups in either rat study, at 40 or 80 mg/kg/bid. Considering CNS malformations are not seen at high doses in rat and rabbit,

the incidence of CNS malformations is small, and there is a lack of a dose-response; ***the finding of CNS malformations is equivocal and not clearly vortioxetine related***. Therefore, I do not recommend that the finding of CNS malformations be included in labeling.

In a pre- and post-natal development study, effects of oral vortioxetine were assessed in offspring of pregnant Sprague-Dawley rats dosed with 5, 20, and 60 mg/kg/bid starting from implantation (GD6) and lasting till weaning of offspring (PND20). Maternal toxicity (F_0 dams) occurred at 20 and 60 mg/kg/bid and consisted of decreased body weight gain and feed consumption for dams during gestation at 60 mg/kg/bid. The NOAEL for maternal toxicity was 5 mg/kg/bid. Decreased number of live-born pups and increased number of still born pups was also seen at 20 and 60 mg/kg/bid.

Pup developmental toxicity (F_1 generation) occurred at 20 and 60 mg/kg/bid based on decreased survival and little to no milk in the stomach from birth to weaning, decreased body weight at birth to weaning (60 mg/kg/bid only), and some indication of delayed development based on percent eyes opened at PND15 (the Sponsor did not report the percent of eyes opened after PND15). Body weights remained decreased for high dose male and mid and high dose female F_1 generation rats post weaning. There were no effects of vortioxetine on learning, auditory function, locomotor activity, sexual development, mating, and fertility in F_1 generation rats. The NOAEL for pup development is 5 mg/kg/bid and for post-weaning development, sexual development, mating, and fertility is 60 mg/kg/bid.

Impurities

A genotoxic impurity, (b) (4) has been identified in the (b) (4) for drug substance. The current limit proposed by the Sponsor is not more than (NMT) (b) (4) for starting material and no limit is proposed for key intermediates or final drug substance. It is unclear if the Sponsor has measured this impurity in key intermediates or in the final drug substance, but it is unlikely because there are no limits. ***The exposure to this impurity in humans will need to be limited to not more than*** (b) (4) ***prior to approval from a pharm/tox perspective***.

Conclusions

Vortioxetine is a potent and selective inhibitor of the serotonin transporter, with at least 70-fold selectivity over the norepinephrine transporter and at least 600 selectivity over the dopamine transporter. In addition, vortioxetine is a potent antagonist at 5-HT3 receptor, a moderate antagonist at 5-HT1D receptor, a moderate agonist at 5-HT1A receptor, a moderate to weak partial agonist at 5-HT1B, and a weak antagonist at 5-HT7 receptor.

Although the mechanism of the antidepressant effect of vortioxetine is not fully understood, it is most likely related to its inhibition of the serotonin transporter, which is consistent with other SSRIs. However, based on data from rodent occupancy studies, acute microdialysis studies, and serotonin neuronal firing in the dorsal raphe nucleus, the Sponsor would like to claim a “multimodal” mechanism of action: “direct modulation

of receptor activity and inhibition of the serotonin transporter." However, the data presented do not make a compelling case that the mechanism of action for vortioxetine's antidepressant effect is different from other SSRIs. Although, there is mechanistic data for 5-HT3, 5-HT1B, and 5-HT1A receptors, it is all in rodent and not in human. Considering that the affinity of vortioxetine for these human receptors is different than the affinity for the rodent receptors and that the relevance of rodent studies to human antidepressant treatment is still unclear, it is difficult to put much weight on these rodent studies, especially without clear differences in clinical efficacy or safety of vortioxetine treatment compared with other SSRIs.

Nonetheless, it is reasonable to put binding to relevant receptors in the Mechanism of Action and/or Pharmacokinetics sections of labeling. Because binding and functional activity for human 5-HT3 (Ki = 3.7 nM; cIC50 = 3.5 nM) is similar to human 5-HTT (Ki = 1.6 nM; cIC50 = 5.4 nM), I agree that binding and functional activity at 5-HT3, can be included after the SSRI activity in the Mechanism of Action section. However, functional activity at 5-HT1D (Ki = 54.2 nM; cIC50 = 25 nM), 5-HT1B (Ki = 33 nM; EC50 = 120 – 460 nM), and 5-HT1A (Ki = 15 nM; EC50 = 199 nM) is moderate at best compared to 5-HTT activity; therefore, I do not agree that these three receptors should be included in Mechanism of Action, but because of the moderate functional activity can be included in the Pharmacokinetics section of labeling.

However, for the 5-HT7 receptor the data are not compelling. Although, binding to the human 5-HT7 receptor is reasonable (Ki = 18 nM), functional activity at the human 5-HT7 receptor suggest only weak antagonist activity for vortioxetine (IC50 = 1271 nM; cIC50 = 450). In addition to weak antagonist activity, no further rodent occupancy or *in vivo* functional activity assays were performed for 5-HT7 receptor. This may be due to the low binding vortioxetine has for the rat 5-HT7 receptor compared to human (5 – 11-fold less than to the human). However, without any additional data and an 84-fold difference in the cIC50 from the 5-HTT, the 5-HT7 receptor should not be included in the Mechanism of Action and Pharmacokinetics sections of labeling.

The four major human metabolites; M3 (Lu AA39835 glucuronide), M4(b) (Lu AA34443 glucuronide), Lu AA34443, and M12; are considered qualified and no additional studies are needed.

General toxicities seen in rat or dog that might have clinical relevance are convulsions, kidney and liver pathology, and pupillary dilation. Convulsions were noted after acute dosing in rats and dogs at 195 and 16 times, respectively, the maximum recommended human dose (MRHD) of 20 mg on a mg/m2 bases; but no convulsions were seen in chronic rat and dog studies at doses 39 and 12 times, respectively, the MRHD. In rat, kidney and liver pathology related to the presence of vortioxetine metabolite crystals was noted at doses 39 times the MRHD in the 26-week general toxicology study and 20 times the MRHD in the 2-year carcinogenicity study. In dog, pupillary dilation occurred at doses 8 and 12 times the MRHD in males and females, respectively.

Vortioxetine was not genotoxic. Polypoid adenomas of the rectum occurred in female rats at 39 times the MRHD, but not in male rats at up to 20 times the MRHD and in mice at up to 12 times the MRHD.

Vortioxetine had no effect on male and female fertility at doses up to 58 times the MRHD. There were no findings of teratogenicity in rat and rabbit that were clearly vortioxetine related at doses up to 78 and 58 times, respectively, the MRHD. However, some developmental toxicity was seen in rat and rabbit at 15 and 10 times, respectively, the MRHD. Decreased live-born puts, increased postnatal pup mortality, and slight delayed development were seen in a pre- and post-natal development study in rats at doses up to 20 times the MRHD.

Safety margins, based on body surface area, for toxicities seen in toxicology studies are shown in Table 90 below. I have based safety margins on surface body area rather than exposure multiples because of misconduct at (b) (4) and the extensive metabolization of vortioxetine *in vivo*. Due to misconduct at (b) ( ) not all exposure data is reliable. In addition, vortioxetine is highly metabolized in animals and humans. The Sponsor did not measure exposures to total circulating drug-related compounds in rats, mice, dogs, rabbits, and humans, but measured exposures to parent and usually just the major-human metabolite, Lu AA34443. Therefore, using exposures for just vortioxetine will most likely underestimate exposure multiples. Although using exposures for vortioxetine and Lu AA34443 might be better than vortioxetine alone, it still will most likely be inaccurate. From the PK data, it does not appear that the percent of Lu AA34443 of total circulating is the same across species. In addition, there are three other major-human metabolites that are not measured so there will still most likely be an underestimate of exposure multiples. Therefore, basing safety margins on surface body area seems to be the most appropriate calculation.

Table 90: Margins of safety for toxicities observed in nonclinical studies

Toxicity	Species	NOAEL (mg/kg/day) M/F	Safety Margin Based on Surface Area M/F
General	Rat kidney and liver pathology -26 week kidney and liver pathology-2 years Dog pupillary dilation	 20 14/10 3.75/5	 10 7/5 6/8
Carcinogenicity	Rat Mouse	40/30 50/100	20/15 12/24
Reproductive & Developmental Fertility Embryo-fetal development Pre- and postnatal development	 Rat Rat Rabbit Rat	 60/60 80 (maternal) 10 (fetal) ND (maternal) 2 (fetal) 10 (maternal and offspring)	 58/58 39 (maternal) 5 (fetal) ND (maternal) 2 (fetal) 5 (maternal and offspring)

ND = not determined

The only Pharmacology/Toxicology issue that could prevent approval of this NDA is the genotoxic impurity, (b) (4). This impurity will need to be limited so that the maximum daily clinical dose does not exceed (b) (4) prior to approval from a pharm/tox perspective.

Antonia Dow, Ph.D., Pharmacologist *{see appended electronic signature page}*
Linda Fossom, Ph.D., Supervisory Pharmacologist *{see appended electronic signature page}*

12 Appendix/Attachments

Table 91: Histopathology Inventory for NDA 204447

Study Species	26-Week Rat	52-Week Dog	104-Week Rat	104-Week Mouse
Adrenals	X*	X*	X	X
Aorta – thoracic	X	X	X	X
Brain	X*	X*	X	X
Cecum	X	X	X	X
Colon	X	X	X	X
Duodenum	X	X	X	X
Epididymides	X*	X*	X	X
Esophagus	X	X	X	X
Eyes	X	X	X	X
Femurs	X	X	X	X
Gall Bladder		X		X
Harderian glands	X		X	X
Head			#	#
Heart	X*	X*	X	X
Ileum	X	X	X	X
Jejunum	X	X	X	X
Kidneys	X*	X*	X	X
Lachrymal glands	X	X	X	X
Larynx		X	X	X
Liver	X*	X*	X	X
Lungs	X*	X*	X	X
Lymph nodes - mandibular	X*	X	X	X
Lymph nodes - mesenteric	X*	X	X	X
Mammary area – caudal	X	X	X	X
Nictitans glands		X		
Optic nerves	X	X	X	X
Ovaries	X*	X*	X	X
Pancreas	X	X*	X	X
Pituitary	X*	X*	X	X
Preputial/clitoral gland			X	X
Prostate	X*	X*	X	X
Rectum	X	X	X	X
Salivary glands (submandibular/sublingual)	X*	X*	#	#
Sciatic nerves	X	X	X	X
Seminal vesicles	X*		X	X

Skeletal muscle	X	X	X	X
Skin	X	X	X	X
Spinal cord	X	X	X	X
Spleen	X*	X*	X	X
Sternum	X	X	X	X
Stomach	X	X	X	X
Testes	X*	X*	X	X
Thymus	X*	X*	X	X
Thyroid with parathyroids	X*	X*	X	X
Tongue	X	X	X	X
Trachea	X	X	X	X
Ureters		X	X	X
Urinary bladder	X	X	X	X
Uterus and cervix	X*	X*	X	X
Vagina	X	X	X	X
Zymbals gland with external ear			#	#

X = histopathology performed; * = organ weight obtained; # = examined if effects suspected during the study

Table of Tables

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/s/

ANTONIA L DOW
06/04/2013

LINDA H FOSSOM
06/04/2013
I agree with the conclusions of this very thorough and thoughtful review.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 204447 **Applicant:** Takeda Pharmaceuticals, Inc **Stamp Date:** October 2, 2012

Drug Name: Vortioxetine **NDA Type:** 505(b)(1); Standard

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?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
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)?	X		A juvenile animal study was submitted, but may not be reviewed during this cycle because the approval for MDD will be based on clinical trials in adults. I have contacted the Stats reviewer about the carcinogenicity data sets. I will be working with the Biopharm reviewer during the review process for any major human metabolites (>10% of total circulating drug species).
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).	X		The formulation to be marketed is a tablet, while the formulation used in the toxicology studies was a solution in 5 – 10% hydroxypropyl-ß-cyclodextrin (HPßCD). However, the route of administration (oral) is the same. The Sponsor used HPßCD in the toxicology studies to enhance solubility and oral bioavailability. I am not aware of any novel excipients in the formulation to be marketed, but will be working with the CMC reviewer during the review process.
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?	X		

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
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?	X		Yes, except for the bionalytical data for 12 nonclinical studies (including chronic rat and dog general tox studies; mice and rat carc studies; fertility, embryo-fetal development, and pre and post-natal development rat studies; and an in vivo genotox rat study). GLP is not claimed for the bioanalytical data of these 12 studies because of misconduct by the contract organization (b) (4)
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		No special studies were requested during development.
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?	X		In general yes, except the established pharmacological class is not in the Indications and Usage Section. Vortioxetine is an SSRI with additional activity at five 5-HT receptors.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		I will be working with the CMC reviewer during the review process to determine if there are any impurities and/or degradants present that may need to be qualified.
11	Has the applicant addressed any abuse potential issues in the submission?		X	Vortioxetine is an SSRI, 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. None at this time.

Antonia Dow, Ph.D. — November 13, 2012
Reviewing Pharmacologist — Date

Linda Fossom, Ph.D. — November 13, 2012
Team Leader/Supervisor — Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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/s/

ANTONIA L DOW
11/13/2012

LINDA H FOSSOM
11/13/2012