CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

212102Orig1s000

NON-CLINICAL REVIEW(S)

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration

Division of Neurology
Division of Pharmacology/Toxicology
Office of Neuroscience
Center for Drug Evaluation and Research

Date: June 22, 2020

From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 212-102 (fenfluramine)

NDA 212-102 was originally submitted by Zogenix, Inc. as a 505(b)(2) application on February 5, 2019, to support marketing approval of fenfluramine for the treatment of seizures associated with Dravet syndrome in patients 2 years of age and older. However, on February 25, 2019, the sponsor changed the application from a 505(b)(2) to a 505(b)(1) application. A Refuse-to-File (RTF) letter was issued on April 5, 2019, because of nonclinical and clinical deficiencies. The nonclinical deficiency was the lack of chronic toxicity studies of fenfluramine in rodent (6-month) and nonrodent (9-month).

Following the RTF action, the sponsor requested a Type A meeting, which was held on May 30, 2019. Regarding the nonclinical deficiency, the sponsor asked if the NDA were resubmitted as a 505(b)(2) application and published literature (e.g., Gilbert, 1971) were referenced, would the division agree that no new chronic toxicity studies would be needed. (b) (4) The division responded that, "upon further internal discussion, a decision has been made to consider the lack of chronic toxicity studies a review issue rather than a filing issue."

The NDA was resubmitted on September 25, 2019, as a 505(b)(2) application and filed on November 22, 2019.

Fenfluramine, approved in 1973 for exogenous obesity, was withdrawn from the market for reasons of safety or effectiveness in 2015 (FR Notice Vol 80, No. 188, September 29, 2015) following postmarketing reports of valvular heart disease and, therefore, cannot be used as a Listed Drug to support a (b)(2) application. The nonclinical support for NDA 212-102 consists of studies conducted by the sponsor, including pharmacology and PK/ADME studies, a 13-week GLP toxicity study in rat, a juvenile animal toxicology study in rat, and in vitro and in vivo

genetic toxicology assays. (b) (4)

The nonclinical studies were reviewed in detail by Dr. Fisher (Pharmacology/Toxicology NDA Review and Evaluation, NDA 212102, June 13, 2020). Based on the review, Dr. Fisher concluded that the application is "…considered acceptable from a nonclinical perspective." As described in Dr. Fisher's review, adverse effects in juvenile rats administered fenfluramine by oral gavage for 10 weeks, beginning on postnatal day 7, consisted of reductions in body weight, learning and memory deficits, and reductions in bone and brain size. A no-effect dose for adverse effects on postnatal development was not identified. Fenfluramine was negative in in vitro (Ames) and in vivo (rat micronucleus/comet) assays.

Dr. Fisher noted the sponsor submitted published literature to address the chronic toxicity of fenfluramine; none of these were sufficient or available to the sponsor to support the NDA. However, the clinical team has determined the available human safety data support approval of the NDA, with a black box warning and availability through a restricted program (FINTEPLA REMS). (b) (4)

Reproductive and developmental toxicology studies were not included in the original NDA submission or in the resubmission following the RTF. The Division had previously agreed to the sponsor's proposal to submit these (and carcinogenicity) studies post-approval (IND 125797, PreIND Written Responses, May 16, 2015). However, the sponsor submitted a standard battery of reproductive and developmental toxicology studies to the NDA on March 24, 2020. Because of the late submission, there was insufficient time to conduct a thorough review of the studies. Therefore, it is recommended that they be included as PMRs in the action letter, as well as PMRs for carcinogenicity studies in two species.

Recommendation

There is no objection to approval of the NDA, with appropriate labeling and PMRs for the reproductive and developmental toxicology studies and for the carcinogenicity studies in two species.

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/s/

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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:	212102
Supporting document:	011
Applicant's letter date:	9/25/2019 (resubmission after refusal to file)
CDER stamp date:	9/25/2019
Product:	ZX008 (fenfluramine hydrochloride)
Indication:	Dravet Syndrome
Applicant:	Zogenix
Review Division:	DN2
Reviewer:	Ed Fisher
Supervisor:	Lois Freed
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Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of N212102 are owned by Zogenix or are data for which Zogenix has obtained a written right of reference. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application are for descriptive purposes only and are not relied upon for approval of N212102.

Note: All figures and tables in this review were excerpted from the sponsor's submission

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1 Executive Summary

1.1 Discussion of Nonclinical Findings

An RTF letter was issued on April 5, 2019 after the initial NDA submission on February 5, 2019. Issues included the failure to submit “nonclinical studies to assess the potential for chronic administration of fenfluramine to result in novel or more severe toxicity compared to shorter duration studies.” Subsequently, the Division agreed at a Type A meeting on May 30, 2019 to accept the NDA as a 505(b)(2) application without including new chronic toxicity studies. According to the sponsor, the lack of chronic toxicity studies has been addressed in the resubmission (dated September 25, 2019) by the inclusion of 9 nonclinical literature studies describing the effects of fenfluramine and 5-HT2B agonists on heart valves in rats and mice. The Division determined that the adequacy of this information to support long-term safety would be a review issue.

Fenfluramine (FEN) is a racemic compound containing dexfenfluramine (d-FEN) and levofenfluramine (l-FEN). The mechanism of the anticonvulsant activity of FEN is not well-understood but is thought to involve, at least in part, its 5-HT agonist activity. Generally, agents that elevate 5-HT levels in the brain inhibit seizures, while depletion of 5-HT lowers seizure threshold. It is unclear which 5-HT receptor subtypes are involved. From a safety standpoint, concern has focused on activity at 5-HT2B receptors, since that receptor subtype seems to be most important for the valvulopathy associated with Pondimin (fenfluramine) that resulted in its being taken off the market. But for efficacy, 5-HT1D and 5-HT2C are thought to be most important. 5-HT2B receptors are primarily expressed in the periphery, although there are some in the CNS, while 5-HT2C receptors are found primarily in the CNS.

In literature studies, FEN decreased seizure activity in 2 zebrafish models of Dravet syndrome (Zhang 2015). In the scn1a mutant zebrafish model, administration of FEN or agonists of serotonin 5-HT1D, 5-HT2A, and 5-HT2C receptor subtypes reduced seizure activity, and FEN efficacy was reduced by 5-HT1D or 5-HT2C (but not 5-HT2A) antagonists or a sigma-1 agonist (Sourbron, 2016, 2017). d-FEN, nordexfenfluramine (d-NFEN, major metabolite), and S1RA (a sigma-1-receptor antagonist) reportedly reduced seizures in an NMDA-induced seizure model in CD-1 mice (Rodriguez-Munoz, 2018). A sigma-1 agonist and a serotonin 5-HT2A antagonist partially reversed the ability of d-FEN and d-NFEN to decrease seizure activity. The serotonin 5-HT2A antagonist did not reverse the effect of S1RA. These results were thought to support a mechanism involving sigma-1 receptor antagonism, in addition to 5-HT2C and 5-HT1D (and possibly 5-HT2A) agonism.

No safety pharmacology studies were conducted by the sponsor. Both FEN and NFEN have been shown to produce 5-HT-mediated neurotoxicity in numerous animal studies reported in the literature. They cause dose-related, long-lasting reductions in 5-HT axonal markers in all animal species tested and with all routes of administration used, at clinically-relevant doses (McCann et al., 1997). In a published cardiovascular safety pharmacology study in dogs (Franko, 1965), dose-related pressor effects (increased blood pressure,

heart rate, cardiac output, and myocardial contractility) were reported at single oral doses of 5 and 10 mg/kg (HED 2.7 and 5.6 mg/kg).

The sponsor relied primarily on literature for ADME information but conducted in vitro plasma protein binding and metabolite characterization studies. Following incubation of FEN with rat, dog, and human liver and intestinal S9 fractions, 7 metabolites were detected. These were formed by N-dealkylation, oxygenation, and dehydrogenation, or a combination thereof, with and without glucuronide conjugation. Norfenfluramine (NFEN) and its subsequent N−oxygenation product were the only metabolites detected in human liver S9 fractions, and NFEN was the only metabolite detected in the human intestinal S9 fractions. NFEN showed greater affinity and agonist activity at serotonin 5HT2 receptors than FEN. The metabolites detected in human liver and intestinal S9 fractions were observed in both rat and dog. Evidence of hydroxylation, dehydrogenation, and glucuronidation was observed in the rat and dog but not human test systems. No human-specific metabolites were detected. Both rat and dog S9 fractions showed good coverage of the human metabolites.

However, comparative in vivo metabolism data reported in the literature (Marchant et al., 1992) indicate significant species differences. In plasma, the major circulating radiolabeled compound following oral administration of [14C]-FEN (1 mg/kg) to humans was unchanged FEN, which accounted for approximately 24 and 38% of total drug-related material in plasma at 3 and 6 hr after dosing. NFEN accounted for 22 and 12% of the total at these time points. In addition, an unconjugated diol metabolite not detected at 3 hr reportedly accounted for 17% of total drug-related material in an 8-hr plasma sample. Other metabolites in plasma included 3 conjugation products. In rats administered a high SC dose of FEN (24 mg/kg BID) for 4 days, the 3-hr plasma sample contained 38% FEN, 33% NFEN, 13% collective conjugated metabolites, and 5% of the unconjugated diol.

The unconjugated diol metabolite was not measured in the nonclinical or clinical studies submitted in the initial (February 2, 2019) NDA. The sponsor estimated the amount of the diol in human and rat plasma based on its reported abundance relative to FEN in the published study and calculated a 3-4-fold exposure margin at the HD of 20 mg/kg in the 13-week rat toxicity study. The resubmission (September 25, 2019) included the results of a study in which the diol metabolite was quantified in plasma samples collected for a clinical QTc study conducted in healthy adult subjects administered 15 mg BID (MRHD) for 7 days. These data indicated that the diol accounted for less than 10% of total drug-related material (average 7.8% of sum of FEN, NFEN, and diol at steady state). Plasma diol concentrations measured in rats administered oral doses of 20 mg/kg FEN in this study were approximately equal to those in humans receiving the MRHD. Overall, the information provided does not suggest that humans would be exposed to significantly greater levels of the unconjugated diol metabolite compared to the maximum exposures seen in the rat toxicity studies.

The toxicology package consisted of 5 studies conducted by the sponsor: a 13-week oral (gavage) toxicity study in adult rats ((b) (4) study no. 8001991), oral (gavage) dose range-finding and 10-week definitive toxicity studies in juvenile rats ((b) (4) study nos. 9000468 and 9000406), an Ames assay ((b) (4) study no. 9601196), and a

combined in vivo bone marrow micronucleus test/comet assay in rat ((b) (4) study no. 9800312). TK analyses were included in the adult and juvenile rat toxicity studies.

In the 13-week toxicity study in adult rats, daily oral (gavage) administration of FEN (0 (water vehicle), 3.5, 5, 8, 13, or 20 mg/day; 10 mL/kg) for 91 days resulted in decreased body weight and microscopic findings in the nasal cavity, liver, and epididymis. Dose-dependent decreases in body weight (BW) and BW gain were noted at all doses. Cytoplasmic vacuolation of the nasal cavity olfactory epithelium was seen at ≥3.5 mg/kg/day and dose-related increases in the severity of eosinophilic globules in the olfactory epithelium were observed at all doses. Centrilobular hepatocellular hypertrophy was seen at ≥ 5 mg/kg/day, with dose-related increases in incidence and severity. Microvesicular vacuolation of the epithelium of the epididymis was observed at ≥ 13 mg/kg/day. There was no evidence of drug-related neuronal or axonal degeneration or necrosis or myelin loss in the brain and spinal cord following evaluation with H&E, Kluver Barrera, and Holmes (silver) stains. Mitral and aortic valves were present in all sections of heart examined. There were no drug-related microscopic changes in cardiac valves. The lowest effect dose (3.5 mg/kg) was associated with Day 89 plasma FEN exposures (AUC) of 683 and 1880 ng.h/mL and NFEN exposures of 2790 and 3830 ng.h/mL in males and females, respectively.

In the pivotal juvenile rat toxicity study, FEN (0 (water vehicle), 3.5, 9, or 20 mg/day) was administered daily by oral gavage for 10 weeks, from PND 7 to 76. Drug-related mortality was seen at the HD (4 animals either found dead or euthanized moribund during the pre- and postweaning dosing period). Clinical signs included tremor and uncoordination at the MD and HD and abnormal gait, decreased activity, hyperreactivity, dehydration, and salivation at all doses. Food consumption, BW gain, and BW were dose-dependently decreased in all treatment groups during the dosing period, but BWs were similar among groups at the end of the recovery period. Neurobehavioral changes consisting of decreased locomotor activity and learning and memory deficits were seen in both sexes at all doses at the end of the treatment period and remained, although to a lesser extent, after the recovery period, indicating long-term impairment. There were no clear drug-related effects on reproductive performance. At the end of the dosing period, dose-dependent decreases in bone and brain measurements were seen in both sexes. Partial or complete recovery was seen for these endpoints. Histopathology findings were limited to eosinophilic globules in the olfactory epithelium at all doses. No changes were seen in aortic or mitral cardiac valves. The LOAEL of 3.5 mg/kg/day was associated with PND 76 plasma AUCs of 662 and 764 ng.h/mL for FEN and 1930 and 2400 ng.h/mL for NFEN in males and females, respectively.

The developmental neurotoxicity of FEN observed in the juvenile rat study is in general agreement with the neurobehavioral impairment, including learning and memory deficits, that has been reported in the literature. Following exposure of neonatal rats to d-FEN, spatial learning and memory deficits in the Morris maze and sequential learning deficits in the Cincinnati water maze were observed at doses ≥ 20 mg/kg/day (Morford et al., 2002). The authors remarked on the severity of the d-FEN-induced learning impairments, noting that even after 24 trials during each test phase of the Morris maze, the treated

animals never reached control levels of performance. This performance deficit was said to be more severe than that previously seen with methamphetamine or MDMA in the same lab.

FEN was negative for genotoxicity in an in vitro Ames test and a combined in vivo bone marrow micronucleus test/comet assay in rat. Carcinogenicity studies of FEN have not been conducted by the sponsor or reported in the literature.

The sponsor referenced literature for additional nonclinical safety support. A single paper describing the nonclinical studies conducted by A.H. Robins (Gilbert et al.,1971) for their FEN products (Pondimin tablets, Ponderex capsules, NDA16-618) was the primary literature source for general and reproductive/developmental toxicity information. These included 5-week, 27-week, and 85-week toxicity studies in rats, a 55-week toxicity study in dogs, and reproductive/developmental toxicity studies in mouse, rat, rabbit, and monkey. The publication is brief, and the studies are not described in detail; therefore, it is inadequate to rely on for the safety assessment. The studies predate GLP requirements, the number of animals per group was smaller than currently expected, and dietary dosing was used in the repeated-dose toxicity studies in rats. According to the publication, oral doses up to 24 and 20 mg/kg/day were well-tolerated in rats and dogs, respectively. Dose-dependent decreases in activity, food consumption, and body weight gain were reported in both species. Bradycardia and decreased blood pressure were observed in dogs. There were no histopathological findings reported in the general toxicity studies. The reproductive and developmental toxicity study results were not clearly described, but no teratogenic effects were reported.

The re-submitted NDA also included publications of studies examining cardiotoxicity in animal models. These studies using a variety of nonclinical models used for predicting the human response, including in vitro binding and functional assays, valvulopathy effects assessed by echocardiographic measurements and valve histology in rats chronically treated with 5-HT or other drugs active at the 5-HT2B receptor, indicate that exposures to FEN and its major metabolite NFEN present a significant risk to humans, supporting the clinical evidence. The failure to observe heart valve effects in the 3-month general toxicity and 10-week juvenile animal studies in rats conducted for this application could be due to inadequate doses or duration of dosing in adult rats, differences in response between adult and juvenile rats, or the species difference in metabolism.

1.2 Recommendations

This application would normally be considered not approvable from a pharmacology/toxicology standpoint due to the absence of important safety information, i.e., reproductive/developmental toxicity and carcinogenic potential. However, given the serious indication of Dravet syndrome, the Division agreed that these studies could be conducted postmarketing, provided no new safety issues arose during the review of the available nonclinical or clinical data (PIND 125797 WRO dated May 16, 2015). This is now considered acceptable from a nonclinical perspective. The significant risks for valvular heart disease and adult and developmental neurotoxicity should be described in labeling.

2 Drug Information

2.1 Drug

CAS Registry Number	404-82-0 (HCl Salt)
Generic Name	Fenfluramine hydrochloride (ZX008)
Chemical Name	N-ethyl-α-methyl-3-(trifluoromethyl)phenethylamine hydrochloride
Molecular Formula/Molecular Weight	$C_{12}H_{16}F_3N$ • HCl/267.72 g/mol
Pharmacologic Class	amphetamine derivative, sympathomimetic stimulant

2.2 Relevant IND

125797

2.3 Proposed Clinical Population and Dosing Regimen

ZX008, an oral solution of fenfluramine hydrochloride, is intended for the treatment of seizures associated with Dravet syndrome in patients 2 years of age and older. The proposed clinical doses are between 0.2 to (b) (4) mg/kg/day administered orally in 2 divided doses (BID), with a maximum total daily dose of (b) (4) mg, regardless of weight.

3 Studies Reviewed

Pharmacology
- In vitro binding assays
- Primary and secondary pharmacology literature
- Safety pharmacology literature

Pharmacokinetics
- In vitro metabolism
- Comparative plasma diol metabolite levels
- Toxicokinetics in rat studies
- ADME literature

Repeat-Dose General Toxicity
- 13-week oral toxicity study in adult rat
- General toxicity literature
- Cardiovascular toxicity literature

Genotoxicity
- In vitro and in vivo assays

Reproductive and Developmental Toxicity
- 10-week oral juvenile development study in rat
- Reproductive and developmental toxicity literature

4 Pharmacology

4.1 Primary Pharmacology

FEN is a racemic mixture of 2 enantiomers ((+)-FEN or d-FEN and (-)-FEN or l-FEN). FEN is thought to reduce seizures in Dravet syndrome by releasing serotonin, by acting as an agonist at the 5-HT1D and 5-HT2C receptors, and by acting on the sigma-1 receptor. FEN may also exert anti-seizure activity through the 5-HT1A and 5-HT2A receptors. FEN is a potent 5-HT (serotonin) releaser. Serotonin is able to activate multiple 5-HT receptor subtypes, of which 14 have been described in humans. The N-dealkylated metabolite, norfenfluramine (NFEN), displays high affinity and activity at the 5-HT2B and 5-HT2C receptor subtypes. It is unknown which 5-HT receptor subtypes are involved in the anti-epileptic effect of FEN.

In a receptor binding assay (Study Report XS-0691), the receptors and ion channels that showed moderate to strong binding (defined as an inhibition ratio greater than 30%) were the β-adrenergic receptor (non-selective), the β2 adrenergic receptor, the muscarinic M1 receptor, the sodium ion channel (non-selective for specific subunits), the serotonin 5-HT1A receptor, and the sigma receptor. No other serotonin receptors were tested in this assay. Ki values for these six receptors (Table 1) show a similar pattern, with Ki's for the 5-HT1A receptor and the sigma receptor having the greatest competitive inhibition.

Table 1. Binding of Racemic Fenfluramine and Norfenfluramine

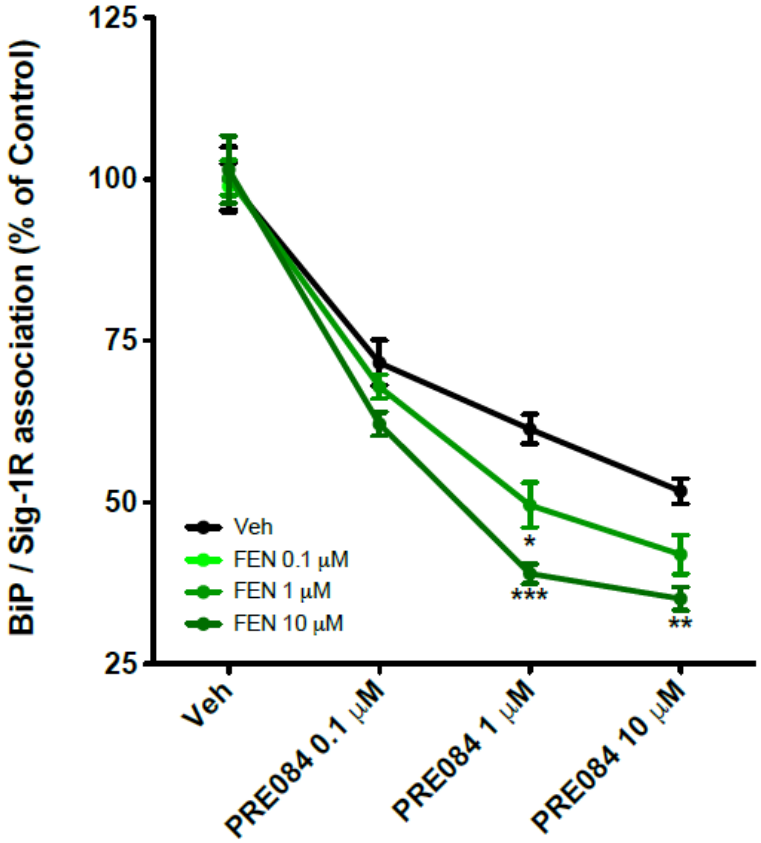
Assay System	Substance	Ki (mol/L)
β-Adrenergic (Non-selective) (Rat brain)	(±)-fenfluramine	1.75×10^{-5}
	(±)-norfenfluramine	1.20×10^{-5}
	(±)-Propranolol*	3.90×10^{-9}
β2-Adrenergic (Human recombinant)	(±)-fenfluramine	1.26×10^{-5}
	(±)-norfenfluramine	8.77×10^{-6}
	(±)-Propranolol*	5.12×10^{-10}
Muscarinic M1 (Rat cerebral cortex)	(±)-fenfluramine	1.13×10^{-5}
	(±)-norfenfluramine	3.74×10^{-6}
	Atropine*	9.80×10^{-10}
Na channel (Rat brain)	(±)-fenfluramine	4.84×10^{-6}
	(±)-norfenfluramine	4.74×10^{-6}
	Dibucaine*	1.19×10^{-7}
Serotonin 5-HT1A (Rat cerebral cortex)	(±)-fenfluramine	3.27×10^{-7}
	(±)-norfenfluramine	6.73×10^{-7}
	Serotonin*	1.01×10^{-9}
Sigma Non-selective (Guinea pig brain)	(±)-fenfluramine	2.66×10^{-7}
	(±)-norfenfluramine	2.92×10^{-6}
	Haloperidol*	1.31×10^{-9}

*=positive control

In an in vitro functional assay examining the agonist or antagonist activity of racemic FEN and NFEN and their enantiomers on the beta-1 adrenergic, beta-2 adrenergic, muscarinic M1, 5-HT1A and nonspecific sigma receptors (Study Report 100026029), no agonist activity was found at any of the receptors tested. Racemic FEN and both of its enantiomers acted as antagonists at the beta-2 receptor only at concentrations (49 to 64 µM) an order of magnitude greater than those expected clinically (estimated to be 2 to 8 µM). The same was true for d-FEN, which acted as an antagonist at the muscarinic M1 receptor at a concentration of 83 µM, racemic NFEN and l-NFEN which acted as antagonists of the beta-2 adrenergic receptor in the concentration range of 67-70 µM, and l-NFEN, which acted as an antagonist at the muscarinic M1 receptor at a concentration of 95 µM.

In an assay examining the effect of FEN on the ability of the sigma-1 receptor agonist PRE084 to dissociate the sigma-1 receptor chaperone – HSP5A (BiP) complex in CHO cells in vitro (Study Reports AM334 and AM335), FEN was a positive allosteric modulator of PRE084 at concentrations of 1 uM or greater (Figure 1) but was without effect by itself.

Figure 1. Effect of Fenfluramine on PRE084-induced BiP–Sig-1R dissociation



When racemic FEN and NFEN and the corresponding d- and l-isomers were evaluated for sodium channel activity (Study Report ZOG121515-1), l-FEN, racemic NFEN, and its enantiomers altered activity only at the hNav1.5 channel, with IC50s in the range of 21.9 to 39.2 µM.

In a study reported in the literature (Rothman et al., Circulation. 2000 Dec 5;102(23):2836-41), FEN [(+/-)-, (+)-, (-)-FEN] and its major active metabolite NFEN [(+/-)-, (+)-, (-)-NFEN] were screened for activity at 11 cloned serotonin receptor subtypes by use of ligand-binding methods and functional assays. FENs and NFENs (racemates and enantiomers) had Ki values ranging from 673 to 1950 nmol/L and lacked agonist activity at the 5-HT1A receptor. Both had very low affinity for the 5-HT1D, 5-HT1B, h5HT1D/1B, and 5-HT1E receptors. FENs had micromolar affinity for the 5-HT2A receptor. NFENs were moderately potent at the 5-HT2C receptor and were full agonists. FENs were also full agonists at the 5-HT2C receptor but were significantly less potent than the NFENs. The NFENs had high affinity (10 to 50 nmol/L) for the 5-HT2B receptor, and functional studies demonstrated that they were full agonists at the 5-HT2B receptor. The FENs, in contrast, bound to the 5-HT2B receptor, with Ki values of 5 µmol/L. Both the FENs and NFENs were inactive at the 5-HT5 and 5-HT6 receptors but had moderate affinity at the 5-HT7 receptor. The pathogenesis of valvulopathy is thought to result from activation of 5-HT2B receptors expressed on heart valve leaflets, while the anorectic effect appears to be primarily due to stimulation of central 5-HT2C receptors.

4.2 Secondary Pharmacology

Secondary pharmacology other than receptor binding (above) was not investigated by the sponsor.

4.3 Safety Pharmacology

Safety pharmacology studies were not conducted by the sponsor. Both FEN and NFEN have been shown to produce 5-HT-mediated neurotoxicity in numerous animal studies reported in the literature. They have been reported to cause dose-related, long-lasting reductions in 5-HT axonal markers in all animal species tested and with all routes of administration used, at clinically-relevant doses (McCann et al., JAMA, 1997;278(8):666-672).

In a CV study conducted by Robins and reported in the literature (Franko et al., J Pharm Pharmacol, 1965;17:222-226), FEN (1, 2, 4, 8, or 16 mg/kg iv and 5 or 10 mg/kg po; HEDs 0.6, 1.1, 2.2, 4.4, or 8.8 mg/kg, and 2.7 or 5.6 mg/kg) administered orally or iv to dogs increased arterial blood pressure, heart rate, myocardial contractile force, cardiac output, and total peripheral resistance at all doses. FEN was said to be qualitatively like dexamphetamine in its cardiovascular effects, but 10 to 20 times less potent as a pressor agent.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Comparative PK data from published studies in which racemic FEN (Caccia) or l-FEN (Spinelli) were orally administered are shown in Table 2. TK data are included under toxicology.

Table 2. Comparative PK and exposure data in mice, rats, dogs, and humans

Species (Formulation)	Dose mg/kg	dexfenfluramine	levofenfluramine	dexnorfenfluramine	levonorfenfluramine	Reference
T_{max} (hr)						
Male CD1-COBS mice (formulation not described)	20 mg/kg PO	0.25	0.25	4.0	4.0	Caccia 1982
Male CD-COBS Sprague-Dawley rats levofenfluramine in water	2.5 mg/kg PO	NA	0.3±0.1	NA	2.0±1.0	Spinelli 1988 (Table 2)
Male CD-COBS Sprague-Dawley rats (formulation not described)	5 mg/kg PO	0.5	0.25	4.0	2.0	Caccia 1982
Male CD-COBS Sprague-Dawley rats levofenfluramine in water	6.25mg/kg PO	NA	0.6±0.3	NA	4.0±0.4	Spinelli 1988 (Table 2)
Male Beagle dogs T_{max} taken from Table 3 in Caccia 1982	20 mg/kg PO	3.0	3.0	4.0	4.0	Caccia 1982 (Table III)
Healthy adult humans T_{max} taken from Table 4 in Caccia 1982	40 mg PO (~0.67 mg/kg)	4.0	4.0	8.0	8.0	Caccia 1982 (Table IV)
Cmax (µg/mL) ± SEM						
Male CD1-COBS mice (formulation not described)	20 mg/kg PO	0.27 ± 0.04	0.26 ± 0.05	0.03 ± 0.00	0.05 ± 0.00	Caccia 1982 (Table V)
Male CD-COBS Sprague-Dawley rats levofenfluramine in water	2.5 mg/kg PO	NA	0.05±0.02 *	NA	0.019±0.02 *	Spinelli 1988 (Table 2)
Male CD-COBS Sprague-Dawley rats (formulation not described)	5 mg/kg PO	0.06 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.07 ± 0.01	Caccia 1982 (Table V)
Male CD-COBS Sprague-Dawley rats levofenfluramine in water	6.25mg/kg PO	NA	0.12±0.07 *	NA	0.32±0.05 *	Spinelli 1988 (Table 2)
Male Beagle dogs (formulation not described)	20 mg/kg PO	0.30 ± 0.02	0.30 ± 0.02	0.28 ± 0.01	0.29 ± 0.01	Caccia 1982 (Table V)
Healthy adult humans	40 mg PO (~0.67 mg/kg)	0.018 ± 0.002	0.018 ± 0.002	0.006 ± 0.001	0.006 ± 0.001	Caccia 1982 (Table V)
AUC (µg/mL x hr) ± SEM						

Male CD1-COBS mice (formulation not described)	20 mg/kg PO	1.39	1.10	0.45	0.79	Caccia 1982 (Table V)
Male CD-COBS Sprague-Dawley rats levofenfluramine in water	2.5 mg/kg PO	NA	0.06±0.15 **	NA	2.88±0.03 **	Spinelli 1988 (Table 2)
Male CD-COBS Sprague-Dawley rats (formulation not described)	5 mg/kg PO	0.39	0.08	0.80	1.28	Caccia 1982 (Table V)
Male CD-COBS Sprague-Dawley rats levofenfluramine in water	6.25mg/kg PO	NA	0.20±0.07 **	NA	6.51±1.41 **	Spinelli 1988 (Table 2)
Male Beagle dogs	20 mg/kg PO	1.37 ± 0.08	1.49 ± 0.12	6.03 ± 0.80	7.61 ± 1.22	Caccia 1982 (Table V)
Healthy adult humans	40 mg PO (~0.67 mg/kg)	0.43 ± 0.04	0.43 ± 0.04	0.33 ± 0.04	0.34 ± 0.04	Caccia 1982 (Table V)
$T_{1/2}$ (hr)±SEM						
Male CD1-COBS mice (formulation not described)	20 mg/kg PO	4.3	3.7	7.7	7.7	Caccia 1982 (Table V)
Male CD-COBS Sprague-Dawley rats levofenfluramine in water	2.5 mg/kg PO	NA	0.9±0.3	NA	11.1±1.3	Spinelli 1988 (Table 2)
Male CD-COBS Sprague-Dawley rats (formulation not described)	5 mg/kg PO	2.6	1.1	12.0	12.6	Caccia 1982 (Table V)
Male CD-COBS Sprague-Dawley rats levofenfluramine in water	6.25mg/kg PO	NA	1.1±0.2	NA	11.9±3.2	Spinelli 1988 (Table 2)
Male Beagle dogs	20 mg/kg PO	2.5 ± 0.2	2.7 ± 0.4	12.8 ± 2.2	14.9 ± 2.0	Caccia 1982 (Table V)
Healthy adult humans	40 mg PO (~0.67 mg/kg)	17.8 ± 0.9	18.4 ± 0.9	31.7 ± 1.4	33.1 ± 1.7	Caccia 1982 (Table V)

NA = Not Applicable; data not reported in the publication
* Spinelli 1988 reported plasma concentrations in nmol/mL, these were converted to µg/mL to enable comparison with other results from the literature.
** Spinelli 1988 reported AUC in nmol/mL·min, these were converted to µg/mL·hr to enable comparison with other results from the literature.

Absorption

The sponsor conducted an in vitro study of the bidirectional permeability of FEN and NFEN across Caco-2 cells (Protocol XT158035). Efflux of both FEN and NFEN was less than 2, indicating that they are not actively transported by the P-gp transporter. The apparent permeability (Papp) of both in comparison to known high and low permeable compounds indicated that they can be classified as highly permeable compounds.

Distribution

In an in vitro protein binding assay in rat, dog, and human plasma conducted by the sponsor (Protocol XS-0688), FEN and NFEN exhibited approximately 50% plasma protein binding across species.

Literature studies indicated that FEN and NFEN distribute into rat brain tissue at concentrations 15 to 60-fold greater than those in plasma. Within the brain, both were reportedly found in the highest concentrations in the cortex, hippocampus and striatum. The highest tissue levels of fenfluramine were found in the gastrointestinal tract.

Metabolism

In an in vitro metabolism study conducted by the sponsor (Study Report XT154063), NFEN and its subsequent N−oxygenation product (C2) were the only metabolites detected in human liver S9 fractions. Evidence of hydroxylation, dehydrogenation, and glucuronidation was observed in the rat and dog. NFEN and 6 other metabolites (C1-C6) were found in rat liver S9, while NFEN and 5 other metabolites (C2-C6) were found in

dog liver. No human-specific metabolites were detected. Both rat and dog S9 fractions showed good coverage of the human FEN metabolites.

However, the comparative in vivo metabolism of FEN in mouse, rat, dog, and humans (2 healthy male volunteers), as reported in the literature (Marchant et al., 1992), indicates that there are significant species differences. In plasma, the major circulating radiolabeled product following oral administration of [14C]-FEN (1 mg/kg) to humans was unchanged FEN, which accounted for approximately 24 and 38% of total drug-related material in plasma at 3 and 6 hr after dosing. NFEN accounted for 22 and 12% of the total at these time points. In addition, an unconjugated diol metabolite not detected at 3 hr reportedly accounted for 17% of total drug-related material in an 8-hr plasma sample. Other metabolites in plasma included 3 conjugation products. Eleven metabolites were found in mouse plasma, which included all the metabolites found in rat, dog, and human in addition to 2 that appeared to be specific to mice. The 3-hr plasma sample from rats given 24 mg/kg FEN sc for 4 days contained 38% FEN, 33% NFEN, 13% collective conjugated metabolites, and 5% unconjugated diol. Dog plasma contained 14% diol glucuronide and very small quantities of FEN and NFEN (4% and 1%, respectively) in addition to 3 other metabolites (1 conjugate at 32%) not produced by humans.

The unconjugated diol metabolite was not measured in nonclinical or clinical studies submitted with the initial (2/5/19) NDA. The sponsor attempted to estimate the amount of the diol in human and rat plasma based on its reported abundance relative to FEN (Table 3). The rat FEN exposure used in the calculation was the level measured at the HD of 20 mg/kg in the 13-week toxicity study. It is not clear where the human FEN exposure came from, but the AUC used is lower than that measured in clinical study ZX008-1505 at a dose of 0.8 mg/kg, i.e., 1600 ng.h/mL (corresponding NFEN: 779 ng.h/mL).

Table 3. Calculated exposures to diol metabolite in rat and human

Row	Parameter	Rat	Human	Source
A	Fenfluramine (% of total)	38	38	Marchant 1992
B	Diol metabolite (% of total)	5	17	Study Report 8001991
C	Diol metabolite (% of fenfluramine)	13%	45%	Row B divided by Row A
D	Mean Fenfluramine AUC, ng*h/mL (male and female rat NOAEL [on Study Day 89, following an 89-day continuous dosing]and human clinical dose)	15150	1110	Study Report 8001991 and Section 2.7.2
E	Inferred diol AUC, ng*h/mL (rat NOAEL and human clinical dose)	1990	500	Row C times Row D
F	Margin of exposure for diol metabolite (rat/human)	4-fold		-

The resubmission (9/25/2019) included the results of a study in which the diol metabolite was quantified in plasma samples collected for a clinical QTc study conducted in healthy adult subjects administered 15 mg BID (MRHD) for 7 days (ZX008-1603; Table 4). According to the sponsor, these data indicate that the diol accounted for less than 10% of drug-related material (average 7.8% of sum of FEN, NFEN, and diol at steady state)

and, thus, should not be considered a metabolite requiring nonclinical characterization based on the ICH M3(R2) guideline (January 2010) and CDER guidance on Safety Testing of Drug Metabolites (March 2020). But it is not clear that the average of diol metabolite concentrations determined at 3 time points after dose administration during steady state is an adequate measure of metabolite exposure. The guidance refers to drug metabolites of toxicological concern for which nonclinical characterization is warranted as those present at greater than 10% of total drug-related exposure, where exposure is generally understood to mean AUC, and for which exposure would be expected to be significantly greater in humans than the maximum exposure seen in the toxicity studies.

Table 4. Levels of FEN, NFEN, and diol metabolite, and diol % of sum at steady state

Time Point	Subject	Concentration (ng/mL) at Time Point			Maximum Possible Diol Level (% of Total Drug-Related Material)
		Fenfluramine	Norfenfluramine	Diol	
1 hour	3	31.1	39.4	4.24	5.7%
	11	33.5	15.7	3.04	5.8%
	16	42.5	26.6	7.63	9.9%
	18	42	16.7	5.96	9.2%
	19	40.8	14.3	7.45	11.9%
	24	20.2	12.5	2.54	7.2%
	26	30.9	23	5.37	9.1%
	30	31.3	17.6	7.82	13.8%
4 hour	3	44.3	40.1	6.15	6.8%
	11	42.6	16.7	3.95	6.2%
	16	56.5	28.6	7.62	8.2%
	18	52.1	17.4	6.87	9.0%
	19	46.6	15.2	7.75	11.1%
	24	27.9	13.4	2.61	5.9%
	26	39.4	23.8	4.78	7.0%
	30	40.3	18.5	6.1	9.4%
8 hour	3	38.8	41.5	3.62	4.3%
	11	46.3	19	3.72	5.4%
	16	52.6	29.3	5.67	6.5%
	18	49.8	18.7	5.77	7.8%
	19	48.9	16.6	6.81	9.4%
	24	30.7	16.4	1.85	3.8%
	26	38.6	27.5	3.16	4.6%
	30	36.7	18.9	5.51	9.0%
Overall Averages				**5.25**	**7.8%**

Source: ZX008-1603 CSR for fenfluramine and norfenfluramine, Appendix 1 of this report for the diol.

Plasma diol concentrations (pooled samples said to reflect the AUC0-24h) measured in rats administered oral doses of 20 mg/kg FEN in this study were approximately equal to those in humans receiving the MRHD (Table 5). Again, the guidance states that comparison between human and animal exposure is generally based on AUC. However, taken together, the information provided does not suggest that humans would be exposed to significantly greater levels of the unconjugated diol metabolite compared to the maximum exposures seen in the rat toxicity studies.

Table 5. Levels of diol metabolite in rat, dog, and human plasma

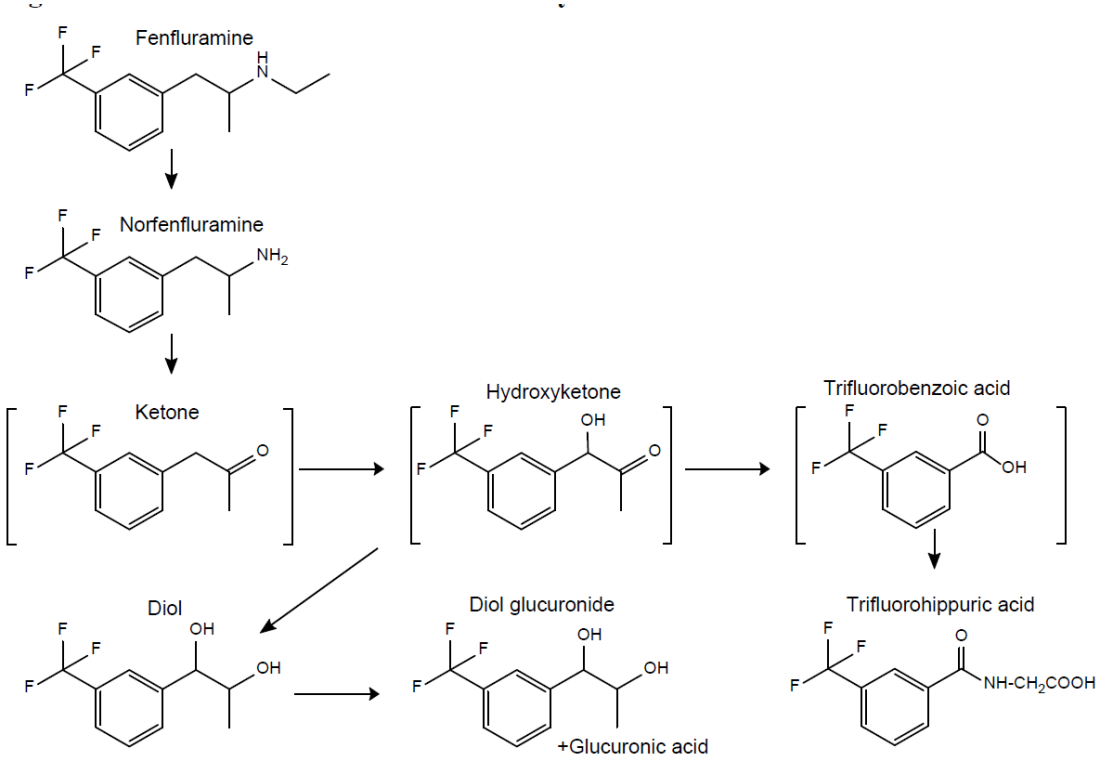
Sex/Species	Dose	Plasma Concentration (ng/mL)					Animal/ Human Ratio
		Animal Plasma				Human Plasma Mean	
		Replicate			Mean		
Male Rats	20 mg/kg/day	7.32	6.95	6.63	6.97	5.25	1.33
Female Rats		6.14	6.16	6.39	6.23		1.19
Male Dogs	20/10 mg/kg/day*	19.3	20.7	22.2	20.7		3.95

Source: Appendix 1

* Animals received 20 mg/kg/day fenfluramine HCl on Day 1 and 10 mg/kg/day fenfluramine HCl on Days 2, 3 and 4. Samples obtained on Day 4

Based on a study of human urinary metabolism in which the diol was identified as a major breakdown product (Brownsill et al.,1991), a metabolic pathway was proposed (Figure 1).

Figure 1. Proposed FEN biotransformation pathways in humans (Brownsill et al., 1991)



Excretion

Based on information reported in the literature (Marchant et al., 1992), the primary route of excretion of radioactivity in mice, rats, dogs, and humans following a single oral dose of [14C]-FEN (1 mg/kg) was in urine (>80%), with small amounts found in the feces.

6 General Toxicology

6.1 Repeat-Dose Toxicity

Study title: A 13-Week Oral Gavage Study of Fenfluramine Hydrochloride in the Rats

Study no.:	8001991
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	05 Apr 2017
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	97343-04, 100.1%

Key Study Findings

Daily oral (gavage) administration of FEN (0 (water vehicle), 3.5, 5, 8, 13, or 20 mg/day; 10 mL/kg) to SD rats for 91 days resulted in decreased body weight and microscopic findings in the nasal cavity, liver, and epididymis. Dose-dependent decreases in body weight (BW) and BW gain were noted at all doses. Cytoplasmic vacuolation of the nasal cavity olfactory epithelium was seen at ≥ 3.5 mg/kg/day, and dose-related increases in the severity of eosinophilic globules in the olfactory epithelium were observed at all doses. Centrilobular hepatocellular hypertrophy was seen at ≥ 5 mg/kg/day, with dose-related increases in incidence and severity. Microvesicular vacuolation of the epithelium of the epididymis was observed at ≥ 13 mg/kg/day. There was no evidence of drug-related neuronal or axonal degeneration and necrosis or myelin loss in the brain and spinal cord following evaluation of H&E, Kluver Barrera, and Holmes (silver) stains. Mitral and aortic valves were present on all sections of heart examined. There were no drug-related microscopic changes in cardiac valves.

Methods

Doses:	0 (vehicle), 3.5, 5, 8, 13, or 20 mg/kg/day
Frequency of dosing:	QD
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	ultra pure water
Species/Strain:	Rat / Crl:CD(SD)
Number/Sex/Group:	15/sex/group
Age:	6 Weeks
Weight:	M: 182-265 gm, F: 145 to 208 g
Satellite groups:	3-9/sex/grp TK
Unique study design:	None
Deviation from study protocol:	None that impacted study quality or integrity

The following parameters were evaluated: clinical signs, body weights, food consumption, ophthalmology, clinical pathology parameters (hematology, coagulation, clinical

chemistry, and urinalysis), toxicokinetic parameters (parent and metabolite desethyl fenfluramine), gross necropsy, organ weights, and histopathologic examinations. Necropsy was conducted on Day 92, one day following the last day of dosing. The histopathology examination of a comprehensive list of tissues was conducted in C and HD rats; target tissues and gross lesions were examined in the lower dose groups. For examination of the brain, eight levels (olfactory bulb, rostral forebrain, forebrain, thalamus, midbrain 1, midbrain 2, cerebellum/pons, and medulla/cerebellum) were stained with standard H&E, as well as Kluver Barrera and Holmes (silver) stains. Microscopic evaluation of the heart included aortic and mitral valves.

Gr. No.	Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Animals			
					Main Study		Toxicokinetic	
					Males	Females	Males	Females
1	Reference Item	0	10	0	15	15	3	3
2	Fenfluramine hydrochloride	3.5		0.35	15	15	9	9
3	Fenfluramine hydrochloride	5.0		0.5	15	15	9	9
4	Fenfluramine hydrochloride	8.0		0.8	15	15	9	9
5	Fenfluramine hydrochloride	13		1.3	15	15	9	9
6	Fenfluramine hydrochloride	20		2.0	15	15	9	9

Dose selection was based on the results of a dose-range finding study in juvenile rats (Study (b) (4)9000468), a 10-week study in juvenile rats (Study (b) (4)9000406), and a micronucleus study in adult rats (Study (b) (4)9800312). In these studies, orally-administered doses ranged from 3.5 to 100 mg/kg/day.

Observations and Results

Mortality

There were no unscheduled deaths during the course of the study.

Clinical Signs

An increased incidence of red staining on the fur (mostly at the muzzle, cranium, periorbital and/or lower jaw) was observed from Week 6 through the end of the study at ≥ 13 mg/kg/day. One HD male was noted intermittently with non-sustained convulsions, hyperreactivity, hypersensitivity, erected fur, partly closed eyes and salivation, at the start of Week 12 then continuing through the end of Week 13. The condition of the animal was relatively stable until the scheduled euthanasia (these clinical signs were noted occasionally), and the animal gained weight similarly to others from the same group. These clinical signs were consistent with serotonergic activity.

Body Weights

Dose-dependent decreases in body weight (BW) and BW gain were noted at all doses, starting the first week of dosing and reaching statistical significance on most occasions thereafter (Table 1).

Table 1. Percent mean cumulative (Days -1 – 91) BW gain reduction compared to control.

Group	2		3		4		5		6	
Dose (mg/kg/day)	3.5		5.0		8.0		13		20	
Sex	M	F	M	F	M	F	M	F	M	F
Body weight gain (% reduction of controls)	**17**	15	15	**23**	**21**	**25**	**26**	**26**	**30**	**32**

Numerical values indicate fold changes of the Test Item-treated mean group value relative to the Reference Item group mean value. Bolded values are statistically significant at $p \leq 0.05$.

Food Consumption

Food consumption was dose-dependently decreased at all doses compared to C (7-36% in males and 1-29% in females).

Hematology

On Day 92, there were mild increases in differential white cell counts at all doses and in platelets at the 2 highest doses. These changes were indicative of chronic inflammation but had no microscopic correlate.

Clinical Chemistry

On Day 92, there were slight increases in ALT in both sexes (but not D-R in females) at all doses and in ALP in males at ≥ 8 mg/kg/day (Table 2), correlating with hepatocyte hypertrophy seen histopathologically at all but the LD (Table 3). There were also slight increases in urea (males at ≥ 13 mg/kg/day, females at ≥ 8 mg/kg/day) and creatinine (males at ≥ 13 mg/kg/day). These changes had no microscopic correlate and were thought to reflect dehydration.

Table 2. Clinical Chemistry Changes - Day 92

Group	2		3		4		5		6	
Dose (mg/kg/day)	3.5		5.0		8.0		13		20	
Sex	M	F	M	F	M	F	M	F	M	F
ALT	**1.32**	**1.40**	**1.39**	**1.87**	**1.52**	**1.57**	**1.58**	**1.47**	**1.70**	1.27
ALP	–	–	–	1.38	**1.30**	1.26	**1.28**	1.26	**1.36**	–
UREAN	–	–	–	–	–	1.21	**1.24**	1.13	**1.46**	1.17
CREAT	–	–	–	–	–	–	**1.16**	**–**	**1.18**	–
CHOL	–	–	–	–	–	–	–	1.15	–	1.19
TPROT	–	–	–	–	**1.05**	–	**1.06**	–	**1.06**	–
GLOB	–	–	–	–	1.09	–	**1.10**	–	**1.12**	–
CL	–	–	–	–	–	–	**0.98**	**0.98**	0.98	**0.98**

M = Males F = Females
A dash (—) indicates absence of change. Numerical values indicate fold changes of the Test Item-treated group mean value relative to the Reference Item group mean value.
ALT= alanine aminotransferase, ALP= alkaline phosphatase, UREAN= urea, CREAT=creatinine, TPROT= total protein, GLOB= globulin, CHOL= cholesterol, CL=chloride.
Bolded values are statistically significant at $p \leq 0.05$.

Urinalysis

There were no drug-related changes in urinalysis parameters.

Gross Pathology

There were no drug-related macroscopic observations.

Organ Weights

Liver weight relative to body weight was increased (SS) in HD females.

Histopathology

Vacuolation of the nasal cavity olfactory epithelium was seen at all doses in both sexes (Table 3). According to the report, “these changes were characterized by one to several discrete, large, round, colorless cytoplasmic vacuoles that displaced the nucleus. Eosinophilic globules in sustentacular cells of the olfactory epithelium were also noted at all doses in both sexes. This change was said to be “characterized by intracytoplasmic subnuclear and/or, less frequently, supranuclear accumulation of brightly eosinophilic, homogenous, globular material with mild distortion of the cells.” According to a literature reference provided by the sponsor (Harkema, 1990), “eosinophilic globules in the sustentacular cells of the olfactory epithelium are occasionally seen in the nasal cavity of rodents and can be exacerbated by chemical agents, possibly associated with enzyme induction associated with compound metabolism.”

Microvesicular vacuolation of the epididymis was seen in males at ≥ 13 mg/kg/day. This finding had not been reported previously in studies of FEN. According to

the report, “epithelial vacuolation was of low severity and not associated with any degenerative changes (i.e. inflammation or necrosis) within the epididymis.”

Neither the nasal cavity nor epididymis finding was considered adverse.

Centrilobular hepatocellular hypertrophy was observed in males and females at all but the LD.

There was no evidence of drug-related neuronal or axonal degeneration and necrosis or myelin loss in the brain and spinal cord following evaluation of HE, Kluver Barrera, and Holmes (silver) stains.

Mitral and aortic valves were present on all sections of heart examined. There were no drug-related microscopic changes in cardiac valves.

Table 3. Summary of Microscopic Findings – Scheduled Euthanasia (Day 92)

	Males						Females					
Group	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
Dose (mg/kg/day)	**0**	**3.5**	**5.0**	**8.0**	**13**	**20**	**0**	**3.5**	**5.0**	**8.0**	**13**	**20**
No. Animals Examined	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**
Body cavity, nasal (No. Examined)	15	15	15	15	15	15	15	15	15	15	15	15
Eosinophilic globules, olfactory epithelium	(3)[a]	(15)	(15)	(15)	(15)	(15)	(3)	(15)	(15)	(15)	(15)	(15)
Minimal	3	14	4	3	1	0	3	13	3	4	2	0
Mild	0	1	11	12	14	15	0	2	12	11	13	15
Vacuolation, olfactory epithelium	(0)	(9)	(13)	(7)	(11)	(9)	(0)	(11)	(10)	(11)	(11)	(8)
Minimal	0	9	13	7	11	9	0	11	10	11	11	8
Epididymis (No. Examined)	15	15	15	15	15	15	—	—	—	—	—	—
Vacuolation, epithelial	(0)	(0)	(0)	(0)	(3)	(14)	—	—	—	—	—	—
Minimal	0	0	0	0	3	9	—	—	—	—	—	—
Mild	0	0	0	0	0	5	—	—	—	—	—	—
Liver (No. Examined)	15	15	15	15	15	14	15	15	15	15	15	15
Hypertrophy, centrilobular	(0)	(0)	(2)	(3)	(9)	(9)	(0)	(0)	(2)	(3)	(9)	(10)
Minimal	0	0	2	3	6	5	0	0	2	3	9	6
Mild	0	0	0	0	3	4	0	0	0	0	0	4

[a] Numbers in parentheses represent the number of animals with the finding.

Toxicokinetic

TK data are shown in Table 4. Exposure margins (rat/human) for fenfluramine and norfenfluramine are shown in Table 5.

Table 4.

Dose (mg/kg)	0 (Control)		3.5		5.0		8.0		13		20	
Number of Animals/Sex/Group:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Main:	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**
Toxicokinetic:	**3**	**3**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**
Fenfluramine - Day 1												
AUC_{0-t} (hr.ng/mL)	BQL	BQL	97.0	119	169	239	446	516	2090	2560	5150	4830
C_{max} (ng/mL)	BQL	BQL	31.2	34.7	41.4	73.3	110	131	268	227	360	317
Fenfluramine - Day 89												
AUC_{0-t} (hr.ng/mL)	BQL	BQL	683	1880	2540	2430	5090	5740	9590	10400	13600	16700
C_{max} (ng/mL)	BQL	BQL	148	232	251	275	386	630	786	738	1120	1170
Norfluramine - Day 1												
AUC_{0-t} (hr.ng/mL)	BQL	BQL	1480	1470	1940	1980	3650	3140	6020	4960	7760	7410
C_{max} (ng/mL)	BQL	BQL	95.7	91.9	122	118	228	169	296	260	436	350
Norfenfluramine - Day 89												
AUC_{0-t} (hr.ng/mL)	BQL	BQL	2790	3830	4560	4870	7410	10300	14400	16500	20300	23600
C_{max} (ng/mL)	BQL	BQL	162	194	263	247	364	519	772	821	1040	1220

Table 5. Exposure (AUC) comparisons between rat and human

	Rat Mean $AUC_{(0-t)}$ on Day 89 (ng*h/mL) at NOAEL 20 mg/kg		Human Mean $AUC_{(0-t)}$ at High Clinical Dose of 0.8 mg/kg/day of Fenfluramine Hydrochloride (ng*h/mL)*	Safety Factor	
	Males	Females		Human Mean AUC / Male Rat Mean AUC	Human Mean AUC / Female Rat Mean AUC
Fenfluramine	13600	16700	1110	12	15
Norfenfluramine	20300	23600	785	26	30

*Section 2.7.2

7 Genetic Toxicology

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

Study title: Fenfluramine Hydrochloride Bacterial Reverse Mutation Test in Salmonella typhimurium and Escherichia coli

Study no.:	9601196
Study report location:	4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	09 November 2015
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	Fenfluramine hydrochloride, 96668-02, 100.3%

Methods and Results

FEN was negative (compared to water vehicle) when evaluated for mutagenic potential in Salmonella typhimurium strains (TA1535, TA1537, TA98, TA100) and Escherichia coli strain WP2 uvrA at a range of concentrations up to 5000 µg/plate, in the presence and absence of an Arochlor rat liver fraction (S9 mix), using the plate incorporation version of the bacterial mutation test. No increases in revertant colony numbers were obtained with any of the tester strains in either the presence or absence of S9 mix.

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

Study title: Fenfluramine Hydrochloride Combined Mammalian Erythrocyte Micronucleus Test in Rat Bone Marrow and Comet Assay in Liver

Study no:	9800312
Study report location:	4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	03 Nov 2015
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	Fenfluramine hydrochloride, 96668-02, 100.3%

Methods and Results

SD rats (5/sex/group) received oral (gavage) doses of FEN (0(water), 17.5, 35, or 70; 10 mL/kg) at 48, 24, and 3 hours prior to the collection of the bone marrow for the micronucleus test and the liver for the comet assay. The positive controls received a single oral dose of cyclophosphamide (20 mg/kg), 24 hours prior to sampling, followed by

2 oral doses of ethyl methanesulfonate (200 mg/kg), 24 and 3 hours prior to sampling. There was no mortality. Clinical signs included salivation and fur staining. In the micronucleus test, animals dosed with FEN did not show increases in micronucleated immature erythrocytes (% MIE). In addition, there were no substantial decreases in the proportion of immature erythrocytes. The proportion of immature erythrocytes and % MIE in the vehicle control group were within the laboratory historical control range. CP and EMS induced a clear increase in % MIE. In the comet assay, no increases were seen in the proportion of DNA in the tail extracted from the nuclei (% tail DNA) in the liver tissue of FEN-treated animals. Individual and group mean values for FEN-treated animals all fell within the laboratory historical control range. The positive controls CP and EMS induced a clear increase in the % tail DNA. The mean % tail DNA in the vehicle control group was within the laboratory historical control range. In conclusion, FEN did not induce chromosome damage in rat bone marrow immature erythrocytes and did not show evidence of induction of DNA damage in the liver, at oral dose up to 70 mg/kg/day.

8. Carcinogenicity

Carcinogenicity studies of FEN have not been conducted by the sponsor or reported in the literature.

9 Reproductive and Developmental Toxicology

9.1-3 Fertility and embryofetal and pre- and postnatal development studies were not included in the original NDA submission.

9.4 Juvenile Animal Toxicity Studies

Study title: A 10-Week Oral Gavage Toxicity Study of Fenfluramine Hydrochloride in the Juvenile Albino Rats with a 4-week Recovery Period

Study no.:	9000406
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	21 May 2015
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Lot no. 150101, 100.1%

Key Study Findings

Juvenile SD rats were administered FEN (0 (water vehicle), 3.5, 9, or 20 mg/day) daily by oral gavage for 10 weeks, on PNDs 7 to 76. Drug-related mortality was seen at the HD (4 animals either found dead or euthanized moribund during the pre- and postweaning dosing period). Clinical signs included tremor and uncoordination at the MD and HD and abnormal gait, decreased activity, hyperreactivity, dehydration, and salivation at all doses. Food consumption, BW gain, and BW were dose-dependently decreased in all treatment groups during the dosing period, but BWs were similar among groups at the end of the recovery period. Neurobehavioral changes, consisting of decreased locomotor activity and learning and memory deficits, were seen in both sexes at all doses at the end of the treatment period and remained, although to a lesser extent, after the recovery period, indicating long-term impairment. There were no clear drug-related effects on reproductive performance. At the end of the dosing period, dose-dependent decreases in bone and brain size measurements were seen in both sexes. Partial or complete recovery was seen for both of these endpoints. Histopathology findings were limited to eosinophilic globules in the olfactory epithelium at all doses. No changes were seen in aortic or mitral cardiac valves. The LOAEL of 3.5 mg/kg/day was associated with PND 76 plasma AUCs of 662 hr*ng/mL for males and 764 hr*ng/mL for females for FEN and 1930 hr*ng/mL for males and 2400 hr*ng/mL for females for desethyl FEN.

Methods

Doses:	0 (vehicle), 3.5, 9, 20 mg/day
Frequency of dosing:	QD
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	Ultra pure water
Species/Strain:	Rat, Sprague-Dawley (Crl:CD[SD])
Number/Sex/Group:	10/sex/group main/recovery, 16/sex/group neurobehavioral/reproductive, 18+9/sex/grp TK (see table below)
Age:	Postnatal Days 7 to 76

Group No.	Test Item	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)
1	Vehicle Control[a]	0	10	0
2	Fenfluramine Hydrochloride	3.5	10	0.35
3	Fenfluramine Hydrochloride	9	10	0.9
4	Fenfluramine Hydrochloride	20	10	2.0

[a] Reference Item/Vehicle: ultra pure water

Group No.	Test Item	Number of Animals							
		Main/Recovery Subsets		Neurobehavioral/ Reproductive Subset		Toxicokinetic Subset		Dosed Spares[@]	
		Males	Females	Males	Females	Males	Females	Males	Females
1	Vehicle Control	10/10	10/10	16	16	3+3	3+3	2	2
2	Fenfluramine Hydrochloride	10/10	10/10	16	16	18+9	18+9	3	3
3	Fenfluramine Hydrochloride	10/10	10/10	16	16	18+9	18+9	3	3
4	Fenfluramine Hydrochloride	10/10	10/10	16	16	18+9	18+9	3	3

[@] Data collected from the dosed spares unassigned to a subset were retained with the raw data, but were not reported.

Endpoints included: clinical signs, body weight (BW), body weight changes, food consumption, clinical pathology (hematology, coagulation, clinical chemistry, and urinalysis), ophthalmology, growth (crown-rump length), sexual maturation, behavioral and reproductive function assessments, toxicokinetic analysis, gross necropsy, organ weights, histopathology (including a thorough sectioning and examination of the brain and peripheral nervous system and aortic and mitral cardiac valves), brain and long bone measurements.

Dose selection was based on the results of an oral gavage range-finding study in juvenile rats ((b) (4) Study No. 9000468) in which animals received FEN (0, 12, 35, or 100 mg/kg QD or 50 mg/kg BID (100 mg/kg/day)) on PNDs 7 to 27. Due to clinical signs and early sacrifice of 1 animal on PND 14, the HD (100 mg/kg/day, given QD or BID) was decreased to 50 mg/kg/day (given QD and as 25 mg/kg BID) starting on PND 12/15. After

dose reduction, administration of FEN resulted in mortality in the two HD groups; clinical signs that included tremor and incoordination at ≥ 35 mg/kg/day, and decreased activity, piloerection, abnormal gait in the two HD groups; and dose-dependent reductions in body weight and food intake, with marked differences at the HD. Histopathological examination of the heart (mitral and aortic valves were present in all sections examined and the tricuspid valve was present in approximately 2/3 of animals with similar distribution across groups) showed no drug-related microscopic changes in cardiac valves.

Observations and Results

Mortality

Seven animals were found dead or euthanized in poor condition: 3 preweaning and 4 postweaning. One HD female (No. 451-5, Neurobehavioral/Reproductive subset) was euthanized on PND 17 due to poor condition, 1 HD male (463-3, TK subset) was euthanized on PND 14 due to poor condition, and 1 HD male (463-2, TK subset) was found dead on PND 18. All 3 exhibited similar clinical signs prior to death (decreased activity, uncoordination, tremor, weakness, dehydration, skin pallor, thinness, and cold to touch starting on PND 12). During the postweaning period, 1 C male (1028), 2 MD males (3022 and 3025), and 1 HD male (4023), all from the Neurobehavioral/Reproductive subset, were found dead on PNDs 99, 63, 95, or 79, respectively. No abnormal clinical signs were noted for these animals prior to death. In addition, 2 LD rats (male 265-2 from the Main subset and male 264-3 from the Neurobehavioral/Reproductive subset) were euthanized on PND 17 due to external observations that were not drug-related. Only the HD deaths were attributed to drug.

Clinical Signs

Dose-related clinical signs included tremor and uncoordination at the MD and HD and abnormal gait, decreased activity, hyperreactivity, dehydration, and salivation at all doses. Tremor and/or incoordination starting on PND 7 at the HD and PND 11 at ≤ 9 mg/kg/day; these observations persisted until PND 31. Animals appeared to develop tolerance with repeating dosing. Other less frequent observations included abnormal gait in all treated groups and piloerection at ≥ 9 mg/kg/day during the pre-weaning dosing period and dehydration, hyperreactivity, hypersensitivity, ungroomed fur and/or decreased activity at all doses during the pre-and postweaning dosing period.

Body Weights

Dose-dependent decreases in BW gain and BW (20 and 19% below C in HDM and HDF on PND 21) were noted during the preweaning dosing period for both sexes in all treatment groups (Tables 1). These decreases persisted during the post weaning dosing period (BW 14 and 10% below C in HDM and HDF on PND 77; Table 2). During the recovery period, BW rebounded, and BW gains were increased compared to C. By the end of the recovery periods (PND 105 or 134), BWs were similar among groups. However, BW gain during pregnancy was decreased at the HD (17% compared to C) in Reproductive subset females.

Table 1. Preweaning body weight

Males

Group 1 - Vehicle Control
Group 2 - Fenfluramine Hydrochloride 3.5 mg/kg/day
Group 3 - Fenfluramine Hydrochloride 9 mg/kg/day
Group 4 - Fenfluramine Hydrochloride 20 mg/kg/day

Group	Summary Information	Day Post Partum 4	7	10	14	17	21
1	Mean	12.25	18.96	25.57	35.44	42.49	58.92
	SD	1.34	2.05	2.83	3.30	3.87	6.08
	N	36	36	36	36	36	36
2	Mean	11.64	18.82	24.79	32.13 C	38.01 C	52.75 C
	SD	1.18	1.65	2.51	4.07	4.88	6.37
	N	36	36	36	36	36	36
3	Mean	11.66	18.73	24.51	31.69 C	37.16 C	51.03 C
	SD	1.14	1.45	2.23	3.06	4.23	5.10
	N	36	36	36	36	36	36
4	Mean	12.12	19.37	23.61 B	29.62 C	34.79 C	46.81 C
	SD	1.21	1.96	2.56	3.77	3.93	4.97
	N	36	36	36	36	36	36

Significantly different from control group (Group 1) value: A - $P \leq 0.05$ B - $P \leq 0.01$ C - $P \leq 0.001$ (Dunnett)
D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

Females

Group 1 - Vehicle Control
Group 2 - Fenfluramine Hydrochloride 3.5 mg/kg/day
Group 3 - Fenfluramine Hydrochloride 9 mg/kg/day
Group 4 - Fenfluramine Hydrochloride 20 mg/kg/day

Group	Summary Information	Day Post Partum 4	7	10	14	17	21
1	Mean	11.40	18.05	24.51	33.79	40.50	56.53
	SD	1.02	1.64	2.33	3.32	3.35	5.75
	N	36	36	36	36	36	36
2	Mean	11.74	18.77	24.80	31.87	37.42 A	52.54 B
	SD	1.09	1.69	2.50	3.94	4.51	5.45
	N	36	36	36	36	36	36
3	Mean	11.32	18.21	23.88	30.70 B	36.76 B	50.14 C
	SD	1.13	1.77	2.90	4.34	5.07	6.11
	N	36	36	36	36	36	36
4	Mean	11.42	18.30	22.39 B	28.33 C	33.06 C	45.66 C
	SD	1.21	2.06	3.00	3.81	4.54	4.92
	N	36	36	36	36	36	35

Significantly different from control group (Group 1) value: A - $P \leq 0.05$ B - $P \leq 0.01$ C - $P \leq 0.001$ (Dunnett)
D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

Table 2. Postweaning body weights

Males

Group 1 - Vehicle Control
Group 2 - Fenfluramine Hydrochloride 3.5 mg/kg/day
Group 3 - Fenfluramine Hydrochloride 9 mg/kg/day
Group 4 - Fenfluramine Hydrochloride 20 mg/kg/day

Group	Summary Information	Day Post Partum 21	24	28	31	35	38	42	49	56	63	70	77
1	Mean	59.0	73.7	100.3	125.8	163.1	195.6	236.3	308.5	380.6	431.4	478.0	506.5
	SD	6.1	7.6	9.2	12.3	16.2	21.2	26.3	33.9	42.4	49.8	56.7	64.3
	N	36	36	36	36	36	36	36	36	36	36	36	36
2	Mean	52.9 C	67.6 B	94.4 A	119.2 A	155.6	185.3	223.3 A	292.2	358.0 A	403.6 A	446.1 A	474.6 A
	SD	6.3	7.7	9.9	12.5	16.1	20.3	23.6	29.9	35.8	43.8	50.3	61.1
	N	36	36	36	36	36	36	36	36	36	36	36	36
3	Mean	51.1 C	65.9 C	92.9 B	117.1 B	151.9 B	180.6 B	215.6 C	282.6 C	345.8 C	382.4 C	423.7 C	451.3 C
	SD	5.0	6.7	7.8	10.4	13.8	17.2	20.1	26.3	34.4	42.5	46.0	50.7
	N	36	36	36	36	36	36	36	36	36	36	35	35
4	Mean	46.9 C	60.4 C	87.4 C	110.3 C	145.1 C	172.0 C	207.1 C	271.5 C	331.0 C	372.5 C	406.7 C	434.9 C
	SD	5.0	7.3	8.9	11.4	14.8	17.7	21.9	28.3	34.8	38.5	44.4	48.4
	N	36	36	36	36	36	36	36	36	36	36	36	36

Group	Summary Information	Day Post Partum 84	91	98	105	112	119	126	133	134
1	Mean	551.2	574.5	591.6	594.3	624.3	647.7	666.1	685.7	690.6
	SD	65.4	67.7	65.6	68.6	59.0	68.7	66.3	70.1	70.4
	N	26	26	26	25	15	15	15	15	15
2	Mean	529.3	553.4	571.5	575.4	612.6	631.0	650.9	674.9	682.4
	SD	67.7	73.5	73.8	79.4	82.7	88.6	92.0	96.0	93.5
	N	26	26	26	26	16	16	16	16	16
3	Mean	505.4 A	531.8	545.9	554.0	562.4	585.4	606.8	625.4	631.1
	SD	55.7	59.5	64.3	64.9	61.2	68.7	68.7	74.3	75.0
	N	25	25	24	24	14	14	14	14	14
4	Mean	495.6 B	528.4 A	556.1	564.8	591.8	614.2	637.5	662.0	668.9
	SD	47.4	54.2	58.4	61.3	69.6	76.1	75.9	86.0	83.3
	N	25	25	25	25	15	15	15	15	15

Significantly different from control group (Group 1) value: A - $P \leq 0.05$ B - $P \leq 0.01$ C - $P \leq 0.001$ (Dunnett)
D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

Females

Group 1 - Vehicle Control
Group 2 - Fenfluramine Hydrochloride 3.5 mg/kg/day
Group 3 - Fenfluramine Hydrochloride 9 mg/kg/day
Group 4 - Fenfluramine Hydrochloride 20 mg/kg/day

Group	Summary Information	Day Post Partum 21	24	28	31	35	38	42	49	56	63	70	77
1	Mean	56.6	69.8	92.0	111.6	138.6	158.2	177.8	210.4	239.3	258.1	279.2	283.0
	SD	5.8	7.0	9.1	11.6	13.9	15.9	15.7	18.7	23.9	25.4	28.0	29.7
	N	36	36	36	36	36	36	36	36	36	36	36	36
2	Mean	52.6 B	66.3	88.5	108.8	136.0	156.1	177.3	210.5	238.8	259.2	277.5	283.9
	SD	5.5	7.1	8.3	10.5	12.9	13.8	14.9	17.1	21.5	26.9	24.3	28.1
	N	36	36	36	36	36	36	36	36	36	36	36	36
3	Mean	50.3 C	63.2 C	84.7 B	103.9 A	129.5 A	147.9 B	166.1 B	198.3 A	224.9 A	241.0 A	256.2 B	265.7 A
	SD	6.1	7.3	8.4	10.7	13.3	13.9	15.0	19.9	22.0	25.1	25.4	28.0
	N	36	36	36	36	36	36	36	36	36	36	36	36
4	Mean	45.8 C	57.7 C	79.9 C	97.5 C	122.8 C	140.9 C	159.8 C	189.8 C	214.3 C	229.9 C	242.7 C	254.6 C
	SD	4.9	7.2	8.3	11.0	13.1	14.4	15.4	21.2	23.7	28.6	30.4	32.4
	N	35	35	35	35	35	35	35	35	35	35	35	35

Group	Summary Information	Day Post Partum 84	91	98	105
1	Mean	304.8	313.0	318.3	318.2
	SD	31.2	35.6	33.0	36.9
	N	26	26	26	24
2	Mean	307.6	316.5	323.9	326.5
	SD	28.1	30.9	28.7	27.9
	N	26	26	26	25
3	Mean	295.5	302.6	310.5	313.4
	SD	27.6	28.2	32.6	33.7
	N	26	26	26	25
4	Mean	287.7	305.2	315.2	317.3
	SD	32.8	38.7	40.2	41.5
	N	25	25	25	23

Significantly different from control group (Group 1) value: A - $P \leq 0.05$ B - $P \leq 0.01$ C - $P \leq 0.001$ (Dunnett)
D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

Food Consumption

Food consumption was dose-dependently decreased during the postweaning dosing period but was similar among groups during the recovery period.

Physical and Sexual Development

Crown-rump length was decreased during the dosing period at the HD (SS on PNDs 14, 17, and 21 pre-weaning in both sexes, and PNDs 24, 28, 31, and 38 for males and 24, 31, and 35 for females postweaning). There were no drug-related effects on mean day to development of preputial separation in males or vaginal opening in females.

Ophthalmic Examination

No drug-related ophthalmic findings were noted.

Clinical Pathology

Hematology

Drug-related changes in hematology and coagulation parameters observed on PND 77 consisted of increased platelet (+19% to +31%) and reticulocyte counts (+30% at HD) and slightly decreased activated partial thromboplastin time (-9% in F). These changes had no microscopic correlate in bone marrow.

Clinical chemistry

Slight increases in globulin (7-11%), cholesterol (34%), and potassium (9%), and decreases in glucose (18%), sodium (2%), and chloride (2-3%) seen in treatment groups at the end of the dosing period on PND 77 had no microscopic correlate and were no longer present at the end of the recovery period on PND 105.

Urinalysis

There were no drug-related changes observed in urinalysis parameters.

Neurobehavioral assessment (end of treatment and after recovery)

FOB

Toward the end of the dosing period (PND 58), there was a decrease (SS) in the incidence of rearing in the arena in HD males. Decreased (SS) body temperatures were noted on PND 58 in males at all doses and in HD females and after recovery (PND 91) at the MD and HD in both sexes. A reduction (SS) in hindlimb splay seen in HD females on PND 58 was attributed, at least in part, to the smaller size of the females.

Locomotor activity

Ambulation was decreased in males at all doses at the end of the treatment period and in both sexes at all doses during the recovery period (Tables 3-4).

Table 3. Motor activity on PND 56-58

Male - Day 56-62 pp

Ambulation

Group	Summary Information	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	All Intervals Combined	
Group 1 - Vehicle Control	LSMean	668.75	331.50	137.44	102.31	82.75	79.12	233.65	
	SELSM	43.458	61.906	33.257	34.242	20.835	24.168	24.585	
	N	16	16	16	16	16	16	96	
Group 2 - 3.5 mg/kg/day	LSMean	542.69	120.31	86.56	108.63	53.06	71.31	163.76	A
	SELSM	47.994	23.389	33.413	48.244	22.215	23.192	13.227	
	N	16	16	16	16	16	16	96	
Group 3 - 9 mg/kg/day	LSMean	551.75	171.06	83.75	100.88	67.69	56.31	171.91	
	SELSM	51.550	33.853	24.016	43.476	31.276	18.148	16.444	
	N	16	16	16	16	16	16	96	
Group 4 - 20 mg/kg/day	LSMean	468.63	139.25	61.81	96.81	50.81	61.31	146.44	A
	SELSM	59.523	33.042	18.879	29.203	26.047	25.052	16.132	
	N	16	16	16	16	16	16	96	

Significantly different from Group 1: A - P <= 0.05 (Dunnett's test)

Table 4. Motor activity on PND 91-97

Male - Day 91-97 pp

Ambulation

Group	Summary Information	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	All Intervals Combined	
Group 1 - Vehicle Control	LSMean	569.31	291.00	197.56	188.87	148.69	131.31	254.46	
	SELSM	41.164	30.040	29.993	28.125	25.392	23.876	17.197	
	N	16	16	16	16	16	16	96	
Group 2 - 3.5 mg/kg/day	LSMean	521.00	200.47	110.20	114.13	51.80	78.40	179.33	B
	SELSM	42.514	31.025	30.977	29.048	26.225	24.659	17.761	
	N	15	15	15	15	15	15	90	
Group 3 - 9 mg/kg/day	LSMean	493.40	177.20	121.40	95.80	103.13	69.67	176.77	B
	SELSM	42.514	31.025	30.977	29.048	26.225	24.659	17.761	
	N	15	15	15	15	15	15	90	
Group 4 - 20 mg/kg/day	LSMean	412.93	112.73	156.27	60.87	55.73	63.33	143.64	C
	SELSM	42.514	31.025	30.977	29.048	26.225	24.659	17.761	
	N	15	15	15	15	15	15	90	

Significantly different from Group 1: B - P <= 0.01 C - P <= 0.001 (Dunnett's test)

Female - Day 91-97 pp

Ambulation

Group	Summary Information	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	All Intervals Combined	
Group 1 - Vehicle Control	LSMean	493.81	280.63	174.75	164.25	173.44	171.06	242.99	
	SELSM	32.912	29.713	25.759	26.042	32.210	23.401	14.460	
	N	16	16	16	16	16	16	96	
Group 2 - 3.5 mg/kg/day	LSMean	465.25	182.94	91.88	117.63	82.19	104.62	174.08	B
	SELSM	32.912	29.713	25.759	26.042	32.210	23.401	14.460	
	N	16	16	16	16	16	16	96	
Group 3 - 9 mg/kg/day	LSMean	399.69	158.88	74.75	57.56	86.19	67.81	140.81	C
	SELSM	32.912	29.713	25.759	26.042	32.210	23.401	14.460	
	N	16	16	16	16	16	16	96	
Group 4 - 20 mg/kg/day	LSMean	409.67	184.67	123.60	70.93	77.93	119.20	164.33	C
	SELSM	33.992	30.688	26.604	26.896	33.266	24.168	14.934	
	N	15	15	15	15	15	15	90	

Significantly different from Group 1: B - P <= 0.01 C - P <= 0.001 (Dunnett's test)

Auditory startle habituation

There were no clear drug-related effects on auditory startle habituation (startle at start, maximum startle, time of maximum startle and average startle).

Learning and memory (Cincinnati maze)

When tested at the end of the dosing period (PNDs 56 to 69), swimming ability was unaffected by treatment, but increases in time to complete the maze, errors, and number of animals failing to complete the maze were seen in both sexes at all doses, primarily in the more difficult Path B in males, but in both paths in females (results for latencies and errors in Path B shown in Tables 5-6).

When tested during the recovery period (PNDs 91-102), the learning and memory impairment appeared to remain in both sexes at all doses, although only in females did latencies on Path A and errors on both Paths A and B reach SS (results for latencies and errors in Path B shown in Tables 7-8).

Table 5. Time to complete the maze (sec) on PNDs 56-69

Path B - Between Post Partum Days 56 and 69
Males

Group 1 - Vehicle Control
Group 2 - Fenfluramine Hydrochloride 3.5 mg/kg/day
Group 3 - Fenfluramine Hydrochloride 9 mg/kg/day
Group 4 - Fenfluramine Hydrochloride 20 mg/kg/day

Group	Summary Information	Trial Number 1	2	3	4	5	6
1	Mean	271.9	138.7	64.7	44.0	28.0	18.8
	SD	56.7	108.2	66.6	58.9	19.0	8.6
	N	20	20	20	20	20	20
2	Mean	235.5	153.3	137.9 D	70.4	32.3	19.2
	SD	84.8	112.6	107.8	69.8	23.5	9.0
	N	20	20	20	20	20	20
3	Mean	263.4	198.3	165.5 D	80.6	62.7	36.9
	SD	89.0	102.9	116.0	101.4	84.2	65.2
	N	20	20	20	20	20	20
4	Mean	283.0	197.6	144.1 D	109.5	59.0	49.7
	SD	60.0	120.7	105.2	115.7	69.0	71.7
	N	20	20	20	20	20	20

Significantly different from control group (Group 1) value: A - P ≤ 0.05 B - P ≤ 0.01 C - P ≤ 0.001 (Dunnett)
D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

F1 Generation Adults
Path B - Between Post Partum Days 56 and 69
Females

Group 1 - Vehicle Control
Group 2 - Fenfluramine Hydrochloride 3.5 mg/kg/day
Group 3 - Fenfluramine Hydrochloride 9 mg/kg/day
Group 4 - Fenfluramine Hydrochloride 20 mg/kg/day

Group	Summary Information	Trial Number 1	2	3	4	5	6
1	Mean	213.6	115.8	75.9	36.2	23.5	26.2
	SD	96.7	82.5	69.9	21.6	11.3	23.2
	N	20	20	20	20	20	20
2	Mean	272.9 D	184.9	131.2	82.1	55.0	20.1
	SD	73.6	113.4	108.1	100.6	64.7	11.4
	N	20	20	20	19	20	20
3	Mean	232.0	132.3	92.3	45.8	33.1	25.8
	SD	93.8	87.7	67.1	34.3	25.8	18.5
	N	20	20	20	20	20	20
4	Mean	276.4 E	154.8	128.7	66.5	48.8	21.2
	SD	67.9	101.6	100.6	82.6	61.4	10.5
	N	20	20	20	20	20	20

Significantly different from control group (Group 1) value: A - P ≤ 0.05 B - P ≤ 0.01 C - P ≤ 0.001 (Dunnett)
D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Table 6. Errors - PNDs 56 to 69

Path B - Between Post Partum Days 56 and 69
Males

Group 1 - Vehicle Control
Group 2 - Fenfluramine Hydrochloride 3.5 mg/kg/day
Group 3 - Fenfluramine Hydrochloride 9 mg/kg/day
Group 4 - Fenfluramine Hydrochloride 20 mg/kg/day

Group	Summary Information	Trial Number 1	2	3	4	5	6
1	Mean	19.5	7.7	3.0	1.4	0.9	0.1
	SD	8.0	6.0	3.2	2.6	1.7	0.2
	N	20	20	20	20	20	20
2	Mean	18.4	8.9	9.5 E	5.0	1.3	0.1
	SD	7.0	8.5	7.5	6.7	1.9	0.4
	N	20	20	20	20	20	20
3	Mean	20.6	13.4	11.6 E	5.5	3.9	2.0
	SD	8.0	7.9	10.0	8.6	6.5	5.5
	N	20	20	20	20	20	20
4	Mean	25.3 D	13.7	11.5 F	8.1 D	4.1 D	2.7 D
	SD	7.6	9.3	7.6	10.8	5.2	5.6
	N	20	20	20	20	20	20

Significantly different from control group (Group 1) value: D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

Path B - Between Post Partum Days 56 and 69
Females

Group 1 - Vehicle Control
Group 2 - Fenfluramine Hydrochloride 3.5 mg/kg/day
Group 3 - Fenfluramine Hydrochloride 9 mg/kg/day
Group 4 - Fenfluramine Hydrochloride 20 mg/kg/day

Group	Summary Information	Trial Number 1	2	3	4	5	6
1	Mean	18.5	8.2	4.4	1.3	0.3	0.7
	SD	10.3	8.3	5.5	2.6	0.6	1.8
	N	20	20	20	20	20	20
2	Mean	24.0	13.6	8.7	4.5	3.0	0.6
	SD	8.6	10.0	8.1	6.1	5.0	1.8
	N	20	20	20	19	20	20
3	Mean	22.7	9.9	7.4	2.8	1.3	0.5
	SD	11.1	8.9	7.1	3.8	1.9	0.9
	N	20	20	20	20	20	20
4	Mean	24.6	13.8	11.6	5.0 D	4.1 D	1.0
	SD	9.9	10.1	10.8	6.7	7.0	1.8
	N	20	20	20	20	20	20

Significantly different from control group (Group 1) value: D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

Table 7. Time to complete the maze (sec) – PNDs 91-102

Path B - Between Post Partum Days 91 and 102
Males

Group 1 - Vehicle Control
Group 2 - Fenfluramine Hydrochloride 3.5 mg/kg/day
Group 3 - Fenfluramine Hydrochloride 9 mg/kg/day
Group 4 - Fenfluramine Hydrochloride 20 mg/kg/day

Group	Summary Information	Trial Number 1	2	3	4	5	6
1	Mean	240.0	90.5	77.4	24.3	19.0	19.8
	SD	78.0	88.1	61.6	15.7	11.5	9.0
	N	15	15	15	15	15	15
2	Mean	274.8	121.9	108.4	44.0	24.8	17.5
	SD	62.9	77.8	89.7	52.9	19.3	6.8
	N	16	16	16	16	16	16
3	Mean	237.7	166.1	119.0	44.9	23.0	19.7
	SD	95.4	104.6	104.6	72.8	8.0	6.3
	N	14	14	14	14	14	14
4	Mean	213.0	145.2	78.7	44.5	36.8	18.7
	SD	104.5	125.5	81.7	56.1	67.5	8.7
	N	15	15	15	15	15	15

Significantly different from control group (Group 1) value: A - $P \leq 0.05$ B - $P \leq 0.01$ C - $P \leq 0.001$ (Dunnett)
D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

Path B - Between Post Partum Days 91 and 102
Females

Group 1 - Vehicle Control
Group 2 - Fenfluramine Hydrochloride 3.5 mg/kg/day
Group 3 - Fenfluramine Hydrochloride 9 mg/kg/day
Group 4 - Fenfluramine Hydrochloride 20 mg/kg/day

Group	Summary Information	Trial Number 1	2	3	4	5	6
1	Mean	202.5	101.7	76.3	25.4	23.1	21.8
	SD	91.9	86.1	67.3	11.6	13.3	20.8
	N	16	16	16	16	16	16
2	Mean	241.3	153.8	96.4	30.3	35.1	20.4
	SD	88.0	104.4	83.6	15.1	21.3	6.0
	N	16	16	16	16	16	16
3	Mean	254.7	146.5	87.1	42.4	25.9	20.4
	SD	67.1	109.0	79.1	27.3	11.8	12.9
	N	16	16	16	16	16	16
4	Mean	247.5	168.8	152.0	65.5	31.8	23.9
	SD	88.6	95.5	98.0	75.4	26.0	11.5
	N	15	15	15	15	15	15

Significantly different from control group (Group 1) value: A - $P \leq 0.05$ B - $P \leq 0.01$ C - $P \leq 0.001$ (Dunnett)
D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

Table 8. Errors - PNDs 91 to 102

Path B - Between Post Partum Days 91 and 102
Males

Group 1 - Vehicle Control
Group 2 - Fenfluramine Hydrochloride 3.5 mg/kg/day
Group 3 - Fenfluramine Hydrochloride 9 mg/kg/day
Group 4 - Fenfluramine Hydrochloride 20 mg/kg/day

Group	Summary Information	Trial Number 1	2	3	4	5	6
1	Mean	11.4	3.7	2.0	0.1	0.3	0.1
	SD	5.6	4.9	1.9	0.3	0.9	0.5
	N	15	15	15	15	15	15
2	Mean	14.0	5.4	4.7	0.8	0.3	0.1
	SD	3.7	4.5	4.2	1.0	0.8	0.3
	N	16	16	16	16	16	16
3	Mean	14.4	7.1	4.5	0.9	0.1	0.0
	SD	7.0	5.4	4.7	2.7	0.5	0.0
	N	14	14	14	14	14	14
4	Mean	13.1	8.7	4.3	1.9	1.3	0.4
	SD	7.7	8.7	5.9	4.7	4.1	1.1
	N	15	15	15	15	15	15

Significantly different from control group (Group 1) value: D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Path B - Between Post Partum Days 91 and 102
Females

Group 1 - Vehicle Control
Group 2 - Fenfluramine Hydrochloride 3.5 mg/kg/day
Group 3 - Fenfluramine Hydrochloride 9 mg/kg/day
Group 4 - Fenfluramine Hydrochloride 20 mg/kg/day

Group	Summary Information	Trial Number 1	2	3	4	5	6
1	Mean	12.3	5.3	2.6	0.1	0.0	0.1
	SD	7.3	6.9	3.7	0.3	0.0	0.3
	N	16	16	16	16	16	16
2	Mean	14.9	5.8	3.3	0.5	0.6	0.2
	SD	6.9	4.9	4.4	1.2	1.6	0.4
	N	16	16	16	16	16	16
3	Mean	16.6	7.9	4.4	1.3 D	0.7	0.3
	SD	6.1	6.8	7.1	1.6	1.0	1.0
	N	16	16	16	16	16	16
4	Mean	18.1	9.6	10.1	3.8 D	1.1	0.4
	SD	12.4	6.0	10.4	6.7	2.9	1.1
	N	15	15	15	15	15	15

Significantly different from control group (Group 1) value: D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Reproductive Performance

When animals were mated on PND 104 or 105, there were no clearly drug-related effects on fertility indices or C-section parameters (implantations, corpora lutea, early/late

resorptions, number of live/dead fetuses, or % pre- or post-implantation loss). Days to mating were increased, and mating and fertility indices were somewhat decreased in HD animals compared to C, but the differences were not SS.

Table 9. Reproductive Performance

Group 1 - Vehicle Control
Group 2 - Fenfluramine Hydrochloride 3.5 mg/kg/day
Group 3 - Fenfluramine Hydrochloride 9 mg/kg/day
Group 4 - Fenfluramine Hydrochloride 20 mg/kg/day

Group	Number Placed for Mating: Males	Number Placed for Mating: Females	Number Males Mating	Number Females Mating	Mean (SD) Day to Mating	Number of Males Producing a Pregnancy	Number Females Pregnant	Mating Index (%)	Fertility Index (%)	Conception Rate (%)
1	15	16	15	16	3.6 3.1 (N = 16)	13	14	100.0	86.7	87.5
2	16	16	15	15	3.8 3.0 (N = 14)	15	15	93.8	93.8	100.0
3	14	16	14	16	3.7 3.0 (N = 15)	13	15	100.0	92.9	93.8
4	14	15	12	12	4.7 4.4 (N = 11)	11	11	85.7	78.6	91.7

Significantly different from control group (Group 1) value: D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn - day to mating only)
* - $P \leq 0.05$ ** - $P \leq 0.01$ *** - $P \leq 0.001$ (Fisher's)

Necropsy

Gross observations

No drug-related gross findings were noted.

Organ weights

At the end of the dosing period (PND 77), drug-related decreases in adrenal weights were seen in both sexes at all doses (up to 30%, SS at MD and HD). There were no gross or microscopic correlates to this adrenal weight change. Organ weight differences were not observed at the end of the recovery period (PND 105).

Bone measurements

Bone size measurements at the end of the dosing period (PND 77) showed dose-dependent decreases in both sexes (SS for femur width and tibia length in MD and HD

males). Small D-D decreases were still seen at the end of the recovery period (PND 105) but were not SS.

Brain measurements

Small decreases in some brain measurements (cerebral length, brain length, cerebral width) were seen at the end of treatment (cerebral width SS in HD females; Table 10). Slight decreases in cerebral width remained in females after the recovery period, but SS was not reached.

Table 10. Brain measurements – PND 77

Main Subset
Females

Group 1 - Vehicle Control
Group 2 - Fenfluramine Hydrochloride 3.5 mg/kg/day
Group 3 - Fenfluramine Hydrochloride 9 mg/kg/day
Group 4 - Fenfluramine Hydrochloride 20 mg/kg/day

Group	Summary Information	Cerebral Lenght mm	Brain Lenght mm	Cerebral Width mm
1	Mean	15.168	21.695	15.310
	SD	0.399	2.885	0.349
	N	10	10	10
2	Mean	15.026	20.534	15.393
	SD	0.536	0.554	0.181
	N	10	10	10
	% Diff (G1)	-1	-5	1
3	Mean	15.178	20.630	15.117
	SD	0.206	0.354	0.313
	N	10	10	10
	% Diff (G1)	0	-5	-1
4	Mean	15.051	20.426	14.975 A
	SD	0.435	0.656	0.322
	N	10	10	10
	% Diff (G1)	-1	-6	-2

Significantly different from control group (Group 1) value: A - P <= 0.05, B - P <= 0.01, C - P <= 0.001 (Dunnett)
D - P <= 0.05, E - P <= 0.01, F - P <= 0.001 (Dunn)

Histopathology

Drug-related histopathology changes were seen in the nasal cavity at all doses, consisting of eosinophilic globules in the olfactory epithelium of minimal to mild severity (Table 11). This change was characterized by hypertrophy of sustentacular cells of the olfactory epithelium, which contained abundant eosinophilic material. This finding remained at the end of recovery but with a decrease incidence and/or severity (Table 12). No degenerative changes were present at either time. No histopathological findings were seen in aortic or mitral cardiac valves.

Table 11. Microscopic Findings – PND 77

	Males				Females			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Dose (mg/kg/day)	**0**	**3.5**	**9**	**20**	**0**	**3.5**	**9**	**20**
No. Animals Examined	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Body cavity, nasal (No. Examined)								
Eosinophilic globules; olfactory epithelium	(0)[a]	(0)	(10)	(10)	(0)	(7)	(10)	(10)
Minimal	0	0	4	0	0	7	0	0
Mild	0	0	6	10	0	0	10	10

[a] Numbers in parentheses represent the number of animals with the finding.

Table 12. Microscopic Findings – PND 105

	Males				Females			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Dose (mg/kg/day)	**0**	**3.5**	**9**	**20**	**0**	**3.5**	**9**	**20**
No. Animals Examined	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Body cavity, nasal (No. Examined)								
Eosinophilic globules; olfactory epithelium	(0)[a]	(0)	(9)	(9)	(0)	(1)	(10)	(10)
Minimal	0	0	9	8	0	1	8	8
Mild	0	0	0	1	0	0	2	2

[a] Numbers in parentheses represent the number of animals with the finding.

Toxicokinetics

TK parameters for FEN and desethyl FEN are shown in Tables 13 and 14.

Table 13. Fenfluramine TK Parameters – PNDs 7 and 76

Analyte	Day pp	Sex	Dose (mg/kg)	T_{max} (hr)	C_{max} (ng/mL)	C_{max}/D (ng/mL/(mg/kg))	$AUC_{(0-t)}$ (hr*ng/mL)	$AUC_{(0-t)}/D$ (hr*ng/mL/(mg/kg))	$T_{1/2}$ (hr)
Fenfluramine	7	Male	3.5	0.5	95.2 ± 65.6	27.2	1060 ± 164	304	4.62
		Male	9	0.5	622 ± 161	69.1	3910 ± 172	435	6.32
		Male	20	2	944 ± 87.1	47.2	15200 ± 598	761	10.2
		Female	3.5	1	161 ± 24.3	46.1	679 ± 86.0	194	*
		Female	9	0.5	398 ± 54.3	44.2	4400 ± 519	489	5.37
		Female	20	4	1140 ± 12.9	56.8	17000 ± 964	852	NC

Analyte	Day pp	Sex	Dose (mg/kg)	T_{max} (hr)	C_{max} (ng/mL)	C_{max}/D (ng/mL/(mg/kg))	$AUC_{(0-t)}$ (hr*ng/mL)	$AUC_{(0-t)}$/D (hr*ng/mL/(mg/kg))	$T_{1/2}$ (hr)	R_{AUC}
Fenfluramine	76	Male	3.5	0.5	147 ± 77.9	42.0	662 ± 316	189	*	0.622
		Male	9	1	456 ± 15.2	50.7	3480 ± 277	387	3.25	0.889
		Male	20	1	1020 ± 67.0	51.2	12100 ± 793	607	5.38	0.798
		Female	3.5	1	166 ± 2.62	47.3	764 ± 55.4	218	3.36	1.12
		Female	9	1	620 ± 75.6	68.9	4680 ± 366	520	3.17	1.06
		Female	20	4	1210 ± 99.0	60.4	14500 ± 726	724	NC	0.850

*= Result not reported because extrapolation exceeds 20%, or R-squared is less than 0.800
NC = Not Calculable

Table 14. Desethylfenfluramine TK Parameters - PNDs 7 and 76

Analyte	Day pp	Sex	Dose (mg/kg)	T_{max} (hr)	C_{max} (ng/mL)	C_{max}/D (ng/mL/(mg/kg))	$AUC_{(0-t)}$ (hr*ng/mL)	$AUC_{(0-t)}$/D (hr*ng/mL/(mg/kg))	$T_{1/2}$ (hr)
Desethylfenfluramine	7	Male	3.5	4	110 ± 6.69	31.4	1900 ± 131	544	NC
		Male	9	24	212 ± 11.0	23.5	4490 ± 197	498	NC
		Male	20	8	355 ± 21.0	17.7	7240 ± 345	362	NC
		Female	3.5	8	110 ± 24.6	31.5	2210 ± 262	630	NC
		Female	9	24	254 ± 72.3	28.2	5330 ± 626	592	NC
		Female	20	4	371 ± 22.0	18.6	7620 ± 476	381	NC

Analyte	Day pp	Sex	Dose (mg/kg)	T_{max} (hr)	C_{max} (ng/mL)	C_{max}/D (ng/mL/(mg/kg))	$AUC_{(0-t)}$ (hr*ng/mL)	$AUC_{(0-t)}$/D (hr*ng/mL/(mg/kg))	$T_{1/2}$ (hr)	R_{AUC}
Desethylfenfluramine	76	Male	3.5	4	170 ± 38.0	48.7	1930 ± 147	552	NC	1.02
		Male	9	8	270 ± 3.98	30.0	4470 ± 193	497	NC	0.996
		Male	20	8	652 ± 15.4	32.6	13300 ± 474	667	NC	1.84
		Female	3.5	8	138 ± 1.64	39.5	2400 ± 70.7	686	NC	1.09
		Female	9	8	353 ± 43.5	39.2	6210 ± 479	690	NC	1.17
		Female	20	4	823 ± 7.65	41.1	16700 ± 1320	835	NC	2.19

NC = Not Calculable

10. Integrated Summary and Safety Evaluation

Fenfluramine (FEN) is an amphetamine derivative and a sympathomimetic stimulant with appetite-suppressant property. It stimulates the release and blocks the reuptake of serotonin (5-HT) by disrupting neurotransmitter vesicular storage and transporter function. FEN (previously marketed as Pondimen tablets and Ponderex capsules, NDA16-618) is a racemic mixture of 2 enantiomers. The dextro enantiomer (d-FEN) was marketed under the trade name Redux. FEN and d-FEN are metabolized to (±)-norfenfluramine (NFEN) and (+)-norfenfluramine (d-NFEN), respectively. Results from in vitro assays reported in the literature (Rothman et al., JPET**,** 305:1191-1199, 2003) confirmed that these drugs are potent substrates for 5-HT transporters: d-FEN, l-FEN, d-NFEN, and l-NFEN released [3H]5-HT from synaptosomes with EC50 values of 52, 147, 59, and 287 nM, respectively. In addition, d-FEN and l-NFEN released [3H]NE with EC50 values of 302 and 73 nM.

In addition to their effects on 5-HT release and reuptake, FEN and NFEN have direct agonist activity at certain 5-HT receptors, in particular members of the 5-HT2 receptor family (Rothman et al., 2000). The anorectic effect of FEN is thought to primarily be due to stimulation of central 5-HT2C receptors by NFEN. After reports of heart valve disease and pulmonary hypertension, including cardiac fibrosis, FEN was withdrawn from the U.S. market in 1997 due to safety reasons. Data reported by Rothman et al. (Circulation, 102:2836-41, 2000) and others (reviewed in Elangbam, Toxicol Path, 38: 837-848, 2010) indicate that the 5-HT2B receptor plays a critical role in the pathogenesis of FEN-associated valvulopathy.

The molecular mechanism by which FEN produces its anticonvulsant actions appears to primarily involve 5-HT activity; however, the sigma-1 receptor has also been implicated in the activity of FEN in some seizure models including the mutant scn1Lab zebrafish model of Dravet syndrome (Polster, 2019). The majority of Dravet syndrome patients have a mutation in the SCN1A gene, which codes for a neuronal voltage-gated sodium channel subunit and has been proposed as the cause. Zebrafish larvae that are homozygous for mutant scn1Lab, the zebrafish ortholog of human SCN1A, exhibit epileptiform motor activity that is attenuated in the presence of FEN. Pharmacologic investigation of the model suggested that the anticonvulsant activity of FEN is mediated by 5-HT1D and 5-HT2C (and possible 5-HT2A) receptors (Sourban et al., 2017). However, a more recent paper reporting findings in the scn1lab zebrafish model (Griffin et al., Brain Commun. 2019; 1(1): fcz008) concludes that “5-HT2B receptors are a critical mediator in the mechanism of seizure suppression observed in Dravet syndrome patients treated with 5-HT modulating drugs” such as FEN. The interaction of FEN with the sigma-1 receptor has been further characterized using the dizocilpine-induced learning impairment mouse model of spontaneous alternation in a Y-maze (Martin et al., 2017). In this model, both FEN and the specific sigma-1 agonist PRE-084 attenuated the learning deficit in a dose-related manner.

The sponsor relied primarily on literature for ADME information but conducted in vitro studies to compare species differences in metabolic stability, plasma protein binding, red

blood cell/plasma partition coefficient, and metabolite characterization. After single oral doses of racemic FEN to animals (male rat, mouse, and dog) and humans, d-FEN had a longer t1/2 and greater AUC than the l-isomer in mouse and rat, consistent with stereoselective N-deethylation of l-FEN (Caccia et al., Archives Internationales de Pharmacodynamie et de Therapie, 258:15-28, 1982). Thus, in both species the plasma and brain AUCs of l-NFEN were twice that of d-NFEN. In dog and human, slight or no differences were seen between the kinetic and metabolic profiles of the isomers. The t1/2 of d-FEN was 4.3 hr in mouse, 2.6 hr in rat, 2.5 hr in dog, and 17.8 hr in human. The deethylated metabolite NFEN was present in plasma, brain, or both, of all species examined as a major metabolite. At the oral doses of FEN tested, the ratio of the AUC for d-FEN to d-NFEN was 4.4, 2, 0.8, and 0.3 in the dog, rat, human, and mouse respectively. The t1/2 of the metabolite was longer than that of parent drug in all species.

Following incubation of FEN with rat, dog, and human liver and intestinal S9 fractions, 7 metabolites (the N-dealkylation metabolite NFEN and C1 through C6) were detected. These were formed by N-dealkylation, oxygenation, and dehydrogenation, or a combination thereof, with and without glucuronide conjugation. Norfenfluramine (NFEN) and its subsequent N−oxygenation product were the only metabolites detected in human liver S9 fractions, and norfenfluramine was the only metabolite detected in human intestinal S9 fractions. NFEN shows greater affinity and agonist activity at serotonin 5HT2 receptors than fenfluramine. The metabolites detected in human liver and intestinal S9 fractions were observed in both rat and dog incubation samples. Evidence of hydroxylation, dehydrogenation, and glucuronidation was observed in the rat and dog but not human test systems. No human-specific metabolites were detected. Both rat and dog S9 fractions showed good coverage of the human metabolites.

Comparative in vivo metabolism data reported in the literature (Marchant et al., Xenobiotica, 22:11, 1251-1266, 1992) indicated significant species differences. In plasma, the major circulating radiolabeled products following oral administration of [14C]-FEN (1 mg/kg) to humans (2 healthy male volunteers) were unchanged FEN (24 and 38% of total drug-related material in plasma at 3 and 6 hr after dosing) and the major metabolite NFEN (22 and 12% of total radioactivity at these time points). However, an unconjugated diol metabolite not detected at 3 hr reportedly accounted for 17% of total drug-related material in an 8-hr plasma sample. The 3-hr plasma sample from rats given 24 mg/kg FEN SC for 4 days contained 38% FEN, 33% NFEN, 13% collective conjugated metabolites, and 5% unconjugated diol. Thus, it appeared, based on these limited data, that rats had relatively low circulating levels of the unconjugated diol compared to humans. The diol was also identified as a significant human metabolite in a published study of human urinary metabolism (Brownsill et al., J Chromatogr, 562: 267-277, 1991).

The unconjugated diol metabolite was not measured in nonclinical or clinical studies submitted with the initial (February 5, 2019) NDA, but the sponsor attempted to estimate the amount of the diol in human and rat plasma based on its reported abundance relative to FEN in the Marchant et al. (1992) publication. The calculated values indicated an exposure margin (3X using the higher clinical AUC for FEN reported in clinical study ZX008-1505 after a single oral dose of 0.8 mg/kg, ie, 1600 ng.h/mL (corresponding

NFEN: 779 ng.h/mL) in the 13-week rat toxicity study, but this needed to be confirmed with actual PK/TK data.

The NDA resubmission (September 25, 2019) included the results of a study in which the unconjugated diol metabolite was quantified in plasma samples collected for a clinical QTc study conducted in healthy adult subjects administered 15 mg BID (MRHD) for 7 days. According to the sponsor, these data indicate that the diol accounted for less than 10% of drug-related material (average 7.8% of sum of FEN, NFEN, and diol at steady state) and, thus, should not be considered a metabolite requiring nonclinical characterization based on the ICH M3(R2) guideline (January 2010) and CDER guidance on Safety Testing of Drug Metabolites (March 2020). But it is not clear that the average of diol metabolite concentrations determined at 3 time points after dose administration during steady state is an adequate measure of metabolite exposure. The guidance refers to drug metabolites of toxicological concern for which nonclinical characterization is warranted as those present at greater than 10% of total drug-related exposure, where exposure is generally understood to mean AUC, and for which exposure would be expected to be significantly greater in humans than the maximum exposure seen in the toxicity studies. Plasma diol concentrations (pooled samples said to reflect the AUC0-24) measured in rats administered oral doses of 20 mg/kg FEN in this study were approximately equal to those in humans receiving the MRHD. Again, the guidance states that comparison between human and animal exposure is generally based on AUC, which was not used for either the rat or human. However, overall, the information provided does not suggest that humans would be exposed to significantly greater levels of the unconjugated diol metabolite compared to the maximum exposures seen in the rat toxicity studies.

In the 13-weeks toxicity study in adult rats, daily oral (gavage) administration of FEN (0 (water vehicle), 3.5, 5, 8, 13, or 20 mg/day; 10 mL/kg) for 91 days resulted in decreased body weight and microscopic findings in the nasal cavity, liver, and epididymis. Dose-dependent decreases in body weight (BW) and BW gain were noted at all doses. Cytoplasmic vacuolation of the nasal cavity olfactory epithelium was seen at ≥3.5 mg/kg/day, and dose-related increases in the severity of eosinophilic globules in the olfactory epithelium were observed at all doses. Centrilobular hepatocellular hypertrophy was seen at ≥ 5 mg/kg/day, with dose-related increases in incidence and severity. Microvesicular vacuolation of the epithelium of the epididymis was observed at ≥ 13 mg/kg/day. There was no evidence of drug-related neuronal or axonal degeneration and necrosis or myelin loss in the brain and spinal cord following evaluation of HE, Kluver Barrera, and Holmes (silver) stains. Mitral and aortic valves were present on all sections of heart examined. There were no drug-related microscopic changes in cardiac valves. The lowest effect dose (3.5 mg/kg) was associated with Day 89 plasma FEN exposures (AUC) of 683 and 1880 ng.h/mL and NFEN exposures of 2790 and 3830 ng.h/mL in males and females, respectively. Human AUCs at the MRHD of 0.8 mg/kg were reported (clinical study ZX008-1505) as 1600 and 779 ng.h/mL for FEN and NFEN, respectively.

In the pivotal juvenile rat toxicity study, FEN (0 (water vehicle), 3.5, 9, or 20 mg/day) was administered daily by oral gavage for 10 weeks, on PNDs 7 to 76. Drug-related mortality

was seen at the HD (4 animals either found dead or euthanized moribund during the pre- and postweaning dosing period). Clinical signs included tremor and uncoordination at the MD and HD and abnormal gait, decreased activity, hyperreactivity, dehydration, and salivation at all doses. Food consumption, BW gain, and BW were dose-dependently decreased in all treatment groups during the dosing period, but BWs were similar among groups at the end of the recovery period. Neurobehavioral changes, consisting of decreased locomotor activity and learning and memory deficits in the Cincinnati maze, were seen in both sexes at all doses at the end of the treatment period. These changes were still evident, although to a lesser extent, after the recovery period, indicating long-term impairment. There were no clear drug-related effects on reproductive performance. At the end of the dosing period, dose-dependent decreases in bone and brain size measurements were seen in both sexes. Partial or complete recovery was seen for both of these endpoints. Histopathology findings were limited to eosinophilic globules in the olfactory epithelium at all doses. No changes were seen in aortic or mitral cardiac valves. The LOAEL of 3.5 mg/kg/day was associated with PND 76 AUCs of 662 and 764 ng.h/mL for FEN and 1930 and 2400 ng.h/mL for NFEN in males and females, respectively.

FEN was negative in an adequate Ames test and an adequate in vivo mammalian cell genotoxicity assay (combined rat bone marrow micronucleus/liver comet assay). A report in the literature describes a positive response in an in vivo SCE assay in mice at FEN doses of 1.5 mg/kg or greater (Agarwal K et al. Environ Mol Mutagen, 19: 323-6, 1992). Carcinogenicity studies of FEN have not been conducted by the sponsor or reported in the literature.

In the late 1990s, cases of vascular heart disease were reported in patients without a history of cardiac disease who had been treated with FEN and phentermine in combination or FEN or d-FEN alone (Connolly et al., 1997, Cannistra et al., 1997, Graham & Green, 1997). Subsequently, reports of valvulopathy associated with FEN or its d-enantiomer increased. Most hypotheses implicate 5-HT in the pathogenesis of valvular heart disease (VHD), but the exact mechanism is uncertain (Rajamani et al., British J Pharmacol, 129:843–852, 2000). The histologic picture described in patients treated with FEN was virtually indistinguishable from that of VHD induced by ergotamine or methysergide, indicating 5-HT involvement. Rothman et al. (2000) demonstrated that FEN, NFEN, and other drugs known to produce VHD have preferentially high affinities for the 5-HT2B receptor subtype, which is known to be capable of stimulating mitogenesis. High doses of FEN also directly result in pulmonary hypertension, which secondarily induces ischemic cardiac injury (Hunsinger & Wright, 1990).

There are no validated animal models or preclinical/toxicologic screens to accurately predict drug-induced valvulopathy in humans (Elangbam, Toxicol Path, 38:837-848, 2010). The only nonclinical evidence of valvular changes associated with FEN was reported by Bratter et al. (European J Pharmacol, 369: R1–R3, 1999). In a study in which pregnant rats were infused (osmotic minipump) with phentermine plus d-FEN on GDs 3 through 17, 25% of the pups showed what was described as mitral valve thickening. There

were no effects of prenatal drug treatment on offspring survival, birth weight, or motor coordination assessed at PND 11.

Gustafsson et al. (Circulation, 111, 1517–1522, 2005) showed that daily SC administration of 5-HT (20 mg/kg) to SD rats for 3 months resulted in valvular changes that were described as morphologically and echocardiographically similar to those seen in human carcinoid heart disease. In a separate study, Droogmans et al. (European Heart Journal, 28:2156–2162, 2007) reported that rats given 5-HT (20 mg/kg/day SC) or pergolide (0.5 mg/kg IP) for 5 months had valvular regurgitation and microscopic changes similar to those seen in anorexigen-induced valvulopathy in humans. A subsequent study by Droogmans et al. (Am J Physiol Heart Circ Physiol, 296:H1940–H1948, 2009) showed that cyproheptadine (5-HT2B receptor antagonist) co-treatment prevented the development of pergolide-induced valvulopathy in rats with a reduced number of 5-HT2BR-positive valvular cells, supporting the idea that 5-HT2B receptors play an important role in the pathogenesis of valvulopathy (Elangbam, Toxicol Path, 38:837-848, 2010). Most recent publications assume that the mechanism of this cardiac risk involves the chronic, excessive stimulation of 5-HT2B receptors in cardiac valve leaflets (Cavero and Guillon, J Pharmacol Toxicol Methods 69: 150–161, 2014). Overstimulation of 5-HT2B receptors up-regulates cardiac valve pro-fibrotic mechanisms that lead to structural changes resulting in leaky (regurgitation) and narrowed (stenosis) valves and may over time produce pulmonary hypertension and cardiac failure (ibid).

In an effort to find patterns of 5-HT2B receptor functional selectivity that might be useful for identifying compounds likely to induce VHD, Huang et al. (Mol Pharmacol, 76:710–722, 2009) screened approximately 2200 approved or investigational drugs at concentrations of 3–10 μM for 5-HT2B receptor agonist activity using calcium-based high-throughput screening. Of these 2200 compounds, 27 were 5-HT2B receptor agonists (hits); 14 of these had previously been identified as 5-HT2B receptor agonists, including 7 known valvulopathogens (cabergoline, ergonovine, ergotamine, dihydroergotamine, methylergonovine, pergolide, and NFEN). Six of the hits (guanfacine, quinidine, xylometazoline, oxymetazoline, fenoldopam, and ropinirole) are marketed approved drugs. Twenty-three of the hits were then “functionally profiled” (i.e., assayed in parallel for 5-HT2B receptor agonism using multiple readouts to test for functional selectivity). Concentration-response isotherms were generated for the selected, putative 5-HT2B agonists to obtain estimates of potency (pEC50) and efficacy relative to 5-HT (Emax). In these assays, the known valvulopathogens were efficacious at concentrations as low as 30 nM, whereas the other compounds were less so. Hierarchical clustering analysis of the pEC50 data revealed that ropinirole (which is not associated with valvulopathy) was clearly segregated from known valvulopathogens, while NFEN was clustered on a node with 5-HT, indicating a similar pattern of functional selectivity for these two known valvulopathogens.

Cavero and Guillon (2014) calculated safety margins (SM) for lorcaserin, d-NFEN, and pergolide by using binding affinity (Ki) and potency (ED50) data for receptors mediating the therapeutic activity (on-target) and the adverse effect (off-target: 5-HT2B). The receptors mediating clinical activity were 5-HT2C for lorcaserin and d-NFEN and the D2

dopamine receptor for pergolide. For these 3 drugs, the purported pharmacological mechanism responsible for valvulopathy is the 5-HT2B receptor agonism. Additionally, the SMs obtained by using Ki and IP accumulation EC50 values and the human free plasma concentration for efficacy were reported. The nonclinical SM values for lorcaserin calculated from affinity data for on-target receptors (5-HT2C) mediating the clinical beneficial effects (anorectic) and off-target receptors (5-HT2B) mediating valvular disease is 11, whereas the SM calculated from a cell functional assay measuring IP accumulation was 61. The corresponding nonclinical SMs for d-NFEN were only 1 to 3 and 1.4, respectively, while the SMs for pergolide were 0.3 to 3 and 33, respectively. SMs calculated from either off-target binding affinity Ki or in vitro ED50 from a functional assay measuring IP accumulation and available free plasma concentration values were 3 and 45 for lorcaserin, 4 and 0.4 for d-NFEN, and 0.1 and 0.5 for pergolide. When MAPK2 readout was used as the functional assay, the SMs for pergolide and d-NFEN were still below 0. The sponsor has stated that the lower doses of FEN needed for the current indication (MRHD 0.8 mg/kg/day or 30 mg/day compared to 120 mg/day for obesity) result in a more favorable SM.

Cavero and Guillon (2014) have recommended that the screening process for VHD risk include, in addition to in vitro binding and functional assays, valvulopathy effects assessed by echocardiographic measurements and valve histology in rats chronically treated with the candidate drug. It appears that this has never been performed for FEN; however, human data now exist. Although chronic rat and dog toxicity studies were conducted by Robins as reported in Gilbert et al. (1971), they would not have included echocardiographic measurements and it is unlikely that the histopathology examinations would have adequately assessed possible heart valve effects. As pointed out by Elangbam (Toxicol Path, 38:837-848, 2010), unless specified for a particular rodent study, it is not customary or mandatory practice to examine and record heart valve findings, resulting in underdiagnosing and underreporting of valvular effects. However, the heart valves were specifically examined in the adult and juvenile rat toxicity studies conducted by the sponsor for the current application, and no changes were reported. It is possible that the duration of dosing was inadequate (13 and 10 weeks, respectively). Although Gustafsson et al. (2005) reported valvular changes in rats dosed with 5-HT for 3 months, in the rat model proposed by Droogmans et al. (2007), significant echocardiographic valvular regurgitation was first seen after 20 weeks of dosing with pergolide, whereas it was seen after 10 weeks of 5-HT administration. Another possible reason for not seeing valve changes in the rat studies conducted with FEN for this application is the species difference in metabolism.

FEN is a well-known adult neurotoxicant. Both FEN and NFEN have been shown to produce 5-HT neurotoxicity in numerous animal studies reported in the literature. They have been reported to cause dose-related, long-lasting reductions in 5-HT axonal markers in all animal species tested and with all routes of administration used, at clinically relevant doses (McCann et al., JAMA, 1997;278(8):666-672). The developmental neurotoxicity of FEN has been less well-studied. However, in general agreement with the findings in the juvenile rat study conducted for this application, neurobehavioral impairment, including learning and memory deficits, has been reported by Morford et al.

(Eur J Neuroscience,16:491-500, 2002) following exposure of neonatal rats to d-FEN (0, 20, 40, or 60 mg/kg/day on PND 11-20). When animals were tested in the Cincinnati and Morris water mazes as adults beginning on PND 52, spatial learning and memory deficits in the Morris maze and sequential learning deficits in the Cincinnati water maze were observed at all doses. The authors remarked on the severity of the d-FEN-induced learning impairments, noting that even after 24 trials during each test phase of the Morris maze, the treated animals never reached control levels of performance. This performance deficit was said to be more severe than that previously seen with methamphetamine or MDMA in the same lab.

There are very few investigations of possible FEN effects on prenatal development reported in the literature, and none were conducted by the sponsor for this application. The Gilbert et al. (1971) paper briefly discusses the results of reproductive/developmental studies in mouse, rat, rabbit, and monkey. The study designs and results were not clearly described, but no teratogenic effects were reported. Two more detailed literature reports describe studies conducted in mice and rats. In a study focusing primarily on cardiac toxicity, female mice were administered placebo and 2 doses of either FEN or d-FEN in the diet from 2 weeks before mating until GD 15 (Rayburn et al., Drug Chem Toxicol, 23:419-31, 2000). The mice ingested average doses of 10.5 and 31.8 mg/kg/d for FEN and 5 and 16.2 mg/kg/d for d-FEN. According to the report, the maternal and offspring hearts, including mitral and aortic valves, of FEN-exposed mice were indistinguishable from the placebo-exposed mice, and there were no effects on litter and developmental endpoints (offspring BW, body length, head circumference, early functional testing, and postnatal growth measured as BW to PND 120). A behavioral teratology study was conducted by Vorhees et al. (Science, 205: 1220-1225, 1979) in which pregnant S-D rats were administered FEN (20 mg/kg) orally (by gavage) on GDs 7-20 and offspring were evaluated postnatally. Decreases in preweaning viability and BW, neurobehavioral abnormalities (swimming development, locomotor activity), and decreased adult (PND 70) brain weights were observed in the prenatally FEN-exposed offspring.

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