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APPLICATION NUMBER:

216675Orig1s000

NON-CLINICAL REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	216675
Supporting document/s:	1, 7 and 9
CDER stamp date:	06/28/2022, 01/17/2023 and 02/24/2023
PDUFA date:	06/28/2023
Product:	Perfluorohexyloctane Ophthalmic Solution, 100%
Indication:	Treatment of the signs and symptoms of Dry Eye Disease (DED) associated with Meibomian Gland Dysfunction (MGD)
Applicant:	Bausch & Lomb Incorporated
Review Division:	Division of Pharm/Tox for Rare Diseases, Pediatrics, Urologic and Reproductive Medicine/ Specialty Medicine (DPT-RPURM/SM) in support of Division of Ophthalmology
Reviewer:	Aling Dong, Ph.D., DABT
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Division Director:	Mukesh Summan, Ph.D., DABT
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Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

The Applicant, Bausch & Lomb Incorporated (B&L) submitted NDA 216675 under the 505(b)(1) pathway for Perfluorohexyloctane Ophthalmic Solution, 100% (Miebo™), for treatment of the signs and symptoms of Dry Eye Disease (DED) associated with Meibomian Gland Dysfunction (MGD), which included the proposed labeling text to comply with the "Pregnancy and Lactation Labeling Rule".

1.2 Brief Discussion of Nonclinical Findings

- Ocular instillation of perfluorohexyloctane ophthalmic solution, 100% (code name: F6H8) at the dose of 427.8 mg/day, four times daily bilaterally (~3 hours apart) for up to 26 weeks in New Zealand White (NZW) rabbits was well tolerated and did not induce any ocular or systemic signs of toxicity. The systemic no-observed-adverse-effect level (NOAEL) was 427.8 mg/animal/day whereas the ocular NOAEL was 213.92 mg/eye/day.

- Ocular instillation of F6H8 at doses up to 428.8 mg/day bilaterally into the conjunctival sac of NZW rabbits four times daily (~3 hours apart) for four weeks was well-tolerated, with no systemic toxicity or F6H8 related histopathological findings. The systemic NOAEL was 428.8 mg/day, and the ocular NOAEL was 214.4 mg/eye/day (the highest dose tested).

- Once daily oral administration of F6H8 at doses up to 2000 mg/kg/day to Wistar rats for 28 consecutive days were well tolerated and did not produce F6H8 related toxicity. The NOAEL of F6H8 was 2000 mg/kg/day, the highest dose tested in this study.

- CMC requested Pharm/Tox to evaluate the Applicant-proposed limit of NMT (b) (4)% for the impurity (b) (4). Based on the nonclinical studies, the proposed impurity limit of (b) (4)% can't be supported. Instead, impurity limit of (b) (4)% for (b) (4) was supported by nonclinical data and recommended. Consequently, CMC sent an IR and requested that the limit for (b) (4) be revised to NMT (b) (4)%. The Applicant agreed.

- Daily oral administration of F6H8 at doses up to 2000 mg/kg/day to pregnant Wistar rats during the period of organogenesis (from GD6 to GD17 inclusive) was well tolerated with no toxicological effects on maternal or embryofetal parameters. Thus, the NOAEL for maternal and embryofetal toxicity was the highest dose tested, 2000 mg/kg/day (HED=19459 mg/day) in this embryofetal developmental (EFD) toxicity study.

- Following daily oral administration of F6H8 at doses of 0 (saline), 250, 500 and 1000 mg/kg/day to pregnant female NZW rabbits during the period of organogenesis (from GD6 to GD19 inclusive), there were abortions in all treated group (6, 3 and 3 females in the 250, 500 and 1000 mg/kg/day groups, respectively), compared with none in the control group.
- During the dosing period, dose-depended reduced mean body weight (BW) gain was noted in all treated groups (as -28%, -53% and -93% in the low, mid and high dose group, respectively). Reduced food consumption was also noted at a dose-related trend. There were higher incidences of reduced fecal output, soft feces and/or absent urine in all groups during the dosing period compared with the control group, which was consistent with the reduced food consumption. Thus, a NOAEL for maternal toxicity could not be established in rabbits.
- Consistent with the maternal toxicity, mean fetal weight was reduced in all treated groups (up to -12% in the 1000 mg/kg/day group) compared with the concurrent control group and the Testing Facility's historical control data (HCD).
- There were 1 (1), 7 (5) and 6 (5) fetuses (litters) with fetal malformations (external, visceral and/or skeletal) in the low, mid and high dose treatment groups as compared with 1 (1) in the control group. Although the findings were within the range of Testing Facility's HCD (historical control data, when verified individually), considering the significantly increased incidences of these findings (when taken together) in the mid and high dose groups compared with the concurrent control group, this reviewer agrees with the Applicant on that "*based on the maternal systemic exposure saturation, the developmental NOAEL can be more conservatively established at 250 mg/kg/day for safety margin calculations*". As such, the fetal NOAEL was the low dose, 250 mg/kg/day (HED= 4865 mg/day) in this EFD toxicity study.

- Sections 8.1, 8.2, 12.1, and 13 of Applicant-proposed labeling text were revised to comply with "Pregnancy and Lactation Labeling Final Rule" (PLLR).

1.3 Recommendations

1.3.1 Approvability

Approval is recommended.

1.3.2 Additional Nonclinical Recommendations

None.

1.3.3 Labeling

This reviewer recommends the following editorial changes in Sections 8.1, 8.2, 12.1, and 13 of the Applicant-proposed labeling, according to PLLR.

Reviewer Comments

- This reviewer edited the Applicant-proposed labeling according to PLLR, and based upon the systemic exposure margins calculated below.

Table 1 Systemic Exposure Margins to Support Labeling Edits

Clinical Exposure Margins (Based on Dose)			
Species/Type of Study	NOAEL (mg/kg/day)	HED (mg/day)	Exposure Margins (based on a MRHD of 120 mg/day)
Rat EFD	2000	19459	162X
Rabbit EFD	250 (for fetal)	4865	40.54X

NOAEL = no-observed-adverse-effect level
MRHD = maximum recommended human dose
HED = human equivalent dose (based on body surface area)

2 Drug Information

2.1 Drug

Generic Name

Perfluorohexyloctane; 1-(perfluorohexyl)octane; semifluorinated alkane perfluorohexyloctane; NovaTears®, EvoTears®

Code Name

NOV03, F6H8

CAS Registry Number

133331-77-8

Chemical Name

1,1,1,2,2,3,3,4,4,5,5,6,6-tridecafluorotetradecane

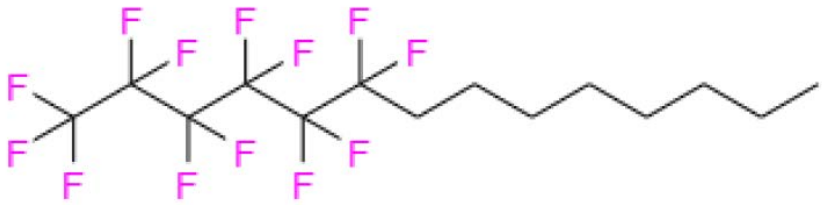
Molecular Formula

$C_{14}H_{17}F_{13}$

Molecular Weight

432.26 g/mol

Structure or Biochemical Description



Pharmacologic Class: Semiflluorinated alkane

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 130558, the parent IND for the current NDA.

2.3 Drug Formulation

The proposed drug product consists of the perfluorohexyloctane (drug substance) as the single ingredient in the formulation.

Table 2 Qualitative and quantitative composition of drug product

Component	Reference to Quality Standard	Function	Concentration (g/mL)	Quantity per Unit (g/3 mL)
Perfluorohexyloctane	In-house	Active	1.338	4.014

2.4 Comments on Novel Excipients

None.

2.5 Comments on Impurities/Leachable of Concern

- On 8/11/2022, CMC team emailed Pharm/Tox (P/T) team regarding impurity (b) (4)

  I wanted to alert you that one related substance ((b) (4)) in the perfluorohexyloctane drug substance specifications is proposed at a limit of NMT (b) (4)%, which is above the ICH Q3A qualification threshold of 0.15%. When you get some time, can you let me know if the proposed limit of NMT (b) (4)% for (b) (4) related substance impurity in the drug substance specifications for the perfluorohexyloctane drug substance is acceptable based on non-clinical studies?.

- This reviewer conducted the following assessments:

- The proposed dosing regimen is: "*Instill one drop of <TRADENAME> four times daily into each eye*". At the drop size of 11µL, the maximum recommended human dose (MRHD) of perfluorohexyloctane (F6H8) will be 60 mg/eye/day or 120 mg/day. Thus, at the proposed limit of (b) (4)%, the clinical TDI (total daily intake) will be (b) (4) mg/eye/day or (b) (4) mg/day for impurity (b) (4).

- Through the current NDA and the parent IND submissions, this reviewer identified the following ocular toxicology studies in which the test articles contained the impurity (b) (4)

- In a 26-week GLP topical ocular toxicity study with F6H8 in rabbits (Study #: AB21993), the animals were well tolerated and did not induce any ocular or systemic signs of toxicity, with the NOAEL identified as 427.8 mg/day or 213.92 mg/eye/day. Per Analysis Certificate: the impurity (b) (4) was detected at (b) (4)%.

(b) (4)

Thus, the TDI was (b) (4) mg/eye/day or (b) (4) mg/day for impurity (b) (4) in this study, which is less than the clinical TDI of (b) (4) mg/eye/day or (b) (4) mg/day with the proposed limit of (b) (4)% for impurity (b) (4)

As such, the 26-week ocular toxicity study does not support the proposed (b) (4)% limit for (b) (4).

- In a 4-week GLP topical ocular toxicity study with F6H8 in rabbits (Study #: AB21563), the animals were well tolerated with NOAEL identified as 428.8 mg/day or 214 mg/eye/day. Per Analysis Certificate: the impurity (b) (4) was detected at (b) (4)%.

(b) (4)

Thus, the TDI was (b) (4) mg/eye/day (or (b) (4) mg/day) for impurity (b) (4) in this study, which is greater than the clinical TDI of (b) (4) mg/eye/day (or (b) (4) mg/day) at the proposed impurity limit of (b) (4)%.

However, the study duration was only 4 weeks for this ocular toxicity study, which precludes the use of this study as safety support for the proposed chronic human use.

- As such, none of the nonclinical studies tested support the proposed limit of NMT (b) (4)% for (b) (4). This reviewer informed CMC team the P/T assessment conclusion, CMC sent an information request (IR) subsequently.

- On 1/18/2023, CMC team forwarded the Applicant-provided IR response (to the CMC IR), requested P/T that "*if the proposed limit is adequately justified*?"

- In the IR response, the Applicant justified that "*both ocular and systemic studies have evaluated levels of the impurity at the NOAEL doses (with favorable results) that exceed the (b) (4)% impurity level and therefore support the qualification of the (b) (4)% impurity specification*", based upon the 28-day ocular toxicity study in rabbits (# AB21563), 7-day oral toxicity study in rats (# AB21526), and the 28-day oral toxicity study in rats (# AB21563).

- This reviewer disagrees. Data in 7-day or 28-day oral or ocular toxicity studies are not sufficient to support a chronic ocular use in human due to the limitation of the study durations.

- Based on the discussion above, in the 26-week ocular toxicity study, the maximum TDI was (b) (4) mg/eye/day or (b) (4) mg/day for impurity (b) (4) Comparing with the clinical TDI of (b) (4) mg/eye/day or (b) (4) mg/day with the proposed limit of (b) (4)%, this study will support the impurity limit of up to (b) (4)% in human chronical use.

- As such, P/T provided the following comment to CMC:

  From the sponsor's nonclinical studies, Nonclinical can support (b) (4) impurity at (b) (4)%.

  Consequently, CMC sent an additional IR and r uested that the limit for (b) (4) be revised to NMT (b) (4)%. The Applicant agreed.

2.6 Proposed Clinical Population and Dosing Regimen

Per the proposed labeling:

- The proposed indication is: "*for treatment of the signs and symptoms of dry eye disease associated with meibomian gland dysfunction".*

- The proposed dosing regimen is: "*Instill one drop of <TRADENAME> four times daily into each eye*."

2.7 Regulatory Background

- On 10/13/2017, the original IND Sponsor Novaliq GmbH submitted a new IND for its NOV03 (perfluorohexyloctane) ophthalmic solution, for the treatment of dry eye syndrome. The 30-day safety review by this reviewer was archived in DARRTS dated 11/02/2017.

- On 12/08/2018, the Sponsor submitted an EOP2 meeting request, seeking the Agency's advice for the same product to treat "dry eye disease (DED) due to

meibomian gland dysfunction (MGD)". The meeting minutes was archived in DARRTS dated 04/30/2019.

- On 09/20/2021, the current Sponsor Bausch & Lomb Inc. submitted a pre-NDA meeting request for development of the same drug product. P/T review was completed by this reviewer and archived in DARRTS dated 12/15/2021.
- During the Pre-NDA industry meeting dated 12/15/2021, the Agency provided guidance to the Sponsor; the meeting minutes was archived in DARRTS dated 01/14/2022.
- On 06/28/2022, Bausch & Lomb submitted the current NDA #216675 via the 505(b)(1) pathway, for Perfluorohexyloctane Ophthalmic Solution, 100%, for "*treatment of the signs and symptoms of Dry Eye Disease (DED) associated with Meibomian Gland Dysfunction (MGD)*".

3 Studies Submitted

3.1 Studies Reviewed

- Embryo-Fetal Development Toxicity Study of F6H8 in Rat (Study # 20270706)
- Embryo-Fetal Development Toxicity Study of F6H8 in Rabbit (Study # 20270730)
- Perfluorohexyloctane as a long-term vitreous tamponade in the experimental animal (Zeana, et.al., 1999)
- All other nonclinical studies were previously reviewed (under IND #130558 and/or IND # (b) (4)).

3.2 Studies Not Reviewed

None.

3.3 Previous Reviews Referenced

- Two nonclinical reviews under IND #130558 by Dr. Aling Dong, dated 11/02/2017 and 12/15/2021.
- A nonclinical review under IND # (b) (4) by Dr. Muriel Saulnier dated 03/16/2021.

4 Pharmacology

4.1 Primary Pharmacology

- NOV03 is a clear ophthalmic solution, contain a single component of F6H8. F6H8 is physically, chemically and physiologically inert, and has no known pharmacology activity.
- F6H8 supplements the lipid layer of the tear film, an effect related to its physicochemical properties, such as spreading and film-building properties. Per the submission:

  After eye drop application, perfluorohexyloctane presumably forms a film on the lipid layer of the tear film, which results in stabilization of the tear film and reduction of evaporation of the aqueous phase.

All primary pharmacology studies were previously reviewed by this reviewer under IND 130558 (dated 11/02/2017). In summary:

- Two (2) ex-vivo eye irritation tests (EVEIT) on rabbit corneas showed that F6H8 did not interfere with the corneal healing process after mechanical induction of a corneal erosion, thus is suitable for ocular surface application. (Studies # EVEIT2010 and # EVEIT2016)
- Two (2) in vivo non-GLP rabbit studies showed after four times daily ocular instillations of F6H8 into the right eye of NZW rabbits for 7 days, a trend with an increase of TFBUT (tear film breakup time) 15 minutes after the last administration of F6H8 at all timepoints (Day 1, 3 and 7) were observed when comparing against untreated eyes. (Studies # N38D17113 and # N38D31813)

4.3 Safety Pharmacology

Per the P/T review by this reviewer under IND 130558 dated 11/02/2017:

- CNS (Central Nervous System) safety pharmacology was assessed using Irwin test as part of a 7-day oral maximum tolerated dose (MTD) toxicity study in rats at 1 hour after oral dosing of F6H8 at doses up to 5000 mg/kg/day and did not cause CNS effects. (Study # AB21526)
- No additional safety pharmacology endpoints were assessed.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Two PK/ADME studies (# 8352938 and # 8352939) were previously reviewed by this reviewer under IND 130558 (dated 11/02/2017). In summary:

- Following single or repeated topical ocular dose of ^{14}C-perfluorohexyloctane, low levels of radioactivity were detected in blood and plasma. The concentrations were at least 2-fold higher in the repeat dose group, compared to the single dose group, suggesting possible systemic accumulation of ^{14}C-perfluorohexyloctane. Concentrations declined over time and were approximately 3-fold lower by 24 hours post the last dose compared to the maximal concentration.
- Following the repeated topical ocular dose of ^{14}C-perfluorohexyloctane, rank order of highest mean concentrations (>5000 ng equivalents ^{14}C-F6H8/g) was: tears > meibomian glands > cornea > palpebral conjunctiva > accessory lacrimal gland > main lacrimal gland > bulbar conjunctiva. Low levels of radioactivity were observed in the vitreous humor, choroid-RPE, and retina. The exposure (AUC0-t), with the exception of the aqueous humor, choroid-RPE, iris-ciliary body, posterior sclera, and vitreous humor, was greater than plasma, indicating that substance-related radioactivity was associated with the tissues.
- Single or repeated oral dose of ^{14}C-perfluorohexyloctane to rats resulted in little absorption. The majority of the administered dose was eliminated in feces (up to 70%). Very low levels of radioactivity were also recovered in urine (less than 0.1%), and in bile (less than 0.04%), which indicated that biliary and renal excretion was not a route of elimination for the compound.
- The low percentage recoveries of radioactivity in tissues (less than 0.6%) showed that while low levels of compound were distributed into tissues, there was no notable retention.

TK assessments in two Embryo-Fetal Developmental (EFD) toxicity studies were reviewed by this reviewer under the current NDA. In summary:

- Following daily oral (gavage) administration of F6H8 at doses of 300, 1000 and 2000 mg/kg/day to pregnant Wistar rats during the period of organogenesis (from GD6 to GD17 inclusive), T_{max} was generally observed at 2 hours post-dose. On both GD6 and GD17, C_{max} remained similar despite the increasing dose from 300 to 2000 mg/kg/day while the AUC_{0-24} increased in a less than dose proportional manner from 300 to 1000 mg/kg/day but remained approximately similar between 1000 and 2000 mg/kg/day. The Cmax and AUC_{0-24} increased by up to 2-fold on GD 17 when compared to GD 6 at all dose levels suggesting minimal accumulation. (Study # 20270706)
- Following daily oral (gavage) administration of F6H8 at doses up to 2000 mg/kg/day to pregnant New Zealand White rabbits during the period of organogenesis (from GD6 to GD19 inclusive), T_{max} was between 4 and 8 hours post-dose. There were no marked changes in systemic exposure between GD6 and GD19. C_{max} and AUC_{tlast} values were almost invariant with doses, suggesting

that F6H8 absorption approached saturation at the low dose, which was consistent with its hydrophobic physicochemical properties (Study # 20270730)

6 General Toxicology

6.1 Single-Dose Toxicity+

No studies were conducted.

6.2 Repeat-Dose Toxicity

Two repeat-dose toxicity studies were reviewed by this reviewer under IND 130558 (dated 11/02/2017). In summary:

- Ocular instillation of F6H8 at doses up to 428.8 mg/day bilaterally into the conjunctival sac of New Zealand White (NZW) rabbits four times daily (~3 hours apart) for 4 consecutive weeks was generally well-tolerated, with no systemic toxicity or F6H8 related histopathological findings. The no-observed-adverse-effect level (NOAEL) was 214.4 mg/eye/day (~10 µL dosing volume per eye, QID), or 428.8 mg/day, the highest dose tested in this study. At the NOAEL, systemic exposure achieved 39.4 ng/mL and 17.4 ng/mL for C_{max}, and 99.5 ng.h/mL and 28.4 ng.h/mL for AUC_{0-t}, in males and females, respectively, on Day 24. (Study # AB21563)

- Once daily oral administration of F6H8 at doses up to 2000 mg/kg/day to Wistar rats for 28 consecutive days were well tolerated and did not produce F6H8 related toxicity. The NOAEL of F6H8 was 2000 mg/kg/day, the highest dose in this study. At the NOAEL, systemic exposure achieved 5660 ng/mL for C_{max}, and 129000 ng.h/mL for AUC_{0-t}, males and females combined on Day 27. (Study # AB21624)

One repeat-dose toxicity study (# AB21993) was reviewed by Dr. Saulnier Muriel under IND (b) (4) (dated 03/16/2021). In summary:

- Ocular instillation of F6H8 at the dose of 427.8 mg/animal/day, four times daily bilaterally (~3 hours apart) for up to 26 weeks in NZW rabbit was well tolerated and did not induce any ocular or systemic signs of toxicity. The systemic NOAEL was 427.8 mg/animal/day after 13 or 26 weeks of treatment whereas the ocular NOAEL was 160 µL/eye/day or 213.92 mg/eye/day at a concentration F6H8 of 1337 mg/mL, the highest dose tested in this study.

- At the NOAEL, corresponding C_{max} was 89 ng/mL and 15 ng/mL, and AUC_{0-t} was 702 ng.h/mL and 62 ng.h/mL, in males and females, respectively, in Week 13; and the corresponding C_{max} was 36 ng/mL and 22 ng/mL, AUC_{0-t} was 183 ng.h/mL and 64 ng.h/mL, in males and females, respectively, in Week 26.

7 Genetic Toxicology

- Two in vitro genetic toxicology studies (# 16G12005 and # 17G12002) have been reviewed by this reviewer under IND 130558 (dated 11/02/2017). In summary:

  F6H8 was considered not to induce a mutagenic effect with the Ames test or induce structural chromosome aberrations in cultured human lymphocytes.

- One in vivo clastogenicity assay (# 517990) has been reviewed by Dr. Saulnier Muriel under IND (b) (4) (dated 03/16/2021). In summary:

  F6H8 was not clastogenic or aneugenic in the bone marrow micronucleus test of male rats up to a dose of 2000 mg/kg.

8 Carcinogenicity

No studies were conducted.

9 Reproductive and Developmental Toxicology

9.2 Embryonic and Fetal Development+

9.2.1 Study Title: Embryo-Fetal Developmental Toxicity Study of F6H8 by the Oral (Gavage) Route in the Rat

Study no.:	20270706
Study report location:	Module 4.2.3.5
Conducting laboratory and location:	(b) (4)
GLP compliance:	Y
Drug, lot #, and % purity:	Perfluorohexyloctane (or F6H8), lot # 202050, 100% purity; Saline for injection (or: NaCl 0.9%), lot # 2002090.

Key Study Findings

- Daily oral (gavage) administration of F6H8 at doses up to 2000 mg/kg/day to pregnant Wistar rats during the period of organogenesis (from GD6 to GD17 inclusive) were well tolerated with no toxicological effects on maternal or fetal parameters. In addition, there were no malformations attributed to F6H8 in any of the treated groups.
- Thus, the no observed adverse effect level (NOAEL) for maternal and embryofetal toxicity was the highest dose tested, 2000 mg/kg/day (HED=19459 mg/day), which corresponded to a mean C_{max} and AUC_{0-24} of 3460 ng/mL and 47000 hr*ng/mL on GD6, respectively, and 7270 ng/mL and 74100 hr*ng/mL on GD17, respectively.

Methods

Doses:	0 (saline), 300, 1000 and 2000 mg/kg/day
Frequency of dosing:	Once daily
Number/Sex/Group:	22 pregnant females/group
Dose volume:	See Table 3 below
Formulation/Vehicle:	NaCl 0.9%
Route of administration:	ORAL GAVAGE
Species:	Rat
Strain:	Wistar
Study Design and Conduct:	An EFD study was conducted with dosing during organogenesis (from Gestation Day [GD] 6 to GD17). Caesarean examination was conducted on GD21; systemic exposure of F6H8 was assessed under the defined experimental conditions.

Table 3. Experimental Design of Embryo-Fetal Development Toxicity Study of F6H8 in Rat (Study # 20270706)

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume[a, b] (mL/kg/day)	Dose Concentration (mg/mL)	Number (and Identification) of Animals	
					Main Animals	Toxicokinetics Animals
1	Control item[c]	0	1.5	0	22 (1 to 22)	3 (101 to 103)
2	F6H8	300	0.22	1338	22 (23 to 44)	6 (104 to 109)
3	F6H8	1000	0.75	1338	22 (45 to 66)	6 (110 to 115)
4	F6H8	2000	1.5	1338	22 (67 to 88)	6 (116 to 121)

[a]: Based on the most recent body weight measurement.
[b]: Rounded value based on fixed dose concentration and dose level.
[c]: Saline for injection (NaCl 0.9%) was used as control item.

Observations and Results

F_0 Dams

Mortality (at least twice daily)

No unscheduled death in any group.

Clinical Signs (once daily; detailed clinical observation on each weighing day)

No test article-related effects.

Body Weight (GD0, GD3, GD6, GD9, GD12, GD15, GD18, and GD21)

No test article-related effects.

Feed Consumption (GD0, GD3, GD6, GD9, GD12, GD15, GD18, and GD21)

No test article-related effects.

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

No test article-related effects.

- Pre-implantation data (numbers of corpora lutea, implantations and the percentage pre-implantation loss) were comparable in all groups.
- The mean number of resorptions and percentage post-implantation loss in each of the treated groups were comparable with, or lower than, those in the control group.
- At the terminal caesarean examinations, there were 21, 19, 18 and 19 pregnant females in the control, 300, 1000 and 2000 mg/kg/day groups, respectively, all of

which had live fetuses except for 1 female (# 30) in the 300 mg/kg/day group (which was incidental).

Necropsy/ Histopathology (GD21)

No test article-related effects.

Toxicokinetics (Blood samples collected from maternal animals on GD 6 and GD 17)

Table 4. Blood Sampling Schedule in Pregnant Rat (Study # 20270706)

		Time Postdose on GD6 and GD17					
Group Nos.	**Number of Females**	**0hr[a]**	**1hr**	**2hr**	**4hr**	**8hr**	**24hr**
1	All females	X	-	X	-	X	-
2 to 4	3/group	X	-	X	-	X	-
	3/group	-	X	-	X	-	X

X: Sample collected on toxicokinetic females; -: Not collected.
[a]: Before dosing.

- Following oral administration of F6H8, T_{max} was generally observed at 2 hours postdose at all dose levels on both TK days, except on GD6 at 2000 mg/kg where T_{max} was at 1 hour postdose.
- $T_{1/2}$ was calculated only at 300 mg/kg/day on GD6 and at 1000 mg/kg on GD17, and was 2.82 and 8.91 hours, respectively.
- C_{max}, remained similar despite the increasing dose from 300 to 2000 mg/kg/day.
- AUC_{0-24} increased with increasing dose in a less than dose proportional manner from 300 to 1000 mg/kg/day. From 1000 to 2000 mg/kg/day, the AUC_{0-24} remained approximately similar despite the increasing dose on both GD6 and GD17.

Table 5. TK Parameters in Pregnant Rat Whole Blood on GD6 and GD17 (Study # 20270706)

GD	Dose (mg/kg/day)	C_{max} (ng/mL)	C_{max}/Dose (ng/mL/ (mg/kg/day))	t_{max} (hr)	t_{last} (hr)	AUC_{0-24} (hr*ng/mL)	AUC_{0-24}/Dose (hr*ng/mL/ (mg/kg/day))	$T_{1/2}$ (hr)	R_{AUC}
6	300	3080	10.3	2	24	21200	70.6	2.82	NA
	1000	3860	3.86	2	24	42500	42.5	NRR	NA
	2000	3460	1.73	1	24	47000	23.5	NRR	NA
17	300	6640	22.1	2	24	26200	87.3	NRR	1.24
	1000	8180	8.18	2	24	58000	58.0	8.91	1.36
	2000	7270	3.64	2	24	74100	37.1	NRR	1.58

NA: Not Applicable; hr: Hours; GD: Gestation Day.
R_{AUC} = Accumulation ratio, GD17 AUC_{0-24}/GD6 AUC_{0-24}.
NRR: No reportable result (Rsq was less than 0.8 and/or the extrapolation of the AUC to infinity represented more than 20% of the total area).
As t_{last} was 24 hours for all profiles, AUC_{tlast} was equivalent to AUC_{0-24}.

F_1 Offspring

Terminal Observations (embryofetal viability, fetal weight and sex ratio)

No test article-related effects.

Fetal Malformations/ Variations (external, visceral, skeletal)

No test article-related effects.

9.2.2 Study Title: Embryo-Fetal Developmental Toxicity Study of F6H8 by the Oral (Gavage) Route in the Rabbit

Study no.:	20270730
Study report location:	Module 4.2.3.5
Conducting laboratory and location:	(b) (4)
GLP compliance:	Y
Drug, lot #, and % purity:	Perfluorohexyloctane (or F6H8), lot # 202050, 100% purity; Saline for injection (or: NaCl 0.9%), lot # 2002090.

Key Study Findings

- Following daily oral (gavage) administration of F6H8 at doses of 0 (saline), 250, 500 and 1000 mg/kg/day to pregnant female NZW (New Zealand White) rabbits

during the period of organogenesis (from GD6 to GD19 inclusive), there were abortions in all treated group {6, 3 and 3 females in the 250, 500 and 1000 mg/kg/day groups, respectively}, compared with none in the control group.

- During the dosing period, dose-depended reduced mean body weight (BW) gain was noted in all treated groups. The difference in mean BW gain between treated and control groups was more than -10% (-28%, -53% and -93% in the low, mid and high dose group, respectively), indicating maternal toxicity at all doses. {Recovery was noted in all treated groups during the post-dose period (GD20 to GD29)}
- Reduced food consumption was also noted at a dose-related trend. There were higher incidences of reduced fecal output, soft feces and/or absent urine in all groups during the dosing period compared with the control group, which was consistent with the reduced food consumption. {Recovery was noted in all treated groups during the post-dose period (GD20 to GD29)}
- Thus, a no observed adverse effect level (NOAEL) for maternal toxicity could not be established in rabbits.
- Consistent with the maternal toxicity, mean fetal weight was reduced in all treated groups (up to -12% in the 1000 mg/kg/day group) compared with the control group and the Testing Facility's historical control data (HCD). Note, in the absence of any obvious delay in skeletal ossification, the study report authors considered this finding non-adverse. In addition, there was no increase in embryofetal death in any treated groups.
- In total, there were 1 (1), 7 (5) and 6 (5) fetuses (litters) with external, visceral and/or skeletal malformations in the low, mid and high dose treatment groups as compared with 1 (1) in the concurrent control group. Although the findings were within the range of Testing Facility's HCD (when verified individually), considering the mid and high dose groups have significant increased incidences of these findings (when taken together) compared with the concurrent control group, this reviewer can't totally exclude the potential test article-related effect; thus, agrees with the Applicant on that "*based on the maternal systemic exposure saturation, the developmental NOAEL can be more conservatively established at 250 mg/kg/day for safety margin calculations*".
- As such, the fetal NOAEL was the low dose, 250 mg/kg/day (HED= 4865 mg/day), which corresponded to a mean C_{max} and AUC_{tlast} of 709 ng/mL and 9230 hr*ng/mL on GD6, respectively, and 999 ng/mL and 11600 hr*ng/mL on GD19, respectively.

Methods

Doses:	0 (saline), 250, 500 and 1000 mg/kg/day
Frequency of dosing:	Once daily
Number/Sex/Group:	22 pregnant females/group
Dose volume:	See Table 6 below
Formulation/Vehicle:	NaCl 0.9%
Route of administration:	ORAL GAVAGE
Species:	Rabbit
Strain:	New Zealand White
Study Design and Conduct:	An EFD study was conducted with dosing during organogenesis (from Gestation Day [GD] 6 to GD19). Caesarean examination was conducted on GD29; systemic exposure of F6H8 was assessed under the defined experimental conditions.

Table 6. Experimental Design of Embryo-Fetal Development Toxicity Study of F6H8 in Rabbit (Study # 20270730)

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume[a,b] (mL/kg/day)	Dose Concentration (mg/mL)	Number (and Identification) of Animals
1	Control item[c]	0	0.75	0	22 (1 to 22)
2	F6H8	250	0.19	1338	22 (23 to 44)
3	F6H8	500	0.37	1338	22 (45 to 66)
4	F6H8	1000	0.75	1338	22 (67 to 88)

[a]: Based on the most recent body weight measurement.
[b]: Rounded value based on fixed dose level and dose concentration.
[c]: Control item: NaCl 0.9%.

Observations and Results

F_0 Dams

Mortality (at least twice daily)

Table 7. Applicant-provided Summary of the Mortalities in Rabbits (Study # 20270730)

Dose (mg/kg/day)	Animal No.	Day of Death	Reason for Euthanasia	Associated Signs	Test Item-Related
0	20	GD11	Accidental trauma (fracture)	Swollen and lame left forepaw/ forelimb and abnormal gait	No
250	30	GD16	Abortion	Markedly reduced food consumption and body weight loss (-4%) from GD9	Yes
	34	GD19	Abortion	Reduced food consumption from GD6, body weight loss (-18% from arrival), thin and decreased activity on GD18/19	Yes
	35	GD19	Abortion	Low food consumption and body weight loss (-14%) from arrival (GD0)	No*
	37	GD21	Abortion	Markedly reduced food consumption from GD12 and body weight loss (-6% from GD6)	Yes
	38	GD19	Abortion	Markedly reduced food consumption and body weight loss (-12%) from GD12	Yes
	41	GD24	Abortion	Reduced food consumption and body weight loss (-7%) from GD12	Yes
500	50	GD21	Ethical reasons	Negligible food consumption and body weight loss >20% from arrival (GD0)	No*
	52	GD26	Abortion	Markedly reduced food consumption between GD15 and GD20 and body weight loss (-8% from GD23)	Yes
	54	GD22	Abortion	Low food consumption and body weight loss (-16%) from arrival (GD0)	No*
	64	GD16	Moribund	Laboured breathing and decreased activity just after dosing (likely due to reflux) Oesophagus intact at necropsy	No
	66	GD19	Abortion	Markedly reduced food consumption from GD9 and body weight loss (-14% from arrival)	Yes
1000	77	GD25	Abortion	Reduced food consumption and body weight loss (-8%) from GD18	Yes
	78	GD22	Abortion	Reduced food consumption between GD15 and GD20 and body weight loss (-3% between GD18 and GD20)	Yes
	80	GD26	Ethical reason	Reduced food consumption from GD6 and body weight loss >20%	Yes
	85	GD23	Abortion	Reduced food consumption and body weight loss (-5%) from GD15	Yes

GD: Gestation Day.
*: Cause of the death was mainly related to a difficult acclimatization, possibly exacerbated by F6H8.

- There was one (1) female in each of the 500 and 1000 mg/kg/day groups which was euthanized due to marked body weight loss (greater than 20%) following a difficult acclimatization (animal # 50) or F6H8-related toxicity (animal # 80) and 1 female in each of the control and 500 mg/kg/day groups euthanized following accidental trauma associated with the dose administration procedure.

- One (1) female in each of the control and 500 mg/kg/day groups (animals #20 and #64) were euthanized following trauma associated with the dosing procedure (i.e., fracture after excessive struggling during handling) or with possible reflux of the test item following dosing. The cause of death in the 500 mg/kg/day group was unrelated to F6H8.
- There were 6, 3 and 3 females that aborted in the 250, 500 and 1000 mg/kg/day groups, respectively, compared with none in the control group.

Clinical Signs (once daily; detailed clinical observation on each weighing day)

Higher incidences of reduced fecal output, soft feces and/or absent urine were noted in all treated groups during the dosing period, compared with the control group. The finding was consistent with the reduced food consumption.

Body Weight (GD0, GD3, GD6, GD9, GD12, GD15, GD18, GD20, GD23, GD26 and GD29)

Table 8. Changes in Body Weight Gain and Terminal Body Weight (in Grams, Percent of Difference from the Control)

Period/Dose	0 mg/kg/day	250 mg/kg/day	500 mg/kg/day	1000 mg/kg/day
GD6-GD20	183.4	131.2 (-28%)	85.9 (-53%)	12.5** (-93%)
GD20-GD29	126.7	148.1 (+17%)	122.0 (-4%)	151.0 (+19%)
Terminal Body Weight	4057.9	3917.3 (-3%)	3885.9 (-4%)	3799.4 (-6%)

**: $p \leq 0.01$; GD: Gestation Day.

- Dose-related lower mean body weight gains in all treated groups were noted compared with the control group. The difference in mean body weight gains between treated and control groups was -28%, -53% and -93% in the low, mid and high dose group, respectively, indicating maternal toxicity at all doses.
- However, recovery was noted in all treated groups during the postdose period (GD20 to GD29) such that terminal mean body weight in all treated groups was comparable, or minimally lower than that of the control group.

Feed Consumption (Daily to GD29)

Table 9. Changes in Food Consumption (in Grams, Percent of Difference from the Control)

Period/Dose	0 mg/kg/day	250 mg/kg/day	500 mg/kg/day	1000 mg/kg/day
GD6-GD20	141.76	112.73 (-20%)	104.29* (-27%)	95.62** (-33%)
GD20-GD29	112.25	114.17 (+2%)	105.44 (-6%)	101.22 (-10%)

*: $p \leq 0.05$; **: $p \leq 0.01$; GD: Gestation Day.

- During the dosing period (GD6 to GD20), there was a lower mean food consumption in all treated groups (with a dose-related trend) compared with the control group.
- Recovery was noted in all treated groups during the postdose period (GD20 to GD29) such that mean food consumption in all treated groups was comparable with, or minimally lower that of the control group.

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

- At the terminal caesarean examinations, there were 17, 13, 17 and 18 pregnant females in the control, 250, 500 and 1000 mg/kg/day groups, respectively, all of which had live fetuses.
- No test article-related effects were noted in pre-implantation data (numbers of corpora lutea, implantations and the percentage pre-implantation loss).
- There was an increased mean number of late resorptions at 1000 mg/kg/day (0.9/litter) compared with the concurrent control group (0.1/litter) and the HCD (historical control data) range at the Test Facility (0.1 to 0.6/litter for main EFD studies).
    - Per the study report, the increased mean value was mainly due to two (2) atypical females (animals #73 and #79) in the 1000 mg/kg/day group with a high number of late resorptions (9 and 5, respectively) that disproportionately influenced the mean value.
    - This was consistent with marked maternal toxicity since the 2 females had negligible food consumption (less than 3 g/animal/day during GD15-20 and GD12-18) and lost weight during the dosing period (reduced 190 g and 309 g, respectively).
    - After exclusion of these females, the mean number of late resorptions (0.2/litter) for this treatment group was comparable with the concurrent control group and historical control data.
- The mean number of resorptions were increased in all treated groups, and percentage post-implantation loss in each of the treated groups were higher than

those in the control group. However, number of live fetuses were comparable in all groups.

- Per the study report, "*all values were within the historical control data range at the Test Facility (2.4% to 11.4% for main EFD studies), with the exception of an atypically low value for the concurrent control group. Therefore, the difference from the concurrent control was considered incidental".*

Necropsy/ Histopathology (GD29)
No test article-related effects.

Toxicokinetics (Blood samples collected from maternal animals on GD 6 and GD 19)

Table 10. Blood Sampling Schedule in Pregnant Rabbit (Study # 20270730)

Group No.	Number of females	Time Postdose on GD6 and GD19					
		0hr[a]	1hr	2hr	4hr	8hr	24hr
1 to 4	3/group	X	-	X	-	X	-
	3/group	-	X	-	X	-	X

X: sample collected.
-: not applicable.
Hr: Hour.
[a]: before dosing.

Table 11. TK Parameters in Pregnant Rabbit Whole Blood on GD6 and GD19 (Study # 20270730)

GD	Dose level (mg/kg)	t_{max} (hr)	C_{max} (ng/mL)	C_{max}/Dose (ng/mL)/(mg/kg)	t_{last} (hr)	AUC_{tlast} (hr*ng/mL)	AUC_{tlast}/Dose (hr*ng/mL)/(mg/kg)	R_{AUC}
6	250	8	709	2.84	24	9230	36.9	NA
	500	4	644	1.29	24	11100	22.3	NA
	1000	8	752	0.752	24	8910	8.91	NA
19	250	4	999	3.99	24	11600	46.2	1.25
	500	4	1100	2.19	24	8960	17.9	0.805
	1000	8	1490	1.49	24	18900	18.9	2.12

NA: Not Applicable; hr: Hours; GD: Gestation Day.
R_{AUC}: Accumulation ratio of AUC.

- On both GD6 and GD19, C_{max} were observed between 4 and 8 hours after administration, and F6H8 concentrations were continued to be detectable at 24 hours postdose.
- From GD6 to GD19, the accumulation ratios based on AUC were 1.25, 0.805 and 2.12 at 250, 500 and 1000 mg/kg/day respectively, suggesting no systemic drug accumulation.

- There was no dose proportionality in C_{max} and AUC_{tlast} values which were almost invariant with the dose on GD6 and GD19, suggesting that the absorption of the test item approached saturation already at the low dose, and is consistent with its hydrophobic physicochemical properties.

F_1 Offspring

Terminal Observations (embryofetal viability, fetal weight and sex ratio)

- There was no test article-related effect on embryo-fetal survival and sex ratio in any group.
- There was a reduced mean fetal weight in all treated groups (35.4 g to 37.8 g), with no clear dose-relationship, compared with the concurrent control group (40.2 g) and HCD range at the Test Facility (38.4 g to 43.2 g for main EFD studies). The reduced fetal weight was most pronounced at 1000 mg/kg/day (-12%). The finding was consistent with the maternal toxicity as discussed above.

Table 12. Summary of Mean Fetal Weights in Rabbit (Study # 20270730)

Sex: Female Day(s) Relative to Mating (Litter: A)		0 mg/kg /day Group 1	250 mg/kg /day Group 2	500 mg/kg /day Group 3	1000 mg/kg /day Group 4
Mean Fetal Weight all (g) [G]	Mean	40.15	36.42	37.83	35.36
	SD	4.12	5.10	5.23	6.55
	N	17	13	17	18
	%Diff	-	-9.29	-5.78	-11.92
Mean Fetal Weight males (g) [G]	Mean	40.65	36.53	38.53	35.01**
	SD	4.18	4.92	4.95	6.73
	N	16	13	17	18
	%Diff	-	-10.13	-5.22	-13.89
Mean Fetal Weight females (g) [G]	Mean	39.28	36.61	37.19	35.43
	SD	4.48	5.71	5.81	6.85
	N	16	13	17	18
	%Diff	-	-6.79	-5.32	-9.80

- Per the study report, in the absence of any obvious delay in skeletal ossification, this finding was considered to be non-adverse. In addition, there was no increase in embryofetal death in any dose group.

Fetal Malformations/ Variations (external, visceral, skeletal)

- In total, there were 1 (1), 1 (1), 7 (5) and 6 (5) fetuses (litters) with external, visceral and/or skeletal malformations. However, these findings were incidental and/or noted within the HCD range, thus were not F6H8-related.

Table 13. Summary of Fetal Malformations in Rabbit (Study # 20270730)

Dose Level (mg/kg/day)	Female No.	Fetus No.	Malformation(s)[#]
0	13¤	10	Interrupted aortic arch.
250	32	8	Malformed lumbar vertebrae (misshapen centrum, misaligned and small centrum, fused centra and absent right arch), scoliosis
500	46	1	Absent left kidney and ureter.
		7	Malformed thoracic vertebrae (misshapen centrum, small and misaligned centrum, small and misshapen arch), scoliosis
	47	1	Short tail, fused frontals
		5	Malpositioned and misshapen left testis, fetroesophageal subclavian artery, fused frontals
	48¤	4	Dilated aortic arch
	57	9	Malpositioned left kidney
	60	1	Narrowed aortic arch, ventricular septum defect, dilated pulmonary trunk
1000	68	3	Intestinal fistula
		10	Intestinal fistula
	70	7	Forepaw hyperflexion, severely fused sternebrae
	81	2	Severe retinal fold
	84	10	Spina bifida, splayed lumbar and sacral arches
	88	1	Short tail, Dilated aortic arch, atretic pulmonary trunk, three-chambered heart, malpositioned right subclavian artery, left kidney and ureter absent Malformed thoracic, sacral and caudal vertebrae (incomplete ossification, misshapen, fused and/or small centrum; misshapen, fused and/or absent arches; absent, branched, fused and/or short ribs)

[#]: Including external, visceral and skeletal examinations.
¤: Female Nos. 13 and 48 had the same parents.

- External findings: There were 1 and 3 fetuses from different litters in the 500 and 1000 mg/kg/day groups with external malformations, respectively, compared with none in the control and the 250 mg/kg/day groups.
    - At 1000 mg/kg/day, 1 fetus (from litter # 84) had spina bifida, 1 fetus (from litter # 70) had bilateral hyperflexion of the forepaws and 1 fetus (from litter # 88) had a short tail. The fetus at 500 mg/kg/day (from litter # 47) also had a short tail.
    - Study Report authors stated:
        - *Short tail was not observed in the historical control data at the Test Facility. However, absent tail (i.e., acaudia), considered a most severe finding but of similar nature, was previously noted at similar incidences. Therefore, the short tail was considered incidental.*
        - *Other malformations noted in the high dose only were isolated cases and/or are within the historical control data range for this strain of*

rabbit. Therefore, they were considered incidental and not test item-related.

- This reviewer agrees based upon the same considerations.

- Visceral findings: There were 1 (1), 5 (5) and 4 (3) fetuses (litters) in the control, 500 and 1000 mg/kg/day groups, respectively, with visceral malformations. No visceral malformations were noted at 250 mg/kg/day.
  - One fetus in each of the 500 and 1000 mg/kg/day groups (litters #60 and #88) had a malformed heart associated with malformed great blood vessels (three-chambered heart or ventricular septum defect, dilated or narrowed aortic arch, atretic or dilated pulmonary trunk and/or malpositioned right subclavian artery origin).
  - In the 500 mg/kg/day group, 1 fetus (litter # 47) had a retroesophageal right subclavian artery, 1 other fetus (litter # 48) had a dilated aortic arch; and 1 control fetus (litter # 13) had an interrupted aortic arch.
  - Study Report authors stated:
    - *These findings in the heart and great blood vessels are part of the background data for this strain of rabbit at similar incidences and were therefore considered as incidental. In addition, it is of note that both fetuses presenting abnormalities of the aortic arch (from Litters # 13 and 48) had the same genealogy (i.e., same parents) and therefore a genetic cause was possible.*

Exam Type: FreshVisBody		0 mg/kg /day Group 1	250 mg/kg /day Group 2	500 mg/kg /day Group 3	1000 mg/kg /day Group 4
Number of Fetuses Examined:		153	117	141	156
Number of Fetuses Evaluated:		153	117	141	156
Number of Litters Examined:		17	13	17	18
Number of Litters Evaluated:		17	13	17	18
Heart					
Heart, Three-chambered heart - Malformation	Fetuses N(%)	0(0.00)	0(0.00)	0(0.00)	1(0.79)
	Litters N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.6)
Heart, Memb Ventricular Septal Defect - Malformation	Fetuses N(%)	0(0.00)	0(0.00)	1(0.74)	0(0.00)
	Litters N(%)	0(0.0)	0(0.0)	1(5.9)	0(0.0)
Ventricle, Small - Variation	Fetuses N(%)	0(0.00)	0(0.00)	2(1.58)	0(0.00)
	Litters N(%)	0(0.0)	0(0.0)	2(11.8)	0(0.0)
Innominate artery					
Innominate artery, Absent - Variation	Fetuses N(%)	0(0.00)	0(0.00)	0(0.00)	2(1.11)
	Litters N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.6)
Intestine					
Intestine, Fistula - Malformation	Fetuses N(%)	0(0.00)	0(0.00)	0(0.00)	2(1.23)
	Litters N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.6)

[Fetuses %] - Kruskal-Wallis & Dunn

- This reviewer identified a [redacted] (b) (4) historical control database (HCD) online [1] which includes data from the performing laboratory in [redacted] (b) (4) for NZW Rabbits (2001 to 2018). Per this HCD database, the mean litter incidence for these cardiovascular findings was 7.39% (with range of 4.76 to 15.00%), which provides additional support that the findings were not test article related.

- Among the fetuses with malformations of the heart and/or great blood vessels, several also presented abnormalities of the urogenital tract (kidney and/or ureters). One fetus in each of the 500 and 1000 mg/kg/day groups (litters #46 and #88) had an absent kidney and ureter. A malpositioned kidney was also noted for 1 other fetus at 500 mg/kg/day (litter # 57).

Table 14. Fetal (Litter) Incidences of Major Malformations of the Urogenital Tract (Number of Affected Fetuses, % of Affected Fetuses, % of Affected Litters in Bracket)

	HCD Incidence Ranges	Doses (mg/kg/day)			
		0	250	500	1000
Number of fetuses (litters)	5360 (579)	153 (17)	117 (13)	141 (17)	156 (18)
Kidney and Ureters					
Absent kidney	1 0-0.5% (0-5.3%)	-	-	1 0.53% (5.9%)	1 0.79% (5.6%)
Malpositioned kidney	6 0-1.7% (0-5.6%)	-	-	1 0.84% (5.9%)	-
Genital					
Malpositionned testis	3[#] 0-0.6%	-	-	1 0.65% (5.9%)	-
Misshapen testis	-	-	-	1 0.65% (5.9%)	-

HCD: Historical Control Data.
-: No abnormalities detected; [#]: Count and fetal incidence range from 2013-2015 HCD.

- As shown in Table 14 above (and verified per the online database), these findings were previously noted at similar incidences in the HCD (historical control data). Thus, this reviewer agrees that they were incidental.

- A fetus {fetus # 5} in the 500 mg/kg/day group (litter # 47) had a mishappen and malpositioned testis. Per the study report:

1 [redacted] (b) (4)

- *Three cases of malpositioned testis were noted in previous historical control data (2013 to 2015) at a similar fetal incidence (up to 0.6%) and therefore this finding was considered as incidental*.

- Two fetuses from the same litter at 1000 mg/kg/day (litter # 68) had an intestinal fistula. Per the study report:
  - *Even if the malformation was not previously noted in the historical control data, the presence of 2 cases within a single litter suggests a genetic cause*.
- One fetus in the 1000 mg/kg/day (litter # 81) had a severe retinal fold. This isolated case was noted in the online HCD database; thus, was considered incidental.

- Skeletal findings: There were 1 (1), 3 (2) and 4 (4) fetuses (litters) in the 250, 500 and 1000 mg/kg/day groups, respectively, with skeletal malformations compared with none in the control.
  - One fetus in each of the 250, 500 and 1000 mg/kg/day had malformed vertebrae (lumbar, thoracic, sacral and/or caudal), associated or not with scoliosis. These types of abnormalities are part of the background data for this strain of rabbit (as verified per the online HCD database) and were considered incidental.

Table 15 Fetal (Litter) Incidences of Major Skeletal Changes of the Body (Number of Affected Fetuses, % of Affected Fetuses, % of Affected Litters in Bracket)

	HCD Incidence Ranges	Doses (mg/kg/day)			
		0	250	500	1000
Number of fetuses (litters)	5269 (570)	153 (17)	117 (13)	141 (17)	156 (18)
Malformed vertebrae (general)	1[#] 0-0.8% (0-5.6%)	-	1 0.77% (7.7%)	1 0.53% (5.9%)	1 0.79% (5.6%)

HCD: Historical Control Data.
-: No abnormalities detected.
[#]: Due to a change in the method of examination in 2019, this abnormality was also previously recorded as malformed vertebrae (without mention of general) in 2 fetuses (fetal range: 0-1.3% and litter range: 0-5.3%).

- *At 1000 mg/kg/day, consistent with the spina bifida seen externally, splayed lumbar and sacral arches were noted for 1 fetus (litter # 84). in addition, severely fused sternebrae were noted for 1 fetus (litter # 70). These isolated findings are part of the background data for this strain of rabbit and were therefore considered incidental.*

- *At 500 mg/kg/day, 2 fetuses from the same litter (# 47) had fused frontal bones. These isolated findings, also noted in the historical control data, were incidental and the presence in the same litter suggests a genetic cause.*
- *Incidences of the less severe skeletal variations were comparable with the concurrent control and/or historical control data.*

Table 16 Fetal (Litter) Incidences of Selected Less Severe Skeletal Variations (Number of Affected Fetuses, % of Affected Fetuses, % of Affected Litters in Bracket)

	HCD Incidence Ranges	Doses (mg/kg/day)			
		0	250	500	1000
Number of fetuses (litters)	5269 (570)	153 (17)	117 (13)	141 (17)	156 (18)
Incomplete Ossification					
Phalanges (forepaws)	23[#] 0-2.9% (0-20.0%)	-	5 4.48% (30.8%)	7 5.09% (17.6%)	6 3.52% (16.7%)
Metacarpal	11 0-1.7% (0-11.1%)	-	2 1.51% (15.4%)	5 3.83 (11.8%)	-
Pubis	123 0-4.8% (0-30%)	-	5 4.81% (38.5%)	3 1.58% (11.8%)	12** 6.43% (44.4%)
Unossified					
Phalanges (forepaws)	16# 0-1.7% (0-15.0%)	-	1 1.10% (7.7%)	1 0.84% (5.9%)	-
Phalanges (hindpaws)		-	2* 1.65% (15.4)	-	-
Talus	4 0-1.0% (0-11.1%)	2 1.31% (5.9%)	5 3.67% (30.8%)	3 1.38% (11.8%)	10 5.08% (16.7%)
Pubis	22 0-2.2% (0-16.7%)	-	1 0.55% (7.7%)	1 0.42% (5.9%)	2 1.26% (11.1%)

HCD: Historical Control Data.
-: No abnormalities detected; *: $p \leq 0.05$; **: $p \leq 0.01$.
[#]: Due to a change in the method of examination in 2019, variations in phalanx were previously recorded, whaterver the paw, as incomplete ossification of phalanx (proximal) and unossified phalanx. The corresponding values are presented.

This reviewer verified the above statement regarding the skeletal findings per the online HCD database by the performing laboratory.

10 Special Toxicology Studies

Perfluorohexyloctane as a long-term vitreous tamponade in the experimental animal (Damiana Zeana, et al., International Ophthalmology 23: 17–24, 1999; Module 4.3) - This study investigated intraocular tolerance to perfluorohexyloctane as a long-term vitreous substitute in the experimental animal.

Methods:

- Chinchilla bastard rabbits (n = 34) were vitrectomized under anesthesia, and the vitreous body was replaced by filling with1.0–1.2 ml perfluorohexyloctane or balanced salt solution (BSS).
- During the observation period of 3 months, the eyes were examined by slit lamp biomicroscopy, fluorescein angiography and ERG (electroretinography). At the end of the observation period, the animals were euthanized and the eyes were processed for light- and electron microscopy examination.

Results:

- Perfluorohexyloctane depicted dispersion beginning between the first and third week. Over a period of 9 weeks, no toxic effect on retina, lens and cornea was noticed. At 14 weeks, ERG showed a slight decrease in amplitude and early morphological changes in the retina.
- The findings suggest that perfluorohexyloctane is tolerated when be filled into vitreous of the rabbit eyes for 9 weeks.

11 Integrated Summary and Safety Evaluation

This NDA was submitted under the 505(b)(1) pathway for Perfluorohexyloctane Ophthalmic Solution, 100% (Miebo™), for treatment of the signs and symptoms of Dry Eye Disease (DED) associated with Meibomian Gland Dysfunction (MGD).

In a 26-week ocular toxicity study in rabbits, ocular instillation of perfluorohexyloctane ophthalmic solution, 100% (code name: F6H8) at the dose of 427.8 mg/day, four times daily bilaterally for up to 26 weeks was well tolerated and did not induce any ocular or systemic signs of toxicity. The systemic no-observed-adverse-effect level (NOAEL) was 427.8 mg/animal/day whereas the ocular NOAEL was 213.92 mg/eye/day. At the proposed clinical dosing regimen, the ocular exposure margin is 3.6X for the MRHD (60 mg/eye/day), and the systemic exposure margin is 39X for the MRHD (120 mg/day).

In a 28-day oral toxicity study in rats, once daily oral administration of F6H8 at doses up to 2000 mg/kg/ for 28 days were well tolerated and did not produce F6H8 related toxicity. The NOAEL of F6H8 was 2000 mg/kg/day, the highest dose tested in this study.

In an embryofetal developmental (EFD) toxicity study in rats, daily oral administration of F6H8 at doses up to 2000 mg/kg/day to pregnant Wistar rats during the period of

organogenesis (from GD6 to GD17 inclusive) was well tolerated with no toxicological effects on maternal or embryofetal parameters. Thus, the NOAEL for maternal and embryofetal toxicity was the highest dose tested, 2000 mg/kg/day (HED=19459 mg/day). At the proposed clinical dosing regimen, the systemic exposure margin is 162X for the MRHD (120 mg/day).

In an EFD toxicity study in rabbits, following daily oral administration of F6H8 at doses of 0 (saline), 250, 500 and 1000 mg/kg/day to pregnant female NZW rabbits during the period of organogenesis (from GD6 to GD19 inclusive), there were abortion in all treated group (6, 3 and 3 females in the 250, 500 and 1000 mg/kg/day groups, respectively), compared with no abortion in the control group.

- During the dosing period, dose-depended reduced mean body weight (BW) gain was noted in all treated groups (as -28%, -53% and -93% in the low, mid and high dose group, respectively). Reduced food consumption was also noted at a dose-related trend. There were higher incidences of reduced fecal output, soft feces and/or absent urine in all groups during the dosing period compared with the control group, which was consistent with the reduced food consumption. Thus, a NOAEL for maternal toxicity could not be established in rabbits.
- Consistent with the maternal toxicity, mean fetal weight was reduced in all treated groups compared with the concurrent control group and the Testing Facility's historical control data (HCD). Note, there was no increase in embryofetal death nor test article-related delay in skeletal ossification in any treated groups.
- There were 1 (1), 7 (5) and 6 (5) fetuses (litters) with fetal malformations (external, visceral and/or skeletal) in the low, mid and high dose treatment groups as compared with 1 (1) in the control group. Although the findings were within the range of Testing Facility's HCD (when verified individually), considering the significant increased incidences of these findings (when taken together) in the mid and high dose groups compared with the concurrent control group, this reviewer agrees with the Applicant on that "*based on the maternal systemic exposure saturation, the developmental NOAEL can be more conservatively established at 250 mg/kg/day for safety margin calculations*".
- As such, the fetal NOAEL was the low dose, 250 mg/kg/day (HED= 4865 mg/day). At the proposed clinical dosing regimen, the systemic exposure margin is 41X for the MRHD (120 mg/day).

Table 17 Systemic Exposure Margins Per the Pivotal Toxicity Studies

Clinical Exposure Margins (Based on Dose)			
Species/Type of Study	NOAEL (mg/kg/day or mg/day)	HED (mg/day)	Exposure Margins (Based on a MRHD of 120 mg/day
Rabbit/26-week ocular	427.8 (mg/day)	4625*	38.54X
Rat/ EFD	2000 (mg/kg/day)	19459	162X
Rabbit/ EFD	250 (mg/kg/day) (for fetal)	4865	40.54X

NOAEL = no-observed-adverse-effect level
MRHD = maximum recommended human dose
HED = human equivalent dose (based on body surface area)
* Calculated based on 1.8 kg body weight for rabbits

Table 18 Ocular Exposure Margins Per the Pivotal Toxicity Studies

Species/Type of Study	NOAEL (mg/eye/day)	Exposure Margins (Based on a MRHD of 60 mg/eye/day)
Rabbit/26-week ocular	213.9	3.6X

NOAEL = no-observed-adverse-effect level
MRHD = maximum recommended human dose

As such, P/T has no objection to the approval of Perfluorohexyloctane Ophthalmic Solution, 100%.

In addition, minor format and language changes were made in Sections 8.1, 8.2, 12.1, and 13 of the Applicant-proposed labeling text to adhere to PLLR guidance and to provide consistency of language across paragraphs of the labeling.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

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Concur with AP