CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

207795Orig1s000

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 REVIEW AND EVALUATION

Application number:	207795
Supporting document/s:	SD 30 (Resubmission)
Applicant's letter date:	February 24, 2017
CDER stamp date:	February 24, 2017
Product:	Latanoprostene bunod (Vyzulta)
Proposed indication:	Reduction of intraocular pressure for patients with open-angle glaucoma of ocular hypertension
Applicant:	Bausch & Lomb Inc. Bridgewater, New Jersey 08807
Review Division:	Division of Transplant and Ophthalmology Products (DTOP), Office of Antimicrobial Products (OAP), CDER, HFD-590
Reviewer:	Andrew J. McDougal, PhD, DABT, DTOP
Supervisor/Team Leader:	Lori E. Kotch, PhD, DABT, DTOP
Division Director:	Renata Albrecht, MD, DTOP
Project Manager:	Lois Almoza

1 Executive Summary

- On July 21, 2015, pursuant to Section 505(b)(1) of the Federal Food, Drug and Cosmetic Act and 21 CFR §314.50, the applicant and sponsor, Bausch & Lomb Incorporated (B & L; a wholly-owned subsidiary of Valeant Pharmaceuticals International)" submitted an original new drug application (NDA #207795) for VYZULTA® (latanoprostene bunod ophthalmic solution) 0.024%, for the reduction of intraocular pressure (IOP) in patients with open-angle glaucoma or ocular hypertension.
- From a nonclinical perspective, no safety issues would have precluded approval (McDougal, 5/20/2016; Jacobs 5/23/2016; NDA 207795).
- A Complete Response letter was conveyed to the Applicant on July 21, 2016. A type A meeting was held between the Division and the Applicant on September 1, 2016. A labeling discussion teleconference was held on September 7, 2016.
- The Applicant's Class 2 Resubmission was received on February 24, 2017. The Applicant has proposed revisions to the nonclinical sections of labeling, which are reviewed below.
- Latanoprost bunod (LBN) is a new molecular entity (NME). IND 73435 is the only predecessor IND for this NDA submission.
- The internal electronic document room (EDR) location is \\CDSESUB1\evsprod\NDA207795\207795.enx

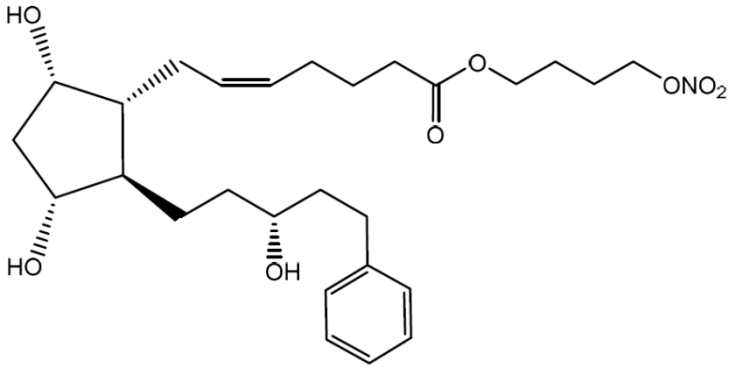
2 Drug Information

2.1 Drug

Chemical Abstracts Service (CAS) Registry Number	860005-21-6
Established name	Latanoprostene bunod
Trade names	• VYZULTA (accepted by CDER) • Vesneo (proposed by the Applicant but rejected by CDER)
Code names	• (b) (4) • BOL-303259-X (Bausch & Lomb) • PF-03187207 (Pfizer) • NCX-116 (Nicox) • (b) (4) • (b) (4)
Chemical name	4-(Nitrooxy)butyl (5Z)-7-{(1R,2R,3R,5S)-3,5-dihydroxy-

	2-[(3R)-3-hydroxy-5-phenylpentyl]cyclopentyl}hept-5-enoate
Molecular formula	$C_{27}H_{41}NO_8$
Molecular weight	507.62 g/mol
Pharmacologic class[1]	prostaglandin F2α analogue

Figure 1: Structure of latanoprostene bunod (LBN)



Note: structure of LBN has 5 chiral centers.

2.2 Notable Cross-Reference

As noted previously (McDougal, 5/20/2016, NDA 207795), NDA 207795 is submitted under the 505(b)(1) pathway. The NDA includes a letter of authorization from Pharmacia & Upjohn Co. for NDA 025097 (Xatalan®, latanoprost ophthalmic solution 0.005%). NDA 207795 included the embryofetal development (EFD) studies for latanoprost, as supporting information.

[1] Note: the pharmacologic class for Xalatan® (NDA 20597) is "prostaglandin F2α analogue". For latanoprost bunod, the Applicant proposed (b) (4) (b) (4)

3 Current Proposed Labeling (SD 30)

- No new nonclinical studies were submitted in the 2/24/2017 resubmission. The Applicant cross-referenced previously submitted information.
- Based on the CDTL review (Boyd, 6/17/2016) and Division Director Review (Chambers, 6/12/2016), the Division conveyed labeling recommendations to the Applicant (Almoza, 6/22/2017, NDA 207795).
- This table compares the DTOP labeling as conveyed to the Applicant, to the Applicant's labeling proposed 2/24/2017, with P/T recommendations.
    - Note: the Applicant's annotated draft labeling did not track changes from the DTOP version to their most recent version.
- *Note:* the top line of each cell is the section of the labeling in which the following statement is found. Then statement being proposed/edited (i.e. the top line text and the statement below it are not necessarily contiguous in the actual labeling).

FDA proposed text (Almoza, 6/22/2016)	Applicant's proposed text with their annotation (2/24/2017)	PT recommendations
8 USE IN SPECIFIC POPULATIONS **8.1 Pregnancy** <u>Risk Summary</u> There are no available human data for the use of VYZULTA during pregnancy to inform any drug associated risks. Latanoprostene bunod (b) (4) (b) (4) (b) (4) has caused miscarriages, abortion, and fetal harm in rabbits. Latanoprostene bunod was shown to be abortifacient and teratogenic when administered intravenously (IV) to pregnant rabbits at exposures ≥ 0.28 times the clinical dose. Doses ≥ 20 µg/kg/day (23 times the clinical dose) produced 100% embryofetal lethality. Structural abnormalities observed in rabbit fetuses included anomalies of the great vessels and aortic arch vessels, domed head, sternebral and vertebral skeletal anomalies,	**8 USE IN SPECIFIC POPULATIONS** **8.1 Pregnancy** <u>Risk Summary</u> There are no available human data for the use of VYZULTA during pregnancy to inform any drug associated risks. Latanoprostene bunod (b) (4) (b) (4) (b) (4) has caused miscarriages, abortion, and fetal harm in rabbits. Latanoprostene bunod was shown to be abortifacient and teratogenic when administered intravenously (IV) to pregnant rabbits at exposures ≥ 0.28 times the clinical dose. Doses ≥ 20 µg/kg/day (23 times the clinical dose) produced 100% embryofetal lethality. Structural abnormalities observed in rabbit fetuses included anomalies of the great vessels and aortic arch vessels, domed head, sternebral and vertebral skeletal anomalies,	Reject.

limb hyperextension and malrotation, abdominal distension and edema.	limb hyperextension and malrotation, (b) (4) abdominal distension and edema.	
8.1 Pregnancy Risk Summary Latanoprostene bunod was not teratogenic in the rat at (b) (4) (b) (4)	**8.1 Pregnancy** Risk Summary Latanoprostene bunod was not teratogenic in the rat when administered IV at 150 mcg/kg/day (87 times the clinical dose). (b) (4)	Accept. "Latanoprostene bunod was not teratogenic in the rat when administered IV at 150 mcg/kg/day (87 times the clinical dose)."
8.1 Pregnancy Data *Animal Data* … (b) (4)	**8.1 Pregnancy** Data *Animal Data* … (b) (4)	Reject. [The Applicant proposed adding the paragraph to the end of the Animal Data.]
12 CLINICAL PHARMACOLOGY **12.1 Mechanism of Action** (b) (4) topical ocular administration, latanoprostene bunod is rapidly metabolized (b) (4) (b) (4)	**12 CLINICAL PHARMACOLOGY** **12.1 Mechanism of Action** (b) (4) topical ocular administration, latanoprostene bunod is rapidly metabolized (b) (4) (b) (4)	Accept.

11 Brief Summary of Nonclinical Points

- For the September 1, 2016 teleconference, minutes are available (Almoza, 9/29/2016, NDA 207795). At the 9/01/2016 teleconference, concurrence was reached that the Applicant would not conduct additional nonclinical toxicology studies to support labeling.
- At the 9/07/2016 meeting, the Applicant expressed understanding of P/T review conclusions for the EFD studies.

Applicant's proposed change	Discussion
P/T had proposed to use the wording 'Latanoprostene bunod was not teratogenic in the rat at (b) (4) (b) (4) to describe the 87x exposure margin. The Applicant proposes to add complexity to the Risk Summary, by specifying the NOAEL.	• The rat EFD studies identified a maternal LOAEL of 1500 µg/kg (870 times the clinical dose based on BSA). • The developmental NOAEL was 150 µg/kg (87x), the lowest dose tested in the rat GLP EFD study (report # 20073521). The developmental LOAEL was 300 µg/kg (174x), the mid-dose. The rat EFD range-finder tested the same and higher doses, but not lower doses. • (b) (4) to describe the 87x exposure margin is technically imprecise ○ One dose level, with a range of exposures, was tested. ○ The NOAEL was above what would generally be considered a (b) (4) (b) (4) • Therefore, the Applicant's proposed revision is acceptable.
In Section 8.1, the Applicant proposes to add the word (b) (4) P/T recommends rejecting this change.	• The authors of the EFD studies considered distended abdomen detected by external examination to be a malformation, and this reviewer concurs. • For the rabbit EFD range-finder (report # 20073522), one fetus (5413-7) in the 5 µg/kg group did exhibit both edema of the neck and abdominal distension. The two observations are linked for this animal. • For the rabbit GLP EFD study (report # 20073523), one mid-dose fetus (1.2 µg/kg) and two high-dose fetuses (6 µg/kg) exhibited distended abdomen without a finding of edema being recorded. Of these, one (high-dose # 2271-R2) had persistent truncus

	arteriosus and ventricular septum defect (i.e. clear cause for the abdominal distension). • Therefore, P/T recommends rejecting the Applicant's proposed (b) (4). • Adding a comma (the Oxford comma) after the word 'distension' would further clarify that the two findings are not necessarily inseparable. This grammar mark is not readability, and therefore is not recommended.
The Applicant proposes (b) (4) (b) (4)	• The Applicant has right-of-reference to the latanoprost EFD study reports (and submitted the reports to the NDA). • (b) (4) • The study reports have been reviewed by P/T previously. • (b) (4) •

	(b) (4). • The labeling does summarize latanoprost nonclinical data when no latanoprostene bunod data are available (i.e. fertility and carcinogenicity). • (b) (4)
The Sponsor made an agreed-upon change to Section (b) (4), deleting the sentence (b) (4) (b) (4)	• This sentence, (b) (4) (b) (4) • At a September 2016 teleconference, this wording was discussed. The Applicant noted that this sentence does not provide information to the reader, and proposed to delete it. DTOP concurred.

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/s/

ANDREW J MCDOUGAL
08/01/2017

LORI E KOTCH
08/01/2017

Comments on NDA 207795 lantanoprostene bunod

From: A. Jacobs, AD

Date: 5/23/16

1. I concur that there are no pharm-tox approval issues.

2. I have conveyed other comments to the reviewer and they will be addressed as appropriate.

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/s/

ABIGAIL C JACOBS
05/23/2016

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:	207795
Supporting document/s:	• SD 1 (new NDA) • SD 10 (Response to Nonclinical Information Request, received 1/27/2016) • SD 16 (Response to Nonclinical Information Request, received 3/30/2016) • SD18 (Response to Nonclinical Information Request, received 4/27/2016)
Applicant's letter date:	July 21, 2015
CDER stamp date:	July 21, 2015
Product:	Latanoprostene bunod (Vyzulta)
Proposed indication:	Reduction of intraocular pressure for patients with open-angle glaucoma of ocular hypertension
Applicant:	Bausch & Lomb Inc. Bridgewater, New Jersey 08807
Review Division:	Division of Transplant and Ophthalmology Products (DTOP), Office of Antimicrobial Products (OAP), CDER, HFD-590
Reviewer:	Andrew J. McDougal, PhD, DABT, DTOP
Supervisor/Team Leader:	Lori E. Kotch, PhD, DABT, DTOP
Division Director:	Renata Albrecht, MD, DTOP
Project Manager:	Lois Almoza

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1 Executive Summary

1.1 Introduction

- "Pursuant to Section 505(b)(1) of the Federal Food, Drug and Cosmetic Act and 21 CFR §314.50, the applicant and sponsor, Bausch & Lomb Incorporated (B & L; a wholly-owned subsidiary of Valeant Pharmaceuticals International) is submitting an original new drug application (NDA #207795) for VESNEO™ [name changed to VYZULTA®] (latanoprostene bunod ophthalmic solution) 0.024%, for the reduction of intraocular pressure (IOP) in patients with open-angle glaucoma or ocular hypertension."
- The internal electronic document room (EDR) location is \\CDSESUB1\evsprod\NDA207795\207795.enx
- Latanoprost bunod (LBN) is a new molecular entity (NME).
- The PDUFA goal date is July 21, 2016.
- IND 73435 is the only predecessor IND for this NDA submission.
- This NDA for LBN was submitted under the 505(b)(1) pathway, and no listed drug product is referenced. Notably, (b) (4) (b) (4) NDA 20597 for latanoprost are cross-referenced, as their nonclinical data provides some support for the characterization of LBN. Letters of authorization were provided, and selected (but not all) nonclinical study reports from (b) (4) NDAs were submitted to the current NDA (#207795).

1.2 Brief Discussion of Nonclinical Findings

Background - class

- Prostaglandin F2α analogues are a class of drugs which includes latanoprost[1], travoprost[2], bimatoprost[3], tafluprost[4] (the ophthalmic prostaglandins), and carboprost[5] (intramuscular administration for specific non-ophthalmic indications).
- Latanoprost was approved in 1996, and is indicated for the reduction of elevated intraocular pressure in patients with open-angle glaucoma or ocular hypertension. Latanoprost is an isopropyl ester prodrug and a prostaglandin F2α analogue; it is absorbed through the cornea and is hydrolyzed to latanoprost

[1] NDA 20597/S-045 and S-048 label for latanoprost (Xalatan®)'s 2014 label was accessed via: http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/020597s045s048lbl.pdf
[2] NDA 21257/S-025 label for travoprost (Travatan®)'s 2011 label accessed via: http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/021257s025lbl.pdf . Travoprost is also the subject of NDA 21994 and NDA 204822.
[3] NDA 22184/S-005 for bimatoprost (Lumigan®)'s 2014 label accessed via http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/022184s005lbl.pdf . Bimatoprost is also the subject of NDA 21275 and NDA 22369.
[4] NDA 202514 for trafluporst (Zioptan®)'s 2015 label accessed via: http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/202514s003s004lbl.pdf
[5] NDA 017989 for carboprost tromethamine (hemabate)'s 2013 label was accessed via: http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/017989s019lbl.pdf

acid. Latanoprost acid is the active metabolite, a prostaglandin F receptor (FP receptor) agonist. Ocular exposure to latanoprost acid activates the FP receptor, increasing uveoscleral outflow, and thereby reducing intraocular pressure (IOP).

LBN: putative mechanisms of action for IOP lowering

- Latanoprostene bunod (LBN, PF-0318707, BOL-303259-X) is a pro-drug consisting of latanoprost acid covalently bound by an ester linkage to 4-hydroxybutyl nitrate (butanediol mononitrate [BDMN]; nitrooxy butyl alcohol [NOBA]).
    - The Applicant has shown that topical ocular administration of LBN results in rapid appearance of latanoprost acid (a prostaglandin receptor agonist). LBN has IOP-lowering activity in animals attributable to latanoprost acid.
    - The Applicant proposes that LBN is metabolized by esterases to latanoprost acid and to free BDMN. The Applicant further proposes that BDMN releases nitric oxide (NO), at sufficient local concentrations to relax the trabecular meshwork of the eye, further lowering IOP.
    - However, no experimental work was submitted to determine whether LBN releases any NO in ocular tissues.
- At sufficient local concentrations, nitric oxide (and other organic nitrates) are known to reduce IOP. A fundamental question for this NDA is whether the amount of NO (if any) resulting from topical ocular LBN dosing is enough to meaningfully affect IOP, local toxicity, and systemic toxicity.

Nonclinical data for LBN:

In vitro pharmacodynamics (PD)	No clear proof-of-concept. The data support the biological plausibility of LBN might target the trabecular meshwork cells (to cause relaxation → increased aqueous humor outflow → decreased IOP).
In vivo PD	• LBN had slight IOP lowering activity (-0.45 to -1.23 mm Hg), in a FP receptor knock-out mouse model. Because latanoprost acid is inactive in this model, the decrease may be attributable to NO activity on the trabecular meshwork. From a P/T perspective, the potential clinical significance of this magnitude of IOP lowering is unclear. • LBN appeared more active than latanoprost for IOP lowering in dog and rabbit models for IOP, but the reliability of the reporting is low, due to unexplained data omissions.

	• In monkeys that underwent lasering of the trabecular meshwork to increase IOP; LBN was not more effective than latanoprost at decreasing IOP. These findings do not support or refute the Applicant's proposed mechanism of action for additional IOP lowering via NO relaxation of the trabecular meshwork.
Safety pharmacology	• Electrocardiography (ECG) was measured in the 28-day and 9-month topical ocular toxicity studies. No safety concerns were identified. • No other safety pharmacology endpoints were assessed nonclinically.
Ocular distribution	• Following topical ocular distribution, LBN could not be detected in monkey eyes (lower limit of detection [LLOQ] = 5 mg/ml in aqueous humor and 0.1 to 0.5 ng for tissues). • A bridging study in rabbits showed that ocular distribution of latanoprost acid from LBN was similar for the Phase 1/2 formulation (phosphate- buffered) and the Phase 3 formulation (citrate-buffered).
Topical ocular dosing	• Transient minimal-to-moderate ocular inflammation was observed for 1 or a few days after the first dose (rabbits and monkeys). • For the 28-day monkey study, the high-dose, 0.04% bid (24 µg/eye/day) [Phase 1/2 phosphate-buffer formulation] was the NOAEL for ocular and systemic toxicity. • A second 28-day monkey study was performed, to qualify 2 impurities [Phase 3 citrate-buffer formulation]. The only dose tested was 0.04%, 2 drops/dose bid OD (48 µg/eye/day). Gross pathology for one treated monkey (1/8)

	observed uncollapsed lung, without microscopic correlate. The significance of this lesion is unclear; this reviewer presumes it is related to the lung toxicity observed in the 9-month study (i.e. grossly-apparent pleural fibrosis changing the appearance of the lung). No other treatment-related effects were observed. • For the 9-month monkey study, no ocular NOAEL was identified [Phase 3 citrate-buffer formulation]. The systemic NOAEL was the low-dose (14.4 µg/eye/day, equivalent to 3.6 µg/kg/day, or 43.2 µg/m^2), and the systemic LOAEL was the mid-dose (24.0 µg/eye/day, equivalent to 6.8 µg/kg/day, or 81.5 µg/m^2), o Iris hyperpigmentation was observed at the low-dose (0.024% bid) and the high-dose (two 0.040% drops/dose, bid). Because iris hyperpigmentation is considered potentially adverse, this study did not identify an ocular NOAEL. o Aside from iris hyperpigmentation, the ocular NOAEL would be the high-dose (48 µg/eye/day). o Minimal-to-slight perivascular lymphocyte /macrophage infiltrates of the episclera was observed in all LBN-dose groups, but was not considered adverse. o For pleural/subpleural chronic fibrosis/inflammation, the NOAEL was the low-dose. In males, the LOAEL was the mid-dose (minimal-to-slight severity); severity and incidence increased in the male high-dose group (1 minimal, 1 slight, 1 moderate).

Embryofetal toxicity	• Prostaglandin analogs (i.e. latanoprost, travoprost, bimatoprost, tafluprost, carboprost).are expected to be abortifacient (i.e. primary pharmacology resulting from systemic exposure), Travoprost and tafluprost were associated with structural teratogenesis in rats. • In rabbits, LBN was abortifacient and teratogenic at the lowest-dose tested (0.24 mg/kg iv), with clear dose-responses for developmental toxicity. • In rats, the developmental NOAEL was 150 µg/kg. LBN increased embryofetal lethality and produced malformations at 300 and 1500 µg/kg. • The lack of adequate systemic TK limits the approaches for calculating human equivalent doses (HEDs) for these studies.
Genotoxicity	• Negative in the reverse mutation assay in bacteria (Ames). • Positive in the *in vitro* chromosomal aberration assay in mammalian cells. • Negative in the *in vivo* rat micronucleus assay.

<u>Exposure margin considerations for LBN</u>

LBN is an ophthalmic solution, intended for topical ocular administration.

The 9-month topical ocular toxicity study in monkeys (report # 6348-415) observed systemic toxicity (target organ was the lung); systemic TK evaluation was only performed for latanoprost acid (i.e. not LBN, BDMN or NO).

The rat GLP embryofetal (EFD) study (report # 20073521) administered LBN by daily intravenous (iv) injection, and analyzed plasma for LBN, latanoprost acid, and BDMN. All three were detected systemically, latanoprost acid > BDMN > LBN. The study did not perform systemic TK for NO.

The rabbit GLP EFD study (report # 200753523) also administered LBN by iv injection, and analyzed plasma for LBN, latanoprost acid, and BDMN (but not NO). The

rabbit GLP EFD study tested a lower range of doses than the rat GLP EFD study; and systemic exposures were lower (as expected). LBN was not detected in plasma, with a lower limit of quantitation (LLOQ) of 10.0 pg/ml. BDMN was detected in one serum sample (LLOQ of 1 ng/ml), and latanoprost acid was detected sporadically (LLOQ = 30 pg/ml).

Taken together, the nonclinical data indicate the systemic toxicity caused by LBN (in the 9-month monkey study, and in the EFD studies) are due to systemic exposure to the parent LBN compound or other metabolites (i.e. BDMN and NO), and not entirely due to the latanoprost acid metabolite.

For the nonclinical data presented in the label, this reviewer proposes labeling based on exposure margins calculated from dose, on a body surface area (BSA) basis. One alternative approach, labeling with exposure margins based on either LBN or BDMN exposure and quantitation limits, would dramatically over-predict the actual risk (due to the limitations of the analytical methods used). Another alternative approach, labeling with exposure margins based only on latanoprost acid, would under-predict the actual risk shown by the animal studies.

Exposure margins based on BSA are not ideal, and more sensitive analytical methods may have allowed by exposure margin calculations based on exposure(s). However, requiring additional embryofetal studies or additional ocular toxicology studies to further refine the exposure margin estimates would not be warranted. P/T does not expect that such studies would result in meaningful changes to the exposure margins that would be helpful for physicians or patients.

1.3 Recommendations

1.3.1 Approvability: P/T identified no safety issues that would preclude approval. P/T has no objection to approval.

1.3.2 Additional Non Clinical Recommendations

The NDA was submitted 7/21/2015. The Applicant had, or should have had, draft reports for the embryofetal (EFD) range-finder studies at the time of the NDA submission. The Applicant had, or should have had, Safety Reportable results (i.e. abortion data and fresh visceral malformations) from the GLP EFD studies.

Table 1: Available nonclinical data relevant to safety, withheld from the original NDA submission of July 21, 2015 (embryofetal study results submitted January 27, 2016)

Type	Report #	Date initiated	Date in-life completed	Date of the unaudited draft report
Rat EFD range	20073520	3/17/2015	3/31/2015	5/26/2015

finder				
Rat EFD study	20073521	5/11/2015	6/04/2015	1/15/2016
Rabbit EFD range finder	20073522	3/24/2015	4/15/2015	6/10/2015
Rabbit EFD study	200753523	5/11/2015	6/05/2015	1/15/2015

- These four study reports were submitted on 1/27/2016 as unsigned, unaudited draft reports, in response to a P/T Information Request (IR).
- For these four studies, the final reports were submitted on March 30, 2016. Although changes were not annotated, the end of each document had a listing of changes.
- Failure to submit all available information relevant to safety in the original NDA at the time of filing appears to be a regulatory violation.
- The Applicant has not submitted these draft reports to the IND (IND # 73435); this appears to be a Safety Reporting violation. Per 21CFR312.32(c)(1)(iii) CFR (http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=312.32) says "The sponsor must report any findings from animal or *in vitro* testing, whether or not conducted by the sponsor, that suggest a significant risk in humans exposed to the drug, such as reports of mutagenicity, teratogenicity, or carcinogenicity, or reports of significant organ toxicity at or near the expected human exposure. Ordinarily, any such findings would result in a safety-related change in the protocol, informed consent, investigator brochure (excluding routine updates of these documents), or other aspects of the overall conduct of the clinical investigation."

P/T defers to the Division, regarding the appropriate regulatory follow-up.

1.3.3 PT Labeling Recommendations

- This reviewer references the August 2015 labeling review tool (http://inside.fda.gov:9003/downloads/cder/officeofnewdrugs/immediateoffice/labelingdevelopmentteam/ucm457434.doc)
- The Applicant submitted draft labeling in the original NDA (7/21/2015), and updated draft labeling in the clinical safety update (11/18/2015).

Table 2: P/T sections of the Applicant's proposed labeling (as of 7/21/2015)

Applicant's proposed text	Reviewer's recommendations
1 INDICATIONS AND USAGE	**1 INDICATIONS AND USAGE**

TRADENAME™ (latanoprostene bunod ophthalmic solution) 0.024% is (b) (4) (b) (4) (b) (4) indicated for the reduction of intraocular pressure in patients with open-angle glaucoma or ocular hypertension.	**VYZULTA** (latanoprostene bunod ophthalmic solution) 0.024% is a prostaglandin F2α receptor agonist indicated for the reduction of intraocular pressure in patients with open-angle glaucoma or ocular hypertension.
8.1 Pregnancy Risk Summary There are no (b) (4) (b) (4)	**8.1 Pregnancy** Risk Summary **There are no available human data for the use of VYZULTA during pregnancy to inform any drug associated risks.**
	Based on animal studies with latanoprostene bunod *[see Animal Data]*, VYZULTA may cause miscarriage, abortion, and fetal harm (b) (4). **Latanoprostene bunod was shown to be abortifacient and teratogenic when administered to pregnant rabbits (IV) at exposures ≥ 0.28 times the clinical dose, in the absence of maternal toxicity. Doses ≥ 20 µg/kg/day (23 times the clinical dose) produced 100% embryofetal lethality. Structural abnormalities observed in rabbit fetuses included anomalies of the great vessels and aortic arch vessels, domed head, sternebral and vertebral skeletal anomalies, limb hyperextension and malrotation, abdominal distension and edema. Latanoprostene bunod was not teratogenic in the rat (b) (4) (b) (4).** **Advise pregnant women of the**

(b) (4)	**potential risk to the fetus.** **The background risk of major birth defects and miscarriage for the indicated population is unknown. However, the background risk in the U.S. general population of major birth defects is 2 to 4%, and of miscarriage is 15 to 20%, of clinically recognized pregnancies.**
Data	***Animal Data*** **Embryofetal studies were conducted in pregnant rabbits administered latanoprostene bunod daily by intravenous injection on gestation days 7 through 19, to target the period of organogenesis. The doses administered ranged from 0.24 to 80 micrograms µg/kg/day. No maternal toxicity was observed at any dose. Abortion occurred at doses ≥ 0.24 µg/kg/day latanoprostene bunod (0.28 times the clinical dose, on a body surface area basis, assuming 100% absorption). Embryofetal lethality (resorption) was increased in latanoprostene bunod treatment groups, as evidenced by increases in early resorptions at doses ≥ 0.24 µg/kg/day and late resorptions at doses ≥ 6 µg/kg/day (6.95 times the clinical dose). No fetuses survived in any rabbit pregnancy at doses of 20 µg/kg/day (23 times the clinical dose) or greater. Latanoprostene bunod produced structural abnormalities at doses ≥ 0.24 µg/kg/day (0.28 times the clinical dose). Malformations included anomalies of sternum, coarctation of**
(b) (4)	

(b) (4)	**the aorta with pulmonary trunk dilation, retroesophageal subclavian artery with absent brachiocephalic artery, domed head, forepaw hyperextension and hindlimb malrotation, abdominal distention/edema, and missing/fused caudal vertebrae. A no observed adverse effect level (NOAEL) was not established in this study.** **An embryofetal study was conducted in pregnant rats administered latanoprostene bunod daily by intravenous injection on gestation days 7 through 17, to target the period of organogenesis. The doses administered ranged from 150 to 1500 µg/kg/day. Maternal toxicity was produced at 1500 µg/kg/day (870 times the clinical dose, on a body surface area basis, assuming 100% absorption), as evidenced by reduced maternal weight gain. Embryofetal lethality (resorption and fetal death) and structural anomalies were produced at doses ≥ 300 µg/kg/day (174 times the clinical dose). Malformations included anomalies of the sternum, domed head, forepaw hyperextension and hindlimb malrotation, vertebral anomalies and delayed ossification of distal limb bones. A NOAEL was established at 150 µg/kg/day (87 times the clinical dose) in this study.**
8.2 Lactation Risk Summary (b) (4)	**8.2 Lactation** Risk Summary **There are no data on the presence of**

in human milk. (b) (4) (b) (4)	**VYZULTA in human milk, the effects on the breastfed infant, or the effects on milk production.** (b) (4) (b) (4)
	The developmental and health benefits of breastfeeding should be considered, along with the mother's clinical need for VYZULTA, and any potential adverse effects on the breastfed infant from VYZULTA or from the underlying maternal condition.
(b) (4)	

(b) (4)

12 CLINICAL PHARMACOLOGY **12.1 Mechanism of Action** (b) (4)	**12.1 Mechanism of Action** (b) (4) Latanoprostene bunod is thought to lower intraocular pressure **by increasing the outflow of aqueous humor. Studies in animals and man with latanoprost acid suggest that its main mechanism of action is increased uveoscleral outflow.** Intra-ocular pressure is a major modifiable risk factor for glaucoma progression. Reduction of intraocular pressure reduces risk of glaucomatous visual field loss. *[Reviewer note: P/T defers to Clinical and Clinical Pharmacology regarding a decision to retain the language contained in the last two sentences of this section.]*
13 NONCLINICAL TOXICOLOGY **13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility** Latanoprostene bunod was not mutagenic in bacteria and did not induce micronuclei formation in the in vitro rat bone marrow micronucleus assay. Chromosomal aberrations were observed in vitro with human lymphocytes, (b) (4) (b) (4)	**13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility** Latanoprostene bunod was not mutagenic in bacteria and did not induce micronuclei formation in the *in **vivo*** rat bone marrow micronucleus assay. Chromosomal aberrations were observed *in vitro* with human lymphocytes **in the absence of metabolic activation**. Latanoprostene bunod has not been

Latanoprostene bunod has not been tested for carcinogenic activity in (b) (4) (b) (4)	tested for carcinogenic potential in long-term animal studies. **Latanoprost acid is a main metabolite of latanoprostene bunod. Exposure of rats and mice to latanoprost acid, resulting from oral dosing with latanoprost in lifetime rodent bioassays, was not carcinogenic.**
Fertility studies have not been conducted with latanoprostene bunod; (b) (4) the potential to impact fertility can be characterized by (b) (4), latanoprost acid (b) (4) (b) (4) found not to have any effect on male or female fertility in (b) (4) (b) (4)	Fertility studies have not been conducted with latanoprostene bunod. The potential to impact fertility can be **partially** characterized by **exposure to** latanoprost acid**, a common metabolite of both latanoprostene bunod and latanoprost**. Latanoprost **acid has not been found to have any effect on male or female fertility in animal studies.**
(b) (4)	**13.2 Animal Toxicology and/or Pharmacology** **A 9-month toxicology study administered topical ocular doses of**

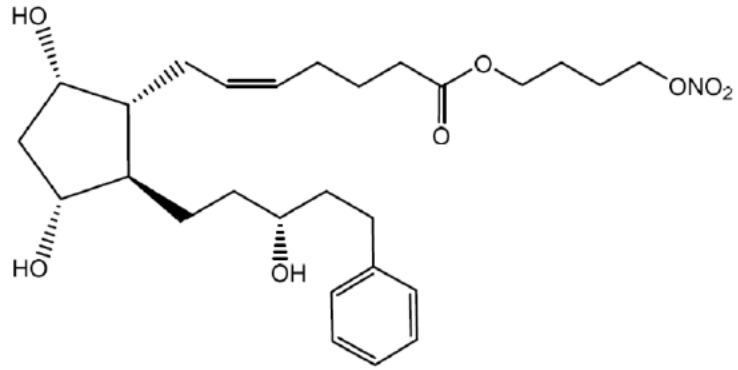
	latanoprostene bunod to one eye of cynomolgus monkeys: control (vehicle only), a low-dose (one drop of 0.024% bid), mid-dose (one drop of 0.040% bid) and high-dose (two drops of 0.040% per dose, bid). The systemic exposures are equivalent to 4.2-fold, 7.9-fold, and 13.5-fold the clinical dose, respectively, on a body surface area basis (assuming 100% absorption). Microscopic evaluation of the lungs after 9 months observed pleural/subpleural chronic fibrosis/inflammation in the mid- and high-dose male groups, with increasing incidence and severity compared to controls. Lung toxicity was not observed at the low-dose.

2 Drug Information

2.1 Drug

Chemical Abstracts Service (CAS) Registry Number	860005-21-6
Established name	Latanoprostene bunod
Trade names	• VYZULTA (accepted by CDER) • Vesneo (proposed by the Applicant but rejected by CDER)
Code names	• (b) (4) • BOL-303259-X (Bausch & Lomb) • PF-03187207 (Pfizer) • NCX-116 (Nicox) • (b) (4) • (b) (4)
Chemical name	4-(Nitrooxy)butyl (5Z)-7-{(1R,2R,3R,5S)-3,5-dihydroxy-2-[(3R)-3-hydroxy-5-phenylpentyl]cyclopentyl}hept-5-enoate
Molecular formula	$C_{27}H_{41}NO_8$
Molecular weight	507.62 g/mol
Pharmacologic class[6]	prostaglandin F2α analogue

Figure 1: Structure of latanoprostene bunod (LBN)



Note: structure of LBN has 5 chiral centers.

[6] Note: the pharmacologic class for Xalatan® (NDA 20597) is "prostaglandin F2α analogue". For latanoprost bunod, the Applicant proposed (b) (4) (b) (4)

2.2 Relevant INDs, NDAs, BLAs and DMFs

The Applicant, on for 356, cross-references: IND 73435, [redacted], NDA 20597, DMF [redacted], DMF [redacted], DMF [redacted], DMF [redacted], DMF [redacted], DMF [redacted], and DMF [redacted].

Letters of Authorization of the NDA are provided in NDA section 1.4.1 Letter of Authorization.

- [redacted]
- For NDA 025097 (Xalatan®, latanoprost ophthalmic solution 0.005%), a "letter of authorization or right of reference and cross reference" is provided "by Pharmacia & Upjohn Co [sic], a division of Pfizer Inc." (Vogt/DTOP, 4/25/2015). The letter notes, "Latanoprostene bunod (also known as BOL-303259-X) (the "Product") was the subject of a Research, Option, Development and License Agreement, dated August 25, 2004", that included "right of reference to NDA 020597, to Bausch & Lomb." The letter also notes, "Pfizer will receive certain milestones and royalties [redacted], [redacted] for the Product."

Note: The Applicant submitted study reports for [redacted] latanoprost that had previously been submitted to these NDAs. However, the Applicant did not mention other data in those NDAs (e.g. [redacted] the intravenous toxicity data for latanoprost).

2.3 Drug Formulation

From the NDA's module 2.3.P.1 Description and Composition of Drug Product:

Table 3: Composition of latanoprostene bunod ophthalmic solution, 0.024%

Component	Reference to Quality Standard	Function	Concentration (mg/ml)
Latanoprostene bunod	In-house	Active	0.24
Benzalkonium chloride (BAK), [redacted]	NF	[redacted] preservative	[redacted]
Polysorbate 80	NF	[redacted]	[redacted]
Edetate disodium [redacted]	USP		
Sodium citrate [redacted]	USP	Buffering agent	[redacted]
Citric acid, [redacted]	USP	Buffering agent	
Glycerin	USP	[redacted]	[redacted]
Water [redacted]	USP		

NF = National Formulary. USP = United States Pharmacopeia

2.4 Comments on Novel Excipients

None of the excipients are novel for topical ocular administration.

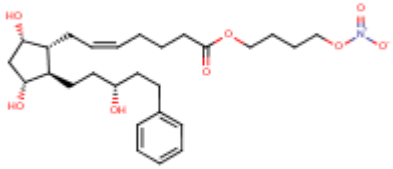
2.5 Comments on Impurities/Degradants of Concern

These acceptance criteria are not concerning:

Table 4: Acceptance criteria for impurities in LBN

Impurity	Acceptance Criteria in Specification	Origin
(b) (4)		

Table 5: Chemical names and structures for LBN and impurities in LBN

LBN is 4-(Nitrooxy)butyl (5Z)-7-{(1R,2R,3R,5S)-3,5-dihydroxy-2-[(3R)-3-hydroxy-5-phenylpentyl]cyclopentyl}hept-5-enoate.	

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(b) (4)

- The presence of (b) (4) as impurities is not a safety concern.
  - (b) (4) is a starting material for the manufacture of LBN. (b) (4)
  - (b) (4).
- A 28-day monkey study (report # 8273344) was conducted to qualify two impurities: (b) (4). Moreover, the test article contained (b) (4) which is also qualified by the toxicology study.
  - The Applicant reports (NDA module 3.2.S.3.2 Impurities) that (b) (4) (b) (4)
  -
  -
- LBN was shown to be negative in the Ames and *in vitro* micronucleus test, but positive in the *in vitro* clastogenicity assay.
  - (b) (4), this reviewer expects (b) (4) (b) (4) to exhibit similar activity (i.e. negative in Ames, positive for *in vitro* clastogenicity). However, this has not been tested experimentally.

2.6 Proposed Clinical Population and Dosing Regimen

- Proposed clinical population: patients with open-angle glaucoma or ocular hypertension
- Proposed dose: one drop (0.024%) in the affected eye(s) once daily in the evening.
  - 0.024% is the same as 0.24 µg/µl of LBN
  - Assuming one drop = 35 µl, the one drop has 8.4 µg/drop of LBN
  - One drop per day means that the dose is 8.4 µg/eye/day.
  - For a 60 kg patient, the daily dose would be 0.14 µg/kg/day if only one eye is dosed once daily, and 0.28 µg/kg/day if both eyes are dosed once daily.

- To convert human doses from a body weight basis (µg/kg) to a body surface area basis (µg/m^2), the default approach[7] is to multiply by a k_m factor of 37 kg/m^2, assuming 100% absorption. The daily dose would be 5.18 µg/m^2 if one eye is dosed (10.36 µg/m^2 if both eyes dosed.

2.7 Regulatory Background

- Pfizer submitted the original IND 73435 for LBN (PF-03187207) on February 20, 2007. The file was transferred to NiCox (in 2009), and then (in 2010) to Bausch and Lomb (B&L), the current Sponsor.
- The regulatory background regarding the embryofetal studies for LBN is remarkable. Please refer to Appendix 1.1 of this review for more details.

3 Studies Submitted

3.1 Studies Reviewed

Table 6: List of Primary Pharmacology Study Reports

Report #	Report title
PH13015	The effect of latanoprost on endothelin-1 induced myosin light chain-2 phosphorylation in primary human trabecular meshwork cells
PH13023	The effect of latanoprostene bunod on cGMP generation in human trabecular meshwork cells
PH13025	The effect of latanoprostene bunod on endoethelin-1 induced myosin light chain-2 phosphorylation in primary human trabecular meshwork cells
PH13028	Effect of latanoprostene bunod on actin cytoskeleton dynamics in primary human trabecular meshwork cells
PH13031	The effect of the soluble guanylate cyclase inhibitor ODQ on latanoprostene bunod-induced cGMP generation in human trabecular meshwork cells
PH13033	Effect of latanoprostene bunod on endothelin-1-induced primary human trabecular meshwork cell contractility
PH14006	Effect of latanoprostene bunod on IOP in ocular hypertensive non-human primates
PH14007	The effect of latanoprostene bunod on IOP in wild type and FP receptor knockout C57BL/6 mice
PH14008	Effect of latanoprostene bunod on IOP in glaucomatous dogs

[7] CDER 200 Guidance for Industry. Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. Accessed via: http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm078932.pdf

PH14009	Effects of latanoprostene bunod (NCX 116) on saline-induced transient IOP increase in rabbits
PH14010	Effect of latanoprostene bunod (NCX 116) on cGMP levels in PC-12 cells and iNOS levels in RAW macrophages
PH14011	Effect of latanoprostene bunod (NCX 116) on cGMP levels in HEK 293 cells
PH14005 *	Effect of latanoprostene bunod on aqueous humor dynamics in ocular hypertensive primates

* Submitted to the NDA on January 27, 2016.

Table 7: Listing of the Secondary Pharmacology Study Report

Report #	Report title
757076a	*In vitro* pharmacology: tier 0 profile – study of PF-03187207-00-

Table 8: List of Distribution Study Reports

Report #	Report title
9400106	Tissue distribution of tritium labelled latanoprost in the cynomolgus monkey after topical administration on the eyes studied by whole body autoradiography
BL10008	Investigation of the ocular pharmacokinetics of latanoprost acid following topical ocular administration of BOL-303259-X in various formulations to Dutch Belted rabbits
PF03187207-PDM-007	Pharmacokinetics of PF-03187207 in Dutch Belted rabbits following topical administration
PF03187207-PDM-008	Pharmacokinetics of PF-03187207 in cynomolgus monkeys following topical administration

Table 9: Listing of the Metabolism Study Report

Report #	Report title
PF03187207-PDM-006	*In vitro* characterization of PF-03187207

Table 10: Listing of the Single-Dose Toxicity Study Report

Report #	Report title
06AM024	Acute intravenous toxicity study with 6 days observation period of PF-03187207 in rats

Table 11: Repeat-Dose Toxicity Study Reports

Report #	Report title
05NCX001	*In vivo* toleration topical ocular irritation toxicity study of NCX116 (PF-03187207) in female pigmented rabbits
6750-267	28-day twice daily ocular instillation toxicity and toxicokinetic study with PF-03187207 in cynomolgus monkeys
6348-415	9-month topical ocular instillation toxicity and toxicokinetic study with PF-03187207 in cynomolgus monkeys
8273344	28-Day ocular instillation toxicity and toxicokinetic study with latanoprostene bunod (BOL-303259) in cynomolgus monkeys (impurity qualification)

Table 12: Listing of Genotoxicity Study Reports

Report #	Report title
06AA026	Bacterial reverse mutation assay of PF-3187207
06AA133	*In vitro* structural chromosome aberration assay of PF-03187207 in human peripheral lymphocytes
07GR073	*In vivo* bone marrow micronucleus assay of PF-03187207

Table 13: Listing of embryofetal studies for LBN

Report #s*	Report title
20073520	A dose range-finding embryo-fetal development study of latanoprostene bunod (LBN) by intravenous (bolus) in rats
20073521	An embryo-fetal development study of latanoprostene bunod (LBN)
20073522	A dose range-finding embryo-fetal study of latanoprostene bunod (LBN) by intravenous (bolus) in rabbits
200753523	An embryo-fetal development study of latanoprostene bunod (LBN) by

* Unaudited reports were submitted to the NDA on January 27, 2016. Final reports were submitted to the NDA on March 30, 2016.

3.2 Studies Not Reviewed

- Note: The code name PhXA41 (as well as PhXA 41, XA 41, and XA41) refers to latanoprost. These code names are public knowledge.
- [redacted] (b) (4)

Table 14: Analytical methods and validation reports

Report #s*	Report title
1009.063015	The GC/MS/MS quantification of 1,4-butanediol mononitrate (BDMN) in New Zealand White rabbit plasma between 1.00 and 150 ng/ml
10033.072015	The GC/MS/MS quantification of 1,4-butanediol mononitrate (BDMN) in Sprague Dawley rat plasma between 1.00 and 150 ng/ml
150200VCPM-BRN	Method validation for the quantitation of BOL-303259-X and latanoprost free acid in treated rat plasma by (b) (4) LC/MS/MS
150203VCPM_BRN	Method validation for the quantitation of BOL-303259-X and latanoprost free acid in treated rabbit plasma by (b) (4) LC/MS/MS

* Reports were submitted to the NDA on March 30,2016, with the final EFD study reports.

Table 15: List of Safety Pharmacology Study Reports - Latanoprost

Report #	Report title	Year
9600118 (SR-9411)	Latanoprost (PhXA41) – General Pharmacological Study	1995
L411 C037	Effects of intravenous administration of PhXA41 on airway resistance and regional blood flow in vital organs of the cynomolgus monkey	1991
L41 C048	PhXA41 Evaluation of the effects on the cardiovascular system and on respiration in the conscious cynomolgus monkey	1992

Table 16: List of Pharmacokinetics – Analytical Methods and Validation Reports

Report #	Report title
130437VSMB_BRN	Method validation for the quantitation of latanoprost free acid and BOL-303259-X in monkey plasma by (b) (4) LC/MS/MS
8418.051514	The GC/MS/MS quantification of 1,4-butanediol mononitrate (BDMN) in cynomolgus monkey plasma between 0.200 and 50.0 ng/ml
9400174	Bioanalysis of the acid of latanoprost (PhXA85) by radioimmunoassay
9400068	Extraction and separation of tritium labelled latanoprost (PhXA41) and its metabolites in plasma, urine and faeces
ARLAT1	Method validation report for the determination of latanoprost acid in monkey plasma (K_2EDTA) using LC-API/MS/MS

ARLAT2	Method validation report for the determination of latanoprost acid in rat plasma (K_2EDTA) using LC-API/MS/MS

Table 17: List of Absorption Study Reports - Latanoprost

Report #	Report title	Year
9300006	(^{3}H)-PhXA41: Absorption, distribution and excretion following oral, intravenous and ocular administration to the cynomolgus monkey	1993
9400423	Tritium labelled latanoprost, (^{3}H)-phxa41 - plasma levels and excretion of radioactivity following intravenous administration to the dog	1994
9400424	Tritium labelled latanoprost, (^{3}H)-phxa41 - plasma levels and excretion of radioactivity following ocular and intravenous administration to the rabbit	1994
9400458	Tritium labelled latanoprost. (^{3}H)-Phxa41: absorption, distribution and excretion following oral and intravenous administration to the rat	1994
9400459	Tritium labelled latanoprost. (^{3}H)-Phxa41: absorption and excretion following repeat ocular administration to the cynomolgus monkey	1994

Table 18: List of Metabolism Study Reports - Latanoprost

Report #	Report title	Year
9300779	PhXA41: induction/inhibition of hepatic cytochrome P-450 in the rat	1993
9400104	Metabolism of [13,14-^{3}H]-latanoprost in the dog after intravenous administration	1994
9400105	Metabolism of [13,14-^{3}H]-13,14-dihydro-17-phenyl-18,19,20-trinor-$PGF_{2\alpha}$-isopropyl ester ([13,14-^{3}H]-PhXA41) in the rat after a single intravenous or oral administration	1994
9400110	The metabolism of [13,14-^{3}H]-latanoprost in the cynomolgus monkey after repeated topical administration on the eye	1994
9400260	The ocular pharmacokinetics and metabolism of [^{3}H]-13,14-dihydro-17-phenyl-18,19,20-trinor-$PGF_{2\alpha}$-isopropyl ester ([13,14-^{3}H]-PhXA41) in the rabbits after topical administration	1994
9400531	Metabolism of [13,14-^{3}H]-latanoprost in the rabbit after intravenous or topical administration on the eye	1994
L411 C056	Metabolism of latanoprost in the cynomolgus monkey after single intravenous, oral or topical administration on the eye	1994
Pharm-002	Binding of XA84 to melanin *in vitro*	1994

Table 19: List of Repeat-Dose Toxicity Study Reports - Latanoprost

Report #	Report title	Year
9400368-397/30	PhXA41: 13 week oral (gavage administration) sub-chronic toxicity study in the mouse	1994
9400302	Determination of PhXA84 (free acid of PhXA410 in plasma samples collected from toxicological study with PhXA41 ((b) (4) study no. 397/518)	1994
9400311	Determination of PhXA84 (free acid of PhXA410 in plasma samples collected from toxicological study with PhXA41 ((b) (4) study no. 397/503)	1994
9400425	PhXA41 – 52 week ocular toxicity study in the cynomolgus monkey [study # 397/503)	1994
9400426	PhXA41: 52 week ocular toxicity study in the cynomolgus monkey [study # 397/518)	
9600213	PhXA41 – two year repeated administration ocular toxicity study in the rhesus monkey with an interim sacrifice, followed by a 2 year treatment-free period	1996
9400427	PhXA41: 52 week ocular toxicity study in the rabbit	1994
9400304	Determination of the immunoreactive PhXA85 (free acid of PhXA41) in plasma samples collected from toxicological study with PhXA41 ((b) (4) study no 397/29)	1994
9400367-397/29	PhXA41: 13 week oral (gavage administration) subchronic toxicity study in rat	1994

Table 20: List of Genetic Toxicity Study Reports - (b) (4)

Report #	Report title	Year
(b) (4)		

Table 21: List of Genetic Toxicity Study Reports - Latanoprost

Report #	Report title	Year
L411 S017	Study to determine the ability of PhXA41 to induce mutations in four histidine-requiring strains of *Salmonella typhimurium* and two tryptophan-requiring strains of *Escherichia coli*	1991
L411 S024 [2MLREPHS 006; PHS	Study to determine the ability of PhXA41 to induce mutations to 6-thioguanine resistance in mouse lymphoma L5178Y cells using a fluctuation assay	1991

6/ML]		
L411 S027	Study to evaluate the chromosome damaging potential of PhXA41 by its effects on cultured human lymphocytes using an *in vitro* cytogenetics assay	1992
9400244	Study to evaluate the potential of PhXA41 to induce unscheduled DNA synthesis in rat liver using an *in vivo/in vitro* procedure	1994
L411 S023	Study to evaluate the potential of PhXA41 to induce micronuclei in polychromatic erythrocytes of CD-1 mice	1991

Note: this reviewer could not verify the study # L411 S024 from the study report; the other study numbers listed on the report are presented in brackets.

Table 22: List of Carcinogenicity Study Reports - (b) (4)

Report #	Report title	Year
(b) (4)		

Table 23: List of Carcinogenicity Study Reports - Latanoprost

Report #	Report title	Year
9400620	PHXA41 oral (gavage administration) carcinogenicity study in the mouse	1994
9400614-397-16	PHXA41 oral (gavage administration) carcinogenicity study in the rat	1994
9400397	Determination of immunoreactive PhXA85 (free acid of PhXA41) in rat plasma samples collected from toxicological study with PhXA41 (study No. 397/16)	1994

Table 24: List of DART and Fertility Study Reports - (b) (4)

Report #	Report title	Year
(b) (4)		

Table 25: List of DART and Fertility Study Reports - Latanoprost

Report #	Report title	Year
9200027-397/513	Fertility study by intravenous route in the rat (Segment I)	1993
9300279	Teratology study by intravenous route in the rat (Segment II)	1993
9300280	Teratology study by intravenous route in the rabbit (Segment II)	1993
9300281	Developmental toxicity study by intravenous route in the rat (Segment III)	1993

Table 26: List of Local Tolerance and Other Study Reports - Latanoprost

Report #	Report title	Year
9200028	Test to determine the index of primary cutaneous irritation in the rabbit	1993
9600119	Antigenicity study in mice sensitized with PhXA41	1996
9400059	PhXA41 - evaluation of the potential to induce immediate hypersensitivity. Passive cutaneous anaphylaxis (PCA) and induced anaphylactic shock in the guinea-pig	1994
9400529	Test to evaluate sensitizing potential in the guinea pig	1994

3.3 Previous Reviews Referenced

(b) (4)

(b) (4) This review references:

- For IND 073435 (Bausch and Lomb Inc.'s IND for LBN):
  - P/T pre-IND review (Ellis, 1/20/2006)
  - P/T review of the original IND submission (Ellis, 4/27/2007)
  - P/T review in response to Sponsor questions (Ellis, 8/03/2007)
  - P/T review of the 9-month monkey study (Lansita, 11/14/2011)
  - P/T end-of-phase 2 review (McDougal, 10/18/2012a)
  - P/T review of genotoxicity studies (McDougal, 10/18/2012b; McDougal, 11/10/2013; McDougal, 12/06/2013)
  - P/T pre-NDA review (McDougal, 2/23/2015)
  - DTOP's review related to Sponsor questions (McDougal, 3/10/2015; Chambers, 3/25/2015; Lwin, 3/25/2015; Lwin, 5/11/2015)

(b) (4)

(b) (4)

4 Pharmacology

- DARRTS does not record previous review of the pharmacology studies for latanoprostene bunod (e.g. for the IND).

4.1 Primary Pharmacology

- None of the primary pharmacology/pharmacodynamics (PD) studies were conducted in compliance with U.S. Good Laboratory Practices (GLP). Deficiencies in the designs (e.g. positive and negative controls, checking cell viability) and reporting (data omissions) limit the usefulness of these reports to understand LBN.
- No safety issues were identified in review of the primary pharmacology study reports.
- The *in vitro* and *in vivo* PD studies provided some support for the Applicant's proposed mechanism of action, but were not incontrovertible proof of concept.

Table 27: Summary of the usefulness of the primary PD studies

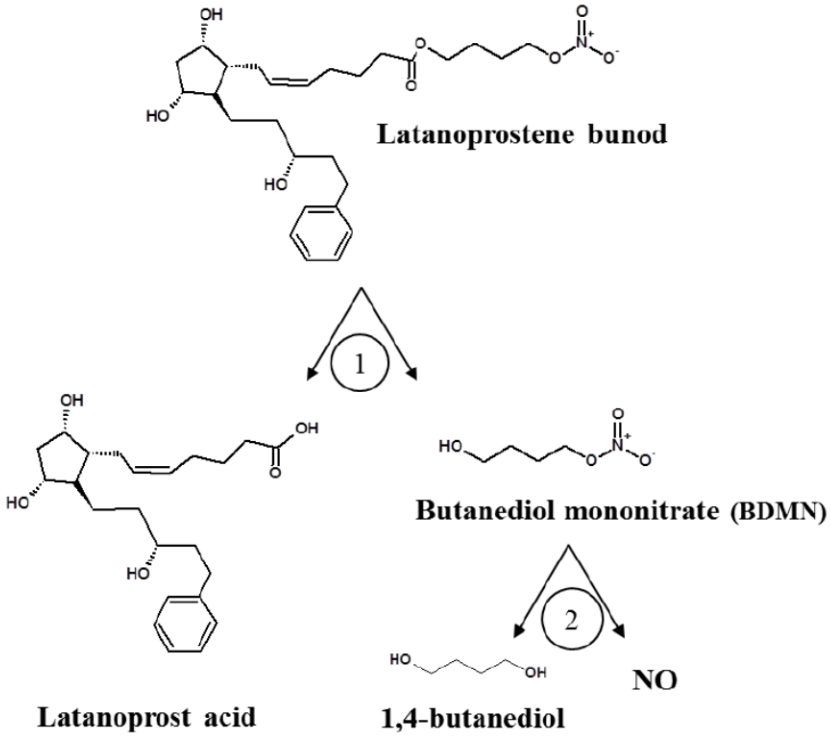
Study #	Description	Notes on the usefulness for proof-of-concept
PH13015	*In vitro* assay with latanoprost	-
PH13025	*In vitro* assays in human trabecular meshwork cells (HTMC)	No apparent difference between LBN and latanoprost
PH13033		LBN was more active at 45 µM but not at 30 µM; biological relevance of this difference is unclear
PH13028		Not useful for regulatory review
PH13023		
H13031		Results indicate that LBN has activity mediated through cGMP (i.e. supports proof-of-concept for LBN)

PH14010	*In vitro* assays in rat adrenal tumor cell line	LBN clearly more active than latanoprost; biological relevance unclear (supports proof-of-concept)
PH14011	*In vitro* assays in a human embryonic kidney cell line	
PH14006	Study in monkeys with ocular hypertension (laser-damaged trabecular meshwork)	No apparent difference between LBN and latanoprost
PH14007	Study in FP receptor knock out mice	Weakly suggestive of LBN activity (i.e. not via the FP receptor)
PH14008	Study in dogs with glaucoma	LBN was clearly more active than latanoprost, but the reliability of the study is low due to reporting omissions
PH14009	Study in rabbits with elevated IOP induced by injecting hypertonic saline	LBN was slightly more active than latanoprost, but the reliability of the study is low due to reporting omissions

4.1.1 Summary of the Proposed Mechanisms of Action

- The primary pharmacology studies demonstrate the latanoprostene bunod has activity comparable to latanoprost, but failed to show additional activity attributable to the nitric oxide-donating moiety.
- Latanoprost is a prostaglandin F2α (FP) receptor agonist indicated for the reduction of elevated IOP in patients with open-angle glaucoma or ocular hypertension. Latanoprost is believed to reduce IOP by increasing the outflow of aqueous humor (increased uveoscleral outflow).
- LBN is a new chemical entity, comprised of the latanoprost moiety covalently linked by an ester group to butanediol mononitrate (BDMN). The Applicant report that "after topical ocular administration, LBN is hydrolyzed by cellular esterases to the active moieties latanoprost acid (LA) and" BDMN, which "is further metabolized to 1,4-butanediol and the active signaling molecule, NO."
    - The Applicant hypothesizes that the latanoprost acid moiety will exhibit the same activity (i.e. IOP lowering) as is observed for latanoprost.
    - Additionally, the Applicant hypothesizes that the release of NO will cause relaxation of the trabecular meshwork, thereby increasing outflow and lowering IOP through an alternate pathway.
- The following figure was copied from the NDA (Module 2.4 Nonclinical Overview).

Figure 2: Depiction of the metabolism of LBN to latanoprost acid and NO



- Guanosine 3',5'-cyclic monophosphate (cGMP) is a key intracellular messenger molecule. Soluble guanylate cyclase (sGC) and membrane bound guanylate cyclase convert GTP into cGMP.
- Organic nitrate compounds which donate NO can activate soluble guanylate cyclase (sGC) to produce cyclic GMP (cGMP), which is associated with vaso-relaxation. In smooth muscle cells, NO-induced cGMP causes relaxation via cGMP-dependent protein kinases: decreased intracellular calcium, activation of potassium channels, and activation of myosin light chain phosphatase (which dephosphorylates myosin light chains)[8].
- The Applicant cited several journal articles (in NDA module 2.6.2 Pharmacology Written Summary) reporting that nitric oxide-releasing agents, such as nitroglycerin, were shown to have IOP-lowering activity under the conditions tested. Although this reviewer did not find a detailed explanation in the NDA, presumably the Applicant is proposing that cGMP relaxes trabecular meshwork cells via the same mechanisms that it relaxes smooth muscle cells. The Applicant provided several studies showing that LBN exposure results in dephosphorylation of myosin light chain 2 (MLC-2) in trabecular meshwork cells. . The relevance of these *in vitro* experiments to IOP lowering *in vivo* was not directly investigated.

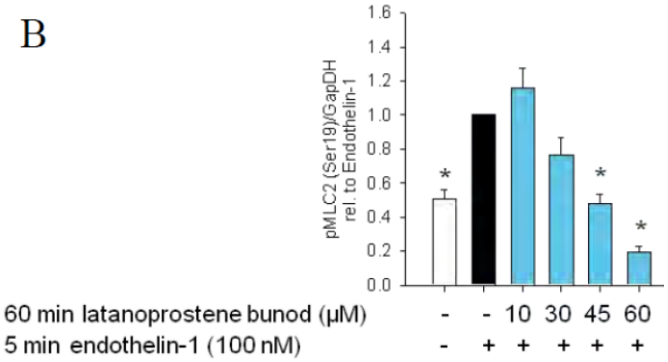
[8] Carvajal JA, Germain AM, Huidobro-Toro JP, and Wiener CP. 2000. Molecular mechanisms of cGMP-mediated smooth muscle relaxation. J. Cell Physiol. 184(3):409-420. Accessed via: http://www.ncbi.nlm.nih.gov/pubmed/10911373

4.1.3 Primary review of the submitted PD study reports

Title	**The effect of latanoprost on endothelin-1 induced myosin light chain-2 phosphorylation in primary human trabecular meshwork cells**	
Report #	PH13015	
Key findings	• This study tested latanoprost, not latanoprostene bunod. The results of this study are only relevant to the NDA, insofar as they serve as a comparator for the next PD study (#PH13023, reviewed below).	
Study details	Laboratory	Bausch + Lomb, Preclinical Pharmacology 1400 North Goodman Street Rochester, NY 14609
	Report date	November 12, 2013
	Test article	LBN, lot # 6889 (no purity or formulation information provided)
Rationale	• As the Xalatan® label notes, "latanoprost is a prostaglandin F2α analogue indicated for" IOP… "Studies … suggest that the main mechanism of action if increased uveoscleral outflow." • Endothelin 1 (ET-1) is a vasoconstrictor. ET-1 activation of endothelin receptor A (ET_A) results in vasoconstriction. The authors cite several papers which claim that ocular dosing with ET-1 increases IOP, and that ET_a antagonists decrease IOP. o Myosin light chain-2 (MLC-2) is present in smooth muscle (as well as cardiac and skeletal muscle). Muscle contraction is regulated by phosphorylation of MLC-2. o The authors claim that MLC-2 is expressed in trabecular meshwork (TM) cells, and that dosing of TM cells with ET-1 causes increased phosphorylation of MLC-2. o The authors consider increased MLC-2 phosphorylation to be evidence of increased contractility, and decreased MLC-2 phosphorylation to indicate relaxation. • The report is not clear regarding the mechanism of action (i.e. how latanoprost interacts with ET-1 signaling) • The authors propose that relaxation of HTMB cells *in vitro* may predict relaxation of trabecular meshwork cells *in vivo*, and that relaxation of these cells may increase outflow, thereby lowering IOP.	
Method notes	Cell type	• Primary human trabecular meshwork cells (HTMC) • Purchased from a commercial vendor • Cells were "isolated from juxtacanalicular and corneoscleral regions."
	Dosing	• Cells were pre-treated with 0 to 60 µM of latanoprost for 60 minutes • Then cells were co-treated with ET-1 (100 nM) +

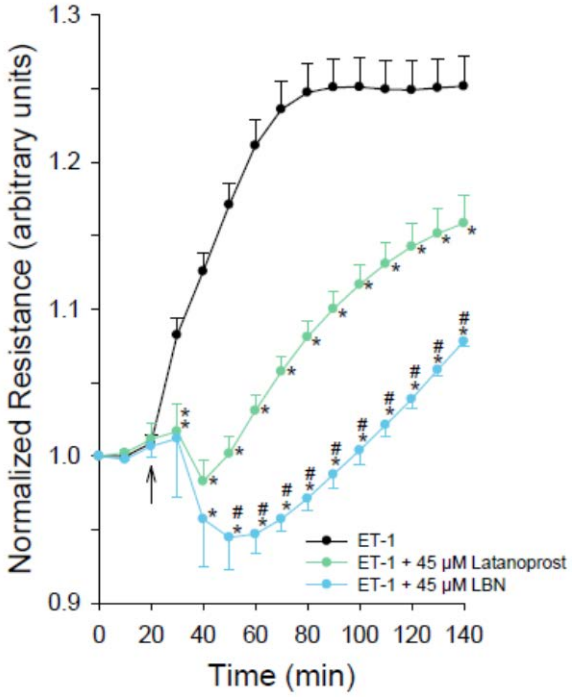
		latanoprost (at the previous concentration) for 5 minutes.
	Data collection	• Cells were lysed; lysate was used in a Western Blot analysis against phosphorylated MLC-2 • Blots were quantitated using densitometry • Results were reported graphically (but numerical data were not provided)
Results	• ET-1 alone increased MLC-2 phosphorylation compared to vehicle control • ET-1 + a low concentration of latanoprost (10 μM) increased MLC-2 phosphorylation compared to ET-1 alone. • ET-1 + higher concentrations of latanoprost (30, 45, 60 μM) decreased MLC-2 phosphorylation back toward control. • A dose-response was apparent (comparing the 10 μM concentration versus the higher concentrations)	
Conclusion	• The authors conclude that low exposure to latanoprost might increase trabecular meshwork contractility (i.e. increase IOP), while higher exposures might relax the trabecular meshwork (i.e. lower IOP). • As the Xalatan® label notes, "latanoprost is absorbed through the cornea were the isopropyl ester prodrug is hydrolyzed to the acid form to become biologically active." Because the rate of hydrolysis (if any) in this *in vitro* experiment is unclear, the concentrations tested are not directly relevant to *in vivo* exposure.	

Title	**The effect of latanoprostene bunod on endothelin-1 induced myosin light chain-2 phosphorylation in primary human trabecular meshwork cells**	
Report #	PH13025	
Key findings	• LBN exhibited activity comparable to latanoprost (report # H 13015) on an *in vitro* biomarker: ET-1 induced phosphorylation of MLC-2 • Comparing the two studies (# PH13015 and PH13025), no difference between latanoprost and latanoprostene bunod is apparent. • Note: the authors propose that latanoprostene bunod was more active than latanoprost, and therefore, the NO-release moiety contributed to the intended pharmacology. However, the results presented in the report do not support this interpretation.	
Study details	Laboratory	Bausch + Lomb, Preclinical Pharmacology 1400 North Goodman Street Rochester, NY 14609
	Report date	November 14, 2013
	Test article	LBN, lot # 6889 (no purity or formulation information provided)
Rationale	• In addition to the prostaglandin receptor pathway and the ET_A	

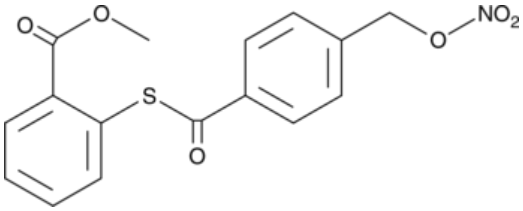
	receptor pathways intended to reduce IOP, the authors propose that NO increases cGMP levels in TM cells, which would result in decreased MLC-2 phosphorylation, thereby causing the TM cells to relax further, thereby further increasing outflow.
Methods	• Same as for study # PH13015 (above) • The concentrations of latanoprostene bunod (0, 10, 30, 45, 60 µM) were the same (to allow equimolar comparison)
Results:	• Results from 2 (presumably representative) experiments (out of "at least 3 experiments") were reported. The graph from one experiment is copied below, to illustrate the results:  **Figure 3: Representative results from *in vitro* study # PH13025**
	• ET-1 alone increased MLC-2 phosphorylation compared to vehicle control • ET-1 + a low concentration of latanoprostene bunod (10 µM) increased MLC-2 phosphorylation compared to ET-1 alone. • Dosing with higher concentrations of latanoprostene bunod + ET-1 decreased the MLC-2 phosphorylation compared to ET-1 alone, and a dose-response was apparent (toward the vehicle control without ET-1)
Conclusions:	• The authors concluded, "Taken together, these data [presented in the report] suggest that the NO-donating moiety of LBN provides additional effects in reducing HTML MLC-2 phosphorylation, and therefore cell relaxation as compared to latanoprost alone." • This reviewer disagrees – the results of two different experiments were provided for both latanoprost and latanoprostene bunod. Comparing the four studies together, there is no clear difference between the two test articles.

Title	**Effect of latanoprostene bunod on endothelin-1-induced primary human trabecular meshwork cell contractility**	
Report #	PH13033	
Key findings	• LBN exhibited activity in HTMC cells at 30 and 45 µM, but not at 10 µM, on an *in vitro* endpoint. The pharmacological relevance of the endpoint is unclear. The study design and reporting are not adequate to demonstrate a proof-of-concept for intended pharmacology. o LBN was not different than latanoprost at 30 µM. o LBN was more active than latanoprost at 45 µM; it is not clear whether this difference is biologically relevant. • The reported results appear to overlap with a published paper, Cavet et al. 2015[9].	
Report details	Study laboratory	Bausch + Lomb, Preclinical Pharmacology 1400 North Goodman Street Rochester, NY 14609
	Report date	April 1, 2014
	Test article	LBN, lot # 6889 (no purity or formulation information provided)
Test method	• The authors claim that contractility in HTMC cells can be measured *in vitro* using an ECIS system, which uses arbitrary units of measurement. Data are double-normalized (first to pre-treatment resistance, then to untreated control values). No additional information regarding the endpoint was provided in the report. • The paper (Cavet et al. 2015) reports that ECIS is "electric cell substrate impedance sensing": cells are grown on a small electrode until confluent. A larger electrode is placed into the culture medium, and the electric impedance value is measured, to calculate resistance and capacitance over 2 hours. It is not clear to this reviewer, from the study report or the paper, if or how this endpoint relates to contractility.	
Rationale	• ET-1 caused a rapid increase in HTMC resistance, reaching a maximum by 60 minutes post-dose • Latanoprost and LBN partially inhibit the increase induced by ET-1. • The authors propose that the nitric oxide released by LBN will relax the HTMC cells *in vitro*, and similarly will relax trabecular meshwork cells *in vivo*, increasing outflow and thereby reducing IOP	
Methods	• Confluent cell cultures were serum-starved overnight, then treated with 100 nM ET-1 + 0, 10 µM, 30 µM, or 45 µM of either latanoprost or LBN.	

[9] This paper was submitted to the NDA as a Clinical literature reference (NDA module 5.4) but not as a Nonclinical literature reference (NDA module 4.3). The citation is: Cavet ME, Vollmer TR, Harrington KL, VanDerMEdi K, Richardson ME. 2015. Regulation of endothelin-1-induced trabecular meshwork contractility by latanoprostene bunod. Invest. Ophthalmol. Vis. Sci. 56(6):4108-4116.

	• ECIS data were collected for 20 minutes pre-treatment, to 120 minutes post-treatment.
Results:	• At 10 µM, neither LBN nor latanoprost inhibited the activity of ET-1. • At 30 µM, both LBN and latanoprost exhibited the activity of ET-1 for 2 hours. No difference between the two compounds was apparent. • At 45 µM, LBN appeared more active than latanoprost for inhibition of ET-1 activity. **Figure 4: BLN inhibition of ET-1 activity on HTMC cells *in vitro* (report # PH13033)**  *: statistically different from control , p< 0.05, by two-way ANOVA-Tukey Kramer test #: statistically different from latanoprost, p < 0.05 by two-way ANOVA-Tukey Kramer test

Title	**Effect of latanoprostene bunod on actin cytoskeleton dynamics in primary human trabecular meshwork cells**
Report #	PH13028
Key findings	• This report is not useful to support regulatory review, because insufficient data were provided to allow for meaningful review. • The authors report that LBN decreased ET-1 induction of actin stress fibers and vinculin localization to focal adhesions in HTMC cells *in vitro*.

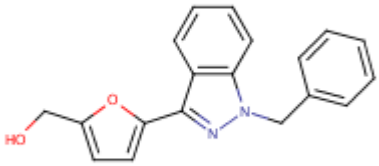
Report details	Study laboratory	Bausch + Lomb, Preclinical Pharmacology 1400 North Goodman Street Rochester, NY 14609
	Report date	April 1, 2014
	Test article	LBN, lot # 6889 (no purity or formulation information provided)
Rationale	• The purpose of the experiment was to compare LBN and latanoprost for activity on HTMC actin cytoskeleton dynamics. The authors proposed that NO would affect MLC-2 phosphorylation, and thereby alter the cytoskeleton dynamics, showing a relaxation of the cell which would predict increased outflow and lower IOP *in vivo*. • The results of this report were also published in the Cavet et al. 2015 paper.	
Methods	• SE 175 was used as a NO donating positive control. The identity and mechanism of action of SE 175 is public knowledge: SE 175 is 2-[[4-[(nitrooxy)methyl]benzoyl]thio]-benzoic acid, methyl ester (CAS # 258278-64-7). Commercial vendors[10],[11],[12] report that SE 175 exhibits vasorelaxing properties similar to nitroglycerin, via transformation of the nitrate group to nitric oxide. **Figure 5: Structure of the NO-releasing positive control, SE 175**  	
	• HTMC cells were treated, than fixed and stained for immunohistochemistry: for actin using labeled phalloidin, and for focal adhesions using antibody against vinculin). • The authors report that treatment for 1 hour with 0.1 µM ET-1 increased stress fiber formation and vinculin localization at stress fibers. • Multiple experiments were conducted, testing LBN and latanoprost at 30 or 45 µM, either treating cells with the test agents first, followed by ET-1, or co-treating cells with test article + ET-1. • The report states “two images were taken from each treatment	

[10] Caymen Chemicals. Product Information SE 175. Accessed via https://www.caymanchem.com/pdfs/82340.pdf
[11] Sigma-Aldrich. SE 175. Accessed via http://www.sigmaaldrich.com/catalog/product/sigma/s2189?lang=en&region=US
[12] Santa Cruz Biotechnology. Product block. SE 175. Accessed via http://www.scbt.com/datasheet-205506-se-175.html

	group". It is not clear if the different treatment groups used more than one well/treatment group.
Results	• Results were presented as microscopy images only (40x magnification), with staining for actin, vinculin, or both. • The report did not provide quantitative data. The report does not explicitly claim that the photographs provided were representative. Therefore, the data are not reviewable. • The authors report that latanoprost had "minimal effect" on inhibiting ET-1 induced actin and vinculin localization, while LBN "dramatically altered" the actin cytoskeleton and focal adhesion dynamics in HTMC cells.

Title	**The effect of latanoprostene bunod on cGMP generation in human trabecular meshwork cells**	
Report #	PH13023	
Key findings	• This study is not useful for regulatory purposes; the results were too variable and inconsistent for interpretation. • The authors concluded that LBN increased cGMP in HTMC cells more than latanoprost did • This study did not co-treat cells with YC-1 and other test articles; later studies (reviewed below) did use YC-1 co-treatment.	
Report details	Study laboratory	Bausch + Lomb, Preclinical Pharmacology 1400 North Goodman Street Rochester, NY 14609
	Report date	October 30, 2013
	Test article	LBN, lot # 6889 (no purity or formulation information provided)
Rationale	NO has been shown to activate sGC in muscle cells, and thereby elevate cGMP. These experiments were intended to determine whether release of NO by LBN activates this pathway in HTMC cells	
Methods	• 3-isobutyl-1-methylxanthine (IBMX) was used as a phosphodiesterase inhibitor • 1,4-butanediol (BDMN) was also tested. LBMS is the NO-donating moiety of LBN. • 3-(5-hydroxymethyl-2-furyl)-benzylindazole (YC-1, CAS # 154453-18-6) was used as an activator of sGC, as a positive control. o The authors note that YC-1 does not release NO. o This reviewer found several published papers, which support the author's claim that YC-1 activates sGC.[13],[14],[15].	

[13] Stone JR, Marletta MA. 1998. Synergistic activation of soluble guanylate cyclase by YC-1 and carbon monoxide: implications for the role of cleavage of the iron-histidine

	YC-1 binds directly to sGC, and stabilizes the binding of sGC to both NO and CO. o The chemical structure of YC-1 was obtained from the National Institutes of Health (NIH)'s ChemIDplus database (chem.sis.nlm.nih.gov): **Figure 6: Chemical structure of YC-1, an activator of cyclic guanylate cyclase (cGC)**  • SE 175, a NO-donor, was used as another positive control
	• HTMC cells were pre-treated with 100 µM IBMX. Although not explained in the NDA, this reviewer understands that the purpose of the IBMX is to prevent metabolism of cGMP, to allow for accumulation of detectable levels. The duration of IBMX pre-treatment was not reported. • The effect of IBMX on cell viability was not reported.
	• After IBMX pre-treatment, cells were treated with various test agents, including LBN (1, 3, 10, 30, or 100 µM), BDMN (10 or 100 µM), and latanoprost (30 or 100 µM) for 30 minutes. • Cells were lysed, and cMPG was measured using a commercial enzyme immunoassay (EIA) kit. The lower limit of quantitation (LLOQ) was not reported, but graphical data are presented in increments < 5 femtomol/ml, which seems implausible.
Results	• Across multiple experiments, results were inconsistent, with positive controls only sometimes exhibiting expected results. • In some assays, cGMP was increased by latanoprost, LBN, and

bond during activation by nitric oxide. Chem. Biol. 5(5):255-261. Abstract accessed via: http://www.ncbi.nlm.nih.gov/pubmed/9646941

[14] Friebe A, Koesling D. 1998. Mechanism of YC-1-induced activation of soluble gyanylyl cyclase. Mol. Pharmacol. 53(1):123-127. Paper accessed via: http://www.ncbi.nlm.nih.gov/pubmed/9443939

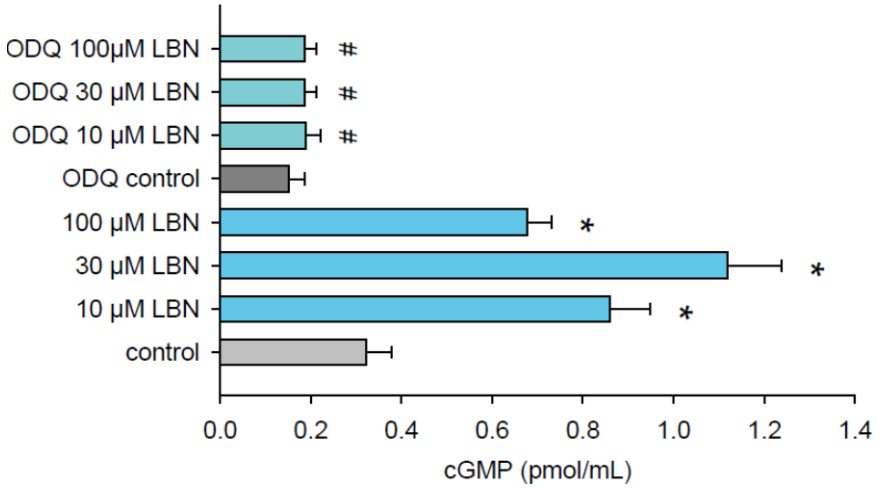
[15] Pal B, Kitagawa T. 2010. Binding of YC-1/BAY 41-2272 to soluble guanylate cyclase: A new perspective to the mechanism of activation. Biochem. Biophys. Res. Commun. 397(3):375-379. Paper accessed via: http://www.ncbi.nlm.nih.gov/pubmed/20513359

	BDMN. Only one experiment observed a dose response for LBN; the same experiment observed less activity for latanoprost.

Title	**The effect of the soluble guanylate cyclase inhibitor ODQ on latanoprostene bunod-induced cGMP generation in human trabecular meshwork cells**	
Report #	PH13031	
Key findings	• An inhibitor of sGC blocked the effect of LBN on cGMP levels in HTMC cells under the conditions tested *in vitro*. • This provides some support for the Applicant's proposed mechanism of action (i.e. that LBN releases NO, which activates sGC to produce cGMP). • These experiments used YC-1 to bind sGC. Therefore, the potential relevance of these results to *in vivo* activity is unclear.	
Study details	Study laboratory	Bausch + Lomb, Preclinical Pharmacology 1400 North Goodman Street Rochester, NY 14609
	Report date	January 10, 2014
	Test article	LBN, lot # 6889 (no purity or formulation information provided)
Rationale	• The author reported that 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one(ODQ) is a highly selective, irreversible, heme-site inhibitor of sGC. This reviewer identified several published papers which support this claim[16],[17]. • The inhibition of cGMP activity induced by LBN (+ YC-1) indicates that at LBN is affecting sGC in HTMC cells.	
Methods	• HTMC cells were pretreated with 100 µM IBMX, with or without 1 µM ODX (duration of pre-treatment not specified). • Cells were then treated with 10 µM YC-1 + IBMX, with or without LBN (10, 30 or 100 µM) and with or without ODQ for 30 minutes. • cGMP was assayed using an EIA kit.	
Results	• In the presence of YC-1, LBN induced cGMP. No dose-response was	

[16] Zhao Y, Brandish PE, Di Valentin M, Schelvis JP, Babcock GT, Marletta MA. 2000. Inhibition of soluble guanylate cyclase by ODQ. Biochemistry. 39(35):10848-10854. Abstract accessed via: http://www.ncbi.nlm.nih.gov/pubmed/10978171

[17] Feelisch M, Kotsonis P, Siebe J, Clement B, Schmidt HH. 1999. The soluble guanylyl cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3,-a] quinoxalin-1-one is a nonselective heme protein inhibitor of nitric oxide synthase and other cytochrome P-450 enzymes involved in nitric oxide donor bioactivation. Mol Pharmacol. 56(2):243-253. Paper accessed via: http://www.ncbi.nlm.nih.gov/pubmed/10419542

	apparent. • Pre-treatment followed by co-treatment with ODQ prevented this increase in cGMP by LBN. **Figure 7: LBN induced cGMP production in HTMC cells; ODQ inhibited this cGMP production (report # PH13031)**  * = statistically different from control, $p < 0.05$ by two-way ANOVA and Tukey-Kramer test. # = statistically different from LBN without ODQ, $p < 0.05$ by two-way ANOVA and Tukey-Kramer test.

Title	**Effect of latanoprostene bunod (NCX 116) on cGMP levels in PC-12 cells and iNOS levels in RAW macrophages**	
Report #	PH14010	
Key findings	• In a rat adrenal tumor cell line (PC-12) primed with YC-1, LBN clearly induced more cGMP than latanoprost • The iNOS assay in RAW cells was not useful for regulatory review.	
Study details	Study laboratory	NicOx (further location details not provided in the report)
	Report dates	• "Version 2" was edited on February 11, 2005 • Bausch + Lomb added a cover sheet and assigned a new study number on June 11, 2014. • Data were published by Krauss et al. 2011[18]

[18] Krauss, A.H., Impagnatiello, F., Toris, C.B., Gale, D.C., Prasanna, G., Borghi, V., Chiroli, V., Chong, W.K., Carreiro, S.T., and Ongini, E. 2011. Ocular hypotensive activity of BOL-303259-X, a nitric oxide donating prostaglandin F2alpha agonist, in

	Test article	LBN, laboratory code NCX116, purity ≥ 95.0%
Methods for the cGMP study	• PC-12 cells were obtained from a rat pheochromocytoma of the adrenal medulla. • In PC-12 cells, LBN has "only marginal effects of cGMP accumulation (data not shown)." • In the presence of 20 µM YC-1, the authors tested a series of 9 LBN concentrations (from ~ 1 nM to ~ 50 µM). • The method of cGMP detection was not reported • A positive control compound (NCX 4016) was also tested over a range of concentrations, and latanoprost was tested at one concentration (~ 50 µM).	
Results for the cGMP study	• LBN EC_{50} = 2.49 µM • Latanoprost did not increase cGMP, under the conditions tested.	
iNOS study	• Neither latanoprost nor LBN exhibited activity in the experiment. • The model was lipopolysaccharide (LPS) induction of inducible nitric oxide synthase (iNOS) in a rat macrophage cell line, RAW 264.7 cells. • Although the authors did not explain the rationale, this reviewer infers that the goal was to investigate whether or not the effects of LBN on cGMP could be attributable to indirect NO production, rather than NO released from LBN.	

Title	**Effect of latanoprostene bunod (NCX 116) on cGMP levels in HEK 293 cells**	
Report #	PH14011	
Key findings	LBN was more active than latanoprost for induction of cGMP in a human embryonic kidney cell line, HEK 293 cells, primed with YC-1.	
Report details	Study laboratory	• Bausch + Lomb, Preclinical Pharmacology 1400 North Goodman Street Rochester, NY 14609 • Note: results were published in the Krauss et al. 2011 paper
	Report date	June 11, 2014
	Test article	LBN (NCX 116); no lot, purity, or formulation information
Methods	• HEK 293 cells are a human embryonic kidney cell line. • HECK 293 cells were treated with IBMX (100 µM), YC-1 (50 µM), and test article for 30 minutes. • A range of 5 concentrations of latanoprost and LBN were tested, with a highest concentration of 50 µM. • Intracellular cGMP accumulation was assayed using an EIA kit.	

preclinical models. Exp Eye Res 93:250-255. Paper submitted to the NDA in module 4.3 (Nonclinical Literature References).

Results	• LBN EC_{50} = 8.8 µM • Latanoprost exhibited weaker activity than LBN at each concentration level. The authors did not calculate and EC_{50} value for latanoprost.

Title	**Effect of latanoprostene bunod on IOP in ocular hypertensive non-human primates**	
Report #	PH14006	
Key findings	• Both LBN and latanoprost lowered IOP in the left eyes of monkeys who underwent lasering of the trabecular meshwork to increase IOP. • No difference between LBN and latanoprost is apparent o The results are difficult to interpret, due to variability in baseline and inconsistent IOP-lowering following application of the vehicle. o The authors concluded that LBN exhibited greater efficacy than latanoprost at 0.03% and 0.12%. However, this conclusion is not supported by the results provided.	
Report details	Study laboratory	• The study was conducted in the laboratory of Dr. Carol Toris at the University of Nebraska Medical Center, Omaha, NE • Note: results were published in the Krauss et al. 2011 paper • The Applicant, Bausch + Lomb, prepared the report from unpublished and published information provided to them.
	Report date	May 21, 2014
	Test article	LBN (NCX 116); no lot, purity, or formulation information
Disease model	• Eight female cynomolgus monkeys (4-15 years old) received OS laser treatment of the trabecular meshwork, 0.2 to 8.3 years before this study, to cause ocular hypertension. o This reviewer presumes that at least several of the monkeys had been used in previous experiments (i.e. not treatment-naïve). o IOP measurements were made OU without sedation, with topical proparacaine, and using a pneumatometer	
Methods	• Vehicle was: (b) (4) % tween-80, (b) (4), 0.2 mg/ml BAK, in PBS, pH 6.7 • Treatment was masked. The left eyes received one 30 µl drop of test article or vehicle, and then 3 days later, received the opposite (either vehicle or test article) • Five different experiments were conducted, comparing test articles with vehicle: o 0.012% LBN	

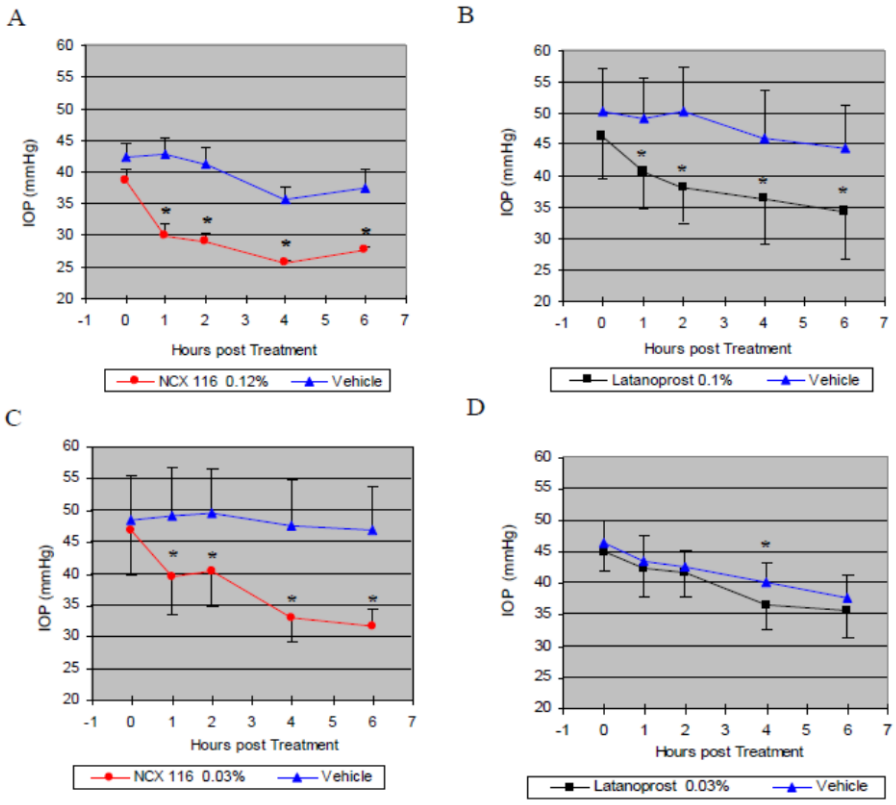
	o 0.01% latanoprost o 0.03% LBN o 0.03% latanoprost o 0.12% LBN o 0.1% latanoprost. Note: no explanation was provided for why latanoprost was tested at 20% lower concentrations than LBN (0.01% versus 0.012%, and 0.1% versus 0.12%). • The washout period between experiments was not reported. • OD IOP was measured; it is not clear if the right eyes received no treatment, or vehicle.
Results	All of the report results are copied below, which includes all the monkey results from the published paper (Krauss et al. 2011). **Figure 8: IOP graphical results from the monkey PD study (report # PH14006)**  Time-course of IOP effect (mean ± SEM; N = 6) of latanoprostene bunod (NCX 116) in ocular-hypertensive nonhuman primates is shown in Panels A (36 µg) and C (9 µg) and of latanoprost in Panels B (30 µg) and D (9 µg). Drug and vehicle emulsions were administered topically as single 30 µL drops in a crossover fashion immediately after the baseline IOP measurement at time 0 h. * p <0.05 (drug vs. vehicle; mixed-model ANOVA analysis).

Table 28: IOP time course data for 0.03% LBN in monkeys (report # PH14006)

Table 6-1 IOP after treatment with 0.03% latanoprostene bunod (NCX 116)

Time (h)	IOP (± SEM; mm Hg)			
	0.03% (9 µg) LBN (NCX 116)		Vehicle	
	OD	OS	OD	OS
0	25.38 ± 2.13	46.83 ± 7.14	23.87 ± 2.01	48.30 ± 6.91
1	25.35 ± 1.97	39.38 ± 6.05	23.83 ± 2.39	49.00 ± 7.55
2	25.60 ± 1.97	40.30 ± 5.46	25.65 ± 1.92	49.48 ± 6.85
4	24.70 ± 2.22	33.07 ± 3.81	23.52 ± 2.20	47.42 ± 7.47
6	26.72 ± 2.24	31.60 ± 2.72	24.18 ± 1.72	46.73 ± 6.92

Table 29:IOP time course data for 0.03% latanoprost in monkeys (report # PH14006)

Table 6-2 IOP after treatment with 0.03% latanoprost

Time (h)	IOP (± SEM; mm Hg)			
	0.03% (9 µg) latanoprost		Vehicle	
	OD	OS	OD	OS
0	28.48 ± 1.49	44.97 ± 3.10	25.97 ± 1.34	46.23 ± 3.60
1	27.80 ± 1.73	42.23 ± 4.49	27.25 ± 1.14	43.27 ± 4.09
2	27.25 ± 1.13	41.55 ± 3.77	28.12 ± 1.09	42.53 ± 2.64
4	27.27 ± 1.50	36.45 ± 3.86	27.08 ± 1.94	40.08 ± 3.12
6	26.80 ± 1.71	35.50 ± 4.33	28.28 ± 1.21	37.55 ± 3.48

Note: time course data were not provided for the other concentrations compared (i.e. 0.01% versus 0.012%, and 0.1% versus 0.12%).

Table 30: Summary change in IOP data for monkeys treated with

LBN or latanoprost (report # PH14006)

Table 6-3 IOP lowering profile of LBN and latanoprost in OHT non-human primates

Treatment Group	Concentration	Basal IOP (mm Hg)	Δ_{max} (mm Hg)[a]	Δ_{max} (%)[a]
Vehicle (latanoprost)	(0.01)	48.2 ± 3.0	NE	NE
	(0.03)	46.2 ± 3.6	-8.7 ± 2.2	-18.7
	(0.10)	50.2 ± 6.7	-5.8 ± 2.5	-11.5
Latanoprost	0.01	46.2 ± 2.5	NE	NE
	0.03	44.9 ± 3.1	-9.5 ± 1.9	-21.0
	0.10	46.1 ± 6.6	-11.9 ± 3.8*	-25.8
Vehicle (LBN)	(0.012)	45.0 ± 4.5	NE	NE
	(0.030)	48.3 ± 6.9	-1.6 ± 2.2	-3.0
	(0.120)	42.0 ± 4.5	-6.5 ± 1.9	-15.0
LBN	0.012	42.3 ± 4.6	NE	NE
	0.030	46.8 ±7.1	-15.2 ± 4.9*	-31.0
	0.120	39.0 ±3.0	-13.5 ± 3.0*	-35.0

[a] Δ_{max} refers to maximal changes from baseline values within the experimental period (0- 6 h).
NE, not effective
*P<0.05 vs. respective vehicle using a mixed model ANOVA analysis incorporating animal-to-animal and period-to-period variability.

Discussion:

- Although the authors conclude that latanoprost was less active than LBN, this reviewer disagrees.
- The differences in baseline IOP, and the decrease in IOP for vehicle, indicate that the numerical differences between the LBN and latanoprost groups are not meaningful, under the conditions tested.

Title	**Effect of latanoprostene bunod on aqueous humor dynamics in ocular hypertensive primates**	
Report #	PH14005	
Key findings	• Previous studies using latanoprost acid the pro-drug have demonstrated that latanoprost acid lowers IOP by increasing uveoscleral outflow of aqueous humor. • In monkeys, LBN lowered IOP, attributed to increased uveoscleral outflow (i.e. presumably via latanoprost acid). • This study raises no safety concerns for LBN.	
Report details	Study laboratory	(b) (4)
	Report dates	• July 22, 2015 (b) (4) report) • January 8, 2016 (B&L cover pages)
	Test article	LBN (not lot #, formulation, or purity data provided)

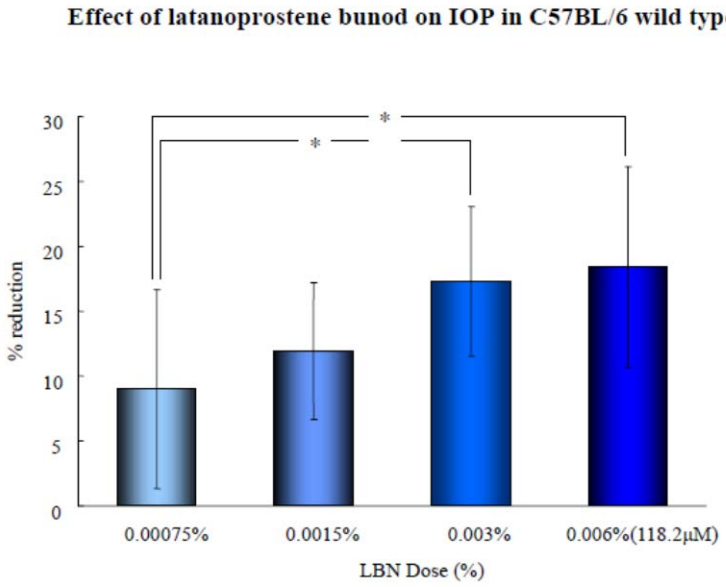
	Submission date	This report was submitted to the NDA on 1/22/2016.
Disease model	• Adult female cynomolgus monkeys (7 to 15 years of age). Previously (4 to 8 years earlier), these monkeys had had laser treatment of the OS or OU trabecular meshwork to raise IOP (i.e. by decreasing/preventing outflow through the trabecular meshwork). • These monkeys were not treatment naïve (washout period not specified) • The authors report that lasering raises IOP by 5 to 10 mm Hg. • Note: although not explicitly stated, this reviewer presumes that these are the same monkeys as were used in study # PH14006.	
Rationale	• The rationale was to show that lasering the trabecular meshwork to increase IOP does not obviate the effect of therapies on uveoscleral outflow. • As this has already been demonstrated for latanoprost acid, this reviewer infers that the authors were demonstrating the sensitivity of their lasered females to LBN therapy, rather than investigating LBN activity *per se* [i.e. these studies were not intended to investigate the contribution of NO to IOP lowering]	
Methods	• Eyes were treated with vehicle or 0.024% LBN: two 25 µl drops per dose (5 minutes apart), QD. • IOP was measured with or without iv ketamine anesthesia. • Fluorescein was applied topically in some experiments, and the aqueous flow was measured using a scanning ocular fluorophotometer. [Details of the method are unclear.]	
Results	• 0.024% LBN: o Did not affect aqueous flow, as measured by fluorophotometry, in normotensive or hypertensive eyes. o Did not decrease IOP in normotensive eyes. o Decreased IOP in hypertensive eyes in two different experiments: by 12.4 mm Hg in one experiment, and by 3 mm Hg in another experiment. ▪ The authors claim that by tonography calculations, aqueous humor was decreased in the hypertensive eyes by increasing uveoscleral outflow. No calculations or other relevant data were submitted, to allow for review of this claim.	

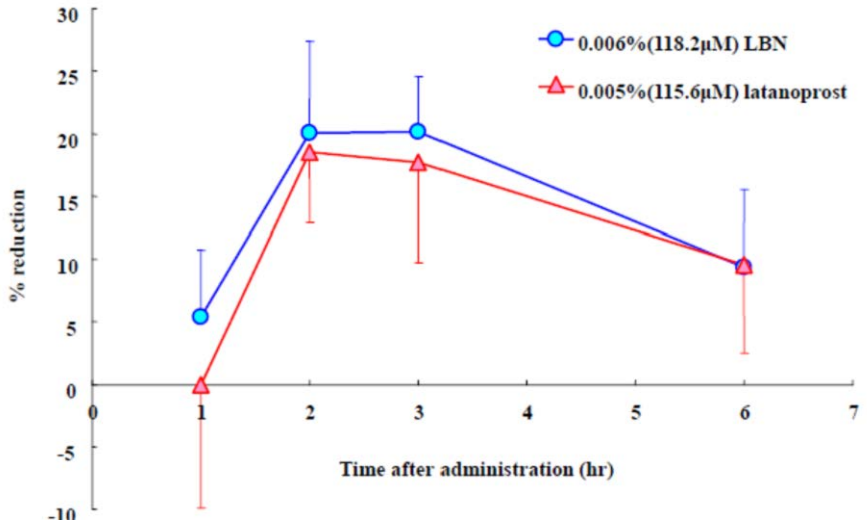
Title	**The effect of latanoprostene bunod on IOP in wild type and FP receptor knockout C57BL/6 mice**	
Report #	PH14007	
Key findings	• LBN lowered IOP in wild-type mice, comparable with latanoprost. • FP receptor knockout mice are resistant to latanoprost. LBN exhibited a small but detectable decrease in IOP in this model (-0.45 to -1.23 mm Hg), indicating that LBN exhibits IOP separate from the FP receptor (i.e. via NO donation). • The report is 9 pages total. The lack of details regarding the model and the results limit the regulatory usefulness of these experiments.	
Report details	Study laboratory	• These experiments were performed in the laboratory Dr. Araie at Tokyo University, Bunkyo-ku, Japan. • Note: the results were published in an abstract: Saeki, T., Tsuruga, H., Aihara, M., Araie, M., Rittenhouse, K. 2009. Dose-response profile of PF-03187207 (PF-207) and peak IOP lowering response following single topical administration to FP receptor knockout mice vs. wild type mice. Invest. Ophthalmol Vis Sci:E-abstract 4064.
	Report date	May 19, 2014
	Test article	LBN; no lot, purity, or formulation information
Animal model	• Male wild type C57BL/6 mice • Male FP receptor knock-out (FPKO) mice - C57BL/67FPKO mice. o The authors cite two papers, Crowston et al. 2004[19] and Ota et al. 2005[20] for background information on the model. This reviewer identified one additional paper by Dr. Araie's laboratory with this model, Ota et al. 2007[21]. o In FPKO mice, lack of IOP-lowering activity has previously been observed for latanoprost, travoprost, bimatoprost, and unoprostone.	
Methods	• Groups of 8 C57BL/6 mice received a single 3 µl topical ocular dose of 0.00075%, 0.0015%, 0.003%, or 0.006% LBN to one eye. IOP was	

[19] Crowston JG, Lindsey JD, Aihara M, Weinreb RN. 2004. Effect of latanoprost on intraocular pressure in mice lacking the prostaglandin FP receptor. Invest Ophthalmol Vis Sci. 45(10):3555-3559. Abstract accessed via: http://www.ncbi.nlm.nih.gov/pubmed/15452062

[20] Ota T, Aihara M, Narumiya S, Araie M. 2005. The effects of prostaglandin analogues on IOP in prostanoid FP-receptor-deficient mice. Invest Ophthalmol Vis Sci. 46(11):4159-4163. Abstract accessed via: http://www.ncbi.nlm.nih.gov/pubmed/16249494

[21] Ota T, Aihara M, Saeki T, Narumiya S, Araie M. 2007. The IOP-lowering effects and mechanism of action of tafluprost in prostanoid receptor-deficient mice. Br J Ophthalmol. 91(5):673-676. Paper accessed via: http://www.ncbi.nlm.nih.gov/pubmed/17124244

	measured 3-hours post-dose • In a separate experiment, Groups of 8 C57BL/6 mice or 8 FPKO mice received a single 3 µl topical ocular dose of 0.006% LBN or 0.005% latanoprost to one eye. • The micro-needle method was used to measure IOP pre-dose, and 1, 2, 3 and 6 hours post-dose.
Results	• A dose-response was reported for wild-type mice treated with LBN, with IOP measured 3 hours post-dose. **Figure 9: LBN lowered IOP in wild type mice (report # PH14007)** 
	• In wild-type mice, both LBN (0.005%) and latanoprost (0.005%) lowered IOP. No difference between the two treatments was observed throughout the time course (1-6 hours post-dose). **Figure 10: LBN was not more active than latanoprost for IOP lowering in wild type mice (report # PH 14007)**



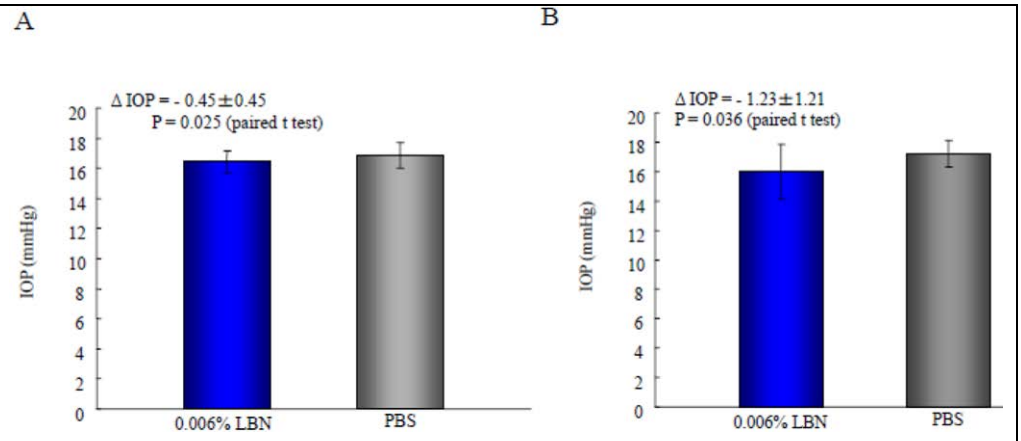
- FPKO mice had a baseline IOP of ~ 16 mm Hg. The authors report that latanoprost had no effect.
- The authors provided a single data point for 2 experiments at 3 hours post-dosing. Without standard error values reported, and without the raw IOP data, it is not possible to determine the statistical significance or biological relevance of these data.

Table 31: LBN had a small IOP lowering effect in prostaglandin F (FP) receptor knock out mice (FPKO mice) (report # PH 14007)

Experiment #	IOP lowering at 3 hours post-dose, versus baseline, for a single topical ocular dose of 0.006% LBN
# A	- 0.45 mm Hg
# B	- 1.23 mm Hg

For experiment A, the control was the contralateral eye (treated with PBS at the same time). For experiment B, the control was the same eye as treatment, re-treated with PBS after a 2-week washout. The same data presented graphically:

Figure 11: LBN had a small IOP lowering effect in prostaglandin F (FP) receptor knock out mice (FPKO mice) (report # PH 14007)

Title	**Effect of latanoprostene bunod on IOP in glaucomatous dogs**	
Report #	PH14008	
Key findings	• In beagle dogs with inherited primary moderate open-angle glaucoma, 0.03% LBN was clearly more active for IOP lowering than 0.03% latanoprost. • This report is the strongest proof-of-concept submitted in the nonclinical data package, for the proposed mechanism of additional IOP lowering via NO. • This reviewer considers this study to be of questionable reliability, due to unexplained missing data.	
Report details	Study laboratory	• The experiment was conducted in the laboratory of Drs. ME Kalberg and K.N. Gellatt at the University of Florida. • Note: results were published in the Krauss et al. 2011 paper • The Applicant, Bausch + Lomb, prepared the report from unpublished and published information provided to them.
	Report date	June 23, 2014
	Test article	LBN (NCX 116); no lot, purity, or formulation information
Disease model	• The dogs all "exhibited primary open angle glaucoma in the moderate stage (narrow iridocorneal angle and ciliary cleft opening), and all animals demonstrated clinical vision. IOP measurements were recorded with an applanation tonometer". • The authors cite Gelatt and MacKay 2001[22] for the model.	

[22] Gelatt KN, MacKay EO. 2001. Effect of different dose schedules of latanoprost on intraocular pressure and pupil size in the glaucomatous Beagle. Vet. Ophthalmol. 4(4):283-288. Abstract accessed via: http://www.ncbi.nlm.nih.gov/pubmed/11906665

<table>
<tr><td>Methods</td><td>

Vehicle: (b) (4) % Tween-80; (b) (4); 0.2 mg/ml BAK in PBS pH 6.7
Dosing: dog received a single topical ocular drop (15 µg) of either 0.03% LBN (15 µg/eye) or 0.03% latanoprost
IO was measured pre-dose, and at 1, 2, 3, 4 and 6 hours post-dose
The report does not indicate the design, e.g.:

o if dosing was one eye treated with test article, other eye treated with vehicle
o # of dogs/treatment (presumably 4/dose, but perhaps 8/dose if a washout period was used)

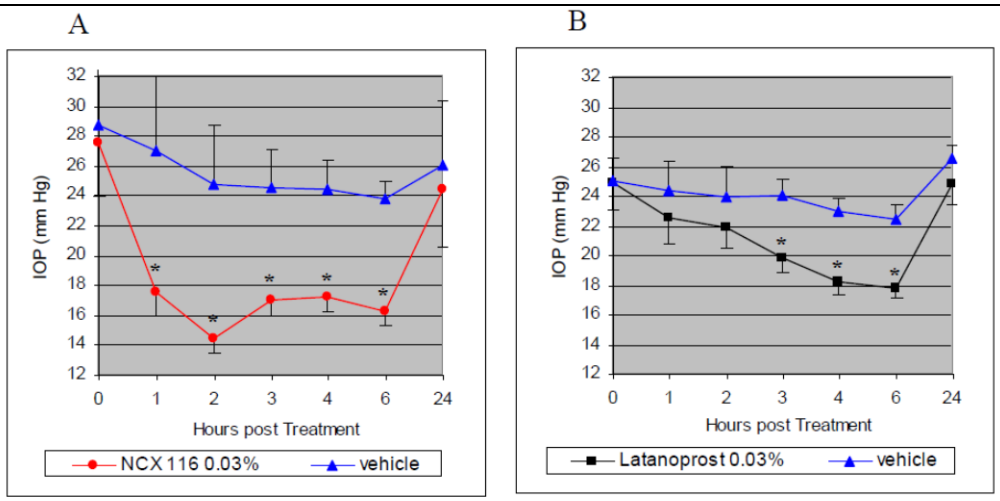
</td></tr>
<tr><td>Results</td><td>

LBN and latanoprost lowered IOP. LBN was clearly more active.
The report provided limited numerical data, and two graphs.

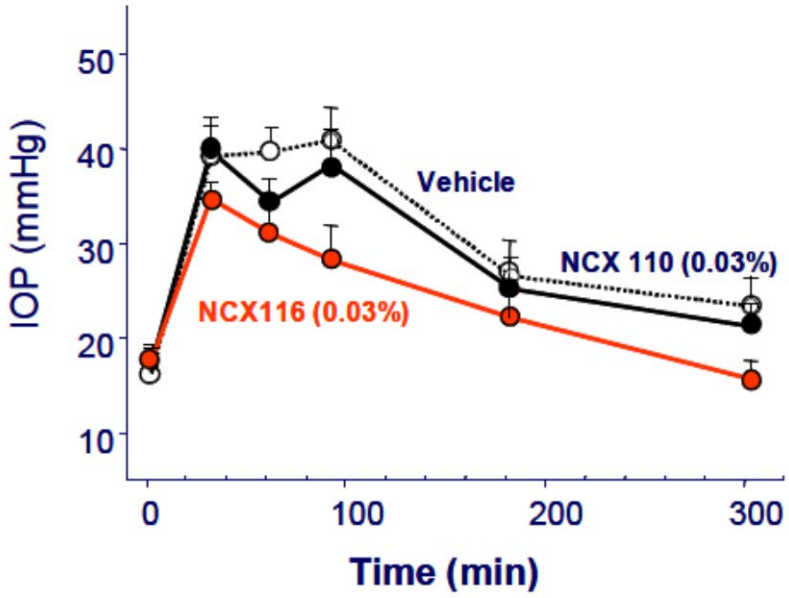
Table 32: IOP data reported for the dog PD study (report # PH 14008)
<table>
<tr><td></td><td colspan="4">IOP (mm Hg)</td></tr>
<tr><td>Time point</td><td>LBN vehicle group</td><td>LBN 0.03% group</td><td>latanoprost vehicle group</td><td>latanoprost 0.03% group</td></tr>
<tr><td>Pre-dose</td><td>28.5 ± 3.5</td><td>27.8 ± 2.7</td><td>25.1 ± 2.5</td><td>25.1 ±2.0</td></tr>
<tr><td>2 hours</td><td>24.5 ± 1.0</td><td>13.2 ± 1.5</td><td>-</td><td>-</td></tr>
<tr><td>6 hours</td><td>-</td><td>-</td><td>22.5 ± 1.0</td><td>18.0± 1.8</td></tr>
</table>
Note: this table was prepared by this reviewer from the author’s text.

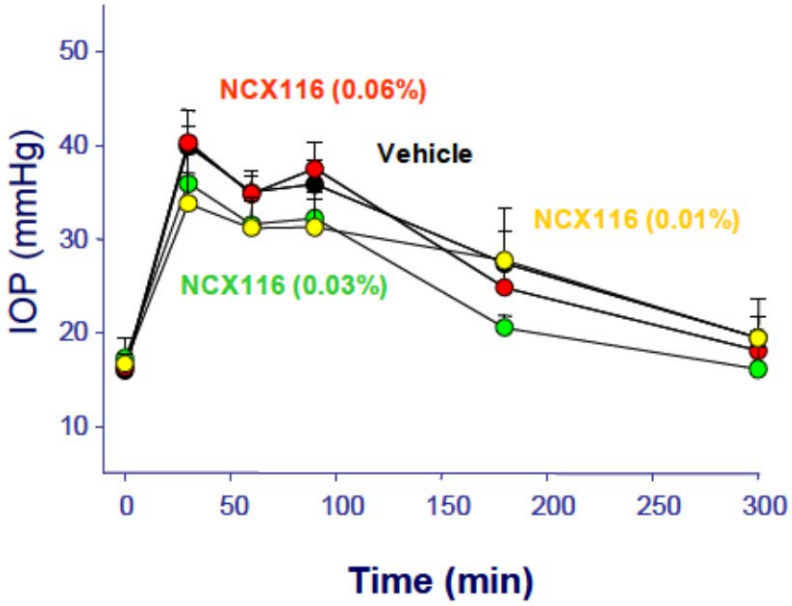
The authors report that the following figure used an N of 6. Since the study used a total of 8 dogs, this reviewer presumes that the same dogs were used for both the 0.03% LBN (window A below) and for 0.03% latanoprost (window B), with a wash out period in between.
The lack of data for the remaining 2/8 dogs is a notable study limitation.

Figure 12: IOP graphical data in the dog PD study (report # PH 14008)
</td></tr>
</table>

	 Note: NCX116 is a code-name for LBN. Data presented as means ± standard error *: statistically different, p < 0.05, by "mixed-model ANOVA analysis"

Title	**Effects of latanoprostene bunod (NCX 116) on saline-induced transient IOP increase in rabbits**	
Report #	PH14009	
Key findings	• This study used a rabbit model of saline-induced IOP. LBN appeared somewhat more active than equimolar latanoprost at lowering IOP in this model. • Like the dog PD study (# PH 14008) reviewed above, this reviewer considers the reliability of this report to be low, due to unexplained missing data.	
Report details	Study laboratory	• Note: results were published in the Krauss et al. 2011 paper • The Applicant, Bausch + Lomb, prepared the report from unpublished and published information provided to them.
	Report date	June 11, 2014
	Test article	LBN; no lot, purity, or formulation information
Disease model	• Male New Zealand White (NZW) rabbits, 2 to 2.5 kg, received an injection of 0.1 ml of hypertonic saline (5%) into the anterior chamber of both eyes. • IOP was measured using a tonometer.	
Methods	• Vehicle: (b) (4) % Tween-80; (b) (4) ; 0.2 mg/mL BAK in phosphate buffer saline pH 6.7 • Individual eyes were randomly assigned to treatment groups • Treatment groups were: o 0, 0.01%, 0.03%, 0.06% LBN	

	o 0.03% latanoprost o Drop size not reported • Group sizes were not reported in the study design section. • IOP was measured pre-dose, and post-dose at 30, 60, 90, 180 and 300 minutes
Results	• Comparing 0.03% LBN with 0.03% latanoprost, LBN appeared more active: **Figure 13: 0.03% LBN decreased IOP more than 0.03% latanoprost in a rabbit model of ocular hypertension (report # PH14009)**  Note: NCX116 is LBN. NCX 110 is a code-name for latanoprost. • No dose-response was apparent for LBN. The authors reported for LBN that "solubility issues (at 0.06% the solubility is close to maximal possible in this vehicle) might have affected the compound availability at the higher dose tested." **Figure 14: The rabbit PD study did not observe a clear dose response for IOP lowering (report # PH14009)**

	 Note: NCX 116 is a code name for LBN.
Discussion note	• The number of eyes/group reported ranged from 4 to 9, without explanation. It is not clear if, or why, data were omitted from the report.

4.2 Secondary Pharmacology

The Applicant submitted one secondary pharmacology study, an *in vitro* screening assay. The results were not concerning. The NDA did not provide secondary pharmacology data for latanoprost for comparison.

Title	***In vitro* pharmacology: tier 0 profile. Study of PF-03187207-00-**	
Report #	757076a	
Key findings	• LBN exhibited inhibition of the human norepinephrine (NE) transporter, with an IC_{50} of 11 µM. • Weak inhibition was observed for 9 other targets: cannabinoid receptor, cholecystokinin 1, muscarinic acetylcholine receptors M1 and M2, neurokinin 1 receptor, serotonin receptors 5-HT2A, 5-HT2B, urea-tryptophan-1 receptor, and dopamine transporter. • This Applicant concluded, and this reviewer concurs, that these results are not concerning.	
Report details	Study laboratory	(b) (4)
	Report date	August 24, 2005

<table>
<tr><td rowspan="3"></td><td>Study period</td><td>August 1 to August 23, 2005</td></tr>
<tr><td>Method</td><td>• A panel of 60 proteins (receptors, channels, or transporters) and 8 enzymes were assayed
• Competitive binding assays were performed, using a radiolabelled ligand specific for each target protein. The specific ligand binding to the target protein is defined as the difference between the total binding and the nonspecific binding determined in the presence of an excess of unlabeled ligand.
• The potential of latanoprostene bunod (PF-03187207-00) to inhibit specific ligand binding was tested at a single concentration, 10 µM
• The authors consider inhibition of natural ligand binding of <20% to be not significant, 20-50% to be weak-to-moderate interaction, and inhibition > 50% to warrant further investigation (i.e. potentially biologically relevant)</td></tr>
<tr><td>Test compound</td><td>PF-03187207-00, batch # PF-03187207-00-0002</td></tr>
<tr><td>Results</td><td colspan="2">Table 33: Selected in vitro secondary pharmacology data (report # 757076a)
<table><tr><th>Target</th><th>% inhibition by 10 µM LBN</th></tr><tr><td>NE transporter(h)</td><td>53</td></tr><tr><td>5-HT_{2b}(h)(agonist site)</td><td>49</td></tr><tr><td>DA transporter(h)</td><td>38</td></tr><tr><td>M1(h)</td><td>33</td></tr><tr><td>5-HT_{2A}(h)(agonist site)</td><td>29</td></tr><tr><td>CB_2(H)</td><td>27</td></tr><tr><td>M2(h)</td><td>22</td></tr><tr><td>UT1(h)</td><td>29</td></tr><tr><td>CCK_A(h)</td><td>20</td></tr></table>Note: the designation (h) refers to human protein</td></tr>
<tr><td>Follow-up Experiment</td><td colspan="2">• For the NE transporter only, the authors repeated the experiment with a range of 8 concentrations of LBN (100 µM to 10 pM)
• No activity was observed ≤ 1 µM.
• The high concentration (100 µM) completely inhibited binding of the NE transporter to its reference compound (protriptyline)
• The 10 µM concentration inhibited binding by ~ 50%.
• The authors calculated an IC_{50} of 11 µM for LBN inhibition of the NE transporter.</td></tr>
<tr><td>Discussion</td><td colspan="2">The Applicant considers these results unlikely to be pharmacologically relevant, and this reviewer concurs. For reference:</td></tr>
</table>

	• The norepinephrine (NE) transporter is expressed by noradrenergic neurons, and is important for the reuptake of extracellular norepinephrine. • 5-Hydroxytryptamine receptor 2B (5-HT_{2B}; serotonin receptor 2B) and 5-HT_{2A} are target receptors for the neurotransmitter serotonin. • The muscarinic M1 receptor (M1) and muscarinic M2 receptor (M2) are important for the parasympathetic nervous system. • The dopamine (DA) transporter is expressed in the brain, and is important for cognition and other brain functions. • Urotensin-II receptor (UT1) binds urotensin II, and is expressed in the cardiovascular system. • Cholecystokinin A receptor (CCK1) is expressed in the gastrointestinal tract and central nervous system, and is important for regulation of stomach pH, gall bladder activity, and gut motility.

4.3 Safety Pharmacology

- No stand-alone safety pharmacology studies for LBN were submitted.
- Electrocardiography (ECG) was measured in the 28-day and 9-month topical ocular toxicity studies. No safety concerns were identified.
- P/T defers to Clinical regarding review of the clinical data. The Applicant reports (NDA module 2.7.4 Summary of Clinical Safety) that LBN did not affect heart rate, systolic or diastolic blood pressure in patients.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The results of the three ocular distribution studies were consistent. Following topical ocular administration to rabbit and monkey eyes, distribution of LBN was cornea > aqueous humor > iris/ciliary body. The Applicant provided a bridging study, comparing ocular distribution of the LBN in both the citric acid-buffered vehicle and in the phosphate-buffered vehicle.

A metabolism study using cornea (rabbit and monkey) and aqueous humor (rabbit only) found that LBN disappeared more quickly than latanoprost in the cornea and aqueous humor. The experiment did not investigate which enzymes were responsible for the disappearance.

(b) (4)

, no nonclinical metabolism experiments were submitted that directly investigated the effects of esterase activity on LBN.

5.1.1 Distribution studies

<table>
<tr><td>Title</td><td colspan="2">Pharmacokinetics of PF-03187207 in Dutch Belted Rabbits Following Topical Administration</td></tr>
<tr><td>Report #</td><td colspan="2">PF03187207-PDM-007</td></tr>
<tr><td>Key findings</td><td colspan="2">• The purpose of the study was to compare the ocular PK of latanoprost acid, following topical ocular administration of LBN or latanoprost to rabbits
• The authors considered the results comparable between LBN and latanoprost, and this reviewer concurs.
• Distribution was cornea > aqueous humor > iris/ciliary body.</td></tr>
<tr><td rowspan="4">Report details</td><td>Study laboratory</td><td>Pfizer Global R&D
La Jolla Laboratories
La Jolla, California</td></tr>
<tr><td>Report date</td><td>February 5, 2007</td></tr>
<tr><td>Test article</td><td>PF-03187207 (LBN), lot # PF-03187207-00-0001 (purity not reported; formulation not reported in the study report)</td></tr>
<tr><td>Species</td><td>Dutch Belted rabbits (ages and sexes not reported)</td></tr>
<tr><td>Methods</td><td colspan="2">• Groups of 8 rabbits received a single topical ocular dose of 30 µl to both eyes (OU) of either 0.01% LBN, or 0.01% latanoprost [i.e. 3 µg/eye]
• 2 rabbits/dose were sacrificed at 0.5, 1, 3 or 6 hours post-dose. Each eye was enucleated.
• The cornea, aqueous humor, and iris/ciliary body were collected, weighed, stored frozen, and then analyzed for latanoprost acid by lC-MS/MS.
• The lower limit of detection (LLOQ) was 1 ng/ml for aqueous humor, and 1 ng for tissue.</td></tr>
<tr><td>Results</td><td colspan="2">Table 34: Rabbit ocular distribution results for latanoprost acid following topical ocular dosing with LBN or latanoprost (report # PF03187207-PDM-007)
<table>
<tr><th>Tissue</th><th>Test compound</th><th>C_{max} (ng/ml or ng/g tissue)</th><th>T_{max} (h)</th><th>$AUC_{0\text{-}6h}$ (ng*h/ml)</th><th>$t_{1/2}$ (h)</th></tr>
<tr><td rowspan="2">Cornea</td><td>LBN</td><td>1526 ± 834</td><td>0.5</td><td>3824</td><td>1.8</td></tr>
<tr><td>latanoprost</td><td>2033 ± 845</td><td>0.5</td><td>5810</td><td>1.7</td></tr>
<tr><td rowspan="2">Aqueous humor</td><td>LBN</td><td>93 ± 31</td><td>1</td><td>360</td><td>2.1</td></tr>
<tr><td>latanoprost</td><td>60 ± 22</td><td>1</td><td>228</td><td>3.3</td></tr>
<tr><td rowspan="2">Iris/ciliary body</td><td>LBN</td><td>43 ± 18</td><td>1</td><td>175</td><td>4.6</td></tr>
<tr><td>latanoprost</td><td>60 ± 26</td><td>1</td><td>255</td><td>2.6</td></tr>
</table>
Data are means ± standard deviations</td></tr>
</table>

<table>
<tr><td>Title</td><td colspan="2">Pharmacokinetics of PF-03187207 in Cynomolgus Monkeys Following Topical Administration</td></tr>
<tr><td>Report #</td><td colspan="2">PF03187207-PDM-008</td></tr>
<tr><td>Key findings</td><td colspan="2">• Like report # PF03187207-PDM-007 reviewed above (the rabbit study), the purpose of the study was to compare the ocular PK of latanoprost acid, following topical ocular administration of LBN or latanoprost in monkeys
• The authors also attempted to measure latanoprost and LBN, but the concentrations were below LLOQ.
• The authors concluded that exposure was higher following latanoprost dosing (+17% to + 2.7-fold), and this reviewer concurs.
• Distribution was cornea > aqueous humor > iris/ciliary body.</td></tr>
<tr><td rowspan="4">Report details</td><td>Study laboratory</td><td>Pfizer Global R&D
La Jolla Laboratories
La Jolla, California</td></tr>
<tr><td>Report date</td><td>February 5, 2007</td></tr>
<tr><td>Test article</td><td>PF-03187207 (LBN), lot # PF-03187207-00-0001 (purity not reported; formulation not reported in the study report)</td></tr>
<tr><td>Species</td><td>Cynomolgus monkeys (ages and sexes not reported)</td></tr>
<tr><td>Methods</td><td colspan="2">• Groups of 4 monkeys received a single topical ocular dose of 30 µl to both eyes (OU) of either nominally 0.01% LBN, or 0.01% latanoprost
o The actual dose was 0.0117% LBN [3.5 µg/eye], and 0.01% latanoprost (3 µg/eye]
• 2 monkeys/dose were sacrificed at 0.5, 1, 3 or 6 hours post-dose. Each eye was enucleated
• The cornea, aqueous humor, and iris/ciliary body were collected, weighed, stored frozen, and then analyzed for latanoprost acid by lC-MS/MS.
• For all three analytes (latanoprost free acid, latanoprost, and LBN), the LLOQ was 5 mg/ml for aqueous humor and 0.1 to 0.5 ng for tissues.
•</td></tr>
<tr><td>Results</td><td colspan="2">• The levels of intact LBN and latanoprost were below the lower detection limit in all monkey tissues.
• The actual difference is more pronounced than these results appear, because the eyes received 10% more LBN (i.e. 0.0117% formulation, rather than 0.1%]

Table 35: Monkey ocular PK results for latanoprost acid following topical ocular dosing with LBN or latanoprost (report # PF03187207-PDM-008)
<table>
<tr><th>Tissue</th><th>Test compound</th><th>C_{max} (ng/ml or ng/g</th><th>T_{max} (h)</th><th>AUC_{0-6h} (ng*h/ml)</th><th>$t_{1/2}$ (h)</th></tr>
</table></td></tr>
</table>

			tissue)			
Cornea	LBN	1313	0.5	2611	1.8
	latanoprost	1863	0.5	3743	1.1
Aqueous humor	LBN	101	0.5	275	2.3
	latanoprost	155	0.5	322	1.5
Iris/ciliary body	LBN	37	1	27	NC
	latanoprost	52	0.5	73	NC

Data are means ± standard deviations
NC = not calculable.

Title	**Investigation of the ocular pharmacokinetics of latanoprost acid following topical ocular administration of BOL-303259-X in various formulations to Dutch Belted rabbits**	
Report #	BL1008	
Key findings	• The purpose of this study was to bridge from the phosphate-buffered vehicle used in Phase 3 clinical trials, to the citric acid-buffered vehicle, for LBN. • The authors concluded that the ocular distribution to the aqueous humor, iris/ciliary body, and cornea of latanoprost acid were comparable. This reviewer concurs. • Distribution of latanoprost acid after topical ocular LBN exposure: cornea > aqueous humor > iris/ciliary body	
Report details	Study laboratory	(b) (4)
	Bioanalytical laboratory	
	Report date	• The amended report is dated June 27, 2014 • The original report was dated November 29, 2010 • In-life was conducted August 2007
	Report status	Not GLP, signed
Formulations	Phase 1/2 formulation	• BLN, 0.024% to 0.04% • (b) (4) phosphate buffer at pH 5.5 • (b) (4) • (b) (4) mg/mL Tween 80 • 0.2 mg/mL Benzalkonium chloride • Sterile water for injection
	Phase 3 formulation	• BLN, (b) (4) % • (b) (4) mM citric acid buffer adjusted to pH 5.5 • (b) (4) % EDTA • (b) (4) % Glycerin

<table>
<tr><td></td><td></td><td>• (b) (4) mg/mL Tween 80
• 0.2 mg/mL Benzalkonium chloride
• Sterile water for injection.</td></tr>
<tr><td></td><td>3 other formulations</td><td>3 other formulations were also investigated, but were not tested clinically (results not presented in this review)</td></tr>
<tr><td>Methods</td><td colspan="2">• This study was conducted over 3 separate experiments, to test a total of 10 different groups (varying the concentration and formulation).</td></tr>
<tr><td></td><td>Test species</td><td>Male Dutch Belted rabbits</td></tr>
<tr><td></td><td>Group size</td><td>8 male rabbits/group</td></tr>
<tr><td></td><td>Dosing</td><td>• Single topical ocular dose, OU
• 30 µl volume
• The single dose was administered into the conjunctival sac. Eyelids were held closed for several seconds after dosing to reduce run-off.</td></tr>
<tr><td></td><td>Safety endpoint</td><td>• Cage side observations only</td></tr>
<tr><td></td><td>Sample harvest</td><td>• 2 rabbits/group per time point were harvested at 0.5, 1, 3, and 6 hours post-dose.
• Aqueous humor, cornea, and the iris-ciliary body were collected and analyzed for latanoprost acid by LC/MC/MS.
• [Other tissues were also harvested, but not analyzed.]</td></tr>
<tr><td>Cage side observations</td><td colspan="2">• The authors report all animals appeared healthy, and no adverse reactions were noted.</td></tr>
<tr><td>Ocular PK results</td><td colspan="2">
Table 36: Latanoprost acid ocular distribution following LBN dosing in rabbits, comparing the Phase 1/2 and Phase 3 formulations (report # BL1008)
<table>
<tr><th rowspan="2">Tissues</th><th rowspan="2">PK para-meter</th><th colspan="2">Phosphate-buffered formulation (Phase 1/2)</th><th colspan="3">Citrate-buffered formulation (Phase 3)</th></tr>
<tr><th>7.2 µg/eye</th><th>12 µg/eye</th><th>4.2 µg/eye</th><th>7.2 µg/eye</th><th>12 µg/eye</th></tr>
<tr><td colspan="7"></td></tr>
<tr><td rowspan="4">Cornea</td><td>C_{max} (µg/g)</td><td>3.29 ± 0.771</td><td>5.23 ± 0.559</td><td>1.81 ± 0.224</td><td>3.74 ± 1.16</td><td>6.80 ± 2.57</td></tr>
<tr><td>C_{max}/ dose</td><td>0.46</td><td>0.44</td><td>0.43</td><td>0.52</td><td>0.57</td></tr>
<tr><td>T_{max} (h)</td><td>0.5</td><td>0.5</td><td>0.5</td><td>1</td><td>0.5</td></tr>
<tr><td>AUC_{0-6} (µg*h/g)</td><td>7.30 ± 0.342</td><td>11.6 ± 0.888</td><td>4.16 ± 0.327</td><td>9.88 ± 0.782</td><td>15.8 ± 1.72</td></tr>
</table>
</td></tr>
</table>

	AUC_{0-6} / dose	1.01	0.97	0.99	1.37	1.32
	$T_{1/2}$ (h)	1.74	1.46	1.90	1.69	1.45
Aqueous humor	C_{max} (µg/ml)	0.149 ± 0.0233	0.198 ± 0.0362	0.0883 ± 0.0450	0.168 ± 0.0624	0.308 ± 0.147
	C_{max}/ dose	0.021	0.017	0.021	0.023	0.026
	T_{max} (h)	1	1	1	1	3
	AUC_{0-6} (µg*h/ml)	0.552 ± 0.0298	0.801 ± 0.0475	0.291 ± 0.0291	0.573 ± 0.0425	1.25 ± 0.187
	AUC_{0-6} / dose	0.077	0.067	0.069	0.080	0.11
	$T_{1/2}$ (h)	2.21	2.03	2.66	2.35	2.43
Iris / ciliary body	C_{max} (µg/g)	0.113 ± 0.0317	0.173 ± 0.0226	0.0709 ± 0.0331	0.121 ± 0.0339	0.209 ± 0.101
	C_{max}/ dose	0.016	0.014	0.017	0.017	0.017
	T_{max} (h)	1	0.5	1	1	0.5
	AUC_{0-6} (µg*h/g)	0.438 ± 0.243	0.625 ± 0.0302	0.242 ± 0.0222	0.457 ± 0.0335	0.812 ± 0.116
	AUC_{0-6} / dose	0.061	0.052	0.058	0.064	0.068
	$T_{1/2}$ (h)	2.20	2.27	2.42	2.35	2.52

Data presented as mean ± standard error.
Note: the report (page 12) notes that the units for the aqueous humor for C_{max} is µg/ml, and for AUC is µg*h/ml. However, the summary tables (i.e. NDA module 2.6.4 Pharmacokinetics Written Summary) present the units as grams rather than milliliters.

5.1.2 Metabolism study

Title	***In Vitro* Characterization of PF-03187207**	
Report #	PF03187207-PDM-006	
Key findings	• The purpose of the experiment was to evaluate the conversion of LBN and latanoprost to latanoprost acid in various tissues • LBN consistently converted more quickly	
Report details	Study laboratory	Pfizer Global R&D La Jolla Laboratories La Jolla, California
	Report date	February 5, 2007
	Test article	PF-00914731, lot # PF-00914731-00-0001 (purity and formulation not reported)
Methods	Tissue samples	• Rabbit and monkey corneal homogenate (prepared from frozen corneas) • Rabbit aqueous humor (obtained commercially) • Plasma from human, monkey, dog, and rabbit

<table>
<tr><td></td><td></td><td>(frozen samples obtained commercially)</td></tr>
<tr><td></td><td>Incubation</td><td>• Tissue samples were incubated with either10 µM of LBN or 10 µM of latanoprost at 37^{o}C.
• Aliquots were taken at 0, 1, 2, 3, 5, 10, 15, 30, 45 and 60 minutes, and analyzed for LBN and latanoprost acid by LC-MS/MS.</td></tr>
<tr><td>Results</td><td colspan="2">Table 37: LBN converted to latanoprost free acid in vitro (report # PF03187207-PDM-006)
<table>
<tr><th>Species</th><th>Matrix</th><th>LBN $t_{1/2}$ (minutes)</th><th>Latanoprost $t_{1/2}$ (minutes)</th></tr>
<tr><td>Rabbit</td><td rowspan="2">Cornea</td><td>0.05</td><td>0.28</td></tr>
<tr><td>Monkey</td><td>0.40</td><td>5.20</td></tr>
<tr><td>Rabbit</td><td>Aqueous humor</td><td>2.8</td><td>20.0</td></tr>
<tr><td>Human</td><td rowspan="4">Plasma</td><td>1.6</td><td>6.9</td></tr>
<tr><td>Monkey</td><td>22</td><td>> 240</td></tr>
<tr><td>Dog</td><td>> 240</td><td>> 240</td></tr>
<tr><td>Rabbit</td><td>0.3</td><td>6.0</td></tr>
</table></td></tr>
<tr><td>Discussion</td><td colspan="2">• Under the conditions tested, LBN apparently converted to latanoprost free acid faster than latanoprost.
• Although the results were presented as elimination half-lives, these values were calculated from latanoprost free acid and LBN (but not latanoprost). No details were presented regarding how the results were calculated. It is not clear how much these data reflect enzymatic metabolism, versus non-enzymatic degradation.</td></tr>
<tr><td></td><td colspan="2">• This reviewer presumes the dog and monkey plasma samples are not representative (i.e. the > 240 minute results appear artifactual)
• The report details the sources of each tissue matrix. It is not clear that the results are comparable across species. Without details regarding storage, the apparent differences may be artifactual).</td></tr>
</table>

5.1.3 Discussion of metabolism

The Applicant provided a Pharmacokinetic Written Summary (NDA module 2.6.4), noting :

- that the *in vivo* studies detected latanoprost acid, but not LBN or latanoprost, after LBN administration.
- that the metabolism of latanoprost acid is well-understood.

The Applicant cites 3 papers (Govoni et al. 2006[23]; Vree et al. 1978[24]; Irwin 1996[25]) regarding the metabolism of BDMN. The Applicant reports:

- BDMN is metabolized by glutathione-S-transferase to inorganic nitrogen oxides (NOx) and 1,4-butanediol
- Metabolism of 1,4-butanediol proceeds by oxidation (alcohol dehydrogenase/ aldehyde dehydrogenase) in the liver to γ-hydroxybutyric acid. Further oxidative reactions ultimately lead to formation of succinic acid, which enters the TCA cycle.
- This reviewer notes that Govoni et al. 2013[26] updates the findings reported in Govoni et al. 2006.

6 General Toxicology

6.1 Single-Dose Toxicity

The Applicant submitted one single-dose toxicity study for LBN; rats received a single intravenous dose of LBN (report # 06AM024). The NOAEL was the high dose, 2 mg/kg.

Title	**Acute intravenous toxicity study with 6 days observation period of PF-03187207 in rats**
Report #	06AM024
Key findings	• No NOAEL was identified. Rats were dosed once iv. On D7 (the day of euthanasia), all main-group rats were bled from the sublingual vein under light anesthesia [not specified], and subsequently euthanized with CO_2. Histopathology was only performed for the control and high-dose group. The incidence and severity of tongue hemorrhage was higher in male and female high-dose rats, compared to controls. o Presumably, the procedure (i.e. vein puncture, and/or holding the tongue with forceps) affected these observations. o Because the incidence and severity increased with dose, the

[23] Govoni M, Casagrande S, Maucci R, Chiroli V, Tocchetti P. 2006. In vitro metabolism of (nitrooxyl)butyl ester nitric oxide-releasing compounds: comparison with glyceryl trinitrate. The Journal of Pharmacology and Experimental Therapeutics. 317:752-761. Paper accessed via: http://www.ncbi.nlm.nih.gov/pubmed/16424150

[24] Vree TB, Dalen RV, Kleijn EVD, Gimbrere JSF. 1978. Pharmacokinetics of butanediol 1,4 and 4-hydroxybutyric ethylester in man, rhesus monkey and dog. Pages 66-73.

[25] National Toxicology Program (NTP). NTP report series # 54. NTP summary report on the metabolism, disposition, and toxicity of 1,4-butanediol (CAS No. 11-63-4). NIH Publication 96-3932. May 1996.

[26] Govoni M, Tocchetti P, Lundberg JO. 2013. Metabolism and pathways for denitration of organic nitrates in the human liver. J Pharmacol Exp Ther. 346(1):96-104. http://www.ncbi.nlm.nih.gov/pubmed/23596058

	effects are presumed treatment-related. ○ Because no histopathology was performed for the low- and mid-dose groups, no NOAEL was identified. • For all other endpoints, the NOAEL was the high-dose, 2 mg/kg of LBN by intravenous injection. This study supports the Applicant's decision to forgo systemic-route repeat-dose toxicology studies with LBN to support topical ocular dosing. • The study rationale noted that latanoprost was previously tested in a single-dose intravenous rat toxicity study, and 2 mg/kg (the maximum feasible dose) was the NOAEL. ○ The endpoints were adequate to identify a NOAEL.	
Study details	Study laboratory	Pfizer Global Research & Development Amboise Safety Sciences Europe Route des Industries, Pocé-sur-Cisse 37400 Amboise, France
	Report date	November 10, 2006
	Study initiation Day of dosing End of in-life	March 21, 2006 March 22, 2006 March 30, 2006
	GLP status and Quality Assurance	Yes, signed
	Test article	PF-03187207 (BLN), lot # 102287-24-48c6. Purity 96.82%
Methods notes	Test species	Sprague Dawley rats Approximately 7 weeks of age at time of dosing Males: 225.7 to 311.8 g Females: 151.6 to 197.1 g
	Dose groups:	0, 0.003, 0.1, or 2 mg/kg
	Group size:	• Main-group: 10/sex/dose level (sacrificed on D7) • TK-satellite group: 3/sex/dose level
	Route of administration	Slow intravenous injection (over 20 seconds) into the caudal (tail) vein
	Dose volume	5 ml/kg
	Vehicle	Benzalkonium chloride (0.2 mg/mL), Tween 80 (b) (4) mg/mL) in buffer ((b) (4) sterile water for injection (qs).
Endpoints	Mortality check	Daily
	Clinical signs	• On the day of dosing, checked pre-dose, and after dosing at 5 minutes, 10 minutes, and 1 hour • Once daily from days 2-6
	Body weight	Measured on D1 and D6
	Food	Measured on D6 only

	consumption	
	Hematology, clinical chemistry, and urinalysis	• Animals were fasted overnight (17 hours) prior to Day 7, with access to water. • On Day 7, blood samples were collected for hematology (standard battery of 24 measured or calculated endpoints), coagulation (3 endpoints), and clinical chemistry (standard battery, 18 measured or calculated endpoints) via puncture of the sublingual vein under light anesthesia. • Overnight urine was collected in a metabolism cage on D7, and analyzed (standard battery of 11 endpoints)
	TK	• Blood was collected from the sublingual vein on D1 only, at 5, 10 and 30 minutes, and 1, 2 and 4 hours post-dose • Plasma concentrations of latanoprost acid were assayed
	Gross pathology	"Full necropsy" was performed on all animals; results were provided for the standard list of systemic tissues and organs.
	Organ weights	At necropsy, weights were collected for the adrenal, brain, epididymis, heart, kidney, liver, ovary, prostate, spleen, testes, and thymus.
	Histopathology	• Histopathology was performed for control and high-dose animals only • Standard battery of systemic tissues and organs, including the eye, optic nerve, Harderian gland, injection site, and any gross abnormalities. • A histopathology peer review was performed.
Toxicity results	• The authors concluded that no treatment-related effects were apparent for any endpoint. • This reviewer notes that the histopathology observation of tongue hemorrhage was clearly increased for the high-dose group, compared to controls. o Blood was collected from the sublingual vein of main-group rats on D7, prior to euthanasia, for hematology and clinical chemistry. [Tongues were not evaluated for the TK animals, which were bled sublingually on D1] o A cursory literature review[27],[28] by this reviewer notes that the sublingual blood collection procedure may require clamping the	

[27] Heinmann M, et al. 2009. Blood collection from the sublingual vein in mice and hamsters: a suitable alternative to retrobulbar technique that provides large volumes and minimizes tissue damage. Laboratory Animals. 43:255-260. Accessed via: http://lan.sagepub.com/content/43/3/255.full.pdf

[28] Zeller W, et al. 1998. Refinement of blood sampling from the

tongue with fine forceps (to extend the tongue), which might cause blunt trauma. It is not clear to this reviewer whether the observed tongue hemorrhage might have been at the site of puncture, or at the site of holding the tongue.

- o No other treatment-related findings were apparent (e.g. no increases in hemorrhage detected in other tissues; food consumption, body weight, hematology, clinical chemistry, or urinalysis endpoints). In the absence of correlating endpoints, the biological relevance of the tongue hemorrhage observations is unclear.
- o Since this finding was not observed in the 28-day or 9-month topical ocular studies, this reviewer concludes that this finding is not relevant to topical ocular exposure.
- o This finding may be artifactual (e.g. if the control animals had slightly more time to recover, after the light anesthesia for blood collection, and prior to CO_2 euthanasia).

Table 38: Intravenous BLN associated with increased tongue hemorrhage in rats (report # 06AM024)

Tongue hemorrhage	Males				Females			
Dose level	0	0.003 mg/kg	0.1 mg/kg	2 mg/kg	0	0.003 mg/kg	0.1 mg/kg	2 mg/kg
# examined	10	0	0	10	10	0	0	10
Minimal hemorrhage	1	-	-	1	1	-	-	3
Mild hemorrhage	0	-	-	4	1	-	-	5

TK results

- The authors did not attempt to measure parent LBN, or latanoprost.
- Plasma levels of latanoprost acid were assayed by LC-MS/MS; the LLOQ was 2.0 ng/ml.
- No latanoprost acid was detected in any low-dose sample.
- The authors could not calculate elimination half-life, clearance, of Vss for the mid-dose.
- At 2 mg/kg, exposure appeared higher in males than in females.

Table 39: Plasma latanoprost acid TK for rats following single iv dose of LBN (report # 06AM024)

Dose (mg/kg)	Sex	C_0 (ng/ml)	$AUC_{0\text{-}4hr}$ (ng*hr/ml)	$T_{1/2}$ (minutes)	Cl (ml/hr/kg)	Vss (ml/kg)

sublingual vein of rats. Laboratory Animals 32:369-376. Accessed via: http://lan.sagepub.com/content/32/4/369.full.pdf

	0.1	M	73 ± 14	6.7 ± 0.7	-	-	-
		F	64 ± 19	5.2 ± 0.9	-	-	-
	2	M	3240 ± 620	242 ± 33	5.1 ± 1.5	8400 ± 1200	490 ± 120
		F	2020 ± 370	153 ± 32	4.3 ± 0.1	13600 ± 2870	760 ±120
	"-": not calculable. Values presented as means ± standard deviation. Values rounded by this reviewer for significance and readability.						

6.2 Repeat-Dose Toxicity

Title	***In vivo* toleration topical ocular irritation toxicity study of NCX116 (PF-03187207) in female pigmented rabbits**	
Report #	05NCX001	
Key findings	• This study was conducted prior to the single-dose rabbit study (report # 06AM024) reviewed above. The objective was to assess tolerability (limited endpoints) and systemic TK. • Topical ocular LBN 0.12% was well-tolerated, causing transient mild (grade 1) conjunctival redness. • LBN was not detected in plasma post-dose; latanoprost acid was detected in plasma at 10 and 30 minutes after dosing with topical ocular LBN.	
Report details	Study laboratory	Pfizer Global Research & Development Safety Sciences La Jolla, CA
	Report date	February 1, 2006
	Study initiation	June 27, 2005
	End of in-life	June 30, 2005
	GLP status	Not GLP
	Test article	PF-03187207 (NCX 116; LBN), lot # 24JUN2005NicOX-116
Study details	Test species	Female Dutch Belted rabbits 14 weeks of age Weight 1.87 to 2.13 kg
	Vehicle	Aqueous formulation: (b) (4) mg/ml tween 80, 0.2 mg/ml benzalkonium chloride, (b) (4) phosphate, pH 6.7, (b) (4) mOsm
	Doses	0 or 0.12% LBN
	Dosing	• Left eye (OS) received vehicle • Right eye (OD) received 0.12% LBN

<table>
<tr><td></td><td></td><td>

Dosing was by topical ocular administration
Volume was 0.05 ml/dose (i.e. 60 µg of LBN per dose)
three times daily (tid; i.e. 180 µg of LBN per day), approximately 2 hours apart, for 4 consecutive days
Rabbits were sacrificed on D5.

</td></tr>
<tr><td></td><td>Endpoints</td><td>

Ocular clinical observations, scored based on Draize guidelines for swelling, redness and opacity

approximately 5 minutes after each dose
approximately 2-3 hours after the last daily dose.

Blood collection for PK, prior to the first daily dose on D2 and D4.
Gross necropsy and histopathology of the eye, surrounding lid and conjunctival tissue.
Note: post-dose body weight was not measured.

</td></tr>
<tr><td>Toxicity results</td><td colspan="2">

Mild (grade 1) conjunctival redness was observed in all eyes, somewhat more frequently in LBN-treated eyes than vehicle-treated eyes.

The redness generally resolved by 2-3 hours after the last dose.
No redness was noted on D5 (prior to sacrifice).

No swelling or opacity was noted for any eye.
The authors concluded that no treatment-related gross or microscopic findings were reported (no tabulated or individual data were presented, only the author’s conclusion).

</td></tr>
<tr><td>Systemic TK results</td><td colspan="2">

LBN was not detected in plasma. LLOQ = 1 ng/ml
Latanoprost acid was not detected on D2, but was detected on D4 (at 10 and 30 minutes post-dose). LLOQ = 5 ng/ml

Table 40: Serum latanoprost free acid concentrations after tid single eye dosing with 0.12% LBN (report # 05NCX001)
<table>
<tr><th>Time point</th><th>Concentration of latanoprost free acid (ng/ml)</th></tr>
<tr><td>D2</td><td>Below LLOQ</td></tr>
<tr><td>D4: pre-dose</td><td>Below LLOQ</td></tr>
<tr><td>D4: 10 minutes</td><td>18.6 ± 5.2</td></tr>
<tr><td>D4: 30 minutes</td><td>6.5 ± 0.3</td></tr>
<tr><td>D4: 60 minutes</td><td>6.3 ± 0.6
[incorrectly reported as 4 ± 3 ng/ml by the authors]</td></tr>
</table>
Mean ± standard deviation
</td></tr>
</table>

	Note: for the D4: 10 and 30 minute time points, latanoprost free acid was detected in all samples (5/5 rabbits). For the D4: 60 minute time point, latanoprost free acid was only detected in 3/5 samples.

Study title: 28-Day Twice Daily Ocular Instillation Toxicity and Toxicokinetic Study with PF-03187207 in Cynomolgus Monkeys

Study no.:	6750-267
Study report location:	NDA module 4.2.3.2 (Toxicology – Nonhuman primate – topical – short) \\cdsesub1\evsprod\nda207795\0000\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\stf-6750-267\6750-267.pdf
Conducting laboratory and location:	(b) (4)
Report date:	January 25, 2007
Date of study initiation:	March 6, 2006
GLP compliance and QA statement:	Yes, signed
Drug, lot #, and % purity:	LBN, lot # NKN102287-24-48C6, purity 97.62%

Key Study Findings

- NOAEL was 0.04% twice daily (24 µg/eye/day), the highest dose tested, for both local ocular and systemic toxicity.
- TK was attempted for latanoprost acid only.

Methods

Doses:	• 0, 0.003%, 0.017%, or 0.04%, one (30 µl) drop per dose, 2 doses per day. • Equivalent to: 0, 1.8, 10.2, and 24 µg/eye/day
Frequency of dosing:	• Controls received vehicle in the left eye twice daily (4 hours ± 15 minutes apart apart), and nothing in the right eye • Test-article groups received LBN in the right eye, and vehicle in the left eye, twice daily (4 hours ± 15 minutes apart)
Route of administration:	Topical ocular instillation
Dose volume:	30 µl
Formulation/Vehicle:	Aqueous solution: • (b) (4) • 0.2 mg/ml benzalkonium chloride • (b) (4) mg/ml Tween 80 (w/v)

	• pH 5.5 [Note: this is the phosphate-buffered formulation, used clinically for Phase 1 and 2, but not for Phase 3 trials]
Species/Strain:	cynomolgus monkeys (*Macaca fascicularis*)
Number/Sex/Group:	3/sex/dose
Age:	3 to 5 years
Weight:	• males 2.1 to 2.8 kg at start of dosing • females 2.0 to 2.7 kg at start of dosing
Deviation from study protocol:	Protocol deviations were recorded in the report; none were important to interpretation of the study.

Observations and Results

Mortality

- No premature mortality or morbidity was apparent.
- Animals were observed twice daily for mortality, abnormalities, and signs of pain or distress.

Clinical Signs

- No treatment-related cage-side observations were apparent.
- Cage side observations were made 1 hour ± 15 minutes after each daily dose (i.e. twice daily).

Body Weights

- No treatment-related effects on body weight were apparent.
- Body weight was measured pre-dose, on D1, weekly thereafter, and on the day of necropsy.

Feed Consumption

- No remarkable food consumption observations were made.
- Food consumption was assessed qualitatively, on a daily basis.

Ophthalmoscopy

- No treatment-related effects on ophthalmology endpoints were apparent.
- Ophthalmic examinations were performed pre-dose, on D1 (after each dose), on D3, and weekly thereafter.
    - Indirect ophthalmoscopy to examine the ocular fundus, including the macula lutea, optic disk and retina
    - Slit lamp examination to examine the anterior and medium segments of the eye, including: the conjunctiva, cornea, anterior chamber, iris, lens, and vitreous body
    - Tonometry (tonometer method not specified)

- Note: the authors did not provide a summary table (or figures) for IOP; this appears to be an oversight. This is not a study limitation, because the individual animal IOP data were provided in Appendix 8.

Electroretinography (ERG)

- No treatment-related effects on ERG endpoints were apparent.
- ERG was performed once pre-dose and once during the last week of dosing (within 30 minutes of the second dose)
    - Analysis for a- and b-waves

Electrocardiogram (ECG) and Blood Pressure Measurements

- No treatment-related effects on ECG endpoints were apparent.
- ECG was measured once pre-dose and once during the last week of dosing (within 30 minutes of the second dose. "Leads I, II, III, aVR, aVL, aVF, V1, V2, V3, and V4 were used. A heart rate correction for the QT interval (QTc) was calculated."
- An inflatable cuff was positioned over the base of the tail, to measure arterial, systolic and diastolic blood pressure, and heart rate, once pre-dose and once during the last week of dosing (within 30 minutes of the second dose).

Hematology and Clinical Chemistry

- No treatment-related effects on hematology or clinical chemistry endpoints were apparent.
- Blood was collected pre-dose and on D29, with fasting overnight. Standard hematology, coagulation, and clinical chemistry parameters were determined.

Urinalysis

- No treatment-related effects on urinalysis endpoints were apparent.
- Overnight urine specimens were collected from cage pans (on the same days as blood collection for hematology and clinical chemistry). Standard urinalysis endpoints were determined.

Gross Pathology

- No treatment-related gross pathology effects were apparent.
- On D29, animals were necropsied. Gross pathology consisted of examining "the external features of the carcass; external body orifices; abdominal, thoracic, and cranial cavities; organs; and tissues."

Organ Weights

- No treatment-related differences in organ weights were apparent.
- At necropsy, weights for the adrenals (2), brain, heart, kidneys (2), liver with gallbladder (drained), spleen, testis(2) and thymus were measured.
- Note: no explanation was provided for why a full panel of organ weights was not measured, and the lack of adequate organ weight data (e.g. epididymides, prostate, ovaries, uterus) is a minor study limitation.

Histopathology

Adequate battery?	Yes.
Histopathology of the eyes and adnexa:	• The left and right eyes were preserved in modified Davidson's fixative. Eyes were sectioned to include the macula in the section. • The superior eyelid (2), inferior eyelid (2), optic nerve (2), and lacrimal gland [presumably both left and right; the text of the report does not specify] were evaluated.
Full systemic histopathology:	The following were preserved and examined microscopically: adrenal (2), aorta, brain, cecum, cervix, colon, duodenum, epididymis (2), esophagus, femur with bone marrow (articular surface of the distal end), gallbladder, heart, ileum, jejunum, kidney (2), grossly-observed lesions, liver, lung with large bronchi, lymph node (axillary), lymph node (mesenteric), mammary gland, ovary (2), oviduct (2), pancreas, Peyer's patches (gut associated lymphoid tissue), pituitary gland, prostate, salivary gland [mandibular (2)], sciatic nerve, seminal vesicle, skeletal muscle (thigh), skin/subcutis, spinal cord (cervical, thoracic, and lumbar), spleen, sternum with bone marrow, stifle joint, stomach, testis (2), thymus, thyroid (2 lobes) with parathyroid, tongue, trachea, ureter, urinary bladder, uterus, vagina
Peer review?	Yes • The histopathology evaluation was performed by (b) (4) (b) (4). • Histopathology peer-review was performed by (b) (4). (b) (4).

- No treatment-related histopathology effects were apparent.
  - For context (i.e. with the 9-month study and the impurity qualification study): no effects on lung, adrenal (medulla or cortex), spinal cord, optic nerves, sciatic nerve, heart or aorta were noted for any animal.

Toxicokinetics

- Blood was collected for TK on D1 (after the first dose, at 5, 15, 30, 60 and 120 minutes post-dose) and on D28 (after the second dose, also at 5, 15, 30, 60 and 120 minutes post-dose)
- Plasma samples were analyzed for latanoprost acid only. The failure to measure for LBN, BDMN, or NO is a study limitation.
- Latanoprost acid concentrations were below the lower limit of quantitation (LLOQ = 0.500 ng/ml) for all samples in the low-dose group (1.8 µg/eye/day).
- T_{max} was 5 minutes (when measurable).

Table 41: Plasma latanoprost acid TK for the 28-day topical ocular monkey toxicity study (report # 6750-267)

Day	Dose (µg/eye)	C_{max} (ng/ml)	$AUC_{0\text{-}2\ hr}$ (ng*hr/ml)
1	1.8	Below LLOQ	
28			
1	10.2	0.545 ± 0.290	0.0681 ± 0.0363
28		0.499 ± 0.414	0.0624 ± 0.0518
1	24	1.61 ± 0.488	0.309 ± 0.0945
28		1.05 ± 0.598	0.182 ± 0.120

Results reported for both sexes combined, means ± standard deviations.

Dosing Solution Analysis

- Vehicle and dosing formulations were prepared weekly, and stored refrigerated.
- Concentration verification, homogeneity testing, and stability testing (for 15 days only) were performed.

Study title: 28-Day Ocular Instillation Toxicity and Toxicokinetic Study with Latanoprostene Bunod (BOL-303259) in Cynomolgus Monkeys (Impurity Qualification)

Study no.:	8273344
Study report location:	NDA module 4.2.3.7.6 (Toxicity – other toxicity studies – impurities) \\cdsesub1\evsprod\nda207795\0000\m4\42-stud-rep\423-tox\4237-other-tox-stud\42376-imp\stf-8273344\8273344.pdf
Conducting laboratory and location:	(b) (4)
Study report:	December 5, 2014
Date of study initiation:	January 29, 2014
GLP compliance and QA statement:	Yes, signed
Drug, lot #, and % purity:	LBN lot # 130818314 (93.6% pure) was used to prepare the test article (lot # RPG-140201-01): • (b) (4) % LBN • (b) (4) • (b) (4) • Note: analytical testing detected (b) (4) % (b) (4) in the test article.

Key Study Findings

- One LBN dose level was tested, 0.04% (2 drops/dose, 2 doses/day for 28 days).
- The BLN test article intentionally contained two impurities: (b) (4) % (b) (4) (b) (4), and (b) (4) % (b) (4) to qualify these two impurities.
- Additionally, because the test article had (b) (4) % (b) (4), this exposure is also qualified by the study.
- Although systemic histopathology was limited to select organs/tissues, the study design is acceptable.
- One treated male exhibited uncollapsed lung grossly. This reviewer considers this finding treatment-related; therefore, this study has no NOAEL.
  - This reviewer presumes that this finding is consistent with the treatment-related lung toxicity observed in the 9-month monkey study (report # 6348-415).
  - Although no histopathologic correlate was observed, this reviewer presumes that the cause of the uncollapsed lung was (undetected) pleural/subpleural fibrosis/inflammation.
  - No other remarkable findings were noted.

Methods	
Nominal doses:	• 0 or 0.04% LBN • Equivalent to 0 or 48 µg/eye/day
Frequency of dosing:	Daily for 28 days • Both controls and the treated group received vehicle in the left eye. • The treated group received LBN in the right eye only. The control group right eye was untreated. • Two doses per day, approximately 4 hours apart • Each dose was two 30 µl drops, administered 5 to 15 minutes apart (total of 4 drops/day/eye)
Route of administration:	Topical ocular instillation • Using a pipette • The upper eye lid was extended, and the drop was placed directly on the cornea • After dosing, the eye was allowed to close naturally.
Dose volume:	Two 30 µl drops per dose
Formulation/Vehicle:	The citrate-buffered formulation (i.e. same as for the 9-month toxicology study, and the Phase 3 trial formulation): • (b) (4) polysorbate 80 (w/v) • benzalkonium chloride (w/v) • (b) (4) citric acid/sodium citrate (pH 5.5) • (b) (4) EDTA (w/v), • (b) (4) glycerin (w/v) • in (b) (4) Water (b) (4) USP
Species/Strain:	cynomolgus monkeys (*Macaca fascicularis*)
Number/Sex/Group:	4/sex/dose
Age:	4 to 5 years of age
Weight:	○ Males 4.8 to 7.3 kg ○ Females 3.3 to 4.3 kg
Deviation from study protocol:	Minor study deviations were documented in the report; they do not change the interpretation of the results.

Observations and Results

Mortality

- No early mortalities occurred.

- Animals were checked twice daily for mortalities, abnormalities, and signs or pain or distress.

Clinical Signs

- No treatment-related clinical signs were apparent.
- Cage side observations were made once daily. Detailed observations were made pre-dose, and weekly during dosing (D1, 8, 15, 22 and 29).

Body Weights

- No treatment-effect on body weight was apparent.
- Body weight was measured pre-dose, and weekly during dosing (D1, 8, 15, 22 and 29).

Feed Consumption

- No treatment-effect on food consumption was apparent.
- Food consumption was assessed qualitatively, once daily.

Ophthalmoscopy

- No treatment-related ophthalmology findings were apparent.
- Ophthalmology was assessed pre-dose, on D1 (after the last dose), and on D7, 13 and 23 (after the first dose, prior to the second daily dose)
- Ophthalmology examinations consisted of slit lamp biomicroscopy (adnexa and anterior portions of the eye), indirect ophthalmoscopy (ocular fundus of each eye), and tonometry (using an applanation tonometer). Results were graded using a modified Hackett-McDonald scoring system.

Hematology and Clinical Chemistry

- No treatment-related effects on hematology, coagulation, or clinical chemistry were apparent.
- Blood was sampled pre-dose and on D23. Standard hematology, coagulation, and clinical chemistry parameters were tested.

Urinalysis

- No treatment-related effects on urinalysis were apparent.
- Overnight urine samples were collected once pre-dose and on G23. Standard urinalysis endpoints were tested.

Gross Pathology

- All monkeys were necropsied on D29. No remarkable gross pathology findings were reported.

- "Macroscopic examinations were conducted, consisting of an examination of the external features of the carcass; external body orifices; abdominal, thoracic, and cranial cavities; organs; and tissues."

Table 42: 28-day monkey impurity qualification study: gross observation of uncollapsed lung (report # 8273344)

Gross observation	Males		Females	
	0	LBN 0.04%	0	LBN 0.04%
Lung uncollapsed	0/4	1/4	0/4	1/4

- Uncollapsed lung was noted for one treated male (# I01390). The individual animal data (page 1038) describes the observation as "lung: uncollapsed; lobes, multiple; present; collected/lobes on right side". The significance of this finding is unclear. The uncollapsed lung was examined microscopically, no histopathology finding was detected (pages 1020 and 1039).
  - Hematology, coagulation, clinical chemistry, and urinalysis for this monkey (# I01390) were unremarkable.
- An information request was sent to the Applicant, and the Applicant responded on 4/27/2016. The Applicant reported:
  - The historical control incidence at (b) (4) for this gross observation in non-human primates is 1.41% (15/1061) for females, and 1.64% (18/1099) for males, respectively.
  - "generally, the lung collapses when the normal negative pressure is lost in the thoracic cavity when opened during necropsy." The Applicant considered this gross observation to be incidental.
  - The routine processing of lungs for histopathology would have been "from the left apical lobe and the right caudal lobe."
    - Since the gross observation was made only for the right side, it is reasonable to expect that the left lobe histopathology would have been unremarkable.
    - The right caudal lobe is relatively smaller than the right upper and middle lobes. If the gross finding was limited to the upper and middle lobes, then it would be reasonable for histopathology of the right caudal lobe to be unremarkable.
    - This reviewer concludes that the lack of histopathology findings does not mitigate the potential concern for the gross observation. This reviewer considers the lung not collapsing normally to be treatment-related and potentially adverse.

Organ Weights

- No treatment-related changes for organ weights were apparent.

- At necropsy, the following organs/tissues were weighed: adrenal (2), brain, gall bladder (drained), heart, kidney (2), liver, spleen, testis (2), and thymus.
- The failure to weigh the standard battery of organs (i.e. uterus, ovaries, pancreas, lung) is a study limitation.

Histopathology

Adequate battery?	Yes. • The eye (2) with bulbar conjunctivae, eyelid (2) (upper and lower with palpebral conjunctivae), lacrimal gland (2), and optic nerve (2) were preserved and examined microscopically. • Only partial systemic histopathology was performed: adrenal, brain, gall bladder, heart, kidney, gross lesions, liver, mandibular lymph node, sciatic nerve, spleen, testis, and thymus. • Note: the failure to examine all lungs for histopathology findings is a study limitation (based on the results of the 9-month monkey study identifying the lungs as a target organ)
Peer review?	No. Histopathology was evaluated by (b) (4), (b) (4).
Findings	• No treatment-related histopathology findings were apparent. • No findings in right eyes (local histopathology) or in LBN-treated animals (systemic histopathology) were remarkable.

Toxicokinetics

- Blood samples were collected on D1 and D28, prior to the first daily dose, after the first daily dose (at 5, 15 and 30 minutes), and after the second daily dose (at 1, 4, 8 and 20 hours).
- Samples were analyzed for LBN, latanoprost acid, and BDMN.
  - No LBN was detected. The LLOQ was 10.0 pg/ml.
  - No BDMN was detected. The LLOQ was 0.200 ng/ml.
  - Latanoprost acid was detected from 5 minutes to 1 hour after the second daily dose (LLOQ = 30.0 pg/ml).
- Latanoprost acid was detected in one control female (# I01394) on D28, at a concentration of 55.9 pg/ml. "There was no in-life or analytical reasons for this value."

Table 43: Latanoprost acid TK for the 28-day monkey impurity qualification study (report # 8273344)

Latanoprost acid serum TK	Males		Females	
	D1	D28	D1	D28
C_{max} (pg/ml)	812 ± 459	460 ± 279	1180 ± 337	942 ± 310
AUC_{0-4} (pg*hr/ml)	239 ± 168	146 ± 48.3	327 ± 168	262 ± 135
AUC_{0-t} (pg*hr/ml)	199 ± 135	127 ± 40.1	272 ± 121	222 ±105

Values reported as mean ± standard deviation

Dosing Solution Analysis

- "A fresh unopened bottle was used for each daily dosing interval." (page 19).
- Analytical testing (concentration analysis) was performed on 8/15/2013 (i.e. prior to the start of dosing, which was 2/27/2014). No stability or homogeneity analysis was performed.

Study title: 9-Month Topical Ocular Instillation Toxicity and Toxicokinetic Study with PF-03187207 in Cynomolgus Monkeys	
Study no.:	6348-415
Study report location:	NDA module 4.2.3.2 (Toxicology – Nonhuman primate – topical – long) \\cdsesub1\evsprod\nda207795\0000\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\stf-6348-415\6348-415.pdf
Conducting laboratory and location:	(b) (4)
Report date:	April 21, 2011
Date of study initiation:	October 26, 2007
GLP compliance and QA statement:	Yes, signed
Drug, lot #, and % purity:	LBN (PF-03187207), lot # AA0002168, purity 99.7%

Key Study Findings

- No NOAEL was identified for local ocular toxicity.
  - Treatment-related iris hyperpigmentation was observed in two low-dose monkeys and one high-dose female. This effect is a known class effect of topical prostaglandin analogs. Although the effect has no functional consequence, P/T considers it adverse.
- For systemic toxicity, the NOAEL is the low-dose (0.024%), and the LOAEL is the mid-dose (0.040%).
  - For pleural/subpleural chronic fibrosis/inflammation, the NOAEL was the low-dose. In males, the LOAEL was the mid-dose (minimal-to-slight severity); severity and incidence increased in the male high-dose group (1 minimal, 1 slight, 1 moderate).
    - Reversibility was not assessed.
    - The authors did not consider these observations treatment-related.
  - Minimal multifocal axon degeneration of the sciatic nerve was observed in one mid-dose male and one high-dose male.
- Minimal-to-slight perivascular lymphocyte/macrophage infiltrates of the episclera of the eye was observed across all LBN- treated groups. The authors considered

this treatment-related only at the high-dose, and not adverse. This reviewer concurs that this effect is not adverse.

- A transient vehicle effect was apparent. On D2, several eyes treated with vehicle or LBN exhibited minimal-to-mild inflammation (grade 1 or 2 effects conjunctival congestion, conjunctival discharge, corneal opacity) that had resolved by D5. No treatment-related effects on irritation were observed thereafter.
- Plasma TK was measured for latanoprost acid only. AUC and C_{max} values were calculated.

Methods

Doses:

Doses for the monkey 9-month study			
Group	Conc. %	# of drops/ eye/day	µg/eye/day
Control	0	2	0
Low	0.024	2	14.4
Mid	0.040	2	24.0
High	0.040	4	48.0

Frequency of dosing: Twice daily dosing, approximately 4 hours apart for 273 days

- controls: left eye dosed with vehicle, right eye untreated
- Low- and mid-dose: left eyes treated, right eyes dosed with vehicle
- High-dose: left eye received twice-daily dosing as divided doses, administered 5 to 15 minutes apart (4 drops total/day); right eyes were treated on the same schedule with vehicle.

Route of administration: Topical ocular instillation

- Using a positive displacement pipette
- The head was position so that the cornea was directed upward, and the eyelids were held open.
- After dosing, the eyelids were allowed to close naturally.

Dose volume: 30 µl

Formulation/Vehicle: The citrate-buffered formulation: (b) (4) (w/v) polysorbate 80, (b) (4) (w/v) benzalkonium chloride, (b) (4) citric acid/sodium citrate (pH 5.5), (b) (4) (w/v) edetate disodium (EDTA), and (b) (4) (w/v) glycerin

	prepared in (b) (4) Water (b) (4), USP
Species/Strain:	cynomolgus monkeys (*Macaca fascicularis*)
Number/Sex/Group:	4/sex/dose group
Age:	3 to 4 years old at start of dosing
Weight:	• Males 2.4 to 4.3 kg at start of dosing • Females 2.1 to 3.1 kg at start of dosing
Deviation from study protocol:	Minor study deviations were documented in the report; they do not change the interpretation of the results.

Observations and Results

Mortality

- Animals were checked twice daily for mortality, morbidity, and signs of pain or distress.
- Two premature mortalities occurred; no cause of death was identified for either:
  - A control male (# I04596) was found dead on D222 (individual animal data on page 736).
  - A low-dose female (# I04616) was found dead on D206. Individual animal data on pages 775-776. This animal had minimal heart hemorrhage. Several gastrointestinal tissues were autolyzed and unreadable.

Clinical Signs

- No treatment-related clinical signs were apparent.
- Cage side observations were made daily. Detailed observations were made pre-dose, on D1, and weekly thereafter.

Body Weights

- No treatment-related effect on body weight was apparent.
- Body weight was measured pre-dose, on D1, and weekly thereafter.

For the purposes of systemic NOAEL and LOAEL calculation, the male monkey body weights are important:

Table 44: Calculation of dose (µg/kg and µg/m^2) for the 9-month monkey study (report # 6348-415)

	Control	Low-dose (14.4 µg/eye/day)	Mid-dose (24.0 µg/eye/day)	High-dose (48.0 µg/eye/day)
D1 (kg)	3.4 ± 0.74	3.4 ± 0.22	3.1 ± 0.56	3.6 ± 0.51
D134 (kg)	3.9 ± 0.94	3.9 ± 0.33	3.5 ± 0.50	4.0 ± 0.43
D274 (kg)[a]	5.0 ± 0.64	4.7 ±0.29	4.0 ± 0.34	4.8 ± 0.39
Average (kg)	4.10	4.00	3.533	4.133

Dose (µg/kg, using the average body weight)	0	3.60	6.79	11.61
Dose (µg/m^2, using the average body weight)	0	43.2	81.51	139.35

[a] Note: D134 is approximately half-way through dosing. Including or omitting the D134 value does not change the average meaningfully.
[b] Using the default conversion factor of 12 for cynomolgus monkeys

Feed Consumption

- No treatment-related effect on food consumption was apparent.
- Food consumption was assessed qualitatively, on a daily basis.

Ophthalmoscopy

- Methods:
    - Indirect ophthalmology (using a slit lamp) was performed pre-dose, after dosing on D1, and prior to dosing on D2, 5, 7; and during weeks 2, 4, 8, 12, 16, 20, 24, 28, 43, and 36.
        - Irritation was scored using a modified McDonald-Shadduck scoring system.
        - Irises were photographed to evaluate color/pigment.
    - Intraocular pressure (IOP) was measured using a TonoPen (i.e. an applanation tonometer) pre-dose, after dosing on D1, and every 4 weeks thereafter (as part of the ophthalmology exam).
    - Corneal thickness (pachymetry) was measured pre-dose, after dosing on D1, and every 4 weeks thereafter (as part of the ophthalmology exam).
    - Pupil diameter was measured pre-dose, and during weeks 20 and 36 (within 30 minutes after the first daily dose).
    - Eyelash length was measured pre-dose, and during weeks 20 and 36.
    - Electroretinography (ERG) was measured pre-dose, during week 13, and every 12 weeks thereafter.
        - Scotopic (at least 2 hours dark adaptation), photopic (at least 10 minutes light adaptation), and visual evoked potential tests were conducted.

Results:

- On D2, several eyes treated with vehicle or LBN exhibited minimal-to-mild inflammation (grade 1 or 2 effects conjunctival congestion, conjunctival discharge, corneal opacity) that had resolved by D5. No treatment-related effects on irritation were observed thereafter.
- Treatment increased iris pigmentation was present in 3/12 LBN-treated eyes.
- All irises appeared normal up to D222

- On D247 (the last assessment), 3 monkeys exhibited iris hyperpigmentation in the treated (OD) eye: one low-dose male, one low-dose female, and one high-dose female. The individual animal data (pages 383, 427, 458 respectively) described the effect as subtle iridal/iris pigment mottling, with the rest of the iris appearing normal.
    - Recovery for iris hyper-pigmentation was not assessed.

Treatment was weakly associated with decreased IOP (the intended pharmacology) at some time points. The authors graphed the data:

Note: Group 1 was control (left eye vehicle, right eye untreated). Groups 2-4 received 14.2, 24, or 48 µg/eye/day of LBN into the right eye, respectively, and vehicle into the left eye daily.

Figure 15: Male monkey IOP data from the 9-month topical ocular study (report # 348-415)

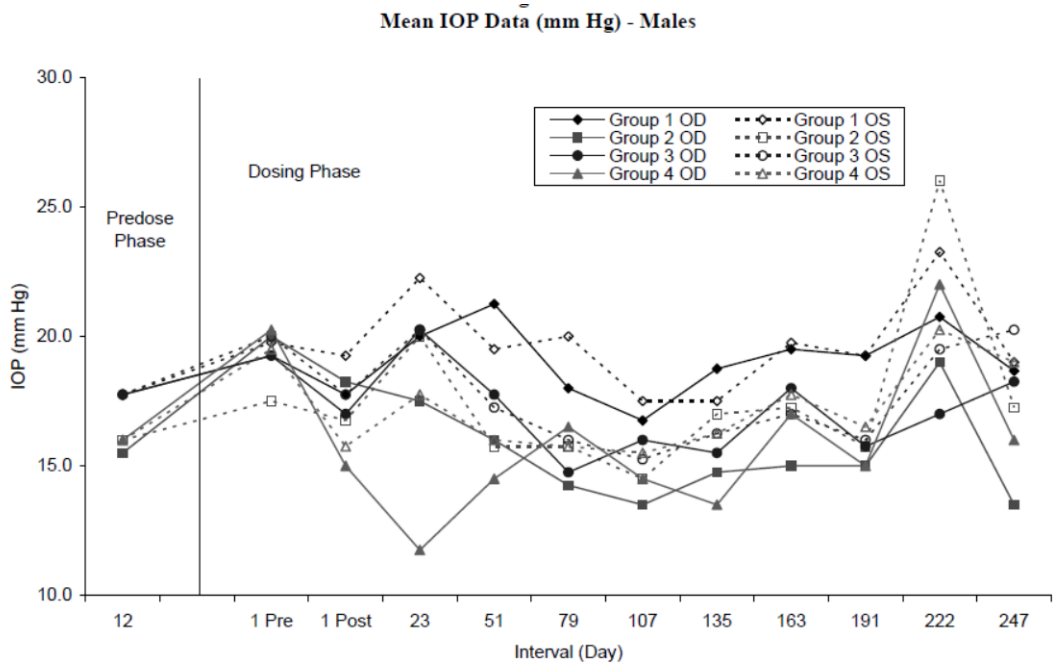
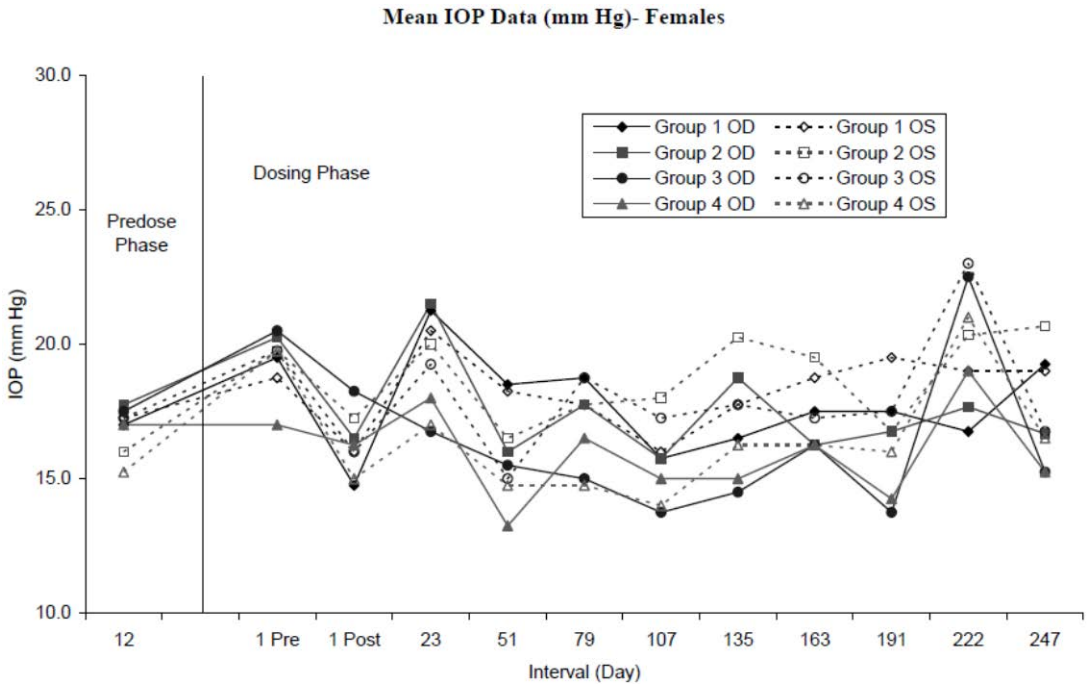


Figure 16: Female monkey IOP data from the 9-month topical ocular study (report # 348-415)



Electrocardiogram (ECG) examination

- No treatment-related effects on ECG were apparent.

Five lead-ECG was measured pre-dose, during week 15, and during week 36 of dosing (approximately 1 hour after the second daily dose). Animals were anesthetized, and measurements (RR, heart rate, QRS, PR, QT, QTc) were taken using Leads I, II, aVF, CV5RL, and CV6LL.

At 36 weeks, mid- and high-dose males had slightly slower heart rate (with corresponding increases in RR interval and QT interval, but without changed QT_C) compared to controls and to previous time points (baseline and week 15). The authors concluded, and this reviewer concurs, that the observations appear incidental.

Hematology and Clinical Chemistry

- No effects on hematology, coagulation, or clinical chemistry endpoints were apparent.
- Blood was collected for hematology, coagulation, and chemical chemistry from fasted animals pre-dose, during week 20, and during week 40.
- Standard hematology endpoints were assessed/calculated: red blood cell (erythrocyte) count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet

count, red cell distribution width, white blood cell (leukocyte) count, differential blood cell count, reticulocyte count.
- Standard coagulation endpoints were assessed: prothrombin time, fibrinogen, activated partial thromboplastin time.
- Standard clinical chemistry endpoints were assessed/calculated: urea nitrogen, creatinine, total protein, albumin, globulin, albumin/globulin ratio, cholesterol, total bilirubin, alanine aminotransferase, alkaline phosphatase, gamma glutamyltransferase, aspartate aminotransferase, calcium, inorganic phosphorus, sodium, potassium, chloride.

Urinalysis

- No effect on urinalysis endpoints was apparent.
- Urine samples were collected from fasting animals pre-dose and during week 40.
- Standard urinalysis endpoints were assessed: appearance (clarity and color), specific gravity, pH, protein, glucose, ketones, bilirubin, urobilinogen, blood, microscopic examination of sediment.

Gross Pathology

No effects on macroscopic observations at necropsy were apparent.
After 39 weeks of dosing, necropsy was performed on each surviving animal, consisting of “examination of the external features of the carcass; external body orifices; the abdominal, thoracic, and cranial cavities; organs; and tissues.”

Organ Weights

No effect on organ weights was apparent at necropsy.
At necropsy, a limited panel of organs were weighed: adrenal (2), brain, heart, kidney (2), spleen, testis (2), thymus, and liver with gallbladder (drained).

Histopathology

Adequate battery	Yes: Histopathology of the eyes and ocular adnexa Full standard systemic histopathology
Peer review	Yes: • Histopathology was evaluated by (b) (4) (b) (4). • Peer review was conducted by (b) (4), (b) (4)
Histopathology method notes	At necropsy, “Eyes (including bulbar conjunctiva) and optic nerves from each animal were preserved in the half-strength Karnovsky’s fixative for approximately 24 hours and transferred to 10% neutral-buffered formalin.” Testes were preserved in modified Davidson’s fixative. Remaining tissues were preserved in 10% neutral-buffered formalin.

	"The eyes were trimmed and embedded along a plane approximately horizontal allowing for eye sections to be in a plane, including the optic nerve, optic disc, and macula. In addition to the sagittal (longitudinal) section of bulbar optic nerve, a cross (transverse) section of bulbar optic nerve was prepared. Tissues were examined microscopically by a board-certified veterinary pathologist."
	Full systemic histopathology was performed. The following tissues/organs were evaluated: adrenal (2), aorta, brain, cecum, cervix, colon, duodenum, epididymis (2), esophagus, eye [to include bulbar conjunctiva (2)], eyelids [upper and lower to include palpebral conjunctiva (2)], femur with bone marrow (articular surface of the distal end), gallbladder, heart, ileum, jejunum, kidney (2), lacrimal gland (2), grossly-observed lesions, liver, lung with large bronchi, lymph node (axillary), lymph node (mandibular), lymph node (mesenteric), mammary gland (females only), optic nerve (2), ovary (2), oviduct (2), pancreas, Peyer's patch (gut associated lymph tissue), pituitary gland, prostate, rectum, salivary gland [mandibular (2)], sciatic nerve, seminal vesicle, skeletal muscle (thigh), skin/subcutis, spinal cord (cervical, thoracic, and lumbar), spleen, sternum with bone marrow, stomach, testis (2), thymus, thyroid (2 lobes) with parathyroid, tongue, trachea, ureter (2), urinary bladder, uterus, vagina.
Authors noted:	• Treatment-related minimal-to-slight perivascular lymphocyte/macrophage infiltrates in the episclera of the right eye (LBN-treated). Based on the lack of ophthalmology observations, the authors considered this finding non-adverse. • Minimal multifocal axon degeneration in the sciatic nerve was observed in two males (one mid-dose and one high-dose). The authors noted, "a possible relationship to PF-03187207 cannot be ruled out." • Note: no treatment-related effects were detected in the left and right optic nerves, or the spinal cord sections.
This reviewer notes:	Lung: dose response in males (incidence and severity increasing with increasing dose) for lung "fibrosis/inflammation, chronic, pleural/subpleural" Other histology findings noted in the lungs (granuloma, bronchiolar epithelium hyperplasia, macrophage infiltrates) appear incidental to treatment. o Historical control data were not provided. Chamanza et al. 2010[29] reported lung focal pleural

[29] Chamanza R, Marxfeld HA, Blanco AI, Naylor SW, Bradley AE. 2010. Incidences and range of spontaneous findings in control cynomolgus monkeys (Macaca fasciularis)

	fibrosis/ pleuritis in 16 males and 9 females out of a total of control 285 monkeys/sex (i.e. 4.4%; group range 0 to 50%).
	The incidence of minimal infiltrates into the ciliary body is notable. As it occurred in both right (treated) and left (vehicle treated) eyes, the observation may reflect a vehicle effect (i.e. high-dose received a total of 4 drops/eye/day). No other treatment-related effects were apparent. Because the authors provide severity summaries for only selected tissues, this reviewer verified that no dose-responses were apparent for severity for some incidental observations (tabulated below).
Information Request (IR) and Applicant's response	An information request was sent regarding the pleural/subpleural chronic fibrosis/inflammation. The Applicant responded on 4/27/2016, reporting a historical control incidence at (b) (4) for cynomolgus monkeys of: • Males: mean 3.04%, range 1 to 50% (1433 males in 293 studies) • Females: mean 2.48%, range 0-40% (1424 females in 291 studies). The Applicant's response did not address the treatment-relatedness of the lung toxicity observed in this study. This reviewer notes that the incidences observed in LBN-treated monkeys are far above the historical control means reported, and concludes that the findings are treatment-related.

Table 45: Selected scheduled-termination histopathology results for the 9-month topical ocular monkey toxicity study (report # 6348-415)

Observation	Severity	Males				Females			
		0	low	mid	high	0	low	mid	high
# examined	-	3	4	4	4	4	3	4	4
Right-eye									
Infiltrate, lymphocyte / macrophage, perivascular, episclera	Minimal	0	1	1	1	0	0	2	1
	slight	0	0	2	1	0	0	0	0
Infiltrate, lymphocytes / plasma cells, ciliary body	Minimal	0	0	1	1	0	1	1	0

used in toxicology studies. Toxicologic Pathology. 38:642-657. Accessed via: http://tpx.sagepub.com/content/38/4/642.full.pdf

Left eye									
Infiltrate, lymphocyte / macrophage, perivascular, episclera	Minimal	0	1	0	0	0	0	0	0
Infiltrate, lymphocytes / plasma cells, ciliary body	Minimal	0	0	0	1	0	0	0	2
Sciatic nerve									
# examined	-	4	4	4	4	4	4	4	4
Degeneration, axon, multifocal	Minimal	0	0	1	1	0	0	0	0
Brain									
Inflammation, subacute, focal	Minimal	0	1	0	0	0	0	0	0
Infiltrate, lymphocytes / macrophages, perivascular	Minimal	0	1	2	0	1	0	1	1
Lung									
Fibrosis / inflammation, chronic, pleural / subpleural	Minimal	1[a]	0	**2**	**1**	1	0	0	0
	Slight	0	0	**1**	**1**	0	0	0	0
	moderate	0	0	0	**1**	0	0	0	0
Aorta									
Thickening, intima	Minimal	0	0	0	1	0	0	0	0
Heart[b]									
Hematocyst	present	0	1	0	0	0	0	0	0
Inflammation, subacute, focal / multifocal	Minimal	2[a]	3	2	2	3	2	3	4
Liver									
Fibrosis, capsule	Minimal	0	0	0	1	1	0	0	1
Hyperplasia, arteriolar	Minimal	0	0	0	1	0	0	0	0
Inflammation, subacute, focal /	Minimal	2	1	1	0	1	0	1	1

multifocal									
Inflammation, granulomatous / eosinophilic, focal	Minimal	0	0	0	0	0	0	1	0
Necrosis, focal	Minimal	0	0	0	0	0	0	1	0
Adrenal [b]									
Cortex congestion / hemorrhage, unilateral	slight	0	0	1	0	0	0	0	0
Medulla mineralization	Minimal	0	1	0	1	0	0	0	0
Stomach									
Hemorrhage	Slight	0	0	0	1	0	0[b]	0	0
Colon									
Hemorrhage	Minimal	0	0	0	0	0	0	0	1
Cervix									
Hemorrhage	Minimal	-				0	0	1	0

[a] In addition to these scheduled sacrifices, control male # I05496 was found dead on D222. This male also exhibited minimal lung fibrosis / inflammation, chronic, pleural / subpleural, and heart minimal focal/multifocal subacute inflammation (pages 735-736).
[b] Mid-dose female # I04616 was found dead; this monkey exhibited heart minimal hemorrhage, slight stomach hemorrhage, slight adrenal cortex congestion/hemorrhage, with several GI tissues autolyzed (pages 775-776).

Toxicokinetics – Latanoprost acid

- Blood was collected for TK via the femoral vein on D1, and during weeks 13, 26, and 39 at 5, 15, 30 and 60 minutes following the last daily dose. Plasma was analyzed only for latanoprost acid (LLOQ = 0.500 ng/ml).
- Additionally, blood samples were collected immediately (± 1 minute) of the ECG reading at week 36.
- The TK analysis report is signed, with signed GLP-compliance and QA statements.
- Results:
    - Latanoprost acid was only detectable at 5 and 15 minutes post-dose.
    - No sex-difference was apparent.
    - The high-dose TK results are only slightly above the mid-dose, indicating that < 100% of the high-dose was absorbed. This is consistent with how the high-dose was administered i.e. two drops spaced 5-15 minutes apart per dose).

Table 46: Latanoprost acid plasma TK results for the 9-month topical ocular monkey toxicology study (report # 6348-415)

TK parameter	Time point	Low-dose (14.4 µg/eye/day)	Mid-dose 24 µg/eye/day)	High-dose (48 µg/eye/day)
C_{max} (ng/ml)	Day 1	0.755 ± 0.370	1.77 ± 1.29	1.88 ± 0.703
	Week 13	0.444 ± 0.382	0.954 ± 0.635	1.36 ± 0.664
	Week 26	0.293 ± 0.338 [a]	1.23 ± 0.401	1.66 ± 0.396
	Week 39	0.382 ± 0.484	0.954 ± 0.687	1.33 ± 0.753
$AUC_{(0\text{-}1h)}$ (ng*h/ml)	Day 1	0.0943 ± 0.0463	0.409 ± 0.233	0.380 ± 0.159
	Week 13	0.0555 ±0.0478	0.158 ±0.145	0.228 ± 0.160
	Week 26	0.0366 ±0.0423 [a]	0.217 ± 0.120	0.286 ± 0.125
	Week 39	0.0599 ±0.0458	0.162 ±0.148	0.229 ±0.149

Data presented as means ± standard deviation.
[a] Female mean value only; not enough male values > LLOQ for calculation

Dosing Solution Analysis

- Dosing formulations were prepared at approximately 3-month intervals, and stored refrigerated.
- Homogeneity testing was performed: samples were taken from the top, middle and bottom of the BLN formulations on D1, and analyzed.
- Concentration analysis was performed 3 times (for each formulation preparation).
- Stability testing was performed, on the day of dosing, and after 45 days, 3 months, and 9 months.
- The results of the homogeneity analysis were acceptable (98.7 to 100.0% of nominal)
- The results of the concentration analysis were acceptable (98.9 to 107% of nominal).
- The results of stability testing were acceptable (97.2 to 100% of nominal).

7 Genetic Toxicology

The genetic toxicology study reports were submitted under IND 73435 and were reviewed. P/T concluded that LBN is not genotoxic overall (McDougal, 12/06/2013, IND 73435). These studies were re-reviewed for this NDA, with the same overall conclusion.

- The Ames study was previously reviewed as part of the original IND submission (Ellis, 4/27/2007, IND 73435)
- LBN was positive in the *in vitro* chromosomal aberration study. A draft report was submitted to the original IND and reviewed (Ellis, 4/27/2007, IND 73435). The final report was also reviewed (McDougal 10/18/2012, IND 73435).
- The *in vivo* micronucleus test (report # 07GR073) was amended in response to a P/T Information Request (IR). P/T reviewed in consultation with CDER's Genetic Toxicology Subcommittee of the Pharmacology/Toxicology Coordinating Committee (PTCC). P/T's final conclusion is that the study is negative (McDougal, 12/06/2013, IND 73435).

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

Study title: Bacterial reverse mutation assay of PF-3187207	
Study no.:	06AA026
Study report location:	NDA module 4.2.3.1 (*In vitro* genotoxicity) \\cdsesub1\evsprod\nda207795\0000\m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\stf-06aa026\06aa026.pdf
Conducting laboratory and location:	Pfizer Global Research & Development Drug Safety Research & Development 2800 Plymouth Rd Ann Arbor, MI, USA
Date report signed:	February 7, 2007
Date of study initiation:	March 24, 2006
GLP compliance and QA statement:	Yes, signed
Drug, lot #, and % purity:	LBN (PF-3187207), lot # 102287-24-48C6, purity 97.62%

Key Study Findings

- LBN was not genotoxic under the conditions tested, up to 5000 µg/plate, in five different strains of bacteria.
- The test methods were adequate.

Methods

Strains:
- *Salmonella typhimurium* strains TA-98, TA-100, TA-1535, and TA-1537
- *Escherichia coli* strain WP2*uvrA* pkM101

Concentrations in definitive study: 312.5, 625, 1250, 2500, and 5000 µg/plate

Basis of concentration selection: Preliminary mutagenicity test

Negative control: Vehicle

Positive control:

Strain	-S9	+ S9
TA-98	2-nitrofluorene	Benzo[a]pyene
TA-1535	Sodium azide	2-aminoanthracene
TA-1537	9-aminoacridine	
TA-100	Sodium azide	
WP2*uvrA*	4-nitroquinoline-N-oxide	

Formulation/Vehicle: Dimethylsulfoxide (DMSO), at a final volume of 0.1 ml/plate

Incubation & sampling time:

Study Validity

The study design appears appropriate.[30,31]

- Strain selection was appropriate. TA-1535 and WP2*uvrA* detect base-pair substitution mutations. TA-1537 and TA-98 detect frameshift mutation. TA-100 detects both types of mutation.
- Only the plate incorporation method was used (for both the preliminary mutagenicity test, and the definitive mutagenicity test).
- The criteria for a positive assay were appropriate:
  - ≥ 2-fold increase for TA-98, TA-100, and WP2*uvrA*
  - ≥ 3-fold increase for TA-1535 and TA-1537
- S9 liver homogenate from male rats treated with Aroclor 1254 was obtained commercially. The use of benzo[a]pyrene to confirm the activity of the S9 is appropriate.
- An automated colony counter was used to count colonies per plate.
- The concentrations of the test article were analyzed, and were within 97-115% of nominal.

[30] This reviewer consulted OECD 471. OECD [Organization for Economic Cooperation and Development] guideline for the testing of chemicals. Bacterial reverse mutation test. Adopted 1997. Accessed via: http://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en

[31] This reviewer consulted ICH S2(R1). International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). ICH Harmonized Tripartite Guideline. Guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use. 2011. Accessed via: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S2_R1/Step4/S2R1_Step4.pdf

Results

- No cytotoxicity was apparent at any dose.
- LBN was not positive in any strain.
- Slight to moderate precipitation was observed ≥ 1250 µg/plate.
- Positive controls gave the expected responses.

Table 47: Results of the bacterial reverse mutation assay's definitive study (report # 06AA026)

Revertant Frequency

Table 2. Definitive Mutagenicity Assay – Plate Incorporation[a]

Phase	Dose (µg/plate)	TA-1535[b] Mean[c]	TA-1535[b] Fold of Control	TA-1537[b] Mean[c]	TA-1537[b] Fold of Control	TA-98[b] Mean[c]	TA-98[b] Fold of Control	TA-100[b] Mean[c]	TA-100[b] Fold of Control	WP2uvrA[b] Mean[c]	WP2uvrA[b] Fold of Control
S9-	Vehicle Control[d]	14.3	n/a	5.3	n/a	20.3	n/a	85.7	n/a	231.0	n/a
	312.5	15.7	1.1	8.7	1.6	25.0	1.2	70.0	0.8	272.3	1.2
	625	17.3	1.2	5.7	1.1	21.0	1.0	50.7	0.6	251.3	1.1
	1250	15.0	1.0	3.7	0.7	20.0	1.0	45.0	0.5	253.7	1.1
	2500	18.3	1.3	6.3	1.2	19.7	1.0	46.0	0.5	256.0	1.1
	5000	15.3	1.1	7.3	1.4	22.7	1.1	40.3	0.5	248.7	1.1
	Positive Control[e]	365.7	25.6	541.7	102.2	192.7	9.5	962.3	11.2	1903.7	8.2
S9+	Vehicle Control[d]	14.0	n/a	9.0	n/a	25.3	n/a	81.7	n/a	248.0	n/a
	312.5	14.7	1.1	10.0	1.1	20.0	0.8	72.7	0.9	250.3	1.0
	625	17.3	1.2	7.3	0.8	25.7	1.0	76.7	0.9	257.3	1.0
	1250	15.0	1.1	9.3	1.0	24.7	1.0	77.3	0.9	246.0	1.0
	2500	21.3	1.5	9.7	1.1	24.0	0.9	72.3	0.9	243.0	1.0
	5000	16.3	1.2	9.7	1.1	22.3	0.9	72.7	0.9	235.3	0.9
	Positive Control[e]	276.7	19.8	199.7	22.2	562.7	22.2	1704.3	20.9	1427.0	5.8

n/a = Not applicable
[a] Fold of control refers to comparison with corresponding vehicle control.
[b] Data represents the mean of 3 plates.
[c] Mean refers to the mean number of colonies/plate.
[d] DMSO
[e] See page 11 for positive control compounds.

7.2.1 *In Vitro* Assays in Mammalian Cells

Study title: *In vitro* structural chromosomal aberration assay of PF-03187207 in human peripheral lymphocytes	
Study no.:	06AA133
Study report location:	NDA module 4.2.3.3 (*In vitro* genotoxicity) \\cdsesub1\evsprod\nda207795\0000\m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\stf-06aa133\06aa133.pdf
Conducting laboratory and location:	Purity 99.7%
Date report signed:	June 14, 2007
Date of study initiation:	November 2, 2006
GLP compliance and QA statement:	Yes, signed
Drug, lot #, and % purity:	LBN (PF-03187207), lot # AA0001418, 99.5% pure

Key Study Findings

- The authors conclude, and this reviewer concurs, that PF-03187207 was positive for inducing structural chromosomal aberrations in human peripheral lymphocytes
    - Although the authors concluded that PF-03187207 did not induce numerical chromosome aberrations, this conclusion is not supported by the data. Polyploidy counts showed clear increases for PF-3187207 treated flasks versus either the negative or positive controls for all three assays. As the guidance notes, these increases in polyploidy counts observed suggest that test article [PF-3187207 in this case] has the potential to inhibit mitosis and to induce numerical chromosome aberrations.

Methods

Cell line:	Human peripheral lymphocytes cultured from venous blood from a healthy male volunteer
Concentrations in definitive study:	• 0, 150, 275 and 350 µg/ml for 3 hours + S9 • 0, 150, 175 and 225 µg/ml for 3 hours –S9 • 0, 150, 175 and 200 µg/ml for 24 hours –S9
Basis of concentration selection:	Range finding assay: 50% decrease in mitotic index observed at 150 to 200 µg/ml
Negative control:	Vehicle
Positive control:	• Cyclophosphamide + S9 (7.5 or 10 µg/ml) • Mitomycin C – S9 (0.4 µg/ml for 3 hours, 0.1 µg/ml for 24 hours)
Formulation/Vehicle:	dimethyl sulfoxide (DMSO)

Study Validity

- Study design appears adequate[32,33]
- Dose selection appears adequate (high dose at or above 50% reduction in mitotic index). Although the highest concentration tested at 3 hours (–S9) did not induce $\geq$ 50% reduction of mitotic index, the assay is acceptable because the results were positive.

[32] This reviewer consulted OECD 473. OECD Guideline for the Testing of Chemicals. *In vitro* mammalian chromosome aberration test. Adopted July 1997. Accessed online via: http://www.oecd.org/chemicalsafety/assessmentofchemicals/1948434.pdf
[33] This reviewer consulted ICH S2(R1)

Results
PF-3187207 was negative at 3 hours +S9, positive at 3 and 24 hours –S9

Table 48: Results of the *in vitro* structural chromosomal aberration assay (report # 06AA133)

Treatment	Mean % mitotic suppression	Mean % of gaps	Mean % abnormal cells [a]	Fisher p-value [b]	Mean % PE cells [c]
3 hours treatment + S9					
DMSO	0	1%	1.5	-	0.05
PF03187 (b) (4) 150 µg/ml	24.1%	1%	2.5	0.36	0.10
PF03187 (b) (4) 275 µg/ml	46.7%	2.5%	4	0.11	0.05
PF03187 (b) (4) 350 µg/ml	53.1%	1%	4	0.11	0.40
Cyclophosphamide	53.1%	3%	32	< 0.001*	0.10
3 hours treatment – S9					
DMSO	0	1%	1	-	0.10
PF03187 (b) (4) 150 µg/ml	45.4%	2.5%	3.5	0.087	0.35
PF03187 (b) (4) 175 µg/ml	42.9%	0.5%	6	0.006*	0.60
PF03187 (b) (4) 225 µg/ml	47.9%	1.5%	4.5	0.031*	0.65
Cyclophosphamide	11.7%	1%	38	< 0.001*	0.0
24 hours treatment – S9					
DMSO	0	0.5%	1	-	0.0
PF03187 (b) (4) 150 µg/ml	21.0%	3%	4	0.052	0.30
PF03187 (b) (4) 175 µg/ml	45.3%	0.5%	3.5	0.087	0.25
PF0318 (b) (4) 200 µg/ml	50.0%	2%	4.5	0.031*	0.25
Cyclophosphamide	22.3%	0%	37	< 0.001*	0.0

a – number of abnormal cells excluding gaps. Two replicates of 100 cells counted per dose.
b – Data analyzed by one-tailed Fisher's exact test for increases in abnormal cells excluding gaps, compared to the vehicle control.
c – PE = cells with polyploidy or endoreduplication. Two replicates of 10000 cells counted per dose
* - statistically significantly different from control, $p \leq 0.05$

Some values rounded by this author for readability.

7.2.2 *In Vitro* Assays in Mammalian Cells

Report # 06AA133 (the *in vitro* chromosomal aberration study reviewed above) noted (report page 11) that dose selection was "based on data from an *in vitro* micronucleus assay conducted with CHO cells (Pfizer Global Research & Development, La Jolla, CA)".

- The Applicant has reported that the report is not available; they did not receive these data when the IND file was transferred.
- Pfizer had provided a summary table to B&L, stating that LBN was "equivocal" – S9 and negative + S9 in the CHO micronucleus study.
- The Applicant did not propose to mention this study in labeling, and P/T concurs that the omission is appropriate (because the reviewed report # 06AA133 represents a worse-case).

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

Study title: *In vivo* bone marrow micronucleus assay of PF-03187207	
Study no:	07GR073
Study report location:	NDA module 4.2.3.3.2 (in vivo genotoxicity) \\cdsesub1\evsprod\nda207795\0000\m4\42-stud-rep\423-tox\4233-genotox\42332-in-vivo\stf-07gr073\07gr073.pdf
Conducting laboratory and location:	Pfizer Global Research & Development Drug Safety Research & Development Groton, CT USA
Report dates:	• Report Amendment No. 1 dated July 17, 2013 • Original report dated August 13, 2007
Initiation date:	March 19, 2007
GLP compliance and QA statement:	Yes, signed
Drug, lot #, and % purity:	PF-031870207, lot AA0001418, purity 99.5%

Key Study Findings

- Based on the lack of a dose-response relationship, and because the % MN-PCE values fell within the historical control range, P/T considers the assay negative overall.
- A weak positive effect was apparent for three of the treated groups.

Methods	
Doses in definitive study:	• Nominal: 0, 0.5, 1, or 2 mg/kg/dose • Actual: 0, 0.389, 0.82, 1.55 mg/kg/dose

Frequency of dosing:	Twice daily (8 hours apart) for 2 consecutive days (4 doses total)
Route of administration:	Slow bolus intravenous injection
Dose volume:	5 ml/kg/dose (10 ml/kg/day)
Formulation/Vehicle:	A solution of 0.2 mg/ml of benzalkonium chloride (b) (4) mg/ml of polysorbate 80 in buffer ((b) (4))
Species/Strain:	Sprague-Dawley rats ((b) (4) :CD®[SD]) from (b) (4) • Approximately 6 to 7 weeks at arrival • Males 150 – 200 g at arrival • Females 125 – 175 g at arrival
Number/Sex/Group:	5/sex/dose were analyzed for bone marrow nuclei
Basis of dose selection:	4 mg/kg/day was considered by the authors to be a maximum feasible dose (MFD) based on solubility and maximum dose volume
Negative control:	Vehicle
Positive control:	Cyclophosphamide, by oral gavage, two daily doses of 10 mg/kg/day

Method notes:

- PF-03187207 was received as an oil; it was mixed with (b) (4) pre-concentrate (10x)" (polysorbate 80) with heating and agitation, then mixed with 9 parts of the rest of the vehicle. The high dose was diluted to make the lower doses, and then each was filtered (b) (4).
- The highest concentration (nominally 2 mg/kg in 5 ml, equal to 0.4 mg/ml) is listed as 0.4 mg/ml of PF-03187207, 0.2 mg/ml of benzalkonium chloride (b) (4) mg/ml of polysorbate 80, in buffered water, pH 5.55
- Analytic sampling found that the actual doses were more than 10% lower than nominal. The authors attributed this to incomplete dissolving of the oil into the formulation vehicle.
- Animals were dosed twice per day (actual doses: 0. 0.778, 1.64 or 3.1 mg/kg/day) for 2 days. Approximately 14 to 18 hours after the last dose, animals were euthanized, femoral bone marrow was removed and fractionated. Flow cytometry was used to measure the number of polychromatic erythrocytes (PCE), normochromatic erythrocytes (NCE), micronucleated PCE and micronucleated NCE. Approximately 20,000 PCE per animal were counted.

Study Validity

- The report, as revised, is acceptable.[34,35,36]
- This micronucleus study was conducted by Pfizer in 2007, but was not submitted to IND 73435 until 2012, after B&L had assumed sponsorship of the IND. In response to a P/T information request, the Sponsor submitted a report revised by (b) (4)
    - The report has multiple "protocol amendments", changing the responsible personnel, changing the laboratory to B&L, and changing the statistical analysis. There is no suggestion that (b) (4) (or B&L) have access to the original study notebooks, or any other study materials archived by Pfizer.
- In the original study report, the authors claim a MFD was used.
    - P/T had requested clarification, to understand "why dosing with (b) (4) PF-03187207 as supplied was considered infeasible" and "if the (b) (4) PF-03187207 supplied was (b) (4) PF-03187207, (b) (4) product could have been supplied."
    - The revised report clarifies tha (b) (4) PF-03187207 is an oil, not water soluble. The laboratory did work with different formulations, but determined that the highest feasible concentration is 0.4 mg/ml. The highest dose in the acute IV rat study was 2 mg/kg (at 5 ml/kg).
    - This explanation is acceptable.
- No statistical analysis was provided in the original study report, and P/T requested analyses. The revised report used:
    - Square-root transformation of data
    - % MNPCE analyzed by one-way ANOVA
    - Pair wise comparison by Dunnett's test (Student t-test)
    - P/T requested a Statistics Consult (described in the P/T review: McDougal, 10/18/2012, IND 73435). The CDER Statistical reviewer concurred with the report's statistical analysis.
    - The revised report's statistical analysis is acceptable.

Results

- No premature mortalities; no clinical signs observed for any animals.
- No apparent changes in body weight; no reductions in % PCE for the PF-031870207 treated animals (indicating no bone marrow toxicity)
- The data suggest an increase in % MN-PCE for PF-031870207 compared to

[34] This reviewer consulted OECD Guidelines for the Testing of Chemicals. 474. Mammalian erythrocyte micronucleus test. Accessed online via: http://www.oecd.org/chemicalsafety/assessmentofchemicals/1948442.pdf

[35] This reviewer consulted FDA/CFSAN 2000. Toxicology Principles for the Safety Assessment of Food Ingredients. Chapter IV.C.1.d. Mammalian Erythrocyte Micronucleus Test. Accessed online via: http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodIngredientsandPackaging/Redbook/ucm078338.htm

[36] This reviewer consulted ICH S2(R1).

controls:

- For males, % MP-PCE increased by 21%, 37% and 32% for the low-, mid-, and high-doses respectively compared to controls.
- For females, % MP-PCE increased by 53%, 71% and 10% for the low-, mid-, and high-doses respectively compared to controls.
- These differences were not statistically significant.

- The historical control data provide support for concluding that LBN was negative in this study:
  - The male negative control historical range overlaps with the positive control historical range, reducing confidence in their usefulness compared to the concurrent negative control data.
  - Although the female negative control historical range does not overlap with the positive control historical range, the upper bound of the female negative control range is only 9% less than the lower bound of the female positive control range, indicating that the female historical negative control data are of limited usefulness compared to the concurrent negative control data.

Table 49: Results of the in vivo rat micronucleus study (report # O7GR073)

Dose	Males	Females
	% MN-PCE	% MN-PCE
Vehicle	0.19 ± 0.04	0.21 ± 0.05
Cyclophosphamide	1.41 ± 1.01	1.56 ± 0.52
1.56 mg/kg PF-03187207 (total dose over 2 days)	0.23 ± 0.05	0.32 ± 0.04
3.28 mg/kg PF-03187207 (total dose over 2 days)	0.26 ± 0.09	0.36 ± 0.10
6.2 mg/kg PF-03187207 (total dose over 2 days)	0.25 ± 0.04	0.23 ± 0.03
Historical data: negative controls mean	0.23 ± 0.10 (range 0.08 to 0.81)	0.24 ± 0.1 (range 0.09 to 0.57)
Historical data: cyclophosphamide mean	1.52 ± 0.54 (range 0.53 to 3.83)	1.54 ± 0.53 (range 0.62 to 3.37)

Data presented as means ± standard deviation

7.4 Supporting Genetic Toxicity Study Data

- In addition to the tests for LBN, the Applicant also provided genotoxicity study reports for latanoprost (b) (4).
- Latanoprost received original approval on June 5, 1996; Pharmacia and Upjohn filed NDA 020597 for latanoprost (Xalatan®). The current latanoprost label[37] notes the

(b) (4)

Figure 17: Supporting genotoxicity and carcinogenicity data for latanoprost (b) (4)

(b) (4)

Assay	LBN	Latanoprost
Reverse mutation assay in bacteria (Ames)	Negative	Negative
In vitro chromosomal aberration assay in mammalian cells	Positive	Positive
In vivo rodent micronucleus assay	Negative	Negative
Mouse lymphoma assay	Not tested	Negative
Unscheduled DNA synthesis assay		Negative [a]
Mouse carcinogenicity study		Negative
Rat carcinogenicity study		Negative

[a] The latanoprost UDS study was not adequate for inclusion in labeling (P/T review by Dr. Shriver, 1/11/1996, NDA 20-597).

8 Carcinogenicity

DTOP concluded that carcinogenicity studies were not warranted to support the indicated route of administration and indication. Therefore, DTOP waived the requirement, for this particular indication and route of exposure, for nonclinical carcinogenicity studies with LBN.

[37] Label dated 3/13/2012. Accessed online via: http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/020597s044lbl.pdf

The Applicant addressed the carcinogenicity potential for LBN in the NDA, module 2.4 Nonclinical Overview), noting:

- Negative Ames results and negative *in vivo* micronucleus study results
- LBN plasma concentrations in healthy volunteers was below the LLOQ
- Guidance from the International Conference of Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidance
- The supporting genotoxicity and carcinogenicity data for latanoprost (b) (4) (b) (4) (tabulated above).

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

No study to evaluate the effect of LBN on fertility and early embryonic development was submitted to the NDA. During development (i.e. under IND), the Sponsor proposed not conducting fertility or pre- post-natal studies for LBN, and P/T concurred.

As supporting data, the Applicant provided the fertility studies for latanoprost (report # 9200027-397/513) (b) (4)

- Intravenous dosing with latanoprost did not affect fertility in male or female rats (b) (4)

9.2 Embryonic Fetal Development

- The annotated draft labeling submitted in the original NDA (7/21/2015; \\cdsesub1\evsprod\nda207795\0000\m1\us\annotated-draft-labeling-text.pdf ; Section (b) (4).1) notes, "As agreed with FDA, Embryofetal studies are being conducted with latanoprostene bunod in two species. Reports will be submitted during the NDA review cycle."
- The mid-cycle meeting was held December 14, 2015 (minutes by Boyd, 1/04/2016, NDA 207795). At the meeting, P/T inquired into the status of the embryofetal studies. A follow-up information request (IR) was sent on January 6, 2016 regarding the range-finding EFD studies.
- The Applicant sent unaudited draft reports (reviewed below) on January 27, 2016, and noted "The final reports for the above referenced draft reports [#20073520, 20073521, 20072522, 20072523] will be submitted by the end of February, 2016."
- On March 30, 2016, the Applicant sent final reports for the four embryofetal studies. A P/T Information Request (IR) was conveyed on 4/04/2016, which included questions about the interpretation of these studies.

9.2.1 Rat embryofetal (EFD) range-finder

Study title: A dose range-finding embryo-fetal development study of latanoprostene bunod (LBN) by intravenous (bolus) in rats	
Study no.:	20073520
Study report location:	NDA module 4.2.3.5 Reproductive and Developmental Toxicity • Draft report location: \\cdsesub1\evsprod\nda207795\0009\m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\stf-audited-draft-report-20073520\20073520.pdf • Final report location: \\cdsesub1\evsprod\nda207795\0015\m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\stf-20073520\20073520.pdf
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 17, 2015
Date in-life completed	March 31, 2015
Report date and status	• Draft report (unsigned and unaudited) dated May 26, 2015 • Final report dated February 9, 2016
GLP compliance:	Not GLP
QA statement:	None
Drug, lot #, and % purity:	LBN, batch # 3U001, purity 99.7%

Key Study Findings

- The purpose of this study was to select doses for the GLP rat embryofetal study, and to provide a preliminary evaluation of maternal toxicity, and also developmental toxicity from implantation to closure of the hard palate (period of organogenesis).
- Maternal NOAEL = 0.3 mg/kg. The maternal LOAEL = 1.5 mg/kg, associated with decreased weight gain, decreased food consumption.
- Cesarean Section Data: post-implantation loss, increased early and late resorptions.
- The study design was not fully adequate to definitely identify a developmental NOAEL/LOAEL.
    - No developmental toxicity was apparent at 0.3 mg/kg, the nominal NOAEL.
    - At 1.5 mg/kg, post-implantation loss and reduced fetal weight were apparent, the nominal LOAEL.

- This study did not include TK measurements.
- Based on the results of the study, the authors recommended 1.5 mg/kg/day as the high-dose for the GLP study.
  - The 0.3 mg/kg/day animal dose (9000 µg/m^2) provides an exposure margin of 170X from the clinical dose (10.36 µg/m^2), on a body surface area basis.
- Rats were dosed from GD7 to GD17. The lack of dosing on GD6 is a study limitation; LBN might have exhibited more toxicity if dosing had started one day earlier.
  - The authors refer to GD0 for mating (i.e. the day of mating was GD0, not GD1).
  - Per ICH S5(R2)[38] note 2 (1.2) Timing conventions, "In this guideline the convention for timing of pregnancy is to refer to the day that a sperm positive vaginal smear and/or plug is observed as day 0 of pregnancy even if mating occurs overnight. Unless shown otherwise it is assumed that, for rats, mice and rabbits implantation occurs on day 6-7 of pregnancy, and closure of the hard palate on day 15-18 of pregnancy. "

Methods	
Doses:	0, 0.15, 0.3, 1.5, and 3 mg/kg/day
Frequency of dosing:	Once daily from gestation day (GD) 7 to 17
Dose volume:	2 ml/kg
Route of administration:	Slow intravenous (iv) injection via the lateral tail vein (over 1-2 minutes)
Formulation/Vehicle:	Aqueous solution: • (b) (4) • (b) (4)% polysorbate 80 (b) (4) • pH 5 (b) (4) • sterile water for injection
Species/Strain:	Pregnant female Sprague-Dawley rats • Time-mated • Age approximately 79 days at start of dosing (GD7)
Number/Sex/Group:	6 females/dose group (sacrificed on GD21)
Notes regarding the study design:	• Rats were naturally bred at the supplier, and shipped to arrive at the test facility on GD2. • Rats were co-housed (2/cage) until the day

[38] ICH S5(R2) Detection of toxicity to reproduction for medicinal products & toxicity to male fertility. Addendum incorporated 2005. Accessed via: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S5/Step4/S5_R2__Guideline.pdf

	of scheduled euthanasia • The acute rat iv study (report # 06AM024) done by Pfizer reported that 2 mg/kg was the maximum feasible dose. The formulations are slightly different.

Note regarding the draft and final statuses:

- The report was unsigned, and was missing the following: figure 1 (maternal body weights), Appendix 2 (test article characterization), Appendix 3 (dose formulation analysis report), and Appendix 11 (historical control data).
- The final report is signed, and has these previously-missing appendices. Additionally, Appendix 12 is "differences from the submitted draft report versus the final report."

Observations and Results

Mortality

- Viability checks were performed at least twice daily.
- One (1/6) high-dose rat (#1277, 3 mg/kg/day group) was found dead on GD11, 72 minutes after dosing. The cause of death was not determined. The authors presumed that the cause was test-article related.
    - Food consumption and body weight were unremarkable prior to death
    - No notable clinical signs prior to death
    - This rat had 13 embryos in utero; the authors were unable to assess their viability.

Clinical Signs

- General appearance was assessed daily. Additionally, post-dose observations were made at least once on days of dosing.
- At the 1.5 mg/kg group, one rat (#1269) exhibited mild dehydration on one day (GD17). The authors presumed this effect to be treatment-related, because the observation was also observed in the 3 mg/kg group animals.
- For the high-dose (3 mg/kg/day):
    - 4/5 surviving rats exhibited 'red perivaginal substance' (9 occurrences, ranging from GD14 to GD19),
        - The timing of these observations was consistent with the C-section outcomes.
        - For example, rat # 1278 was the only dam without this finding; she had 11 viable fetuses (and 1 early resorption, 1 late resorption).
    - 2/5 exhibited mild dehydration (both GD 18-19). One of these rats also exhibited hunched posture (GD18-19).

Body Weight

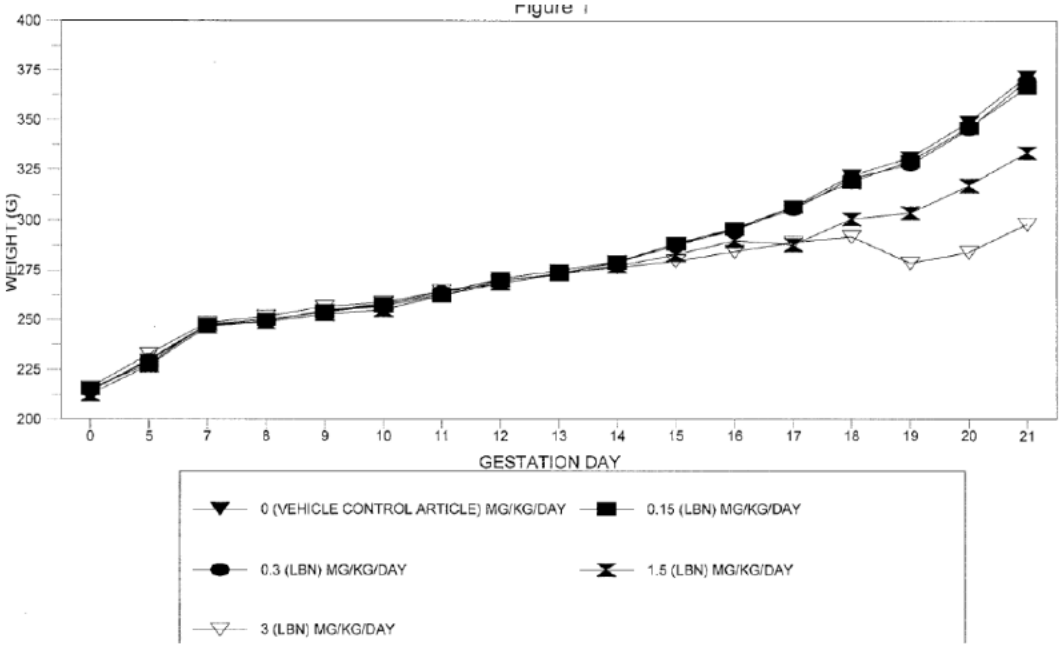
- Body weights were measured pre-dose, and daily from GD7 onward.
- Weight gain was reduced for the 3 mg/kg group from D17 onward.

- A less-pronounced reduction in weight gain was apparent for the 1.5 mg/kg group from D17 onward.
- No effect on body weight was apparent for the 0.15 or 0.3 mg/kg groups.

Table 50: Selected body weight data for the rat EFD range-finder (report # 20073520)

Gestation day (GD)	Body weight (% control for value)			
	0.15 mg/kg	0.3 mg/kg	1.5 mg/kg	3 mg/kg
15	100%	100%	98%	97%
16	100%	100%	98%	97%
17	100%	100%	94%	94%
18	99%	100%	93%	91%
19	99%	99%	92%	84%
20	99%	99%	91%	81%
21	99%	99%	90%	80%

Figure 18: Maternal body weights for the rat EFD-range finder (report # 20073520)



Feed Consumption

- Food consumption was measured on GD7, 10, 12, 15, 18, and 21. The results are reported quantitatively, in 3-day blocks.
  - Note: rats were pair-housed (presumably with another rat from the same dose-level).

- The authors noted that food consumption was reduced in the 1.5 and 3 mg/kg groups. However, this reviewer notes that food consumption was reduced in all groups beginning on GD12-15. A dose-response is apparent for severity.

Table 51: Selected food consumption data for the rat EFD range-finder (report # 2003520)

Gestation day (GD)	Food consumption (% control for value)			
	0.15 mg/kg	0.3 mg/kg	1.5 mg/kg	3 mg/kg
12-15	87%	87%	88%	69%
15-18	92%	95%	83%	74%
18-21	91%	90%	81%	52%

Necropsy

- On GD21, surviving females were sacrificed. Rats were examined for gross lesions. Pregnancy status and uterine contents were recorded.
- One rat (#1273) in the 1.5 mg/kg group was found to be not pregnant (no fetuses, no resorptions, no implantation sties or corpora lutea).
- No gross observations were recorded for the maternal necropsy.

Cesarean Section Data

- "The ovaries and uterus were examined for number and distribution of corpora lutea, implantation sites, placentae (size, color or shape), live and dead fetuses, and early and late resorptions."
- The one non-pregnant 1.5 mg/kg animal, and the one 3 mg/kg animal (# 1277) found dead on GD11 were excluded from these data (i.e. not included in the mean ± standard deviation calculations).
    - Rat # 1277 had 13 fetuses in the uterus; viability and other points were not evaluable for these offspring.
- Two rats in the 3 mg/kg group had total loss of litters (100% early resorptions)
- The 1.5 and 3 mg/kg groups exhibited increased incidences of early resorptions/litter, mean number of late resorptions, and % resorbed conceptuses/litter.
- No dead fetuses were detected.
- No placental abnormalities were detected.
- The authors noted that no treatment-related effect was apparent for # of corpora lutea, implantations, % preimplantation loss, or % live male fetuses.

Table 52: Cesarean section data for the rat EFD range-finder (report # 20073520)

Endpoint	Value (N, mean ± SD, or %)	0	0.15 mg/kg	0.3 mg/kg [NOAEL]	1.5 mg/kg [LOAEL]	3 mg/kg
Rat pregnant and	N	6	6	6	5	5

evaluated on GD 21						
Corpora lutea	Mean	12.7 ±1.2	13.2 ±1.2	12.2 ±1.8	10.4 ±3.4	13.2 ±1.9
Implantations	Mean	12.2 ±1.2	12.7 ±0.8	11.7 ±1.9	10.0 ±3.3	13.2 ±1.9
% preimplantation loss	Mean	3.8 ± 4.2	3.5 ± 5.7	4.0 ± 6.4	3.9 ± 5.7	0.0 ± 0.0
Litter size	Mean	11.5 ±1.4	12.3 ±0.5	11.3 ±1.8	**7.4 ±4.1**	**3.2 ± 4.5**
Live fetus	N	6	74	68	**37**	**16**
	Mean	11.8 ±1.4	12.3 ± 0.5	11.3 ±1.8	7.4 ± 4.1	3.2 ±4.5
Dead fetuses	N	0	0	0	0	0
Resorptions	Mean	0.7 ± 0.5	0.3 ± 0.8	0.3 ± 0.5	**2.6 ± 2.3**	**10.0 ± 5.1**
Early resorptions	N	4	2	2	**13**	**48**
	Mean	0.7 ± 0.5	0.3 ± 0.8	0.3 ± 0.5	**2.6 ± 2.3**	**9.6 ± 5.6**
Late resorptions	N	0	0	0	0	**2**
	Mean	0	0	0	0	**0.4 ± 0.5**
% post-implantation loss	Mean	5.6 ± 4.4	2.4 ± 5.8	2.7 ± 4.1	**28.0 ± 9.8**	**75.2 ±35.0**
Dams with any resorptions	N	4	1	2	4	5
	Mean	66.7%	16.7%	33.3%	80%	100%
Dams with all conceptuses resorbed	N	0	0	0	0	**2** (40%)
Dams with viable fetuses	N	6	6	6	5	**3**
	%	100%	100%	100%	100%	40%
Placenta appeared abnormal	N	0	0	0	0	0

Bolding used to emphasize treatment-related differences from control

Offspring Data

- Fetuses were examined to assess viability, sex, external abnormalities, and body weight.
- Definition notes (page 17 of the draft report, page 18 of the final report): “A live fetus was defined as one that responds to stimuli; a dead fetus was defined as a term fetus that did not respond to stimuli and that was not markedly autolyzed; dead fetuses demonstrating marked to extreme autolysis were considered to be late resorptions. A conceptus was defined as a late resorption if it was grossly evident that organogenesis had occurred; if that was not the case, the conceptus was defined as an early resorption.”

- Fetal body weight was decreased in the 1.5 and 3 mg/kg groups compared to controls.

Table 53: Offspring data for data for the rat EFD dose range-finder (report # 20073520)

Endpoint	Value (N, mean ± SD, or %)	0	0.15 mg/kg	0.3 mg/kg [NOAEL]	1.5 mg/kg [LOAEL]	3 mg/kg
Litters with one or more live fetuses	N	6	6	6	5	**3**
Implantations	Mean	12.2 ±1.2	12.7 ±0.8	11.7 ±1.9	10.0 ±3.3	13.0 ±1.0
Live fetuses	N	69	74	68	37	16
	Mean	11.5 ±1.4	12.3 ±0.5	11.3 ±1.8	7.4 ±4.1	5.3 ±4.9
Live male fetuses	N	38	28	35	16	6
Live male fetuses/litter	Mean	54.2 ±18.3	38.0 ±11.2	51.5 ±11.0	43.9 ±8.4	61.6 ±41.1
Live fetal body weight /litter (both sexes combined)	Mean	5.76 ±0.33	5.46 ±0.43	5.66 ±0.27	5.08 ±0.46	**4.41 ±0.51**
Live male fetal body weight/litter	Mean	5.86 ±0.39	5.71 ±.052	5.78 ±0.22	5.04 ±0.47	**4.54 ±0.47**
Live female fetal body weight/litter	Mean	5.69 ±0.33	5.36 ±0.39	5.51 ±0.37	5.09 ±0.47	**4.18 ±1.04**
% dead or resorbed conceptuses/litter	Mean	5.6 ±4.4	2.4 ±5.8	2.7 ±4.1	**28.0 ±29.8**	**58.7 ±37.9**

Bolding used to emphasize treatment-related differences from control

Dosing Solution Analysis

- Samples were collected for concentration analysis, osmolarity, and PH analysis. Results were provided in the final report, and are acceptable.
  - Certificates of analysis were provided for the test article (lot # 3U001) on April 30, 2014 and again on November 30, 2016. The test article appears stable. The only notable difference is a small increase in (b) (4) (from (b) (4)% to (b) (4)%)
  - Dose formulation analysis was measured on March 17, 2015 and again on April 22, 2015. Doses were 99.2% to 102.5% of nominal.
- The authors report that they plan to rely upon the Sponsor's reporting for homogeneity and stability (i.e. no additional testing conducted for this study).

9.2.2 Rat EFD GLP study

Study title: An embryo-fetal development study of latanoprostene bunod (LBN) by intravenous bolus administration in rats	
Study no.:	20073521
Study report location:	NDA module 4.2.3.5 Reproductive and Developmental Toxicity • Draft report location: \\cdsesub1\evsprod\nda207795\0009\m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\stf-unaudited-revised-draft-report-20073521\20073521.pdf • Final report location: \\cdsesub1\evsprod\nda207795\0015\m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\stf-20073521\20073521.pdf
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 11, 2015
End of in-life:	June 4, 2015
Report date and status:	• The final report is dated March 1, 2016 • The previous (unaudited revised draft report) was dated January 15, 2016
GLP compliance:	• Yes, signed
QA statement:	• Yes, signed
Drug, lot #, and % purity:	LBN, batch "C (also known as 3U001)", purity 99.7% [same as for the rat EFD range-finder, and the rabbit EFD studies]

Key Study Findings

- The purpose of this study was to evaluate the toxicity of LBN on pregnant rats and on embryofetal development from implantation to closure of the hard palate.

- The NOAEL for maternal toxicity was 0.3 mg/kg. The maternal LOAEL was 1.5 mg/kg, based on decreased weight gain and food consumption (as predicted by the rat EFD range-finder).
  - Food consumption was decreased for all LBN groups from GD18-21 (not adverse).

- The NOAEL for developmental toxicity was 0.15 mg/kg:
  - The NOAEL for C-section data was 0.15 mg/kg.

- 0.3 mg/kg is the LOAEL, based on two (2/21) lost litters (one due to all dead fetuses, the other due to all early resorptions).
- At 1.5 mg/kg, 10/21 litters were lost, due to 100% post-implantation loss. The 1.5 mg/kg dose also caused increased numbers of early resorptions and late resorptions. As a corollary, gravid uterine weight was reduced at 1.5 mg/kg.

- LBN was structurally teratogenic at 0.3 and 1.5 mg/kg:
  - At 0.3 mg/kg, one (dead) fetus had domed head (externally observed malformation). This malformation was observed once in the historical control data (1075 litters with a total of 14,352 live fetuses examined over 47 studies). Because the death of this fetus appears treatment-related, the malformation in this fetus is also considered treatment-related.
  - Note: no further examination of the domed head was performed (e.g. to verify the presence of correlating intracranial abnormalities)
  - At 1.5 mg/kg, external malformations (forepaw hyperextension, malrotated hindlimbs), and skeletal malformations of the sternebra (split sternebra, fused sternebra) and vertebra (lumbar and sacral hemi-vertebra; fused thoracic, lumbar, and sacral vertebra; and supernumerary lumbar vertebrae) were observed.
- The 1.5 mg/kg dose also caused reduced fetal weights, skeletal variations and sites of delayed ossification.

- Blood samples were taken for TK of LBN and the metabolites latanoprost acid and BDMN. All three (LBN, latanoprost acid, and BMDN) were detected systemically, with T_{max} values of 5 minutes post-dose. C_{max} values were reported, and AUC values were calculable for the high-dose (for all the analytes) and for latanoprost acid at all three dose levels.
- The 1.5 mg/kg dose is the equivalent of 0.9 mg/m^2, which is 87 x the maximum clinical dose (16.8 µg/patient/day, equivalent to 0.01036 mg/m^2) on a body surface area basis.

Methods	
Doses:	0, 0.15, 0.3, 1.5 mg/kg/day
Frequency of dosing:	Once daily from GD7 through GD17
Dose volume:	2 ml/kg
Route of administration:	Slow intravenous injection via the lateral tail vein over 1 to 2 minutes
Formulation/Vehicle:	Aqueous solution: • (b) (4) • (b) (4) • % polysorbate 80 (b) (4) • pH 5. (b) (4)

	• sterile water for injection [Same as for the rat EFD range-finder and the rabbit EFD studies]
Species/Strain:	Pregnant female Sprague-Dawley rats
Number/Sex/Group:	• Approximately 69 to 80 days on arrival • Main-group: 22 females/dose (sacrifice GD21) • TK: 6 controls, 18 per LBN dose group
Notes on the study design:	• Female rats were naturally bred at the Supplier, and shipped to arrive on GD1-5 • Rats were co-housed (2/cage) until the day of sacrifice • Statistical analyses were performed.

Note regarding the draft status versus the final report:

- In the draft report, the certificate of analysis (Appendix 2) and the Analysis of the test articles (Appendix 3) were signed and dated.
- The bioanalytical report for LBN and latanoprost acid (Appendix 17) was unsigned in the draft report, and is signed in the final report.
- The bioanalytical report for BDMN (Appendix 18) had been finished and signed for the draft report (dated December 30, 2015).
- The final study report has the final signed TK report (Appendix 19), which had not been included in the draft report.

Observations and Results

Mortality

- Viability checks were performed twice daily.
- Two rats were found dead. The authors consider both deaths incidental to treatment. This reviewer concurs that the death of rat # 6447 appears incidental. The potential effect of treatment on the death of rat # 6364 is unclear.
    - One 0.3 mg/kg main group rat (# 6364) was found dead on GD21.
        - No remarkable findings for body weight, food consumption, clinical signs, or gross necropsy for noted.
        - The rat was pregnant; 9 dead fetuses were found *in utero*.
        - The authors did not identify a cause of death.
        - Because no other deaths were observed at 0.3 or 1.5 mg/kg, the authors considered this death unrelated to treatment.
    - One 0.3 mg/kg TK rat (# 6447) was found dead on GD8, 58 minutes after blood collection for TK.
        - After the blood collection, a veterinary examination was needed; the rat exhibited decreased motor activity, low carriage, tachypnea, impaired righting reflex, and paleness.
        - The animal was pregnant (fetuses not counted for evaluated).

- Body weight was unremarkable for this rat (food consumption not assessed, since she was in a TK group).
- The death "was presumed to be due to a procedural error during the blood collection process."

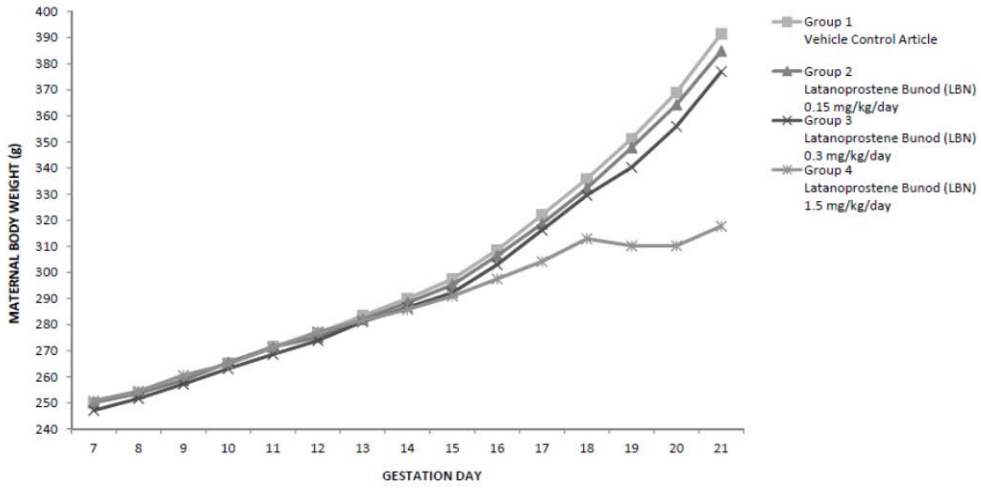
Clinical Signs

- No treatment-related clinical signs were apparent.
- General appearance was assessed once daily.
- Additionally, during the dosing period, a post-dose observation was made (1 to 2 hours post-dose).
- Note: based on the incidental findings, this reviewer understands that this monitoring was less robust and detailed than clinical signs for general toxicology studies.

Body Weight

- Body weight was measured pre-dose, and then daily from GD7 onward.
- At 0.15 and 0.3 mg/kg, no effect on maternal body weight or gravid uterine weight was apparent.
- At 1.5 mg/kg, weight gain was reduced, beginning on GD15 (-2% compared to controls), and continuing to GD21 (-19% compared to controls).

Figure 19: Maternal weight gain for the GLP rat EFD study (report # 20073521)



Feed Consumption

- Food consumption was measured quantitatively per cage, on GD7, 10, 12, 15, 18 and 21.
  - Note: rats were pair-housed (presumably with another rat from the same dose-level), except for the day of sacrifice (GD21).

- The authors noted that food consumption was decreased in the high-dose group from GD15 onward.
- This reviewer also notes that food consumption was decreased for all treated groups for GD18-21.

Table 54: Selected cage food consumption data for the GLP rat EFD study (report # 20073521)

Food consumption (units not reported, presumably g/cage)		0	0.15 mg/kg	0.3 mg/kg	1.5 mg/kg
GD15-18	Reported data	25.8 ± 1.9	24.8 ± 2.3	24.7 ± 1.1	22.8 ± 2.5
	% control	100%	96%	96%	88%
GD18-21	Reported data	26.5 ± 2.0	23.1 ± 2.9	22.6 ± 3.3	20.2 ± 2.7
	% control	100%	87%	85%	76%

Data presented as means ± standard deviation, rounded by this reviewer.

Necropsy

- Methods:
  - On GD21, surviving main-group females were sacrificed. A gross necropsy (thoracic, abdominal, and pelvic viscera) was performed.
  - The gravid uterus was weighed.
  - Select tissues were collected and preserved for possible future histopathology, if warranted.
- Results:
  - No gross observations were made for any rat.
  - Gravid uterine weight was lower for the 1.5 mg/kg group

Table 55: Gravid uterine weight data for the GLP rat EFD study (report # 20073521)

Gravid uterine weight	0	0.15 mg/kg	0.3 mg/kg	1.5 mg/kg
g	101.1 ± 12.9	104.0 ± 17.9	95.4 ± 25.3	37.7 ± 38.4*
(% control	100%	104%	95%	37%

Data presented as mean ± standard deviation
* : statistically different from control, $p \leq 0.01$ by ANOVA and Dunn test

Cesarean Section Data

- Methods:

- "The ovaries and uterus were examined for number and distribution of corpora lutea, implantation sites, placentae (size, color or shape), live and dead fetuses, and early and late resorptions."
- For non-pregnant animals, the presence/absence of implantation sites was verified by pressing the uterus between two glass plates.
- Definition note: (page 25 of the draft report, page 28 of the final report): "A live fetus was defined as a term fetus that responded to stimuli. An early resorption was defined as one in which organogenesis was not grossly evident. A late resorption was defined as one in which the occurrence of organogenesis was grossly evident. Late resorptions were differentiated by the degree of autolysis present; marked to extreme autolysis indicated that the fetus was a late resorption"

- Results:

Narrative results - Cesarean section data	
Complete loss of litter: 0.3 and 1.5 mg/kg	This reviewer considers the C-section results for one mid-dose (0.3 mg/kg) female to be clearly-treatment related (1/22 litters): • # 6355 exhibited 13/13 early resorptions o The authors did not consider the loss of 1 litter to early resorptions to be treatment-related. Because the effect is consistent with the observations at the high-dose, this reviewer concludes that the early resorptions are clearly treatment-related. • Rat # 6364 was found dead on GD21. Examination found all (9) fetuses dead. One of these dead fetuses (R02) had a domed head malformation o The authors considered the death of this female incidental, and therefore considered the death of her fetuses to be incidental. o This reviewer concurs that the deaths of the fetuses may have been incidental, and no direct treatment-relationship can be inferred.
	The high-dose (1.5 mg/kg) group has 10/21 litters with no live fetuses due to 100% post-implantation loss. • Only one dead fetus was observed in the high-dose group (in one litter, for female # 6371). This rat also had 13 early resorptions, and no live fetuses). • Notably, female # 6371 was listed as 100% early resorption. Since the 1 dead fetus is by definition not an early resorption, this appears to be a data audit problem. • The dead fetus had subcutaneous edema of the entire body, and a skeletal variation (bipartite 12th thoracic centrum)

	The authors reported "There were no dead fetuses." (Page 35). This statement is directly contradicted by the summary tables and individual data (pages 267, 273, 276). The author's statement appears to be an error, and does not affect the overall interpretation of the data. • One control female (# 6321) had one dead fetus (1/12) • One 0.3 mg/kg female (# 6364) was found dead, with all fetuses found dead (9/9) • One 1.5 mg/kg female (#6371) was found with one dead fetus (1/1, no live fetuses)
	The historical control incidences for dead fetuses (page 371 of the final report) and dams with all conceptuses resorbed (page 372 of the final report) are both 0.0, for 45 full studies (1084 litters) and 27 dose-range finding studies (190 litters).
Other effects at 1.5 mg/kg	Increased incidences compared to control for: • Mean # of early resorptions/litter (statistically significant) • Mean # of late resorptions litter (statistically significant)
Calculated differences at 1.5 mg/kg	As a corollary of the increased early and late resorptions, the following parameters were different for the 1.5 mg/kg compared to controls (statistically significant): • Higher mean total for resorptions • Higher % of pre-implantation loss [the authors erroneously report this as not affected by treatment (page 31 of the draft report; page 35 of the final report). This is a clear audit error] • Higher % of post-implantation loss • Lower mean litter size • Fewer live males/litter • Fewer live females/litter
Parameters not affected by treatment	The authors report: • No abnormal placentas detected • No effect on litter means for: o Corpora lutea o Implantations o Sex ratio

Table 56: Cesarean section data for the GLP rat EFD study (report # 20073521)

Endpoint	Value (N, mean ± SD)	0	0.15 mg/kg	0.3 mg/kg	1.5 mg/kg
# pregnant	N	22	21	22	21
	%	100%	96%	100%	96%
# not pregnant	N	0	1	0	1
Litters with live fetuses	N	22	21	20	11
Litters with all dead or resorbed	N	0	0	**2**	**10**
Litters with any abortions	N	0	0	0	0
Unscheduled euthanasia	N	0	0	1	0
Litters with total resorptions	N	0	0	**1**	**9**
Litters with at least one resorption	N	7	9	7	**18**
Dams with normal placenta	N	22	21	21	**12**

Bolding used to emphasize treatment-related differences from control

Table 57: Cesarean section data for the GLP rat EFD study (report # 20073521)

Endpoint	Value (N or litter mean ± SD)	0	0.15 mg/kg	0.3 mg/kg	1.5 mg/kg
# of corpora lutea	Mean	13.3 ± 1.5	13.8 ± 2.2	13.3 ± 2.6	12.6 ± 2.9
# of implants	Mean	13.0 ± 1.5	13.4 ± 2.2	12.9 ± 2.0	11.8 ± 2.3
N of litters with any pre-implantation loss	N	3	4	2	**9**
% pre-implantation loss	Mean	1.58 ± 4.85	2.27 ± 5.35	2.00 ±7.72	**5.36 ±8.33**
Live male fetuses	Mean	6.6 ± 1.9	7.2± 2.3	5.2 ± 2.3	**2.3 ± 2.7** [a]
	%	53.1%	55.3%	44.2%	53.2%
Live female fetuses	Mean	5.9 ± 2.1	5.7 ± 1.7	6.7 ± 2.7	**1.9 ± 2.3** [a]

# of litters with any dead fetuses	N	1	0	1	**1**
# live fetuses	Mean	12.5 ± 1.8	12.9 ± 2.3	11.9 ± 3.3	**4.1 ± 4.6** [b]
# dead fetuses	Mean	0.0± 0.2	0 ± 0	0 ± 0 [d]	0.0 ± 0.2
# of dead fetuses/ dose level	N	1	0	9	1
Total # fetuses/litter	Mean	12.5 ± 1.8	12.9 ± 2.3	12.5 ± 2.0	**7.3 ± 3.7** [b]
# resorptions	Mean	0.5 ± 1.0	0.5 ± 0.7	1.0 ± 2.8	**7.6 ± 5.0** [b]
# early resorptions	Mean	0.5 ± 1.0	0.4 ± 0.7	1.0 ± 2.8	**7.2 ± 5.3** [b]
# late resorptions	Mean	0 ± 0	0.1 ± 0.3	0 ± 0	**0.4 ± 0.9** [c]
% post-implantation loss	Mean	4.27 ± 7.23	3.96 ± 5.06	7.55 ± 71.78	**63.35 ± 40.42** [b]
# of litters with any early resorptions	N	7	7	7	**17**
Total # of early resorptions/ dose level	N	11	9	21	**152**
# of litters with any late resorptions	N	0	2	0	**5**
Total # of late resorptions/ dose level	N	0	2	0	**8**
Fetal body weight (both sexes combined)	Mean	5.94 ± 0.34	5.80 ± 0.40	5.82 ± 0.37	**5.23 ± 0.79** [c]

Bolding used to emphasize treatment-related differences from control
[a] p≤ 0.01 (ANOVA and Dunnett's)
[b] p≤ 0.01 (ANOVA and Dunn's test)
[c] p≤ 0.05 (ANOVA and Dunn's test)
[d] Note: This table omits the dead fetuses for rat # 6364 in the 0.3 mg/kg group, because their deaths are not clearly related to direct LBN-toxicity. For context, one litter in the control group (# 6321) had one dead fetus, and one litter in the 1.5 mg/kg group (# 6371) had one dead fetus.

Offspring Data

- Methods:
  - Fetuses were examined for sex and external abnormalities.
  - Fetal body weights were measured. Only live fetus weights were used to calculate means.

- ~ half of each litter was processed, and examined for visceral and head anomalies. Each fetus was fixed in Bouins solution; and heads were subsequently "examined by free-hand sectioning" [citing Wilson 1965, no further details provided]
- The remaining ~ half of each litter was stained with alizarin red S and examined for skeletal anomalies.
- Definitions (page 31 of the draft report; page 35 of the final report):
  - The authors defined malformations as "irreversible changes that occur at low incidences in this species and strain"
  - The authors defined variations as "common findings in this species and strain and reversible delays or accelerations in development"

- Results:
  - Decreased fetal body weight at 1.5 mg/kg,

Fetal anomalies – narrative discussion of results	
External examination	In the 1.5 mg/kg group, "forepaw, both, hyperextension – malformation" was observed in 2 fetuses (# R05) and in 1 litter (# 372) • The authors considered these treatment-related, and this reviewer concurs. • Neither of these fetuses had other abnormalities reported (e.g. skeletal observations). However, the effect on the forepaw is consistent with the decreased ossification in forelimb phalanges and metatarsals observed in other 1.5 mg/kg fetuses. • The authors note that paw hyperextension was not reported in the historical control data. o This reviewer notes that paw(s) flexed has 1 observation in 1075 litters (14,352 fetuses)

	For the fetuses found dead:
	• The control fetus (dam # 6321, R03) had no external (or skeletal) abnormalities.
	• For the 0.3 mg/kg group female # 6364, 1 of the dead fetuses had an external 'head, domed – malformation'.
	  o For this fetus (#6364 R02), the head was fixed but no abnormalities were detected (page 336 of the final report). The body was also evaluated for visceral abnormalities (none detected).
	  o The historical control incidence (page 374 of the final report) was 1 observation in 1075 litters (14,352 fetuses)
	  o The other 8 dead fetuses in this litter had no external abnormality detected
	• For the 1.5 mg female # 6371, the dead fetus exhibited external 'entire body, subcutaneous edema – localized – variation'
	  o This reviewer considers this effect secondary to the death

	One 1.5 mg/kg fetus (dam # 6372, fetus L14) had an external malformation. Both hindlimbs were malrotated (medially) (page 344 of the final report). The historical control incidence of fore and/or hindlimb(s) rotated (page 375 of the final report) was 2 fetuses in different litters (out of 1075 litters; 14,352 fetuses). • This fetus had multiple skeletal variations (cervical and thoracic vertebrae) noted, but no skeletal malformations noted by alizarin red evaluation: o Cervical arch (7^{th}) – bilateral misshapen (variation), "accelerated development of the transverse process" o Incomplete ossification of two cervical arches (3^{rd} right, 6^{th} left) o Thoracic centrum, bipartite ossification of 7 arches (#1-5, 7 and 9) o
Visceral examination	• No treatment-related effects apparent • Only two visceral abnormalities were detected (retinal fold in 1 fetus, small kidneys in another fetus), among all the fetuses examined. The authors considered both observations incidental to treatment.
Skeletal examination	Treatment-related effects apparent only for the 1.5 mg/kg group • Malformations: o Fused split sternebra o Fused centra in sacral vertebrae o Fused centra in thoracic vertebrae o Hemivertebra present (lumbar or sacral vertebrae) o Fused lumbar vertebrae • Variations: o Nodulated ribs o Asymmetric sternebrae o Fused sternebrae o Misshapen sternebrae o Misshapen arches of lumbar vertebrae (statistically significant difference from controls) o Misshapen arches of sacral vertebrae • Incomplete ossifications: o Sternebrae o Unilaterally ossified caudal centra o Small arches in lumbar vertebrae o Arches in lumbar vertebrae o Centra in thoracic vertebrae o Bipartite centra in lumbar or thoracic vertebrae (statistically significant difference from controls) o Caudal vertebra o Cervical arches o Lumbar arches

	○ Sacral arches • Reduced number of ossification sites: ○ Hindlimb phalanges ○ Forelimb phalanges ○ Forelimb metatarsals

Table 58: Selected external fetal observations for the GLP rat EFD study (report # 20073521)

External fetal observation	Measure-ment	0	0.15 mg/kg	0.3 mg/kg	1.5 mg/kg
Number examined	Fetuses	276	271	259	88
	Litters	22	21	21	12
Head, domed (M)	Fetuses (%)	0	0	1[a] (0.4%)	0
	# of litters	0	0	1 (4.8%)	0
Hindlimb, malrotated, both, medially (M)	Fetuses (%)	0	0	0	1 (1.1%)
	# of litters	0	0	0	1 (8.3%)
Forepaw, hyperextension (M)	Fetuses (%)	0	0	0	2 (2.3%)
	# of litters	0	0	0	1 (8.3%)
Edema, subcutaneous, entire body	Fetuses (%)	0	0	0	1[a] (1.1%)
	# of litters	0	0	0	1 (8.3%)

M: malformation
[a] Dead fetus

Table 59: Selected fetal skeletal findings for the GLP rat EFD study (report # 20073521)

Skeletal Observation (alizarin red)	Measure-ment	0	0.15 mg/kg	0.3 mg/kg	1.5 mg/kg
Number examined for skeletal abnormalities	Fetuses	144	140	135	46
	Litters	22	21	21	12
Skeletal malformations (total)	Fetuses (%)	0	1 (0.7%)	0	5 (10.9%)
	# of litters	0	1	0	4 *

Skeletal variations (total)	Fetuses (%)	16 (11.1%)	10 (7.1%)	15 (11.1%)	19 (41.3%)
	# of litters	9	8	9	9
Rib					
Rib, incomplete ossification (V)	Fetuses (%)	0	0	1 (0.7%)	1 (2.2%)
	Litters (%)	0	0	1 (4.8%)	1 (8.3%)
Rib, nodulated (V)	Fetuses (%)	0	0	1 (0.7%	2 (4.3%)
	Litters (%)	0	0	1 (4.8%)	2 (16.7%)
Sternebra					
Sternebra, asymmetric (V)	Fetuses (%)	0	0	0	2 (4.3%)
	Litters (%)	0	0	0	2 (16.7%)
Sternebra, fused (V)	Fetuses (%)	0	0	0	4 (8.7%)
	Litters (%)	0	0	0	2 (16.7%)
Sternebra, incomplete ossification (V)	Fetuses (%)	0	0	0	2 (4.3%)
	Litters (%)	0	0	0	2 (16.7%)
Sternebra, misshapen (V)	Fetuses (%)	0	0	0	1 (2.2%)
	Litters (%)	0	0	0	1 (8.3%)
Sternebra, split (M)	Fetuses (%)	0	0	0	1 (2.2%)
	Litters (%)	0	0	0	1 (8.3%)
Vertebra					
Caudal centrum, unilateral ossification (V)	Fetuses (%)	0	0	0	1 (2.2%)
	Litters (%)	0	0	0	1 (8.3%)
Caudal centrum, incomplete ossification (V)	Fetuses (%)	0	0	0	2 (4.3%)
	Litters (%)	0	0	0	2 (16.7%)
Cervical arch, Misshapen (V)	Fetuses (%)	3 (2.1%)	0	2 (1.5%)	3 (6.5%)
	Litters (%)	3 (13.6%)	0	2 (9.5%)	3 (25.0%)

Lumbar arch, incomplete ossification (V)	Fetuses (%)	0	0	0	1 (2.2%)
	Litters (%)	0	0	0	1 (8.3%)
Lumbar arch, misshapen (V)	Fetuses (%)	0	1 (0.7%)	0	3 (6.5%)
	Litters (%)	0	1 (4.8%)	0	3 (25.0%)
Lumbar arch, small (V)	Fetuses (%)	0	0	0	1 (2.2%)
	Litters (%)	0	0	0	1 (8.3%)
Lumbar arch, unossified (V)	Fetuses (%)	0	0	0	1 (2.2%)
	Litters (%)	0	0	0	1 (8.3%)
Lumbar centrum, bipartite ossification (V)	Fetuses (%)	0	0	0	3 (6.5%)
	Litters (%)	0	0	0	2 (16.7%)
Lumbar vertebra, fused (M)	Fetuses (%)	0	0	0	1 (2.2%)
	Litters (%)	0	0	0	1 (8.3%)
Lumbar vertebra, hemivertebra (M)	Fetuses (%)	0	0	0	1 (2.2%)
	Litters (%)	0	0	0	1 (8.3%)
Lumbar vertebra, supernumerary (M)	Fetuses (%)	0	1 (0.7%)	0	1 (2.2%)
	Litters (%)	0	1 (4.8%)	0	1 (8.3%)
Sacral arch, incomplete ossification (V)	Fetuses (%)	0	0	0	2 (4.3%)
	Litters (%)	0	0	0	2 (16.7%)
Sacral arch, misshapen (V)	Fetuses (%)	0	0	0	1 (2.2%)
	Litters (%)	0	0	0	1 (8.3%)
Sacral centrum, fused (M)	Fetuses (%)	0	0	0	1 (2.2%)
	Litters (%)	0	0	0	1 (8.3%)
Sacral vertebra, hemivertebra (M)	Fetuses (%)	0	0	0	1 (2.2%)

	Litters (%)	0	0	0	1 (8.3%)
Thoracic centrum, bipartite ossification (V)	Fetuses (%)	3 (2.1%)	5 (3.6%)	4 (3.0%)	13 (28.3%)
	Litters (%)	3 (13.6%)	5 (23.8%)	4 (19.0%)	8 * (66.7%)
Thoracic centrum, fused (M)	Fetuses (%)	0	0	0	1 (2.2%)
	Litters (%)	0	0	0	1 (8.3%)
Thoracic centrum, unilateral ossification (V)	Fetuses (%)	0	0	0	1 (2.2%)
	Litters (%)	0	0	0	1 (8.3%)
Thoracic centrum, Unossified (V)	Fetuses (%)	0	0	0	2 (4.3%)
	Litters (%)	0	0	0	2 (16.7%)

M – Malformation
V – Variation
***** - statistically significantly difference from controls, $p < 0.01$ (Fisher's exact 2 sided test)

Table 60: Selected ossification site data for the GLP rat EFD study (report # 20073521)

Site	0	0.15 mg/kg	0.3 mg/kg	1.5 mg/kg
N (# of litters)	22	21	20	12
Forelimb phalanges	8.00 ± 1.04	8.35 ± 0.57	8.12 ± 0.84	7.07 ± 1.30
Hindlimb phalanges	6.87 ± 1.28	6.80 ± 1.03	6.45 ± 1.13	5.47± 0.99 [a]
Metatarsals	4.91 ± 0.18	4.91 ± 0.16	4.83 ± 0.25	4.62 ± 0.44

Data presented as mean number of ossification sites per fetus per litter, ± standard deviation
[a] - statistically significant difference from controls, $p \leq 0.01$ (Dunnett's)

Toxicokinetics

- This study had two TK groups: half (3 control rats, 9/LBN group) for LBN and latanoprost analysis, and half (3 control and 9/LBN group) for BDMN analysis.
- Blood samples were collected from both groups on GD7 and GD17, 3 rats/time points (i.e. 0 for all groups; and for the LBN-treated groups post-dose at 5, 15, 30 minutes, then 1, 2, 6 and 24 hours).

- TK animals were sacrificed on GD18. Pregnancy status was recorded, and no further evaluation was performed.
- No TK results were provided in the draft report.
- TK results were provided in the final report. Analysis of LBN and latanoprost free acid was conducted under GLP by (b) (4) (b) (4) (Appendix 17). Analysis of BDMN was conducted under GLP by (b) (4) (b) (4) (Appendix 18). The TK evaluation report was conducted under GLP by (b) (4) (b) (4) (Appendix 19).
    - LBN (the parent compound), latanoprost acid, and BDMN were each detected systemically, with T_{max} values at 5 minutes post-dose.
    - For LBN:
        - LLOQ = 15 pg/ml
        - LBN was generally detectable at 5, 15 and 30 minutes post-dose (and was detected in one sample at 6 hours post-dose)
        - The authors considered the C_{max} to show dose-proportionality between 0.15 and 0.3 mg/kg, but less than dose-proportionality between 0.3 and 1.5 mg/kg.
        - A comparison of the D7 and D17 data suggests slower clearance by D17 (e.g. possibly an adaptive response)

Table 61: LBN serum TK for the GLP rat IV EFD study (report # 20073521)

GD	Dose (mg/kg)	C_{max} (pg/ml)	C_{max}/dose	AUC_{0-t} (hr*pg/ml)
7	0.15	15.0 ± 7.52	100	-
	0.3	37.9 ± 2.75	126	-
	1.5	142 ±18.5	94.7	47.3 ± 3.89
17	0.15	27.4 ± 1.34	183	-
	0.3	54.9 ±5.30	183	-
	1.5	223 ±28.4	148	61.5 ±3.68

Data reported as mean ± standard error
AUC values were not calculated for the low- or mid-dose groups
Summary table located on page 985 of the final report.

    - For latanoprost acid
        - LLOQ = 45 pg/ml
        - Systemic exposure to latanoprost acid was higher than systemic exposure to LBN.
        - Latanoprost acid was generally detectable up to 2 hours post-dose, and was detectable in some samples at 6 or 24 hours post-dose
        - For the high-dose (1.5 mg/kg), the C_{max} and AUC were clearly higher on GD17 compared to GD7.

Table 62: Latanoprost acid serum TK for the GLP rat IV EFD study (report # 20073521)

GD	Dose (mg/kg)	C_{max} (ng/ml)	C_{max}/dose	AUC_{0-t} (hr*ng/ml)	AUC/dose	$T_{1/2}$ (hr)
7	0.15	55.4 ± 2.85	369	8.77 ± 0.46	58.5	-
	0.3	98.6 ± 2.83	329	17.7 ± 1.75	58.9	0.249
	1.5	455 ± 116	303	78 ±14.8	52.0	0.295
17	0.15	80.2 ± 3.95	535	12.1 ± 0.651	80.9	-
	0.3	160 ± 22.3	532	28.5 ± 5.20	94.9	0.190
	1.5	1,040 ± 33.3	696	154 ±6.04	102	0.689

Data reported as mean ± standard error.
Note: units for this table are ng (not pg).
$T_{1/2}$ values were not considered reliable enough to report
Summary table located on page 986 of the final report.

- for BDMN
  - LLOQ = 1 ng/ml
  - The low- and mid-dose were detectable systemically at 5 and 15 minutes; the high-dose was detectable at 5, 15 and 30 minutes (but not 1 hour post-dose).
  - AUC could only be calculated for the high-dose group.

Table 63: BDMN serum TK for the GLP rat IV EFD study (report # 20073521)

GD	Dose (mg/kg)	C_{max} (pg/ml)	C_{max}/dose	AUC_{0-t} (hr*pg/ml)
7	0.15	10900 ± 1060	72,400	-
	0.3	24000 ± 5920	80,100	-
	1.5	108000 ± 12400	72,000	11500
17	0.15	18200 ±3580	121,000	-
	0.3	25700 ±7350	85,500	-
	1.5	141000 ±19000	94,000	14200

Data reported as mean ± standard error
AUC values were not calculated for the low- or mid-dose groups
Summary table located on page 987 for the final report.

Dosing Solution Analysis

- LBN concentrations were 101.9% to 107.0% of nominal, which is acceptable.

- Test article dosing formulations were prepared once, and stored refrigerated, and removed from the refrigerator 30 minutes prior to dosing. Samples were taken at preparation, and after the last dose, for each dose level.
    - All samples were analyzed for concentration.
    - The first samples (but not the end samples) were analyzed for osmolarity and pH. Osmolarity and pH results were not identified in the draft report.
- No analysis was performed for homogeneity or stability. The authors report that the Applicant will separately address the homogeneity and stability of LBN. This is a minor study limitation, since concentration data are available.
    - The clinical formulation for topical ocular dosing has BAK preservative (b) (4) polysorbate 80 (this iv formulation tested for this study ha (b) (4) polysorbate 80), and (b) (4) glycerin (b) (4) and edetate disodium (b) (4)
    - Without BAK preservative and without testing, the potential for microbial contamination of the formulation, followed by metabolism of the test article, cannot be fully assessed. The authors of the analysis report noted (page 122) that "The ample solutions were observed … No cloudiness or floating particles were found that would indicate microbial growth."
- The test article (BLN lot # 3U001) was 99.7% pure, and had two identified impurities: (b) (4).

9.2.3 Rabbit EFD range-finder

Study title: A dose range-finding embryo-fetal study of latanoprostene bunod (LBN) by intravenous (bolus) in rabbits	
Study no.:	20073522
Study report location:	NDA module 4.2.3.5 Reproductive and Developmental Toxicity \\cdsesub1\evsprod\nda207795\0009\m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\stf-audited-draft-report-20073522\20073522.pdf
Conducting laboratory and location:	(b) (4)
Report date:	11 February 2016
Report status:	final report
Date of study initiation:	March 24, 2015
End of in-life:	April 15, 2015
GLP compliance:	Not GLP
QA statement:	No; the draft report explicitly notes that the report is unaudited (page 20)
Drug, lot #, and % purity:	LBN, batch # 3U001, purity 99.7% [same as for the rat EFD studies]

Key Study Findings

- Maternal weight gain and food consumption were decreased at all LBN-dose levels, but no dose response was apparent and the magnitude of the effects were too small to be considered adverse.
- Intravenous dosing with 20 or 80 µg/kg was not compatible with pregnancy. 80 µg/kg was more toxic, although the outcome (abortion) was more severe for the 20 µg/kg group:
  - 80 µg/kg caused total litter loss due to early resorptions (100% in 4/4) rabbits, with one aborting (GD19) and the others being C-sectioned on GD29.
  - A dose of 20 µg/kg caused all pregnant rabbits (4/4) to abort (GD19 or GD20). The conceptuses were predominantly late resorptions, with some early resorptions. Two implantation sites (of one rabbit) were not accounted for; the authors presume they correspond to cannibalized conceptuses or early resorptions..
  - The general observation of "red substance in cage pan" was made earlier (GD11-14) for two 80 µg/kg rabbits, and on GD19 for two others (not observed for the 5th rabbit at 80 µg/kg). In contrast, 4/4 rabbits at 20 µg/kg exhibited "red substance in cage pan" on GD19-20. This reviewer concludes that the increased toxicity at 80 µg/kg killed fetuses earlier (i.e.

preventing the possibility of late resorption, and decreasing the possibility of abortion).

- No developmental NOAEL was established in this study. At the low-dose (1 µg/kg), one fetus was found dead, and another fetus (from a different litter) had a grossly apparent malformation, domed head.
  - This reviewer considers the observation of domed head in this study to support the conclusion the rat domed head malformation is treatment-related.
  - No cranial examination was performed on the fetus with domed head (e.g. to characterize potential brain malformation)
- The study design did not include TK evaluation.

Methods

Doses:	0, 1, 5, 20, 80 µg/kg/day
Frequency of dosing:	Once daily from GD 7 through 19 (sacrificed on GD29)
Dose volume:	2 ml/kg
Route of administration:	Slow intravenous injection via the marginal ear vein over 1-2 minutes
Formulation/Vehicle:	Aqueous solution: • (b) (4) • [redacted] • (b) (4)% polysorbate 80 (b) (4) • sterile water for injection • pH 5. (b) (4) [same as for the rat EFD studies]
Species/Strain:	Female time-mated New Zealand White (NZW) rabbits Approximately 4-6 months of age at time of arrival to the testing facility
Number/Sex/Group:	5 females/dose
Notes on the study design:	• Rabbits were naturally bred by the supplier, and shipped to arrive at the testing facility on GD2 • Rabbits were housed individually • Statistical analyses were not performed.
Deviation from study protocol:	Not reported in the draft

Note regarding the status of the draft report versus the final report:

- The final report is signed. The draft report was unsigned.
- The final report includes the maternal body weight figure (Figure 1), the Protocol and Amendments (Appendix 1), test article characterization (Appendix 2), the dose formulation analysis report (Appendix 3), and historical control data (Appendix 11), that were omitted from the draft report.

- Individual fetal weights were reported (pages 111-112 of the final report), but mean fetal weights were not tabulated (in either the draft or the final report).

Observations and Results

- Note: one 20 µg/kg rabbit was not pregnant (# 5417), and was excluded from analyses.

Mortality and Clinical Observations

- Viability checks were made at least twice daily. General appearance was recorded daily. Additionally, post-dose observations were made (on the days of dosing, 1-2 hours after dosing).
- No rabbits were found dead.
- One 80 µg/kg rabbit (1/5, # 5424) aborted on GD19, and four 20 µg/kg rabbits (4/4) aborted on GD19 or GD20. These rabbits were therefore euthanized.
    - The authors considered each abortion to be treatment-related, and mechanistically related to the observation of early resorptions observed at 80 µg/kg.
    - All of these rabbits had been pregnant. The high-dose litters consisted of all early resorptions (7/7). The litters for the affected 20 µg/kg rabbits consisted of early and late resorptions, with 2 conceptuses (from the same litter) presumed lost to cannibalism or early resorption, given that the number of implantation sites exceeded the number of late resorptions detected.
    - Clinical signs for these rabbits were scant, soft or liquid feces (GD17-20), red substance in the cage pan (GD19 or 20), lacrimation (GD16), and ungroomed coat (GD19)
    - Maternal weight gain and food consumption were decreased for these rabbits, compared to controls.
- Red substance in the cage was observed for all 80 µg/kg rabbits (consistent with the Cesarean section findings).
- No other remarkable clinical signs were apparent.

Body Weight

- Body weight was measured pre-dose, and daily after the start of dosing (GD7).
- Control rabbits gained weight normally.
- Beginning on GD10, treated rabbits either gained weight more slowly, or lost weight. After accounting for the lost litters, no dose-response is apparent.
- At GD29, mean body weights for the pregnant survivors in the 1, 5, and 80 µg/kg groups were 96%, 92%, and 95% of the control value.

Feed Consumption

- Food consumption was measured pre-dose, and daily after the start of dosing (GD7).

- Food consumption was reduced in all treated groups (to 72 to 85% of control values, for GD7-20).

Necropsy

- Rabbits were sacrificed on GD29. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed for each rabbit. Pregnancy status was recorded. The uterine contents and ovaries were examined.
- Liver changes were the only remarkable necropsy finding. Based on the lack of liver findings in other LBN toxicology studies, this reviewer concludes that the liver observations are incidental to treatment.
    - Note: the liver was not identified as a target organ by the topical ocular toxicity monkey studies for LBN. Likewise, the liver was not identified as a target organ in the intravenous EFD studies conducted with latanoprost (submitted to this NDA).
    - Because the rabbits that were euthanized early (due to abortion) were necropsied prior to the GD29 necropsy, the observations of small liver lobes in 3/5 of the prematurely euthanized rabbits were made without concurrent control observations for comparison. The severity of the observations was not reported.

Table 64: Small livers noted at necropsy for the rabbit EFD range-finder (report # 20073522)

Liver effect	0	1 µg/kg	5 µg/kg	20 µg/kg	80 µg/kg
N	5	5	5	5	5
Small	0	0	**1**	0	0
All lobes, small	0	0	0	3	0
Gallbladder: white area (3x1.5 cm)	0	**1**	0	0	0

Cesarean Section Data

- At necropsy, "the ovaries and uterus were examined for number and distribution of corpora lutea, implantation sites, placentae (size, color or shape), live and dead fetuses, and early and late resorptions."
- For the one non-pregnant rabbit, lack of pregnancy was confirmed by pressing the uterus between glass plates to confirm the absence of implantation sites.
- Definitions (page 19 of both the draft report and the final report): "A live fetus was defined as one that responds to stimuli; a dead fetus was defined as a term fetus that did not respond to stimuli and that was not markedly autolyzed; dead fetuses demonstrating marked to extreme autolysis were considered to be late resorptions. A conceptus was defined as a late resorption if it was grossly evident that

organogenesis had occurred; if that was not the case, the conceptus was defined as an early resorption."

- Results:
  - The 1 µg/kg and 5 µg/kg doses had no apparent effect on C-section parameters.
  - The 80 µg/kg dose increased preimplantation loss and early resorptions.
  - This reviewer concludes that the 80 µg/kg dose was more toxic than the 20 µg/kg dose (i.e. the losses earlier in pregnancy prevented subsequent abortions)
  - Note: the rabbits with abortions (GD19 or 20) were euthanized and examined. Their data is not included in the table below, which only tabulates GD29 C-section results. The authors reported (page 20):
    - the four 20 µg/kg rabbits had all early or late resorptions (100% total litter loss)
    - the one 80 µg/kg rabbit with aborted litter had all early resorptions.
  - Note: the high-dose group had early resorptions; placental tissue was not available for evaluation.

Table 65: Pregnancy outcome details for aborted litters, for the rabbit EFD range-finder (report # 20073522)

Endpoint	Value	20 µg/kg	80 µg/kg
# of aborted litters	N	4	1
# of fetuses detected	N/dose	43	7
	N/litter	10.75 ± 2.22	7
# of litters with any early resorptions	N	2	1
# of early resorptions	Litter mean	2.5 ± 4.36	7 ± 0
# of litters with any late resorptions	N	3	0
# of late resorptions	Litter mean	7.75 ± 5.32	0
# of cannibalized fetuses	N/dose	2	0

Values tabulated by this reviewer (from data on pages 78-79).
Note: abortions occurred on GD19 or GD20

Table 66: C-section data (GD29) for the rabbit EFD range-finder (report # 20073522)

Finding	N or Mean ± SD	0	1 µg/kg	5 µg/kg	20 µg/kg	80 µg/kg
Rabbits tested	N	5	5	5	5	5
# pregnant	N	5	5	5	4	5
# aborted and euthanized	N (%)	0 (0%)	0 (0%)	0 (0%)	4 (100%)	1 (20%)
# of rabbits C-sectioned on GD29	N	5	5	5	0	4
Corpora lutea	Litter mean	11.8 ± 3.6	11.2 ± 2.2	10.4 ± 1.8		8.2 ± 2.1

Implantation	Litter mean	11.0 ± 1.9	11.0 ± 2.2	10.0 ± 1.6		**7.0 ± 2.2**
% implantation loss	Litter mean	4.4 ± 9.9	1.8 ± 4.1	3.5 ± 4.8		**16.0 ±13.8**
Litter size	Litter mean	10.0 ± 1.2	10.2 ± 2.3	9.0 ±1.0		**0**
# of live fetuses	Per dose group (N)	50	50	45		**0**
	Litter mean	10.0 ± 1.2	10.0 ± 2.5	9.0 ±1.0		**0**
# of dead fetuses	Per dose group (N)	0	1	0		**0**
	Litter mean)	0	0.2 ± 0.4	0		**0**
Resorptions	Litter mean	1.0 ± 1.0	0.8 ± 0.8	1.0 ± 1.7		**7.0 ± 2.2**
Early resorptions	Total (N)	4	1	4		**28**
	Litter mean	0.8 ± 1.8	0.2 ± 0.4	0.8 ± 1.8		**7.0 ± 2.2**
Late resorptions	Total (N)	1	3	1		0
	Litter mean	0.2 ± 0.4	0.6 ± 0.9	0.2 ± 0.4		0
% post-implantation loss	Litter mean	7.9 ± 12.5	9.3 ± 12.3	8.5 ±14.4		"0"
Does with any resorptions	N (%)	2 (40%)	3 (60%)	2 (40%)		4 (100%)
Does with all fetuses resorbed	N (%)	0 (0%)	0 (0%)	0 (0%)		4 (100%)
Does with viable fetuses	N (%)	5 (100%)	5 (100%)	5 (100%)		0 (0%)
Placenta appeared normal	N (%)	5 (100%)	5 (100%)	5 (100%)		0 (0%)

Table 67: Alternate presentation of the pregnancy outcome data (abortions & C-section data) for the rabbit EFD range-finder (report # 20073522)

Dose group	Rabbit #	Corpora lutea	Live fetus	Dead fetus	Early resorp-tion	Late resorp-tion	Empty implantation site, conceptus presumed cannibalized
0	5401	11	11	0	0	0	0
	5402	9	8	0	0	1	0
	5403	18	10	0	4	0	0
	5404	10	10	0	0	0	0
	5405	11	11	0	0	0	0

1 µg/kg	5406	10	7	**1**	0	2	0
	5407	11	9	0	1	0	0
	5408	15	14	0	0	1	0
	5409	10	10	0	0	0	0
	5410	10	10	0	0	0	0
5 µg/kg	5411	8	8	0	0	0	0
	5412	12	10	0	0	1	0
	5413	11	10	0	0	0	0
	5414	9	9	0	0	0	0
	3851	12	8	0	4	0	0
20 µg/kg	5416	14	0	0	0	**12**	**2**
	5417	0	Not pregnant				
	5418	10	0	0	1	**8**	0
	5419	10	0	0	**9**	0	0
	3852	10	0	0	0	**10**	0
80 µg/kg	5421	8	0	0	**8**	0	0
	5422	6	0	0	**4**	0	0
	5423	11	0	0	**10**	0	0
	5428	8	0	0	**8**	0	0
	5425	8	0	0	**8**	0	0

This table was tabulated by this reviewer from the individual fetal evaluation data, as a check for the unaudited draft report. Bolding used to emphasize treatment-related effects.

- This method of tabulation highlights several notable points:
  - 3851 and 3852 were replacement females, for two animals that had low food consumption prior to start of dosing.
  - For female #5416, two fetuses were presumed cannibalized (i.e. could have been dead fetuses or late resorptions).
  - The high-dose group had fewer corpora lutea (a chance effect, since dosing was started after mating). The effect on the study outcome is unclear.

Offspring Data

- Fetuses were examined for external abnormalities and sexed, to the extent possible. Fetuses were weighed, and examined internally to verify sex.
- Results:
  - This reviewer calculated fetal body weights. No clear dose-relationship is apparent.

- For the GLP rabbit EFD study (report # 20073523), the authors noted (page 20) that for this rabbit EFD range-finding study, "slight treatment-related effects were observed (low fetal weights) in dams treated with 1 and 5 µg/kg/day."
- One fetus in the 1 µg/kg group (5406-9) was found dead at C-section. Her weight (22.2 g) was the lowest fetal weight measured in this study.
- One fetus in the 1 µg/kg group (5407-10) had domed head.
- One fetus in the 5 µg/kg group (5413-7) had edema of the neck and abdominal distension.

Table 68: Fetal data for the for the rabbit EFD range-finder (report # 20073522)

Finding	N or Mean ± SD	0	1 µg/kg	5 µg/kg	20 µg/kg	80 µg/kg
Litters evaluated	N	5	5	5	0	0
Litters with live fetuses	N	5	5	5	0	0
Fetuses evaluated	N	50	51	45	0	0
Live fetuses	N	50	50	45	0	0
Dead fetuses	N	0	**1**	0	0	0
Fetal body weight [a] (grams)	Litter mean	43.48 ± 4.72	37.36 ± 5.8	40.10 ± 5.33		
Sum fetal body weight [a] (grams)	Sum total	2119	1912	1793		
Domed head	Litter incidence	0	**1** 20%)	0		
	Fetal incidence	0	1 (2.0%)	0		
Body (neck) edema and abdominal distension	Litter incidence	0	0	1 (20%)		
	Fetal incidence	0	0	1 (2.2%)		
	Fetal incidence	0	0	1 (2.2%)		

[a] Calculated by this reviewer, by finding the mean fetal weight for each litter, and then averaging those per treatment group.
Bolding added to emphasize apparent treatment-related toxicity.

Dosing Solution Analysis

- Dose formulation analysis was performed on March 24, 2015 (the first day of dosing) and on April 22, 2015 (one week after the completion of in-life). The control and dose-results were confounded (i.e. artificially high).
  - Samples were analyzed using a gradient UPLC procedure with UV detection and external standard calibration.
  - The authors report than an extractable from the vial septum resulted in a peak at the same retention time as LBN.

- The author's explanation is reasonable. Because the experimental formulation values may be unreliable, the nominal concentrations are used in this review.

9.2.4 Rabbit EFD GLP study

Study title*: An embryo-fetal development study of latanoprostene bunod (LBN) by intravenous (bolus) in rabbits	
Study no.:	200753523
Study report location:	NDA module 4.2.3.5 Reproductive and Developmental Toxicity \\cdsesub1\evsprod\nda207795\0009\m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\stf-unaudited-revised-draft-report-20073523\20073523.pdf
Conducting laboratory and location:	(b) (4)
Report date:	• The final report is dated 09 March 2016 • The unaudited revised draft report was dated 15 January 2016
Report status:	Final report
Date of study initiation:	May 11, 2015
End of in-life date:	June 5, 2015
GLP compliance:	Yes, signed
QA statement:	Yes, signed
Drug, lot #, and % purity:	LBN, batch "C (also known as 3U001)", purity 99.7% [same as for the rat EFD studies and the rabbit EFD range-finder]

*The title is missing the word 'injection'.

Key Study Findings

- The purpose of this study was to evaluate the toxicity of LBN on pregnant rabbits and on rabbit embryofetal development from implantation to closure of the hard palate (period of organogenesis).

- No maternal toxicity was apparent (separate from treatment-related abortions, described below).

- No NOAEL was observed for reproductive toxicity. The LOAEL was the lowest dose tested, 0.24 µg/kg/day iv. Abortion was observed at the all LBN-dose levels.

  - Three (2/20) treatment-related abortions occurred at 0.24 µg/kg. One litter was lost due to 100% post-implantation loss (including 3 early resorptions); another other litter was lost due to 1 early resorption and 8 pre-implantation losses. For the third (rabbit # 2221), one fetus was dead and the other nine fetuses were found live at Cesarean section.
  - One female (# 2259) in the 1.2 µg/kg was euthanized early (GD21), based on the clinical observation of "material, aborted tissue, red" on GD21. This female was found to have 9 corpora lutea, one implantation site, and one early resorption. Because this rabbit had fewer-than-expected implantations (prior to the start of dosing), the pregnancy status does not appear normal. Therefore, this reviewer considers the relationship of LBN exposure to abortion to be unclear. However, the authors considered the abortion in this animal to be treatment-related, and consistent with the other treatment-related abortions observed.
  - Two (2/20) treatment-related abortions occurred at 6 µg/kg. Both litters were lost due to post-implantation losses (one with 4 early resorptions detected, the other with no early resorptions detected).
  - The laboratory was alerted to the losses of pregnancy by observing red aborted tissue material in the cage, upon daily observation checks.
  - Notably, no treatment-related effects on C-section results were apparent in the does that did not abort.

- No NOAEL was observed for developmental toxicity. LBN produced structural anomalies at the lowest dose tested, 0.24 µg/kg/day iv.
  - A total of 3 fetuses (one at the 0.24 µg/kg dose and two at the 1.2 µg/kg dose) exhibited a malformation characterized by 3 related observations: absence of the innominate artery, retroesophageal subclavian artery, with the subclavian artery origin malpositioned.
  - Additionally, the one 0.24 µg/kg fetus mentioned above also exhibited two other malformations: dilated pulmonary trunk, and narrow aorta.
  - Nitric oxide (NO) is a known vasodilator. Although these malformations may not be life-threatening, they indicate irreversible activity of NO on the developing vasculature.
  - Split sternebra was observed in 3 fetuses at 0.24 µg/kg, 1 fetus at 1.2 µg/kg, and 2 fetuses at 6 µg/kg. Based on the apparent-dose response and the historical control incidence, this reviewer concludes that these malformations are treatment-related.
- Additional external malformations were noted at higher doses:
  - One fetus at 1.2 µg/kg exhibited hindlimbs malrotated and forepaw hyperextension.
    - Because these findings are rare, and consistent with the malformations observed in the rat EFD study, this reviewer deems them treatment-related.
  - One fetus at 1.2 µg/kg and two fetuses at 6 µg/kg exhibited distended abdomen.

  - An additional skeletal malformation was observed at 6 µg/kg: absent caudal vertebra, in one fetus. The authors considered this effect to be treatment-related.
- Note: although the authors considered the incidence of fused sternebra at 6 µg/kg (5 fetuses in 2 litters) to be treatment-related, this reviewer disagrees. These observations are in line with the historical control data (litter incidence and fetal incidence).
- Plasma TK analysis was attempted.
  - LBN was below the LLOQ for all samples. BDMN was only detected in one sample from a high-dose animal, near the LLOQ.
  - Latanoprost acid was detected in samples at all LBN dose-levels; latanoprost acid C_{max} values were calculated for all 3 dose levels, and latanoprost AUC was calculable only for the high-dose.

Methods

Doses:	0, 0.24, 1.2, or 6 µg/kg/day
Frequency of dosing:	Once daily from GD 7 through 19 (sacrificed on GD29)
Dose volume:	2 ml/kg
Route of administration:	Slow intravenous injection via the marginal ear vein over 1-2 minutes [same as the rabbit EFD range-finder]
Formulation/Vehicle:	Aqueous solution: • (b) (4) • [redacted] • (b) (4)% polysorbate 80 (b) (4) • sterile water for injection • pH 5 (b) (4) [same as for the rat EFD studies and the rabbit EFD range-finder]
Species/Strain:	Female time-mated New Zealand White (NZW) rabbits Approximately 4-5 months of age at time of arrival to the testing facility
Number/Sex/Group:	20 females/dose
Study design:	• Rabbits were naturally bred at the Supplier, and shipped to arrive at the testing facility on GD1-4. • Rabbits were individually housed. • Statistical analyses were performed.

Notes comparing the draft and final reports:

- The final report has the bioanalysis report for BDMN (Appendix 17), that was omitted from the draft report.
- The TK evaluation report by (b) (4) (Appendix 18) is final and signed.

Observations and Results

Mortality

- Rabbits were checked twice daily for viability.
- No rabbits were found dead.
- A total of 7 rabbits were suspected of having aborted between GD17 and 25, and were subsequently euthanized: 3 low-dose, 1 mid-dose, and 3 high-dose. The authors considered these abortions treatment-related.
    - Note: low-dose rabbit # 2221 and high-dose rabbit # 2272 had live fetuses. It is appropriate to cull does when cage observations indicate possible abortion, to prevent cannibalism (i.e. loss of study data).
    - Note: for 3 rabbits (two in the low dose, and one in the high dose), more implantation sites were counted than were accounted for (i.e. by live fetuses, dead fetuses, early resorptions, or late resorptions). The authors presumed that the conceptuses were cannibalized. The status of the conceptus (e.g. early or late resorption) was therefore unknown.
- No abnormal findings were noted at necropsy for any of the 7 does.

Table 69: Author's summary data for the rabbits that aborted in the GLP rabbit EFD study (report # 20073523)

Dose levels:	0.24 µg/kg			1.2 µg/kg	6 µg/kg		
Individual rabbit #	2221	2222	2227	2259	2265	2272	2277
Day euthanized	GD 23	GD 25	GS 20	GD 21	GD 21	GD 19	GD 20
Clinical observations							
Red aborted tissue	GD 23	GD 25	GD 20	GD 21	GD 21	GD 19	GD 20
Red liquid material in cage pan	-	GD 24 and 25	-	-	-	-	-
Decreased fecal output	GD10 to 12	GD 10 to 11	-	-	-	-	-
Fur loss	-	-	-	-	-	GD 18 and 19	-
Body weight change							
Body weight change (kg) [author's	-3.2% from GD7	-2.7% from GD23	-2.6% from GD18 to	-5.3% from GD18 to	-2.6% from GD20	-5.9% from GD15	-3.0% from D19

summary'	to 23	to 25	GD20	21	to 21	to 19	to 20
Uterine contents							
Implantation sites	10	10[a]	1[a]	1	10[a]	9	12
Live fetuses	9	0	0	0	0	9	0
Dead fetuses	1	0[a]	0[a]	0	0[a]	0	0
Early resorptions	0	3	0	1	0	0	4
Late resorptions	0	0[a]	0[a]	0	0[a]	0	8
# of corpora lutea [b]	11	10	8	9	10	9	12
Pre-implantation loss %[b]	9.1%	0%	87.5%	88.9%	0%	0%	0%
Post-implantation loss %[b]	10%	100%	'100%' [12.5%][c]	'100%' [11.1%][d]	100%	0%	100%

[a] Authors noted that the implantation sites were empty; the authors presumed that the conceptuses aborted and were cannibalized.
[b] These rows were compiled by this reviewer from the individual ovarian and uterine content data. The other rows were compiled by the authors (page 31 of the final report).
[c] For rabbit #2227, the authors reported 100% post-implantation loss (page 296 of the final report). This appears to be an error. This reviewer calculates a post-implantation loss of 12.5% (i.e. 1/8).
[d] For rabbit # 2259, the authors report 100% post-implantation loss (page 302 of the final report). This appears to be a pre-audit error. This reviewer calculates a post-implantation loss of 11.1% (i.e. 1/9).

- For does # 2227 and 2259, the detection of only 1 implant site for each indicates that these rabbits had reproductive difficulty prior to dosing. No fetal assessment was possible for either litter. This reviewer considers their reproductive performance irrelevant to LBN, and excludes them from consideration of potential treatment-related effects.

Clinical Signs

- Rabbits were observed for general appearance pre-dose, and daily beginning with the start of dosing, until sacrifice. Additionally, post-dose observations were made 1-2 hours after dosing.
- Aside from the observations noted above (for the rabbits suspected of aborting), decreased feces and soft feces were noted at all LBN-treatment levels (page 42 of the final report).
- One high-dose rabbit (# 2263) was observed to have red liquid material in the cage, from GD15-21.

- Although these effects are not adverse, they support the conclusion that LBN was active at all dose levels.

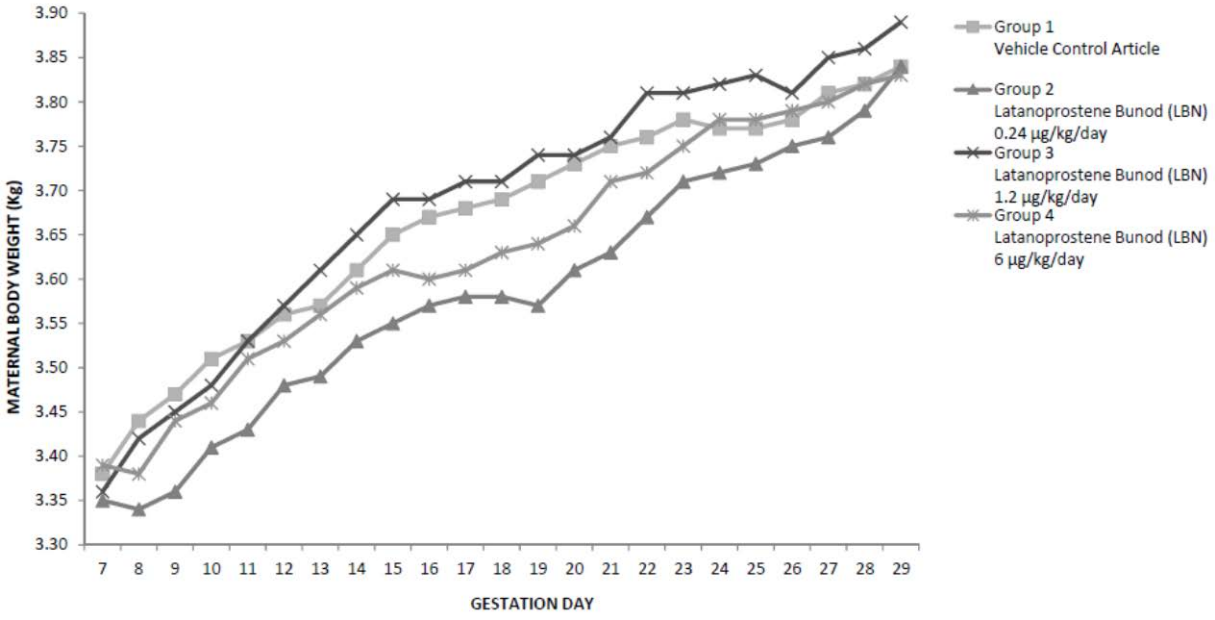
Table 70: Selected clinical sign data for the GLP EFD rabbit study (report # 20073523)

Observation	Duration and # affected	0	0.24 µg/kg	1.2 µg/kg	6 µg/kg
Material, aborted tissue, red	# of animals	0	3	1	3
	# of observations	0	3	1	3
	Days (from-to)	-	20-25	21	19-21
Material, liquid, red	# of animals	0	1	0	1
	# of observations	0	2	0	6
	Days (from-to)	-	24-25	-	15-21
Feces, output decreased	# of animals	0	6	4	4
	# of observations	0	21	8	8
	Days (from-to)	-	10-25	8-10	9-22
Feces, soft	# of animals	0	1	1	1
	# of observations	0	2	2	2
	Days (from-to)	-	25-26	25-26	5-7

Body Weight

- Body weight was measured pre-dose, and daily beginning with the start of dosing, until sacrifice.
- The authors concluded that no treatment-related effect on maternal body weight, weight gain, or gravid uterine weight was apparent, and this reviewer concurs.
    - Although the low-dose group appears to have exhibited slower weight gain, no dose-response is apparent. Therefore, this reviewer concurs that no treatment-effect on maternal body weight is apparent.

Figure 20: Maternal body weight graph for the GLP EFD rabbit study (report # 20073523)



Feed Consumption

- Food consumption was recorded daily beginning with the receipt of the rabbits at the study laboratory.
- The authors concluded that no treatment-effect on food consumption was apparent, and this reviewer concurs.
    - Although the low-dose group appears to have exhibited slightly reduced food consumption during dosing, no dose-response is apparent. Therefore, this reviewer concurs that no treatment-effect on food consumption is apparent.

Necropsy

- Rabbits were sacrificed on D29. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed for each rabbit.
- No gross lesions were detected for any animal.

Cesarean Section Data

- “The ovaries and uterus were examined for number and distribution of corpora lutea, implantation sites, placentae (size, color, and shape), live and dead fetuses, and early and late resorptions.”
- The gravid uterus was weighed.
- For nonpregnant rabbits, the uterus was pressed between glass plates to verify the absence of implantation sites.

- Definition notes (page 26 of both the draft report and final report): "A live fetus was defined as one that responds to stimuli; the dead fetus was defined as a term fetus that did not respond to stimuli and that was not markedly autolyzed; the dead fetuses demonstrating marked to extreme autolysis are considered to be late resorptions. A conceptus was defined as a late resorption if it was grossly evident that organogenesis had occurred; if that was not the case, the conceptus was defined as an early resorption"
- Results:
  - The high-dose group had fewer pregnant animals, and lower pre-implantation loss (chance occurrences, since treatment was initiated on GD7).
  - The authors report that no placenta had detectable abnormalities (page 33 of the final report).
  - For the rabbits sacrificed on GD29, the authors report that there were no treatment-related findings for ovarian and uterine parameters.

Table 71: Numbers of pregnant rabbits evaluated on GD29 for the GLP EFD rabbit study (report # 20073523)

Observation	N/dose, or litter mean ± SD	0	0.24 µg/kg	1.2 µg/kg	6 µg/kg
# of females dosed	N	20	20	20	20
# of females euthanized early for suspected abortion	N	0	3	1	3
# of females with pregnancy confirmed	N	19	19	20	**15**
# of females sacrificed on GD 29	N	20	17	19	17
# of females found not pregnant on GD 29	N	1	1	0	5

Table 72: GD29 ovarian and uterine findings for the GLP EFD rabbit study (report # 20073523)

GD 29 Observations	N/dose, or litter mean	0	0.24 µg/kg	1.2 µg/kg	6 µg/kg
# of pregnant females	N	19	16	19	12
Corpora lutea	mean	10.3 ± 2.4	10.5 ± 2.0	10.6 ± 2.3	10.3 ± 1.5
# of implants	Mean	9.9 ± 2.4	10.3 ± 2.2	10.3 ± 2.7	10.3 ± 1.5

Pre-implantation loss %	Mean	3.8 ± 8.8	2.6 ± 7.8	4.8 ± 13.4	**0.83** ± 2.89
Live males %	Mean	49 ± 18	50 ± 13	46.4 ± 12	47 ± 12
Live male fetuses	Mean	4.4 ± 1.7	4.7 ± 1.5	4.3 ± 2.0	4.3 ± 1.0
Live female fetuses	Mean	4.9 ± 1.9	4.9 ± 2.0	5.0 ± 2.1	5.0 ± 1.7
# of live fetuses	Mean	9.3 ± 2.9	9.6 ± 2.5	9.3 ± 3.2	9.3 ± 1.6
# of dead fetuses	Mean	0	0	0	0
Total # of fetuses	Mean	9.3 ± 2.9	9.6 ± 2.5	9.3 ± 3.2	9.3 ± 1.6
# of early resorptions	Mean	0.3 ± 0.8	0.5 ± 1.5	0.8 ± 2.1	0.6 ± 0.9
# of late resorptions	Mean	0.3 ± 0.6	0.2 ± 0.4	0.2 ± 0.5	0.4 ± 0.5
# of resorptions	Mean	0.6 ± 0.9	0.7 ± 1.5	0.9 ± 2.1	1.0 ± 1.0
Post-implantation loss %	Mean	7.8 ± 14.1	6.5 ± 14.9	8.7 ± 19.2	9.7 ± 8.3
Fetal body weight (both sexes average) (g)	Mean	39.9 ± 4.4	39.8 ± 6.7	40.6 ± 4.4	40.5 ± 4.5

Means are shows ± standard deviation
Some values rounded by this reviewer for readability.

Offspring Data

- Fetuses were examined for external abnormalities, and sexed and weighed.
- "The fetuses in each litter will be examined for visceral abnormalities by using a modification of the microdissection technique of Staples. A single cross-section will be made between the parietal and frontal bones (i.e., coronal section), and the brain will be examined in situ. … Each fetus will be examined for skeletal abnormalities after staining with alizarin red S."
    - Note: a single coronal section may miss treatment-related brain damage, if it occurs anterior or posterior to the cut.
    - Unlike the human coronal suture (which is relatively linear), the rabbit coronal suture curves. The protocol (e.g. page 98 of the final report) does not report what landmarks were used to make the section; therefore, it is unclear what fetus-to-fetus variability may have occurred.
- Definition notes:
    - The authors define malformation as "irreversible changes that occur at low incidences in this species and strain" (page 33 of the final report).
    - The authors define variation as "common findings in this species and strain, and reversible delays or accelerations in development" (page 33 of the final report).
    - Therefore, this reviewer understands that anomalies classified as malformations had the appearance of irreversible changes (i.e. abdominal distension, hyperextended paws, malrotated limbs), rather than apparently reversible observations.
- Results:

Author's conclusions on fetal data	P/T review concurrence & notes
One high-dose fetus (#2271-R2) exhibited abdominal distension (at external examination) and the presence of a clear red fluid in the thorax (at fresh visceral examination). • This fetus also had persistent truncus arteriosus with interventricular septal defect (M); this does not appear treatment-related *per se*, based on the concurrent control incidence	Concur that these effects (abdominal distension, red fluid in thorax, and septal defect) are not clearly treatment-related. They may be artifacts of handling and processing. • The causal relationship of the observations is unclear. Two other fetuses had persistent truncus arteriosus (without abdominal distension or thorax fluid). An additional two fetuses had abdominal distension (without truncus arteriosus or thorax fluid).
Observation in 1 fetus of absent caudal vertebrae (# 2268-R01) considered treatment-related.	This reviewer concurs. This fetus had caudal vertebrae fused (1^{st} and 2^{nd}, 5^{th} – 8^{th}) and caudal vertebra absent (8 of 16), and fused sternebra (2^{nd}-5^{th}). Although this fetus is clearly malformed, the other abnormalities fall within the reported historical control range.
Fused sternal centra (in 6 fetuses from 3 litters, versus 1 fetus for the control group) considered treatment-related	This reviewer notes that the evidence for treatment-relatedness is weak, based on the reported historical control range (observed in 0-24% of litters, and 0-4.4% of fetuses [page 397 of the final report]).
The authors did not identify any treatment-related differences in fetal ossification site averages.	Concur

- This reviewer identified additional treatment-related findings, not noted by the author.
    - Note: Appendix 15 has historical control data (June 2011-September 2014) for 39 full studies (6242 fetuses from 718 litters) and 19 dose-ranging studies (1025 fetuses from 119 litters).

Review focus: selected fetal anomalies		
Abnormality description	Dose level	Historical control information
Three fetuses exhibited a malformation, characterized in each by detection of 'innominate artery, absent', 'subclavian artery, retroesophageal', and 'subclavian artery origin, malpositioned'	• 1 at 0.24 µg/kg (# 2233-L9) • 2 at 1.2 µg/kg (# 2243-R4; # 2251-L11) [Note – none of these fetuses had the truncus arteriosus persistent and interventricular septal defects observations]	• Innominate artery absent in 3 fetuses (0.05%) from 3 different litters (0.42%) [page 390 of the final report] • Right subclavian artery passes dorsal to trachea and esophagus in 2 fetuses (0.03%) from 2 litters (0.28%) [p 390]
• Pulmonary trunk [artery] dilated (M); and • Aorta, narrow (M)	Both observations noted for one fetus, at 0.24 µg/kg (# 2233-L9, noted above)	[page 390] • Pulmonary trunk distended in 1 fetus (0.02%) from 1 litter (0.14%) • No listing for narrow aorta
External observations: • hindlimb malrotated (M); • and forepaw hyperextension (M)	1 fetus at 1.2 µg/kg (# 2243 R4, noted above)	[page 388 of the final report] • Paw(s) flexed/rotated in 1 fetus (0.02%) in 1 litter (0.14%) • Limb(s) flexed in 5 fetuses (0.08%) in 5 litters (0.70%)
External observation – trunk, distended abdomen (M)	• 1 fetus at 1.2 µg/kg (# 2256-L7) • 2 fetuses at 6 µg/kg (# 2271-R2, who also exhibited fluid-filled thorax, persistent truncus arteriosus, and ventricular septum defect; and #2275-L9)	[page 388] • Body – abdominal distension in 2 fetuses (0.03%) in 2 litters (0.28%)
Skeletal observations in one fetus: • caudal vertebra absent (M) [described as "8 present excluded from average"] • fused caudal vertebra	1 fetus as 6 µg/kg (# 2268-R1)	[pages 392 and 393 of the final report] • absent caudal vertebra – not reported • fused caudal vertebra: 3 fetuses (0.05%) from 3 litters (0.28%) • fused sternebra: 101

(M) [described as "1st and 2nd, 5th-8th"] • fused sternebra (V) [described as "2nd-5th"]		fetuses (1.62%) from 77 litters (10.72%) Note: only the absent caudal vertebra appears treatment-related, based on these data.
Sternebra, fused (V)	• 1 control fetus (2206-L4) • 2 litters (2 fetuses) at 0.24 µg/kg (2236-R4; this fetus also has a split sternebra) (2239-L4) • 2 litters (5 fetuses) at 1.2 µg/kg (2243-4, this fetus also had malrotated hindlimb, paw hyperextension, and retro-esophageal subclavian artery) (2254-R1, -R5, –L8, and –L12) • 3 litters (6 fetuses) at 6 µg/kg (2261-R3) (2268-R1, R-6, L10) (2274-R1, -R3)	[page 397 of the final report] • fused sternebra: 101 fetuses (1.62%) from 77 litters (10.72%) • The range per study is 0-8 fetuses (0-4.4%) and 0-5 litters (0-23.8%) Notably, this variation was observed in multiple fetuses for 3 of the rabbits. Generally, the appropriate 'experimental unit' for EFD studies is the litter, rather than the fetus. This reviewer concludes that these observations are not clearly related to treatment.
Sternebra, split (M)	• 3 fetuses at 0.24 µg/kg (2232-R4) (2233-L6) (2236-R4) • 1 fetus at 1.2 µg/kg (2257-R1) • 2 fetuses at 6 µg/kg (2261-R6) (2274-R3)	[page 397] • Split sternebra: 1 fetus (0.02%) in 1 litter (0.14%)
The only brain anomaly reported was in one fetus: bilateral "lateral ventricle, dilated, moderate – variation". [Note: this head of this fetus was not reported as domed.]	(2225-R09 in the 0.24 µg/kg)	[page 389 of the final report] • Brain moderate ventricular dilation: 3 fetuses (0.42%) in 3 litters (0.05%) The single observation is not clearly related to treatment.

Table 73: Selected external and visceral fetal findings for the GLP EFD rabbit study (report # 20073523)

Observation	N (%)	0	0.24 µg/kg	1.2 µg/kg	6 µg/kg
#examined (for external, visceral, and skeletal) malformations	Fetuses (N)	177	153	177	111
	Litters (N)	19	19	19	12
External					
• Hindlimb, malrotated (M) • Forepaw, hyperextension (M)	Fetuses (%)	0	0	1 (0.6%)	0
	Litters (%)	0	0	1 (5.3%)	0
Trunk, distended abdomen (M)	Fetuses (%)	0	0	1 (0.6%)	2 (1.8%)
	Litters (%)	0	0	1 (5.3%)	2 (16.7%)
Fresh Visceral					
Aorta, narrow (M)	Fetuses (%)	0	1 (0.7%)	0	0
	Litters (%)	0	1 (6.3%)	0	0
• Great vessels – truncus arteriosus, persistent (M); • Heart - ventricular septum defect (M)	Fetuses (%)	1 (0.6%)	1 (0.7%)	0	1 (0.9%)
	Litters (%)	1 (5.3%)	1 (6.3%)	0	1 (8.3%)
• Innominate artery, absent (V) • Subclavian artery, retroesophageal (M) • Subclavian artery origin, malpositioned (V)	Fetuses (%)	0	1 (0.7%)	2 (1.1%)	0
	Litters (%)	0	1 (6.3%)	2 (10.5%)	0
Pulmonary trunk [artery] dilated (M)	Fetuses (%)	0	1 (0.7%)	0	0
	Litters (%)	0	1 (6.3%)	0	0
Thorax, fluid filled (V)	Fetuses (%)	0	0	0	1 (0.9%)
	Litters (%)	0	0	0	1 (8.3%)

V = variation
M = malformation

Table 74: Selected skeletal fetal findings for the GLP EFD rabbit study (report # 20073523)

Observation	N (%)	0	0.24 µg/kg	1.2 µg/kg	6 µg/kg
#examined	Fetuses (N)	177	153	177	111
	Litters (N)	19	19	19	12
Pelvic girdle					
Pubis, Incomplete ossification (V)	Fetuses (%)	0	2 (1.3%)	0	1 (0.9%)
	Litters (%)	0	2 (12.5%)	0	1 (8.3%)
Pubis, Unossified (V)	Fetuses (%)	0	0	0	1 (0.9%)
	Litters (%)	0	0	0	1 (8.3%)
Rib					
Rib, absent (M)	Fetuses (%)	0	0	1 (0.6%)	0
	Litters (%)	0	0	1 (5.3%)	0
Rib, fused (M)	Fetuses (%)	0	1 (0.7%)	0	0
	Litters (%)	0	1 (6.3%)	0	0
Rib, unossified (V)	Fetuses (%)	0	1 (0.7%)	0	0
	Litters (%)	0	1 (6.3%)	0	0
Supernumerary rib – cervical, short (V)	Fetuses (%)	0	2 (1.3%)	0	1 (0.9%)
	Litters (%)	0	2 (12.5%)	0	1 (8.3%)
Skull					
Fontanelle, Large, Minimal (V)	Fetuses (%)	0	0	1 (0.6%)	1 (0.9%)
	Litters (%)	0	0	1 (5.3%)	1 (8.3%)
Nasal, Isolated ossification site (V)	Fetuses (%)	0	0	1 (0.6%)	0
	Litters (%)	0	0	1 (5.3%)	0
Parietal, left, hole (V)	Fetuses (%)	0	0	1 (0.6%)	0
	Litters (%)	0	0	1 (5.3%)	0

Sternebra					
Sternebra, fused (V)	Fetuses (%)	1 (0.6%)	2 (1.3%)	5 (2.8%)	6 (5.4%)
	Litters (%)	1 (5.3%)	2 (12.5%)	2 (10.8%)	3 (25%)
Sternebra, misshapen (V)	Fetuses (%)	2 (1.1%)	0	0	5 (4.5%)
	Litters (%)	1 (5.3%)	0	0	2 (16.7%)
Sternebra, split (M)	Fetuses (%)	0	3 (2.0%)	1 (0.6%)	2 (1.8%)
	Litters (%)	0	3 (18.8%)	1 (5.3%)	2 (16.7%)
Vertebra					
• Caudal vertebra, absent (M); • Caudal vertebra, fused (M)	Fetuses (%)	0	0	0	1 (0.9%)
	Litters (%)	0	0	0	1 (8.3%)
• Cervical centrum, fused (M) • Cervical hemivertebra (M) • Cervical arch, misshapen (V) • Cervical centrum, bipartite ossification (V)	Fetuses (%)	0	1 (0.7%)	0	0
	Litters (%)	0	1 (6.3%)	0	0
• Lumbar centrum, fused (M) • Lumbar centrum misshapen (V)	Fetuses (%)	0	0	1 (0.6%)	0
	Litters (%)	0	0	1 (5.3%)	0
Thoracic centrum, fused (M)	Fetuses (%)	0	2 (1.3%)	1 (0.6%)	0
	Litters (%)	0	1 (6.3%)	1 (5.3%)	0

V = variation
M = malformation

- Note: this reviewer carefully reviewed the incidences of herniated umbilicus, malpositioned liver, and malpositioned kidney, and concluded that no treatment-related effect was apparent for these visceral anomalies.

Toxicokinetics

- On GD7 and GD19, blood was collected from groups of over 8 time points (post-dose 0, 5, 15, 30 minutes; 1, 2, 6 and 24 hours). A group of 3/dose were sampled 8 times for analysis of LBN and latanoprost acid (target volume of 2.0 ml/time point). A second group of 3/dose were sampled 8 times for analysis of BDMN (target volume of 1.0 ml/time point). [I.e. total of 16 ml collected from each of the first TK animals over 24 hours, and 8 ml collected from each of the second group over 24 hours.]
- Dual samples were collected: LBN and latanoprost acid were analyzed in one set of samples (data reported), and BDMN was analyzed in the other set.
  - Determination of LBN and latanoprost acid was performed by (b) (4) (b) (4) Results were reported in Appendix 16. Analyses were GLP and QA.
  - Determination of BDMN was performed by (b) (4), (b) (4). Results were reported in Appendix 17. Analyses were GLP and QA.
  - Appendix 18 was the TK evaluation report, prepared by (b) (4) (b) (4) (b) (4) The TK evaluation was GLP and QA'ed.
- The analysis for LBN, latanoprost acid, and BDMN has signed GLP and QA statements (Appendix 18).
  - No LBN (LLOQ = 10.0 pg/ml) was detected in any plasma sample.
  - For BDMN, the LLOQ = 1 ng/ml.
    - BDMN was detected in one sample for a high-dose female (#2278) at 5 minutes post dose on D19. The result was **1.17 ng/ml** (page 708 of the final report). This animal was not pregnant.
    - However, the authors incorrectly report (page 863 of the final report) that "Plasma concentrations of 1,4-butanediol mononitrate were below the limit of quantitation (LOQ = 1 ng/mL) in all analyzed control group samples and all treated group samples."
    - This error does not affect the overall interpretation of the data.
  - Latanoprost acid was detected (LLOQ = 30 pg/ml) in some samples for the 0.24 µg/kg group (at 5 minutes only), at 1.2 µg/kg (5 and 15 minutes only) and 6 µg/kg (up to 30 minutes post-dose).
  - The authors explained (report page 861), "Animals Nos. 2267, 2276 and 2278 were not pregnant and therefore they were not included in the TK evaluation."
  - For 6 µg/kg, latanoprost acid was only detected in 2/3 samples at the 5 minute time point, and therefore standard deviations could not be calculated.
    - For latanoprost acid, this resulted in individual TK data for only 2 animals at the high-dose (pages 880-881 of the final report).
    - For BDMN, this resulted in data for only one high-dose rabbit (#2277 pages 888-889). However, (b) (4) (Appendix 17, pages 707-709) reported BDMN TK data for all three high-dose rabbits (# 2276, # 2277, # 2278), including the one with detectable BDMN.

Table 75: Latanoprost acid serum TK for the GLP rabbit EFD study (report # 20073523)

TK parameters	0.24 µg/kg	1.2 µg/kg	6 µg/kg
GD7			
C_{max} (pg/ml)	118 ± 35.5	368 ± 286	2880
AUC_{0-t} (pg*hr/ml)	NC	NC	467
GD19			
C_{max} (pg/ml)	105 ± 50.1	253 ± 177	2490
AUC_{0-t} (pg*hr/ml)	NC	NC	406

Values are mean ± standard deviation
NC = not calculable

Table 76: TK rabbit ID #s (report # 20073523)

TK rabbits	0	0.24 µg/kg	1.2 µg/kg	6 µg/kg
Rabbits sampled for LBN and latanoprost acid TK (on both GD7 and GD19)	2206, 2207, 2208	2226, 2227, 2228	2246, 2247, 2248	2266, 2268
Rabbits sampled for BDMN TK (on both GD7 and GD19)	2216, 2217, 2218	2236, 2237, 2238	2236, 2237, 2238	2277

- The failure to measure TK in enough high-dose rabbits was not due to the early euthanasia. Rabbits # 2227 (0.24 µg/kg) and # 2277 (6 µg/kg) were euthanized on GD20 due to suspected abortion, based on the clinical observation of "red aborted tissue" in the cage pan on GD20.
    - Having 2/5 of the abortions being among the 21/80 rabbits undergoing blood collection for TK is unremarkable (2-sided Fisher's exact test p value = 0.63).
    - This reviewer concludes that no effect is apparent between the blood draws and the abortion outcomes or fetal anomaly observations.

Dosing Solution Analysis

- The vehicle and test article dosing formulations were prepared once, stored refrigerated, and removed from the refrigerator 30 minutes prior to dosing (i.e. same as the rat GLP EFD study).
- The signed dose formulation analysis was provided in Appendix 3.
- Samples were collected from each dosing solution at first preparation and at the end of the dose period. The concentrations ranged from 91.9% to 109.5% of nominal. These results are acceptable.

- Osmolarity and pH were measured when the formulations were prepared, but the results were not reported ("documented in raw data.")
- No homogeneity analysis or stability analysis were performed; the authors rely upon the Sponsor's claim that LBN solution will be homogenous and stable.

9.2.5 Notes for the Latanoprost EFD Data

- As was noted above, the Applicant provided a letter of authorization of right of reference and cross-reference to NDA 20-597, from Pharmacia & Upjohn Co, a division of Pfizer Inc.

- The Applicant submitted two latanoprost EFD study reports to this NDA, as support for LBN:
  - Teratology study by intravenous route in the rat (segment II) (report # 9300279)
  - Teratology study by intravenous route in the rabbit (segment II) (report # 9300280)

- Compared with latanoprost, latanoprostene bunod is:
  - More embryocidal in the rat
  - Approximately equally abortifacient in the rabbit
  - More teratogenic in both the rat and rabbit

- The Xalatan 2014 label[39] states, "Teratogenic Effects: Pregnancy Category C. Reproduction studies have been performed in rats and rabbits. In rabbits, an incidence of 4 of 16 dams had no viable fetuses at a dose that was approximately 80 times the maximum human dose, and the highest nonembryocidal dose in rabbits was approximately 15 times the maximum human dose."

- The P/T review of the original NDA 20-597 for is referenced (Shriver, 1/11/1996, NDA 20597).

9.3 Prenatal and Postnatal Development

- No study to evaluate the effect of LBN on prenatal and postnatal development was submitted to the NDA. During development (i.e. under IND), the Sponsor proposed not conducting fertility or pre- post-natal studies for LBN, and P/T concurred.
- The available nonclinical data suggest that metabolites of LBN, if present in serum, will enter into milk.

[39] NDA 20-597/S-045 and S-048 was accessed via: http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/020597s045s048lbl.pdf

- No data were identified, regarding the distribution of LBN (the parent compound) into milk, after dosing by any route.
- Because latanoprost acid has been detected in rat milk following dosing with latanoprost, and because latanoprost acid has been detected in serum following topical ocular dosing with LBN, it is reasonable to expect that topical ocular dosing with LBN will result in latanoprost acid in milk.

(b) (4)

- As supporting data, the Applicant submitted a Segment III study in the rat with latanoprost (report # 9300281), (b) (4) (b) (4)
  - The Applicant reports that latanoprost did not affect peri- and postnatal development

(b) (4)

- Notably, the Applicant did not submit the latanoprost report # 9200137, "Dose range-finding peri- and post-natal study (segment III) by intravenous route in the rat." Both latanoprost pre- and post-natal development reports have previously been reviewed by P/T (Shriver, 1/11/1996, NDA 20597):
  - For the range-finder (report # 9200137), P/T concluded, "Daily intravenous dosing with 1 µg/kg to dams during the peri- and post-natal (day 15 of gestation to day 21 of lactation) was the no effect dose. Doses of 3 µg/kg and greater caused deaths within the litters and doses of 10 µg/kg or greater caused a decrease in the number of male pups delivered."
  - For the submitted study (report # 9300281), P/T concluded, "There were no treatment-related effects to dams of the F0 generation or F1 and F2 pups at intravenous doses of 1, 3 or 10 µg/kg." A pdf search of the text of the report found no mention of milk, or any milk analysis.

- A literature search for additional information regarding distribution of latanoprost acid into milk found a summary[40] of the nonclinical data for Xalatan, which mentions TK analysis of milk collected from pregnant or lactating rats dosed with tritium-labelled latanoprost:

  "The feto-placental transfer and lacteal secretion of latanoprost was investigated in rats. The concentrations of radioactivity of latanoprost and PhXA85 (acid of latanoprost) were measured in plasma and milk. The

[40] Pfizer Canada Inc. 2014. Product monograph. PrXalatan*. Latanoprost ophthalmic solution, 50 µg/ml. Submission control # 173373. Accessed via http://www.pfizer.ca/sites/g/files/g10017036/f/201410/Xalatan_0.pdf

concentration of radioactivity was analysed in tissues after single intravenous administration of tritium labelled latanoprost at a dose of 200 µg/kg to pregnant or lactating rats. On the 12th-gestation day, the concentration of radioactive latanoprost in the fetus was 0.00006% of the dose at 1 hour. The value of radioactivity in the fetus at 24 hours was below the limit of detection. On the 18th gestation day, the concentration of radioactive latanoprost in the fetus was 0.018% (at 1 hour) and 0.005% (at 4 hour). Again, at 24 hours there was no radioactivity measured. In the milk the concentration of radioactive latanoprost was shown to be eliminated more slowly than for plasma. Of the low levels remaining in milk at 2 hours and 8 hours, only 5.5% and 15% respectively was the acid of latanoprost. The more polar metabolites formed the rest of the radioactivity in the milk."

(b) (4)

11 Integrated Summary and Safety Evaluation

The Applicant submitted nonclinical reports for LBN, investigating *in vitro* and *in vivo* PD, distribution, acute intravenous toxicity, repeat-dose topical ocular toxicity, developmental toxicity, and genotoxicity. The Applicant also provided selected nonclinical study reports for latanoprost (b) (4). Moreover, the Applicant has right-of-reference to the NDAs for (b) (4) latanoprost (b) (4) (b) (4)

Following topical ocular dosing, two separate monkey studies identified the lung as a target organ.

The embryofetal development (EFD) studies demonstrated that intravenously-administered LBN is abortifacient and teratogenic in both rabbits and rats.

LBN was clastogenic *in vitro*, but negative in the Ames assay and in vivo micronucleus test.

11.1 Exposure margins for topical ocular data

The VYZULTA draft label states that the dosage is "one drop in the affected eye(s) once daily in the evening." The formulation (0.024%) is 0.24 µg/µl of LBN. Assuming 35 µl drop size, the clinical dose is 8.4 µg/eye,

Table 77: Exposure Margins for local ocular effects, based on dose/eye (µg/eye/day)

Report #	Species, duration	LBN Dose [a]	LBN dose para-meters	Effect	Dose per day (µg/eye /day)[b]	Exposure margins (based on a clinical dose of 8.4 µg/eye/day)
***In vivo* PD** (topical ocular dosing)						
PH14006	Ocular hypertensive monkey	0.012% (3.6 µg/eye)	one 30 µl drop, once	IOP lowering	3.6	0.43 x
		0.12% (36 µg/eye)			36	4.3 x
PH14005		0.024% (12 µg/eye)	Two 25 µl drops per dose, qd		12	1.43 x
PH14007	Wild-type mouse	0.003% (0.09 µg/eye)	One 3 µl drop, once	IOP lowering	0.09	0.011 x
		0.006% (0.18 µg/eye)			0.18	0.021 x
	FP receptor KO mouse	0.006% (0.18 µg/eye)		IOP lowering	0.18	0.021 x
PH14008	Naturally-glaucomatous dogs	0.03% (45 µg/eye)	One 50 µl drop, once	IOP lowering	45	5.36 x
PH14009	Rabbit (saline induced ocular hyper-tension)	0.03% (10.5 µg/eye)	Drop size not reported, presume to be 35 µl, once	IOP lowering	10.5	1.25 x
		0.06% (21 µg/eye)		No effect on IOP	21	2.5 x
Non-GLP toxicology (topical ocular dosing)						
05NCX001	Rabbit (tolerability study – Phase 1/2 formulation)	0.12% (60 µg/eye)	one 60 µl drop per dose, tid	Well-tolerated; transient mild conjunctival redness	180	21.4 x
GLP toxicology (topical ocular dosing)						
6750-267	28-day monkey study (GLP; Phase 1/2 formulation)	0.04% (12 µg/eye)	one 30 µl drop per dose, bid	NOAEL	24	2.86 x
8273344	28-day monkey study	0.04% (24 µg/eye) [c]	two 30 µl drops per	No ocular toxicity	48	5.71 x

	(GLP, Phase 3 formulation)		dose, bid			
6348-415	9-month monkey study (GLP, Phase 3 formulation)	0.024% (7.2 µg/eye) [lowest dose tested = LOAEL] [c]	one 30 µl drop per dose, bid	Iris hyper-pigmentation; minimal-to-slight perivascular lymphocyte/macrophage infiltrates of the episclera (observed at histopathology, considered non-adverse)	14.4	1.71 x
		0.040% (24 µg/eye) [c]	two 30 µl drop per dose, bid	No other ocular toxicity than listed above for the 0.024% dose	48	5.71 x

[a] Value accounts for the drop size and # drops/dose
[b] Accounting for the # of doses/day (i.e. qd, bid, or tid)
[c] See below for discussion of systemic toxicity

11.2 HED values and exposure margins for systemic effects – based on body surface area (BSA) conversion

As noted above, the VYZULTA draft label states that the dosage is "one drop in the affected eye(s) once daily in the evening." The formulation (0.024%) is 0.24 µg/µl of LBN. Assuming 35 µl drop size, the clinical dose is 8.4 µg/eye, or 16.8 µg/person/day of LBN if both eyes are treated. This dose is equivalent to 0.28 µg/kg (assuming a body weight of 60 kg), or to 10.36 µg/m^2 (assuming a body surface area of 1.62 m^2).

Table 78: Dose conversion based on body surface area

Default factors for converting animal doses to human equivalent doses (HED) based on body surface area[41]		
Species	Reference body weight (kg)	To convert dose from mg/kg to dose by mg/m^2, multiple by k_m:
Human [adult]	60	37
Rat	0.150	6
Rabbit	1.8	12
Monkey	3	12

As noted in Table 44 above, the average male monkey body weights (4.0 kg for the low-dose, 3.53 kg for the mid-dose, and 4.13 kg for the high-dose) were used to estimate

[41] CDER 2005. Guidance for Industry. Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. Accessed via: http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm078932.pdf

the doses for the 9-month monkey study (report # 6348-415), rather than the default monkey body weight of 3 kg.

Table 79: Systemic toxicity exposure margins based on body surface area (µg/m^2) [assuming 100% absorption for topical ocular dosing and for intravenous dosing]

Report # [GLP status]	Species, route, duration	Dose/day [a]	Effect at that dose	HED - Dose based on body surface area µg/m^2)	Exposure Margins (based on clinical exposure of 10.36 µg/m^2)
06AM024 [GLP]	Rat acute iv toxicity study	LOAEL = 2 mg/kg (no NOAEL)	Increased incidence and severity of hemorrhage near the pre-terminal blood draw site	12,000	1158 x
		2 mg/kg	NOAEL for all other endpoints	12,000	1158 x
07GR073 [GLP]	Rat micronucleus study (iv dosing, 2 days)	4 mg/kg/day	No mortality or clinical signs observed	24,000	2316 x
6750-267 [GLP]	Monkey 28-day topical ocular toxicity study	24 µg/eye/day = 8 µg/kg/day	NOAEL (high-dose) for ocular and systemic toxicity	96	9.27 x
8273344 [GLP]	2nd monkey 28-day topical ocular toxicity study	48 µg/eye/day = 16 µg/kg/day	Uncollapsed lung (gross observation)	192	18.53 x
6348-415[b] [GLP]	9-month monkey topical ocular toxicity study	14.4 µg/eye/ day = 3.60 µg/kg/day	Systemic NOAEL	43.2	4.17 x
		24.0 µg/eye/ day = 6.85 µg/kg/day	Systemic LOAEL: lung fibrosis/ inflammation, chronic/ pleural / subpleural [2 minimal, 1 slight]	81.51	7.87 x
		48 µg/eye/ day = 11.61 µg/kg/day	Lung fibrosis/ inflammation, chronic/ pleural / subpleural [1 minimal, 1 slight, 1 moderate]	139.35	13.45 x
20073520 [not GLP]	Rat EFD range-finder (iv, GD7-17)	0.3 mg/kg	Maternal NOAEL; nominal developmental NOAEL	1800	173.7 x

		1.5 mg/kg	Maternal LOAEL (↓ weight gain and food consumption); nominal developmental LOAEL - ↑ post-implantation loss, ↑ early and late resorptions	9000	868.7 x
20073521 [GLP]	Rat EFD study (iv, GD7-17)	0.15 mg/kg	Developmental NOAEL	900	86.9 x
		0.3 mg/kg	Maternal NOAEL; Developmental LOAEL (↑ dead fetuses, early resorptions, structural teratogenicity)	1800	173.7 x
		1.5 mg/kg	Maternal LOAEL (↓ weight gain and food consumption)	9000	868.7 x
20073522 [not GLP]	Rabbit EFD range-finder (iv, GD7-19)	1 µg/kg	Developmental LOAEL (dead fetus and structural teratogenicity)	12	1.158 x
		20 µg/kg	Mid-dose (no fetuses survived)	240	23.17 x
		80 µg/kg	Maternal LOAEL (abortion in 1 rabbit)	960	92.66 x
200753523 [GLP]	Rabbit EFD study (iv, GD7-19)	0.24 µg/kg	Maternal LOAEL (abortions); Developmental LOAEL (↑ early resorption; structural teratogenicity)	2.88	0.278 x
		6 µg/kg	High dose (↑ late resorptions)	72	6.95 x

[a] Using the default species body weight (for the topical ocular studies)
[b] Topical ocular effects were observed: iris pigmentation (low- and high-dose only) and perivascular lymphocyte/macrophage infiltrates of the episclera (all dose levels)

Note regarding the rat tongue hemorrhage

- The acute iv rat study (report # 06AM024) observed increased incidence and severity of tongue hemorrhage for the high-dose group (2 mg/kg) compared to controls.
    - These animals had undergone sublingual bleeding (for hematology and clinical chemistry endpoints) prior to euthanasia. These observations may be at least partially procedure-related.

- The laboratory did not evaluate tongue histopathology for the low- or mid-dose groups.
- P/T discussed this finding with Clinical (personal communication, Boyd/McDougal, 4/07/2016). The Clinical review discipline noted that the nonclinical and clinical ocular endpoints evaluated in the ocular studies are adequate and robust to detect potential effects related to increased risk of wound-healing of the cornea and conjunctiva. Because no treatment-related findings after ocular dosing raise a concern for increased hemorrhaging, these observations following iv dosing are considered not relevant to topical ocular dosing with LBN.

Note regarding the use of 35 µl for the clinical drop volume

- The Applicant (in NDA module 2.6.6 Toxicology Written Summary table 2.6.6.9-1, footnote) used a volume of 35 µl for the clinical dose volume to estimate exposure margins.
- This reviewer did not identify a clinical drop volume, or information regarding the drop size produced by the container (e.g. NDA module 3.2.P.7 Container Closure System; NDA module 2.7.2 Summary of Clinical Pharmacology Studies).
- Therefore, the default of 35 µl is used.
- Note: the nonclinical topical ocular studies used pipetting, to accurately deliver the intended dose volumes (50µl/drop for the rabbit study; 30µl/drop for the monkey studies).

11.3 Considerations for calculating systemic exposure margins values based on available serum TK results

Clinical PK summary

P/T defers to Clinical Pharmacology regarding review of the clinical PK results.

Clinical Pharmacology (Zhang, 4/07/2016, NDA 207795) reviewed two clinical trials:

- a clinical trial in 22 healthy patients (study # 809) who received topical ocular administration of Vyzulta once daily (one drop bilaterally in the morning) for 28 days. The Clinical Pharmacology review noted:
  - "There were no quantifiable plasma concentrations of latanoprostene bunod (lower limit of quantitation, LLOQ, of 10.0 pg/mL) or butanediol mononitrate (LLOQ of 200 pg/mL) post dose on Day 1 and Day 28."
  - "Latanoprost acid concentrations were quantifiable … The mean maximal plasma concentrations (C_{max}) of latanoprost acid were 59.1 pg/mL and 51.1 pg/mL on Day 1 and Day 28, respectively. The mean time of maximal plasma concentration (T_{max}) for latanoprost acid was approximately 5 min post administration on both Day 1 and Day 28."
  - "The elimination of latanoprost acid from human plasma is rapid as latanoprost acid plasma concentration dropped below the LLOQ (30.0 pg/mL)

in most of subjects by 15 min post the ocular administration of Vyzulta 0.024% in humans."

- a separate study (#874) to assess systemic NO exposure by measuring "the potential change in percentage of systemic methemoglobin (% MetHb), after a single and 7-day once-daily repeated topical bilateral ocular administration of LBN 0.024% in healthy subjects."
    - "There were no significant changes from baseline in %MetHb for LBN treated subjects on Day 1 and Day 7, and there was also no change in %MetHb between the vehicle- and LBN-treated groups when directly compared, indicating that the NO systemic exposure is likely to be limited and/or minimal following repeated once daily dosing of LBN 0.024%."

<u>P/T serum TK data</u>

- For the non-EFD toxicology studies:
    - The rat acute iv toxicity study (06AM024) detected latanoprost acid in serum, but did not attempt to measure LBN or latanoprost.
    - The 28-day monkey topical ocular study (05NCX001) did not detect LBN (LLOQ = 1 ng/ml), and did detect latanoprost acid. The study did not attempt to detect latanoprost.
    - The second 28-day monkey topical ocular study (6750-267) detected latanoprost acid, and did not attempt to detect LBN or latanoprost.
    - The 9-month monkey topical ocular study (6348-415) detected latanoprost acid, and did not attempt to detect LBN or latanoprost.
    - The rat micronucleus test (7GR073) did not measure TK.
- TK was not measured in the rat (20073520) or rabbit (20073522) EFD range-finding studies.
- For the pre-NDA meeting, P/T encouraged the Applicant to include "adequate evaluation of toxicokinetics" in the EFD studies. In post-meeting correspondence, P/T recommended that the Applicant "consider also measuring the % methemoglobin". The Applicant did not measure % methemoglobin in any of the nonclinical studies.
- For the rat GLP EFD study (20073521), this reviewer considers using HED values based on BSA to be the most appropriate and accurate for conveying risk.
    - Serum TK for this study detected LBN, BDMN and latanoprost acid systemically. This study reported LBN C_{max} values for all 3 dose levels, but could only calculate LBN AUC for the high-dose (not useful for exposure margin estimates, since no clinical AUC is available).
    - Use of the clinical LLOQ for LBN or BDMN would overpredict risk (see Table 80).
- Latanoprost iv was more toxic (i.e. lethal) to pregnant rats, compared to LBN iv. The Applicant did not submit any comparison of the rat EFD data (i.e. LBN versus latanoprost). Because death (of the pregnant animals, and of the conceptus) can mask other developmental toxicities, a clear understanding of toxic effect by exposure level is important. It is not clear from the LBN data how much (if any) of the observed developmental toxicity was due to latanoprost acid exposure. Therefore, this reviewer concludes that the nonclinical data submitted to NDA

207795 are not sufficient to rely upon the metabolite TK results as a surrogate for all LBN and LBN-metabolite exposure and toxicity. This conclusion should be revisited, if/when the Applicant provides additional EFD data and/or analyses for review.

Table 80: Exposure margin calculations based on serum TK for the GLP rat EFD study (report # 20073521)

	0.15 mg/kg[1]	0.3 mg/kg	1.5 mg/kg
Exposure margins for LBN based on BSA (mg/m^2)	**86.9**	**173.7**	**868.7**
LBN C_{max} (pg/ml) – GD7	15.0	37.9	142
Exposure margin for LBN (compared to the clinical LLOQ of 10.0 pg/ml)	1.5 x	3.79 x	14.2 x
BDMN C_{max} (pg/ml) – GD7	10,900	24,000	108,000
Exposure margin for BDMN (compared to the clinical LLOQ of 200 pg/ml)	54.4 x	120 x	540 x
Latanoprost acid C_{max} (pg/ml) – GD7	55,400	98,600	455,000
Exposure margin for latanoprost acid (compared to the clinical mean C_{max} of 51.1 pg/mL)	1084 x	1930 x	8904 x

[1] *Developmental NOAEL*
Bolded text – Margin of exposure used for labeling

- The rabbit GLP EFD study (# 20073523) detected BDMN (in one serum sample, taken from a non-pregnant rabbit in the high-dose group (LLOQ = 1 ng/ml). LBN was not detected in any serum sample (LLOQ = 10.0 pg/ml). Latanoprost acid was detected.
    - This reviewer concludes that using HED values based on BSA is appropriate.

- For LBN, this study and the clinical data have the same LLOQ (10 pg/ml). Therefore, a meaningful exposure multiple cannot be calculated.
- Whereas latanoprost appeared more toxic to pregnant rats than LBN, neither was maternally toxic (excepting abortion) at the doses tested in the EFD studies. LBN-related developmental toxicity was apparent at lower doses, compared to the latanoprost doses associated with developmental toxicity.
- The Applicant did not submit any comparison of the EFD data (LBN versus latanoprost). As noted above, because death (of the pregnant animals, and of the conceptus) can mask other developmental toxicities, a clear understanding of toxic effect by exposure level is important. It is not clear from the LBN data how much (if any) of the observed developmental toxicity was due to latanoprost acid exposure. Therefore, this reviewer concludes that the nonclinical data submitted to NDA 207795 are not sufficient to rely upon the metabolite TK results as a surrogate for all LBN and LBN-metabolite exposure and toxicity. This conclusion should be revisited, if/when the Applicant provides additional EFD data and/or analyses for review.

Table 81: Exposure margin calculations based on serum TK for the GLP rabbit EFD study (report # 20073523)

	0.24 µg/kg[1]	1.2 µg/kg	6 µg/kg
Exposure margin for LBN based on BSA (µg/m^2)	**0.278 x**	**1.39 x**	**6.95 x**
BDMN detected above LLOQ	-	-	1170 pg/ml
Exposure margin for BDMN (compared to the clinical LLOQ of 200 pg/ml)	-	-	8.85 x
Latanoprost acid C_{max} (pg/ml) – GD19	105	253	2490
Exposure margin for latanoprost acid (compared to the clinical mean C_{max} of 51.1 pg/mL)	2.05x	4.95x	48.72x

[1] No developmental NOAEL established in the rabbit; this dose represents developmental LOAEL.
Bolded text – Margins of exposure used for labeling

(b) (4)

11.4 Discussion of the proposed mechanisms of action, and pharmaceutical class

- The Applicant summarized the proposed mechanism of action in the Pharmacology Written Summary (NDA module 2.6.2) (b) (4)
- LBN, like latanoprost, is a pro-drug that will be metabolized to latanoprost acid, which will activate FP receptor (i.e. same mechanism of action as latanoprost).
- The Applicant proposes that (a) the metabolism of LBN also releases the BDMN moiety, (b) BDMN will release NO, (c) released NO will target the trabecular meshwork, causing trabecular meshwork cells to relax, increasing outflow, and thereby further lowering IOP.
- Published data show that NO, and organic nitrates, reduce IOP at sufficiently high local concentrations. A pivotal P/T question for this NDA, is whether or not topical ocular dosing with LBN results in sufficient NO (or other active organic nitrogen moieties) at the trabecular meshwork. This reviewer concludes that the nonclinical information submitted to the NDA are not adequate to support the conclusion that LBN has a different (or additional) mechanism of action than latanoprost.
- Notably, the Applicant provided no nonclinical data showing that LBN is metabolized to BDMN, or that NO is released. (b) (4) (b) (4)
- As reviewed above (section 4.1 of this review), the submitted primary pharmacology studies clearly demonstrated that LBN has activity similar to latanoprost.
- The PD study with FP receptor knock out mice indicates that LBN may have additional IOP lowering activity (i.e. NO activity) of – 0.45 to -1.23 mm Hg. The reliability of this report is questionable. The clinical significance of this magnitude of additional IOP lowering is unclear.

- For the [redacted] (b) (4) the Applicant proposed (7/21/2015, original NDA submission):

[redacted] (b) (4)

- Under IND 73435, the Sponsor requested and was granted a Type B pre-NDA meeting. The Applicant's regulatory question 2 asked about the pharmacological class of LBN, "… Does the Agency anticipate that the compound [LBN] could be evaluated as a distinct pharmacological class, as per CDER MAPP 7400.13 Determining the Established Pharmacologic Class for Use in the Highlights of Prescribing Information?" DTOP responded, "No. Metabolism to latanoprost acid suggests a similar if not the same pharmacologic class. Until proven otherwise, the same Warnings/Precaution and Dosing recommendations as the prostaglandin analogs will be considered during the review."
- [redacted] (b) (4)

- The Review Team discussed these issues at the internal NDA mid-cycle meeting (December 8, 2015). The Clinical and Clinical Pharmacology reviewers do not expect that the clinical data will support (b) (4)
- P/T recommends that the LBN label be consistent with that latanoprost (Xalatan®) label.
- Reference is made to:
  - MAPP 7400.13 Determining the Established Pharmacologic Class for Use in the Highlights of Prescribing Information accessible via http://www.fda.gov/downloads/aboutfda/centersoffices/officeofmedicalproductsandtobacco/cder/manualofpoliciesprocedures/ucm361380.pdf .
  - The list of established classes in an easy-to-read format is accessible via: http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/lawsactsandrules/ucm428333.pdf
  - The formal current list of established pharmacological classes at http://www.fda.gov/forindustry/datastandards/structuredproductlabeling/ucm162549.htm
  - The 2009 Guidance for Industry and Review Staff Labeling for Human Prescription Drug and Biological Products — Determining Established Pharmacologic Class for Use in the Highlights of Prescribing Information Good Review Practice is accessible via http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM186607.pdf]

(b) (4)

[redacted]

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11.7 LBN EFD summary – comparison with latanoprost EFD data

The ophthalmic prostaglandins have abortifacient activity, but have not shown teratogenicity. In contrast, LBN exposure was associated with teratogenicity in both rats and rabbits.

As noted above, the label for NDA 020597 (Xalatan®) was revised 11/2014[42]. Section 8.1 of the label states:

> "Teratogenic Effects: Pregnancy Category C.
> Reproduction studies have been performed in rats and rabbits. In rabbits, an incidence of 4 of 16 dams had no viable fetuses at a dose that was approximately 80 times the maximum human dose, and the highest nonembryocidal dose in rabbits was approximately 15 times the maximum human dose.
>
> There are no adequate and well-controlled studies in pregnant women. XALATAN should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus."

No additional information regarding these studies was presented in Section 13 of the label.

[42] NDA 20597/S-045 and S-048 label accessed via: http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/020597s045s048lbl.pdf

Although the P/T review is not available via Drugs@FDA or via DARRTS, this reviewer accessed a previously redacted and published copy[43] of the review by Dr. David A. Shriver (finalized 2/19/1996). The following is extracted from the review:

PhXA41 – dose range-finding fertility and reproductive study by intravenous route in female rat	• Report # L411 S037 R001 • "This study is uninterpretable" due to inconsistent reporting. • Deaths at 300 µg/kg
PhXA41 – dose range-finding fertility and reproductive study by intravenous route in the male rat	• Report # L411 S036 R001 • Male rats were dosed iv with 0, 5, 50, or 300 µg/kg for 71 days (prior to mating, and up to 14 days of mating). • The review concluded that latanoprost did not affect testis or epididymis weight, caesarean data or fetal data.
Fertility study by intravenous route in the rat (segment I)	• Report # 9200027 • IV doses of 0, 5, 35 or 250 µg/kg • Male rats were dosed beginning 9 weeks prior to mating, and through mating. Female rats were dosed from 2 weeks prior to mating, through GD7. • "Conclusion: Latanoprost at an intravenous dose (250 µg/kg, i.v.) that killed both male and female rats had no effect on fertility, reproductive performance or fetuses."
PhXA41 – dose range-finding study by intravenous route in the pregnant rat	• Report # L411 S034 R 001 • No signs of maternal or fetal toxicity with any of the doses tested, up to 300 µg/kg iv (GD6 to 15, killed on GD20)
Teratology study by intravenous route in the rat (segment II)	• Report # 9300279 • IV doses of 5, 50 or 250 µg/kg from GD6 to 15, evaluation on GD20 • "Conclusion: Latanoprost administered by the intravenous route to rats from day 6 to 15 inclusively had no maternal toxic or fetotoxic effect at doses up to 250 µg/kg."
PhXA41 – dose range-finding study by intravenous route in the pregnant rabbit	• Report # L411 S035 R001 • IV doses of 0, 0.1, 1, 5, 50 or 300 µg/kg from GD6 to 19 • Developmental NOAEL = 1 µg/kg • 50 and 300 µg/kg "induced complete early resorptions. A dose of 1 µg/kg, i.v., is a no

[43] Accessed from Pharmapendium via http://www.pharmapendium.com/#/browse/fda/Latanoprost

	effect dose." • 5 µg/kg caused abortion in one litter; no teratogenic effects observed (i.e. at 1 or 5 µg/kg)
Teratology study by intravenous route in the rabbit (segment II)	• Report # 9300280 • IV dosing of 0, 0.2, 1 or 5 µg/kg on GD6 to 18, killed on GD29 • "Conclusion: Latanoprost was not teratogenic at intravenous doses up to 5 µg/kg, but was fetotoxic at this dose. The no effect dose in this study was 1 µg/kg."
Dose range-finding peri- and post-natal study (segment III) by intravenous route in the rat	• Report # 9200137 • Rats were dosed from GD15 to lactation day 21, with 0, 1, 3, 10 or 100 µg/kg of latanoprost iv. • Two high-dose females (100 µg/kg) died 5 days after parturition; the cause of death was not determined. • "Conclusion: Daily intravenous dosing with 1 µg/kg to dams during the peri- and post-natal (day 15 of gestation to day 21 of lacation) was the no effect dose. Doses of 3 µg/kg and greater caused deaths within the litters and doses of 10 µg/kg or greater caused a decrease in the number of male pups delivered." [sic]
Developmental toxicity study by intravenous route in the rat (segment III)	• Report # 9300281 • F0 females were dosed from GD6 until weaning, with 0, 1, 3, or 10 µg/kg latanoprost iv. The F1 generation was untreated. • "Conclusion: There were no treatment-related effects to dams of the F0 generation or F1 and F2 pups at intravenous doses of 1, 3 or 10 µg/kg."

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/s/

ANDREW J MCDOUGAL
05/20/2016

LORI E KOTCH
05/20/2016

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA 207795

NDA Number: 207795 **Applicant:** **Stamp Date: 7/21/2015**

Drug Name: VESNEO™ (latanoprostene bunod ophthalmic solution) 0.024%

NDA Type: 505(b)(1) original new drug application (NDA)

On **initial** overview of the NDA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	☑		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	☑		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	☑		
4	Are all required and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?		☒	No issues were identified that would prevent filing. • P/T recommended submission of two embryofetal studies (McDougal, 2/23/2015, IND 73435). This recommendation was conveyed at the type B pre-NDA meeting held on February 9, 2015 (minutes by Milstein, 3/09/2015, IND 73435), "FDA recommends testing latanoprostene bunod in GLP embryofetal studies in two relevant nonclinical species (with adequate evaluation of toxicokinetics) to support the NDA submission." • In post-meeting follow-up correspondence, the Sponsor asked "Assuming the rest of the NDA is satisfactory for filing, will the absence of the embryo-fetal study for the parent compound at initial submission, by itself, lead to a refusal-to-file?" The Division responded, "…Assuming the rest of the NDA is satisfactory for filing, the absence of the embryo-fetal study for the parent compound at the initial submission would not in itself lead to a refusal to-file. … If the proposed labeling includes the potential for embryotoxicity due to the latanoprost acid component, the embryo-fetal study for the parent compound would not be required for the initial review of the application." (Lwin, 3/25/2015, IND 73435). • The audited draft reports for the embryofetal studies are expected at "the end

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA 207795

	Content Parameter	Yes	No	Comment
				of December 2015."
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route).	☑		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	☑		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?		☒	• None of the primary pharmacology or secondary pharmacology studies are GLP compliant. Two of the safety pharmacology studies are GLP compliant, but 11 are non-GLP. • This reviewer presumes that the non-GLP studies are not pivotal; however, this will be a review issue. • The statements made in NDA module 2.6.6 Toxicology Written Summary are adequate, when taken together, to check the 'yes' box for the toxicology studies. • The reports for which GLP compliance is claimed may not have been fully reviewed yet by P/T for deviations from GLP and explanations. This will be a review issue.
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?		☒	See comment #4 above.
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m^2 or comparative serum/plasma levels) and in accordance with 201.57?		☒	No issues were identified that would prevent filing. • The request for exposure multiples based on systemic AUC was conveyed at the pre-NDA meeting (minutes by Milstein, 3/09/2015, IND 73435). • The appropriateness of labeling will be a review issue. • The exposure multiples in the draft label are (b) (4)
10	Have any impurity, degradant, extractable/leachable, etc. issues been addressed?	Pending		No issues were identified that would prevent filing.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA 207795

	Content Parameter	Yes	No	Comment
11	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?	Not applicable		
12	If the applicant is entirely or in part supporting the safety of their product by relying on nonclinical information for which they do not have the right to the underlying data (i.e., a 505(b)(2) application referring to a previous finding of the agency and/or literature), have they provided a scientific bridge or rationale to support that reliance? If so, what type of bridge or rationale was provided (e.g., nonclinical, clinical PK, other)?	☑		No issues were identified that would prevent filing. • This NDA is filed under 505(b)(1). • No listed drug products were identified on the Applicant's FDA Form 356h. • The Applicant claims right-of-reference to data submitted under (b) (4) (b) (4) (b) (4) NDA 20597 (Xalatan®).

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ____yes____

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

- None

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/s/

ANDREW J MCDOUGAL
09/01/2015

LORI E KOTCH
09/01/2015