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APPLICATION NUMBER:

215487Orig1s000

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:	215487
Supporting document/s:	SDN 1, 9, 22, and 25
Applicant's letter date and CDER stamp date:	March 24, 2023 (SDN 1); June 26, 2023 (SDN 9); September 29, 2023 (SDN 22); and October 27, 2023 (SDN 25)
Product:	Naloxone Nasal Spray (10 mg in 110 mcL; (b) (4) mg/mL naloxone HCl concentration)
Indication:	For the emergency treatment of known or suspected opioid overdose, as manifested by respiratory and/or central nervous system depression.
Applicant:	Summit Biosciences LLC
Clinical Review Division:	Division of Anesthesiology, Addiction Medicine, and Pain Medicine (DAAP)
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Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

Summit Biosciences LLC is developing Naloxone Nasal Spray for intranasal administration (10 mg in 110 mcL or [redacted] mg/mL naloxone HCl concentration) to deliver 10 mg of naloxone HCl with a maximum daily dose of 20 mg of naloxone HCl. The proposed indication is "for the emergency treatment of known or suspected opioid overdose, as manifested by respiratory and/or central nervous system depression" and "intended for immediate administration as emergency therapy in settings where opioids may be present." It is noted that "the product is not a substitute for emergency medical care." The Applicant is submitting an application via the 505(b)(2) pathway, proposing to rely upon the Agency's previous findings of safety and efficacy of Narcan® injectable product (NDA 16636).

1.2 Brief Discussion of Nonclinical Findings

To support this NDA application, the Applicant submitted intranasal repeat-dose toxicity studies in two species, qualification studies to support a drug product degradant specification as well as assessments for extractables/leachables from the container closure system, elemental impurities, and [redacted]. There are no novel excipients in the formulation and the levels of the excipients at the maximum daily dose are in the FDA Inactive Ingredient Database at comparable levels in acute use FDA-approved nasal formulations. The drug substance specifications are within ICH Q3A(R2) and are acceptable. The drug product specifications meet ICH Q3B(R2) qualification threshold with the exception of [redacted], whose specification is NMT [redacted]%. In support of this specification, the Applicant submitted a negative QSAR evaluation for mutagenic potential as well as a 28-day rat toxicology study (Study 20246992) that includes the high dose naloxone group (36.4 mg/day) alone and with degradants (including [redacted]. Based on the 28-day rat toxicology study, the drug product specification for [redacted] of NMT [redacted]% is qualified for systemic safety but is not qualified from a local safety perspective.

To support the safety of the container closure system, [redacted] unit dose nasal sprayer, extractable leachable and elemental impurities assessments were submitted. All leachable compounds detected were below the [redacted] threshold and as such, do not represent a safety concern. The levels of the [redacted] are below the limit of [redacted] The elementals detected in the elemental impurities assessment meet ICH Q3D PDE limits or were below the qualification threshold of [redacted].

In support of the marketing application, the Applicant submitted a 14-day dog (Study 2950-002) and a 28-day rat toxicology (Study 20246992) study. In both species, irritation and microscopic findings in the nasal cavity and in surrounding tissues were observed (minimal to mild in severity) and were dependent on the number of administrations but were not considered dose limiting. After the recovery period, these

findings in the nasal cavity and its surrounding tissues were absent or lower in incidence and severity in the recovery animals demonstrating recovery or a trend to recovery. The local NOAEL is the mid dose (8 sprays per naris) in dogs and an acceptable local LOAEL is the high dose (an instillation twice daily per nostril) in rats that resulted in a concentration margin of at least 1x to support the local safety of the high naloxone concentration. The systemic NOAEL was the HD evaluated as evidenced by the lack of test article related findings, which resulted in safety margins of 83x (1667 mg/20 mg MDD = 83) and 54x (1084 mg/20 mg MDD = 54) in dogs and rats, respectively, based on a body surface area comparison and using an average human weighing 60 kg.

Taken together, the Applicant submitted two intranasal repeat-dose toxicity studies in rats and dogs that qualified the safety of the higher naloxone concentration and higher maximum daily dose in comparison to the reference product. The drug product did not contain any novel excipients and there are no safety concerns with the container closure system. Drug substance and drug product impurities, elemental impurities, and (b) (4) levels were within respective thresholds with the exception of one drug product degradant, (b) (4) ICH Q3B(R2) qualification thresholds. To qualify the safety of the proposed specification of this degradant at (b) (4)%, the Applicant submitted a negative computational toxicology evaluation that assessed the mutagenic potential of the compound and submitted a 28-day intranasal repeat-dose toxicity rat study that evaluated a naloxone formulation that contained different degradants that included (b) (4)% of (b) (4). Although this study qualified the systemic safety of the degradant, this study tested a lower concentration than the proposed specification of (b) (4)%, resulting in a safety margin of (b) (4) for local safety. The Applicant purported that a higher concentration could not be tested due to several factors, including the degradant could not be synthesized or isolated and the degradant readily degrades. However, it is noted that data and documentation were not provided to support whether the Applicant did their due diligence to synthesize and isolate the drug product degradant. The Applicant also argued that the rat study provided support for the local safety of the proposed specification when normalizing the dose with intranasal surface area (i.e., mg/cm^2) of the species, to result in a (b) (4) safety margin. However, when reviewing the safety of an impurity specification, the supporting nonclinical qualification study should support safety by establishing adequate safety margins using 3 approaches: human equivalent dose (HED) using body surface area, dose normalization to nasal surface area (mg/cm^2), and a comparison of the tested concentration in the toxicity study versus the actual concentration in the drug product. In this case, the safety margin calculated by the concentration approach is below 1x and therefore is not supportive of the proposed specification of (b) (4)% for (b) (4) (b) (4) However, the lack of nonclinical support for the local safety of (b) (4) need not preclude marketing approval in the opinion of this Reviewer for the following reasons: 1) the submitted nonclinical data are from a study that evaluated exaggerated dosing conditions (repeated dosing for 28 consecutive days compared to the clinical use scenario of a single dose) and did not identify a safety signal at the local tissues; 2) adequate safety margins were established for the degradant based on HED conversion using BSA and based on dose normalization using nasal surface area; 3) this product is indicated for an acute, single-use indication; and 4) the drug product is a

potentially life-saving therapy. Therefore, should this NDA be approved, it is recommended that a postmarketing requirement be issued to the Applicant to conduct a nonclinical study that tests an adequate degradant concentration to support the local safety of the proposed specification. (b) (4)

1.3 Recommendations

1.3.1 Approvability

From a nonclinical pharmacology toxicology perspective, NDA 215487 for Naloxone HCl Nasal Spray may be approved with the following postmarketing requirement.

1.3.2 Additional Nonclinical Recommendations

The following nonclinical study is recommended as a postmarketing requirement (PMR) should this PMR be acceptable to the clinical reviewer team and the rest of the review team given the benefit risk of the proposed drug product.

> Conduct a GLP repeat-dose toxicology study of at least 14 days duration in a single species to adequately characterize the toxicological potential of the naloxone degradant, (b) (4) to local tissues.

1.3.3 Labeling

The following labeling recommendations have not been discussed with the entire review team or the Applicant and should not be considered final. The reader is referred to the approval letter for final agreed upon labeling. Suggested deletions are in red crossed-out text. Suggested additions are in blue text.

Proposed	Suggested Revisions	Rationale/Comment
8 USE IN SPECIFIC POPULATIONS 8.1 Pregnancy Risk Summary (b) (4) aloxone hydrochloride at doses equivalent to 2-times and 4-	8 USE IN SPECIFIC POPULATIONS 8.1 Pregnancy Risk Summary (b) (4) In animal reproducti (b) (4) on studies, (b) (4) no embryonic or teratogenic effects were observed in mice and rats (b) (4) administered	Changes were made by DPMH (maternal health) to maintain consistency with the referenced naloxone drug product and also update margin with a human dose of 20 mg/day (two naloxone nasal sprays) based on a body surface area and a 60 kg human. Per DPMH, (b) (4)

times, respectively, a human dose of 20 mg/day (2 sprays of TRADENAME) (b) (4) [redacted] *[see DATA]*.	naloxone hydrochloride during organogenesis at doses equivalent to 2-times and 4-times, respectively, a human dose of 20 mg/day (2 sprays of TRADENAME) (b) (4) [redacted] *see Data)*.	(b) (4) [redacted]
Data *Animal Data* Naloxone hydrochloride was administered (b) (4) [redacted] a human dose of 20 mg (two TRADENAME Nasal Sprays). These studies demonstrated (b) (4) [redacted] naloxone hydrochloride.	Data *Animal Data* Naloxone hydrochloride was administered (b) (4) [redacted] during organogenesis to mice and rats at dose 2-times and 4-times, respectively, a human dose of 20 mg (two TRADENAME Nasal Sprays) based on body surface area comparison. These studies demonstrated no (b) (4) [redacted] mbroytoxic or teratogenic effects due to naloxone hydrochloride.	Changes were made to be consistent with class labeling of products that rely on Narcan injection as the listed drug and to update margins with a human dose of 20 mg/day (two naloxone nasal sprays) based on a body surface area and a 60 kg human.
13 NONCLINICAL TOXICOLOGY 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility	13 NONCLINICAL TOXICOLOGY 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility	
Carcinogenesis Long-term animal studies to evaluate the carcinogenic potential of naloxone have not been completed.	Carcinogenesis Long-term animal studies to evaluate the carcinogenic potential of naloxone have not been completed.	No changes are recommended.
Mutagenesis Naloxone was weakly positive in the Ames mutagenicity and in the in vitro human lymphocyte chromosome aberration tests but was negative in the in vitro Chinese hamster V79 cell HGPRT mutagenicity assay and in the in vivo rat bone marrow chromosome aberration study.	Mutagenesis Naloxone was weakly positive in the Ames mutagenicity and in the in vitro human lymphocyte chromosome aberration tests but was negative in the in vitro Chinese hamster V79 cell HGPRT mutagenicity assay and in the in vivo rat bone marrow chromosome aberration study.	No changes are recommended.

Impairment of Fertility (b) (4)	Impairment of Fertility (b) (4) Reproduction studies conducted in mice and rats at doses 2-times and 4-times, respectively, a human dose of 20 mg/day (from two nasal sprays of BRANDNAME) based on body surface comparison, demonstrated no adverse effects on fertility of naloxone hydrochloride.	Changes were made to be consistent with other class labeling that rely on Narcan injection as the listed drug and also update margin with a human dose of 20 mg/day (two naloxone nasal sprays) based on a body surface area and a 60 kg human.

2 Drug Information

2.1 Drug

CAS Registry Number
51481-60-8

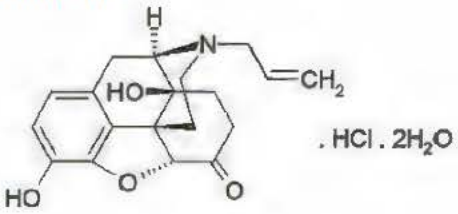
Generic Name
Naloxone hydrochloride dihydrate

Code Name
N/A

Chemical Name
Morphinan-6-one,4,5-epoxy-3,14-dihydroxy-17-(2-propenyl)-,hydrochloride,(5α)-, dihydrate
17-Allyl-4,5-epoxy-3,14-dihydroxymorphinan-6-one hydrochloride, dehydrate
4,5α-Epoxy-3,14-dihydroxy-17-(prop-2-enyl)morphinan-6-one hydrochloride, dihydrate

Molecular Formula/Molecular Weight
$C_{19}H_{21}NO_4 \cdot HCl\ 2H_2O$ / 399.87

Structure



Pharmacologic Class
Opioid antagonist (Established Pharmacological Class)

2.2 Relevant INDs, NDAs, BLAs and MFs

NDA#	Drug Name	Div	Strength (route)	Marketing Status	AP Date	Indication	Company
16636	Narcan (Naloxone HCl)	DAAP	0.4 and 1.0 mg/mL (IV, IM, or SC)	Withdrawn Effective (Not for safety reasons)	August 20, 2010	For the complete or partial reversal of opioid depression, including respiratory depression	Adapt Pharma Operations, Ltd.

IND#	Drug Name	Div	Status	Indication	Company
142850	Naloxone Hydrochloride	DAAP	Active (December 1, 2019)	For the emergency treatment of known or suspected opioid overdose, as manifested by respiratory and/or central nervous system depression	Summit Biosciences Inc.
141634	Naloxone Hydrochloride	DAAP	Presubmission (October 12, 2018)	For the emergency treatment of known or suspected opioid overdose	Summit Biosciences Inc.

MF#	Subject of MF	Holder	Status Date	Reviewer's Comment
		(b) (4)	October 13, 2009 (active)	Referenced by numerous approved products (ANDAs)
			September 14, 2010 (acitve)	Referenced by numerous approved products (NDAs and ANDAs)
			July 26, 1994 (active)	Referenced by numerous approved products (NDAs, BLAs, and ANDAs)
			Sepetember 11, 1995 (active)	Referenced by numerous approved products (NDAs, BLAs, and ANDAs)
			December 14, 2015	Referenced by numerous approved products (NDAs, BLAs, and ANDAs)

2.3 Drug Formulation

The proposed drug product concentration is (b) (4) mg/mL of naloxone hydrochloride (HCl) dihydrate or (b) (4) mg/mL of naloxone HCl in a volume of (b) (4) mcL. Each spray dose delivers a volume of 110 mcL and 10 mg of naloxone hydrocholoride. The following table illustrates the formulation of the proposed product (from the Applicant's submission):

Table 1: Naloxone Hydrochloride Formulation, 10 mg/dose

Component	Grade	Function	Composition		
			mg/mL	% w/w	mg/dose[1]
Naloxone HCl (b) (4) (b) (4)	USP	API	(b) (4)		
Glycerin	USP	(b) (4)			(b) (4)
Sodium Citrate Dihydrate	USP				
Hydrochloric Acid	NF	pH Adjustment	AR	AR	AR
Sodium Hydroxide	NF	pH Adjustment	AR	AR	AR
Purified Water	USP	(b) (4)	QS	QS	QS
		Total:			(b) (4)

[1] (b) (4) AR = As Required to Target pH (b) (4) QS = *quantum satis*

The maximum daily dose is 2 sprayers or 20 mg of naloxone hydrochloride, which confers a maximum daily volume of 0.22 mL/day = 220 mcL/day (110 mcL/spray x 2 spray/day = 220 mcL/day = 0.22 mL/day).

2.4 Comments on Novel Excipients

All excipients listed in the above table are found in the FDA IIG for use in nasal formulations and the levels are comparable to other FDA-approved nasal formulations for acute use. As such, there are no safety concerns with the amounts of the excipients in the formulation.

2.5 Comments on Impurities/Degradants of Concern

Drug Substance

The drug substance specifications are from (b) (4) Type II drug master file (DMF (b) (4)). The following table illustrates the drug substance specification (data from the Applicant's submission):

Test	Method	Release Specification
	Impurities	
Impurity A (Noroxymorphone)	USP Monograph	≤0.15%
Impurity B (3-O-Allynaloxone)		≤0.15%
Impurity C (10-α-Hydroxynaloxone)		≤0.15%
Impurity D (7,8-Didehydronaloxone)	EP Monograph	NMT 75 ppm
Impurity E (2,2'-Bisnaloxone)	USP Monograph	≤0.15%
Impurity F (10β-Hydroxynaloxone)		≤0.15%
Any Individual unspecified impurity		≤0.10%
Total Impurities		≤0.8%
	Residual Solvents	
(b) (4)	--	(b) (4)

(b) (4)		--	(b) (4)
		--	

NMT = no more than

The drug substance specifications for the impurities meet the ICH Q3A(R2) qualification threshold of no more than 0.15% or 1.0 mg per day intake (whichever is lower) for drug products with a maximum daily dose of ≤ 2 g/day. The impurity 7,8-didehydronaloxone (Impurity D) (b) (4) specification of NMT 75 ppm results in (b) (4) which is below the 120 mcg/day threshold for genotoxic impurities in acute products per ICH M7. The specifications for the residual solvents each meet ICH Q3C limits. Thus, the drug substance specifications are acceptable.

Drug Product

The following table illustrates the drug product specifications (from the Applicant's submission):

Table 1: Drug Product Specifications (continued)

Test	Method	Specification
Degradation Products[1]		
Specified Identified Impurities		
(b) (4)	AP-SN-014	(b) (4)
	AP-SN-000 / AP-SN-014	
	AP-SN-000	
Specified Unidentified Impurities		
(b) (4)	AP-SN-014	
Unspecified Unidentified Impurities		
Total Degradation Products[3]	AP-SN-000 / AP-SN-014	

(b) (4)

(b) (4)

Generally, the local safety of a compound for the intranasal route of administration is assessed by comparing the concentration administered (i.e., concentration of the test article that the local tissues are exposed to) to the proposed specification and in this case, this study does not adequately address the local safety of the proposed specification of (b) (4) An information request was sent to the Applicant stating the above on how the Agency reviews data to qualify local safety of an intranasal administered compound and requested further justification for the local safety of the proposed specification for (b) (4) . The Applicant replied that attempts were made to synthesize and isolate the drug product degradant but have not been successful and stated that direct evaluation at the proposed specification cannot be achieved by the inability to

synthesize the impurity or test an aged lot due to degradation. The Applicant also provided calculations to justify local safety by comparing the mg dose administered to the nasal epithelial surface area as shown in the Table below (Adapted from the Applicant's submission):

(b) (4)

When reviewing the safety of an impurity specification, the supporting nonclinical qualification study should result in adequate safety margins using 3 approaches (Emami et al., 2018): human equivalent dose (HED) using body surface area; dose normalization to nasal surface area (mg/cm^2); and concentration comparison of the tested concentration in the toxicity study versus the actual concentration in the drug product. In this case, acceptable margins were calculated based on HED body surface area and dose normalized to nasal surface area. However, when concentrations were used, the resulting margin is below (b) (4) and therefore is not supportive of local safety for the proposed specification of (b) (4) % for (b) (4) (see Table above).

As such, the specification for the drug product degradant (b) (4) of NMT (b) (4) % is not acceptable. However, given the available data to suggest that the degradant does not pose a safety concern and given the indication is potentially life saving, the lack of local support need not preclude approval. Should this NDA be approved, a nonclinical PMR to qualify the local safety of the drug product specification of NMT (b) (4) % for (b) (4) specification will be instituted.

Container Closure System

The container closure is the (b) (4) unit dose sprayer (DMF (b) (4)). The following figure illustrates the container closure system of the proposed drug product (from the Applicant's submission):

Figure 1: Unit Dose Nasal Spray Delivery System

The following table illustrates the components of the proposed container closure system (from the Applicant's submission):

Table 1: Container Closure Component Information

Component	Material Type	Drawing Reference	Summit Part Number	Manufacturer	Type III DMF #
Vial	Clear, type (b) (4) glass vial (b) (4)	(b) (4)			
Plunger (Rubber Stopper)	(b) (4)				
Container Holder (Vial Holder)				France	
Actuator Sub-Assembly	(b) (4)				

The drug product solution is contained in a glass vial and is in direct contact with the rubber stopper. Accordingly, an extractables and leachables assessment of the rubber stopper was submitted. It is noted that the components of the container closure system (both primary and secondary) have been referenced and used in other approved intranasal products (NDAs and ANDAs).

Extraction Studies

The Reviewer's Analytical Evaluation Threshold (AET) is calculated as follows using the (b) (4) threshold of concern and (b) (4) maximum daily volume of the product:

(b) (4)

The extraction studies were conducted on the (b) (4) stoppers. The following tables illustrate the extraction conditions, the instrumental analysis of sample extracts, and the extracts of interest from each analytical method (from the Applicant's submission):

(b) (4)

(b) (4)

Leachables Study:

The leachable study was conducted on 3 lots of Naloxone HCl 10 mg Unit Dose product that were stored for various timepoints (3, 6, 12, 18, 24, and 36 months). The analytical methods used were GC/MS for (b) (4)

(b) (4)

[1] Control of Nitrosamine Impurities in Human Drugs Guidance for Industry. Available at https://www.fda.gov/media/141720/download

As none of these leachable compounds exceed the (b) (4) threshold, a toxicological risk assessment was not needed. As the maximum exposures of the leachable compounds via the maximum daily dose of the proposed product are below (b) (4), there are no safety concerns with the container closure system.

Elemental Impurities
The elemental impurities assessment was conducted in the leachables study.

All elementals were below the limit of detection of the analytical method, (b) (4) which meets all ICH Q3D PDE limits.

(b) (4)

2.6 Proposed Clinical Population and Dosing Regimen

The proposed product is indicated for the emergency treatment of known or suspected opioid overdose, as manifested by respiratory and/or central nervous system depression and is intended for immediate administration as emergency therapy in settings where opioids may be present. Recommended initial dose is one nasal spray (110 mcL) that contains 10 mg of naloxone into one nostril. If the patient does not respond or responds and then relapses into respiratory depression, additional doses of the proposed product may be given every 2 to 3 minutes into alternating nostrils until emergency medical assistance arrives. The proposed product is not a substitute for emergency medical care. Dosing regimen is for both the adult and pediatric populations.

2.7 Regulatory Background

This is a 505(b)(2) application referencing the Agency's previous findings of safety to Narcan® (NDA 16636) injectable product. The pharmaceutical development of the proposed product was conducted under IND 142850.

Several meetings with the Applicant have taken place including a pre-IND meeting on February 2, 2019 (see Meeting Minutes dated March 7, 2019 under IND 141634) as well as written responses on October 27, 2020 and December 22, 2021.

The following nonclinical issues and recommendations were identified to the Applicant during development:

- The proposed product was higher concentration and therefore repeat-dose intranasal toxicity studies in two species should be conducted for the NDA;
- Leachables assessment should be conducted on several batches at multiple time points; and
- Agreed that in silico assessment for [redacted] (b) (4) qualification purposes.

3 Studies Submitted

3.1 Studies Reviewed

The following table illustrates the toxicology studies that were submitted and reviewed:

Study Number	Study Title
2950-002	A 14-Day Intranasal Naloxone HCl Toxicity Study with a 7-Day Recovery Period
20246992	A 28-Day Study of Naloxone HCl by Intranasal Administration in Rats with a 19-Day Recovery Period
2950-001*	A Pilot Study for Intranasal Administration in Dogs*

* = This study was not reviewed using the review template but was summarized

3.2 Studies Not Reviewed

None.

3.3 Previous Reviews Referenced

There were no previous reviews referenced.

4 Pharmacology

4.1 Primary Pharmacology

There were no new primary pharmacology studies with naloxone conducted and submitted in this NDA.

4.2 Secondary Pharmacology

There were no new secondary pharmacology studies with naloxone conducted and submitted in this NDA.

4.3 Safety Pharmacology

There were no new safety pharmacology studies with naloxone conducted and submitted in this NDA.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

There were no new dedicated PK/ADME studies with naloxone conducted and submitted in this NDA.

5.2 Toxicokinetics

There were no separate toxicokinetics studies with naloxone conducted and submitted in this NDA.

6 General Toxicology

In support of the higher concentration of naloxone hydrochloride (b) (4) mg/mL), the Applicant submitted the 28-day rat and 14-day dog toxicology studies. These studies were also conducted to support the drug product degradant (b) (4). The following table illustrates the formulations used in the dog and rat toxicology studies, which is comparable to the clinical formulation (from the Applicant's submission):

Table 4.2.1.1.11.1 – Summary of Formulations Used in Nonclinical, Clinical, and Commercial Formulations

Ingredient	Grade	Dog Intranasal Toxicology Study Formulation			Rat Intranasal Toxicology Study, Clinical, and Commercial Formulation		
		mg/mL	mg/dose	% w/w	mg/mL	mg/dose	% w/w
Naloxone HCl Dihydrate *(corresponding to naloxone hydrochloride)*	USP						
(b) (4)		(b) (4)					(b) (4)
Trisodium Citrate Dihydrate	USP						
Glycerin	USP						
NaOH or HCl	N/A	As required to target pH (b) (4)			As required to target pH (b) (4)		
Purified Water	USP	QS to target batch weight.					

The formulation used in the dog toxicology study slightly differed from the rat toxicology studies. The rat study included a group called SN(7032) that is equivalent to the clinical formulation that also contains several drug product degradants, including (b) (4) among others (see below in the review of the 28-day rat study).

6.2 Repeat-Dose Toxicity

A pilot study in dogs was conducted to determine a maximum feasible volume (MFV) to be used in the 14-day toxicology study in dogs and is summarized below.

A Pilot Study for Intranasal Administration to Dogs (Study 2950-001) EDR link
This pilot study in dogs was non-GLP. There were 2 phases in this pilot study in dogs (Phase A and B). The objective of Phase A of this study was to determine the (b) (4) based formulation when administered by a single-dose nasal spray applicator (also known as Naloxone HCl Placebo Unit Dose Actuators) via sequential device actuations to a beagle dog. The objective of Phase B of this study was to confirm the practicality, feasibility, and tolerability of the MFV of the placebo via intranasal administration on a repeated basis (3 consecutive days to beagle dogs). The following table illustrates the study design of this dog pilot study (from the Applicant's submission):

Study Design

Text Table 1
Experimental Design

Group No.	Actuations/ Naris	Total Actuations	Volume (mL)/ Naris/ Actuation	Number of Dosing Days	Total Dose Volume (mL)	Number of Animals	Identification of Female Animals
Phase A							
1	2[a] to 15	4[a] to 30	0.1	1	0.4	1	1501
Phase B							
2	15	30[b]	0.1	3	3.0	2	2501-2502

[a] This was the starting dose only. The animal was then administered successive intranasal instillations of the placebo volume (0.1 mL/actuator/naris, every 2 - 3 minutes) up to a maximum of 15 actuations/naris/day. The MFV was based on moisture out of nares and/or signs of swallowing or emesis (gag reflex).
[b] Once the MFV was determined in Phase A, 2 animals received successive intranasal instillations of the placebo volume (0.1 mL/actuator/naris, every 2 to 3 minutes) for 3 consecutive days

The dosing during Phase A was in one day while dosing in Phase B was once daily for 3 consecutive days. In-life procedures parameters that were measured and recorded are illustrated in the following table (from the Applicant's submission):

In-life Procedures, Observations, and Measurements

Cage Side Observations:	Observations for morbidity, mortality, injury, and the availability of food and water were conducted twice daily for all animals.
Cageside Clinical Observations:	Cageside clinical observations were conducted on each dosing day during dosing and at 2, 4, 6, and 8 hours after the last actuation. Each animal was examined visually while still in the cage for clinical signs of disease, toxicity, and injury. If warranted, the animal was removed from the cage for further signs of disease, toxicity, and injury. The animals were observed outside of the cage if scheduled observations occurred concurrently with other study related activities.
Detailed Clinical Observations:	Observations for clinical signs were recorded at unscheduled intervals. The observations collected from a companion animal are not reported but are maintained in the study file. The observations included, but were not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, and nervous system effects including tremors, convulsions, reactivity to handling, and unusual behavior.
Body Weights:	The body weight of the animal in Phase A of the study was measured and recorded at transfer. This body weight is not reported but is maintained in the study file. Body weights were not recorded during the study for the animals in Phase B of the study.

All dogs at study termination were returned to the stock colony.

No dogs died during the course of the study. There were no noteworthy individual cageside clinical observations. No actuator device-related observations were noted in

the cageside clinical observation during Phase A or Phase B of the study, indicating that the dogs tolerated the actuators and the actuators performed as intended. There were no treatment-related changes in body weights in both Phase A and Phase B.

During Phase A of the study moisture was noted around the nare after the 15th actuation was administered to the dog. Due to the moisture that was noted after the 15th actuation (30 total actuations), the maximum feasible volume was considered to be 15 actuations/naris (15 actuations x 0.1 mL/actuation for a total volume of 3.0 mL or 1.5 mL/naris).

No effects were noted during Phase B.

Taken together, the maximum feasible volume was determined to be 3.0 mL or 1.5 mL/naris due to the moisture that was noted after the 15th actuation (30 total actuations) during Phase A. During Phase B, repeat dosing at a maximum feasible volume of 3.0 mL/day was tolerated for 3 days.

Study title: A 14-Day Intranasal Naloxone HCl Toxicity Study with a 7-Day Recovery Period

Study no.:	2950-002
Study report location:	EDR Link
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 18, 2019
GLP compliance:	Yes. Signature provided on July 31, 2020.
QA statement:	Yes. Signature provided on July 30, 2020.
Drug, lot #, and % purity:	Naloxone Nasal Spray 10 mg, Lot No. NSNB1901A, 101.1% pure (provided from Summit Biosciences Inc.)

Key Study Findings

- Beagle dogs were administered 0 (saline control or vehicle control), 20, 160, or 300 mg/day naloxone (100 mg/mL) intranasally for 14 consecutive days followed by a 7-day recovery period (no treatment).
- As the doses are reported in mg/day, an average dog weight of 6 kg can be used to calculate mg/kg/day doses. As such, the doses are 3.33 (20/6 = 3.33), 26.7 (160/6 = 26.7), and 50 (300/6 = 50) mg/kg/day, respectively.
- Nasal mucosal irritation was present in the mid- and high dose dogs early in the treatment period but subsided and was not apparent on Day 14.
- Sporadic microscopic findings were observed in the nasal cavity and in its surrounding tissues with the highest incidence observed at the high dose. The

local NOAEL is the mid dose which was 8 sprays per naris given that the slight increases in incidence microscopic findings was reversible after the recovery period in this group. This local NOAEL results in a concentration margin of 1.1x as the formulation used in this toxicology study (100 mg/mL) is a slightly higher concentration of naloxone HCl than the clinical formulation (b) (4) mg/mL),

- The systemic NOAEL was the high dose of 300 mg/day based on no treatment-related microscopic effects outside of the nasal cavity and is equivalent to a human equivalent dose of 1667 mg, which confers a safety margin of 83x based on a body surface are comparison and using an average human weighing 60 kg.

Methods

Doses:	Saline control, Vehicle control, 20, 160, and 300 mg/day of Naloxone (10 mg in 100 mcL or 100 mg/mL)
Frequency of dosing:	15, 15, 1, 8, and 15 actuations per naris, respectively, per day for 14 consecutive days
Route of administration:	Intranasal
Dose volume:	3.0, 3.0, 0.2, 1.6, and 3.0 mL/day total volume, respectively
Formulation/Vehicle:	0.9% saline control; Naloxone HCl placebo vehicle control provided from the Applicant
Species/Strain:	Dog/naïve Beagle Dogs
Number/Sex/Group:	3/sex/group (main study); 2/sex/group (recovery)
Age:	4.5 to 5 months
Weight:	4.55 to 7.20 kg
Satellite groups:	Recovery group
Unique study design:	Olfactory functional assessment and mucosal irritation were included in this study
Deviations from study protocol:	Deviations did not affect the interpretation of the results in this study

The following table illustrates the study design of this study (from the Applicant's submission):

Text Table 2
Experimental Design

Group No.	Test Material	Actuations per Naris	Dose Volume (mL/Naris)	Total Daily Dose Volume (mL)	Dose Conc. (mg/mL)	Total Daily Dose (mg)	Animal No.			
							Main Study		Recovery	
							Male	Female	Male[a]	Female[a]
1	Saline	15	1.5	3.0	0	0	1003-1005	1503-1505	1001-1002	1501-1502
2	Vehicle	15	1.5	3.0	0	0	2003-2005	2503-2505	2001-2002	2501-2502
3	Naloxone[c]	1	0.1	0.2	100	20	3003-3005	3503-3505	3001-3002	3501-3502
4	Naloxone[c]	8	0.8	1.6	100	160	4003-4005	4503-4505	4001-4002	4501-4502
5	Naloxone[c]	15	1.5	3.0	100	300	5003-5005	5503-5505	5001-5002	5501-5502

Conc.: Concentration
[a] Two animals were designated for a 1-week recovery period.
[b] Vehicle = Naloxone HCl Placebo Unit Dose.
[c] Naloxone = Naloxone HCl Nasal Spray, 10mg.

It is noted that the doses used in this dog toxicology study are expressed in mg/day and did not consider the body weights of the dogs. Using an average dog weight of 6 kg (from the body weight data), these doses are 3.33 (20/6 = 3.33), 26.7 (160/6 = 26.7), and 50 (300/6 = 50) mg/kg/day and are only used to calculate a Human Equivalent Dose (HED), which are 111 (3.33/1.8 x 60 kg average human weight = 111), 889 (26.7/1.8 x 60 kg average human weight = 889), and 1667 (50/1.8 x 60 kg average human weight = 1667) mg, respectively. The saline control, vehicle control, and all naloxone dose groups were administered daily for 14 consecutive days via the intranasal route using the Applicant-supplied actuators. Administration of each test compound was performed ± 1 hour from the time of the start of dosing on Day 1. Each actuation is 0.1 mL in volume. The dose levels are 20, 160, and 300 mg/day and the total daily dose volumes to achieve these doses are 0.2 (1 actuation/naris), 1.6 (8 actuations/naris), and 3.0 mL (15 actuations/naris), respectively. The 2 control groups (saline or vehicle) was administered in the same manner as the naloxone dose groups with a total daily volume of 3.0 mL (15 actuations/naris). All test compounds were administered first to the left naris and then administered to the right naris after 2 to 3 minutes. Alternating administration between the left and right naris were performed until the required number of actuations was administered.

The high dose of 300 mg dispersed over 15 actuations and a total daily volume of 3.0 mL was determined from the pilot study in dogs (Study 2950-001). The pilot study in dogs determined the maximum feasible volume to be 3.0 mL or 1.5 mL/naris due to the moisture that was noted after the 15th actuation (30 total actuations) during Phase A. During Phase B, repeat dosing at a maximum feasible volume of 3.0 mL/day was tolerated for 3 days. Accordingly, a maximum feasible volume of 3.0 mL was used in the 14-day toxicology study in dogs.

Observations and Results

Mortality

Mortality/moribundity checks were made twice daily during the study. There were no unscheduled deaths during the study.

Clinical Signs

For cage side observations, all dogs were observed for morbidity, mortality, injury, and the availability of food and water twice daily during the study.

For cage side clinical observations, each dog was examined visually for disease, toxicity, and injury at 2, 4, and 24 hours after the first actuation on each dosing day in the treatment phase as well as once daily during the recovery phase.

Detailed clinical observations of each dog was performed weekly during the study. The observations included, but not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, and nervous system effects such as tremors, convulsions, reactivity to handling, and unusual behavior.

A complete physical examination was conducted by a veterinarian on all dogs pretest and throughout the study.

Trembling was noted during cageside clinical observations in 1/5 females from the 300 mg/day dose group. Tremors and/or trembling were noted during detailed clinical observations in 2/5 females from the 300 mg/day dose group. Trembling was noted during veterinary examination in 1/5 males from the 160 mg/day dose group. In the Pathology Report, trembling and/or tremors were not considered treatment-related as they occurred in 1 or 2 dogs and were transient in nature.

Treatment-related salivation was noted during cageside clinical observations in males and females from the 160 and 300 mg/day dose groups. According to the Pathology Report, salivation was not considered adverse because the dogs were examined by the veterinary staff and had normal hydration, normal lung sounds upon auscultation, and normal gum color/capillary refill time. Other cageside clinical observations including decreased activity, emesis/vomitus, abnormal feces (discolored, mucoid, soft and/or watery), red material in pan/bedding, white discharge, lacrimation, dry skin, sparse hair, and or scabbed area were incidental and not considered treatment-related because the observations only occurred in a few dogs per group, were seen in controls, and/or are observations typically seen in this age and strain of dog per the Pathology Report.

This Reviewer concurs with the conclusions of the Pathology Report.

Body Weights

Body weights for all dogs were measured and recorded weekly during the study. Body weight changes were calculated for all dogs between each weighing interval. There were no treatment-related changes in body weight and body weight gains in this study.

Food Consumption

Food consumption was measured and recorded daily and reported weekly throughout the dosing and recovery period. There were no treatment-related changes in food consumption in this study.

Ophthalmoscopy

Ophthalmological examinations were conducted by an ophthalmologist pretest as well as prior to the terminal and recovery necropsies. There were no treatment-related changes in the ophthalmoscopic examinations in the study.

ECG

Electrocardiographic examinations were performed on all dogs pretest and on all surviving dogs on Day 2 (predose and 4 hours post-dose), during the last week of dosing, and once during recovery. Standard ECGs (10 Lead) were recorded at 50 mm/sec. The RR (time between 2 successive R-waves), PR (time from onset of p-wave to the start of the QRS complex), QT intervals (duration of ventricular electrical systole from the beginning of the QRS complex to the end of the T-wave), and QRS duration (duration of QRS complex, which represents the electrical impulse as it spreads through the ventricles and indicates ventricular depolarization) as well as heart rate were measured and recorded using an appropriate lead. The QRS complex is represented in the following figure:

All dogs were in sinus rhythm or sinus arrhythmia, both of which are normal rhythms in dogs. One male dog (Animal no. 5003) from the 300 mg/day dose group had an atrial premature complex (APC) on Day 2 and terminal postdose ECGs. According to the Pathology Report, infrequent APCs can be a normal variant in dogs and as the arrhythmia was observed infrequently in a single animal, the arrhythmia is not considered treatment-related. Aside from the APCs, all of the electrocardiograms were qualitatively and quantitatively within normal limits per the Pathology Report.

When absolute group mean values were evaluated statistically by sex and compared to

interval matched saline group values, the following significant difference was noted:

- Slower heart rate in females at the Day 2 postdose interval following vehicle

When absolute group mean values were evaluated statistically by sex and compared to interval matched vehicle group values, the following significant differences were noted:

- Higher heart rate in males at the Day 11 predose and postdose intervals following the 160 mg/day dose
- Higher heart rate in females at the Day 2 postdose interval following the 300 mg/day dose
- Shorter RR interval in males at the Day 11 postdose interval following the 160 mg/day dose
- Longer QT interval in males at the Day 11 postdose interval following the 160 mg/day dose

The Pathology Report addressed these changes. As the higher heart rate and shorter RR intervals in males were noted only following the mid dose, the changes are not considered treatment-related. The longer QT interval in males is a normal response to a slower heart rate. The higher heart rate in females, while occurring in the high dose group, is not considered test article related as the change was due as much to a slowing of the heart rate in the vehicle group (significantly slower than saline) as it was to an increase in the test article group and it was observed at only a single post-dose interval.

This Reviewer concurs with the conclusions of the Pathology Report.

Hematology

All dogs were fasted overnight prior to blood and urine collection but had access to drinking water. Blood was collected via the jugular vein and urine was collected using steel pans placed under the cages for at least 16 hours on Days 1 and 14. The following tables illustrate the blood and urine sampling schedule (from the Applicant's submission):

Text Table 4
Bioanalysis Sample Collection Schedule

Group No.	Sample Collection Time Points (Time After the First Actuation) on Days 1 and 14						
	0[a] hr	0.25 hr	0.5 hr	1 hr	2 hr	4 hr	24 hr
1[b]	X	X	X	X	X	X	X
2[b]	X	X	X	X	X	X	X
3	X	X	X	X	X	X	X
4	X	X	X	X	X	X	X
5	X	X	X	X	X	X	X

X - Sample was collected: hr - hour
[a] Sample was collected before dosing.
[b] Only the 1 hour postdose samples were analyzed for these groups.

Text Table 2
Clinical Pathology Sample Collection

Panel	Collection Interval	Group 1	Group 2	Group 3	Group 4	Group 5
Hematology	Twice Pretreatment[a], Terminal, and Recovery	X	X	X	X	X
Coagulation	Twice Pretreatment[a], Terminal, and Recovery	X	X	X	X	X
Clinical Chemistry	Twice Pretreatment[a], Terminal, and Recovery	X	X	X	X	X
Urinalysis	Twice Pretreatment[a], Terminal, and Recovery	X	X	X	X	X

[a] Days -11 and -6.
X - samples were collected

Hematology and coagulation parameters were a routine set used in toxicology studies and were measured from the blood samples.

The following table illustrates the changes in hematology and coagulation (data from the Applicant's submission):

Observation	Day	Saline Control	Vehicle Control	20 mg/day Naloxone	160 mg/day Naloxone	300 mg/day Naloxone
Males						
Erythrocytes (10^6 cells/mcL)	14	5.998	6.228	5.900	6.390	**6.806[a] (13.5%)**
Hemoglobin (g/dL)	14	12.92	13.56	12.98	13.88	**14.76[a,b] (14.2 and 8.85%)**
Hematocrit (%)	14	40.42	42.16	40.02	43.20	**46.74[a,b] (15.6 and 10.9%)**
Absolute Reticulocyte (10^3 cells/mcL)	14	67.26	48.46	66.96	61.54	**90.94[d] (87.7%)**
Females						

Prothrombin Time (sec)	14	7.48	7.42	7.92	7.84	**7.74[c] (3.48%)**
Monocytes (10^3 cells/mcL)	21	0.575	**0.245[a] (-57.4%)**	**0.340[a] (-40.9%)**	**0.455[b,c] (-20.9 and 85.7%)**	**0.245[a] (-57.4%)**

a = different from saline control ($p < 0.01$) using ANOVA with multiple comparisons
b = different from vehicle control ($p < 0.01$) using ANOVA with multiple comparisons
c = different from saline control ($p < 0.05$) using ANOVA with multiple comparisons
d = different from vehicle control ($p < 0.05$) using ANOVA with multiple comparison

In males, there was an increase in erythrocytes by 13.5% in the 300 mg/day dose group compared to the saline control at the end of the dosing period. There were increases in hemoglobin and hematocrit by 14.2 and 15.6%, respectively, in the 300 mg/day dose group compared to the saline control as well as by 8.85 and 10.9%, respectively, compared to the vehicle control at the end of the dosing period. There was an increase in absolute reticulocyte by 87.7% in the 300 mg/day dose group compared to the vehicle control at the end of the dosing period. There were no further treatment-related changes in hematology and coagulation in males during the study.

In females, there was an increase in prothrombin time by 3.48% in the 300 mg/day dose group compared to the saline control at the end of the dosing period. There were decreases in monocytes by 57.4, 40.9, 20.9, and 57.4% in the vehicle control, 20, 160, and 300 mg/day dose groups, respectively, compared to the saline control at the end of the recovery period with an increase of 85.7% in the 160 mg/day dose group compared to vehicle control at the end of the recovery period. The decrease in monocytes compared to the saline control was not dose-dependent and the decrease in monocytes compared to the vehicle control was not dose-dependent as the decrease was only observed in the 160 mg/day dose group. There were no further treatment-related changes in hematology and coagulation in females during the study.

In the Pathology report, changes in hematology and coagulation were considered sporadic, consistent with biologic variation and/or negligible in magnitude, and not treatment-related. This Reviewer concurs with the conclusions of the Pathology Report as these parameters are within normal ranges in this strain of dog (Derelanko, 2008).

Clinical Chemistry

Clinical chemistry parameters were a routine set used in toxicology studies and were measured from the blood samples.

The following table illustrates the changes in clinical chemistry (data from the Applicant's submission):

Observation	Day	Saline Control	Vehicle Control	20 mg/day Naloxone	160 mg/day Naloxone	300 mg/day Naloxone
Females						
Sodium (mEq/L)	14	146.4	147.6	146.6	147.0	**146.3[d] (-0.88%)**
Potassium	21	5.07	4.53	4.75	**4.45[c]**	**4.31[c]**

(mEq/L)					**(-12.2%)**	**(-15.0%)**
Creatinine (mg/dL)	14	0.44	0.52	0.46	0.51	**0.44[d] (-15.4%)**
Triglyceride (mg/dL)	21	34.2	**48.6[c] (42.1%)**	**50.9[c] (48.8%)**	**29.4[d] (-39.5%)**	**29.5[d] (-39.3%)**

c = different from saline control ($p < 0.05$) using ANOVA with multiple comparisons
d = different from vehicle control ($p < 0.05$) using ANOVA with multiple comparison

There were no treatment-related changes in clinical chemistry in males during the study.

In females, there were decreases in sodium and creatinine by 0.88 and 15.4%, respectively, in the 300 mg/day dose group at the end of the dosing period. The decrease in creatinine in the 300 mg/day naloxone dose group was not different from saline control with no dose-dependency. At the end of the recovery period, there were decreases in potassium in the 160 and 300 mg/day dose groups by 12.2 and 15.0%, respectively, compared to saline control. At the end of the recovery period, there was an increase in triglyceride in the vehicle control by 42.1% compared to the saline control as well as increase by 48.8% in the 20 mg/day dose group compared to saline control and decreases of 39.5 and 39.3% in the 160 and 300 mg/day dose groups, respectively compared to vehicle control. There were no further treatment-related changes in clinical chemistry in females during the study.

In the Pathology report, changes in clinical chemistry were considered sporadic, consistent with biologic variation and/or negligible in magnitude, and not treatment-related. This Reviewer concurs with the conclusions of the Pathology Report as these parameters are within normal ranges for this strain of dog (Derelanko, 2008).

Urinalysis

Urinalysis parameters were measured from the urine samples including volume, specific gravity, and pH. There were no treatment-related changes in urinalysis in this study.

Gross Pathology

Dogs were sacrificed at the end of the dosing and recovery phases on Day 15 and Day 22, respectively. Subsequently, necropsy examinations were performed by a veterinary pathologist. The dogs were examined carefully for external abnormalities including palpable masses. Necropsy included evaluation of the carcass and musculoskeletal system, all external surfaces and orifices, cranial cavity and external surfaces of the brain, and examination of cavities (thoracic, abdominal, and pelvic cavities) with their associated organs and tissues.

The following table illustrates the gross pathology changes at the end of the dosing and recovery periods (data from the Applicant's submission):

Observation	Saline Control	Vehicle Control	20 mg/day Naloxone	160 mg/day Naloxone	300 mg/day Naloxone
Dosing Period					

Males					
Kidneys --absent	1/3	-	-	-	-
Lung --focus/foci, red --nodule	1/3 0/3	- -	- -	- -	0/3 **1/3**
Females					
Kidney --mass	-	1/3	-	-	-
Recovery Period					
Males					
Pituitary Gland --cyst	-	1/2	-	-	-
Prostate Gland --small	-	-	-	-	**1/2**
Females					
Brain --dilation	-	-	-	-	**1/2**
Skin, Subcutis --edema	-	-	1/2	-	-
Uterus with Cervix --absent, portion	-	1/2	-	-	-

In males at the end of the dosing period, kidney (absent) and lung (focus/foci, red) were observed in the saline control group and as such, can be dismissed. Lung (nodule) was observed in 1/3 dogs in the 300 mg/day dose group and was correlated to mild subacute/chronic inflammation microscopically.

In females at the end of the dosing period, kidney (mass) was observed in the vehicle control group and as such, can be dismissed.

In males at the end of the recovery period, cyst in the pituitary gland was observed in the vehicle control and as such, can be dismissed. Prostate gland (small) was observed in 1/2 dogs in the 300 mg/day dose group and was identified as immature microscopically.

In females at the end of the recovery period, brain (ventricular dilation) was observed in 1/2 dogs in the 300 mg/day dose group and was correlated to minimal hydrocephalus microscopically. Skin, subcutis (edema) was observed in 1/2 dogs from the 20 mg/day dose group only and as such, can be dismissed. Uterus with cervix (absent, portion) was observed in the vehicle control and as such, can be dismissed.

In the Pathology Report, these macroscopic changes were not treatment-related as they were considered incidental, were commonly observed in beagle dogs, and/or were of similar incidence in control and dosed dogs. This reviewer concurs with the conclusions of the Pathology Report.

Organ Weights

Body weights and organ weights were recorded for all dogs at the scheduled necropsies (at the end of the treatment and recovery phases) along with organ weight ratios (relative to body and brain weights).

The following table illustrates the changes in organ-to-body-weight ratio in males at the end of the treatment period (data from the Applicant's submission):

Organ	Saline Control	Vehicle Control	20 mg/day Naloxone	160 mg/day Naloxone	300 mg/day Naloxone
Spleen/Body Weight ratio (%)	1.07663	1.47158	1.25028	1.18590	**1.17346[d] (-20.3%)**

d = different from vehicle control ($p < 0.05$) using ANOVA with multiple comparisons

In males, there was a decrease in the spleen-to-body-weight ratio by 20.3% in the 300 mg/day dose group compared to the vehicle control; however, there was no difference compared to the saline control. According to the Pathology Report, this difference was considered the result of normal biological variation based on its minimal magnitude, the lack of microscopic correlates, and the absence of statistical significance compared to the saline control. This Reviewer concurs with the conclusions of the Pathology Report. There were no further treatment-changes to the body weight parameters (absolute organ weight, organ-to-body-weight ratio, and organ-to-brain-weight ratio) in males during the study.

There were no treatment-related changes in organ weight parameters in females during the study.

Histopathology

Adequate Battery
The following organs and tissues were collected, weighed, preserved, and examined microscopically (from the Applicant's submission):

Text Table 5
Tissue Weighing, Collection, Processing and Evaluation Table

Organ	Weigh	Macroscopic Evaluation and Collection	Histology Processing	Microscopic Evaluation
Animal ID	-	X	-	-
Artery, aorta	-	X	X	X
Bone marrow, sternum	-	X	X	X
Bone marrow smear	-	X[a]	-	-
Bone, femur	-	X (1)	X (1)	X (1)
Bone, sternum	-	X	X	X
Brain (7 sections)	X	X	X	X
Body cavity nasal (4 sections with olfactory bulb)	-	X	X	X
Carina	-	X	X	X
Epididymis	X (2)	X (2)[b]	X (2)	X (2)
Esophagus	-	X	X	X
Eye	-	X (2)[b]	X (2)	X (2)
Extrapulmonary bronchus	-	X	X	X
Gallbladder	-[e]	X	X	X
Ganglion, dorsal root, lumbar	-	X	-	-
Gland, adrenal	X (2)	X (2)	X (2)	X (2)
Gland, lacrimal	-	X (2)	-	-
Gland, mammary	-	X	X	X
Gland, parathyroid	-[d]	X (2)	X (2)	X (2)
Gland, pituitary	X	X	X	X
Gland, prostate	X	X	X	X
Gland, salivary, submandibular	-	X (2)	X (1)	X (1)
Gland, salivary, sublingual	-	X (2)	-	-
Gland salivary, parotid	-	X (2)	-	-
Gland, thyroid	X (2)	X (2)	X (2)	X (2)
Gut-associated lymphoid tissue[c]	-	X	X	X
Heart	X	X	X	X
Joint, femorotibial	-	X (1)	X (1)	X (1)
Kidney	X (2)	X (2)	X (2)	X (2)
Large intestine, cecum	-	X	X	X
Large intestine, colon	-	X	X	X
Large intestine, rectum	-	X	X	X
Larynx	-	X	X	X
Liver	X	X	X	X
Lung	X	X	X	X
Lymph node, tracheobronchial	-	X	X	X
Lymph node, mandibular	-	X (2)	X (2)	X (2)
Lymph node, mesenteric	-	X	X	X
Muscle, skeletal	-	X	X	X
Nasopharynx	-	X	X	X
Nerve, optic	-	X	X (2)	X (2)
Nerve, sciatic	-	X	X (1)	X (1)
Nerve, tibial	-	X	-	-
Oropharynx	-	X	X	X
Ovary	X (2)	X (2)	X (2)	X (2)
Oviduct	-	X	-	-

Organ	Weigh	Macroscopic Evaluation and Collection	Histology Processing	Microscopic Evaluation
Pancreas	-	X	X	X
Skin	-	X	X	X
Small intestine, duodenum	-	X	X	X
Small intestine, ileum	-	X	X	X
Small intestine, jejunum	-	X	X	X
Spinal cord	-	X	X	X
Spleen	X	X	X	X
Stomach	-	X	X	X
Testis	X (2)	X (2)	X (2)	X (2)
Thymus	X	X	X	X
Tongue	-	X	X	X
Tonsils	-	X	X	X
Trachea	-	X	X	X
Ureter	-	X	-	-
Urinary bladder	-	X	X	X
Uterus/Cervix	X	X	X	X
Vagina	-	X	X	X

X = Procedures conducted. - = Not applicable. (1) = one side. (2) = both sides.
Macroscopic abnormalities in the organs listed and in other organs were sampled at necropsy, processed for histology and examined microscopically.
[a] Bone marrow smears were collected from the 5th to 7th rib at scheduled necropsies (for possible examination). Bone marrow smears were allowed to air dry and were not fixed in formalin.
[b] Eyes and optic nerves were preserved in Davidson's fixative. Testes and epididymides were preserved in modified Davidson's fixative.
[c] From small intestine: Peyer's patch or solitary lymphoid follicle.
[d] Weighed with gland, thyroid.
[e] Weighed with liver.

The table above is an adequate set of tissues and organs that were examined microscopically.

Peer Review
The histopathological findings of this study were peer reviewed.

Histological Findings
Microscopic changes at the end of the treatment period are illustrated in the following table (data from the Applicant's submission):

Observation	Saline Control	Vehicle Control	20 mg/day Naloxone	160 mg/day Naloxone	300 mg/day Naloxone
Males					
Lung					
--Inflammation, subacute/ chronic					
Minimal	1/3	0/3	0/3	1/3	1/3
Mild	0/3	0/3	0/3	0/3	1/3
Moderate	1/3	0/3	0/3	0/3	0/3
Lymph Node, Mesenteric					
--Hyperplasia, lymphoid, germinal center					
Minimal	0/3	0/3	0/3	0/3	1/3
Lymph Node, Tracheobronchial					

--Erythrocytosis/ erythrophagocytosis, sinus					
Minimal	0/3	0/3	1/3	0/3	0/3
Mild	1/3	0/3	0/3	0/3	0/3
Nasal b					
--Infiltration, mononuclear cell, increased					
Minimal	0/3	0/3	0/3	1/3	0/3
Nasal c					
--Degeneration/atrophy, olfactory, epithelium					
Minimal	1/3	1/3	0/3	1/3	**2/3**
--Mineralization					
Minimal	0/3	1/3	0/3	0/3	0/3
--Infiltration, mononuclear cell, increased					
Minimal	0/3	0/3	1/3	1/3	0/3
Tracheal bifurcation					
--Infiltration, mononuclear cell					
Minimal	1/3	0/3	0/3	0/3	0/3
Mild	1/3	0/3	0/3	0/3	0/3
Bronchus					
--Infiltration, mononuclear cell					
Minimal	1/3	0/3	0/3	1/3	1/3
Mild	1/3	0/3	0/3	0/3	0/3
Females					
Lung					
--Alveolar macrophage aggregation					
Minimal	1/3	0/3	0/3	0/3	0/3
--Hemorrhage					
Minimal	1/3	0/3	0/3	0/3	0/3
--Inflammation, subacute/ chronic					
Minimal					
Mild	1/3	0/3	0/3	0/3	0/3
--Fibrin deposition	0/3	0/3	0/3	0/3	**1/3**
Mild					
	1/3	0/3	0/3	0/3	0/3
Lymph Node, Tracheobronchial					
--Erythrocytosis/ erythrophagocytosis, sinus					
Minimal	1/3	0/3	1/3	0/3	0/3
Nasal b					
--Infiltration, mononuclear cell, increased					
Minimal	0/3	0/3	0/3	0/3	**1/3**
Nasal c					
--Degeneration/atrophy, olfactory, epithelium					
Minimal	1/3	1/3	1/3	0/3	1/3
Trachea					
--Infiltration, mononuclear cell					
Minimal	1/3	0/3	0/3	0/3	0/3
Tracheal bifurcation					
--Infiltration, mononuclear cell					

Mild	1/3	0/3	0/3	0/3	0/3
Bronchus --Infiltration, mononuclear cell Mild	1/3	0/3	0/3	0/3	0/3
--Degeneration/atrophy, respiratory epithelium Minimal	0/3	0/3	0/3	1/3	0/3

Microscopic changes in the lung (subacute/chronic inflammation) correlated with macroscopic changes (nodule) in males at both the 160 and 300 mg/day naloxone dose groups as well as in the female 300 mg/day naloxone dose group. However, the severity of the finding was greater in the saline control (moderate) than in the naloxone treatment groups (minimal and mild in males and mild in females) and as such, can be dismissed.

The inflammatory response (e.g. hyperplasia, lymphoid, germinal center; sinus erythrocytosis/erythrophagocytosis; increased mononuclear cell infiltration, degeneration/atrophy of the respiratory epithelium) in the lymph nodes (mesenteric and tracheobronchial in males and tracheobronchial in females), trachea in females, and tracheal bifurcation and bronchus of both sexes that were observed sporadically in all dose groups suggesting healing in these tissues from the irritating test article (proposed naloxone formulation).

In the Pathology Report, the following microscopic findings were present in the nasal cavity at the terminal and recovery necropsies: minimal olfactory epithelium degeneration/atrophy (restricted to the dorso-medial meatus on level c), minimal increased mononuclear cell infiltrate (tips of turbinates), and cyst (focal). In addition, minimal focal mineralization was present at the terminal necropsy only. Level c of the nasal cavity was the most commonly affected. Changes were present across groups, including saline and vehicle control groups with no dose response. Therefore, these findings were considered incidental and not test article related.

The following table illustrates the microscopic changes at the end of the recovery period (data from the Applicant's submission):

Observation	**Saline Control**	**Vehicle Control**	**20 mg/day Naloxone**	**160 mg/day Naloxone**	**300 mg/day Naloxone**
Males					
Galt --Inflammation, acute Mild	0/2	0/2	0/2	0/2	**1/2**
Prostate gland --Immature	0/2	0/2	0/2	0/2	**1/2**
Lung --Inflammation, subacute/ chronic Minimal	2/2	0/2	0/2	0/2	0/2
Trachea --Infiltration, mononuclear cell Minimal	0/2	0/2	1/2	0/2	0/2

Lymph Node, Tracheobronchial --Erythrocytosis/ erythrophagocytosis, sinus Minimal Mild	 1/2 0/2	 0/2 1/2	 1/2 0/2	 0/2 0/2	 1/2 0/2
Nasal b --Infiltration, mononuclear cell, increased Minimal	 0/2	 1/2	 0/2	 0/2	 1/2
Nasal c --Degeneration/atrophy, olfactory, epithelium Minimal --Infiltration, mononuclear cell, increased Minimal	 1/2 0/2	 1/2 0/2	 1/2 0/2	 1/2 1/2	 **2/2** 0/2
Bronchus --Infiltration, mononuclear cell Minimal	 0/2	 0/2	 1/2	 0/2	 **1/2**
Females					
Brain --Hydrocephalus Minimal	 0/2	 0/2	 0/2	 0/2	 **1/2**
Heart --Mineralization, vascular Minimal	 0/2	 0/2	 0/2	 0/2	 **1/2**
Lung --Inflammation, subacute/ chronic Minimal Mild --Giant cells Minimal	 0/2 0/2 0/2	 1/2 0/2 0/2	 0/2 1/2 1/2	 0/2 0/2 0/2	 0/2 0/2 0/2
Lymph Node, Tracheobronchial --Erythrocytosis/ erythrophagocytosis, sinus Minimal Mild	 0/2 0/2	 0/2 0/2	 0/2 0/2	 0/2 1/2	 1/2 0/2
Nasal b --Infiltration, mononuclear cell, increased Minimal	 0/2	 0/2	 1/2	 0/2	 0/2
Nasal c --Degeneration/atrophy, olfactory, epithelium Minimal	 0/2	 1/2	 1/2	 1/2	 0/2
Tracheal bifurcation --Infiltration, mononuclear cell Minimal	 1/2	 0/2	 0/2	 0/2	 0/2
Bronchus --Infiltration, mononuclear cell Minimal	 0/2	 0/2	 0/2	 1/2	 0/2

As shown in the table above, the microscopic changes in the lungs, trachea, lymph nodes (mesenteric and tracheobronchial), tracheal bifurcation, and bronchus subsided

at the end of the recovery period with findings occurring sporadically in all dose groups. In males, mild acute inflammation in the galt and immature prostate were observed in 1/2 recovery males, each, from the 300 mg/day dose group. In females, minimal hydrocephalus and minimal vascular mineralization of the heart were observed in 1/2 recovery females, each, from the 300 mg/day dose group.

According to the Pathology Report, these microscopic findings observed in other tissues at the terminal and recovery necropsies were considered incidental, of the nature commonly observed in beagle dogs, and/or were of similar incidence and severity in control and dosed animals and, therefore, were considered incidental/spontaneous and not treatment-related. The test article (proposed naloxone formulation) is a nasal irritant. This Reviewer concurs with the conclusions of the Pathology Report.

Special Evaluation

Olfactory Functional Assessment
Olfactory functional assessments were conducted on all dogs on Days 2, 7, and 14 during the study. A cotton swab soaked in water was used to acclimate each dog to cotton swab. After 15 to 30 seconds, the water-soaked cotton swab was replaced with an ammonia-soaked cotton swab. A recoil reaction represents an intact olfactory response while the lack of a recoil reaction represents a deficient olfactory response.

The following table illustrates the findings of the olfactory functional assessments (from the Applicant's submission):

Text Table 9
Olfactory Functional Assessments[a]

Group	Dose	Pretest[b]		Day 2		Day 7		Day 14	
		Male	Female	Male	Female	Male	Female	Male	Female
1	Saline	4/5	5/5	3/5	4/5	3/5	4/5	3/5	5/5
2	Vehicle	4/5	5/5	4/5	5/5	4/5	5/5	3/5	5/5
3	20 mg/day	4/5	5/5	5/5	5/5	4/5	5/5	4/5	5/5
4	160 mg/day	4/5	5/5	5/5	3/5	5/5	3/5	5/5	5/5
5	300 mg/day	5/5	5/5	4/5	4/5	4/5	4/5	4/5	4/5

[a] Results are presented as number of animals that passed olfactory functional assessment divided by total number of animals.
[b] Pretest assessments were performed twice (on Days -4 and -1), with only the animals which failed on Day -4 being reassessed on Day -1. Animals that passed the pretest olfactory functional assessment on either Day -4 or Day -1 are reported as passed.

As shown in the table above, the decrease in the number of passed was not dose-dependent and was not dependent on the number of treatment days. According to the Pathology Report, there were no treatment-related effects on the olfactory functional assessment and any changes in individual olfactory function were considered incidental and were either present pretest or were likely due to individual variation. This Reviewer concurs with the conclusions of the Pathology Report.

Evaluation of Mucosal Irritation

Evaluations of mucosal irritations were conducted on all dogs daily during the first week of the study and once on Day 14. The left and right external nares were reflected back and the mucosal lining was observed for gross signs of irritation and any other signs of local or systemic effects. The mucosal lining was scored for skin irritation based upon the Draize scale (from the Applicant's submission):

Text Table 3
Nasal Observations

Score	Observation
0	No evidence of nasal symptoms
1	Nasal rattling or sneezing
2	Nasal discharge on external nares
3	Evidence of mouth breathing
	Maximum possible score = 3

The following tables illustrate nasal irritation in the left side of both sexes (from the Applicant's submission):

Text Table 10
Left Nasal Mucosal Irritation Assessments [a]

Group	Dose	Day 1		Day 2		Day 3		Day 4	
		Male	Female	Male	Female	Male	Female	Male	Female
1	Saline	0	0	0.4	0.8	0	0	0.4	0
2	Vehicle	0	0	0.8	0.8	0	0.4	0	0
3	20 mg/day	0	0	0	0	0	0	0	0
4	160 mg/day	0	0.4	1.6	1.6	0.4	0.8	0.4	0.4
5	300 mg/day	0.8	0	2	0.4	0.8	1.2	0.8	1.2

[a] Results are presented as a mean score for the animals in the specific treatment group.

Text Table 10, Cont.
Left Nasal Mucosal Irritation Assessments [a]

Group	Dose	Day 5		Day 6		Day 7		Day 14	
		Male	Female	Male	Female	Male	Female	Male	Female
1	Saline	0	0	0	0	0	0	0.4	0
2	Vehicle	0	0	0	0	0	0.4	0	0.4
3	20 mg/day	0	0	0	0	0.4	0	0.4	0.4
4	160 mg/day	0.2	0	0.4	1.6	0.4	0.4	0.4	0.4
5	300 mg/day	0.2	0	0.4	0.8	0.4	0.4	0.4	0.4

[a] Results are presented as a mean score for the animals in the specific treatment group.

The following tables illustrate nasal irritation in the right side of both sexes (from the Applicant's submission):

Text Table 11
Right Nasal Mucosal Irritation Assessments [a]

Group	Dose	Day 1		Day 2		Day 3		Day 4	
		Male	Female	Male	Female	Male	Female	Male	Female
1	Saline	0	0	0.4	0.8	0.4	0	0	0
2	Vehicle	0	0	0	0.8	0	0.4	0	0
3	20 mg/day	0	0	0	0	0	0	0	0
4	160 mg/day	0.4	0.4	2	1.6	0.8	0.8	0.4	0.4
5	300 mg/day	1.2	0	1.2	0.8	0.8	0.8	1.6	1.2

[a] Results are presented as a mean score for the animals in the specific treatment group.

Text Table 11, Cont.
Right Nasal Mucosal Irritation Assessments [a]

Group	Dose	Day 5		Day 6		Day 7		Day 14	
		Male	Female	Male	Female	Male	Female	Male	Female
1	Saline	0	0	0	0	0	0	0	0
2	Vehicle	0	0	0	0	0	0	0	0.4
3	20 mg/day	0	0	0	0	0.8	0	0	0.4
4	160 mg/day	0.2	0	0.4	0.4	0	0	0	0
5	300 mg/day	0.2	0	0.4	0.4	0.4	0.4	0.4	0

[a] Results are presented as a mean score for the animals in the specific treatment group.

As shown in the table above, there was treatment-related nasal discharge on external nares as the observation increased in males and females at 160 and 300 mg/day in both right and left sides. According to the Pathology Report, nasal discharge correlated with salivation in both sexes at 160 and 300 mg/day and was not considered adverse because the observation was transient in nature, was observed at lower incidences in the control dogs and in 20 mg/day in both sexes, and the dogs were examined by the veterinary staff and had normal hydration, normal lung sounds upon auscultation, and normal gum color/capillary refill time. This Reviewer concurs with the conclusions of the Pathology Report.

Toxicokinetics

Blood sampling for toxicokinetics were collected on Days 1 and 14 from an appropriate vein according to the following schedule (from the Applicant's submission):

TK Sample Collection Schedule

Group No.	Sample Collection Time Points (Time Postdose) on Days 1 and 14						
	0 h[a]	15 min	30 min	1 h	2 h	4 h	24 h
All	X	X	X	X	X	X	X

X = Sample collected; min = minute
[a] Sample collected prior to administration of the first dose

Blood samples for toxicokinetics were sent to (b) (4) The following table illustrates the toxicokinetic parameters that were measured using Phoenix™ WinNonlin® (from the Applicant's submission):

Parameter	Description of Parameter
C_{max}	The maximum observed concentration measured after dosing.
C_{max}/Dose	Dose-normalized C_{max}, where Dose is the total daily dose.
t_{max}	The time after dosing at which the maximum concentration was observed.
AUC_{last} (AUC_{tlast})	The area under the concentration vs. time curve from the start of dose administration (0 h) to the time of the last quantifiable concentration, calculated using the linear trapezoidal method.
AUC_{last}/Dose	The AUC_{last} divided by the dose administered.
t_{last}	Time of last quantifiable concentration
R_{AUC}	Accumulation ratio based on AUC_{last}, calculated as: AUC_{last} (Day 14)/AUC_{last} (Day 1)

The following table illustrates the TK parameters results in this study with the sexes combined and separately (from the Applicant's submission):

Table 1. TK Parameters of Naloxone Based on Plasma Concentrations in Dogs (Males and Females Combined) on Days 1 and 14

			Study Day	
			1 (n=10)	14 (n=10)
Group	Dose (mg)	Parameter	Mean	Mean
3	20	t_{max} (h)	0.250 (0.250-0.250)	0.250 (0.250-0.250)
		C_{max} (ng/mL)	292	232
		AUC_{last} (h*ng/mL)	180	143
4	160	t_{max} (h)	0.375 (0.250-0.500)	0.500 (0.250-1.00)
		C_{max} (ng/mL)	1250	758
		AUC_{last} (h*ng/mL)	1250	1130
5	300	t_{max} (h)	0.500 (0.250-1.00)	0.750 (0.250-2.00)
		C_{max} (ng/mL)	983	990
		AUC_{last} (h*ng/mL)	2340	2320

Note: t_{max} presented as median (range)

Table 3. TK Parameters of Naloxone Based on Plasma Concentrations in Dogs (Males and Females Separately) on Days 1 and 14

				Gender	
				Female (n=5)	Male (n=5)
Group	Dose (mg)	Study Day	Parameter	Mean	Mean
3	20	1	t_{max} (h)	0.250 (0.250-0.250)	0.250 (0.250-0.250)
			C_{max} (ng/mL)	297	288
			AUC_{last} (h*ng/mL)	199	161
3	20	14	t_{max} (h)	0.250 (0.250-0.250)	0.250 (0.250-0.250)
			C_{max} (ng/mL)	198	265
			AUC_{last} (h*ng/mL)	148	138
4	160	1	t_{max} (h)	0.500 (0.250-0.500)	0.250 (0.250-0.500)
			C_{max} (ng/mL)	1400	1090
			AUC_{last} (h*ng/mL)	1430	1070
4	160	14	t_{max} (h)	0.250 (0.250-0.500)	0.500 (0.250-1.00)
			C_{max} (ng/mL)	864	651
			AUC_{last} (h*ng/mL)	1120	1130
5	300	1	t_{max} (h)	0.500 (0.250-1.00)	0.500 (0.250-1.00)
			C_{max} (ng/mL)	848	1120
			AUC_{last} (h*ng/mL)	1760	2920
5	300	14	t_{max} (h)	0.500 (0.250-2.00)	1.00 (0.250-1.00)
			C_{max} (ng/mL)	921	1060
			AUC_{last} (h*ng/mL)	2420	2220

Note: t_{max} presented as median (range)

The following table illustrates the effect of sex on naloxone exposure in dogs with the sexes combined (from the Applicant's submission):

Table 4. Assessment of Sex Effect on Naloxone Exposure in Dogs on Days 1 and 14

		Parameter			
		C_{max} Female/Male Ratio		AUC_{last} Female/Male Ratio	
		Day		Day	
		1	14	1	14
Group	Dose (mg)	Value			
3	20	1.03	0.749	1.24	1.07
4	160	1.29	1.33	1.33	0.995
5	300	0.758	0.870	0.603	1.09

Note: Female/Male ratios based on mean C_{max} and AUC_{last}

The following table illustrates the dose proportionality of naloxone exposure in dogs with the sexes combined (from the Applicant's submission):

Table 2. Assessment of Dose Proportionality of Naloxone Exposure in Dogs (Males and Females Combined) on Days 1 and 14

Day	Dose Range (mg)	Dose Ratio (High Dose/ Low Dose)	C_{max} Ratio (High Dose/ Low Dose)	AUC_{last} Ratio (High Dose/ Low Dose)
1	20-160	8.00	4.26	6.97
	160-300	1.88	0.790	1.87
	20-300	15.0	3.36	13.0
14	20-160	8.00	3.27	7.86
	160-300	1.88	1.31	2.06
	20-300	15.0	4.27	16.2

Note: Ratios based on mean values

After intranasal administration, naloxone was rapidly absorbed into the systemic circulation and maximum plasma concentrations were observed between 0.250 h and 0.500 h on Day 1 and between 0.250 h and 0.750 h on Day 14.

Total exposure to naloxone (AUC_{last}) increased in a proportional manner with an increase in dose from 20 to 300 mg/day. For AUC_{last}, there was a 15.0-fold increase in dose from 20 mg to 300 mg/day which resulted in a 13.0-fold increase on Day 1 and a 16.2-fold increase on Day 14. The proportional increase over the 20 to 300 mg/day dose range was also apparent in the relatively flat slope of the regression line in the AUC_{last}/Dose vs Dose plots. Mean ratios of AUC_{last} for Day 14/Day 1 ranged from 0.913 to 1.31, indicating little to no accumulation during multiple dosing.

With the exception of Cmax after 160 mg/day naloxone, mean Cmax values were similar on Days 1 and 14. On Day 1, mean Cmax ranged from 292 ng/mL after 20 mg/day to 1250 ng/mL after 160 mg/day and on Day 14, mean Cmax ranged from 232 ng/mL after 20 mg/day to 990 ng/mL after 300 mg/day.

Maximum exposure to naloxone (Cmax) increased in a less than proportional manner with an increase in dose from 20 to 300 mg/day. For Cmax, a 15.0-fold increase in dose from 20 mg to 300 mg/day resulted in only a 3.36-fold increase on Day 1 and a 4.27-fold increase on Day 14. The less than proportional increase over the 20 to 300 mg/day dose range was also apparent in the negative slope of the regression line in the Cmax/Dose vs Dose plots. It is notable that inter-animal variability in Cmax/Dose after 20 mg/day is greater than that reported for the mid and high naloxone doses and the t_{max} reported across groups varied with dose. A slight delay in t_{max} reported for the high dose group may be a function of extended drug delivery rather than a delay in drug absorption. As stated previously, plasma samples were collected at times when dosing was still ongoing and t_{max} and Cmax values may not be comparable across dosing groups, therefore, an assessment of dose proportionality for Cmax may not be meaningful; t_{max} and Cmax values should be interpreted with caution.

Although differences in the TK parameters between males and females were present, no consistent sex-related trends in naloxone exposure were observed across naloxone doses and study days. In general, the trends noted for combined data (males and females) were observed when the data were stratified by gender.

Based on Female/Male (F/M) ratios of mean AUC_{last} and Cmax, differences in the TK profile of naloxone were observed between genders, but the differences were not consistent across dose level or study day. $RAUC_{last}$ (F/M) ranged from 0.603 to 1.33 and Rcmax (F/M) ranged from 0.749 to 1.33.

Dosing Solution Analysis

As the Applicant supplied the naloxone formulation and vehicle control as well as actuators, the Applicant provided documentation on the identity, strength, purity, composition, and stability for the naloxone formulations and vehicle control. No dosing solution analysis was performed at the testing facility on the naloxone formulation and vehicle control. Analysis of the saline control was performed at the testing facility.

Study title: A 28-day Study of Naloxone HCl by Intranasal Administration in Rats with a 19-day Recovery Period

Study no.:	20246992
Study report location:	EDR link
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 13, 2020
GLP compliance:	Yes. Signature provided on September 17, 2021.
QA statement:	Yes. Signature provided on September 17, 2021.
Drug, lot #, and % purity:	Naloxone HCl (b) (4) mg/mL); Lot No. NBDEV070-183; 101% pure (provided from Summit Biosciences Inc.)

Key Study Findings

- Sprague Dawley rats were administered saline control, vehicle control, 1.8 mg/day naloxone, 18.2 mg/day naloxone, 36.4 mg/day naloxone, or 36.4 mg/day naloxone with degradants intranasally for 28 consecutive days followed by a 19-day recovery period (no treatment).
- The rat doses are expressed as mg/day. Using an average rat weight of 0.325 kg, these doses can be expressed as mg/kg/day. As such, the rat doses are 5.54 (1.8/0.325 = 5.54), 56 (18.2/0.325 = 56), 112 mg/kg/day (36.4/0.325 = 112), and 112 mg/kg/day with degradants, respectively.
- Nasal irritation in nasal cavity and findings in its surrounding tissues were observed at doses of 18.2 mg/day and above. An acceptable LOAEL is the high dose of 36.4 mg/day after instillations twice daily per nostril, which results in a concentration margin of 1x as the concentration of naloxone HCl used in this study and in the clinical formulation are both (b) (4) mg/mL. Microscopic findings

present in HD rats were slightly increased in incidence but minimal in severity and were absent after the recovery period.

- A systemic NOAEL was the high dose of 36.4 mg/day or 112 mg/kg/day based no treatment-related microscopic effects outside of the nasal cavity and its surrounding tissues and is equivalent to 1084 mg HED, which confers an exposure margin of 54x based on a body surface area comparison using an average human weighing 60 kg.
- The effects of the degradants [redacted] (b) (4) with high dose naloxone were not worse than high dose naloxone alone.

Methods

Doses:	Saline control, vehicle control, 1.8, 18.2, 36.4, and 36.4 (with degradants) mg/day
Frequency of dosing:	Twice daily (saline and vehicle controls, high dose and high dose with degradants; 4 h apart) or once daily (low- and mid-dose) for 28 consecutive days
Route of administration:	Intranasal
Dose volume:	400, 400, 20, 200, 400, and 400 mcL/day total volume, respectively
Formulation/Vehicle:	0.9% saline control; Naloxone HCl placebo vehicle control provided from the Applicant
Species/Strain:	Rats/Crl:CD(SD) Sprague Dawley rats
Number/Sex/Group:	10/sex/group (main study); 5/sex/group (recovery); 3/sex/group (toxicokinetics controls); 6/sex/group (toxicokinetics Groups 3-6)
Age:	8 weeks old at dose initiation
Weight:	189 to 321 g for males and 166 to 232 g for females at dose initiation
Satellite groups:	Toxicokinetics groups
Unique study design:	The main study and recovery study as well as the toxicokinetics study contains Group 6, which is the naloxone formulation with degradants (see table below). An olfactory functional assessment was included in this study.
Deviations from study protocol:	Deviations did not affect the interpretation of the results in this study

The following table illustrates the study design of the main study and recovery groups (from the Applicant's submission):

Text Table 8
Experimental Design – Main and Recovery

Group No.	Test Material	Doses/nostril/day[a]	Dose Level Naloxone HCl (mg/day)	Dose Volume[b] (µL/nostril/ dose)	Total Volume (µL/day)	Dose Concentration Naloxone HCl (mg/mL)	Animal Numbers			
							Main Study		Recovery Study	
							M	F	M	F
1	Saline Control[c]	2	0	100	400	0	1001-1010	1501-1510	1011-1015	1511-1515
2	Vehicle Control[d]	2	0	100	400	0	2001-2010	2501-2510	2011-2015	2511-2515
3[e]	SN(7032)	1	1.8	10	20	(b) (4)	3001-3010	3501-3510	-	-
4[e]	SN(7032)	1	18.2	100	200		4001-4010	4501-4510	-	-
5[e]	SN(7032)	2	36.4	100	400		5001-5010	5501-5510	5011-5015	5511-5515
6[f]	SN(7032)-SPU	2	36.4	100	400	(b) (4) + degradants	6001-6010	6501-6510	6011-6015	6511-6515

M = Males, F = Females; - = Not applicable.
[a] Because of dosing volume considerations, Groups 1, 2, 5, and 6 animals were dosed twice daily and Groups 3 and 5 were dosed once daily from Days 1 to 28/29.
[b] Dose volume was administered into each nostril.
[c] 0.9% Sodium Chloride for Injection, USP.
[d] SN(7032) Vehicle Placebo (clinical formulation without naloxone API).
[e] Groups 3 to 5 were low, medium, and high doses of the clinical formulation.
[f] Group 6 was the clinical formulation including degradants produced during aging.

It is noted that Groups 3 and 4 (low- and mid-dose naloxone dose groups) were not included in the recovery phase. The rat doses are expressed in mg/day and did not consider the weight of the rats. When considering the rat weights (average of 0.325 kg from the body weight data), these doses are 5.54 (1.8/0.325 = 5.54), 56 (18.2/0.325 = 56), 112 (36.4/0.325 = 112) mg/kg/day, and 112 mg/kg/day with degradants, respectively. These values are used to calculate a Human Equivalent Dose (HED), which are 53.6, 542, 1084, and 1084 with degradants mg/kg/day, respectively, and confers margins based on HED body surface area of 2.7x, 27x, 54x, and 54x with degradants, respectively, using the maximum daily dose of 20 mg/day of the proposed product.

The following table illustrates the study design of the toxicokinetics groups (from the Applicant's submission):

Text Table 9
Experimental Design – Toxicokinetic

Group No.	Test Material	Doses/nostril/day[a]	Dose Level Naloxone HCl (mg/day)	Dose Volume[b] (µL/nostril/ dose)	Total Volume (µL/day)	Dose Concentration Naloxone HCl (mg/mL)	Animal Numbers Toxicokinetic Study Males	Females
1	Saline Control[c]	2	0	100	400	0	1016-1018	1516-1518
2	Vehicle Control[d]	2	0	100	400	0	2016-2018	2516-2518
3[e]	SN(7032)	1	1.8	10	20	(b) (4)	3011-3015, 3116	3511-3516
4[e]	SN(7032)	1	18.2	100	200		4011-4016	4511-4516
5[e]	SN(7032)	2	36.4	100	400		5016-5021	5516-5521
6[f]	SN(7032)-SPU	2	36.4	100	400	(b) (4) + degradants	6016-6021	6516-6521

[a] Because of dosing volume considerations, Groups 1, 2, 5, and 6 animals were dosed twice daily and Groups 3 and 5 were dose once daily from Days 1 to 28/29.
[b] Dose volume was administered into each nostril.
[c] 0.9% Sodium Chloride for Injection, USP.
[d] SN(7032) Vehicle Placebo (clinical formulation without naloxone API).
[e] Groups 3 to 5 were low, medium, and high doses of the clinical formulation.
[f] Group 6 was the clinical formulation including degradants produced during aging.

As shown in the table above, the rat doses are expressed in mg/day. Using an average rat weight of 0.325 kg, these doses can be expressed in mg/kg/day. As such, the rat doses are 5.54 (1.8/0.325 = 5.54), 56 (18.2/0.325 = 56), 112 (36.4/0.325 = 112) mg/kg/day, and 112 mg/kg/day with degradants, respectively.

Group 6, which is the naloxone formulation ((b) (4) mg/mL) with degradants, of which the concentration of each degradant is illustrated in the following table (from the Applicant's submission):

Text Table 3
SN(7032)-SPU Degradant – Concentrations

SN(7032)-SPU Degradant	Concentration (%)	Concentration (mg/mL)
(b) (4)		

Group 6 (36.4 mg/day naloxone with degradants) was administered using a total daily volume of 400 mcL/day. At a concentration of 0.34 mg/mL and using the daily volume of

400 mcL/day, the exposure to [redacted] (b) (4) For the conversion to a human dose, an average rat weight from this study is 0.325 kg is used, which confers an exposure to [redacted] (b) (4)). This confers a human equivalent dose (HED) of 4.05 mg [redacted] (b) (4)

Observations and Results

The following table illustrates the in-life observations performed during this study (from the Applicant's submission):

Text Table 10
General In-life Assessments

Parameter	Population(s)	Frequency (minimum required)	Comments
Mortality	All Main, Recovery, and Toxicokinetic Study animals	At least twice daily[a] (morning and afternoon) beginning upon arrival through termination.	Animals were observed within their cage unless removal was necessary for identification or confirmation of possible findings.
Cage Side Observations	All Main, Recovery, and Toxicokinetic Study animals	At least once daily; from Week -1 and throughout the study (for deviation, see Appendix 1). Cage side observations were not required on the days of detailed clinical observations during the pretreatment (prior to Day 1) and recovery periods, when a postdose observation was recorded, or on the day of scheduled euthanasia.	Animals were observed within their cage unless removal was necessary for identification or confirmation of possible findings.

Postdose Observations	All Main and Recovery Study animals	At least twice daily during the dosing period; prior to the first daily dose (for deviation, see Appendix 1) and 1 to 3 hours post each daily dose (for deviation, see Appendix 1).	Animals were observed within their cage unless removal was necessary for identification or confirmation of possible findings.
Detailed Clinical Observations	All Main and Recovery Study animals	At least once weekly; from Week -1 and throughout the study.	Animals were removed from the cage.
Individual Body Weights	All Main, Recovery, and Toxicokinetic Study animals	At least once weekly; from Week -1 and throughout the study.	A fasted weight was recorded for main and recovery study animals on the day of scheduled euthanasia. Body weights were not collected from animals euthanized moribund.
Food Consumption	All Main and Recovery Study animals	Weekly; from at least Day 1 and throughout the study (for deviation, see Appendix 1).	Quantitatively measured, except for on the day of scheduled euthanasia.

[a] Except on days of receipt and euthanasia where frequency was once daily.

Mortality

There was an unscheduled sacrifice (Animal No. 5503) in 1 female from Group 5 (high dose naloxone). A uterine mass consistent with an endometrial stromal polyp was interpreted as the underlying cause of clinical signs (red fur staining at the urogenitial area and liquid red material present in the urogenital area as well as in the cage) and hematology (markedly low erythrocytes, hemoglobin, and hematocrit that was consistent with bleeding) and was considered to be unrelated to naloxone-treatment, according to the Pathology Report. This Reviewer concurs with the conclusions of the Pathology Report.

Clinical Signs

Clinical signs observed during the dosing and recovery phases were consistent with background findings in this age and strain of rats (e.g., thin fur cover and skin scabs), related to stress of dosing (e.g., red staining of the fur), and/or were seen across control and naloxone treatment groups and were, therefore, not considered treatment-related according to the Pathology Report. This Reviewer concurs with the conclusions of the Pathology Report.

Body Weights

There were no treatment-related changes in absolute body weights in both males and females during the dosing and recovery phases.

In males, there was a decrease in the body weight gains during the last week of the dosing period (Days 22-28) in the 36.4 mg/day dose group by 21.6% compared to vehicle control but not compared to saline control. There were no treatment-related changes in body weight gain during the entire dosing period (Days 1-28), recovery

period (Days 28-46), or study (Days 1-46). In the 36.4 mg/day with degradant group, there were decreases in the body weight gain during the last week of the dosing period (Days 22-28) by 38.5% compared to the vehicle control and there were increases during Days 28-36, 36-43, and 28-46 by 47.1, 47, and 37.1%, respectively, compared to vehicle control as well as by 30.6 and 21.3% compared to the 36.4 mg/day dose group during Days 28-36 and 28-46, respectively.

In females, there was an increase in body weight gain during Days 15-22 by 71.9% in the 36.4 mg/day with degradant dose group compared to the 36.4 mg/day dose group. There were decreases in the body weight gain during Days 22-28 in the 36.4 mg/day with degradant dose group by 1788% compared to vehicle control and by 305% compared to the 36.4 mg/day dose group; however, there were no treatment-related changes during the entire dosing period (Days 1-28).

In the Pathology Report, the changes in body weight gains were not toxicologically meaningful due to the degree of these differences. This Reviewer concurs with the conclusions of the Pathology Report.

Food Consumption

In males, there were decreases in food consumption on Days 1-8, 8-15, and Days 22-28 by 10.6, 8.01, and 6.22%, respectively, compared to vehicle control during the dosing period with no such changes compared to the saline control. There were increases in food consumption on Days 36-43 and 43-46 by 7.75 and 3.61%, respectively, compared to vehicle control during the recovery period with no such changes compared to saline control. Food consumption in the 36.4 mg/day with degradant dose group was decreased on Days 22-28 by 7.54% compared to vehicle control during the dosing period and were increased on Days 28-36, 36-43, and 43-46 by 9.38, 8.01, and 13.3%, respectively, compared to vehicle control during the recovery period with increases by 6.46, 0.238, and 9.33%, respectively, compared to the 36.4 mg/day dose group.

In females, food consumption in the 36.4 mg/day dose group was decreased by 12.7% compared to the vehicle control on Days 1-8 during the dosing period. Food consumption on Days 1-8 during the dosing period was increased in the 36.4 mg/day with degradant dose group by 17.5% compared to the 36.4 mg/day dose group with no such changes compared to the vehicle control.

In the Pathology Report, changes in food consumption corresponded to differences in body weight and body weight gains and were not toxicologically meaningful due to the degree of difference and similarities to the saline control groups. This Reviewer concurs with the conclusions of the Pathology Report.

Ophthalmoscopy

Ophthalmological exams were performed once during pretreatment (Day -4) and during the last week of dosing (Day 26). There were no treatment-related changes in the ophthalmoscopic examinations.

ECG

An ECG was not performed.

Hematology

Blood was collected from the vena cava for hematology, coagulation, and clinical chemistry and urine was collected overnight by urine collection cages for urinalysis according to the schedule illustrated in the following table (from the Applicant's submission):

Text Table 11
Samples for Clinical Pathology Evaluation

Group Nos.	Time Point	Hematology	Coagulation	Clinical Chemistry	Urinalysis
1 to 6[a]	Day 29/30	X	X	X	X
1, 2, 5, 6 (Recovery study animals only)	Day 47	X	X	X	X
Unscheduled euthanasia		X	X	X	-
Fasting[b]:		-	-	Min. of 4 hrs.	Min. of 4 hrs.
Method/Comments:		Venipuncture of the Vena Cava[c]	Venipuncture of the Vena Cava[c]	Venipuncture of the Vena Cava[c]	Urine was collected by urine collection cages containing an alternate water source.
Target Volume (mL):		2	1.8	2	-
Anticoagulant:		(K_2) EDTA	Sodium citrate	None	-
Special Requirements:		-	-	-	-
Processing:		None	Plasma	Serum	-

X = Sample collected; - = Not applicable; Min. = Minimum; hrs. = hours.
[a] Samples were only collected from those animals scheduled for euthanasia on Day 29/30.
[b] For scheduled collection only.
[c] Collected under isoflurane anesthesia.

The following hematology and coagulation parameters were measured (from the Applicant's submission):

Text Table 12
Hematology Parameters

Red blood cell count Hemoglobin concentration Hematocrit Mean corpuscular volume Red blood cell distribution width Mean corpuscular hemoglobin concentration Mean corpuscular hemoglobin Reticulocyte count (absolute) Platelet count	White blood cell count Neutrophil count (absolute) Lymphocyte count (absolute) Monocyte count (absolute) Eosinophil count (absolute) Basophil count (absolute) Large unstained cells (absolute) Other cells (as appropriate)

Text Table 13
Coagulation Parameters

Activated partial thromboplastin time Fibrinogen	Prothrombin time Sample quality

The following table illustrates the changes in hematology and coagulation at the end of the dosing period (data from the Applicant's submission):

Observation	Saline Control	Vehicle Control	1.8 mg/day Naloxone	18.2 mg/day Naloxone	36.4 mg/day Naloxone	36.4 mg/day Naloxone w/ Degradants
Dosing Period (Day 29/30)						
Males						
WBC (10^3/mcL)	6.765	7.628	8.179	7.868	**10.202[a] (33.7%)**	**7.851[d] (-23.0%)**
Lymphocyte (10^3/mcL)	5.293	6.249	6.464	6.261	**8.407[a] (34.5%)**	**6.245[d] (-25.7%)**
Eosinophil (10^3/mcL)	0.046	0.054	0.060	0.053	**0.081[a] (50.0%)**	0.064

a = Significantly different from vehicle control ($p \leq 0.05$) with the T-test
d = Significantly different from the 36.4 mg/day group ($p \leq 0.05$) with the T-test

In males, there were increases in white blood cells (WBC), lymphocytes, and eosinophils by 33.7, 34.5, and 50%, respectively, in the 36.4 mg/day dose group compared to vehicle control at the end of the dosing phase. There were decreases in WBC and lymphocytes by 23.0 and 25.7%, respectively, in the 36.4 mg/day with degradants dose group compared to the 36.4 mg/day dose group at the end of the dosing phase; however, these changes were not different compared to either the saline or vehicle controls. These changes likely indicate immune responses to the nasal irritation of the test compound.

There were no further treatment-related changes in hematology at the end of the dosing period in males. There were no treatment-related changes in hematology in females at the end of the dosing and recovery periods.

In the Pathology Report, these changes were not considered treatment-related due to biological variation, inconsistency of the changes, their low magnitude, and/or lack of a dose response.

There were no treatment-related changes in coagulation in males and females at the end of the dosing period.

The following table illustrates the changes in coagulation in males at the end of the recovery period (data from the Applicant's submission):

Observation	Saline Control	Vehicle Control	36.4 mg/day Naloxone	36.4 mg/day Naloxone w/ Degradants
Recovery Period (Day 47)				
Males				
APTT (sec)	16.72	14.70	17.30	**14.28[d] (-17.5%)**

d = Significantly different from the 36.4 mg/day group ($p \leq 0.05$) with the T-test

There was a decrease in APTT by 17.5% in the 36.4 mg/day with degradants dose group compared to the 36.4 mg/day dose group in recovery males; however, these changes were not different compared to either the saline or vehicle controls and are within normal ranges (Derelanko, 2008). There were no further treatment-related changes in coagulation in recovery males.

There were no treatment-related changes in coagulation in recovery females.

In the Pathology Report, the changes in coagulation are not considered toxicologically relevant.

This Reviewer concurs with the conclusions of the Pathology Report regarding the hematology and coagulation parameters.

Clinical Chemistry

The following clinical chemistry parameters were measured (from the Applicant's submission):

Text Table 14
Clinical Chemistry Parameters

Alanine aminotransferase	Total protein
Aspartate aminotransferase	Albumin
Alkaline phosphatase	Globulin (calculated)
Gamma-glutamyltransferase	Albumin/globulin ratio
Creatine kinase	Glucose
Total bilirubin	Cholesterol
Urea nitrogen	Triglycerides
Creatinine	Sodium
Calcium	Potassium
Phosphorus	Chloride
	Sample quality

There were no treatment-related changes in clinical chemistry during the study.

Urinalysis

The following urinalysis parameters were measured (from the Applicant's submission):

Text Table 15
Urinalysis Parameters

Color	Protein
Appearance/Clarity	Glucose
Specific gravity	Bilirubin
Volume	Ketones
pH	Blood

There were no treatment-related changes in urinalysis during the study.

Gross Pathology

On the day of scheduled sacrifice for the treatment and recovery periods on Day 29/30 and Day 47, respectively, all surviving rats were weighed and a complete necropsy examination was performed. Necropsy included evaluation of the carcass and musculoskeletal system, all external surfaces and orifices, cranial cavity and external surfaces of the brain, and examination of cavities (thoracic, abdominal, and pelvic cavities) with their associated organs and tissues.

The following table illustrates the changes in gross pathology at the end of the treatment period (data from the Applicant's submission):

Observation	Saline Control	Vehicle Control	1.8 mg/day Naloxone	18.2 mg/day Naloxone	36.4 mg/day Naloxone	36.4 mg/day Naloxone w/ Degradants
Treatment Period (Day 29/30)						
Males						
Thymus --Focus, dark	0/10	0/10	0/10	2/10	0/10	0/10

--Discoloration, dark	2/10	0/10	0/10	1/10	0/10	**4/10**
Females						
Thymus --Focus, dark --Discoloration, dark	 0/10 0/10	 0/10 1/10	 1/10 0/10	 3/10 1/10	 1/10 0/10	 1/10 0/10
Recovery Period (Day 47)						
Males						
Thymus --Focus, dark --Discoloration, dark	 0/5 0/5	 0/5 1/5	 -- --	 -- --	 1/5 0/5	 1/5 0/5
Females						
Thymus --Focus, dark --Discoloration, dark	 0/5 0/5	 0/5 0/5	 -- --	 -- --	 0/5 0/5	 0/5 0/5

In the thymus, dark focus was noted in the male 18.2 mg/day naloxone dose group (2/10) as well as in all the female naloxone groups (1/10, 3/10, 1/10, and 1/10 in the low-, mid-, high-, and high dose with degradants, respectively) compared to none in either control group (saline and vehicle). Dark focus in the thymus at the end of the recovery period was observed in 1/5 rats each in the male high dose and high dose with degradants dose groups compared to none in either control. Dark focus in the thymus was not observed in the recovery females. Dark focus in the thymus was not present in the recovery naloxone treatment groups.

Dark discoloration of the thymus at the end of the dosing period was observed in 1/10 each from the 18.2 mg/day naloxone dose group in males and females as well as 4/10 males from the high dose with degradants dose group compared to 2/10 in the male saline control and 1/10 in the female vehicle control.

There were no direct microscopic correlate for these macroscopic changes in the thymus in females. In males, the microscopic finding in the thymus (minimal increased apoptosis in the lymphoid tissue of the cortex) was observed in lower incidence in the high dose with degradants dose group (1/10 rats) and as such, is not likely correlated. In the Pathology Report, the gross findings observed at the end of the dosing and recovery phases were considered incidental, of the nature commonly observed in this strain and age of rats, and/or were of similar incidence in control (saline and vehicle) and treated rats, and therefore, were not considered treatment-related.

This Reviewer concurs with the conclusions of the Pathology Report.

Organ Weights

The following organs were weighed (from the Applicant's submission):

Text Table 20
Organs Weighed at Necropsy

Brain	Kidney[a]
Epididymis[a]	Liver
Gland, adrenal[a]	Ovary[a]
Gland, pituitary	Spleen
Gland, prostate	Testis[a]
Gland, thyroid/Gland, parathyroid[a]	Thymus
Heart	Uterus/Cervix

[a] Paired organ weight.

There were no treatment-related changes in absolute organ weights and organ-to-brain-weight ratio in this study.

There were no treatment-related changes in the organ-to-body-weight ratio in males in this study.

The following table illustrates the changes in the organ-to-body-weight ratio in females at the end of the dosing period (data from the Applicant's submission):

Organ-to-Body-Weight Ratio	Saline Control	Vehicle Control	1.8 mg/day Naloxone	18.2 mg/day Naloxone	36.4 mg/day Naloxone	36.4 mg/day Naloxone w/ Degradants
Adrenal Gland (%)	0.02459	0.02549	0.02535	0.02584	0.02851[a] (11.8%)	0.02691
Pituitary Gland (%)	0.00642	0.00679	0.00656	0.00680	0.00761	0.00628[b] (-17.8%)
Thymus (%)	0.16740	0.17043	0.18723	0.15615	0.13777[a] (-19.2%)	0.16863[c] (22.4%)

a = significantly different from the vehicle control $p \leq 0.05$ (T-test)
b = significantly different from the 36.4 mg/day dose group $p \leq 0.01$ (T-test)
c = significantly different from the 36.4 mg/day dose group $p \leq 0.05$ (T-test)

In females, there was an increase in the adrenal gland-to-body-weight ratio by 11.8% in the 36.4 mg/day naloxone group compared to vehicle control. There was a decrease in the pituitary gland-to-body-weight ratio by 17.8% in the 36.4 mg/day naloxone with degradants dose group compared to the 36.4 mg/day naloxone dose group. There was a decrease in the thymus-to-body-weight ratio by 19.2% in the 36.4 mg/day naloxone dose group compared to vehicle control. Moreover, there was an increase in the thymus-to-body-weight ratio by 22.4% in the 36.4 mg/day naloxone with degradants dose group compared to the 36.4 mg/day naloxone dose group that was not different from the vehicle control group. These changes were without any microscopic correlates.

There were no treatment-related changes in the organ-to-body weight ratio in females at the end of the recovery period.

In the Pathology Report, there were no patterns, trends, or correlating data to suggest that the changes in the organ weight parameters (absolute organ weight, organ-to-body-

weight ratio, organ-to-brain-weight ratio) were toxicologically relevant and as such, they were considered incidental and/or related to difference of sexual maturity and not treatment-related.

This Reviewer concurs with the conclusions of the Pathology Report.

Histopathology

Adequate Battery
The following tissues were collected and preserved from the necropsy and then examined microscopically (from the Applicant's submission):

Text Table 21
Tissue Collection and Preservation

Animal identification[a]	Large intestine, cecum
Artery, aorta	Large intestine, colon
Body cavity, nasal[b]	Large intestine, rectum
Bone marrow, sternum	Larynx
Bone marrow smear[c]	Liver
Bone, femur	Lung
Bone, sternum	Lymph node, tracheobronchial
Brain[e]	Lymph node, mandibular
Epididymis[d]	Lymph node, mesenteric
Esophagus	Muscle, skeletal
Eye[d]	Nerve, optic[d]
Ganglion, dorsal root, lumbar[a]	Nerve, sciatic
Gland, adrenal	Nerve, tibial[a]
Gland, clitoral[a]	Ovary
Gland, lacrimal[a]	Oviduct[a]
Gland, Harderian	Pancreas
Gland, mammary	Skin
Gland, parathyroid	Small intestine, duodenum
Gland, pituitary	Small intestine, ileum
Gland, preputial[a]	Small intestine, jejunum
Gland, prostate	Spinal cord
Gland, salivary, submandibular	Spleen
Gland, salivary, sublingual[a]	Stomach
Gland, salivary, parotid[a]	Testis[d]
Gland, seminal vesicle	Thymus
Gland, thyroid	Tongue
Gland, Zymbal's[a]	Trachea
Gut-associated lymphoid tissue[e]	Ureter[a]
Heart	Urinary bladder
Joint, femorotibial	Uterus/Cervix
Kidney	Vagina

[a] Tissues were collected and preserved but were not histologically processed or microscopically evaluated.
[b] Included nasopharynx and oropharynx (processed into 4 sections for evaluation).
[c] Two bone marrow smears were prepared from each euthanized animal (not refrigerated prior to necropsy) and air dried. Bone marrow smears were not fixed in formalin or evaluated.
[d] Eyes and optic nerves were preserved in Davidson's fixative. Testes and epididymides were preserved in Modified Davidson's fixative.
[d] From small intestine: Peyer's patch or solitary lymphoid follicle.
[e] Brain processed into 7 sections for evaluation.

This is an adequate set of tissues for histopathology.

Peer Review
Yes

Histological Findings
The following table illustrates the microscopic changes in, and in associated with, the respiratory tract at the end of the dosing period in males and females (data from the Applicant's submission)

Observation	Saline Control	Vehicle Control	1.8 mg/day Naloxone	18.2 mg/day Naloxone	36.4 mg/day Naloxone	36.4 mg/day Naloxone w/ Degradants
Dosing Period (Day 29/30)						
Males						
Body Cavity, Nasal						
--Inflammation, neutrophilic; olfactory epithelium						
Minimal	0/10	0/10	0/10	0/10	**2/10**	0/10
--Infiltration, neutrophilic; olfactory epithelium						
Minimal	0/10	0/10	0/10	**1/10**	**3/10**	0/10
--Erosion/ulcer; olfactory epithelium						
Minimal	0/10	0/10	0/10	0/10	**2/10**	**3/10**
--Infiltration, lymphocytic; olfactory epithelium						
Mild	0/10	0/10	0/10	0/10	0/10	**1/10**
--Degeneration; olfactory epithelium						
Minimal	0/10	0/10	0/10	**1/10**	**1/10**	0/10
Lung						
--Mineralization; vascular						
Minimal	--	2/10	--	--	**8/10**	**6/10**
--Infiltration, mixed cell; perivascular						
Minimal	--	0/10	--	--	**1/10**	0/10
Mild	--	0/10	--	--	0/10	0/10
--Alveolar macrophages, increased						
Minimal	--	0/10	--	--	0/10	0/10
--Metaplasia; osseous						
Minimal	--	0/10	--	--	0/10	1/10
Lymph Node, Mandibular						
--Cellularity, increased; lymphoid, medullary sinus						
Mild	--	0/10	--	0/1	0/10	0/10
--Cellularity, increased; plasma cell; medullary sinus						
Mild	--	3/10	--	0/1	**5/10**	3/10
Moderate	--	0/10	--	**1/1**	0/10	0/10
--Congestion; medullary sinus						
Minimal	--	1/10	--	0/1	0/10	1/10
Lymph Node, Tracheobronchial						
--Pigmented macrophage; medullary sinus						

Minimal	--	3/10	--	--	1/10	0/10
--Congestion; medullary sinus						
Minimal	--	0/10	--	--	0/10	1/10
--Cellularity, increased; lymphoid, germinal center						
Minimal	--	0/10	--	--	1/10	1/10
Mild	--	1/10	--	--	0/10	0/10
Females						
Body Cavity, Nasal						
--Inflammation, neutrophilic; olfactory epithelium						
Minimal	0/10	0/10	0/10	0/10	0/9	0/10
--Infiltration, neutrophilic; olfactory epithelium						
Minimal	0/10	0/10	0/10	0/10	0/9	**1/10**
--Erosion/ulcer; olfactory epithelium						
Minimal	0/10	0/10	0/10	0/10	0/9	0/10
--Infiltration, lymphocytic; olfactory epithelium						
Mild	0/10	0/10	0/10	0/10	0/9	0/10
--Degeneration; olfactory epithelium						
Minimal	1/10	0/10	0/10	**2/10**	0/9	0/10
Lung						
--Mineralization; vascular						
Minimal	--	2/10	--	--	**4/9**	**5/10**
--Infiltration, mixed cell; perivascular						
Minimal	--	1/10	--	--	1/9	**2/10**
Mild	--	0/10	--	--	0/9	**1/10**
--Alveolar macrophages, increased						
Minimal	--	0/10	--	--	**2/9**	**1/10**
--Metaplasia; osseous						
Minimal	--	0/10	--	--	0/9	0/10
Lymph Node, Mandibular						
--Cellularity, increased; lymphoid, medullary sinus						
Mild	--	1/10	--	--	0/9	0/10
--Cellularity, increased; plasma cell; medullary sinus						
Mild	--	1/10	--	--	0/9	**2/10**
Moderate	--	0/10	--	--	0/9	0/10
--Congestion; medullary sinus						
Minimal	--	0/10	--	--	0/9	0/10
Lymph Node, Tracheobronchial						
--Pigmented macrophage; medullary sinus						
Minimal	--	4/10	--	--	3/10	3/10
--Congestion; medullary sinus						
Minimal	--	2/10	--	--	1/10	1/10
--Cellularity, increased; lymphoid, germinal center						
Minimal	--	0/10	--	--	0/10	0/10
Mild	--	0/10	--	--	0/10	0/10

In the nasal cavity, signs of nasal irritation and healing (inflammatory response) were observed in the 18.2 and 36.4 mg/day naloxone dose groups as well as the 36.4 mg/day naloxone with degradants and includes minimal neutrophilic inflammation, minimal neutrophilic infiltration, minimal erosion/ulcer, and mild lymphocytic infiltration of the olfactory epithelium in males and minimal neutrophilic infiltration of the olfactory epithelium in the female 36.4 mg/day naloxone with degradant dose group. Moreover, minimal degeneration of the olfactory epithelium was observed in 1/10 males and 2/10 females in the 18.2 mg/day naloxone dose groups and 1/10 in the male 36.4 mg/day naloxone dose group with 1/10 females from the saline control.

According to the Pathology Report, minimal, focal to rarely multifocal changes in the nasal turbinates were present in 1/20 Group 1 rats, 4/20 Group 4 rats, 6/20 Group 5 rats, and 4/20 Group 6 rats. Findings were more common in males (11 total rats between Groups 4, 5, and 6) than females (4 total rats between Groups 1, 4, and 6). Similar microscopic findings were not identified in Group 2 (vehicle control) or Group 3 (low dose naloxone group).

These nasal turbinate findings were limited to the olfactory epithelium (most often in levels 3 [second palatine crest, includes paranasal sinus] and 4 [first molar, includes pharyngeal duct/nasopharynx]) which consisted of minimal epithelial degeneration, minimal neutrophilic infiltration, mild lymphocytic infiltration, and minimal erosion/ ulceration. The neutrophilic and lymphocytic infiltration were indicative of local inflammation. Findings were most common in the dorsal turbinates.

Furthermore, while 1 control female had a nasal cavity change (focal olfactory epithelial degeneration), the higher incidence of microscopic findings in the 14 treated animals (Groups 4, 5, and 6) suggests a possible treatment-related effect. These local changes were of low severity and generally focal changes and were not dose-dependent. These were completely reversible at the end of the recovery period and were considered non-adverse (no clinical findings or differential microscopic changes in other tissues). The incidence of affected animals was similar in the 36.4 mg/day naloxone with degradants and 36.4 mg/day naloxone dose groups; therefore, the presence of the degradants did not alter the microscopic findings.

The Applicant examined microscopically tissues from the vehicle control, 36.4 mg/day naloxone and 36.4 mg/day naloxone with degradants outside of the nasal cavity. Minimal vascular mineralization of the lungs in both males and females were observed (2/10 each in the male and female vehicle controls, 8/10 males and 4/9 females from the 36.4 mg/day naloxone dose group, and 6/10 males and 5/10 females from the 36.4 mg/day naloxone with degradants dose groups). Moreover, minimal osseous metaplasia was observed in 1/10 males from the 36.4 mg/day naloxone with degradants dose group only. The remaining microscopic changes in tissues surrounding the nasal cavity are signs of an inflammatory response to healing and including changes in the lung (perivascular mixed cell infiltration, and increased alveolar macrophages), mandibular lymph node (mild increase lymphoid cellularity in the medullary sinus, minimal increased

plasma cell cellularity in the medullary sinus, and minimal congestion in the medullary sinus), and tracheobronchial lymph node (minimal pigmented macrophage in the medullary sinus, minimal congestion in the medullary sinus, and minimal and mild increased lymphoid cellularity in the germinal center) that was present sporadically in the vehicle control, 36.4 mg/day naloxone dose group, and 36.4 mg/day naloxone with degradants dose group. These adverse lung findings were not considered dose limiting as these findings were not present after the recovery period and were likely the result of the test-article affecting the lungs directly due to intranasal instillation of a solution and given that rats are obligate nose breathers. It is noted that lung findings were not present in dogs which used the intranasal spray.

The following table illustrates the systemic microscopic changes at the end of the dosing period in both males and females (data from the Applicant's submission):

Observation	Saline Control	Vehicle Control	1.8 mg/day Naloxone	18.2 mg/day Naloxone	36.4 mg/day Naloxone	36.4 mg/day Naloxone w/ Degradants
Dosing Period (Day 29/30)						
Males						
Heart						
--Cardiomyopathy	--	2/10	--	--	**4/10**	1/10
Minimal						
Kidney						
--Mineralization						
Minimal	--	0/10	--	--	**1/10**	0/10
--Infiltration, lymphocytic; perivascular	--	1/10	--	--	1/10	1/10
Minimal						
--Infiltration,	--	0/10	--	--	0/10	0/10
lymphocytic; pelvis	--	0/10	--	--	0/10	0/10
Minimal						
Mild	--	0/10	--	--	0/10	0/10
--Inflammation, neutrophilic; pelvis	--	1/10	--	--	**2/10**	**2/10**
Mild						
--Basophilia; tubular						
Minimal						
Spleen						
--Extramedullary hematopoiesis	--	0/10	--	--	1/10	1/10
Minimal						
Thymus						
--Apoptosis, increased; lymphoid, cortex	--	0/10	--	0/3	**1/10**	**1/10**
Minimal						
Urinary Bladder						

--Infiltration, neutrophilic; submucosal Minimal	--	0/10	--	--	0/10	0/10
Epididymis --Infiltration, mononuclear cell; perivascular Minimal	0/1	2/10	--	--	**4/10**	1/10
Prostate Gland --Infiltration, mononuclear cell; perivascular Minimal Mild Moderate	-- -- --	0/10 1/10 1/10	-- -- --	-- -- --	**4/10** -- --	1/10 -- --
Females						
Heart -- Cardiomyopathy Minimal	--	0/10	--	--	0/9	0/10
Kidney --Mineralization Minimal --Infiltration, lymphocytic; perivascular Minimal --Infiltration, lymphocytic; pelvis Minimal Mild --Inflammation, neutrophilic; pelvis Mild --Basophilia; tubular Minimal	-- -- -- -- -- --	1/10 1/10 1/10 1/10 1/10 1/10	-- -- -- -- -- --	-- -- -- -- -- --	1/9 0/9 **2/9** 1/9 0/9 1/9	0/10 1/10 0/10 1/10 0/10 1/10
Spleen -- Extramedullary hematopoiesis Minimal	--	1/10	--	--	0/9	0/10
Thymus --Apoptosis, increased; lymphoid, cortex Minimal	--	0/10	0/1	0/4	0/9	0/10
Urinary Bladder						

--Infiltration, neutrophilic; submucosal Minimal	--	0/10	--	--	**1/9**	0/10

It is noted that for these other microscopic changes, the majority of these findings were observed in the vehicle control, 36.4 mg/day naloxone dose group, and the 36.4 mg/day naloxone with degradants dose group with the occasional examination in the saline control as well as low- and mid dose naloxone groups (up to 4 rats per group). These other microscopic changes in the heart, kidney, spleen, thymus, urinary bladder, epididymis, and prostate gland were observed in lower incidences (4 or less) in the affected dose groups or were of similar incidence in the vehicle control.

In the Pathology Report, the other microscopic findings observed were considered incidental, of the nature commonly observed in this strain and age of rats, and/or were of similar incidence and severity in control (saline and vehicle) and treated animals (36.4 mg/day naloxone and 36.4 mg/day naloxone with degradants) and, therefore, were considered unrelated to administration of 36.4 mg/day naloxone or the degradants. Any apparent increase in incidence of minimal severity spontaneous changes in treated animals was interpreted as non-treatment related.

The following table illustrates the microscopic changes at the end of the recovery period (Day 47) in both males and females (from the Applicant's submission):

Summary of Microscopic Pathology: Study Phase - Recovery

20246992

Removal Reason(s): RECOVERY EUTHANASIA Summary: Incidence	Male				Female			
	0 mg/day Saline Control 1 Group 1	0 mg/day Veh. Control 1 Group 2	36.4 mg/day SN7032 Group 5	36.4 mg/day SN7032SPU Group 6	0 mg/day Saline Control 1 Group 1	0 mg/day Veh. Control 1 Group 2	36.4 mg/day SN7032 Group 5	36.4 mg/day SN7032SPU Group 6
Number of Animals:	5	5	5	5	5	5	5	5
BODY CAVITY, NASAL								
Examined	5	5	5	5	5	5	5	5
No Visible Lesions	5	5	5	5	5	5	5	5
OVARY								
Examined	.	.	.	.	0	1	0	0
Cyst	.	.	.	.	.	1	.	.
SKIN								
Examined	0	0	0	1	.	.	.	.
Ulceration	.	.	.	1	.	.	.	.
.... mild	.	.	.	1	.	.	.	.
Crust	.	.	.	1	.	.	.	.
.... moderate	.	.	.	1	.	.	.	.
THYMUS								
Examined	0	1	1	1	.	.	.	.
No Visible Lesions	.	1	1	1	.	.	.	.

In both males and females, all microscopic changes observed at the of the dosing period were not observed at the end of the recovery period with the exception of mild skin ulceration and moderate crust in the male 36.4 mg naloxone with degradants dose group, which was observed in 1 rat and it is noted that only 1 rat was examined with no examination in any other the other recovery groups and may be dismissed. The Pathology Report noted that none of the microscopic changes observed at the end of the dosing period was observed at the end of the recovery period.

Special Evaluation

An olfactory functional assessment was performed once pretreatment (Day -1), during the last week of dosing (Day 27), and at the end of the recovery period (Day 46).

At the end of the dosing phase (Day 27), a single female (Animal No. 6515) from 36.4 mg/day with degradants failed to react to the noxious olfactory stimulus.

At the end of the recovery phase (Day 46), a single male (Animal No. 5011) from 36.4 mg/day, a single male (Animal No. 6014) from 36.4 mg/day with degradants, and a single female (Animal No. 6515) from 36.4 mg/day with degradants failed to react to the noxious olfactory stimulus. In the individual histopathology data of these rats, the nasal cavity was within normal limits microscopically.

According to the Pathology Report, this observation was not considered to be treatment-related due to its minimal occurrence. This Reviewer concurs with the conclusions of the Pathology Report, given that several failures were noted across groups in the 14-day dog study and that administration of the test-article via nasal instillation may have caused failures in the olfactory test.

Toxicokinetics

Blood was collected from the jugular vein for toxicokinetics according to the schedule illustrated in the following table (from the Applicant's submission):

Text Table 16
Bioanalytical Sample Collection Schedule – Days 1 and 28

Group No.	Subgroup	No. of Animals (Male/Female)	(Time Post first daily dose) on Days 1 and 28					
			0 hr[a]	15 min	30 min	1 hr	4.25 hr[b]	24 hr
1	A	3/3	X	-	-	X	-	-
2	A	3/3	X	-	-	X	-	-
3	A	3/3	X	-	X	-	X	-
	B	3/3	-	X	-	X	-	X
4	A	3/3	X	-	X	-	X	-
	B	3/3	-	X	-	X	-	X
5	A	3/3	X	-	X	-	X	-
	B	3/3	-	X	-	X	-	X
6	A	3/3	X	-	X	-	X	-
	B	3/3	-	X	-	X	-	X
Method/Comments:			Venipuncture of the jugular vein (under isoflurane anesthesia)					
Target Volume (mL):			0.3					
Anticoagulant:			K_2EDTA					
Special Requirements:			Tubes were chilled after blood collection.					
Processing:			Plasma					

X = Sample collected; - = Not applicable; min = minutes; hr = hour(s).

[a] Sample was collected before dosing.

[b] Sample occured 15 minutes (± 3 minutes) from the second daily dose. Based on actual dose timing of second daily dose.

The following toxicokinetics parameters were measured (from the Applicant's submission):

Parameter	Description of Parameter
t_{max}	The time after dosing at which the maximum concentration was observed
C_{max}	The maximum observed concentration measured after dosing
C_{max}/Dose	C_{max} divided by the dose administered, where Dose is the total daily dose
AUC_{last}	The area under the concentration versus time curve from the start of dose administration to the last observed quantifiable concentration calculated using the linear trapezoidal method
AUC_{last}/Dose	The AUC_{last} divided by the dose administered, where Dose is the total daily dose
t_{last}	The time after dosing at which the last quantifiable concentration was observed
R_{AUC}	Accumulation ratio based on AUC_{last}, calculated as: AUC_{last} (Day 28)/AUC_{last} (Day 1)

The following table illustrates the TK parameter results after treatment with intranasal naloxone in toxicokinetics rats (from the Applicant's submission):

Table 1. TK Parameters of Naloxone Based on Mean Plasma Concentrations in Male and Female Rats on Days 1 and 28

			Study Day			
			1		28	
Group	Treatment	Parameter	Female	Male	Female	Male
3	SN(7032) 1.80 mg/day	t_{max} (h)	0.250	0.500	0.500	0.250
		C_{max} (ng/mL)	94.2	64.0	108	46.6
		AUC_{last} (h*ng/mL)	109	60.4	104	41.9
		$RAUC_{last}$	ND	ND	0.954	0.693
4	SN(7032) 18.2 mg/day	t_{max} (h)	0.250	0.250	0.250	0.250
		C_{max} (ng/mL)	774	801	720	417
		AUC_{last} (h*ng/mL)	756	871	1070	627
		$RAUC_{last}$	ND	ND	1.41	0.720
5	SN(7032) 36.4 mg/day	t_{max} (h)	4.25[a]	4.25[a]	0.250	0.250
		C_{max} (ng/mL)	1020	1830	490	1130
		AUC_{last} (h*ng/mL)	12800	22000	3680	5470
		$RAUC_{last}$	ND	ND	0.288	0.248
6	SN(7032)-SPU 36.4 mg/day+SPU degradants	t_{max} (h)	0.250	0.250	0.250	0.250
		C_{max} (ng/mL)	1570	1480	614	664
		AUC_{last} (h*ng/mL)	10100	17300	4280	4680
		$RAUC_{last}$	ND	ND	0.422	0.270

[a] The test articles in Groups 5 and 6 were administered as a divided dose, at 0 h and approximately 4 h. The t_{max} for Group 5 occurred after the second administration.
ND = Not determinable

All rats (male and female) treated with test article were exposed to naloxone systemically following multiple intranasal actuations of SN(7032) Naloxone HCl Nasal Spray at total daily doses of 1.8, 18.2, and 36.4 mg/day of naloxone from Day 1 through Day 28.

Overall, mean naloxone concentrations increased with an increase in SN(7032) dose for males and females on Days 1 and 28. An inconsistent increase in mean concentration with dose was observed at early time points (0.5 and 1 hour) on Day 28.

Cmax increased in a less than proportional manner with an increase in dose for females and increased in an approximately proportional manner with an increase in dose for males on Days 1 and 28. AUC_{last} increased in a greater than proportional manner with an increase in dose for males and females on Days 1 and 28.

AUC_{last} Day 28/Day 1 ratios ranged from 0.288 to 1.41 for females and from 0.248 to 0.720 for males, indicating little to no accumulation during multiple dosing for males and females.

Based on Female/Male (F/M) ratios for Cmax and AUC_{last}, differences in the TK profile of naloxone were observed between males and females, but the differences were not consistent across dose level or study day. The F/M Cmax ratios ranged from 0.560 to 1.47 on Day 1 and from 0.435 to 2.32 on Day 28; the F/M AUC_{last} ratios ranged from 0.580 to 1.81 on Day 1 and from 0.673 to 2.49 on Day 28.

In general, no appreciable differences in naloxone AUC_{last} were observed between Groups 5 (36.4 mg/day) and 6 (36.4 mg/day with degradants) across study days and sex. Additionally, Cmax was similar for Groups 5 and 6 on Day 1 in males and on Day 28 in females; differences in Cmax between Groups 5 and 6 were observed on Day 1 in females and on Day 28 in males.

Dosing Solution Analysis

The Applicant supplied the naloxone formulation (including the naloxone formulation with degradants) and vehicle control as well as actuators. The Applicant provides documentation on the identity, strength, purity, composition, and stability for the naloxone formulations and vehicle control. No dosing solution analysis was performed at the testing facility on the naloxone formulation and vehicle control. Analysis of the saline control was performed at the testing facility.

7 Genetic Toxicology

There were no new genetic toxicology studies with naloxone submitted in this NDA. The label will reflect the data in the referenced product.

8 Carcinogenicity

There are no carcinogenicity studies with naloxone submitted in this NDA. The label will reflect the data in the referenced product.

9 Reproductive and Developmental Toxicology

There were no new reproductive and developmental toxicology studies with naloxone submitted in this NDA. The label will reflect the data in the referenced product.

10 Special Toxicology Studies

There were no special toxicology studies with naloxone submitted in this NDA.

11 Integrated Summary and Safety Evaluation

Summit Biosciences LLC is developing Naloxone Nasal Spray for intranasal administration (10 mg in 110 mcL or (b) (4) mg/mL naloxone HCl concentration) to deliver 10 mg of naloxone HCl with a maximum daily dose of 20 mg of naloxone HCl for the emergency treatment of known or suspected opioid overdose, as manifested by respiratory and/or central nervous system depression and for intended for immediate administration as emergency therapy in settings where opioids may be present. It is noted that "the product is not a substitute for emergency medical care." The Applicant is submitting an application via the 505(b)(2) pathway, proposing to rely upon the Agency's previous findings of safety and efficacy of Mallinckrodt's Narcan® injectable product (NDA 16636).

The maximum daily dose of 2 sprayers or 20 mg of naloxone HCl. The drug substance specifications are acceptable. There are no novel excipients in the formulation and the levels of the excipients at the maximum daily dose of 20 mg of naloxone via 2 sprayers are in the FDA Inactive Ingredient Database at comparable levels in acute use FDA-approved nasal formulations are acceptable and do not represent a safety concern.

The drug product specifications meet ICH Q3B(R2) qualification thresholds of NMT 0.5% or 200 mcg TDI, whichever is lower, for drug products with a maximum daily dose of 10 to 100 mg except for (b) (4), whose specification is NMT (b) (4)%. The specified unidentified impurities meet ICH Q3B(R2) identification (NMT 0.2%) and qualification thresholds (NMT 0.5%). The specification for the SN22-02 mixture (NMT (b) (4)%) exceeds ICH Q3B(R2) identification thresholds, which has been identified, and meets ICH Q3B(R2) qualification thresholds. The specification for unspecified unidentified impurities (NMT (b) (4)%) meet ICH Q3B(R2) identification (NMT 0.2%) and qualification thresholds.

In support of the NMT (b) (4)% specification for the drug product degradant, (b) (4), the Applicant submitted a negative computational toxicology evaluation that assessed the mutagenic potential of the compound and submitted a 28-day intranasal repeat-dose toxicity rat study that evaluated a naloxone formulation that contained different degradants that included (b) (4) Although this study qualified the systemic safety of the degradant, this study tested a lower concentration than the proposed specification of (b) (4)%, resulting in a local safety margin of (b) (4) The Applicant purported that a higher concentration could not be tested due to several factors, including the notion that the degradant could not be synthesized or isolated and the degradant readily degrades. It is noted that data and documentation were not provided to support these assertions. The Applicant also argued that the rat study provided support for the local safety of the proposed specification when normalizing the dose with intranasal surface area (i.e., mg/cm^2) of the species, and results in a (b) (4)

safety margin. However, when reviewing the safety of an impurity specification, the supporting nonclinical qualification study should yield adequate safety margins using 3 approaches: human equivalent dose (HED) based body surface area, dose normalization to nasal surface area (mg/cm^2), and through a comparison of the tested concentration in the toxicity study versus the actual concentration in the drug product. In this case, the safety margin calculated by the concentration approach is below 1x and therefore is not supportive of the proposed specification of (b) (4)% for (b) (4) (b) (4). However, the lack of nonclinical support for the local safety of (b) (4) does not preclude marketing approval for the following reasons: 1) the submitted nonclinical data is from a study that evaluated exaggerated dosing conditions (repeated dosing for 28 consecutive days compared to the clinical use scenario of a single dose) and did not identify a safety signal at the local tissues; 2) adequate safety margins were established for the degradant based on HED conversion using BSA and based on dose normalization using nasal surface area; 3) this product is indicated for an acute, single-use indication; and 4) the drug product is a potentially life-saving therapy. It is recommended that a postmarketing requirement be issued to the Applicant to conduct a nonclinical study that tests an adequate degradant concentration to support the local safety of the proposed specification. (b) (4)

The container closure system Is the (b) (4) unit dose sprayer that has been referenced in other applications including the components in direct contact with the drug formulation (vial and plunger with rubber stopper). In support of the container closure, extractable leachable and elemental impurities assessments were submitted. The extraction studies on the rubber stoppers and used (b) (4)

The leachable study was performed on 3 lots of the proposed Naloxone HCl 10 mg drug product (CSNB1903A, RSNB1904A, and RSNB1905A) that were stored for various time points (3, 6, 12, 18, 24, and 36 months) and analyzed using various analytical methods (b) (4) The leachable study is appropriate as the Analytical Evaluation Threshold (AET) is based on a 1.5 mcg/day threshold. The leachable compounds detected (including the (b) (4) are below the 5 mcg/day threshold and as such, do not represent a safety concern at these levels. The levels of the (b) (4) are below the level of detection that are below the limit of (b) (4)/day in the nitrosamine guidance.

The elementals detected in the elemental impurities assessment meet ICH Q3D PDE limits except for (b) (4) The maximum daily exposures

of (b) (4) via the maximum daily dose of the proposed product is qualified and there no safety concerns at these levels.

In support of the marketing application, the Applicant submitted the 14-day dog (Study 2950-002) and 28-day rat toxicology (Study 20246992) studies.

In the 14-day dog toxicology study (Study 2950-002), Beagle dogs were administered 0 (saline control or vehicle control), 20, 160, or 300 mg/day naloxone (100 mg/mL) intranasally for 14 consecutive days followed by a 7-day recovery period (no treatment). There were no unscheduled deaths during the study. As the doses are reported in mg/day, an average dog weight of 6 kg can be used to calculate mg/kg/day doses. As such, the doses are 3.33 (20/6 = 3.33), 26.7 (160/6 = 26.7), and 50 (300/6 = 50) mg/kg/day, respectively. There were no treatment-related changes in clinical signs, olfactory functional assessment, body weight parameters (absolute body weight and body weight gain), food consumption, ophthalmoscopy, electrocardiographic examinations, hematology and coagulation, clinical chemistry, urinalysis, gross pathology outside the nasal cavity, organ weight parameters (absolute organ weight, organ-to-body-weight ratio, and organ-to-brain-weight ratio), and histopathology outside the nasal cavity. Nasal irritation in nasal cavity (including mononuclear cell infiltration and degeneration/atrophy of the olfactory epithelium) and findings in surrounding tissues (including subacute/chronic inflammation in the lung, erythrocytosis/erythrophagocytosis of the sinus in the tracheobronchial lymph node, and mononuclear cell infiltration in the trachea, tracheal bifurcation, and bronchus) in all doses tested were observed with the highest incidence observed at the high dose but were dependent on the number of actuations administered. These microscopic observations in the nasal cavity and its surrounding tissues were not as numerous in the recovery dogs demonstrating these observations were subsiding in the recovery dogs. It is noted that acute inflammation in the galt and immature prostate gland in the male 36.4 mg/day naloxone with degradants dose group as well as hydrocephalus in the brain and vascular mineralization in the heart in the female 36.4 mg/day naloxone with degradants dose group were observed that were considered incidental and commonly observed in beagle dogs and as such, were not treatment-related. Nasal mucosal irritation appeared to be present in the mid- and high dose dogs early in the treatment period but subsided and was not present on Day 14. The local NOAEL is the mid dose (160 mg/day), which was 8 sprays per naris given that the slight increases in incidence of the microscopic findings was reversed after the recovery period in this group. The local NOAEL resulted in a concentration margin of (b) (4) as the formulation used in this toxicology study (100 mg/mL) is a slightly higher concentration of naloxone HCl than the clinical formulation (b) (4) mg/mL). The systemic NOAEL was the high dose of 50 mg/kg (300 mg/6 kg average dog weight = 50 mg/kg) based on no treatment-related microscopic findings in other tissues and organs, which is equivalent to 1667 mg (50 mg/kg / 1.8 x 60 kg average human weight = 1667 mg) HED, which confers a safety margin of 83x (1667 mg/20 mg MDD = 83) based on a body surface area comparison and using an average human weighing 60 kg.

In the 28-day rat toxicology study (Study 20246992), Sprague Dawley rats were administered saline control, vehicle control, 1.8 mg/day naloxone, 18.2 mg/day naloxone, 36.4 mg/day naloxone, or 36.4 mg/day naloxone with degradants intranasally for 28 consecutive days followed by a 19-day recovery period (no treatment). The rat doses are expressed as mg/day. Using an average rat weight of 0.325 kg, these doses can be expressed as mg/kg/day. As such, the rat doses are 5.54 (1.8/0.325 = 5.54), 56 (18.2/0.325 = 56), 112 mg/kg/day (36.4/0.325 = 112), and 112 mg/kg/day with degradants, respectively. There was an unscheduled sacrifice in a female from the 36.4 mg/day dose group where a uterine mass consistent with an endometrial stromal polyp as well as signs of bleeding (red fur staining and liquid red material present at an in the urogenital area as well as markedly low erythrocytes, hemoglobin, and hematocrit) were observed and these findings were considered unrelated to naloxone treatment. There were no toxicologically meaningful changes in body weight and food consumption. There were no treatment-related changes in clinical signs, ophthalmoscopy, hematology and coagulation, clinical chemistry, and male organ weight parameters (absolute organ weight, organ-to-body-weight ratio, and organ-to-brain-weight ratio). In females, organ-to-body-weight ratios of the adrenal gland, pituitary, and thymus were deemed not toxicologically relevant. In the olfactory functional assessment, a single female from the 36.4 mg/day with degradant dose group at the end of dosing as well as a single male from 36.4 mg/day dose group and 1 male and female from the 36.4 mg/day with degradants dose group at the end of recovery failed to react to the noxious olfactory stimulus; however, there were no microscopic changes in the nasal cavity of these rats. It is also noted that single incidences of failures in olfactory assessments in rats have been previously observed. Microscopic findings in the nasal cavity (neutrophilic inflammation, neutrophilic infiltration, erosion/ ulcer, lymphocytic infiltration, and degeneration of the olfactory epithelium) and in its surrounding tissues (vascular mineralization, perivascular mixed cell infiltration, increased alveolar macrophages, and metaplasia of the osseous in the lung as well as increased plasma cell cellularity, increased lymphoid cellularity and congestion of the medullary sinus in the mandibular lymph node and pigmented macrophage, congestion, and increased lymphoid cellularity in the germinal center of the tracheobronchial lymph node) in the 18.2 and 36.4 mg/day dose groups were observed. These microscopic observations in the nasal cavity and its surrounding tissues were not observed in the recovery rats. Given that the local findings were minimal to mild in severity at low incidences and were fully reversible, an acceptable local LOAEL is the high dose of 36.4 mg/day naloxone (an instillation twice daily per nostril) and results in a concentration margin of 1x as the concentration of naloxone HCl used in this study and in the clinical formulation are both [redacted] (b) (4) mg/mL. For systemic tissues and organs, microscopic examination was not conducted on the 1.8 and 18.2 mg/day naloxone dose groups. No microscopic treatment-related changes were observed at 36.4 mg/day, and therefore, the systemic NOAEL is 36.4 mg/day or 112 mg/kg/day, which confers a human equivalent dose of 1084 mg (112 mg/kg / 6.2 x 60 kg average human weight) and a safety margin of 54x (1084 mg/20 mg MDD = 54). The effects of the degradants [redacted] (b) (4) with 36.4 mg/day of naloxone were not worse than 36.4 mg/day of naloxone alone.

From a nonclinical pharmacology toxicology perspective, NDA 215487 for Naloxone HCl Nasal Spray may be approved with a postmarketing requirement (PMR) to conduct a GLP repeat-dose toxicology study of at least 14 days duration in a single species to characterize the toxicological potential of the naloxone degradant, (b) (4) to local tissues after intranasal administration.

12 Appendix/Attachments

Derelanko MJ. The Toxicologist's Pocket Handbook *2nd Ed*. 2009. Informa Healthcare. New York.

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Lim JH, Davis GE, Wang Z, Li V, Wu Y, Rue TC, and Storm DR. Zicam-Induced Damage to Mouse and Human Nasal Tissue. *PLoS ONE*. 2009, 4(10):e7647.

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