CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208969Orig1s000

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:	208969
Supporting document/s:	SDN 50 (Electronic Document Room Sequence Number 49)
Applicant's letter date:	September 7, 2022 (SDN 50)
CDER stamp date:	September 7, 2022 (SDN 50)
Product:	Naloxone Hydrochloride Spray (4 mg in 0.25 mL; 16 mg/mL concentration)
Indication:	For emergency treatment of opioid overdose
Applicant:	Amphastar Pharmaceuticals, Inc.
Review Division:	Division of Anesthesiology, Addiction Medicine, and Pain Medicine (DAAP)
Reviewer:	Carlic K. Huynh, PhD
Team Leader:	Nikunj S. Patel, PhD
Supervisor:	Newton H. Woo, PhD
Division Director:	Rigoberto Roca, MD
Project Manager:	Namrata Thakkar

Template Version: September 1, 2010

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TABLE OF CONTENTS

1 Executive Summary

1.1 Introduction

Amphastar Pharmaceuticals, Inc. submitted a 505(b)(2) application for Naloxone Hydrochloride Nasal Spray, 4 mg of naloxone in a volume of 0.25 mL (16 mg/mL), for the emergency treatment of opioid overdose relying upon the Agency's previous finding of safety of Narcan (naloxone injection; NDA 16636) and literature. This is the second cycle review for this proposed product. This is a modification from the proposed formulation that was proposed for the first review cycle, (b) (4) . At the end of the first cycle review (see Complete Response Letter dated February 17, 20217), there were no nonclinical deficiencies noted (see the nonclinical review dated January 23, 2017), however, there were quality concerns regarding monitoring of non-volatile extractables/leachables that were noted in the additional comments (see Complete Response Letter dated February 17, 2017).

1.2 Brief Discussion of Nonclinical Findings

There were no new nonclinical studies submitted in this NDA. The formulation contains 4 mg of naloxone hydrochloride in 0.25 mL (16 mg/mL) with no novel excipients. The drug substance and drug product specifications are acceptable. The submitted elemental impurities (b) (4) assessments are acceptable. The extractable leachables evaluation included several time points using three batches of the drug product and after review, the data supports the safety of the container closure system.

Therefore, there are no nonclinical concerns with the proposed 4 mg naloxone hydrochloride nasal spray drug product.

1.3 Recommendations

1.3.1 Approvability

From a nonclinical pharmacology toxicology perspective, NDA 208969 submitted for the proposed 4 mg naloxone intranasal spray drug product may be approved.

1.3.2 Additional Nonclinical Recommendations

None.

1.3.3 Labeling

The following table illustrates the recommendations for labeling. 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
(b) (4)		Changes were made to maintain consistency with the referenced naloxone drug product and also update margin with a human dose of 8 mg/day (two naloxone nasal sprays) based on a body surface area and a 60 kg human.
		Changes were made to maintain consistency with the referenced naloxone drug product, to update margins with a human dose of 8 mg/day (two naloxone nasal sprays) based on a body surface area and a 60 kg human, and to be identical to the referenced Narcan injection label. In Narcan nasal spray however is not in Narcan injection label

(b) (4)	(listed drug) and therefore deleted this detailed language.
	We defer to the clinical and maternal health review teams regarding the human pediatric use labeling.

(b) (4)	
	No changes are recommended.
	No changes are recommended.

(b) (4)	
	In Narcan nasal spray however is not present in Narcan injection label (listed drug) and therefore deleted this detailed language.

2 Drug Information

2.1 Drug

CAS Registry Number
51481-60-8

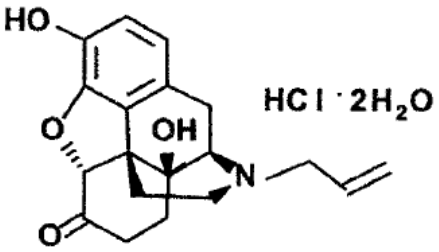
Generic Name
Naloxone HCl Dihydrate

Code Name
None

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

Molecular Formula/Molecular Weight
$C_{19}H_{21}NO_4 \bullet HCl \bullet 2H_2O$ / 399.97 g/mol

Structure or Biochemical Description



Pharmacologic Class
Opioid antagonist (Established Pharmacologic Class)

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND#	Drug	Status	Division	Indication	Status Date	Sponsor
124672	Intranasal naloxone	Active	DAAP	For the treatment of opioid overdose	April 2, 2015	Amphastar Pharmaceuticals, Inc.

NDA	Drug Name	Div	Strength (route)	Marketing Status	AP Date	Indication	Company
16636	Narcan (Naloxone HCl) Injection	DAAP	0.2, 0.4, and 1 mg/mL (Injection)	Withdrawn*	August 20, 2010	Opioid Dependence	Endo Pharmaceuticals, Inc.

*NARCAN was not withdrawn due to issues of safety.

ANDA	Drug Name	Div	Strength (route)	Marketing Status	AP Date	Indication	Company
72076	Naloxone HCl	OGD	1 mg/mL (Injection)	Approved	March 24, 1988	Opioid Dependence	International Medication System

DMF#	Subject of DMF	Holder	Submit Date	Reviewer's Comment
(b) (4)				

(b) (4)

The reader is referred to the quality review for further details regarding the container closure components.

2.3 Drug Formulation

The following table illustrates the composition of Naloxone HCl Nasal Spray (from the Applicant's submission):

Table 23P-3 Unit Dose Compositions of Naloxone HCl Nasal Spray, 4 mg/ 0.25 mL (N002)

Product Strength	Naloxone HCl Nasal Spray, 4 mg/ 0.25 mL		
API:	**Amount per 0.25 mL**	**Amount per mL**	**% w/v**
Naloxone HCl Dihydrate USP*	(b) (4)		
Inactive Ingredients:			
Sodium Chloride, USP	(b) (4)		
Sodium Hydroxide NF	As needed for pH adjustment	As needed for pH adjustment	prn
Water for Injection, USP	QS Ad	QS Ad	QS Ad
(b) (4)			

The maximum daily dose of the proposed product is 8 mg/day, as the proposed product is packaged as 2 spray unit devices in a carton. The maximum daily volume is 0.5 mL/day as each sprayer delivers 0.25 mL.

The inactive ingredients are sodium chloride, sodium hydroxide, and water for injection. These inactive ingredients are in the FDA inactive ingredients database in FDA-approved formulations for nasal products.

2.4 Comments on Novel Excipients

There are no novel excipients in the formulation.

2.5 Comments on Impurities/Degradants of Concern

Drug Substance Specifications

The drug substance is from (b) (4) DMF (b) (4) which is utilized in several other approved naloxone formulations. The following table illustrates the drug substance specifications for the naloxone hydrochloride drug substance (modified from the Applicant's submission):

Test Required	Method	Specifications
Appearance	(b) (4)	
Identification A. IR B. TLC		
Specific Rotation		
Loss on Drying		
Noroxymorphone Hydrochloride and other impurities		
Chloride Content		
Assay		
Related Substance (EP) Noroxymorphone 3-*O*-allylnaloxone 10α-hydroxynaloxone 2,2'-bisnaloxone 10β-hydroxynaloxone Largest Unspecified impurity Total Impurities		
Impurity D* (EP)		
Residual Solvent		

* = 7,8-didehydronaloxone

The maximum daily dose of the proposed product is 8 mg/day. For drug products with a maximum daily dose of ≤2 g/day, the ICH Q3A(R2) qualification threshold is NMT 0.15% or 1.0 mg/day intake (whichever is lower). As shown in the table above, these drug substance specifications exceed ICH Q3A(R2) qualification thresholds. The specification for (b) (4) is NMT (b) (4) ppm or NMT (b) (4) %. The (b) (4) contains a structural alert for mutagenicity and must be reduced to the currently acceptable threshold for potentially genotoxic impurities of NMT (b) (4) mcg/day. The specification set by the Applicant for (b) (4) would result in levels (b) (4) mcg/day when the product is used as labeled (8 mg NLX), and as such, is acceptable. Although these drug substance impurities exceed ICH Q3A(R2) qualification thresholds, the specifications are considered acceptable because DMF (b) (4) is referenced by multiple approved products and no safety concerns have arisen. (b) (4)

(b) (4) as per the manufacturer. Thus, the drug substance specifications are acceptable.

Drug Product Specifications

The following table illustrates the drug product specifications of the proposed naloxone hydrochloride nasal drug product (modified from the Applicant's submission):

Quality Attributes of the Drug Product	Target	Is this a CQA?	Justification
Identification A.RT (HPLC) B. UV Diode Array	(b) (4)	Yes	(b) (4)
pH Determination		Yes	
Assay: Naloxone HCl		Yes	
Osmolality		Yes	
(b) (4)		Yes	
Related Substances		Yes	
(b) (4) Largest unspecified impurity Total impurities			

The maximum daily dose of the proposed product is 8 mg/day. For a drug product with a maximum daily dose of <10 mg, the ICH Q3B(R2) qualification threshold is NMT 1.0% or 50 mcg TDI (total daily intake), whichever is lower. All drug product degradant specifications meet ICH Q3B(R2) qualification thresholds.

Per the CMC reviewer, (b) (4) contains a structural alert. However, upon a preliminary ToxTree QSAR evaluation, no strucutral alerts were flagged for this compound. Moreover, if you consider the specification for (b) (4) of NMT

(b) (4)% and the maximum daily expsoure to naloxone of 8 mg/day, the maximum daily exposure to (b) (4) is (b) (4) mg/day or (b) (4) mcg/day (b) (4) mg/day x (b) (4) = (b) (4) mg/day = (b) (4) mcg/day), which is below the recommendations for acceptable daily intake level for a genotoxic impurity of 120 mcg/day outlined in ICH M7.

Thus, the drug product specifications are acceptable.

Container Closure System

The following table describes the container closure for the proposed product (from the Applicant's submission):

Table 32P11-2 Packaging Components of the Proposed N002 Product

	Medication Container (Primary)	Rubber Stopper (Primary)	N002 Nasal Injector (Secondary)
Description	3 mL (b) (4) glass container	2 mL (b) (4) (b) (4) gray stopper	Each N002 nasal Injector is preassembled with a medication filled, stoppered vial attaching to a vial holder to provide a ready-to-use N002 Nasal Spray Unit
Manufacturer	(b) (4)		International Medication Systems, Limited (IMS)
IMS' Part No.	(b) (4)		

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

Figure 32P2-1 Schematic Drawing for N002 Nasal Spray Unit

Extraction Studies

Briefly, the Applicant performed extraction studies in the 3 mL (b) (4) Glass Vials and 2 mL (b) (4) Stoppers (primary components) as well as the Nasal Injector (secondary component) using acidic water (pH 2), basic water (pH 12), isopropanol:water (50:50), and drug product lab formulation as extraction solvents for 24 h at 60^{O}C. The following tables illustrate the extractable compounds (volatile, semi-volatile, and non-volatile) in the primary component Gray (b) (4) Stoppers (from the Applicant's submission):

(b) (4)

As shown in the tables above, there were no organic extractable compounds detected from the Gray (b) (4) Stoppers. The reader is referred to the quality review for the adequacy of the extraction study methods.

Leachable Studies

For the leachable studies, this Reviewer calculated an AET using a (b) (4) mcg/day threshold and a maximum daily volume of 0.5 mL (0.25 mL/spray x 2 sprays = 0.5 mL) to deliver the maximum daily dose of 8 mg/day:

AET = (b) (4) mcg/day / 0.5 mL/day = (b) (4) mcg/mL

For the leachables evaluation in this review cycle, 3 lots of finished product (Lot # 111920A, 112520A, and 120220A) under various storage conditions (upright or inverted), lengths of time (6- and 12 months), and temperatures were evaluated. The reader is referred to the quality review for the adequacy of the leachable study methods. There were no leachable compounds with the exception of (b) (4) that exceeded the qualification threshold of 5 mcg/day. The concentration reported for (b) (4) was (b) (4) ppm (equivalent to (b) (4) mcg/day), which confers (b) (4) mcg/day (b) (4) ppm = (b) (4) % = (b) (4) mg/mL x (b) (4) mcg/mg = (b) (4) mcg/mL x (b) (4) mL/day (b) (4) mcg/day). (b) (4) is a (b) (4) that is below the established allowable limit of (b) (4) ppm as outlined in ICH Q3C. There are no safety concerns with the proposed container closure system.

Elemental Impurities

The Applicant states that the elemental impurities met ICH Q3D limits as shown in the following table (from the Applicant's submission):

Quality Attributes of the Drug Product	Target	Is this a CQA?	Justification
Elemental Impurities, USP <232>/<233>	(b) (4)		
Other Requirements			

Elemental impurities assessment was performed on 3 lots (Lots 111920A, 112520A, and 120220A), under normal (25^{O}C) and accelerated (40^{O}C) storage conditions, and upright and inverted configuration for a duration of 6 months. The following table illustrates the highest levels of each elemental impurity detected (data from the Applicant's submission):

Elemental Impurity	Highest Amount Detected (ppm)	Maximum Daily Dose (mcg/day)[1]	ICH Q3D Parenteral PDE (mcg/day)	Adequate?
(b) (4)				Adequate, meets ICH Q3D limits
				Adequate, below the 5 mcg/day threshold
				Adequate, see below

(b) (4)	Adequate, below the 5 mcg/day threshold

It is noted in the table above that (b) (4) levels were not included in the elemental impurities assessment. According to the Applicant, (b) (4) In discussions with the Chemistry, Manufacturing, and Controls (CMC) review team, (b) (4) is a (b) (4) per ICH Q3D where risk assessment is not required unless it is intentionally added, which in this case (b) (4) is not used in the synthesis of the active ingredient, and as such, the exclusion of (b) (4) in the elemental impurities assessment is acceptable. This reviewer concurs with the conclusions of the CMC review team.

For (b) (4) the World Health Organization (WHO) recommends (b) (4) mg/kg/day via normal (b) (4) requirements[1]. This confers (b) (4) mg/day in an average human weighing 60 kg ((b) (4) mg/kg/day x 60 kg = (b) (4) mg/day). The amount o (b) (4) detected in the elemental impurities assessment is (b) (4) mcg/day, which is far below the (b) (4) mg/day recommendation. Thus, the levels (b) (4) detected do not pose a safety concern.

Thus, the levels of the elemental impurities are acceptable.

(b) (4)

The Applicant submitted a (b) (4) risk assessment for the proposed container closure system. The following table illustrates the (b) (4) risk from the primary container components (from the Applicant's submission):

Container Closure	IMS P/N	Supplier	Has the supplier risk assessment or statement been received?	Conclusion of the supplier risk assessment:
(b) (4)				

(b) (4)

Container Closure	Treated with	Supplier	Has the supplier risk assessment or statement been received?	Conclusion of the supplier risk assessment:
(b) (4)				

As shown in the tables above, there is an overall no or low risk of presence of (b) (4) from the primary container components. Only the (b) (4) stoppers indicated the potential for (b) (4) presence, however, if you consider the (b) (4) of (b) (4) this level is lower than the limits in the (b) (4) *guidance*[2]. Therefore, there are no safety concerns with regards to the submitted (b) (4) risk assessment.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed clinical population is both adult and pediatric patients. The dose of the proposed drug product should be administered to one nostril. An additional dose of the proposed drug product may be given after 2 minutes if the patient dose not respond using a new intranasal device into the other nostril.

2.7 Regulatory Background

There was a pre-IND meeting on March 12, 2015 and a pre-NDA meeting on November 27, 2015. The reader is referred to the meeting minutes from these meetings for details.

Naloxone hydrochloride (NDA 16636) was originally approved as NARCAN in April 13, 1971 for the treatment of known or suspected narcotic overdose via the IV, IM, or SC route of administration. The original NARCAN NDA (NDA 16636) was withdrawn from the market but not for reasons of safety or efficacy with numerous ANDAs for naloxone hydrochloride that have subsequently become available. Thus, there is an extensive clinical experience with naloxone hydrochloride via the IV, IM, and SC routes of administration.

This is the second cycle NDA review for this proposed product, which is 4 mg of naloxone hydrochloride in 0.25 mL (16 mg/mL) and is a modification from the proposed formulation that was proposed for the first review cycle, which was (b) (4) of naloxone hydrochloride in (b) (4)). At the end of the first cycle review (see Complete

(b) (4)

Response Letter dated February 17, 20217), there were no nonclinical deficiencies noted (see the nonclinical review dated January 23, 2017), however, there were quality concerns regarding monitoring of non-volatile extractables/leachables that were noted in the additional comments (see Complete Response Letter dated February 17, 2017).

3 Studies Submitted

3.1 Studies Reviewed

There were no nonclinical studies with naloxone hydrochloride that were required in this NDA.

3.2 Studies Not Reviewed

N/A

3.3 Previous Reviews Referenced

There were no previous reviews referenced.

4 Pharmacology

4.1 Primary Pharmacology

There are no new primary pharmacology studies with naloxone hydrochloride that were submitted or required to support this NDA.

4.2 Secondary Pharmacology

There are no new secondary pharmacology studies with naloxone hydrochloride that were submitted or required to support this NDA.

4.3 Safety Pharmacology

There are no new safety pharmacology studies with naloxone hydrochloride that were submitted or required to support this NDA.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

There are no new PK/ADME studies with naloxone hydrochloride that were submitted or required to support this NDA.

5.2 Toxicokinetics

There were no toxicokinetics with naloxone hydrochloride that were submitted or required to support this NDA.

6 General Toxicology

There were no general toxicology studies with naloxone hydrochloride submitted or required to support this NDA. The Agency agreed that for this potentially life-saving indication, given the previous clinical experience with off-label use of this injectable drug product intranasally, no intranasal toxicology studies would be required. Clinical monitoring in the human relative bioavailability studies also exists. The reader is referred to the medical officer review.

7 Genetic Toxicology

There were no genetic toxicology studies with naloxone hydrochloride submitted in this NDA. The applicant is relying upon the Agency previous finding of safety for Narcan (naloxone) injection, to support their application. The FDA-approved Narcan injection labeling provides the following information:

> NARCAN was weakly positive in the Ames mutagenicity and in the in vitro human lymphocyte chromosome aberration test 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.

These data will be incorporated into the current drug product labeling.

8 Carcinogenicity

There were no carcinogenicity studies with naloxone hydrochloride submitted in this NDA. The applicant is relying upon the Agency previous finding of safety for Narcan (naloxone) injection, to support their application. The FDA-approved Narcan injection labeling provides the following information:

> Studies in animals to assess the carcinogenic potential of NARCAN have not been conducted.

These data will be incorporated into the current drug product labeling.

9 Reproductive and Developmental Toxicology

There were no new reproductive and developmental toxicology studies with naloxone hydrochloride submitted in this NDA. The applicant is relying upon the Agency previous finding of safety for Narcan (naloxone) injection, to support their application. The FDA-approved Narcan injection labeling provides the following information:

> Reproduction studies conducted in mice and rats at doses 4-times and 8-times, respectively, the dose of a 50 kg human given 10 mg/day (when based on surface area or mg/m^2), demonstrated no embryotoxic or teratogenic effects due to NARCAN.
>
> Use in Pregnancy
> Teratogenic Effects: Pregnancy Category C:
>
> Teratology studies conducted in mice and rats at doses 4-times and 8-times, respectively, the dose of a 50 kg human given 10 mg/day (when based on surface area or mg/m^2), demonstrated no embryotoxic or teratogenic effects due to NARCAN. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, NARCAN should be used during pregnancy only if clearly needed.
>
> Non-teratogenic Effects:
> Risk-benefit must be considered before NARCAN is administered to a pregnant woman who is known or suspected to be opioid-dependent since maternal dependence may often be accompanied by fetal dependence. Naloxone crosses the placenta, and may precipitate withdrawal in the fetus as well as in the mother. Patients with mild to moderate hypertension who receive naloxone during labor should be carefully monitored as severe hypertension may occur.

These data will be incorporated into the current drug product labeling in a PLLR compliant format as appropriate.

A literature review was completed by Dr. Newton Woo (see nonclinical review dated 1/23/2017) in the previous review cycle with several publications that were identified and reviewed.

Collectively these published studies have evaluated the effects of naloxone on reproduction and developmental endpoints and suggest that naloxone can potentially impact the central nervous system. However, in most cases, the doses were significantly higher that which would be produced by this intranasal spray. It is important to note that there was no significant adverse effect identified in these published studies that would negate the benefit of this potentially life-saving therapeutic, given that an opioid agonist itself also have been demonstrated to have adverse impact on brain development. The results of the articles and literature search do not impact the safety or labelling of NARCAN nasal spray.

10 Special Toxicology Studies

In the first review cycle, the Applicant conducted a “Maximization Test for Delayed-Type Hypersensitivity in Hartley Guinea Pigs” (Study 16J0226H-X01G) and an

"Intracutaneous (Intradermal) Reactivity Test in New Zealand White Rabbits" (Study 16J0226H-X02G) and were reviewed (see nonclinical review dated January 23, 2017).

There were no sensitization reactions from the test article (both the saline and the cottonseed oil extract) in the guinea pigs tested or skin irritation from the test article extracted in both saline and cottonseed oil in the rabbits tested.

11 Integrated Summary and Safety Evaluation

There were no new nonclinical studies that were submitted in this NDA. The formulation is a 4 mg of naloxone hydrochloride in 0.25 mL (16 mg/mL) that contains no novel excipients. The drug substance and drug product specifications are acceptable. The submitted extractables leachables assessment used 3 lots of finished product under various storage conditions, lengths of time (6- and 12 months), and temperatures that were evaluated. There were no leachable compounds detected with the exception of (b) (4) that was detected at (b) (4) ppm (equivalent to (b) (4) mcg/day), which may exceed the 5 mcg/day threshold. However, (b) (4) is a (b) (4) that is below the established allowable limit (b) (4) ppm per ICH Q3C. As such, there are no safety concerns with the proposed container closure system. The elemental impurities assessment is acceptable. There is no risk of (b) (4) from most container closure components with the exception of the (b) (4) stoppers, where the levels of (b) (4) is stated to be (b) (4). This is lower than the limits established in the (b) (4) guidance and as such, the specification does not pose a safety concern. Local tolerance studies would normally be required to support a reformulated drug product that employs an alternate route, however, the Division determined that nonclinical studies would not be required given the clinical experience with intranasal naloxone, lack of any novel excipients, the acute use of the drug product, and the potentially life-saving indication and provided that the Applicant conduct nasal examinations before and after administration of the drug product (see clinical review for the human safety data for the proposed formulation). Therefore, there are no nonclinical safety concerns with the proposed naloxone hydrochloride drug product and NDA 208969 may be approved.

12 Appendix/Attachments

References

Ball D, Blanchard J, Jacobson-Kram D, McClellan RO, McGovern T, Norwood DL, Vogel WM, Wolff R, and Nagao L. 2007. Development of Safety Qualification Thresholds and Their Use in Orally Inhaled and Nasal Drug Product Evaluation. Toxicological Sciences 97(2):226-236.

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/s/

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	208969
Supporting document/s:	SDN 1, 13, 17, 19, 23, 25, and 28 (Electronic Document Room Sequence Number 0, 12, 16, 18, 22, 24, and 27)
Applicant's letter date:	April 19, 2016 (SDN 1), August 6, 2016 (SDN 13), September 23, 2016 (SDN 17), October 19, 2016 (SDN 19), November 21, 2016 (SDN 23), November 29, 2016 (SDN 25), and December 15, 2016 (SDN 28)
CDER stamp date:	April 19, 2016 (SDN 1), August 8, 2016 (SDN 13), September 23, 2016 (SDN 17), October 19, 2016 (SDN 19), November 21, 2016 (SDN 23), November 29, 2016 (SDN 25), and December 15, 2016 (SDN 28)
Product:	Naloxone Hydrochloride Spray
Indication:	For emergency treatment of opioid overdose
Applicant:	Amphastar Pharmaceuticals, Inc.
Review Division:	Division of Anesthesia, Analgesia, and Addiction Products (DAAAP)
Reviewer:	Carlic K. Huynh, PhD
Team Leader:	Elizabeth A. Bolan, PhD
Supervisor:	R. Daniel Mellon, PhD
Division Director:	Sharon Hertz, MD
Project Manager:	Shelly Kapoor

Template Version: September 1, 2010

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TABLE OF CONTENTS

Table of Tables

Table of Figures

1 Executive Summary

1.1 Introduction

Amphastar Pharmaceuticals, Inc. submitted a 505(b)(2) application for Naloxone Hydrochloride Nasal Spray, (b) (4) for the emergency treatment of opioid overdose relying upon the Agency's previous finding of safety of Narcan (naloxone injection; NDA 16636) and literature. The volume of the delivered drug product is (b) (4) and as such the final dose of naloxone HCl is (b) (4) The proposed product is provided as an (b) (4) The Applicant notes that their proposed drug product formulation (b) (4)

1.2 Brief Discussion of Nonclinical Findings

There were no required nonclinical studies submitted in this NDA. The formulation is a (b) (4) mg/mL concentration of naloxone hydrochloride in (b) (4) (final dose of (b) (4) in (b) (4) that contains no novel excipients. There are no nonclinical safety concerns with the drug substance and drug product specifications. There are no nonclinical safety concerns with the container closure (b) (4). To support the container closure system, the Applicant submitted the results of the delay-typed hypersensitivity in guinea pigs testing extracts from a component of the container closure system as well as an intracutaneous reactivity test in rabbits, both of which did not demonstrate any skin sensitization or skin irritation of container closure system extractable compounds.

As part of the preNDA advice to the Sponsor, the Agency indicated that the Applicant should submit a review of the literature to determine if there were any findings since the approval of the referenced drug product that would impact labeling. The Applicant did not conduct a literature review or summarize any nonclinical data in the submission. Nonetheless, the Agency has conducted a literature review. Although there have been numerous articles describing the effects on naloxone on reproduction and developmental endpoints which suggest naloxone can potentially impact neuronal development, these findings would not negate the potential benefit of a life-saving therapeutic.

Therefore, there are no additional nonclinical concerns with the proposed naloxone hydrochloride drug product.

1.3 Recommendations

1.3.1 Approvability

From a pharmacology toxicology perspective, the proposed drug product, Naloxone Hydrochloride Nasal Spray, (b) (4) may be approved. If this product is approved, a post-marketing requirement (PMR) to address monitoring non-volatile extractables/leachables per the quality review is recommended. If this product is not approved in this review cycle, the proposed PMR will be a CR issue.

1.3.2 Additional Non Clinical Recommendations

None.

1.3.3 Labeling

The labeling will be reviewed in the next cycle.

2 Drug Information

2.1 Drug

CAS Registry Number
51481-60-8

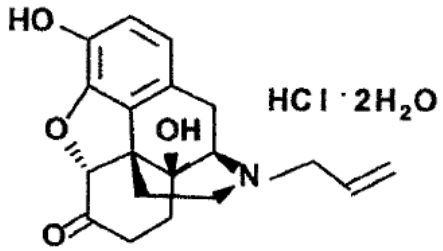
Generic Name
Naloxone HCl Dihydrate

Code Name
None

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

Molecular Formula/Molecular Weight
$C_{19}H_{21}NO_4 \bullet HCl \bullet 2H_2O$ / 399.97 g/mol

Structure or Biochemical Description



Pharmacologic Class
Opioid antagonist (Established Pharmacologic Class)

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND#	Drug	Status	Division	Indication	Status Date	Sponsor
124672	Intranasal naloxone	Active	DAAAP	For the treatment of opioid overdose	April 2, 2015	Amphastar Pharmaceuticals, Inc.

NDA	Drug Name	Div	Strength (route)	Marketing Status	AP Date	Indication	Company
16636	Narcan (Naloxone HCl) Inj	DAAAP	0.2, 0.4, and 1 mg/mL (Injection)	Withdrawn*	August 20, 2010	Opioid Dependence	Endo Pharmaceuticals, Inc.

*NARCAN was not withdrawn due to issues of safety.

ANDA	Drug Name	Div	Strength (route)	Marketing Status	AP Date	Indication	Company
72076	Naloxone HCl	OGD	1 mg/mL (Injection)	Approved	March 24, 1988	Opioid Dependence	International Medication System

DMF#	Subject of DMF	Holder	Submit Date	Reviewer's Comment
(b) (4)				

(b) (4)

The reader is referred to the quality review for the determination of adequacy of the container closure components.

2.3 Drug Formulation

The following table illustrates the composition of Naloxone HCl Nasal Spray (from the Applicant's submission):

Table 1: Composition of Naloxone HCl Nasal Spray

Table 23P-3 Unit Dose Compositions of Naloxone HCl Nasal Spray, (b) (4)

Product Strength	**Naloxone HCl Nasal Spray, (b) (4)**
AMOUNT PER ML	
API:	
Naloxone HCl Dihydrate USP*	(b) (4)
Inactive Ingredients:	
Sodium Chloride, USP	
(b) (4)	pH adjustment as needed
Water for Injection, USP	QS Ad
(b) (4)	

The inactive ingredients are sodium chloride, (b) (4) and water for injection. These inactive ingredients are in the FDA inactive ingredients database in FDA-approved formulations for nasal products. Up to (b) (4) mg/day of naloxone hydrochloride (or (b) (4) mL of the proposed drug formulation) may be given as per the original NARCAN label.

2.4 Comments on Novel Excipients

There are no novel excipients in the formulation.

2.5 Comments on Impurities/Degradants of Concern

Drug Substance Specifications

The drug substance is from (b) (4) DMF (b) (4) The following table illustrates the drug substance specifications for the naloxone hydrochloride drug substance (modified from the Applicant's submission):

Table 2: Drug Substance Specifications

Table 32S41-1 IMS' Specifications for Drug Substance (Naloxone HCl Dihydrate USP)

Test	Method	Specification
Noroxymorphone HCl and other impurities	(b) (4)	
Related Substance (EP) Noroxymorphone 3-*O*-allylnaloxone 10α-hydroxynaloxone 2,2'-bisnaloxone 10β-hydroxynaloxone Largest Unspecified Impurity Total Impurities		
Impurity D* (EP)		
Residual Solvent (b) (4)		

Up to (b) (4) mg/day naloxone hydrochloride may be given as per the label of the referenced product, NARCAN. For drug products with a maximum daily dose of ≤2 g/day, the ICH Q3A(R2) qualification threshold is NMT 0.15% or 1.0 mg/day intake (whichever is lower). The specification for (b) (4) is NMT (b) (4) ppm or NMT (b) (4) %. The (b) (4) contains a structural alert for mutagenicity and must be reduced to the currently acceptable threshold for potentially genotoxic impurities of NMT (b) (4) mcg/day. The specification set by the Applicant for (b) (4) would result in levels (b) (4) mcg/day when the product is used as labeled (up to (b) (4) mg NLX), and is therefore acceptable. Although several drug substance impurities exceed ICH Q3A(R2) qualification thresholds, the specifications will be considered acceptable because DMF (b) (4) is referenced for multiple approved products and no safety concerns have arisen. (b) (4) as per the manufacturer. Thus, there are no nonclinical safety concerns with the drug substance specifications.

Drug Product Specifications

The following table illustrates the drug product specifications of the proposed naloxone hydrochloride nasal drug product (modified from the Applicant's submission):

Table 3: Original Drug Product Specifications

Test	Method	Specifications
pH Determination		(b) (4)
Limit of (b) (4)		
Osmolality		
(b) (4)		
and other Impurity		

For naloxone hydrochloride, up to (b) (4) mg/day may be given as per the original NARCAN label. For a drug product with a maximum daily dose of (b) (4) mg, the ICH Q3B(R2) qualification threshold is NMT (b) (4) mcg TDI (total daily intake), whichever is lower. The specification for (b) (4) ICH Q3B(R2) qualification thresholds; however, the specifications for both (b) (4) and other impurity" exceed the ICH Q3B(R2) qualification thresholds. In the 74-day letter (dated June 29, 2016), the Applicant was tasked with tightening the specification (b) (4) to meet ICH Q3B(R2) qualification thresholds and separating out each component of the specification for " (b) (4) and other impurities" as well as being reminded that all drug product degradants must meet ICH Q3B(R2) qualification thresholds.

The most recent drug product specifications are in SDN 25 (CDER stamp date November 29, 2016) and are illustrated in the table below (from the Applicant's submission):

Table 4: Updated Drug Product Specifications

TESTS REQUIRED	METHOD #	LIMITED RANGE OF VALUES	TEST RESULTS
pH Determination	(b) (4)	(b) (4)	
Osmolality			
(b) (4)			
Related Substances			
(b) (4)			
Largest unspecified impurity			
Total impurities			

The specification for (b) (4) was tightening to NMT (b) (4) %, which meets ICH Q3B(R2) qualification thresholds. The specification for (b) (4) and other

impurities" was [redacted]. All the drug product specifications were NMT [redacted]%, which meet ICH Q3B(R2) qualification thresholds and as such, are considered acceptable. Thus, there are no nonclinical safety concerns with the drug product specifications.

Container Closure System
The following table describes the container closure for the proposed product (from the Applicant's submission):

Table 5: Container Closure Components

Table 32P11-2 Packaging Components of the Proposed N002 Product

[redacted]

The container closure is illustrated in the following diagram (from the Applicant's submission):

Figure 1: Container Closure Components

[redacted]

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(b) (4)

2.6 Proposed Clinical Population and Dosing Regimen

The proposed clinical population is both adult and pediatric patients. The dose of the proposed drug product should be administered to one nostril. An additional dose of the proposed drug product may be given after 2 minutes if the patient dose not respond using a new intranasal device into the other nostril.

2.7 Regulatory Background

There was a pre-IND meeting on March 12, 2015 and a pre-NDA meeting on November 27, 2015. The reader is referred to the meeting minutes from these meetings for details.

Naloxone hydrochloride (NDA 16636) was originally approved as NARCAN in April 13, 1971 for the treatment of known or suspected narcotic overdose via the IV, IM, or SC route of administration. The original NARCAN NDA (NDA 16636) was withdrawn from the market but not for reasons of safety or efficacy as numerous subsequent ANDAs for naloxone hydrochloride have become available. Thus, there is an extensive clinical experience with naloxone hydrochloride via the IV, IM, and SC routes of administration.

3 Studies Submitted

3.1 Studies Reviewed

There were no nonclinical studies with naloxone hydrochloride that were required in this NDA. However, the Applicant conducted a "Maximization Test for Delayed-Type Hypersensitivity in Hartley Guinea Pigs" (Study 16J0226H-X01G) and an "Intracutaneous (Intradermal) Reactivity Test in New Zealand White Rabbits" (Study 16J0226H-X02G.

3.2 Studies Not Reviewed

N/A

3.3 Previous Reviews Referenced

There were no previous reviews referenced.

4 Pharmacology

4.1 Primary Pharmacology

There were no primary pharmacology studies with naloxone hydrochloride submitted or required to support this NDA.

4.2 Secondary Pharmacology

There were no secondary pharmacology studies with naloxone hydrochloride submitted or required to support this NDA.

4.3 Safety Pharmacology

There were no safety pharmacology studies with naloxone hydrochloride submitted or required to support this NDA.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

There were no PK/ADME studies with naloxone hydrochloride submitted or required to support this NDA.

5.2 Toxicokinetics

There were no toxicokinetics with naloxone hydrochloride submitted or required to support this NDA.

6 General Toxicology

There were no general toxicology studies with naloxone hydrochloride submitted or required to support this NDA. The Agency agreed that for this potentially life-saving indication, given the previous clinical experience with off-label use of this injectable drug product intranasally, no intranasal toxicology studies would be required. Clinical monitoring in the human relative bioavailability studies also exists. The reader is referred to the medical officer review.

7 Genetic Toxicology

There were no genetic toxicology studies with naloxone hydrochloride submitted in this NDA. The applicant is relying upon the Agency previous finding of safety for Narcan (naloxone) injection, to support their application. The FDA-approved Narcan injection labeling provides the following information:

> NARCAN was weakly positive in the Ames mutagenicity and in the in vitro human lymphocyte chromosome aberration test 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.

These data will be incorporated into the current drug product labeling.

8 Carcinogenicity

There were no carcinogenicity studies with naloxone hydrochloride submitted in this NDA. The applicant is relying upon the Agency previous finding of safety for Narcan (naloxone) injection, to support their application. The FDA-approved Narcan injection labeling provides the following information:

> Studies in animals to assess the carcinogenic potential of NARCAN have not been conducted.

These data will be incorporated into the current drug product labeling.

9 Reproductive and Developmental Toxicology

There were no reproductive and developmental toxicology studies with naloxone hydrochloride submitted in this NDA. The applicant is relying upon the Agency previous finding of safety for Narcan (naloxone) injection, to support their application. The FDA-approved Narcan injection labeling provides the following information:

> Reproduction studies conducted in mice and rats at doses 4-times and 8-times, respectively, the dose of a 50 kg human given 10 mg/day (when based on surface area or mg/m^2), demonstrated no embryotoxic or teratogenic effects due to NARCAN.
>
> Use in Pregnancy
> Teratogenic Effects: Pregnancy Category C:
>
> Teratology studies conducted in mice and rats at doses 4-times and 8-times, respectively, the dose of a 50 kg human given 10 mg/day (when based on surface area or mg/m^2), demonstrated no embryotoxic or teratogenic effects due to NARCAN. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, NARCAN should be used during pregnancy only if clearly needed.
>
> Non-teratogenic Effects:
> Risk-benefit must be considered before NARCAN is administered to a pregnant woman who is known or suspected to be opioid-dependent since maternal dependence may often be accompanied by fetal dependence. Naloxone crosses the placenta, and may precipitate withdrawal in the fetus as well as in the mother. Patients with mild to moderate hypertension who receive naloxone during labor should be carefully monitored as severe hypertension may occur.

These data will be incorporated into the current drug product labeling in a PLLR compliant format as appropriate.

Although requested by the Agency, the Sponsor did not conduct a literature review to support PLLR labeling requirements. Although normally this could be considered a filing issue, given the nature of the application (potentially life-saving therapy), the Agency did not refuse to file the application.

The following literature review was completed by Dr. Newton Woo:

Published Literature

Published Title: Prenatal naloxone affects survival and morphine sensitivity of rat offspring (Hetta and Terenius, 1980)

Methods: Sprague Dawley (SD) rats were fitted with a subcutaneous minipump during Gestation Day 11 or Day 17 or Postpartum Day 3. Saline or naloxone at 30 or 100

mg/mL was released from the pump at a constant rate of 0.033 or 0.1 mg/h for a period of 7 days. Onset of parturition was noted and the number or pups live or stillborn counted. At 36 h post-delivery, litters were weighed and stillborn or dead pups removed. If number of pups exceeded nine, the litters were culled to nine, equalizing the number of females and males if possible. The pups were weighed weekly with pups weaned on PND 21.

Results: Implantation of pumps to the mothers Postpartum Day 3 did not affect pups with no effects on body growth. Neonatal mortality was significantly increased in the group that received naloxone 0.1 mg/h from Gestational Day 17 compared to saline controls (see Table below). Body weights were slightly decreased by administration of naloxone 0.03 mg/h starting on Gestation Day 17.

Group A: G17; Group B: G17; Group C: G11; Group D: G17; Group E: G17; Group F: G11

Group	Treatment	Litters	Mean number of offspring per litter	
			Delivered	Dead within 36 h
D,E,F	Saline		10.5 ± 1.0	0.5 ± 0.3
A	Naloxone, 0.03 mg/h	8	10.0 ± 1.3	2.6 ± 1.3
B	Naloxone, 0.1 mg/h	12	10.5 ± 0.9	6.3 ± 1.7**
C	Naloxone, 0.03 mg/h	3	11.6 ± 1.1	0.3 ± 0.1

Pups born from dams treated with naloxone did not differ in response to the hotplate test. However pups demonstrated a greater sensitivity to low dose morphine-induced antinociception. The clinical significance of these results is difficult to extrapolate as the dosing was via continuous infusion subcutaneously versus single-administration in the case of NARCAN nasal spray. The authors of this paper stated that the higher dose level of 0.1 mg/h received 2.4 mg/day or approximately 7 mg/kg/day, which corresponds to a HED of 68 mg/day for a 60 kg human based on body surface area.

Publication Title: The interference of naloxone hydrochloride in the teratogenic activity of opiates (Jurand, 1985)

Methods: Naloxone was administered to 8-10 pregnant female JBT/Jd mice on Gestational Day 9 at doses of 25, 40, 80, 120, or 200 mg/kg, IP. In other groups, diamorphine (65 mg/kg), methadone (19 mg/kg), and the synthetic enkephalin analogue FK 33-824 (60 mg/kg) were administered to pretreated pregnant females with either saline or naloxone to determine whether pretreatment with equimolar doses of the antagonist naloxone (see table below) applied 30 min prior to treatment with the opioid agonists antagonizes opioid agonist induced malformations. All pregnant mice were sacrificed on Gestational Day 13 with fetuses dissected and fixed in utero and all malformations were recorded.

Drug tested	Litter LD_{50} (mg/kg)	Teratogenic dose (mg/kg)	Equimolecular dose of naloxone (mg/kg)
Diamorphine hydrochloride	80	65	56
Methadone hydrochloride	22	19	19
Enkephalin analogue FK 33-824	65	60	35
Naloxone hydrochloride	Not embryotoxic up to 200 mg/kg	—	—

Results: Administration of naloxone at doses up to 200 mg/kg was not embryotoxic nor did naloxone produce any teratogenic activity. Pretreatment with equimolar doses of naloxone administered 30 min prior to administration of opioid agonists, resulted in a significant reduction in the occurrence of malformations of the central nervous system, which included kinking of the spinal cord, exencephaly, craniorachischisis, and brachyury (see table below). In contrast, dilatation of the fourth brain ventricle was not affected by pretreatment of naloxone.

Treatment	Total No. of embryos	Percent of normal and malformed embryos						
		Normal	Retarded[2]	Kinking	Exencephaly	Cranio-rachischisis	Dilation of fourth ventricle	Brachyury
No pretreatment								
Control[1]								
a	292	98.6	1.3	—	—	—	—	—
b	74	98.3	1.6	—	—	—	—	—
Diamorphine hydrocholoride								
c	266	66.0	3.0	3.0	21.0	3.2	8.0	2.2
d	57	68.4	1.75	3.5	21.0	3.5	8.8	0.5
Methadone hydrochloride								
c	287	73.5	6.2	5.2	9.5	0.34	5.2	0.34
d	51	70.6	5.1	5.1	11.8	3.9	3.9	1.9
Enkephalin analogue FK 33--824								
c	150	43.0	22.0	21.3	14.0	2.0	11.3	3.3
d	48	48.0	21.0	20.7	16.8	2.1	12.5	2.0

Pretreatment with naloxone								
Control[1]								
a	310	95.4	3.2	0.6	—	—	1.1	—
b	—	—	—	—	—	—	—	—
Diamorphine hydrochloride								
c	298	88.4	2.7	2.7	—	—	6.0	—
d	—	—	—	—	—	—	—	—
Methadone hydrochloride								
c	380	95.2	—	—	—	—	4.7	—
d	—	—	—	—	—	—	—	—
Enkephalin analogue FK 33-824								
c	160	88.7	4.3	—	—	—	6.8	—
d	—	—	—	—	—	—	—	—

[1]No pretreatment with naloxone and no treatment with teratogens or treatment with naloxone only. a = light ether anaesthesia only; b = light ether anaesthesia and intraperitoneal injection of sterile saline according to the body weight; c = light ether anaesthesia before intraperitoneal injection; d = intraperitoneal injection without ether anaesthesia.
[2]Retarded embryos are understood as those that have been found retarded by up to 3 days in growth and development in comparison with the control 13-day-old embryos.

The NOEL identified in this study was 200 mg/kg naloxone, which corresponds to a HED of 16 mg/kg based on body surface area or 975 mg for a 60 kg human.

Publication Title: Behavioral and neuroanatomical sequelae of prenatal naloxone administration in the rat (Shepanek et al., 1989)

Methods: Pregnant Long-Evans Hooded rats received daily subcutaneous injections of either 1 or 5 mg/kg naloxone or vehicle (saline) from Gestational Day 4 to Gestational Day 18. At delivery, litters were culled to 4 males and 4 females. Offspring were assessed for development of righting reflex, negative geotaxis, open field activity, and acquisition of a Warden maze. Offspring sacrificed at Postnatal Day 21 were assessed for several parameters of cerebellar, hippocampal, and motor cortical morphology.

Results: Administration of naloxone to pregnant rats from GD 4 to GD 18 did not produce any effects on maternal weights, pup survival, pup weight, or sex distribution.

PREGNANCY AND BIRTH MEASURES (MEANS ± SEM)

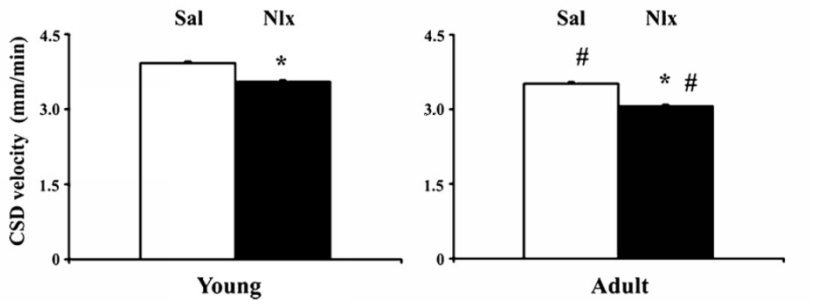
	Saline		1 mg/kg		5 mg/kg	
Number of Litters	9		11		9	
	M	F	M	F	M	F
No. pups/litter	5.3 ± 0.50	5.0 ± 0.78	5.3 ± 0.63	5.3 ± 0.56	6.3 ± 0.68	6.3 ± 0.67
Pup birth wt. (g)	6.74 ± 0.07	6.31 ± 0.08	6.86 ± 0.16	6.34 ± 0.15	6.50 ± 0.09	6.31 ± 0.08
Maternal wt. gain (g)	82.0 ± 3.71		88.5 ± 2.80		88.3 ± 4.00	

Naloxone at 5 mg/kg/day accelerated development of negative geotaxis and right reflex whereas a dose of 1 mg/kg/day resulted in impairments. In a Warden maze, low dose naloxone resulted in females having significantly more errors than controls on the first day of maze learning. No morphological effects in the motor cortex, cerebellum, and hippocampus were observed with the exception of 5 mg/kg/day naloxone, which produced higher concentration of granule cells in the curvature of the dentate gyrus as compared to controls. The results from this study indicate that prenatal exposure to naloxone may later neurobehavioral development in the rat. Doses of 1 and 5 mg/kg/day correspond to HED of 0.16 mg/kg (9.7 mg per 60 kg human) and 0.8 mg/kg (48 mg per 60 kg human), respectively.

Publication Title: Chronic neonatal exposure of rats to the opioid antagonist naloxone impairs propagation of cortical spreading depression in adulthood (Rocha-de-Melo et al., 2008)

Methods: Wistar male rats from Postnatal Day 7 to Postnatal Day 28 were treated daily with a single subcutaneous injection of 10 mg/kg/day naloxone or saline (10 mL/kg). Cortical spreading depression (CSD) was recorded in young pups aged PND 30 to PND 40 and adult rats aged PND 90 to PND 120 that were anesthetized with a mixture of 1 g/kg urethane plus 40 mg/kg chloralose intraperitoneally. A tracheal cannula was inserted and three trephine holes were made on the right side of the skull. One hole was used to apply the stimulus by a 1 min application of a cotton ball soaked with 2% KCl and two other holes were used to record the propagating CSD wave.

Results: CSD propagation velocity was decreased in both young and adult groups that were treated with naloxone as compared to animals treated with saline.



A NOAEL was identified as only one dose of 10 mg/kg/day was evaluated. This does of naloxone corresponds to HED of 1.6 mg/kg or 97 mg naloxone in a 60 kg human based on body surface area comparison.

Publication Title: Effects of opioid agonist and antagonist in dams exposed to morphine during the perinatal period (Sobor et al., 2011)

Methods: Pregnant Wistar rats were administered morphine or saline once daily subcutaneously during gestation and lactation, a period at least 21-22 days. Morphine

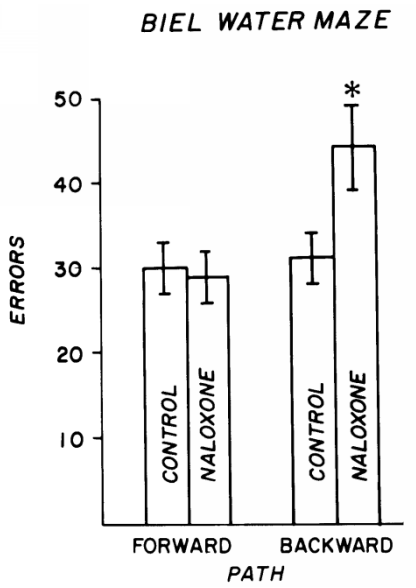
was administered at a dose of 5 mg/kg/day on the first two days and then 10 mg/kg/day afterwards. Physical and behavioral signs of morphine withdrawal were investigated both in the early postpartum period (maternal behavior) and after weaning (physical signs, locomotion, anxiety-like behaviors). Maternal behavior was evaluated after acute challenge with naloxone (3 mg/kg, SC) or morphine (10 mg/kg, SC) and morphine (10 mg/kg, SC) plus naloxone (3 mg/kg, SC).

Results: Maternal behavior was not affected by naloxone (3 mg/kg) alone but impaired maternal behavior in morphine-treated dams. Naloxone precipitated moderate physical withdrawal signals in morphine-treated dams, while anxiety and locomotor activity after administration of naloxone were not changed.

Publication Title: Effects of prenatal naloxone exposure on postnatal behavioral development of rats (Vorhees, 1980)

Methods: Pregnant Sprague-Dawley rats were administered either 40 mg/kg/day of naloxone or saline intraperitoneally in two divided doses (7 h between dosing) on Gestational Day 7 to Gestational Day 20. Dams were weighed weekly during gestation and daily during treatment and at parturition each litter was examined for the presence of dead or malformed pups. Dams and offspring were weighed weekly though weaning (PND 21) and offspring biweekly thereafter. Behavioral testing began on PND 3 and extended into adulthood PND 120. Birth litters with less than 8 progeny were eliminated from the experiment and those with more than 8 were reduced to 4 males and 4 females. Offspring were examined for physical milestones (testicular appearance, incisor eruption, eye opening, vaginal patency) and neurobehavioral measures (surface righting, swimming development, negative geotaxis, pivoting assessment, olfactory orientation, auditory startle, open field, spontaneous alternation, passive avoidance, food grasping, tail flick, activity wheels, rotorod, active avoidance, M-maze and Biel maze).

Results: Prenatal naloxone administration (GD7 to GD 20) had no significant effects on maternal weight, number of small litters, gestation length, litter size, sex distribution, and offspring mortality. However, administration of naloxone resulted in accelerated postweaning growth, upper incisor eruption, righting development, startle development, home scent discrimination, and in directional swimming and as adults, impairments in Biel water maze learning. No differences were reported in other postweaning tests including open field, running wheel, M-maze, spontaneous alternation, active or passive avoidance, rotorod, food grasping or tail flick.



A NOAEL for the impairment in Biel water maze and accelerated development was not identified. The tested dose of 40 mg/kg/day corresponds to a HED of 6.45 mg/kg or 387 mg in a 60 kg human, based on body surface area.

Publication Title: Changes of monoamine and TRH contents in naloxone induced inhibited development of rat cerebrum and cerebellum (Suzuki et al., 1988)

Methods: Newborn Sprague-Dawley rats were administered subcutaneous injections of either 1 or 50 mg/kg naloxone or saline daily until weaning (PND 21). After a week from the last injection, animals were sacrificed and brains and spinal cords rapidly removed and dissected. Levels of monoamines and their metabolites and thyrotropin-releasing hormone (TRH) were measured in different parts of the brain.

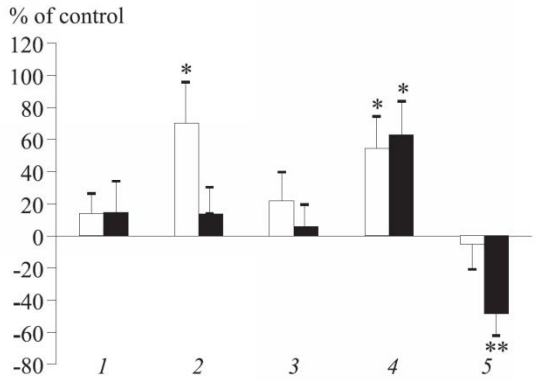
Results: Postnatal administration of naloxone from birth to weaning (PND 1 to PND 21) resulted in a dose-dependent decrease in cerebral and cerebellar weights with a reduction in body weights only observed in the high dose group. However, morphological changes or changes in movement were not observed in naloxone treated animals. Serotonin was decreased in the cerebral cortex and medulla and increased in the post and striatum of naloxone treated animals. Noradrenaline was decreased in the medulla but increased in the pons of naloxone treated animals. TRH was decreased in the cerebellum and hippocampus of naloxone treated animals. The authors suggest that neurotransmitters influence brain development that is modulated by endogenous opioid system. Naloxone doses of 1 and 50 mg/kg/day corresponds to HED doses of 0.16 mg/kg or 9.7 mg for a 60 kg human and 8.1 mg/kg or 484 mg for a 60 kg human based on body surface area, respectively.

Publication Title: Effect of opioid antagonist naloxone on maternal motivation in albino rats (Dobryakova et al., 2005)

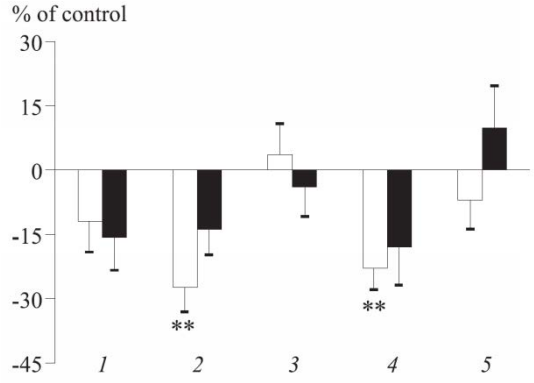
Methods: Maternal behavior was observed on Postnatal Day 4 to Day 6. Ten min prior to testing, females were injected with distilled water (PND 4 and 6) or administered aqueous solution of naloxone (PND 5) either via an intraperitoneal injection at doses of 1 or 5 mg/kg (1 mL/kg) or instillation into the nasal cavity at doses of 0.2, 1.0 and 5.0 mg/kg (100 mcL/animal). Maternal reactions were evaluated in two three sessions. Session 1 included the open field test with spontaneous exploratory activity (running, rearing, grooming etc.) recorded in red light. During Session 2 three rat pups were placed at the center of the arena and latency of the first approach, total number of approaches, number of transfers of pups, and latency of the third pup were recorded under red light. During the last session, the same parameters were evaluated at bright illumination.

Results: A single intraperitoneal injection of naloxone at a dose of 5 mg/kg on PND 5 increased the number of approaches to pups, decreased the latency of their transfer into a new location, which are measures of maternal behavior. Similarly intranasal naloxone at a dose of 1 mg/kg produced similar changes. It was noted that naloxone injected IP modified the number of approaches to the pups while after intranasal administration the number of pup transfers were altered in a more marked manner. The changes in maternal behaviors are not considered adverse by this Reviewer.

Number of pup transfers



Latency of transfer of the last pup



Effect of naloxone (IP versus IN) on maternal behaviors at red dim (light bars) and bright illumination (dark bars). 1) 1 mg/kg intraperitoneally; 2) 5 mg/kg naloxone intraperitoneally; 3) 0.2 mg/kg naloxone intranasally; 4) 1 mg/kg naloxone intranasally; 5) 5 mg/kg naloxone intranasally.

The authors indicate that naloxone administration may enhance maternal motivation in post-partum psychosis and depression. The NOEL was identified to be 1 mg/kg intraperitoneally and 0.2 mg/kg intranasally, which correspond to HED of 9.7 mg IP and 1.9 mg IN, respectively.

Publication Title: Opioid receptors regulate retrieval of infant fear memories: Effects of naloxone on infantile amnesia (Weber et al., 2006)

Methods: Naloxone, naloxone methiodide or saline were subcutaneously administered to Sprague-Dawley rats (PND 17-18) at various timepoints during contextual fear conditioning. Rats were placed into an experimental chamber for 120 seconds and then received a 1 second, 0.6 mA footshock. Rats were removed after 30 seconds and returned to their home cage either directly or after receiving a drug injection.

Results: Subcutaneous injection of naloxone at a dose of 5 mg/kg prior to testing and 7 days prior to testing, but not immediately after training, blocked infantile amnesia. Normally when rats are subjected to a shock in the fear conditioning apparatus, animals freeze when animals are returned to the same context 1 minute after training because they remember and expect a shock. When animals are returned to the apparatus 24 hours later, freezing is reduced indicating that animals do not remember the context in which it was shocked, which describes an active process of infantile amnesia. Because naloxone caused increased freezing as compared to saline when administered prior to testing, the authors believe that endogenous opioids regulate the retrieval of infant fear memories which contributes to an active process known as infantile amnesia. A NOAEL was not identified in this study as the only dose of 5 mg/kg evaluated blocked infantile amnesia. This dose corresponds to an HED of 48 mg for a 60 kg human.

Summary of Reproduction and Development Literature Review

Collectively these published studies have evaluated the effects of naloxone on reproduction and developmental endpoints and suggest that naloxone can potentially impact the central nervous system. However in most cases, the doses were significantly higher that which would be produced by this intranasal spray. It is important to note that there was no significant adverse effect identified in these published studies that would negate the benefit of this potentially life-saving therapeutic, given that an opioid agonist itself also have been demonstrated to have adverse impact on brain development. The results of the articles and literature search do not impact the safety or labelling of NARCAN nasal spray.

10 Special Toxicology Studies

the Applicant conducted a “Maximization Test for Delayed-Type Hypersensitivity in Hartley Guinea Pigs” (Study 16J0226H-X01G) and an “Intracutaneous (Intradermal) Reactivity Test in New Zealand White Rabbits” (Study 16J0226H-X02G). These studies were submitted in SDN 23 (CDER stamp date of November 21, 2016) and are reviewed below.

Title: Maximization Test for Delayed-Type Hypersensitivity in Hartley Guinea Pigs (Study 16J0226H-X01G)

Key Study Findings:

- Hartley guinea pigs were induced (intradermally and topically) and challenged (topically) with test article extracted with saline or cottonseed oil.
- All guinea pigs survived to the scheduled sacrifice.
- There were no test article-related changes in clinical signs and body weights.
- There were no sensitization reactions from the test article (both the saline and the cottonseed oil extract) in the guinea pigs tested.

Methods:
The following table provides information of the test article used in the study (from the Applicant's submission):

Figure 3: Test Article Identification

3.1.1. Test Article Identification

Name:	(b) (4)
Physical Description:	Device
Total Quantity Received for Testing:	5 pouches containing a total of 50 devices
Quantity Used for This Study:	6 devices
Lot Number:	(b) (4)
Sample Code:	Not provided by Sponsor
Part Number:	(b) (4)
Expiration Date:	(b) (4)
Special Handling and/or Precautions:	None
Sterilization Data:	Non-sterile
Storage Conditions:	Room Temperature
Final Intended Use:	Medical Device

Hartley guinea pigs that were young adults and weighed 312 to 450 g (saline control) and 310 to 460 g (cottonseed oil group) were grouped as illustrated in the following table (from the Applicant's submission):

Table 19: Study Design Overview

Text Table 4. Study Design Overview

Extracting Medium	Group	Number of Animals (n)	Induction Phase		Challenge Phase
			1st Induction Route of Administration	2nd Induction Route of Administration	Challenge Route of Administration
Saline	Test	11	Intradermal	Topical	Topical
	Negative Control	6	Intradermal	Topical	Topical
Cottonseed Oil	Test	11	Intradermal	Topical	Topical
	Negative Control	6	Intradermal	Topical	Topical

Guinea pigs (N = 11 for test groups and N = 6 for negative control groups using saline or cottonseed oil as an extracting medium) were used in the study that consisted of 2 major phases (the Induction Phase and the Challenge Phase). In the Induction Phase, guinea pigs will be exposed to either intradermal or topical administration of the test article extracted in saline or cottonseed oil. The negative control animals received control article (saline or cottonseed oil without the test article). Guinea pigs from the test and negative control group were challenged with undiluted test article extract in the Challenge Phase.

Intradermal Administration of the Induction Phase
The following table and figure illustrate the intradermal administration of the test article extracted in either saline or cottonseed oil during the Induction Phase (from the Applicant's submission):

Table 20: Intradermal Injections Used During the Induction Phase

Text Table 5. Induction Phase – Intradermal Injections

Site Location	No. of Sites	Test Group		Negative Control	
		Volume per Site	Dose Preparation	Volume per Site	Dose Preparation
Cranial (A)	2	0.1 mL	1:1 (FCA : SCI/OIL)	0.1 mL	1:1 (FCA : SCI/OIL)
Middle (B)	2	0.1 mL	Test Extract	0.1 mL	Control (SCI/OIL)
Caudal (C)	2	0.1 mL	1:1 (FCA : Test Extract)	0.1 mL	1:1 (FCA : Control SCI/OIL)

FCA – Freund's Complete Adjuvant; SCI – Saline; OIL – Cottonseed Oil

Figure 4: Dosing Sites of the Intradermal Injections (Induction Phase)

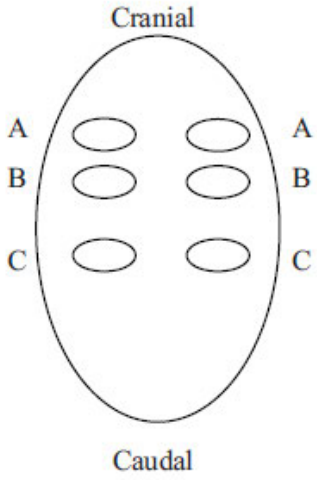


Figure 2: Schematic illustration of dose sites on the dorsum of the animal. A: Equal volumes of FCA and SCI or OIL; B: Test extract or Control; C: Equal volumes of FCA and Test extract or Control

Prior to dosing, the hair on the dorsocranial thorax of each guinea pig was removed by clipping and the injection sites were disinfected with alcohol wipes. There were 3 pairs of 0.1 mL intradermal injection sites (A, B, and C in the figure above). The first pair (A) of injections consisted of an emulsion of Freund's Complete Adjuvant (FCA) in an equal volume of the given vehicle. The second pair (B) of injections consisted of the test article extract. The third pair (C) of injections consisted of an emulsion of the test article extract and an equal volume of FCA. The negative control guinea pigs were similar to the test animals except the test article extract was replaced in the second (B) and third (C) pair of injections with an equal amount of control solution (either saline or cottonseed oil).

Topical Administration of the Induction Phase

Topical application of the Induction Phase occurred 7 ± 1 days after the intradermal injections. For the topical application of the test article extract, the topical areas (the same areas on the dorsocranial thorax used for intradermal injections during the Induction Phase) were clipped and shaved one day prior to topical application. These topical dosing sites were pretreated with 10% sodium dodecyl sulfate (SDS) in petroleum and were left uncovered for 24 ± 2 hours since the test article did not cause irritation. Disks of Whatman #4 filter paper (4.25 cm in diameter) were saturated with 0.3 mL of the undiluted test article extract, applied to application site of each guinea pig, and held in place with surgical tape. The trunks of the guinea pigs were wrapped with 3-inch gauze bandage that was held in place with tape. The trunks of the guinea pigs were then wrapped with light rubber sheeting (dental dam) so that complete occlusion was obtained. The negative control guinea pigs were treated similarly to the test groups except their disks of Whatman #4 filter paper were saturated with either saline or cottonseed oil without the test article. After 48 ± 2 hours, the animals were unwrapped and the disks of Whatman #4 filter paper removed.

Challenge Phase

The Challenge Phase dosing procedure is summarized in the table below (from the Applicant's submission):

Table 21: Challenge Phase

Text Table 6. Challenge Phase

Extraction Medium	Group	No. of Sites	Volume/Site	Article
Saline	Test	1	0.3 mL	Test Extract
		1	0.3 mL	Saline
	Negative Control	1	0.3 mL	Test Extract
		1	0.3 mL	Saline
Cottonseed Oil	Test	1	0.3 mL	Test Extract
		1	0.3 mL	Cottonseed oil
	Negative Control	1	0.3 mL	Test Extract
		1	0.3 mL	Cottonseed oil

The Challenge Phase was performed 14 ± 1 days after the topical application of the Induction Phase. Prior to the Challenge Phase, an area on the right side of each guinea pig (5 x 5 cm) was clipped. On the next day, the right side of each guinea pig was shaved and 2 Hill Top Chambers® (one containing 0.3 mL of the test solution and one containing 0.3 mL of the control solution) was applied to the shaved areas. All guinea pigs (test and control groups) were challenged with the undiluted test article extract. The site exposed to saline or cottonseed oil served as a vehicle control. The trunks of the guinea pigs were wrapped with 3-inch gauze bandage that was held in place with tape and no further wrapping was necessary as the Hill Top Chambers® provided the necessary occlusion. Twenty-four hours after dosing, the guinea pigs were unwrapped and the dosing sites for the saline test group and negative controls were allowed to air dry. The dosing sites for the cottonseed oil test group and its control were gently cleansed with alcohol wipes to remove any chemical residues.

The skin at the challenge dosing sites were scored for skin reaction at 24 ± 2 and 48 ± 2 hours after unwrapping according to the following table (from the Applicant's submission):

Table 22: Magnusson and Kligman Scoring System

Text Table 7. Magnusson and Kligman Scoring System

Patch Test Reaction	Grading Scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

Adopted from ISO 10993–10, *Biological Evaluation of Medical Devices–Tests for Irritation and Skin Sensitization.*

In addition to an evaluation of the skin, mortality/moribundity checks as well as clinical observations of the guinea pigs were performed daily. All guinea pigs were also observed for adverse reactions immediately after dosing and daily until the end of the study. Body weights were measured and recorded prior to the start and at the end of the study.

Results:
All guinea pigs survived to the scheduled sacrifice. There were no test article-related clinical observations noted in any of the groups. The following tables illustrate the results in skin reaction scores and body weights of the guinea pigs (from the Applicant's submission):

Table 23: Skin Reaction Scores and Body Weights (Saline Extraction)

Summary Table 1. Skin Reaction Scores and Animal Weights (Saline Extraction)

Animal Number	Sex	24 Hour Score		48 Hour Score		Weight (g)	
		Test	Control	Test	Control	Pre-Test	Post-Test
Test Group							
30582	M	0	0	0	0	417	576
30583	M	0	0	0	0	410	555
30584	M	0	0	0	0	368	564
30594	M	0	0	0	0	341	445
30595	M	0	0	0	0	424	567
30596	M	1	1	1	1	369	456
30403	F	0	0	0	0	445	549
30604	F	1	1	1	1	312	415
30605	F	1	1	1	1	341	432
30613	F	0	0	0	0	326	452
30614	F	0	0	0	0	328	443
Negative Control Group							
30585	M	0	0	0	0	407	573
30586	M	1	1	1	1	310	487
30587	M	0	0	0	0	423	544
30404	F	1	1	1	1	450	510
30606	F	0	0	0	0	361	491
30607	F	1	0	1	0	334	443

At the 24-hour and 48-hour observation of the saline extraction group, there were 1/6 males and 2/5 females (total of 3/11) with a skin reaction score of 1 (discrete or patchy erythema) in both the test article and control groups. Thus, there were no sensitization reactions from the test article (saline extract) in the guinea pigs tested.

Table 24: Skin Reaction Scores and Body Weights (Cottonseed Oil Extraction)

Summary Table 2. Skin Reaction Scores and Animal Weights (Cottonseed Oil Extraction)

Animal Number	Sex	24 Hour Score		48 Hour Score		Weight (g)	
		Test	Control	Test	Control	Pre-Test	Post-Test
Test Group							
30588	M	0	0	0	0	362	469
30589	M	0	0	0	0	392	546
30590	M	1	1	1	1	385	512
30597	M	0	0	0	0	395	564
30598	M	1	1	1	1	400	501
30599	M	0	0	0	0	371	489
30366	F	1	1	1	1	460	537
30608	F	0	0	0	0	312	456
30609	F	0	0	0	0	327	433
30615	F	0	0	0	0	340	501
30616	F	0	0	0	0	356	505
Negative Control Group							
30591	M	1	1	1	1	407	545
30592	M	0	0	0	0	376	516
30593	M	0	0	0	0	377	513
30610	F	1	1	1	1	322	466
30611	F	0	0	0	0	310	417
30612	F	1	1	1	1	331	489

At the 24-hour and 48-hour observation of the cottonseed oil extraction group, there were 1/6 males and 2/5 females (total of 3/11) with a skin reaction score of 1 (discrete or patchy erythema) in both the test article and control groups. Thus, there were no sensitization reactions from the test article (cottonseed oil extract) in the guinea pigs tested.

All guinea pigs in all groups exhibited similar body weight increases during the study for both the saline and cottonseed oil extract. There were similar body weights from this study and the historical control (see table below). Thus, there were no test article-related changes in body weight.

The following table illustrates the historical control for Hartley guinea pigs in this delayed-type sensitivity study (from the Applicant's submission):

Table 25: Skin Reaction Scores and Body Weights (Historical Control)

Summary Table 3. Skin Reaction Scores and Animal Weights (Data from Historical Positive Control Study)

Animal Number	Sex	24 Hour Score		48 Hour Score		Weight (g)	
		Test	Control	Test	Control	Pre-Test	Post-Test
Test Group							
29575	M	3	0	3	0	389	517
29577	M	3	0	3	0	387	540
29585	M	3	0	3	0	374	491
29599	M	3	0	3	0	324	428
29598	M	3	0	3	0	415	513
29602	M	2	0	2	0	355	428
Negative Control Group							
29576	M	0	0	0	0	376	514
29584	M	0	0	0	0	437	571
29586	M	0	0	0	0	385	510
29600	M	0	0	0	0	364	521
29601	M	0	0	0	0	372	531
29603	M	0	0	0	0	351	469

As shown in the table above, the skin reaction scores from this study (1) was below the historical control skin reaction scores (2-3).

Title: Intracutaneous (Intradermal) Reactivity Test in New Zealand White Rabbits (Study 16J0226H-X02G)

Key Study Findings:

- New Zealand White Rabbits were intracutaneously treated with the test article (Luer-Jet Injector) extracted from either saline or cottonseed oil.
- All rabbits survived to the scheduled sacrifice.
- There were no test article-related changes in clinical signs and body weights.
- There was no skin irritation from the test article extracted in both saline and cottonseed oil in the rabbits tested.

Methods:

The following table provides information of the test article used in the study (from the Applicant's submission):

Figure 5: Test Article Identification

3.1.1. Test Article Identification

Name:	(b) (4)
Physical Description:	Device
Total Quantity Received for Testing:	5 pouches containing a total of 50 devices
Quantity Used for This Study:	2 devices
Lot Number:	(b) (4)
Sample Code:	Not provided by Sponsor
Part Number:	(b) (4)
Expiration Date:	10/13/2020
Special Handling and/or Precautions:	None
Sterilization Data:	Non-sterile
Storage Conditions:	Room Temperature
Final Intended Use:	Medical Device

Adult Female New Zealand White rabbits (N = 3) weighing 2.4 to 2.5 kg were grouped as illustrated in the following table (from the Applicant's submission):

Table 26: Study Design Overview

Text Table 4. Study Design

Group/Extraction Medium	Number of Animals (n)	Route of Administration	Dose/Site	Number of Sites/Animal	
				Test	Control
Saline	3	Intracutaneous	0.2 mL	5	5
Cottonseed Oil		Intracutaneous	0.2 mL	5	5

Prior to the test, the fur of each rabbit was clipped and only rabbits with healthy, intact skin were used in the study. The following figure illustrates the injection site on the back of each rabbit (from the Applicant's submission):

Figure 6: Intradermal Injections Sites

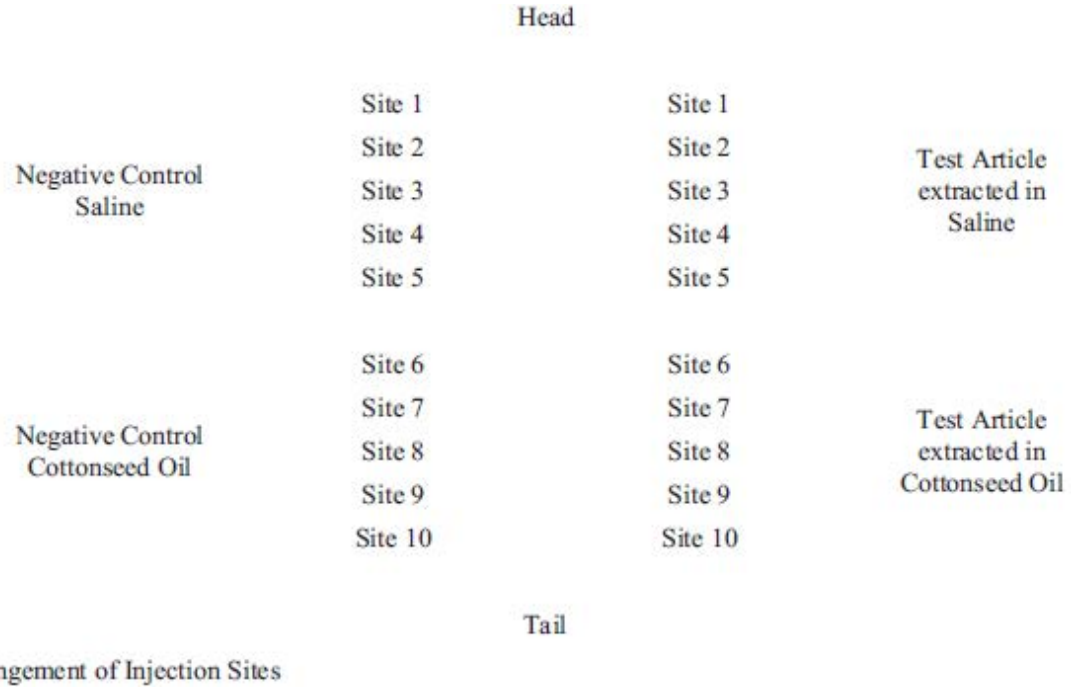


Figure 1: Arrangement of Injection Sites

As shown in the figure above, a volume of 0.2 mL of the test article extracted in saline was injected intracutaneously at five sites on one side of the spinal column, anterior to the dorsal midline, of each of 3 rabbits. A volume of 0.2 mL of the saline control was injected intracutaneously at five sites on the other side of the spinal column, anterior of the dorsal midline, of the same 3 rabbits. This process was repeated on the same rabbits for the test article extracted in cottonseed oil and the cottonseed oil control but posterior of the dorsal midline.

The injection sites were examined immediately after injection and scored for any tissue reactions at 24 ± 2, 48 ± 2, and 72 ± 2 hours post-dosing according to the following table (from the Applicant's submission):

Table 27: Intradermal Skin Reaction Scoring System

Text Table 5. Classification System for Intracutaneous (Intradermal) Reactions

Erythema and Eschar Formation	Score
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet-redness) to eschar formation preventing grading of erythema	4
Edema Formation	Score
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised about 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
Maximal Possible Score for Irritation	8

Table adopted from ISO 10993-10 Biological Evaluation of Medical Devices – Test for Irritation and Skin Sensitization.

In addition to the skin scoring, all rabbits were observed for morbidity and moribundity once daily as well as for daily clinical observations. Body weight measurements were made prior to dosing and at the end of the study.

Results:
All rabbits survived to the scheduled sacrifice. There were no test article-related changes in clinical signs.

The body weight measurements are illustrated in the following table (from the Applicant's submission):

Table 28: Summary of Body Weights

Text Table 6. Body Weights

Animal Number	Initial Body Weight (kg)	Final Body Weight (kg)	Body Weight Change* (kg)
67549	2.5	2.5	0
67550	2.5	2.5	0
67551	2.4	2.4	0

*Initial body weight was subtracted from final body weight.

As shown in the table above, there were no changes in the body weights in the rabbits used in the study.

There was no skin irritation from the test article extracted in saline (see Summary Table 1 from the Applicant):

Table 29: Skin Reaction Scores (Saline Extract)

Summary Table 1. Reaction Scores (Saline Extract)

Animal ID: 67549	Test Sites			Control Sites		
	24 ± 2 hrs	48 ± 2 hrs	72 ± 2 hrs	24 ± 2 hrs	48 ± 2 hrs	72 ± 2 hrs
Erythema	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0
Edema	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0
Total reaction score/observation	0	0	0	0	0	0
Total Mean*	0			0		

Animal ID: 67550	Test Sites			Control Sites		
	24 ± 2 hrs	48 ± 2 hrs	72 ± 2 hrs	24 ± 2 hrs	48 ± 2 hrs	72 ± 2 hrs
Erythema	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0
Edema	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0
Total reaction score/observation	0	0	0	0	0	0
Total Mean*	0			0		

Animal ID: 67551	Test Sites			Control Sites		
	24 ± 2 hrs	48 ± 2 hrs	72 ± 2 hrs	24 ± 2 hrs	48 ± 2 hrs	72 ± 2 hrs
Erythema	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0
Edema	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0
Total reaction score/observation	0	0	0	0	0	0
Total Mean*	0			0		

*Total Mean = Total reaction scores/15. Means are rounded to one decimal place.

Interpretation of Results:

Test Overall Mean Score (Total means for all three animals divided by three): 0 / 3 = 0

Control Overall Mean Score (Total means for all animals divided by three): 0 / 3 = 0

Final Test Score (The difference between Test Overall Mean Score and Control Overall Mean Score): 0 – 0 = 0

The skin reaction scores to evaluate irritation from the test article extracted in cottonseed oil were the same in both the test article and control groups (see Summary Table 2 from the Applicant):

Table 30: Skin Reaction Scores (Cottonseed Oil Extract)

Summary Table 2. Reaction Scores (Cottonseed Oil Extract)

	Test Sites															Control Sites													
Animal ID: 67549	24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs					24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs				
Erythema	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total reaction score/observation	5					5					5					5					5					5				
Total Mean*	1.0															1.0														

	Test Sites															Control Sites													
Animal ID: 67550	24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs					24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs				
Erythema	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total reaction score/observation	5					5					5					5					5					5				
Total Mean*	1.0															1.0														

	Test Sites															Control Sites													
Animal ID: 67551	24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs					24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs				
Erythema	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total reaction score/observation	5					5					5					5					5					5				
Total Mean*	1.0															1.0														

*Total Mean = Total reaction scores/15. Means are rounded to one decimal place.

Interpretation of Results:

Test Overall Mean Score (Total means for all animals divided by three): 3.0 / 3 = 1.0

Control Overall Mean Score (Total means for all animals divided by three): 3.0 / 3 = 1.0

Final Test Score (The difference between Test Overall Mean Score and Control Overall Mean Score): 1.0 – 1.0 = 0

It is noted that were is typically an inflammatory response to the intradermal injection of oil as shown in the erythema score of 1 in both the test article and control groups. Thus, there was no skin irritation from the test article extracted from either saline or cottonseed oil.

The following table illustrates the historical control of this study in New Zealand White Rabbits (from the Applicant's submission):

Table 31: Skin Reaction Scores (Historical Control)

Summary Table 3. Reaction Scores (Data from the Historical Positive Control Study)

	Test Sites														Control Sites													
Animal ID: 67136	24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs					24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs				
Erythema	2	2	2	2	2	2	2	2	2	2	1	2	1	2	2	1	1	1	1	1	1	1	1	1	1	1	0	0	0
Edema	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total reaction score/observation	30					30					28					5					5					2				
Total Mean*	5.9															0.8														

	Test Sites														Control Sites													
Animal ID: 67137	24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs					24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs				
Erythema	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	0	0	0	0	0	0	0
Edema	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total reaction score/observation	30					30					30					5					2					0				
Total Mean*	6.0															0.5														

	Test Sites														Control Sites													
Animal ID: 67138	24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs					24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs				
Erythema	3	3	3	3	3	3	3	3	3	4	3	3	3	3	3	0	0	1	1	1	0	0	0	1	1	0	0	0	1	0
Edema	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total reaction score/observation	35					36					35					3					2					1				
Total Mean*	7.1															0.4														

*Total Mean = Total reaction scores/15. Means are rounded to one decimal place.

Interpretation of Results:

Test Overall Mean Score (Total means for all three animals divided by three): 19 / 3 = 6.3

Control Overall Mean Score (Total means for all animals divided by three): 1.7 / 3 = 0.6

Final Test Score (The difference between Test Overall Mean Score and Control Overall Mean Score): 6.3 – 0.6 = 5.7

The following table illustrates the average skin reaction score for all tested groups as well as the historical control (from the Applicant's submission):

Table 32: Average Skin Reaction Scores

Summary Table 4. Average Reaction Scores at Each Observation Period

Extract	Observation Period	Average Test Score	Average Control Score	Difference
Saline	24 Hr	0	0	0
	48 Hr	0	0	0
	72 Hr	0	0	0
Cottonseed Oil*	24 Hr	1.0	1.0	0
	48 Hr	1.0	1.0	0
	72 Hr	1.0	1.0	0
Positive Control (Historical Data)	24 Hr	6.3	0.9	5.4
	48 Hr	6.4	0.6	5.8
	72 Hr	6.2	0.2	6.0

*Intradermal injection of oil frequently elicits some inflammatory response.

As shown in the historical control and summary tables above, the average skin reaction scores from this study for the saline extract (0) and cottonseed extract (1) were below the skin reaction scores from the historical control (6.3).

11 Integrated Summary and Safety Evaluation

There were no required nonclinical studies submitted in this NDA. The formulation is a (b) (4) mg/mL concentration of naloxone hydrochloride in (b) (4) (final dose of (b) (4) in (b) (4) that contains no novel excipients. There are no nonclinical safety concerns with the drug substance and drug product specifications. (b) (4) To support the container closure system, the Applicant submitted the results of the delay-typed hypersensitivity in guinea pigs testing extracts from a component of the container closure system as well as an intracutaneous reactivity test in rabbits, both of which did not demonstrate any skin sensitization or skin irritation of container closure system extractable compounds. Therefore, there are no additional nonclinical concerns with the proposed naloxone hydrochloride drug product. From a pharmacology toxicology perspective, the proposed drug product, Naloxone Hydrochloride Nasal Spray, (b) (4) is recommended for approval.

12 Appendix/Attachments

References

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I concur.