CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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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

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Product:	Nalmefene Auto-Injector
Indication:	Emergency treatment of known or suspected opioid overdose induced by natural or synthetic opioids in adults and pediatric patients aged 12 years and older
Applicant:	Purdue Pharma L.P.
Review Division:	Division of Pharm/Tox for Neuroscience (DPT-N)
Reviewer:	Min Zhang, PhD
Supervisor:	Newton Woo, PhD
Deputy Division Director:	R. Daniel Mellon, PhD
Clinical Review Division:	Division of Anesthesiology, Addiction Medicine, and Pain Medicine (DAAP)
Clinical Division Director:	Rigoberto Roca, MD
Project Manager:	Sandy Truong, Pharm D

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Executive Summary

1.1 Introduction

Purdue Pharma L.P. submitted a New Drug Application (NDA), via the 505(b)(2) regulatory pathway, for nalmefene HCl auto-Injector 1.5 mg (NAI) with the following indication: Emergency treatment of known or suspected opioid overdose induced by natural or synthetic opioids in adults and pediatric patients aged 12 years and older (b) (4) (b) (4) (b) (4). The NDA relies upon the Agency's previous finding of safety and efficacy for Revex (NDA 20459), which is the listed drug (LD). The drug product, NAI, is a single-dose, (b) (4), disposable automated drug delivery device for intramuscular or subcutaneous administration of nalmefene. The auto-injector houses a prefilled syringe containing 1.5 mg of nalmefene base in 0.5 mL solution that includes 0.94% magnesium chloride. It is estimated that two injections nalmefene (3 mg) results in C_{max} of 16.2 ng/mL and $AUC_{0\text{-}inf}$ of 62 ng*h/mL, which is double the levels achieved after a single IM dose of 1.5 mg (Study NAL1005).

1.2 Brief Discussion of Nonclinical Findings

In support of the nalmefene autoinjector, the Applicant submitted several nonclinical toxicology studies that evaluated the safety of the higher nalmefene concentration and $MgCl_2$ excipient, as well as qualify a drug product degradant specification. The Applicant is seeking approval for an adult and pediatric population, 12 years and older. The Applicant has committed to conducting two juvenile animal studies (JAS), as PREA PMRs.

The Applicant's specifications for the drug substance/drug product impurity and residual solvents are deemed acceptable. The drug substance specification (b) (4), exceeds ICH Q3A(R2) recommended qualification threshold but is qualified by the 14-day repeat-dose and local tolerance studies that evaluated nalmefene, which contained supportive (b) (4) impurity levels. The drug product stability specification (b) (4) exceeds the ICH Q3B(R2) recommended qualification threshold. The Applicant submitted a 14-day IV rat study, local tolerance IM/SC rabbit study, and a (Q)SAR assessment, which were reviewed and adequately qualifies the proposed specification (b) (4). All analyzed elemental impurities are within respective parenteral PDEs recommended in ICH Q3D.

To support safety of the container closure system, the Applicant submitted an extractables/leachables assessment. Issues have been identified by the CMC review team regarding the extractables and leachables data. However, given there are no leachable compounds identified above the 5 mcg/day threshold, components of the autoinjector have been used in other FDA approved products, and that this product is for a life-saving indication, the identified issues do not preclude approval and will be addressed through postmarketing commitment (PMC) studies.

Systemic and local toxicity of the drug product were evaluated in two GLP 14-day repeat-dose general toxicology studies in rats and dogs with IM administration route and in local tolerance studies in rats and rabbits with IM or SC administration route. These studies were conducted to support the higher nalmefene concentration and the levels of $MgCl_2$ as this excipient is not currently in any FDA approved IM/SC products.

In the 14-day repeat-dose toxicity studies, animals received daily IM injections of nalmefene or the vehicle of $MgCl_2$ (0.94%). There were no test article-related adverse systemic effects observed at high exposure multiples compared to the human nalmefene exposure after administration of two doses (see Table 22 for exposure multiples). Test article-related local effects were observed at the IM dosing site, including minimal to mild edema, fibrosis and hemorrhage; minimal to marked myofiber necrosis and associated minimal to moderate inflammatory changes (mixed cell and/or macrophages). Minimal to mild local effects were generally observed in the vehicle (0.94% $MgCl_2$) and the low dose-treated group with similar incidence, but there was a dose-related increase in incidence and severity at the mid-dose and the high dose groups. The local findings at the mid-dose and the high dose of nalmefene were considered exacerbation of vehicle/procedure-related local toxicity. By Day 29, the local changes observed on Day 15 had partially or completely resolved. It is noted that these repeat-dose studies characterized the exaggerated local toxicity profiles given that the nalmefene autoinjector is intended for single acute use.

The local safety of the drug product was primarily characterized in acute local tolerance studies. In these studies that included a 14-day recovery period, rats or rabbits received a single IM or SC administration of saline, several doses of $MgCl_2$ alone or combination of $MgCl_2$ and nalmefene. In the local tolerance rat study, minimal to moderate local effects including necrosis, inflammation and hemorrhage were observed at the IM or SC injection site across all groups. Recovery of the local effects were observed on Day 15. Test article-related minimal to mild local effects were observed at nalmefene concentrations up to 5.2 times and 2.6 times the clinical nalmefene concentration, respectively; and up to 2.8 times and 5 times the clinical $MgCl_2$ concentration, respectively. In the local tolerance rabbit study, minimal to mild local effects were observed at the IM or SC dosing site in all treatment groups with nalmefene or $MgCl_2$ concentration up to 2.5 times the corresponding clinical concentration. These local effects were considered procedure related as opposed to $MgCl_2$ or nalmefene related, given that the local findings are commonly seen secondary to injection procedures, lacked a dose-response in incidence and severity, and occurred at similar incidence and severity across all treatment groups (including the saline group).

Taken together, the submitted pivotal nonclinical studies described above have adequately characterized the local and systemic safety of the nalmefene auto-injector.

1.3 Recommendations

1.3.1 Approvability

From a nonclinical pharmacology toxicology perspective, NDA 218590 for nalmefene HCl auto-Injector 1.5 mg (NAI) may be approved with the following recommended labeling changes and postmarketing requirement (PMR) studies.

1.3.2 Additional Nonclinical Recommendations

The following juvenile animal studies are recommended as a PREA PMRs to support the clinical pediatric study outlined in the agreed Pediatric Study Plan (PSP).

- Conduct juvenile animal studies in rats to support the initiation of clinical studies in pediatric patients from (b) (4) to less than 12 years of age. This study will evaluate the effect of the drug on growth and development, specifically reproductive performance/sexual maturation and central nervous system histopathology and long-term behavioral effects.

- Conduct an acute neurotoxicity study in juvenile rats to address the impact of the drug on neuronal development in children under the age of 3. This study will evaluate the effect of the drug on neuroapoptosis and central nervous system histopathology in neonatal rats during the peak of synaptogenesis.

1.3.3 Labeling

The table below contains the draft labeling proposed by the Applicant, suggested changes by this Reviewer, and the rationale for this Reviewer's changes. For final labeling, the reader is referred to the approval action letter.

Applicant's proposed labeling	Reviewer's proposed changes	Rationale for changes
(b) (4)	**8 USE IN SPECIFIC POPULATIONS** **8.1 Pregnancy** Risk Summary Life-sustaining therapy for opioid overdose should not be withheld (see Clinical Considerations). There are no available data on nalmefene use in pregnant women to evaluate for a drug-associated risk of major birth defects or miscarriage. In animal reproduction studies, no (b) (4) effects (b) (4) (b) (4) on embryo-fetal development were observed in rats and rabbits treated with nalmefene (b) (4)	Per PLLR, the administration route and comparison basis should not be described in the Risk Summary.

(b) (4)	(b) (4) [see Data] The background risk of major birth defects and miscarriage for the indicated population is unknown. All pregnancies have a background risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively.	
	Data *Animal Data* Reproduction studies have been performed in rats (b) (4) and rabbits (b) (4) -by oral administration (b) (4) and in rabbits by intravenous administration of nalmefene (b) (4) . No effects on embryo-fetal development were observed at (b) (4) oral doses up to 1200 mg/m2/day (b) (4) nd up to 2400 mg/m2/day, and (b) (4) (b) (4) intravenous dose up to 96 mg/m2/day, which is 52 times (b) (4) the human dose of 3.0 mg (two TRADENAME (b) (4) administrations) (b) (4) (b) (4) based on body surface area comparison. The treatment in rats did not affect offspring survival.	The Revex drug label describes the reproductive and developmental studies as "Reproduction studies have been performed in rats (up to 1200 mg/m /day) and rabbits (up to 2400 mg/m /day) by oral administration of nalmefene and in rabbits by intravenous administration up to 96 mg/m /day (114 times the human dose).". It is noted that only the exposure multiple in the IV rabbit study is described in the Revex label. (b) (4) (b) (4) Therefore, if the Applicant wishes to add margins for the oral studies then additional PK studies should be conducted and margins be calculated based on AUC.
	8.4 Pediatric Use (b) (4)	New juvenile studies with targeted endpoints up to current standards will be conducted.

(b) (4)	(b) (4)	
	(b) (4) **Pharmacodynamics** Nalmefene reverses the effects of natural and synthetic opioids, including respiratory depression, sedation, and hypotension. Pharmacodynamic studies have shown that nalmefene injection has a longer duration of action than naloxone injection at fully reversing doses. Nalmefene has no opioid agonist activity.	No changes are proposed in this section. The original Revex label has the language, "Nalmefene has no opioid agonist activity." It is noted that nalmefene is an opioid receptor antagonist at mu and delta opioid receptors. In vitro and in vivo studies report that nalmefene is a partial agonist at human kappa opioid receptors (Remmers 1999, Bart 2005), however this has not been uniformly replicated (Toll 1998).
	13 NONCLINICAL TOXICOLOGY **13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility** <u>Carcinogenesis</u> Long-term animal studies to evaluate the carcinogenic potential of nalmefene have not been completed.	No changes are proposed to this section.
	<u>Mutagenesis</u> Nalmefene did not have mutagenic activity in the Ames test with five bacterial strains or the mouse lymphoma assay. Clastogenic activity was not observed in the mouse micronucleus test or in the cytogenic bone marrow assay in rats. However, nalmefene did exhibit a weak but significant clastogenic activity in the human lymphocyte metaphase assay in the absence but not in the presence of exogenous metabolic activation.	No changes are proposed to this section.

(b) (4)	Impairment of fertility Oral administration of nalmefene up to 1200 mg/m^2/day did not affect fertility in rats. (b) (4) (b) (4)	Deleted language that is not relevant to fertility assessment. (b) (4) Therefore, if the Applicant wishes to add margins for the oral studies then additional PK studies should be conducted and margins be calculated based on AUC.

2 Drug Information

2.1 Drug

CAS Registry Number
58895-64-0

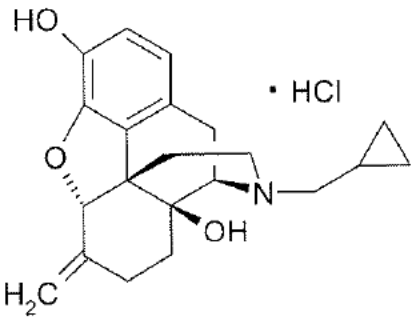
Generic Name
Nalmefene Hydrochloride

Code Name
NA

Chemical Name
(5α)-17-(cyclopropylmethyl)-4,5-epoxy-6-methylenemorphinan-3,14-diol hydrochloride
Morphinan-3,14-diol, 17-(cyclopropylmethyl)-4,5-epoxy-6-methylene-e, hydrochloride, (5alpha)-

Molecular Formula/Molecular Weight
$C_{21}H_{25}NO_3$•HCl/ 375.89 g/mol

Structure



Established Pharmacologic Class
Opioid antagonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

Application	Drug Product	Indication	Division	Status	Company
NDA 20459	Revex	Reversal of opioid drug effect	DAAP	Discontinued	Baxter
ANDA 212955	Nalmefene hydrochloride injection	Reversal of opioid drug effect	Office of Generic Drugs	Active	Purdue Pharma
IND 137597	Nalmefene hydrocholoride autoinjector	Opioid overdose	DAAP	Active	Purdue Pharma
DMF (b) (4)	Nalmefene drug substance			Active	(b) (4)

2.3 Drug Formulation

Nalmefene Hydrochloride Injection, Auto-Injector, 1.5 mg (NAI) is supplied as a single-use, (b) (4), disposable auto-injector that houses a prefilled syringe (PFS) containing a sterile, (b) (4), clear, colorless to light yellow solution containing 1.5 mg of nalmefene (expressed as free base) per 0.5 mL. See Applicant's table below for the drug product composition.

Table 1: Nalmefene Hydrochloride Injection, Auto-Injector, 1.5 mg

Ingredient	Weight/unit (mg/0.5 mL)	% (w/v)	Function	Reference to Standard
Nalmefene Hydrochloride	1.5 mg[1]	(b) (4) Nalmefene HCl (b) (4) Nalmefene	Active ingredient	HSE[2]
Magnesium Chloride Hexahydrate[3]	(b) (4) (4.7 mg as Magnesium Chloride)	(0.94% as Magnesium Chloride)		USP
Hydrochloric Acid	QS to pH (b) (4)	QS to pH (b) (4)	pH adjuster	NF
Water for Injection	QS (b) (4)[5]	QS (b) (4)	(b) (4)	USP/EP
(b) (4)				

[1] Expressed as Nalmefene free-base
[2] HSE: In house standard
[3] Formula: $MgCl_2 \cdot 6H_2O$
[4] (b) (4) HCl is used for pH adjustment.
[5] QS: Quantity sufficient
(b) (4)

2.4 Comments on Novel Excipients

The drug product contains magnesium chloride hexahydrate (see the table below).

Table 1: Acceptability of Excipients in the Drug Product

Excipient	Max. Daily Exposure from Two Doses (mg)*	Amount/Dose Listed in IID (mg)	Acceptability
Magnesium chloride hexahydrate	(b) (4) (9.4 mg as $MgCl_2$)	Not listed in IID for IM or SC route	Yes, See discussion below

$MgCl_2$ is not currently in IM or SC products as depicted in the FDA Inactive Ingredient Database (IID). This excipient is present in products for other parenteral routes including intraperitoneal route. $MgCl_2$ is present in over-the-counter oral supplements with a daily dosage from 200-400 mg. To support the systemic and local safety of $MgCl_2$, the Applicant has conducted 14-day repeat-dose studies with IM administration route in rats and dogs, as well as acute local tolerance studies with IM or SC administration routes in rats and rabbits. As shown in the tables below, no systemic adverse effects were observed with $MgCl_2$ treatment at doses up to 1 times and 3.3 times human exposure to $MgCl_2$ after two doses of NAI (body surface area comparison) in the 14-day rat and dog study, respectively. The systemic safety margin established in the rat study is 1x. Given that $MgCl_2$ is present as an excipient in drug products approved for parental routes including intraperitoneal route at high levels, its use in over-the-counter supplements as well as the safety profile demonstrated in the 14-day repeat-dose studies, this Reviewer has no concerns on systemic safety of $MgCl_2$ at the proposed level for this life-saving drug product. For the local effects, the local tolerance studies with either IM or SC administration route in rats or rabbits demonstrated adequate safety margins. In summary, based upon the totality of the data, this Reviewer concludes that there are no systemic or local safety concerns with $MgCl_2$.

Table 2: Estimated Systemic Safety Margins Reached in Nonclinical Studies Compared to Two Doses in Humans

Excipient	Animal			Human $MgCl_2$ Dose after 2 Doses of NAI (mg/day)	Safety Margin (Animal HED/ Human Dose)
	Study	NOAEL Dose** (mg/kg/day)	HED Based upon BSA* (mg/day)		
$MgCl_2$	14-day Rat (Study 3243-002)	0.94	9.10	9.4	~1
	14-day Dog (Study 3243-003)	0.94	31.33		3.3

*HED: Human equivalent dose calculated based upon conversion factors for the dog and rat, 1.8 and 6.2, respectively, assuming 60 kg human; BSA: body surface area

** NOAEL dose refers to the dose of $MgCl_2$ in the excipient group in the rat and dog study: 9.4 mg/mL * 0.1 mL/kg = 0.94 mg/kg for the NOAEL dose in rats and dogs

Table 3: Estimated Local Safety Margins Reached in Nonclinical Studies

Excipient	Animal			Human	Exposure Margin Based upon Concentration (Animal/Human)
	Animal Study	Route	NOAEL $MgCl_2$ Concentration*	$MgCl_2$ concentration	
$MgCl_2$	Rat Local Tolerance Study (Study 3243-004)	IM	26.4 mg/mL	9.4 mg/mL	2.8
		SC	47 mg/mL		5
	Rabbit Local Tolerance Study (Study 3243-004)	IM	23.5 mg/mL	9.4 mg/mL	2.5
		SC	23.5 mg/mL		2.5

*NOAEL dose for $MgCl_2$ refers to the mid dose of $MgCl_2$ alone treatment for the IM route and the high dose of $MgCl_2$ treatment alone for the SC route in the rat study, and the high dose of $MgCl_2$ treatment alone for IM or SC route in the rabbit study.

2.5 Comments on Impurities/Degradants of Concern

Drug Substance Organic Impurities

A list of DS organic impurity specifications, including comparisons to ICH Q3A(R2) qualification and identification thresholds and acceptability, are summarized in the table below.

Table 4: Drug Substance Organic Impurities

Impurity	Proposed Specification	Max. TDI*	Comparison to ICH Thresholds		Acceptable?
			Identification Threshold	Qualification Threshold	
			Q3A(R2) 0.1% or 1.0 mg/per day whichever is lower	Q3A(R2) 0.15% or 1.0 mg/per day whichever is lower	
(b) (4)	NMT (b) (4) % w/w	(b) (4) mcg/day		Exceeds	Yes; see discussion below
	NMT (b) (4) % w/w	(b) (4) mcg/day		Complies	Yes, per ICH Q3A(R2)
	NMT (b) (4) % w/w	(b) (4) mcg/day	Complies		Yes, per ICH Q3A(R2)

*: TDI: Total daily intake calculated based upon 2 doses of 3 mg/day of nalmefene base

As shown in the table above, the drug substance specification for (b) (4), NMT (b) (4) % (w/w), is greater than 0.15% qualification threshold recommended by ICH Q3A

(R2), whereas the total daily intake (TDI) of (b) (4) after administration of 2 doses of 3 mg nalmefene is less than the qualification threshold of 1 mg/day. (b) (4) (b) (4) In the nonclinical batch (1902000371) used in the 14-day repeat-dose rat study, (b) (4) was present at (b) (4)% (w/w). As shown in the table below, (b) (4) HED at the NOAEL of the study is (b) (4) times the human (b) (4) dose after the administration of 2 doses of nalmefene. Thus the nonclinical repeat-dose rat study provides adequate systemic support for the proposed (b) (4) impurity specification. No adverse effects were observed in the local tolerance studies in rats and rabbits that received IM or SC administration with the test article containing (b) (4) impurity at a concentration (b) (4) times the impurity concentration in the clinical formulation (See Table 6). Based upon these nonclinical data, this Reviewer concludes that there are no safety concerns with the proposed specification for the DS (b) (4) impurity.

Table 5: Systemic Safety Margin of (b) (4) in 14-day Rat Toxicology Study

Impurity	Animal Study	Concentration of (b) (4) in Nonclinical Lot	Nalmefene NOAEL	(b) (4) Dose at NOAEL	(b) (4) HED	Max Human Exposure from 2 doses*	Safety Margin**
(b) (4)	14-day Rat Study	(b) (4) % w/w (lot 1902000371)	(b) (4) mg/kg/day	(b) (4) mg/kg/day	(b) (4) mg/day	(b) (4) mg/day	(b) (4)

*: (b) (4)
**

Table 6: Local Safety Margin of (b) (4) in Local Tolerance Studies

Impurity	Animal Study	Concentration of (b) (4) in Nonclinical Lot	Dosing Rout	Nalmefene NOAEL (mg/mL)	(b) (4) Concentration (mg/mL)	Max (b) (4) Concentration in Drug Formulation at NMT (b) (4) (mg/mL)*	Safety Margin**
(b) (4)	Rat Local Tolerance Study (Study 3243-004)	(b) (4)% w/w Lot 1902000369	IM	(b) (4)		(b) (4)	(b) (4)
			SC				
	Rabbit Local Tolerance Study (Study 3243-005)		IM				
			SC				

* (b) (4)
*

Drug Product Impurities

A list of DP degradant specifications, including comparisons to ICH Q3B(R2) qualification and identification thresholds as well as acceptability, are summarized in the table below.

Table 7: Drug Product Organic Impurities

Impurity	Proposed Specification	Maximum TDI*	Comparison to ICH Thresholds		Acceptable?
			Identification Threshold	Qualification Threshold	
			Q3B(R2) 0.5.% or 20 mcg/per day whichever is lower	Q3B(R2) 1% or 50 mcg /day whichever is lower	
(b) (4)	NMT (b) (4) % (release)	(b) (4) mcg/day		Complies	Yes; per ICH Q3B(R2)
	NMT (b) (4) % (stability)	(b) (4) mcg/day		Exceeds	Yes via nonclinical qualification studies
(b) (4)	NMT (b) (4) % (release; stability)	(b) (4) mcg/day		Complies	Yes; per ICH Q3B(R2)
					Yes; per ICH Q3B(R2)
Individual unspecified Impurities	NMT (b) (4) % (release; stability)	(b) (4) mcg/day	Complies		Yes; per ICH Q3B(R2)

*TDI: Total daily intake calculated based upon two doses of 3 mg nalmefene base.

The proposed specifications for drug product impurities are in compliance with ICH Q3B(R2) guidance, with the exception of the stability specification for (b) (4), which is higher than the Q3B(R2)-recommended qualification threshold. To qualify the safety of the higher level of (b) (4), the Applicant conducted 14-day repeat-dose rat study with (b) (4) administered by IV bolus injection and an acute local tolerance rabbit study with IM and SC administration. As shown in the tables below, there were no adverse systemic effects observed in the 14-day repeat-dose study with (b) (4) at a HED that was (b) (4) times the human dose of (b) (4) after 2 doses of nalmefene administration, and there were no local adverse effects observed in the local tolerance study at the concentration that was (b) (4) times the maximum concentration of (b) (4) in the drug formulation. The Applicant also conducted a (Q)SAR assessment with (b) (4) using a rule-based method and a statistical-based method, which resulted in a negative prediction for the Ames test. According to ICH M7 Note 1, no further genotoxicity studies are needed for safety qualification if the impurity is negative in (Q)SAR and total daily intake

is less than 1 mg. Taken together, the nonclinical studies are considered adequate to support the safety of the proposed specification of NMT (b) (4) % for (b) (4).

Table 8: Systemic Safety Margin of (b) (4) in 14-day Rat Toxicology Study

Impurity	Animal Study	Administration Route	(b) (4) NOAEL	(b) (4) HED Based upon BSA	Max. Human Exposure at NMT (b) (4) % (2 doses)*	Safety Margin**
(b) (4)	14-day Rat Study IV (Study 01352006)	IV	(b) (4) mg/kg/day	(b) (4) mg	(b) (4) mg/day	(b) (4)

(b) (4)

Table 9: Local Safety Margin of (b) (4) in Local Tolerance Study

Impurity	Animal Study	Dosing Rout	(b) (4) NOAEL (mg/mL)	Max (b) (4) Concentration in Drug Formulation at NMT (b) (4) % (mg/mL)*	Safety Margin**
(b) (4)	Local Tolerance Rabbit Study (Study 20457072)	IM	(b) (4)		
		SC			

*:
** (b) (4)

Residual Solvents

As shown in the table below, residual solvents in the DS are Class 2 and Class 3 with limits in compliance to the ICH Q3C guidance. The Applicant states that no residual solvents are used in or generated during the manufacture of the DP.

Table 10: Residual Solvents in the Drug Substance

Residual Solvent	Solvent Class	Acceptance Criteria as per ICH Q3C	Acceptable?
(b) (4)	Class 2	(b) (4) ppm	Yes
	Class 3	ppm	Yes
	Class 2	ppm	Yes
	Class 3	ppm	Yes
	Class 3	ppm	Yes

Elemental Impurities

The elemental impurities were evaluated for several potential sources including the drug substance, excipients, manufacturing equipment, and the drug product including the container closure. The Applicant submitted a report that contains risk assessment for the potential presence of elemental impurities in the finished drug product, according to the ICH Q3D guidance. Based on theoretical cumulative values, all potential elemental impurities are lower than the 30% established parenteral permitted daily exposure (PDE), as per ICH Q3D. Hence, the Applicant concluded that routine analysis and additional controls of elemental impurities for the drug product is not required. There are no concerns on the report of elemental impurity risk assessment from pharmacology toxicology perspective.

(b) (4)

The Applicant stated that a risk assessment was performed for (b) (4) in the drug product. Refer to the CMC review for the adequacy of the (b) (4) risk assessment.

2.6 Container Closure System

The proposed combination product NAI contains a sterile non-pyrogenic nalmefene hydrochloride solution filled into a (b) (4) (b) (4) (b) (4) single auto-injector is placed into a paperboard carton along with the IFU/PI.

(b) (4)

The testing of container closure system included evaluation of extractables with the syringe components (b) (4), leachables evaluation in aged drug products which were developmental samples in prefilled syringes stored at 40°C/75% RH for 6 months, and leachables evaluation of stability registration batches.

Extractable and Leachable Compounds in Protocol P38805.00

Extraction studies were conducted under several conditions (e.g., pure water and 50% IPA/water with agitation at 40ºC for 24 h) to identify potential leachables. See CMC review for adequacy of the extraction data and methodologies. It is noted that the CMC review team has raised concerns regarding the extraction studies (see the CMC review for more details).

In addition to the extraction studies, simulation studies with aged drug product formulation (developmental samples) stored for 6 months at 40°C/75% RH were

evaluated for potential leachables originating from the respective container/closure system. The aged drug product (DP) was analyzed for volatile, semi-volatile, non-volatile, elemental teachable and (b) (4) by HS/GC/MS, DUGCIMS, UHPLC/UV/MS, ICP/MS and ICP/OES, respectively.

The Applicant summarized the simulation data of the aged DP (see table excerpted below). Note that most of the leachables identified in the aged DP were targeted in the stability samples.

Results Table 7: Assessment of Leachable Components from the Autoinjector Device

Components Leached from Aged Drug Product
(b) (4)

Class 1, 2 and 3 metal elements were extracted from the study at levels well below the permitted daily exposures (PDEs), for the parental route, outlined in the ICH Q3D guideline. No Class 1, 2, and 3 metal elements were detected in the aged DP.

Leachable Evaluation in Stability Batches

According to the Applicant, multiple time points within a 24 month period (0, 12, 24 mo) were evaluated for leachables in stability samples of the prefilled syringe at 25°C/ 60%RH storage condition, and 6 months of accelerated stability data from storage at 40°C/75%RH and a single 12 month time point was evaluated for leachables in stability samples at normal conditions from the Nalmefene Hydrochloride Injection Auto-Injector.

Shown below are summarized leachables data collected from 3 batches of prefilled syringes at multiple time points (0, 12, and 24 mo). As shown in the tables below, there were no compounds above the 5 mcg/day threshold at any time point.

Batch 101313-04-01-0001

Test	Specification	Storage Time (months)		
	(b) (4) Sample ID:	0	12	24
		Compound	µg/Syringe	
Semi-volatile Leachables GC/FID (b) (4)	Report Results	(b) (4)		(b) (4)
		Unknown RRT (b) (4)		

Test	Specification	Storage Time (months)								
	(b) (4) Sample ID:	0			12			24		
	Compound	RRT	Mode	µg/ syringe	RRT	Mode	µg/syringe	RRT	Mode	µg/ syringe
Non-volatile Leachable LC/MS	Report Results	(b) (4)								

Batch 101313-04-01-0002

Test	Specification	Storage Time (months)		
	(b) (4) Sample ID:	0	12	24
		Compound	µg/Syringe	
Semi-volatile Leachables GC/FID (b) (4)	Report Results	(b) (4)		

Test	Specification	Storage Time (months)								
	(b) (4) Sample ID:	0			12			24		
	Compound	RRT	Mode	µg/ syringe	RRT	Mode	µg/ syringe	RRT	Mode	µg/ syringe
Non-volatile Leachable LC/MS	Report Results	(b) (4)								

Batch 101313-04-01-0003

Test	Specification	Storage Time (months)		
		0	12	24
	(b) (4) Sample ID:	101313-04-01-0003	101313-04-03-0003	101313-04-04-0003
Semi-volatile Leachables GC/FID (b) (4)	Report Results	Compound	µg/Syringe	(b) (4)
			Replicate 1	Replicate 2
		Unknown RRT (b) (4)	(b) (4)	
		Unknown RRT (b) (4)		

Test	Specification	Storage Time (months)								
		0			12			24		
	(b) (4) Sample ID:	101313-04-01-0003			101313-04-03-0003			101313-04-04-0003		
		Compound	RRT	Mode	µg/ syringe	RRT	Mode	µg/ syringe	RRT	Mode	µg/ syringe
Non-volatile Leachable LC/MS	Report Results	(b) (4)									

The leachables data of three registration batches at the 12-month timepoint for the assembled auto-injector are presented in the Applicant's table below. All the detected leachables are well below the SCT of 5 mcg/day, and below 120 mcg/day limit for genotoxic compounds. See CMC review for the adequacy of the leachables assessment including the detecting methods and limited timepoints.

Test Sample	Compounds	Result (µg/syringe)		
		Drug Product-Lot number I2557	Drug Product-Lot number I2558	Drug Product- Lot number I2559
Semi-volatile Leachables GC/FID (b) (4)	(b) (4)	(b) (4)		
	Unknown RRT			
Non-volatile Leachable (LC/MS)	Unknown RRT (b) (4)			
	Unknown RRT (b) (4)			
	Unknown RRT (b) (4)			
	Unknown RRT (b) (4)			

As there were no compounds above the 5 mcg/day, a toxicological risk assessment was not needed. It is noted that the CMC review team has identified issues with the extractable leachables data that does not preclude approval but will request additional

follow up extractable/leachable data as a postmarketing commitment. Given that no leachable compounds were detected above 5 mcg/day, (b) (4) have been used in other FDA approved products, and that this is a life-saving product, this Reviewer is in agreement that the outstanding CMC issues may be addressed as a postmarketing commitment.

2.7 Proposed Clinical Population and Dosing Regimen

Clinical Population: Adults and pediatric patients aged 12 years and older.

Dosing Regimen
Initial Dosing:
The recommended dose of NAI in adults and pediatric patients aged 12 years and older is 1.5 mg nalmefene base delivered by intramuscular or subcutaneous injection into the anterolateral aspect of the thigh, through clothing if necessary.

Repeat Dosing:
If the desired response is not obtained after 2 to 5 minutes, administer an additional dose of NAI using a new auto-injector. If there is still no response and additional doses are available, administer additional doses of NAI every 2 to 5 minutes until emergency medical assistance arrives.

2.8 Regulatory Background

This is a 505(b)(2) application referencing the approved drug product Revex (NDA 20459) as the listed drug (LD). Revex has been approved for reversal of opioid overdose and reversal of postoperative opioid depression via the IV, IM, or SC route of administration. According to the original Revex drug label, which was initially approved in 1995, the recommended initial dose is 0.5 mg/70 kg, for management of known or suspected opioid overdose. If needed, this may be followed by a second dose of 1.0 mg/70 kg, 2-5 minutes later.

The Division provided comments on the nonclinical development program of the nalmefene auto-injector (refer to PIND meeting minutes (MM) dated 7/19/2018, MM dated 11/3/2021, End-of-Phase 2 (EoP2) MM dated 8/2/2023 and PreNDA MM dated 12/1/2023 under IND 137597). The following key comments were communicated to the Applicant:

- The Division concurred that 14-day repeat-dose studies in the rat and dog via IM administration are appropriate studies to support the systemic safety of the drug product.

- The Division concurred that single-dose local tolerance studies in rats and rabbits via IM and SC routes of administration appear reasonable to support the characterization of local safety for the drug product. We recommended

histopathological evaluation of the injection site on Day 2 and Day 14 to evaluate potential acute and delayed local toxicity.

- The Division concurred that a 14-day repeat-dose toxicity study with IV administration route in rats and an in silico computational analysis for mutagenicity are appropriate to support safety qualification of the drug degradant, (b) (4) at higher levels. The Applicant agreed to conduct a single local tolerance study in rabbits with IM and SC administration route to support the local safety of (b) (4) at higher levels.

- In the PreNDA meeting, we emphasized to conduct toxicology risk assessments based upon the leachables data and the final toxicological risk assessment should be based on the maximum level of each leachable detected in long-term stability samples that include any intended secondary container closure system(s) taking into consideration the maximum daily dose of your product. Detailed recommendations regarding the extractables and leachables evaluations were provided.

The Applicant seeks approval in adults and children, 12 years and older. The Agency concurred that safety and efficacy of nalmefene may be extrapolated to pediatric patients 12-17 years of age. A deferral has been granted by the Agency for clinical studies assessing the pharmacokinetics and pharmacodynamics of nalmefene in patients < 12 years old. The Applicant will conduct two juvenile animal studies to support their pediatric study outlined in the agreed Pediatric Study Plan (PSP). The nonclinical study protocols have been reviewed. The Applicant revised the study protocol based upon the Division's advice and final study protocols have been submitted (SDN 63 under IND 137597).

3 Studies Submitted

The conducted nonclinical studies are summarized in the Applicant's table below.

Table 1. Toxicology Studies to Support Nalmefene Hydrochloride Injection, Auto-injector 1.5 mg

Test Article	Study Type and Species	Route and Duration	Study Number and Submission Information
Systemic and Local Tolerance studies of Nalmefene Hydrochloride in NAI Clinical Formulation			
Nalmefene Hydrochloride in 0.94% $MgCl_2$	Repeat dose toxicity in Rat and Dog	IM injection daily for 14 days	TOX_NAL_N_068 (Rat) TOX_NAL_P_066 (Dog) Section 4.2.3.2
Nalmefene Hydrochloride in 0.94% $MgCl_2$ and $MgCl_2$ in saline	Local tolerance in Rat and Rabbit	IM and SC injection-Single dose	TOX_NAL_N_062 (Rat) TOX_NAL_N_063 (Rabbit) Section 4.2.3.6

Qualification of nalmefene hydrochloride degradant, (b) (4)			
(b) (4) in saline	Repeat dose toxicity (systemic effects) in Rats	Intravenous injection daily for 14 days	TOX_NAL_P_053 Study amendment with histology Section 4.2.3.7.6
(b) (4) in 0.94% $MgCl_2$	Local tolerance in Rabbits	IM and SC injection-single dose	TOX-NAL-N-080 (Rabbits) Section 4.2.3.7.6
(b) (4) in silico Mutagenicity Evaluation	Computational Mutagenicity Analysis of (b) (4)	In silico software DEREK programs	TOX-NAL-N-040 Section 4.2.3.7.6

3.1 Studies Reviewed

All the studies in the table above have been reviewed.

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

IND 137597

4 Pharmacology

4.1 Primary Pharmacology

No primary studies were conducted with nalmefene by the Applicant. Nalmefene is an opioid receptor antagonist at mu and delta opioid receptors. In vitro and in vivo studies report that nalmefene is a partial agonist at human kappa opioid receptors (Remmers 1999, Bart 2005), although this has not been uniformly replicated (Toll 1998). It is well accepted that the mechanism of action to reverse opioid overdose is the blockade of mu opioid receptors.

4.2 Secondary Pharmacology

The Applicant did not submit secondary pharmacology studies with nalmefene.

4.3 Safety Pharmacology

The Applicant did not submit safety pharmacology studies with nalmefene.

5 Pharmacokinetics/ADME/Toxicokinetics

The Applicant did not submit pharmacokinetics/ADME/toxicokinetic data with nalmefene.

6 General Toxicology

6.1 Single-Dose Toxicity

Several GLP single-dose local tolerance studies have been conducted and are reviewed under Section 10 Special Toxicology Studies.

6.2 Repeat-Dose Toxicity

6.21 Study Title: Nalmefene: A 14-Day Intramuscular Toxicity Study in Rats with a 14-Day Recovery Period

Study no.:	Sponsor Reference No. TOX_NAL_N_068 Testing Facility Study No. 3243-002
Study report location:	Study Report
Conducting laboratory and location:	(b) (4)
Date of study initiation:	3/24/2022
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #:	Nalmefene HCl, 190200371, 98.4% $MgCl_2$, 201453, Not specified

Methods	
Doses:	0, 0.3 (LD), 1.5 (MD) and 7.5 mg/kg/day (HD)
Frequency of dosing:	Once daily
Route of administration:	IM
Dose volume:	0.1 mg/kg
Formulation/Vehicle:	Vehicle: 0.94% $MgCl_2$ solution Dose formulations was prepared by adding nalmefene HCl at required amounts to the vehicle (0.94% $MgCl_2$ solution) adjusted to a pH of 3.9 ± 0.05.
Species/Strain:	Rats/Crl:CD®(SD)
Number/Sex/Group:	See Applicant's table below:

Group No.	Test Article	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Main Study		Recovery Study	
					No. of Males	No. of Females	No. of Males	No. of Females
1	Vehicle	0	0.1	0	10	10	6	6
2	Nalmefene	0.3		3	10	10	6	6
3		1.5		15	10	10	6	6
4		7.5		75	10	10	6	6

Group No.	Test Article	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Toxicokinetic Study	
					No. of Males	No. of Females
5	Vehicle	0	0.1	0	6	6
6	Nalmefene	0.3		3	6	6
7		1.5		15	6	6
8		7.5		75	6	6

Age:	8.5 weeks old at initiation of dosing
Satellite groups:	See the table above
Unique study design:	No
Deviation from study protocol:	No deviations were deemed to significantly impact the data interpretation.

Key Study Findings

- At the end of the dosing phase on Day 15, both sexes at the HD exhibited test article-related, reversible, generally minimally higher (up to 84% increase from the control mean value) aspartate aminotransferase (AST) activity which correlated microscopically with myofiber necrosis and associated inflammation at the dosing site.

- Test article-related microscopic findings on Day 15 were limited to the injection site and adjacent skeletal muscles. By Day 29, test article-related injection site changes observed at Day 15 had largely or completely resolved.

    - The findings at the injection site included minimal to mild edema, fibrosis and hemorrhage; minimal to marked myofiber necrosis and associated

minimal to moderate inflammatory changes (mixed cell and/or macrophages).

- The findings were dose dependent and were considered exacerbation of vehicle-related local toxicity. Minimal local effects were observed in the vehicle and LD-treated groups with similar incidence, but there was a dose-related increase in incidence and severity at the MD and HD.

- The findings in the adjacent skeletal muscle included minimal inflammation, necrosis and muscle degeneration/regeneration.

- For the systemic effects, the NOAEL is the HD. This Reviewer considers the findings of moderate inflammation or moderate/marked necrosis, observed at the MD and HD, to be adverse; therefore, the LD, corresponding to 3 mg/mL, is deemed to be the NOAEL with minimal to slight local effects observed. However, this Reviewers agrees that the local effects are not dosing-limiting toxicity considering the reversibility and clinical monitorability.

Observation and Results

Mortality

No test article-related mortality was observed.

This Reviewer noticed that one MD male rat (Rat 3243-002-1-7001) in the TK group was found dead on Day 1 of the dosing. There was no discussion on the death of this animal in the study report. The death was deemed not test article related by the Applicant.

Clinical Signs

The Applicant stated that there were no test article-related clinical signs during the study. This Reviewer noticed there was a reduction of activity in four HD-treated rats during the first week of treatment, which might be potentially test article related.

Body Weights

No test article-related changes were observed.

Food Consumption

No test article-related changes were observed.

Ophthalmoscopy

No test article-related changes were observed.

ECG

Not conducted

Hematology and Coagulation

The blood samples were taken prior to the designated necropsy in fasted animals.

The following hematology parameters were evaluated:

Hematology Parameters

Red blood cell count	White blood cell count
Hemoglobin concentration	Neutrophil count (absolute and percent)
Hematocrit	Lymphocyte count (absolute and percent)
Mean corpuscular volume	Monocyte count (absolute and percent)
Red blood cell distribution width	Eosinophil count (absolute and percent)
Mean corpuscular hemoglobin concentration	Basophil count (absolute and percent)
Mean corpuscular hemoglobin	Large unstained cells (absolute and percent)
Reticulocyte count (absolute and percent)	Other cells (as appropriate)
Platelet count	Blood smear (preserve and stain)[a]

[a] A blood smear will be prepared from each hematology sample. Blood smear review may be performed on select animals per Testing Facility SOP.

Coagulation Parameters

Prothrombin time	Fibrinogen
Activated partial thromboplastin time	Sample quality

No test article-related findings were observed.

This Reviewer noticed the following inflammatory cell-related changes in the test article-treated groups on Day 15 (see the table below). The test article relevance of the findings remains unclear given that the findings were not consistent across the sexes.

Table 11: Noteworthy Changes (% Change from Controls) in Hematology Assessment

Treatment	Nalmefene Hydrochloride							
Sex	M				F			
Group #	1	2	3	4	1	2	3	4
Dose (mg/kg/day)	0	0.3	1.5	7.5	0	0.3	1.5	7.5
Number of Animals								
Toxicity	10	10	10	10	10	10	10	10
HEMATOLOGY / WHOLE BLOOD								
Basophils (10^9/L)								
Day 15	0.043	+9	+23	+56	0.042	-10	-2	+19
Eosinophils/Leukocytes (%)								
Day 15	0.50	+36	-2	-12	0.71	-20	-21	-35
Large Unstained Cells/Leukocytes (%)								
Day 15	0.82	+23	+24	+44	1.21	-5	-12	+2
Large Unstained Cells (10^9/L)								
Day 15	0.091	+30	+35	+60	0.113	-12	-13	+7
Monocytes/Leukocytes (%)								
Day 15	2.63	+12	-6	+2	2.99	-22	-27	-31

Clinical Chemistry

The following clinical chemistry parameters were evaluated:

Clinical Chemistry Parameters

Alkaline phosphatase	Albumin/globulin ratio (calculated)
Total bilirubin[a]	Glucose
Aspartate aminotransferase	Total cholesterol
Alanine aminotransferase	Triglycerides
Urea nitrogen	Electrolytes (sodium, potassium, chloride)
Creatinine	Calcium
Total protein	Phosphorus
Albumin	Sample quality
Globulin (calculated)	

[a] When total bilirubin is > 0.5 mg/dL, direct bilirubin will also be measured, and indirect bilirubin will be calculated.

At the end of the dosing interval on Day 15, both sexes at the HD exhibited test article-related, reversible, generally minimally higher (up to 84% increase from the control mean value) aspartate aminotransferase (AST) activity which correlated microscopically with myofiber necrosis and associated inflammation at the dosing site.

This Reviewer also noticed a 45% increase of alanine aminotransferase (ALT) in the HD male group. However, the increase appears mainly associated with a single HD male rat (Rat 4011) without correlated microscopic findings. The test article relevance of the finding is not clear.

Table 12: Noteworthy Changes (% Change from Controls) in Clinical Chemistry Assessment

Treatment	Nalmefene Hydrochloride							
Sex	M				F			
Group #	1	2	3	4	1	2	3	4
Dose (mg/kg/day)	0	0.3	1.5	7.5	0	0.3	1.5	7.5
Number of Animals								
Toxicity	10	10	10	10	10	10	10	10
CLINICAL CHEMISTRY / SERUM								
Alanine Aminotransferase (U/L)								
Day 15	27.4	+10	+9	+45	23.3	+5	0	+24
Aspartate Aminotransferase (U/L)								
Day 15	81.8	-1	-5	+84	71.1	-8	+6	+78
CLINICAL CHEMISTRY / SERUM								
Alanine Aminotransferase (U/L)								
Day 29	26.2	+2	-2	-6	25.7	-9	+21	+23
Aspartate Aminotransferase (U/L)								
Day 29	69.8	-7	-8	-2	81.0	-27	+18	-15

Urinalysis

The following urinalysis parameters were evaluated:

Urinalysis Parameters

Volume	Bilirubin
Color	Ketones
Appearance	Blood
Specific gravity	Urobilinogen
pH	
Protein	
Glucose	

No test article-related changes were observed.

Terminal Procedures

The scheduled necropsy was conducted on Day 15 and Day 29. Terminal procedures and evaluations were summarized in the Applicant's tables below.

Terminal Animals

Group No.	Scheduled Euthanasia Day	Necropsy Procedures			Histology Processing	Microscopic Evaluation
		Necropsy	Tissue Collection	Organ Weights		
1, 4	15	X	Full List[a]	Full List[a]	Full List[a]	Full List[a]
2, 3	15	X	Full List[a]	Full List[a]	Full List[a]	Full List[a]

Recovery Animals

Group No.	Scheduled Euthanasia Day	Necropsy Procedures			Histology Processing	Microscopic Evaluation
		Necropsy	Tissue Collection	Organ Weights		
1-4	29	X	Full List[a]	Full List[a]	Full List[a]	Full List[a]

Toxicokinetic Animals

	Necropsy	Tissue Collection	Organ Weights	Histology Processing	Microscopic Evaluation
Toxicokinetic Animals (Found Dead or Unscheduled Euthanasia)	X (limited)[b]	-	-	-	-
Toxicokinetic Animals (Scheduled Euthanasia)	-	-	-	-	-

Terminal Procedure Tables Footnotes:
X = Procedure to be conducted; - = Not applicable.
'Histology Processing' = embedded in paraffin, sectioned, mounted on glass slides, and stained with hematoxylin and eosin.
[a] See Tissue Weighing, Collection, Processing and Evaluation Table (Attachment A) for list of tissues applicable to each procedure.
[b] Toxicokinetic animals that die on study or are euthanized for humane reasons will be subjected to a limited necropsy examination, consisting of an evaluation of the organs and tissues in the thoracic, abdominal, and pelvic cavities, with no tissues retained. During the limited necropsy examination, special attention will be directed to evidence of possible procedural-related trauma.

ATTACHMENT A

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
Body cavity, nasal	-	X	-	-
Bone marrow smear	-	X[a]	-	-
Bone marrow, sternum	-	X	X	X
Bone, femur	-	X (1)	X (1)	X (1)
Bone, sternum	-	X	X	X
Brain	X	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)
Ganglion, dorsal root, lumbar	-	X	-	-
Gland, adrenal	X (2)	X (2)	X (2)	X (2)
Gland, clitoral	-	X (2)	-	-
Gland, Harderian	-	X (2)	X (1)	X (1)
Gland, lacrimal	-	X (2)	-	-
Gland, mammary	-	X	X	X
Gland, parathyroid	-	X (2)	X (2)	X (2)
Gland, pituitary	X	X	X	X
Gland, preputial	-	X (2)	-	-
Gland, prostate	X	X	X	X
Gland, salivary, parotid	-	X (2)	-	-
Gland, salivary, sublingual	-	X (2)	-	-
Gland, salivary, mandibular	-	X (2)	X (1)	X (1)
Gland, seminal vesicle	-	X (2)	X (2)	X (2)
Gland, thyroid	-	X (2)	X (2)	X (2)
Gland, thyroid/parathyroid	X (2)	-	-	-
Gland, Zymbal's	-	X (2)	-	-
Gut-associated lymphoid tissue	-	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	-	-
Liver	X	X	X	X
Lung	-	X	X	X

Organ	Weigh	Macroscopic Evaluation and Collection	Histology Processing	Microscopic Evaluation
Lymph node(s) draining administration site(s): inguinal and iliac [c]	-	X (2)	X (1)	X (1)
Lymph node, mandibular	-	X (2)	X (1)	X (1)
Lymph node, mesenteric	-	X	X	X
Macroscopic abnormalities	-	X	X	X
Muscle, skeletal	-	X (2)	X (1)	X (1)
Nerve, optic	-	X (2)[b]	X (2)	X (2)
Nerve, sciatic [c]	-	X (2)	X (1)	X (1)
Nerve, tibial	-	X (2)	-	-
Ovary	X (2)	X (2)	X (2)	X (2)
Oviduct	-	X (2)	-	-
Pancreas	-	X	X	X
Site(s), administration: last dose administration site	-	X (2)	X (2)	X (2)
Skin	-	X	X	X
Small intestine, duodenum	-	X	X	X
Small intestine, ileum	-	X	X	X
Small intestine, jejunum	-	X	X	X
Spinal cord, cervical	-	X	X	X
Spinal cord, thoracic	-	X	X	X
Spinal cord, lumbar	-	X	X	X
Spleen	X	X	X	X
Stomach	-	X	X	X
Testis	X (2)	X (2)[b]	X (2)	X (2)
Thymus	X	X	X	X
Tongue	-	X	X	X
Trachea	-	X	X	X
Ureter	-	X (2)	-	-
Urinary bladder	-	X	X	X
Uterus/Cervix	X	X	X	X
Vagina	-	X	X	X

X = Procedure to be conducted. - = Not applicable. (1) = one side. (2) = both sides.

[a] Bone marrow smears will be collected from the humerus at scheduled and unscheduled necropsies (for possible examination). Smears will not be collected from animals that are found dead or from animals that were euthanized moribund and then stored in the refrigerator prior to necropsy. Bone marrow smears will be allowed to air dry and are not fixed in formalin.

[b] Eyes and optic nerves will be preserved in Davidson's fixative. Testes and epididymides will be preserved in modified Davidson's fixative.

[c] Same side as last injection site (left unless designated otherwise).

Gross Pathology

There are no test article-related changes observed.

Organ Weights

There are no test article-related changes observed. Some organ weight changes were observed on Day 15, but none of them were deemed test article related, considering lack of dose dependency, lack of microscopic changes or individual values in treated groups being generally overlapping with the control values.

Histopathology

Adequate Battery: Yes

Peer Review: Unknown

Test article-related microscopic findings on Day 15 were limited to the injection site and adjacent skeletal muscle (see the table below).

- The findings at the injection site included minimal to mild edema, fibrosis and hemorrhage; minimal to marked myofiber necrosis and associated minimal to moderate inflammatory changes (mixed cell and/or macrophages).
    - The findings were dose dependent and were considered exacerbation of vehicle-related local toxicity.
        - Minimal myofiber necrosis and inflammation were observed in the vehicle and LD-treated groups with similar incidence, but there was a dose-related increase in incidence and severity at the MD and HD.
            - Necrosis was defined as fragmented or swollen myofibers, typically with internal macrophage or satellite nuclei and loss of striations.
- The findings in the adjacent skeletal muscle included minimal inflammation, necrosis and muscle degeneration/regeneration.
    - Applicant: The findings may be a result of possible inadvertent sampling of injection site or limited migration of test article in skeletal muscle near the documented injection site.

Microscopically, by Day 29, test article-related injection site changes observed at Day 15 had largely or completely resolved.

Table 13: Microscopic Findings at the Last Injection Site and in the Adjacent Muscles

Treatment	Nalmefene Hydrochloride							
Sex	M				F			
Group #	1	2	3	4	1	2	3	4
Dose (mg/kg/day)	0	0.3	1.5	7.5	0	0.3	1.5	7.5
TERMINAL SACRIFICE								
MUSCLE, SKELETAL, UNILATERAL	10	10	10	10	10	10	10	10
DEGENERATION/REGENERATION								
1 OF 5	5	1	3	2	0	1	1	2
INFLAMMATION								
1 OF 5	0	0	1	1	1	1	0	2
NECROSIS								
1 OF 5	0	0	0	0	0	1	0	0
REGENERATION								
1 OF 5	0	0	1	0	0	1	0	0
SITE, INJECTION	10	10	10	10	10	10	10	10
EDEMA								
1 OF 5	0	0	0	3	0	0	0	0
2 OF 5	0	0	0	2	0	0	0	1
FIBROPLASIA								

1 OF 5	0	0	0	0	0	0	0	1
2 OF 5	0	0	0	0	0	1	0	0
FIBROSIS								
1 OF 5	0	0	0	0	0	0	0	1
2 OF 5	0	0	0	0	0	0	0	1
FIBROSIS/FIBROPLASIA								
1 OF 5	0	0	0	1	0	0	0	0
HEMORRHAGE								
1 OF 5	0	2	2	1	1	3	3	3
2 OF 5	1	0	0	2	0	0	0	2
INFLAMMATION								
1 OF 5	6	8	8	1	4	6	10	6
2 OF 5	1	0	3	6	0	0	1	6
3 OF 5	0	0	0	3	0	0	0	0
NECROSIS								
1 OF 5	4	5	3	1	2	4	4	0
2 OF 5	0	0	3	3	0	0	3	2
3 OF 5	0	0	1	6	0	0	1	5
4 OF 5	0	0	0	0	0	0	0	1
REGENERATION								
1 OF 5	0	1	1	7	0	2	3	1
2 OF 5	0	0	0	1	0	0	0	5
RECOVERY SACRIFICE								
MUSCLE, SKELETAL, UNILATERAL	6	6	6	6	6	6	6	6
DEGENERATION/REGENERATION								
1 OF 5	0	2	2	0	0	1	1	0
FIBROSIS/FIBROPLASIA								
1 OF 5	0	0	0	1	0	0	0	0
INFILTRATE								
1 OF 5	1	0	1	0	1	0	0	0
INFLAMMATION								
1 OF 5	0	0	0	1	0	0	0	2
REGENERATION								
1 OF 5	0	2	1	2	0	2	1	6
2 OF 5	0	0	0	2	0	0	0	0
SITE, INJECTION	6	6	6	6	6	6	6	6
DEGENERATION/REGENERATION								
1 OF 5	3	3	4	1	3	4	4	3
INFILTRATE								
1 OF 5	0	1	1	0	0	0	1	0

1: minimal; 2: mild; 3: moderate; 4: marked; 5: severe

Special Evaluation

Not conducted

Toxicokinetics

The incurred sample reproducibility data met the acceptance criteria. The TK data are summarized in the Applicant's table below:

Text Table 10
Mean (±SD) TK Parameters on Days 1 and 14 (Males and Females Combined

Dose (mg/kg/day)	Day	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24hr} (hr*ng/mL)
0.3	1	38.6	0.25	35.7
	14	35.1	0.5	39.4
1.5	1	219	0.25	196
	14	171	0.5	163
7.5	1	887	0.25	1330
	14	749	0.25	1110

Dosing Solution Analysis

The dosing solution was prepared daily. The analytical data demonstrated acceptable performance of the method for all reported results, and that dose formulations were prepared at the intended target concentrations except for the Day 14, 3 mg/mL formulations. The actual concentration readings for 3 mg/mL on Day 14 were (b) (4) (b) (4). An investigation was conducted at the Testing Facility and no assignable cause was determined for these high results. This did not impact the interpretation of the study data as a higher dose was given.

6.22 Nalmefene HCl: A 14-Day Intramuscular Toxicity Study in Beagle Dogs with a 14-Day Recovery Period

Study no.:	Testing Facility Study No. 3243-003 Sponsor Reference No. TOX-NAL-P-066
Study report location:	Study Report
Conducting laboratory and location:	(b) (4)
Date of study initiation:	2/9/2022
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, purity:	Nalmefene HCl, 1902000371, 98.4% $MgCl_2$, 201453, not specified

<table>
<tr><th colspan="2">Methods</th></tr>
<tr><td>Doses:</td><td>0, 0.05 (LD), 0.25 (MD) and 2.5 mg/kg/day (HD)</td></tr>
<tr><td>Frequency of dosing:</td><td>Once daily</td></tr>
<tr><td>Route of administration:</td><td>IM</td></tr>
<tr><td>Dose volume:</td><td>0.1 mL/kg</td></tr>
<tr><td>Formulation/Vehicle:</td><td>Vehicle: 0.94%$MgCl_2$ solution

Dose formulations was prepared by adding the nalmefene HCl at required amounts to the vehicle (0.94% $MgCl_2$ solution) adjusted to a pH of 3.9 ± 0.05.</td></tr>
<tr><td>Species/Strain:</td><td>Dog/Beagle</td></tr>
<tr><td>Number/Sex/Group:</td><td>Text Table 1
Experimental Design
<table>
<tr><th rowspan="2">Group No.</th><th rowspan="2">Test Article</th><th rowspan="2">Dose Level (mg/kg/day)</th><th rowspan="2">Dose Volume (mL/kg)</th><th rowspan="2">Dose Concentration (mg/mL)</th><th colspan="2">Terminal Animals</th><th colspan="2">Recovery Animals</th></tr>
<tr><th>No. of Males</th><th>No. of Females</th><th>No. of Males</th><th>No. of Females</th></tr>
<tr><td>1</td><td>Vehicle</td><td>0</td><td>0.1</td><td>0</td><td>3</td><td>3</td><td>2</td><td>2</td></tr>
<tr><td>2</td><td>Nalmefene</td><td>0.05</td><td>0.1</td><td>0.5</td><td>3</td><td>3</td><td>2</td><td>2</td></tr>
<tr><td>3</td><td>Nalmefene</td><td>0.25</td><td>0.1</td><td>2.5</td><td>3</td><td>3</td><td>2</td><td>2</td></tr>
<tr><td>4</td><td>Nalmefene</td><td>2.5</td><td>0.1</td><td>25.0</td><td>3</td><td>3</td><td>2</td><td>2</td></tr>
</table></td></tr>
<tr><td>Age:</td><td>6–7 months old at initiation of dosing</td></tr>
<tr><td>Satellite groups:</td><td>No</td></tr>
<tr><td>Unique study design:</td><td>No</td></tr>
<tr><td>Deviation from study protocol:</td><td>No deviations were deemed to significantly impact the data interpretation.</td></tr>
</table>

Key Study Findings

There were no test article-related systemic effects observed.

Microscopic findings were limited to the injection sites, including minimal to moderate muscle degeneration/necrosis with minimal to moderate mixed cell or neutrophilic inflammation, minimal to mild muscle regeneration and interstitial fibrosis, and minimal ulcer. The local effects were observed across the treatment groups including the vehicle group. The Applicant considered the findings to be procedure related and/or vehicle related. However, some of the findings such as inflammation and necrosis appear to have higher incidence or higher severity rating in the MD or HD group. Local effects observed on Day 15 appear at least partially resolved on Day 29.

For the systemic effects, the HD is the NOAEL. This Reviewer considers the findings of moderate degeneration/necrosis or moderate inflammation, observed in the MD and HD, to be adverse; therefore, the LD, corresponding to 0.5 mg/mL of nalmefene, is deemed to be the NOAEL with minimal to mild local effects observed. However, this Reviewers agrees that the local effects are not dose- limiting toxicity considering the reversibility and clinical monitorability.

Observations and Results

Mortality

No test article-related mortality was observed.

Clinical Signs

No test article-related clinical signs were observed.

Body Weights

No test article-related changes were observed.

Food Consumption

No test article-related changes were observed.

Ophthalmoscopy

No test article-related changes were observed.

ECG

All animals in all groups received an electrocardiography (ECG) examination pretest, predose and 1 to 2 hours postdose on Day 1 and Day 14.

No test article-related changes were observed.

Hematology and Coagulation

The blood samples were taken pretest and prior to terminal and recovery necropsies in fasted animals.

The following hematology parameters were evaluated:

Hematology Parameters

Red blood cell count	White blood cell count
Hemoglobin concentration	Neutrophil count (absolute and percent)
Hematocrit	Lymphocyte count (absolute and percent)
Mean corpuscular volume	Monocyte count (absolute and percent)
Red blood cell distribution width	Eosinophil count (absolute and percent)
Mean corpuscular hemoglobin concentration	Basophil count (absolute and percent)
Mean corpuscular hemoglobin	Large unstained cells (absolute and percent)
Reticulocyte count (absolute and percent)	Other cells (as appropriate)
Platelet count	Blood smear (preserve and stain)[a]

[a] A blood smear will be prepared from each hematology sample. Blood smear review may be performed on select animals per Testing Facility SOP.

No test article-related changes were observed.

Clinical Chemistry

The following clinical chemistry parameters were evaluated:

Clinical Chemistry Parameters

Alkaline phosphatase	Albumin
Total bilirubin[a]	Globulin (calculated)
Aspartate aminotransferase	Albumin/globulin ratio (calculated)
Alanine aminotransferase	Glucose
Gamma glutamyl transferase	Total cholesterol
Urea nitrogen	Triglycerides
Creatinine	Electrolytes (sodium, potassium, chloride)
Total protein	Calcium
	Phosphorus
	Sample quality

[a] When total bilirubin is >1.0 mg/dL, direct bilirubin will be measured, and indirect bilirubin will be calculated.

Urinalysis

The following urinalysis parameters were evaluated:

Urinalysis Parameters

Volume	Bilirubin
Color	Ketones
Appearance	Blood
Specific gravity	Urobilinogen
pH	
Protein	
Glucose	

Terminal Procedures

The scheduled necropsy was conducted on Day 15 and Day 29. Terminal procedures and evaluations were summarized in the Applicant's tables below.

Terminal Animals

Group No.	Scheduled Euthanasia Day	Necropsy Procedures			Histology Processing	Microscopic Evaluation
		Necropsy	Tissue Collection	Organ Weights		
1-4	15	X	Full List[a]	Full List[a]	Full List[a]	Full List[a]

Recovery Animals

Group No.	Scheduled Euthanasia Day	Necropsy Procedures			Histology Processing	Microscopic Evaluation
		Necropsy	Tissue Collection	Organ Weights		
1-4	29	X	Full List[a]	Full List[a]	Full List[a]	Full List[a]

Terminal Procedure Tables Footnotes:
X = Procedure to be conducted; - = Not applicable.
'Histology Processing'= embedded in paraffin, sectioned, mounted on glass slides, and stained with hematoxylin and eosin.
[a] See Tissue Weighing, Collection, Processing and Evaluation Table, Attachment A for list of tissues applicable to each procedure.

ATTACHMENT A

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 smear	-	X[a]	-	-
Bone marrow, sternum	-	X	X	X
Bone, femur	-	X (1)	X (1)	X (1)
Bone, sternum	-	X	X	X
Brain	X	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)
Gallbladder	-	X	X	X
Ganglion, dorsal root, lumbar	-	X	-	-
Gland, adrenal	X (2)	X (2)	X (2)	X (2)
Gland, lacrimal	-	X (2)	-	-
Gland, mammary (females)	-	X	X	X
Gland, parathyroid	-	X (2)	X (2)	X (2)
Gland, pituitary	X	X	X	X
Gland, prostate	X	X	X	X
Gland, salivary, parotid	-	X (2)	-	-
Gland, salivary, sublingual	-	X (2)	-	-
Gland, salivary, mandibular	-	X (2)	X (1)	X (1)
Gland, thyroid	-	X (2)	X (2)	X (2)
Gland, thyroid/parathyroid	X (2)	-	-	-
Gut-associated lymphoid tissue	-	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
Liver	-	X	X	X
Liver/gallbladder	X	-	-	-
Lung	-	X	X	X
Lymph node(s) draining administration site(s): inguinal[c]	-	X (2)	X (1)	X (1)
Lymph node, mandibular	-	X (2)	X (1)	X (1)
Lymph node, mesenteric	-	X	X	X
Macroscopic abnormalities	-	X	X	X
Muscle, skeletal	-	X (2)	X (1)	X (1)
Nerve, optic	-	X (2)[b]	X (2)	X (2)
Nerve, sciatic[c]	-	X (2)	X (1)	X (1)
Nerve, tibial	-	X (2)	-	-

Organ	Weigh	Macroscopic Evaluation and Collection	Histology Processing	Microscopic Evaluation
Ovary	X (2)	X (2)	X (2)	X (2)
Oviduct	-	X (2)	-	-
Pancreas	-	X	X	X
Site(s), administration: last dosing site	-	X (1)	X (1)	X (1)
Skin	-	X	X	X
Small intestine, duodenum	-	X	X	X
Small intestine, ileum	-	X	X	X
Small intestine, jejunum	-	X	X	X
Spinal cord, cervical	-	X	X	X
Spinal cord, thoracic	-	X	X	X
Spinal cord, lumbar	-	X	X	X
Spleen	X	X	X	X
Stomach	-	X	X	X
Testis	X (2)	X (2)[b]	X (2)	X (2)
Thymus	X	X	X	X
Tongue	-	X	X	X
Trachea	-	X	X	X
Ureter	-	X (2)	-	-
Urinary bladder	-	X	X	X
Uterus/Cervix	X	X	X	X
Vagina	-	X	X	X

X = Procedure to be conducted. - = Not applicable. (1) = one side. (2) = both sides.

[a] Bone marrow smears will be collected from the 5th to 7th rib at scheduled and unscheduled necropsies (for possible examination). Smears will not be collected from animals that are found dead or from animals that were euthanized moribund and then stored in the refrigerator prior to necropsy. Bone marrow smears will be allowed to air dry and are not fixed in formalin.

[b] Eyes and optic nerves will be preserved in Davidson's fixative. Testes and epididymides will be preserved in modified Davidson's fixative.

[c] Same side as last injection site (left unless designated otherwise).

Gross Pathology

No test article-related changes were observed.

Animal No. 3501 was noted on macroscopic examination to have multiple white foci up to 1 cm diameter within the spleen and multiple tan nodules up to 0.5 cm along the serosa of the ileum. These macroscopic findings correlated to an incidental microscopic finding of a developmental anomaly most likely arising as a diverticulum from the intestinal tract and adhering to the surface of the spleen.

Animal 3501 was in the MD group.

Organ Weights

There were no test article-related changes observed. Some changes of organ weights were observed on Day 15, but none of them were deemed test article related, considering lack of dose dependency and/or lack of microscopic changes.

Histopathology

Adequate Battery: Yes
Peer Review: Unknown

Histological Findings

Microscopic findings were largely limited to the injection sites, including minimal to moderate muscle degeneration/necrosis with minimal to moderate mixed cell or neutrophilic inflammation, minimal to mild muscle regeneration and interstitial fibrosis, and minimal ulcer. The local effects were observed across the treatment groups including the vehicle group. The Applicant considered the findings to be procedure related and/or vehicle related. However, some of the findings such as inflammation and necrosis appear to have higher incidence or higher severity rating in the HD or MD group. Local effects observed on Day 15 appear at least partially resolved on Day 29.

There were no test article-related systemic effects observed.

Table 14: Microscopic Findings at the Last Injection Site

Treatment	Nalmefene Hydrochloride							
Sex	M				F			
Group #	1	2	3	4	1	2	3	4
Dose (mg/kg/day)	0	0.05	0.25	2.5	0	0.05	0.25	2.5
TERMINAL SACRIFICE								
SITE, APPLICATION, UNILATERAL	3	3	3	3	3	3	3	3
DEGENERATION/NECROSIS								
1 OF 5	1	1	2	2	1	1	1	2
2 OF 5	2	2	0	1	1	1	1	1
3 OF 5	0	0	0	0	0	0	1	0
FIBROPLASIA								
1 OF 5	0	0	0	0	0	1	0	0
FIBROSIS								
1 OF 5	1	2	0	0	0	1	3	1
2 OF 5	0	0	1	1	1	1	0	1
FOREIGN MATERIAL								
1 OF 5	0	1	0	0	0	0	0	0
GRANULOMA								
1 OF 5	0	1	0	0	0	0	0	0
HEMORRHAGE								
1 OF 5	0	0	0	1	1	0	0	0
INFILTRATE								
1 OF 5	3	2	2	1	2	2	3	3
INFLAMMATION								
1 OF 5	2	1	2	2	2	3	2	2
2 OF 5	1	3	1	1	1	1	1	1
3 OF 5	0	0	0	1	0	0	1	1
NECROSIS								
1 OF 5	0	0	0	0	0	0	1	0
REGENERATION								
1 OF 5	1	2	0	2	0	2	2	1
2 OF 5	0	0	0	0	1	1	1	0
ULCER								
1 OF 5	1	2	2	0	0	0	1	1
RECOVERY SACRIFICE								
SITE, APPLICATION, UNILATERAL	2	2	2	2	2	2	2	2
FIBROSIS								
1 OF 5	0	2	1	0	2	2	1	2
INFILTRATE								
1 OF 5	1	0	0	0	1	1	1	0
2 OF 5	0	0	0	0	0	0	0	1
INFLAMMATION								
1 OF 5	0	0	0	0	0	1	0	0
REGENERATION								
1 OF 5	0	2	0	0	1	0	1	2

1: minimal; 2: mild; 3: moderate; 4: marked; 5: severe

Special Evaluation

Not conducted

Toxicokinetics

The incurred sample reproducibility data met the acceptance criteria. The TK data are summarized in the Applicant's table below:

Text Table 10
Mean (±SD) TK Parameters on Days 1 and 14 (Males and Females Combined)

Dose (mg/kg)	Day	Statistic	C_{max} (ng/mL)	T_{max}[a] (hr)	AUC_{0-24hr} (hr*ng/mL)
0.05	1	Mean	14.0	0.25	12.0
		SD	2.73	(0.25-0.25)	1.52
0.05	14	Mean	13.5	0.25	11.8
		SD	2.90	(0.25-0.25)	2.60
0.25	1	Mean	61.2	0.25	51.8
		SD	13.8	(0.25-0.25)	12.0
0.25	14	Mean	62.9	0.25	53.6
		SD	10.1	(0.25-0.25)	9.07
2.5	1	Mean	649	0.25	570
		SD	86.1	(0.25-0.25)	55.0
2.5	14	Mean	674	0.25	603
		SD	150	(0.25-0.25)	124

[a] Median (minimum - maximum), median value only reported if actual collection interval.

Dosing Solution Analysis

The analytical data demonstrated acceptable performance of the method for all reported results, and that dose formulations were prepared at the intended target concentrations.

6.23 A 14-Day Toxicity Study of (b) (4) by Intravenous (Bolus) Injection in Sprague Dawley Rats (Amendment 1)

Study no.:	Testing Facility Study No. 01352006 Sponsor Reference No.TOX_NAL_P_053
Study report location:	Study Report
Conducting laboratory and location:	(b) (4)
Date of study initiation:	5/11/2020
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4)

Methods	
Doses:	0, 0.2 (LD), 1 (MD) and 5 mg/kg/day (HD)
Frequency of dosing:	daily
Route of administration:	IV
Dose volume:	10 mL/kg
Formulation/Vehicle:	Saline
Species/Strain:	Rat/Sprague Dawley
Number/Sex/Group:	See the table below.
Age:	6-10 weeks
Satellite groups:	TK
Unique study design:	No
Deviation from study protocol:	No deviations were deemed to significantly impact the data interpretation.

Text Table 1
Experimental Design – Toxicology Groups

Group Number	Test Material	Dose Level (mg/kg/day)	Dose Volume[a] (mL/kg)	Dose Concentration (mg/mL)	Number of Animals	
					Males	Females
1	Vehicle	0	10	0	10	10
2	(b) (4)	0.2	10	0.02	10	10
3		1.0	10	0.10	10	10
4		5.0	10	0.50	10	10

[a] Based on the most recent body weight measurement. The first day of dosing was based on Day 1 body weights.

Text Table 2
Experimental Design – Toxicokinetic Groups

Group Number	Test Material	Dose Level (mg/kg/day)	Dose Volume[a] (mL/kg)	Dose Concentration (mg/mL)	Number of Animals	
					Males	Females
1	Vehicle	0	10	0	3	3
2	(b) (4)	0.2	10	0.02	6	6
3		1.0	10	0.10	6	6
4		5.0	10	0.50	6	6

[a] Based on the most recent body weight measurement. The first day of dosing was based on Day 1 body weights.

Key Study Findings

There were no test article-related findings on the general toxicology endpoints. The NOAEL is the HD of 5 mg/kg/day.

Observations and Results

Mortality

There were no test article-related deaths.

On Day 6, a HD female (Animal 4516) in the TK group was found dead at approximately 9 minutes postdose. On Day 9, a LD female (Animal 2509) in the toxicology group was found dead after catheter placement prior to dose administration. No cause of death was determined. Microscopic examination was conducted on the LD female Animal 2509 and there were no test article-related findings. In addition, no animals that survived to the terminal sacrifice in (b) (4) treatment groups showed any

signs of toxicity throughout the study. Therefore, given the absence of any test article-related effects and the circumstances around these deaths, the restraint and dosing procedures are deemed to be the likely cause of death of these 2 animals.

Clinical Signs

There were no test article-related findings observed.

Body Weights and Food Consumption

There were no test article-related changes.

Ophthalmoscopy

There were no test article-related changes.

ECG

Not conducted.

Hematology

The blood samples were taken prior to the designated necropsy in fasted animals.

The following hematology parameters were evaluated:

Text Table 11
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[a] Neutrophil count (absolute) Lymphocyte count (absolute) Monocyte count (absolute) Eosinophil count (absolute) Basophil count (absolute) Large unstained cells (absolute) Other cells (as appropriate)

[a] If performed manually, results included platelet estimates and RBC morphology on the individual tables.

Text Table 12
Coagulation Parameters

Activated partial thromboplastin time Fibrinogen	Prothrombin time Sample quality[a]

[a] Included degree of hemolysis, lipemia, and icterus; presented on individual data tables.

There were no test article-related changes observed.

Clinical Chemistry

The blood samples were taken prior to the designated necropsy in fasted animals.

The following clinical chemistry parameters were evaluated:

Text Table 13
Clinical Chemistry Parameters

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

[a] Included degree of hemolysis, lipemia, and icterus; presented on individual data tables.

There were no test article-related changes observed.

Urinalysis

Urine was collected overnight using metabolism cages prior to the designated necropsy. The following urinalysis parameters were evaluated:

Text Table 14
Urinalysis Parameters

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

There were no test article-related changes observed.

Terminal Procedure

Terminal procedures and evaluations are summarized in the Applicant's tables below:

Text Table 17
Terminal Procedures – Toxicology Groups

Group No.	No. of Animals		Scheduled Euthanasia Day	Necropsy Procedures			Histology	Microscopic Evaluation
	Males	Females		Necropsy	Tissue Collection	Organ Weights		
1	10	10	15	X	X	X	Full List[a]	Full List[a]
2	10	9					Full List[a]	Gross Lesions Target Tissues[b]
3	10	10					Full List[a]	Gross Lesions Target Tissues[b]
4	10	10					Full List[a]	Full List[a]
Unscheduled Deaths				X	X	-	Full List[a]	Full List[a]

X = procedure conducted; - = not applicable.
"Histology Processing"= embedded in paraffin, sectioned, mounted on glass slides, and stained with hematoxylin and eosin.
[a] See Tissue Weighing, Collection, Processing, and Evaluation Table for list of tissue applicable to each procedure.
[b] Any target tissues identified by the study pathologist during microscopic evaluation of full list animals were communicated to the Study Director, then processed, evaluated and reported in the non-full list animals.

Text Table 18
Terminal Procedures – Toxicokinetic Groups

Group No.	Necropsy Procedures			Histology	Microscopic Evaluation
	Necropsy	Tissue Collection	Organ Weights		
Unscheduled Deaths	X (limited)[a]	-	-	-	-
Scheduled Euthanasia	-	-	-	-	-

X = procedure conducted; - = not applicable
[a] The toxicokinetic animal that died on study received a complete necropsy in an attempt to determine the cause of death, and was then discarded without tissue collection.

Text Table 19
Organs Weighed at Necropsy

Brain	Liver
Epididymis	Ovary with oviducts
Gland, adrenal	Spleen
Gland, pituitary	Testis
Gland, prostate with gland, seminal vesicle	Thymus
Gland, thyroid with gland, parathyroid[a]	Uterus with cervix
Heart	
Kidney	

[a] Weighed fixed.

Text Table 20
Tissue Collection and Preservation

Animal identification	Large intestine, rectum
Artery, aorta	Larynx
Body cavity, nasal	Liver
Bone marrow, sternum	Lung
Bone marrow, smear (from femur)[a]	Lymph node(s) draining administration site(s):
Bone, femur	inguinal lymph node
Bone, sternum	Lymph node, mandibular (2)
Brain	Lymph node, mesenteric
Epididymis (2)[b]	Muscle, skeletal (2)
Esophagus	Nerve, optic (2)[c]
Eyes (2)[c]	Nerve, sciatic (2)
Ganglion, dorsal root, lumbar	Nerve, tibial (2)
Gland, adrenal (2)	Ovary (2)
Gland, clitoral (2)	Oviduct (2)
Gland, lacrimal (extra-orbital [2])	Pancreas
Gland, Harderian (2)	Site(s) administration: tail
Gland, mammary (female only)	Skin
Gland, parathyroid (2)	Small intestine, duodenum
Gland, pituitary	Small intestine, ileum
Gland, preputial (2)	Small intestine, jejunum
Gland, prostate	Spinal cord
Gland, salivary, submandibular (2)	Spleen
Gland, salivary, sublingual (2)	Stomach
Gland, salivary, parotid (2)	Testis (2)[b]
Gland, seminal vesicle (2)	Thymus
Gland, thyroid (2)	Tongue
Gland, Zymbal's (2)	Trachea
Gut-associated lymphoid tissue[d]	Ureter (2)
Heart	Urinary bladder
Joint, femorotibial	Uterus/Cervix
Kidney (2)	Vagina
Large intestine, cecum	
Large intestine, colon	

[a] Bone marrow smears were obtained at scheduled necropsy, but not placed in formalin; slides were not examined.
[b] Fixed in modified Davidson's solution.
[c] Fixed in Davidson's solution.
[d] From small intestine: Peyer's patch or solitary lymphoid follicle.

Gross Pathology

There were no test article-related changes observed.

Organ Weights

There were no test article-related changes observed.

Histopathology

Adequate Battery: Yes

Peer Review: Unknown

Histological Findings
There were no test article-related changes observed.

Special Evaluation

Not conducted

Toxicokinetics

Following IV administration of [redacted] (b) (4) at 0.2, 1, and 5 mg/kg/day to male and female rats, exposure to [redacted] (b) (4), in terms of AUC_{tlast}, increased as dose level increased from 0.2 to 5 mg/kg/day in a dose-proportional manner in both sexes on Days 1 and 14. Exposure to [redacted] (b) (4), in terms of AUC_{tlast}, was similar in males and females. Minimal accumulation of [redacted] (b) (4) was observed after once daily IV administration for 14 days.

Text Table 24
Summary of Toxicokinetic Parameters

Dosage	AUC_{tlast} (ng•hr/mL)		C_0 (ng/mL)		T_{max} (hr)	
	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14
Males						
0.2 mg/kg/day	32.7	47.3	57.9	54.4	0.17	0.17
1 mg/kg/day	154	276	151	364	0.17	0.17
5 mg/kg/day	754	1180	620	450	0.17	0.17
Females						
0.2 mg/kg/day	28.0	49.3	45.6	55.6	0.17	0.17
1 mg/kg/day	138	185	134	184	0.17	0.17
5 mg/kg/day	626	1310	237	1060	0.33	0.17

Dosing Solution Analysis

The analyzed dosing formulations contained [redacted] (b) (4) of the test article which was within the protocol-specified range of target concentrations for solutions [redacted] (b) (4). The test article was not detected in the analyzed vehicle formulation that was administered to the control group.

7 Genetic Toxicology

The Applicant did not submit genetic toxicology studies with nalmefene.

8 Carcinogenicity

Carcinogenicity studies are not needed for this acute indication.

9 Reproductive and Developmental Toxicology

The Applicant did not submit reproductive and developmental toxicology studies with nalmefene.

10 Special Toxicology Studies

Local Tolerance Studies

Magnesium Chloride (MgCl2) and Nalmefene: A Single Dose Local Tolerance Study in Rats Administered via Intramuscular or Subcutaneous Injection

Study no.:	Testing Facility Study No. 3243-004 Sponsor Reference No. TOX_NAL_N_062
Study report location:	Study Report
Conducting laboratory and location:	(b) (4)
Date of study initiation:	10/13/2021
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Nalmefene HCl, 1902000369, not specified $MgCl_2$, 201453, not specified Saline, 1902089, not specified

Methods	
Doses:	• 0, 6.28 (LD), 18.85 (MD) and 67.15 mg/kg (HD) for $MgCl_2$ treatment alone • 6.28/3.7 (LD), 18.85/11.1 (MD) and 67.15/21.5 mg/kg (HD) for $MgCl_2$/nalmefene combination treatment
Frequency of dosing:	Single dose
Route of administration:	IM or SC
Dose volume:	See the table above
Formulation/Vehicle:	Saline
Species/Strain:	Rats/Crl:CD®(SD)
Number/Sex/Group:	5/male/dose
Age:	9-11 weeks old at initiation of dosing
Satellite groups:	No
Unique study design:	No
Deviation from study protocol:	No deviations were deemed to significantly impact the data interpretation.

Text Table 1
Experimental Design

Group	Test Article	Route[b]	Dose Level $MgCl_2$/Nal (mg/kg)	Dose Volume[a] (mL)	Dose Concentration (mg/mL)	Main Study Males	Recovery Study Males
1	Vehicle (0.9% NaCl)	IM	0	0.25	0	5	5
2	$MgCl_2$ (0.94%)		6.28		8.8	5	5
3	$MgCl_2$ (2.82%)		18.85		26.4	5	5
4	$MgCl_2$ + Nalmefene		6.28/3.7		8.8/5.2	5	5
5	$MgCl_2$ + Nalmefene		18.85/11.1		26.4/15.5	5	5
6	Vehicle (0.9% NaCl)	SC	0	0.5	0	5	5
7	$MgCl_2$ (0.94%)		6.28		4.4	5	5
8	$MgCl_2$ (2.82%)		18.85		13.2	5	5
9	$MgCl_2$ + Nalmefene		6.28/3.7		4.4/2.6	5	5
10	$MgCl_2$ + Nalmefene		18.85/11.1		13.2/7.8	5	5
11	$MgCl_2$ (2.82%)	IM	67.15	0.25	94.0	5	5
12	$MgCl_2$ + Nalmefene		67.15/21.5		94.0/30.1	5	5
13	$MgCl_2$ (2.82%)	SC	67.15	0.5	47.0	5	5
14	$MgCl_2$ + Nalmefene		67.15/21.5		47.0/15.1	5	5

[a] Calculated based on an average bodyweight of 350 g
[b] IM route - Intramuscular; SC route - Subcutaneous

The objective of this study was to determine the potential local tolerance of the test articles, $MgCl_2$ and Nalmefene, when administered via IM or SC injection as a single

dose to rats followed by an observation period of 14 days with histopathological evaluation of the injection sites on Days 2 and Day 15 to evaluate acute and delayed local toxicity.

Key Study Findings
Test article-related effects were observed in microscopic examinations at injection sites. Minimal to mild/moderate local effects including necrosis, inflammation and hemorrhage were observed at the IM or SC injection site on Day 2. Recovery of the local effects were observed on Day 15.

IM administration route

- Minimal to mild local effects were observed in the LD and MD groups received $MgCl_2$ treatment alone or the combination treatment.

- Moderate severity ratings of the local findings were generally observed at the HD of $MgCl_2$ alone or at the HD of combination treatment.

SC administration route

- Moderate findings were only observed in the HD group received the combination treatment. Minimal to mild local effects were observed in other dose groups including the HD of $MgCl_2$ treatment alone.

None of the local effects were deemed adverse by the Applicant. However, this Reviewer conservatively considers moderate local effects adverse; therefore, for the IM administration route, the MD is deemed to be the NOAEL for the local effects of $MgCl_2$ alone or the combination treatment; for the SC administration route, the MD is deemed, by this Reviewer, to be the NOAEL for the combination treatment and the HD to be NOAEL for the $MgCl_2$ alone treatment.

Microscopic Findings at the Dosing Site
Local effects at the IM dosing site
As show in the Applicant's table below, on Day 2 necropsy, there was acute $MgCl_2$ + Nalmefene-related or $MgCl_2$-related microscopic findings at IM injection sites consisted of generally dose-dependent minimal to moderate myofiber degeneration/necrosis, inflammation and myofiber hemorrhage. Additionally, minimal to mild injection site necrosis (muscle, adipose, and collagen degradation) occurred at the HD of $MgCl_2$ alone or high dose combination. Generally, acute $MgCl_2$-related microscopic findings at the IM injection sites consisted of similar findings to those seen in the $MgCl_2$ + Nalmefene dose groups, but at slightly less severity and/or incidence at the LD or MD comparing to the corresponding combination group.

Table 15: IM Injection Site Microscopic Findings on Day 2

Text Table 9

Summary of Test Article-related IM Injection Site Microscopic Findings – Scheduled/Terminal Euthanasia (Day 2)

	Males						
Group	**1**	**2**	**3**	**4**	**5**	**11**	**12**
Dose (mg/kg)	**0**	**6.28**	**18.85**	**6.28/3.7**	**18.85/11.1**	**67.15**	**67.15/21.5**
No. animals examined	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Site, injection, intramuscular (No. Examined)	5	5	5	5	5	5	5
Necrosis	(0)	(0)	(0)	(0)	(0)	(5)	(5)
Minimal	0	0	0	0	0	3	2
Mild	0	0	0	0	0	2	3
Degeneration/necrosis; myofiber	(2)	(3)	(4)	(2)	(5)	(5)	(5)
Minimal	2	3	2	2	1	0	0
Mild	0	0	2	0	4	3	3
Moderate	0	0	0	0	0	2	2
Inflammation, mononuclear cell; myofiber	(3)	(5)	(4)	(4)	(5)	(5)	(5)
Minimal	3	5	2	4	0	0	0
Mild	0	0	2	0	5	5	3
Moderate	0	0	0	0	0	0	2
Inflammation, neutrophilic; myofiber	(0)	(0)	(0)	(0)	(3)	(5)	(5)
Minimal	0	0	0	0	3	3	2
Mild	0	0	0	0	0	2	3
Inflammation, mononuclear cell; subcutaneous tissue	(1)	(0)	(0)	(2)	(0)	(2)	(2)
Minimal	1	0	0	2	0	2	1
Mild	0	0	0	0	0	0	1
Hemorrhage; myofiber	(0)	(1)	(4)	(2)	(3)	(5)	(5)
Minimal	0	1	2	1	0	3	0
Mild	0	0	2	1	3	2	3
Moderate	0	0	0	0	0	0	2

As shown in the Applicant's table below, microscopic findings at the IM injection sites of Day 15 rats at the HD combination group had minimal mononuclear cell infiltrate and minimal fibrosis, suggesting a recovery of local effects.

Table 16: IM Injection Site Microscopic Findings on Day 15

Summary of Test Article-related IM Injection Site Microscopic Findings–Scheduled/Recovery Euthanasia (Day 15)

	Males						
Group	**1**	**2**	**3**	**4**	**5**	**11**	**12**
Dose (mg/kg)	**0**	**6.28**	**18.85**	**6.28/3.7**	**18.85/11.1**	**67.15**	**67.15/21.5**
No. animals examined	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Site, injection, intramuscular (No. Examined)	5	5	5	5	5	5	5
Infiltration, mononuclear cell; myofiber	(0)	(1)	(1)	(0)	(0)	(0)	(5)
Minimal	0	1	1	0	0	0	5
Fibrosis; myofiber	(0)	(0)	(0)	(0)	(0)	(1)	(2)
Minimal	0	0	0	0	0	1	2
Regeneration; myofiber	(0)	(0)	(0)	(0)	(0)	(1)	(1)
Minimal	0	0	0	0	0	1	1

Local effects at the SC dosing site

As shown in the Applicant's table below, acute (Day 2) $MgCl_2$ + Nalmefene SC injection sites of rats had microscopic findings of similar nature to those seen at IM injection sites but largely confined to the subcutaneous tissue. Changes consisted of dose-dependent, minimal to moderate mononuclear inflammation and minimal to mild hemorrhage of the subcutis. Additional changes included minimal to moderate necrosis (adipose, muscle, and collagen degradation), minimal to mild neutrophilic inflammation of subcutaneous tissue, minimal to mild myofiber degeneration/necrosis.

Acute (Day 2) $MgCl_2$-related microscopic findings of SC injection sites were again similar in nature to those seen with $MgCl_2$ + Nalmefene, but most of the findings were limited to the HD group.

Table 17: SC Injection Site Microscopic Findings on Day 2

Text Table 10
Summary of Test Article-related SC Injection Site Microscopic Findings–Scheduled/Terminal Euthanasia (Day 2)

Group	6	7	8	9	10	13	14
Dose (mg/kg)	0	6.28	18.85	6.28/3.7	18.85/11.1	67.15	67.15/21.5
No. animals examined	5	5	5	5	5	5	5
Site, injection, subcutaneous (No. Examined)	5	5	5	5	5	5	5
Necrosis	(0)	(0)	(0)	(0)	(0)	(3)	(5)
Minimal	0	0	0	0	0	2	0
Mild	0	0	0	0	0	1	3
Moderate	0	0	0	0	0	0	2
Degeneration/necrosis; myofiber	(0)	(0)	(0)	(0)	(0)	(1)	(3)
Minimal	0	0	0	0	0	1	2
Mild	0	0	0	0	0	0	1
Inflammation, mononuclear cell; subcutaneous tissue	(3)	(0)	(5)	(4)	(2)	(4)	(5)
Minimal	3	0	2	2	2	1	0
Mild	0	0	3	2	0	3	3
Moderate	0	0	0	0	0	0	2
Inflammation, neutrophilic; subcutaneous tissue	(0)	(0)	(0)	(0)	(0)	(4)	(3)
Minimal	0	0	0	0	0	4	1
Mild	0	0	0	0	0	0	2
Inflammation, mononuclear cell; myofiber	(0)	(1)	(0)	(0)	(0)	(1)	(3)
Minimal	0	1	0	0	0	0	3
Mild	0	0	0	0	0	1	0
Hemorrhage; subcutaneous tissue	(0)	(0)	(0)	(1)	(3)	(4)	(5)
Minimal	0	0	0	1	2	1	0
Mild	0	0	0	0	1	3	5

Microscopic findings observed at the end of the recovery period on Day 15 for SC injection sites are summarized in the Applicant's table below. Only minimal mononuclear cell infiltration or minimal fibrosis in the SC tissue was observed in the test article-treated groups, suggesting a recovery of local effects.

Table 18: SC Injection Site Microscopic Findings on Day 15

Text Table 12
Summary of Test Article-related SC Injection Site Microscopic Findings–Scheduled/Recovery Euthanasia (Day 15)

Group	6	7	8	9	10	13	14
Dose (mg/kg)	**0**	**6.28**	**18.85**	**6.28/3.7**	**18.85/11.1**	**67.15**	**67.15/21.5**
No. animals examined	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Site, injection, subcutaneous (No. Examined)	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Infiltration, mononuclear cell; subcutaneous tissue	(0)	(0)	(1)	(1)	(1)	(3)	(4)
Minimal	0	0	1	1	1	3	4
Fibrosis; subcutaneous tissue	(0)	(0)	(0)	(0)	(0)	(3)	(4)
Minimal	0	0	0	0	0	3	4

$MgCl_2$ and Nalmefene: An Intramuscular and Subcutaneous Injection Local Tolerance Study in Rabbits

Study no.:	Testing Facility Study No. 3243-005 Sponsor Reference No. TOX_NAL_N_063
Study report location:	Study Report
Conducting laboratory and location:	(b) (4)
Date of study initiation:	11/2/2024
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Nalmefene HCl, 1902000369, not specified $MgCl_2$, 201453, not specified

Methods	
Doses:	• 0, 1.57 (LD), and 7.83 (HD) mg/kg for $MgCl_2$ treatment alone • 1.57/0.5 (LD) and 7.83/2.5 (HD) mg/kg for $MgCl_2$/nalmefene combination treatment
Frequency of dosing:	Single dose
Route of administration:	IM or SC
Dose volume:	1 mL/animal
Formulation/Vehicle:	Saline
Species/Strain:	New Zealand White Rabbits
Number/Sex/Group:	3/male/group
Age:	Approximately 6.5 to 7 months
Weight:	2.7-3.5 kg
Satellite groups:	No
Unique study design:	No
Deviation from study protocol:	No deviations were deemed to significantly impact the data interpretation.

Text Table 1
Experimental Design

Group No.	Test Article	Route	Dose Level MgCl2/Nal (mg/kg)[a]	Dose Volume	Dose Concentration (mg/mL)	Terminal Animals No. of Males	Recovery Animals No. of Males
1	Vehicle (0.9% NaCl, pH 3.9)	IM/SC	0/0	1 mL (IM) / 1 mL (SC)	0	3	3
2	$MgCl_2$	IM	1.57	1.0 mL	4.7	3	3
3	$MgCl_2$	IM	7.83	1.0 mL	23.5	3	3
4	$MgCl_2$+Nalmefene	IM	1.57+0.5	1.0 mL	4.7+1.5	3	3
5	$MgCl_2$+Nalmefene	IM	7.83+2.5	1.0 mL	23.5+7.5	3	3
6	$MgCl_2$	SC	1.57	1.0 mL	4.7	3	3
7	$MgCl_2$	SC	7.83	1.0 mL	23.5	3	3
8	$MgCl_2$+Nalmefene	SC	1.57+0.5	1.0 mL	4.7+1.5	3	3
9	$MgCl_2$+Nalmefene	SC	7.83+2.5	1.0 mL	23.5+7.5	3	3

IM = Intramuscular; SC = Subcutaenous; No. = Number.
[a] Calculated based on an average body weight of 3.0 kg.

The objective of this study was to determine the local tolerance of the test articles, $MgCl_2$ alone and a combination of $MgCl_2$ and Nalmefene, when administered via IM or SC injection as a single dose to New Zealand White Rabbits followed by an observation period of 14 days with histopathological evaluation of the injection sites on Days 2 and Day 15 to evaluate acute and delayed local toxicity.

Key Study Findings
The findings were limited to the microscopic examination. There were no test article-related findings observed. The local effects observed at the IM or SC dosing sties were considered injection procedure-related; thus, the NOAEL for the local effects was the HD for the treatment of $MgCl_2$ alone and the $MgCl_2$/Nalmefene combination.

Microscopic Findings at the Dosing Site

Local effects at IM and SC dosing site on Day 2

Microscopic observations at both IM and SC injection sites at termination and recovery (i.e., minimal to mild leukocyte infiltration, myofiber degeneration/necrosis or regeneration, hemorrhage, ulcer, crust, epidermal hyperplasia, and occasional embedded hair foreign material) were observed across the treatment groups. The Applicant considered the findings incidental and generally attributable to injection procedure-related effects, given that these findings are commonly seen secondary to injection procedures, lacked a dose-response in incidence and severity, and occurred at similar incidence and severity across all treatment groups. This Reviewer agrees with this conclusion.

Table 19: Summary of Microscopic Findings on Day 2 at the IM and SC Dosing Site

Summary of Microscopic Pathology: Study Phase - Terminal

3243-005

Removal Reason(s): TERMINAL EUTHANASIA Summary: Incidence	Male								
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Number of Animals:	3	3	3	3	3	3	3	3	3
SITE, INJECTION, INTRAMUSCULAR									
Examined	3	3	3	3	3	0	0	0	0
No Visible Lesions	0	0	0	1	0	.	.	.	.
Infiltration, mixed cell; dermal	3	3	3	0	3	.	.	.	.
.... minimal	3	2	3	0	3	.	.	.	.
.... mild	0	1	0	0	0	.	.	.	.
Infiltration, mixed cell; myofiber	1	2	3	1	0	.	.	.	.
.... minimal	1	2	3	1	0	.	.	.	.
Infiltration, mixed cell; subcutaneous tissue	0	1	3	1	0	.	.	.	.
.... minimal	0	1	3	1	0	.	.	.	.
Crust	2	2	2	2	2	.	.	.	.
.... minimal	2	1	2	2	2	.	.	.	.
.... mild	0	1	0	0	0	.	.	.	.
Foreign material; dermal	1	0	0	0	0	.	.	.	.
.... minimal	1	0	0	0	0	.	.	.	.
Foreign material; myofiber	0	1	0	0	0	.	.	.	.
.... minimal	0	1	0	0	0	.	.	.	.
Hemorrhage; subcutaneous tissue	1	0	1	0	1	.	.	.	.
.... minimal	0	0	0	0	1	.	.	.	.
.... mild	1	0	1	0	0	.	.	.	.
Degeneration/necrosis; myofiber	1	2	3	1	0	.	.	.	.
.... minimal	1	2	2	1	0	.	.	.	.

Removal Reason(s): TERMINAL EUTHANASIA Summary: Incidence	Male								
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Number of Animals:	3	3	3	3	3	3	3	3	3
SITE, INJECTION, INTRAMUSCULAR (Continued...)									
.... mild	0	0	1	0	0	.	.	.	.
Infiltration, mononuclear cell; subcutaneous tissue	1	0	0	0	2	.	.	.	.
.... minimal	1	0	0	0	2	.	.	.	.
Hyperplasia; epidermal	1	1	2	0	1	.	.	.	.
.... minimal	1	1	2	0	1	.	.	.	.
Ulceration	1	1	1	2	1	.	.	.	.
.... minimal	1	0	1	2	1	.	.	.	.
.... mild	0	1	0	0	0	.	.	.	.
SITE, INJECTION, SUBCUTANEOUS									
Examined	3	0	0	0	0	3	3	3	3
No Visible Lesions	1	.	.	.	.	1	0	0	0
Infiltration, mixed cell; dermal	0	.	.	.	.	0	2	0	0
.... minimal	0	.	.	.	.	0	2	0	0
Infiltration, mixed cell; subcutaneous tissue	1	.	.	.	.	0	1	1	0
.... minimal	1	.	.	.	.	0	1	1	0
Infiltration, mononuclear cell; dermal	1	.	.	.	.	1	0	1	0
.... minimal	1	.	.	.	.	1	0	1	0
Infiltration, mononuclear cell; myofiber	0	.	.	.	.	2	0	0	1
.... minimal	0	.	.	.	.	2	0	0	1
Infiltration, mononuclear cell; subcutaneous tissue	1	.	.	.	.	1	1	0	2
.... minimal	1	.	.	.	.	1	1	0	2

Removal Reason(s): TERMINAL EUTHANASIA Summary: Incidence	Male								
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Number of Animals:	3	3	3	3	3	3	3	3	3
SITE, INJECTION, SUBCUTANEOUS (Continued...)									
Crust	1	.	.	.	.	0	2	1	1
.... minimal	1	.	.	.	.	0	2	1	1
Degeneration/necrosis; myofiber	0	.	.	.	.	1	0	0	1
.... minimal	0	.	.	.	.	1	0	0	1
Hemorrhage; subcutaneous tissue	0	.	.	.	.	0	0	0	1
.... minimal	0	.	.	.	.	0	0	0	1
Ulceration	0	.	.	.	.	0	2	1	0
.... minimal	0	.	.	.	.	0	2	1	0
Hyperplasia; epidermal	0	.	.	.	.	0	1	0	0
.... minimal	0	.	.	.	.	0	1	0	0

<u>Local effects at IM and SC dosing site on Day 15</u>

Compatible with the timeframe, these procedure-related findings showed a temporal trend from more acute (Day 2) to more chronic (Day 15), including a shift of cellular composition from frequently mixed leukocyte (neutrophils, lymphocytes, histocytes, eosinophils) to often mononuclear (lymphocytes, histocytes) and decreased incidence of acute observations (hemorrhage, ulceration, crust, myofiber degeneration/necrosis) to occasional chronic changes (fibroplasia and myofiber regeneration); this pattern was consistent with a healing response to the procedure-related effects.

Table 20: Summary of Microscopic Findings on Day 15 at the IM and SC Dosing Site

Summary of Microscopic Pathology: Study Phase - Recovery

3243-005

Removal Reason(s): RECOVERY EUTHANASIA Summary: Incidence	Male								
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Number of Animals:	3	3	3	3	3	3	3	3	3
SITE, INJECTION, INTRAMUSCULAR									
Examined	3	3	3	3	3	0	0	0	0
No Visible Lesions	1	1	0	0	1	.	.	.	.
Infiltration, mixed cell; dermal	0	0	0	0	1	.	.	.	.
.... mild	0	0	0	0	1	.	.	.	.
Infiltration, mixed cell; myofiber	1	0	1	0	0	.	.	.	.
.... minimal	1	0	1	0	0	.	.	.	.
Infiltration, mixed cell; subcutaneous tissue	0	0	1	0	1	.	.	.	.
.... minimal	0	0	1	0	0	.	.	.	.
.... mild	0	0	0	0	1	.	.	.	.
Crust	0	0	1	0	1	.	.	.	.
.... minimal	0	0	1	0	0	.	.	.	.
.... mild	0	0	0	0	1	.	.	.	.
Foreign material; dermal	1	0	0	0	0	.	.	.	.
.... minimal	1	0	0	0	0	.	.	.	.
Foreign material; subcutaneous tissue	0	0	0	0	1	.	.	.	.
.... mild	0	0	0	0	1	.	.	.	.
Hemorrhage; subcutaneous tissue	0	0	0	0	1	.	.	.	.
.... minimal	0	0	0	0	1	.	.	.	.
Infiltration, mononuclear cell; dermal	1	2	1	3	1	.	.	.	.
.... minimal	1	2	1	3	1	.	.	.	.
Infiltration, mononuclear cell; myofiber	1	1	1	1	0	.	.	.	.

Removal Reason(s): RECOVERY EUTHANASIA Summary: Incidence	Male								
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Number of Animals:	3	3	3	3	3	3	3	3	3
SITE, INJECTION, INTRAMUSCULAR (Continued...)									
.... minimal	1	1	1	1	0	.	.	.	.
Infiltration, mononuclear cell; subcutaneous tissue	1	0	1	0	0	.	.	.	.
.... minimal	1	0	1	0	0	.	.	.	.
Hyperplasia; epidermal	0	0	1	1	0	.	.	.	.
.... minimal	0	0	1	1	0	.	.	.	.
Ulceration	0	0	0	0	1	.	.	.	.
.... moderate	0	0	0	0	1	.	.	.	.
Edema; subcutaneous tissue	0	0	0	0	1	.	.	.	.
.... mild	0	0	0	0	1	.	.	.	.
Fibroplasia; dermal	0	0	2	1	0	.	.	.	.
.... minimal	0	0	2	1	0	.	.	.	.
Regeneration; myofiber	0	0	1	0	0	.	.	.	.
.... minimal	0	0	1	0	0	.	.	.	.
SITE, INJECTION, SUBCUTANEOUS									
Examined	3	0	0	0	0	3	3	3	3
No Visible Lesions	0	.	.	.	.	0	1	1	1
Infiltration, mononuclear cell; dermal	2	.	.	.	.	3	1	2	2
.... minimal	2	.	.	.	.	3	1	2	2
Infiltration, mononuclear cell; myofiber	1	.	.	.	.	0	0	0	0
.... minimal	1	.	.	.	.	0	0	0	0
Infiltration, mononuclear cell; subcutaneous tissue	1	.	.	.	.	1	2	0	0

Removal Reason(s): RECOVERY EUTHANASIA Summary: Incidence	Male								
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Number of Animals:	3	3	3	3	3	3	3	3	3
SITE, INJECTION, SUBCUTANEOUS (Continued...)									
.... minimal	1	.	.	.	.	1	2	0	0
Hyperplasia; epidermal	0	.	.	.	.	2	1	1	2
.... minimal	0	.	.	.	.	2	1	1	2

(b) (4) An Intramuscular and Subcutaneous Injection Local Tolerance Study in Rabbits

Study no.: Testing Facility Study No. 20457072
Sponsor Reference No. TOX-NAL-N-080

Study report location: Study Report

Conducting laboratory and location: (b) (4)

Date of study initiation: 7/18/2023

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: (b) (4)

Methods	
Doses:	0, 0.025 (LD), 0.125 (MD), or 0.0625 mg/kg (HD) (0, 0.075, 0.375 or 1.875 mg total)
Frequency of dosing:	Single dose
Route of administration:	SC and IM
Dose volume:	0.5 mL
Formulation/Vehicle:	$MgCl_2$ with 6H2O
Species/Strain:	Rabbit/New Zealand White
Number/Sex/Group:	3/male/dose (see the table above)
Age:	About 4 months old
Weight:	3.2 to 3.5 kg at the first dosing
Satellite groups:	No
Unique study design:	No
Deviation from study protocol:	The mean measured concentration of the 3.75 mg/mL dosing solution was below the acceptance criteria of (b) (4) stated within the protocol. A laboratory investigation (LIR#106662) was initiated, and it was determined that the 3.75 mg/mL dosing solution concentration was low. The reported result for the 3.75 mg/mL solution is the average of four determined concentration from four separate samples. The sample concentrations for the 3.75 mg/mL dosing solution ranged from (b) (4).

Text Table 1
Experimental Design

Group No.	Test Material	Dose Level mg/kg/ (mg@site)[a]	Dose Volume (mL)[b]	Dose Concentration (mg/mL)	Main Study No. of Males	Recovery Study No. of Males
1	Vehicle Control	0	0.5 mL (IM)/ 0.5 mL (SC)	0	3	3
2	(b) (4)	0.025/0.075		0.15	3	3
3		0.125/0.375		0.75	3	3
4		0.625/1.875		3.75	3	3

IM = intramuscular injection; SC = subcutaneous injection.
[a] Calculated based on an average body weight of 3.0 kilograms.
[b] Male rabbits were dosed IM and SC in the same animal.

Dosing Group Nominal Concentration (mg/mL)	Measured (b) (4) Concentrations (mg/mL)
Vehicle	ND
0.15	(b) (4)
0.75	
3.75	

Four groups of male New Zealand White rabbits were administered the vehicle control or (b) (4) at doses of 0.025, 0.125, or 0.625mg/kg (0.075, 0.375, or 1.875 mg total delivered at the injection site) via intramuscular (IM) injection and subcutaneous (SC) injection once on Day 1. Animals designated for the terminal

necropsy (3/group) were euthanized on Day 2, and animals designated for the recovery necropsy (3/group) were euthanized on Day 14 following a 14-day observation period.

The following parameters and endpoints were evaluated in this study: mortality, clinical signs, dermal irritation scores, body weights, body weight gains, food consumption, organ weights, and macroscopic and microscopic examinations. Only dosing sites were evaluated for histopathological changes.

There were no test article-related changes observed on any of the endpoints listed above. Microscopic findings observed on Day 2 and Day 14 were procedure related, incidental, of the nature commonly observed in New Zealand White rabbits, and/or were of similar incidence and severity in control and dosed animals and, therefore, were considered unrelated to administration of (b) (4). The HD, corresponding to the measured concentration of (b) (4) mg/mL, is considered to be the NOAEL for the study.

Juvenile Animal Studies (JAS)

The NDA submission seeks marketing approval in patients who are 12 years or older. The Agency concurred that the safety and efficacy of nalmefene may be extrapolated to pediatric patients 12-17 years. A deferral has been granted by the Agency for clinical studies assessing the pharmacokinetics and pharmacodynamics of nalmefene in patients < 12 years old. The Applicant will conduct two JAS to support their pediatric study outlined in the agreed PSP, which are shown below.

- Conduct a juvenile animal study in rats to support the initiation of clinical studies in pediatric patients from 3 years to less than 12 years of age. This study will evaluate the effect of the drug on growth and development, specifically reproductive performance/sexual maturation and central nervous system histopathology and long-term behavioral effects.

  Conduct a juvenile animal study in rats to support the initiation of clinical studies in pediatric patients from birth to less than 3 years of age. This study will evaluate the effect of the drug on development, specifically neuroapoptosis and central nervous system histopathology.

The Division has reviewed the JAS protocols and provided comments to the Applicant.

11 Integrated Summary and Safety Evaluation

The regulatory pathway for the NDA submission is via 505(b)(2) with Revex as the LD. No additional pharmacology studies were needed to support this NDA. The Applicant conducted 14-day repeat-dose study with IM administration to support the systemic safety and local tolerance studies with IM and SC administration route in rats and rabbits to support the local safety of the drug product. To qualify the safety of the proposed specification for the drug product degradant (b) (4), the Applicant

conducted a14-day repeat-dose study with IV administration of (b) (4) in rats, local tolerance study with IM and SC administration in rabbits and a (Q)SAR assessment. See the tables below for the summary of key findings and resulting exposure margins from the nonclinical studies.

Specifications for the drug substance, drug product impurity and residual solvents are deemed acceptable. The drug substance specification for (b) (4), NMT (b) (4)% (w/w), is greater than ICH Q3A(R2)-recommended qualification threshold of 0.15%, whereas the total daily intake (TDI) of (b) (4) after 2 doses of nalmefene treatment is less than the qualification threshold of 1 mg/day. As discussed in Section 2.5, (b) (4) is an FDA-approved API, and the 14-day repeat-dose rat study and local tolerance studies with IM and SC administration route in rabbits and rats can provide adequate systemic and local support for the proposed higher level of (b) (4). The drug product stability specification for (b) (4), NMT (b) (4)%, is higher than the ICH Q3B(R2)-recommended qualification threshold of 1%. As discussed in Section 2.5, the totality of the data including the nonclinical studies is deemed adequate to support the safety of the proposed specification for (b) (4). The Applicant submitted a report that contains a risk assessment for the potential presence of elemental impurities in the finished drug product, in accordance with ICH Q3D. This Reviewer concurs with the Applicant that routine analysis and additional controls of elemental impurities for the drug product is not required, given that all potential elemental impurities are much lower than 30% of the respective established parenteral PDE (as per ICH Q3D).

To support the safety of the container closure system, the Applicant submitted an extractables leachables assessment. No compounds above the 5 mcg/day threshold were observed at any time points evaluated. It is noted that the CMC review team has identified issues with the extractable leachables data. However, given that there were no leachables above 5 mcg/day, components of the autoinjector have been used in other FDA approved products, and that this product is for a life-saving indication, the identified issues do not preclude approval and will be addressed through postmarketing commitment (PMC) studies.

Safety Assessment of Excipients

The drug product contains magnesium chloride hexahydrate. $MgCl_2$ is not currently in any FDA approved IM or SC drug products. It is used as an excipient in drug products approved for other parenteral administration routes including intraperitoneal route. $MgCl_2$ is widely used in both medical and nutritional contexts to replenish electrolytes. To support the systemic and local safety of $MgCl_2$, the Applicant conducted 14-day repeat-dose studies with IM administration route in rats and dogs, and local tolerance studies with IM and SC administration routes in rats and rabbits which received $MgCl_2$ treatment alone or combination treatment with nalmefene and $MgCl_2$.

In the 14-day repeat-dose studies, as discussed in Section 2.4, there were no adverse systemic effects observed with $MgCl_2$ in rats and dogs up to 1 times and 3.3 times the

human dose of $MgCl_2$ after two doses of the NAI, based upon HED BSA comparison. It is noted that the systemic safety margin established in the rat study is 1x. As discussed previously, $MgCl_2$ is present as an excipient in drug products approved for parental administration routes including intraperitoneal route at high levels. It is used to replenish electrolytes via IV or oral route. The typical dose of $MgCl_2$ in over-the-counter oral supplements for adults ranges from 200 mg to 400 mg. Oral $MgCl_2$ is well absorbed in the gut. Based upon the weight of evidence analysis, this Review concluded that there are no systemic safety concerns with $MgCl_2$ as an excipient in this life-saving drug product.

In the acute local tolerance studies in rats and rabbits, saline, $MgCl_2$ alone or $MgCl_2$ in combination with nalmefene were administered via IM and SC injection as a single dose followed by an observation period of 14 days with histopathological evaluation of the injection sites on Days 2 and Day 15 to evaluate acute and delayed local toxicity. In the rat acute tolerance study, $MgCl_2$-related effects were observed at injection sites, including necrosis, inflammation and hemorrhage observed on Day 2. Recovery of the local effects were observed on Day 15. In rats that received IM administration with $MgCl_2$ alone, moderate local effects were observed at the HD and minimal to mild local effects were observed at the LD and MD. In rats that received SC administration with $MgCl_2$ alone, only minimal to mild local effects were observed. None of the local effects were deemed adverse by the Applicant. However, this Reviewer conservatively considers moderate local effects potentially adverse; therefore, the MD (for IM route) and HD (for SC route) are deemed to be the NOAEL for the local effects corresponding to 2.8 times and 5 times the clinical $MgCl_2$ concentration, respectively.

In the acute tolerance study in rabbits, there were no test article-related findings observed. Microscopic observations at both IM and SC injection sites at termination and at recovery (i.e., minimal to mild leukocyte infiltration, myofiber degeneration/necrosis or regeneration, hemorrhage, ulcer, crust, epidermal hyperplasia, and occasional embedded hair foreign material) were observed across the treatment groups including the saline group. The findings are deemed incidental and generally attributable to injection procedure-related effects, given that these findings are commonly seen secondary to injection procedures, lacked a dose-response in incidence and severity, and occurred at similar incidence and severity across all treatment groups including the saline group. Thus, the high dose of $MgCl_2$ is considered to the NOAEL for both IM and SC treatment route, corresponding to 2.5 times the clinical $MgCl_2$ concentration.

In summary, based upon the totality of the data including the nonclinical studies, this Reviewer concludes that there are no concerns on systemic and local safety with the drug excipient $MgCl_2$ in this life-saving drug product.

Safety Assessment of Nalmefene

Nalmefene in the proposed drug product is in (b) (4), which is higher than nalmefene concentration of 1 mg/mL in the LD, Revex. The nonclinical studies described above for supporting the safety qualification of $MgCl_2$ are also used to support local and systemic

safety of nalmefene. The effects observed in the nonclinical studies and exposure margins of nalmefene reached in the studies are summarized in the tables below.

In the 14-day repeat-dose studies in rats and dogs, the animals received daily IM injection with nalmefene hydrochloride for 14 days with a 14-day recovery period. $MgCl_2$ was used as a vehicle. There were no test article-related systemic findings except that the HD-treated rats exhibited test article-related, reversible, generally minimally higher (up to 84% increase from the control mean value) aspartate aminotransferase (AST) activity at the end of the dosing phase on Day 15. The Applicant stated that the increased AST correlated microscopically with myofiber necrosis and associated inflammation at the dosing site. The effect was not deemed adverse. The HD is deemed to be the NOAEL for the systemic effects in both studies, resulting in high safety margins when comparing to the systemic human exposures after two doses of nalmefene treatment (see Table 22 for exposure multiples).

The local safety of nalmefene was evaluated as a combination with $MgCl_2$ in acute local tolerance studies in rats or rabbits that received a single IM or SC administration. The 14-day repeat-dose studies in rats or dogs that received daily IM administration with nalmefene in 0.94% $MgCl_2$ solution also included histopathological evaluation of the local tissues at the last IM dosing site. The local findings are summarized in Table 21.

In the 14-day repeat-dose study in rats, the local findings at the last IM injection site included minimal to mild edema, fibrosis and hemorrhage; minimal to marked myofiber necrosis and associated minimal to moderate inflammatory changes (mixed cell and/or macrophages). Minimal to mild local effects were generally observed in the vehicle ($MgCl_2$) and the LD-treated groups with similar incidence, but there was a dose-related increase in incidence and severity at the MD and HD. The local findings at the MD and HD were considered exacerbation of vehicle/procedure-related local toxicity by nalmefene. By Day 29 for the recovery assessment, the local changes observed on Day 15 had partially or completely resolved. For the local effects of nalmefene, the HD was considered to be the NOAEL by the Applicant. This Reviewer conservatively considers the findings of moderate inflammation or moderate/marked necrosis, observed at the MD and HD, to be adverse; therefore, the LD in the rat study, corresponding to 1x the clinical nalmefene concentration, is deemed to be the NOAEL with minimal to mild local effects observed. In the 14-day repeat-dose study in dogs, local microscopic findings at the last IM injection sites included minimal to moderate muscle degeneration/necrosis with mixed to neutrophilic inflammation, minimal to mild muscle regeneration/interstitial fibrosis and minimal ulcer. The local effects were observed across the treatment groups including the vehicle (0.94% $MgCl_2$) group. The Applicant considered the findings to be procedure related and/or vehicle related. However, some of the findings such as inflammation and necrosis appear to have higher severity rating in the MD or HD group; thus, exacerbation of local effects by nalmefene cannot be ruled out. Local effects observed on Day 15 were partially or completely resolved on Day 29. The Applicant considered the HD to be the NOAEL. This Reviewer considers the findings of moderate degeneration/necrosis or moderate inflammation, observed in the MD and HD, to be adverse; therefore, the LD, corresponding to 0.2 times the clinical nalmefene

concentration is deemed to be the NOAEL with minimal to mild local effects observed in the dog study.

In the local tolerance studies in rats and rabbits, microscopic examination of the local tissues was conducted on Day 2 for acute effects and on Day 14 for recovery assessment. A saline group was included as a control group in these studies. In the local tolerance study in rats, minimal to moderate local effects including necrosis, inflammation and hemorrhage were observed at the IM or SC injection site on Day 2 across all the groups. Recovery of the local effects were observed on Day 15. Minimal to mild local effects were observed at the LD and MD of nalmefene/$MgCl_2$, and moderate severity ratings of the local findings were generally limited to the HD group; thus the MD, corresponding to 5.2 times and 2.6 times clinical nalmefene concentration, for the IM and SC administration route, respectively, is deemed to be the NOAEL for the rat study, considering the moderate local effects observed at the HD are potentially adverse. In the local tolerance study in rabbits, microscopic observations at both IM and SC injection sites at termination and recovery (i.e., minimal to mild leukocyte infiltration, myofiber degeneration/necrosis or regeneration, hemorrhage, ulcer, crust, epidermal hyperplasia, and occasional embedded hair foreign material) were observed across the treatment groups including the saline-treated group. The findings are considered incidental and generally attributable to injection procedure-related effects, given that these findings are commonly seen secondary to injection procedures, lacked a dose-response in incidence and severity, and occurred at similar incidence and severity across all treatment groups. A recovery trend was demonstrated on Day 15. Thus, the HD is deemed to be the NOAEL for the rabbit study, corresponding to 2.5 times the clinical nalmefene concentration, for both the IM and SC administration route.

Table 21: Nonclinical Findings of Potential Clinical Relevance

Toxicity/Finding	Studies*	Reversible?	Comments
Dosing Site (IM)			
Edema, fibrosis, hemorrhage (minimal to mild) Myofiber necrosis (minimal to marked) Inflammatory changes (minimal to moderate)	14-day Rat (IM)	Yes (partial to complete)	Related to procedure and vehicle ($MgCl_2$) exacerbated by nalmefene treatment
Muscle degeneration/necrosis (minimal to moderate) Mix cell or neutrophilic inflammation (minimal to moderate) Muscle regeneration, interstitial fibrosis (minimal to mild) Ulcer (minimal)	14-day Dog (IM)	Yes (partial to complete)	

Necrosis, inflammation, hemorrhage (minimal to moderate)	Acute Local Tolerance Rat (IM or SC)	Yes	Moderate severity findings were generally limited to HD groups.
Leukocyte infiltration, myofiber degeneration/necrosis or regeneration, hemorrhage, ulcer, crust, epidermal hyperplasia (minimal to mild)	Acute Local Tolerance Rabbit (IM or SC)	Yes	Procedure-related

*14-day rat study: Study 3243-002
14-day dog study: Study 3243-003
Single-dose rat study: Study 3243-004
Single-dose rabbit study: Study 3243-005

Table 22: Systemic Exposure Margins of Nalmefene Reached in the Nonclinical Studies

Animal				Human	
Animal Study	Dose Nalmefene (mg/kg/day)	Exposure (ng*h/mL)		Exposure Margin (Animal/Human)	
		$AUC_{0\text{-}24h}$ ng*h/mL	C_{max} ng/mL	$AUC_{(0\text{-}inf)}$ 62 ng*h/mL*	C_{max} 16.2 ng/mL*
14-day Rat (Study 3243-002)	0.3	39.4	35.1	0.6	2.2
	1.5	163	171	2.7	10.6
	7.5 (NOAEL)	1110	749	18.2	46.2
14-day Dog (Study 3243-003)	0.05	11.8	13.5	0.2	0.8
	0.25	53.6	62.9	0.9	3.9
	2.5 (NOAEL)	603	674	9.9	41.6

*Estimated values for 2 doses of the proposed drug product based upon average C_{max} (8.1 ng/mL) and $AUC_{(0\text{-}inf)}$ (31 ng*h/mL) achieved after IM administration of a single dose of 1.5 mg of the proposed drug product in the clinical study NAL1005

Table 23: Local Exposure Margins of Nalmefene Reached in the Nonclinical Studies

Animal Study	Administration Route (test article)	Nalmefene Concentration (mg/mL)	Exposure Margin*

14-day Rat Study (Study 3243-002)	IM (nalmefene HCL)	3 (NOAEL)	(b) (4)
		15	
		75	
14-day Dog Study (Study 3243-003)	IM (nalmefene HCL)	0.5 (NOAEL)	
		2.5	
		25	
Single-dose Local Tolerance Rat Study (Study 3243-004)	IM (nalmefene+$MgCl_2$)	5.2	
		15.5 (NOAEL)	
		21.5	
	SC (nalmefene+$MgCl_2$)	2.6	
		7.8 (NOAEL)	
		15.1	
Single-dose Local Tolerance Rabbit Study (Study 3243-005)	IM (nalmefene+$MgCl_2$)	1.5	
		7.5 (NOAEL)	
	SC (nalmefene+$MgCl_2$)	1.5	
		7.5 (NOAEL)	

* Exposure margin = Nalmefene concentration in animal study/nalmefene concentration in the DP which is (b) (4)

** Note that these studies evaluated local safety after 14 days of repeatrf dosing whereas the proposed product is for single use.

Drug Labeling

The Applicant proposes to (b) (4) (b) (4) (b) (4). To avoid safety margins that are misleading, the nonclinical review team recommends that margins are only specified for the IV rabbit study and if the Applicant wants to include margins for the oral nonclinical studies then the Applicant should conduct additional nonclinical PK studies in order to calculate safety margins based on AUC.

Overall Summary

In summary, there are no safety concerns with the elemental impurity levels, proposed specifications for drug substance or drug product impurities, and residual solvents.
In the submitted nonclinical studies, there were no systemic adverse effects observed at high exposure multiples of nalmefene. Test article-related local effects at the dosing site

were observed in the repeat-dose studies in rats and dogs and in the local tolerance studies in rats, but not in the local tolerance study in rabbits. Although some of the local effects with severity rating of moderate or above are deemed adverse by this Reviewer, this Reviewer agrees that the local effects are not dose limiting considering the demonstration of recovery process and clinical monitorability. The safety of $MgCl_2$ is supported by the nonclinical data and weight-of-evidence analysis. In support of the safety of the container closure system, no leachable compounds above the 5 mcg/day threshold were reported at any time points tested. Although the CMC review team has identified issues with the E/L evaluation, these issues do not preclude approval and will be addressed in postmarketing commitment studies. Taken together, the submitted nonclinical studies support the safety of the drug product, the excipient $MgCl_2$, and qualifies the safety of the drug product degradant (b) (4).

12 Appendix/Attachments

Bart G, Schluger JH, Borg L, Ho A, Bidlack JM, Kreek MJ. Nalmefene induced elevation in serum prolactin in normal human volunteers: partial kappa opioid agonist activity? Neuropsychopharmacology. 2005 Dec;30(12):2254-62.

Remmers AE, Clark MJ, Mansour A, Akil H, Woods JH, Medzihradsky F. Opioid efficacy in a C6 glioma cell line stably expressing the human kappa opioid receptor. J Pharmacol Exp Ther. 1999 Feb;288(2):827-33.

Toll L, Berzetei-Gurske IP, Polgar WE, Brandt SR, Adapa ID, Rodriguez L, Schwartz RW, Haggart D, O'Brien A, White A, Kennedy JM, Craymer K, Farrington L, Auh JS. Standard binding and functional assays related to medications development division testing for potential cocaine and opiate narcotic treatment medications. NIDA Res Monogr. 1998 Mar; 178:440-66.

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/s/

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