

**CENTER FOR DRUG EVALUATION AND
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PHARMACOLOGY 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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Applicant's letter date: September 23, 2013
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Product: Targiniq ER (oxycodone hydrochloride/naloxone hydrochloride) extended-release tablets
Indication: for the management of pain severe enough to require daily, around-the-clock, long-term opioid treatment and for which alternative treatment options are inadequate.
Applicant: Purdue Pharma, L.P.
One Stamford Forum
Stamford, Connecticut 06901-3431, USA
Review Division: Division of Anesthesia, Analgesia, and Addiction Products
Reviewer: BeLinda A. Hayes, PhD
Supervisor/Team Leader: R. Daniel Mellon, PhD
Division Director: Bob A. Rappaport, MD
Project Manager: Lisa Basham, MS

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1 Executive Summary

1.1 Introduction

Purdue Pharma, L.P. submitted NDA 205777 for Targiniq ER, an extended-release tablet formulation containing oxycodone hydrochloride and naloxone hydrochloride in a fixed 2:1 ratio. Purdue Pharma developed the product in three strengths: 10 mg/5 mg, 20 mg/10 mg, and 40 mg/20 mg oxycodone HCl/naloxone HCl. The proposed indication is for the management of pain severe enough to require daily, around-the-clock, long-term opioid treatment and for which alternative treatment options are inadequate. NDA 205777 was submitted via the 505(b)(2) regulatory pathway with Endo Pharmaceuticals Inc. Narcan (NDA 16636) as the referenced product. Purdue is also cross-referencing their data for oxycodone submitted in NDA 20553 and 22272 (OxyContin). New nonclinical studies were conducted and submitted to this NDA for naloxone, since the total daily dose of naloxone via this drug product exceeds that of any previously FDA-approved naloxone drug product and the referenced drug Narcan is only indicated for acute use. In addition, studies examining the interaction of the two drugs have also been submitted.

1.2 Brief Discussion of Nonclinical Findings

To support the safety of the drug product, the Applicant submitted the full standard battery of nonclinical toxicology studies for naloxone and resubmitted the oxycodone toxicology studies previously completed to support the OxyContin program. In addition, the Applicant submitted 3-month general toxicology studies evaluating the combination of oxycodone and naloxone.

The relative safety of oxycodone alone has been established in the development programs for OxyContin and via post-marketing experience. Characterization of the toxicologic potential of naloxone at the proposed doses and duration required additional studies to support this program. Although the general toxicology studies suggested that high doses of naloxone can produce convulsions in animals, there is an adequate safety margin (>60-fold) for the proposed maximum recommended daily dose of naloxone via this drug product.

The standard ICH battery of genetic toxicology studies were conducted for oxycodone HCl and naloxone HCl. Genetic toxicology studies submitted for oxycodone HCl was previously submitted to support the NDAs for Oxycontin. Oxycodone tested negative in the in vitro bacterial reverse mutation assay for mutagenicity and the in vivo bone marrow micronucleus assay. However, oxycodone was positive in the in vitro chromosomal aberration assay for mutagenicity in the presence of metabolic activation. Likewise, naloxone tested negative in the in vitro bacterial reverse mutation assay and

the in vivo mouse micronucleus assay. However, naloxone also tested positive in the L5178Y mouse lymphoma assay.

No reproductive studies and developmental studies were conducted using the oxycodone and naloxone combination. However, reproductive toxicology studies were performed with naloxone hydrochloride [REDACTED] ^{(b) (4)}. Embryo-fetal developmental studies conducted in pregnant rats treated with 50, 200, and 800 mg/kg/day naloxone hydrochloride by oral gavage during organogenesis. No remarkable treatment-related maternal toxicity was observed at doses up to 800 mg/kg/day. The maternal NOAEL was established at 800 mg/kg/day (192-fold human systemic exposure based on a mg/m² comparison). No developmental toxicity was observed at doses up to 800 mg/kg/day; the NOAEL for developmental toxicity was established at 800 mg/kg/day (192-fold human systemic exposure based on mg/m²).

Embryo-fetal developmental studies were conducted in New Zealand White rabbits treated with 20, 100, or 400 mg/kg/day naloxone hydrochloride by oral gavage during organogenesis. Naloxone was not teratogenic under the conditions of the assay; no significant malformations (external, soft tissue, or skeletal) were noted at doses up to 400 mg/kg/day. The maternal NOAEL was established at 100 mg/kg/day based on a non-statistical decrease in implantation rate, mean number of females per litter, and number of live fetus per dams. The developmental NOAEL is established at > 400 mg/kg/day based on lack of developmental toxicity (192-times the maximum recommended daily dose of 40 mg naloxone, on a body surface area basis).

Pre- and post-natal studies were conducted in pregnant rats treated with 50, 200, and 800 mg/kg/day naloxone hydrochloride by oral gavage from organogenesis through weaning. Evidence of maternal toxicity was indicated by treatment-related mortalities at the 800 mg/kg/day level and decreased body weight gain at the 200 mg/kg/day. The maternal NOAEL was established at 50 mg/kg/day (estimated exposure approximately 192-fold on a mg/m² basis). The developmental NOAEL was established at 200 mg/kg/day based on reduced viability index and newborns per litter from dams orally administered 800 mg/kg/day naloxone.

Collectively, although the existing oxycodone reproductive and developmental toxicology data do not suggest concern for the maximum recommended daily dose of oxycodone via this formulation, and there is an adequate safety margin for any naloxone-mediated effects, there appears to be little reproductive and developmental toxicology risk with this product. However, as there are not studies with the combination, we recommend that the drug product be given a Pregnancy Category C.

No carcinogenicity studies were conducted using the oxycodone and naloxone combination. However, carcinogenicity studies were performed with naloxone hydrochloride. Naloxone was negative in a 26-week Tg.rasH2 mouse carcinogenicity study and in a 2-year dietary rat carcinogenicity study at doses of 4, 20, or 100 mg/kg/day naloxone HCl showed no evidence of treatment-related tumors (24-times the human dose of 40 mg/day on a mg/m² basis). Carcinogenicity data on oxycodone do

not exist and based on OND policy, these studies will not be required for this drug product since the exposures to oxycodone via this formulation do not result in novel exposures compared to the cross-referenced OxyContin drug product.

Adequate safety data for the excipients in the drug has been provided for the maximum recommended daily dose of up to 80 mg oxycodone and 40 mg naloxone vis this drug product. The proposed drug substance and drug product specifications are acceptable for approval at this time. The drug substance impurity [REDACTED] which contains a structural alert for mutagenicity, has historically been limited to not more than (NMT) [REDACTED]^{(b) (4)} % for existing drug product formulations largely based on the relatively low daily exposures to naloxone. However, this drug product results in greater exposure to naloxone, therefore, the Applicant was asked to reduce the level to NMT [REDACTED]^{(b) (4)}. This would require a specification of NMT [REDACTED]^{(b) (4)} %. To date, the drug substance manufacturers are able to reach [REDACTED]^{(b) (4)} % for this impurity, but are not able to reduce it further at this time. Therefore, although not an approval issue, since this is as low as technically feasible, a PMR should be issued to either reduce the levels to NMT [REDACTED]^{(b) (4)} [REDACTED]^{(b) (4)} or to adequately qualify the impurity for safety. This would require an in vivo micronucleus assay and an in vivo comet assay testing both stomach and liver tissue.

It should be noted that this maximum recommended daily dose (MRDD) is not acceptable for single entity controlled release oxycodone drug products, which are taken at much higher levels due to the development of tolerance. This MRDD is based on the presence of the naloxone in the drug product, which is believed to limit the drug product's utility at higher doses. However, should the drug product be deemed appropriate for dosing above the MRDD of 80 mg oxycodone hydrochloride and 40 mg naloxone hydrochloride, further safety justification for the levels of excipients, drug substance impurities, and drug product degradants will be required.

1.3 Recommendations

1.3.1 Approvability

From a pharmacology/toxicology perspective, NDA 205777 may be approved with one post-marketing requirement if the drug product labeling includes a maximum recommended daily dose of 80 mg oxycodone hydrochloride/40 mg naloxone hydrochloride.

1.3.2 Additional Non Clinical Recommendations

The following nonclinical post-marketing requirement (PMR) should be issued:

Conduct a combination in vivo micronucleus and comet assay for [REDACTED]^{(b) (4)}

[REDACTED] The comet assay portion of the study should include assessment of both stomach and liver tissue and include doses of the drug

substance that would be obtained at the maximum recommended daily dose of the drug product and result in adequate toxicity to ensure assay validity.
Alternatively, you may reduce the levels of [REDACTED] (b) (4) to NMT [REDACTED] (b) (4)

1.3.3 Labeling

The table below contains the draft labeling submitted by the Applicant, the changes proposed by the reviewer, and the rationale for the proposed changes. The recommended changes from the proposed labeling are in red (additions) or strikeout font.

| Applicant's proposed labeling | Reviewer's proposed changes | Rationale for changes |
|--|--|--|
| <p>8 USE IN SPECIFIC POPULATIONS</p> <p>8.1 Pregnancy</p> <p style="text-align: center;">(b) (4) <i>Pregnancy</i></p> <p><u>Category C</u></p> <p>There are no adequate and well-controlled studies in pregnant women. (b) (4)</p> <p>(b) (4)</p> | <p>8 USE IN SPECIFIC POPULATIONS</p> <p>8.1 Pregnancy</p> <p>Pregnancy C.</p> <p>Risk Summary</p> <p>There are no adequate and well-controlled studies with TARGINIQ ER in pregnant women. [HUMAN STATEMENT – See Final labeling]. Animal reproduction studies were not conducted with the combination of oxycodone and naloxone, the components of TARGINIQ ER. However, animal data are available from studies conducted with the individual components. Embryo-fetal toxicity was not observed following oral administration of oxycodone to rats and rabbits during the period of organogenesis at doses equal to or 30 times, respectively, the maximum recommended human daily dose (MRDD) of 80/40 mg oxycodone/day based on body surface (b) (4). Embryo-fetal toxicity was not observed following oral administration of naloxone (800 mg/kg) to pregnant rats and rabbits during organogenesis at doses 192 times the MRDD of 80/40 mg naloxone/day of (b) (4). TARGINIQ ER should be used</p> | <p>The format has been changed to comply with the Pregnancy and Lactation Labeling Rule. To comply with this rule, the (b) (4) headings have been replaced with an <i>Animal Data</i> section.</p> |

| | | | |
|--|---------|--|---------|
| | (b) (4) | <p>during pregnancy only if the potential benefit justifies the potential risk to the fetus. All pregnancies, regardless of drug exposure, have a background risk of 2-4% for major birth defects, and 15-20% for pregnancy loss.</p> | |
| | (b) (4) | <p>Clinical Considerations</p> <p><u>Fetal/neonatal adverse reactions</u></p> <p>Prolonged use of opioid analgesics during pregnancy for medical or nonmedical purposes can result in physical dependence in the neonate and neonatal opioid withdrawal syndrome shortly after birth. Observe newborns for symptoms of neonatal opioid withdrawal syndrome, such as poor feeding, diarrhea, irritability, tremor, rigidity, and seizures, and manage accordingly [see Warnings and Precautions (5.3)].</p> | |
| | (b) (4) | | (b) (4) |
| | (b) (4) | | (b) (4) |
| | (b) (4) | | (b) (4) |
| In a peri-/post-natal development study with naloxone in rats, the highest dosage of 800 mg/kg/day (192-times the intake of naloxone at the maximum 40 mg/day) produced mortality and significant toxicity in maternal rats, which was associated with increased pup deaths in the immediate postpartum period. | (b) (4) | | (b) (4) |
| Mild toxic signs were also observed in maternal rats that received 200 mg/kg/day (approximately 48-times the intake of naloxone at the maximum daily dose of on a body surface area basis); however, there were | (b) (4) | | (b) (4) |

no adverse effects on the pups.

(b) (4)
[REDACTED]

In a peri /post natal development study with naloxone in rats, the highest dosage of 800 mg/kg/day (b) (4) - [REDACTED] - 192 times the intake of naloxone at the maximum dose of (b) (4) (b) (4) 40 mg/day of (b) (4) produced mortality and significant toxicity in maternal rats, which was associated with increased pup deaths in the immediate postpartum period. (b) (4)

- Mild toxic signs were also observed in maternal rats that received 200 mg/kg/day (approximately 48 times the intake of naloxone at the maximum (b) (4)) on a body surface area basis); however, there were no adverse effects on the pups.

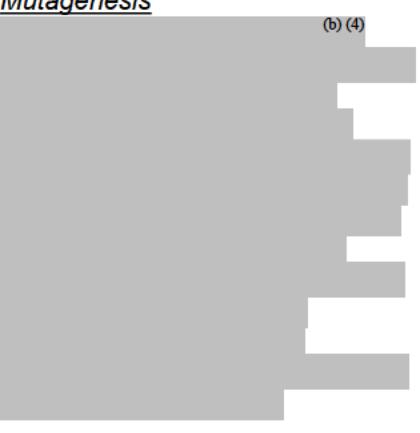
Data

Animal Data

Oxycodone

Studies with oral doses of oxycodone hydrochloride in rats up to 8 mg/kg/day and rabbits up to 125 mg/kg/day, equivalent to 1 and 30 times the maximum

| | | |
|--|---|--|
| | <p>(b) (4) daily dose of 80 mg/day, respectively on a mg/m² basis, did not reveal evidence of harm to the fetus due to oxycodone. In a pre- and postnatal toxicity study, female rats received oxycodone during gestation and lactation. There were no long-term developmental or reproductive effects in the pups.</p> <p>Oxycodone hydrochloride was administered orally to female rats during gestation and lactation in a pre- and postnatal toxicity study. There were no drug-related effects on reproductive performance in these females or any long-term developmental or reproductive effects in pups born to these rats. Decreased body weight was found during lactation and the early post-weaning phase in pups nursed by mothers given the highest dose used (6 mg/kg/day, equivalent to approximately 0.8-times the MRDD of 80 mg/day, on a mg/m² basis). However, body weight of these pups recovered.</p> <p>Naloxone</p> <p>Orally administered naloxone was not teratogenic in the rat or rabbit at the maximum dosages tested (800 mg/kg/day or 400 mg/kg/day, respectively) which were equivalent to approximately 192-times MRDD of 40 mg/day on a mg/m²</p> <p>(b) (4)</p> <p>In a (b) (4) and post-natal development study with naloxone in rats, the highest dosage of 800 mg/kg/day (192 times the MRDD of 40 mg naloxoe/day, (b) (4)</p> | |
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| | | |
|--|--|--|
| | (b) (4) produced mortality and significant toxicity in maternal rats, which was associated with deaths in the immediate postpartum period. Mild toxic signs were also observed in maternal rats that received 200 mg/kg/day (48-times the MRDD of 40 mg naloxone on a body surface area basis); however, there were no adverse effects on the pups. | |
| 13 NONCLINICAL TOXICOLOGY | 13 NONCLINICAL TOXICOLOGY | |
| 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility <u>Carcinogenesis</u> Carcinogenicity studies have not been conducted with oxycodone or the oxycodone and naloxone combination. Naloxone was tested in two carcinogenicity studies in rats and transgenic mice. Naloxone was not carcinogenic in a 2-year rat bioassay at doses as high as 100 mg/kg/day (b) (4)  <u>Mutagenesis</u>  | 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility <u>Carcinogenesis</u> Carcinogenicity studies have not been conducted with oxycodone alone or the oxycodone and naloxone combination. Naloxone was tested in two carcinogenicity studies in rats and transgenic mice. Naloxone was not carcinogenic in a 2-year rat bioassay at doses as high as 100 mg/kg/day (24-times the MRDD 40 mg naloxone/day on a mg/m ² basis) (b) (4)  <u>Mutagenesis</u> Oxycodone was genotoxic in the mouse lymphoma assay. Clastogenicity was observed with oxycodone in the presence of metabolic activation in one | To be consistent with the new Sufenta label. |

| | | | |
|--------------------------------|---------|---|--|
| | (b) (4) | chromosomal aberration assay in human lymphocytes at concentrations greater than or equal to 1250 mcg/mL at 24 but not 48 hours of exposure. In a second chromosomal aberration assay with human lymphocytes, no structural clastogenicity was observed either with or without metabolic activation; (b) (4) oxycodone increased numerical chromosomal aberrations (polyploidy). Oxycodone was not genotoxic in the following assays: Ames S. typhimurium and E. coli test with and without metabolic activation at concentrations up to 5000 mcg/plate, chromosomal aberration test in human lymphocytes (in the absence of metabolic activation) at concentrations up to 1500 mcg/mL, and with activation after 48 hours of exposure at concentrations up to 5000 mcg/mL, and in the in vivo bone marrow micronucleus assay in mice (at plasma levels up to 48 mcg/mL). Naloxone was genotoxic in the mouse lymphoma assay. Naloxone produced a non-dose-related increase in chromosomal aberrations in the presence of metabolic activation that was statistically significant at concentrations of 375 and 1500 mcg/mL but not at 750 or 3000 mcg/mL. In contrast, naloxone was not mutagenic in the S. typhimurium/E. coli bacterial mutagenicity test with or without metabolic activation nor was it genotoxic in in vivo mouse bone marrow micronucleus tests a dose of 500 mg/kg. | |
| <i>Impairment of Fertility</i> | (b) (4) | Impairment of Fertility: (b) (4) | |

| | | |
|---------|--|--|
| (b) (4) | (b) (4) | |
| | <p>Fertility studies to evaluate the combination of oxycodone and naloxone have not been conducted. In a study of reproductive performance, rats were administered a once daily gavage dose of the vehicle or oxycodone hydrochloride doses up to 8 mg/kg (equivalent to the MRDD of 80 mg oxycodone/day on a mg/m² basis). Male rats were dosed for 28 days before cohabitation with females, during the cohabitation and until necropsy (2-3 weeks post-cohabitation). Females were dosed for 14 days before cohabitation with males, during cohabitation and up to Gestation Day 6. Oxycodone hydrochloride did not affect reproductive function in male or female rats.</p> | |

2 Drug Information

2.1 Drug

CAS Registry Numbers

Oxycodone hydrochloride: 124-90-3

Naloxone hydrochloride (b) (4) 51481-60-8

Generic Names

Oxycodone hydrochloride
Naloxone hydrochloride

Code Names

This drug product has been referred to as [REDACTED]^{(b)(4)}, Targiniq, OXN, ONX, and ONU during the course of development.

Chemical Names

Oxycodone hydrochloride: 4,5α-epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one hydrochloride

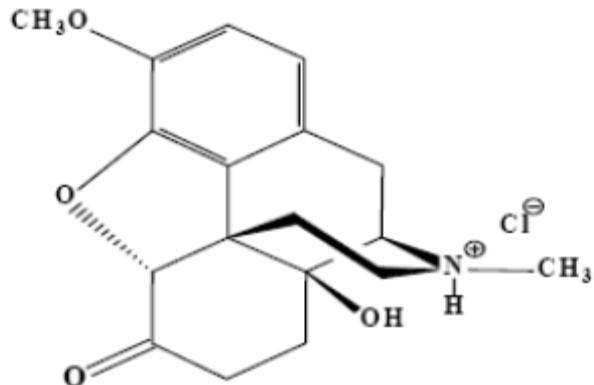
Naloxone hydrochloride: 4,5α-epoxy-3,14-dihydroxy-17-(prop-2-enyl)-6-morphinan-6-one hydrochloride

Molecular Formulas/Molecular Weights

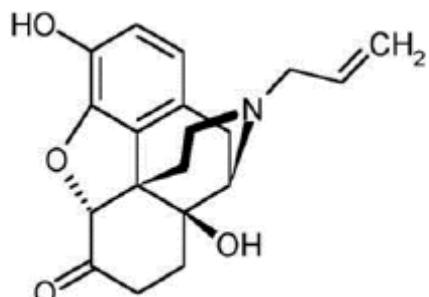
Oxycodone hydrochloride: C₁₈H₂₁N0₄·HCl/351.83 g/mol

Naloxone hydrochloride: C₁₉H₂₁N0₄·HCl/399.87 g/mol

Structures



Oxycodone hydrochloride



Naloxone (depicted as base)

Pharmacologic Classes

Oxycodone hydrochloride: Opioid agonist
Naloxone hydrochloride: Opioid antagonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

| INDs | Status | Division | Indication | Stamp Date | Applicant |
|-------|--------|--|---|------------|---------------------|
| 70851 | Active | Anesthesia, Analgesia and Addiction Products | Analgesia for moderate to severe pain | 03/28/2010 | Purdue Pharma LP |

| NDAs | Drug Product | Status | Division | Indication | Status Date | Applicant |
|-------|--------------|-----------|----------|----------------------------|-------------|-------------------------|
| 16636 | Narcan | Withdrawn | DAAAP | Opioid overdose | 8/20/2010 | Endo Pharmaceuticals |
| 20553 | OxyContin | Withdrawn | DAAAP | Moderate to severe pain | 08/07/2013 | Purdue Pharma LP |
| 22272 | OxyContin | Approved | DAAAP | Moderate to severe pain | 04/05/2010 | Purdue Pharma LP |

| DMFs № | Subject of DMF | Holder |
|---------|-------------------------|---------|
| (b) (4) | Oxycodone hydrochloride | (b) (4) |
| | Naloxone hydrochloride | |
| | Naloxone hydrochloride | (b) (4) |

2.3 Drug Formulation

Targiniq (oxycodone HCl and naloxone HCl) is a fixed 2:1 ratio of oxycodone hydrochloride/naloxone hydrochloride extended-release tablet containing 10/5 mg, 20/10 mg, or 40/20 mg for oral administration. The design of the tablet is to enable absorption of oxycodone with minimal systemic absorption of naloxone while achieving co-extraction of naloxone with oxycodone should the product be crushed and insuflated or manipulated for intravenous injection. The extended release property [REDACTED]^{(b)(4)}

The quantitative composition of Targiniq is described in the table below.

| Component Tablet | Reference | Function | Oxycodone HCl/Naloxone HCl | | |
|------------------------------------|-----------|-------------------|----------------------------|--------|---------|
| | | | 10/5 | 20/10 | 40/20 |
| | | | Quantity (mg/tab) | | |
| Oxycodone HCl | USP | Active Ingredient | 10.0 | 20.0 | 40.0 |
| Naloxone HCl ¹ | USP | Active Ingredient | 5.0 | 10.0 | 20.0 |
| Lactose Monohydrate | NF | | | | (b) (4) |
| Stearyl Alcohol | NF | | | | |
| Ethyl Cellulose ^{(b) (4)} | NF | | | | |
| Providone ^{(b) (4)} | USP | | | | |
| Talc | USP | | | | |
| Magnesium Stearate | NF | | | | (b) (4) |
| Total Film Tablet | | | 127.27 | 142.17 | 284.34 |
| 1: As Naloxone Hydrochloride | | (b) (4) | | | |

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(b) (4)

Determination of the maximum daily dose of oxycodone/naloxone. The maximum recommended daily dose (MRDD) for oxycodone and naloxone via this drug product is essential to establish the drug substances and drug product specifications and the safety of the excipients in this formulation. The Applicant has not provided data to justify their proposed maximum recommended daily dose of their product. However, they are recommending the total daily dose of Targiniq ER not exceed 80 mg oxycodone/40 mg naloxone. The reader is referred to the clinical pharmacology and clinical review for further discussion of the maximum recommended daily dose and why the Division believes that the MRDD proposed is acceptable for this formulation.

2.4 Comments on Novel Excipients

There are no novel excipients in the drug product if the MRDD is 80 mg oxycodone and 40 mg of naloxone per day. All of the above excipients are found in FDA-approved chronic oral drug products within acceptable ranges.

(b) (4) Pink (b) (4) and (b) (4) Yellow (b) (4) are not listed in the Inactive Ingredients Database (IID). (b) (4) Pink (b) (4) is the (b) (4) film coating for the 20/10 dosage form and as per the Applicant, the quantity in this formulation is (b) (4) (b) (4) per tablet. (b) (4) Yellow (b) (4) is the (b) (4) film coating for the 40/20 dosage form and as per the Applicant, the quantity in this formulation is (b) (4) per tablet. Although these (b) (4) are not listed in the IID, the components of these coating are common excipients in oral controlled release tablets previously approved by the Agency. Thus, these excipients are deemed acceptable for use in this drug product.

2.5 Comments on Impurities/Degradants of Concern

Drug Substance Specifications

Table 3. Acceptance criteria specifications for the oxycodone drug substance

| Impurity | Specification | Acceptable? |
|------------------------|---------------|-------------|
| (b) (4) | NMT (b) (4)% | Yes |
| | NMT % | Yes |
| Individual Unspecified | NMT % | Yes |

*: Contains a structural alert for mutagenicity

The specifications for the impurities for oxycodone hydrochloride drug substance obtained from (b) (4) are listed in the table above. The qualification threshold according to the ICH Q3A(R2) guideline for impurities in the drug substance for a MDD of a drug substance ≤ 2 g/day is 0.15% or 1.0 mg/day (whichever is lower). The proposed specification for the impurity (b) (4) exceeds the ICH qualification threshold; (b) (4)

and is therefore deemed acceptable. Per the Applicant, a DEREK evaluation suggested that (b) (4) was not associated with genotoxicity signals. It is deemed adequately qualified, via previous clinical experience with this drug substance and as a minor metabolite of oxycodone. Thus, the specifications for the non-genotoxic impurities are acceptable.

The oxycodone drug substance contains two impurities containing an α , (b) (4) moiety, which is a structural alert for mutagenicity, (b) (4) and (b) (4). The proposed specifications for (b) (4) and (b) (4) are NMT (b) (4)% and (b) (4)%, respectively. For a maximum daily dose of 80 mg oxycodone/day, this specification will result in a total daily intake of (b) (4) for each (b) (4) impurity. Therefore, the proposed specifications for genotoxic impurities are acceptable as the exposure will not exceed NMT (b) (4) limits for potential genotoxic impurities as per current FDA policy and the recently signed ICH M7 guidance.

Table 4. Acceptance criteria specifications for the naloxone hydrochloride drug substance from (b) (4).

| Impurity | Specification | Acceptable? |
|----------|---------------|-------------|
| (b) (4) | NMT (b) (4)% | Yes |
| | NMT % | Yes |

| Impurity | Specification | Acceptable? |
|------------------------|--------------------------|-------------|
| | NMT ^{(b) (4)} % | Yes |
| | NMT % | Yes |
| | NMT % | Yes |
| | NMT ^{(b) (4)} % | No |
| Individual Unspecified | NMT ^{(b) (4)} % | Yes |

Table 5. Acceptance criteria specifications for the naloxone hydrochloride drug substance from [REDACTED]

| Impurity | Specification | Acceptable? |
|------------------------|--------------------------|-------------|
| | NMT ^{(b) (4)} % | Yes |
| | NMT % | Yes |
| | NMT % | Yes |
| | NMT % | Yes |
| | NMT % | Yes |
| | NMT ^{(b) (4)} % | No |
| Individual Unspecified | NMT ^{(b) (4)} % | Yes |

*: Structural alert for mutagenicity

The Applicant is obtaining the naloxone hydrochloride drug substance from two manufacturers, [REDACTED] and [REDACTED]. The specification for the impurities in the naloxone hydrochloride [REDACTED] drug substance obtained from [REDACTED] and [REDACTED] are listed in the tables above. As noted in the tables above, the specifications for these impurities [REDACTED] for both sources of naloxone hydrochloride [REDACTED]. The naloxone drug substance contains [REDACTED] which contains an α, [REDACTED] moiety, a structural alert for mutagenicity. The Agency is aware of data indicating that this compound has been reported to be negative for mutagenicity in the Ames assay but tested positive for clastogenicity in the in vitro chromosome aberration assay. When [REDACTED] was identified as a potential genotoxic impurity in 2002, the Division required this impurity be controlled at NMT [REDACTED] % in part based on the low daily dose of naloxone in the FDA-approved drug products at that time.

The Applicant has set the specifications for [REDACTED] impurity in the naloxone drug substance at NMT [REDACTED] thus meeting the Division's historical established limit of NMT [REDACTED] %. At the MRDD of 40 mg naloxone, the proposed drug substance specification of NMT [REDACTED] will result in a total daily intake of [REDACTED] for this impurity, thus exceeding the FDA recommendation of NMT [REDACTED] limit for potential genotoxic impurities. Therefore, the Applicant should tighten the proposed specification of [REDACTED] or provide adequate safety qualification which must include an in vivo micronucleus assay and an in vivo comet assay which include evaluation of the stomach and liver. This study can be completed as a post-marketing requirement, since the proposed specification is currently as low as technically feasible at this time.

Drug Product Specifications**Table 6. Release and stability specifications for the oxycodone hydrochloride/naloxone hydrochloride (b) (4) drug product**

| Impurities of Oxycodone HCl/Naloxone HCl Drug Product | | |
|---|---------------|---|
| Impurity | Specification | Acceptable? |
| (b) (4) | NMT (b) (4) % | Yes. This is a metabolite of oxycodone |
| | NMT (b) (4) % | Yes. The specification is consistent with ICH Q3B(R2). The degradant is derived from naloxone (MDD of naloxone is 40 mg). |
| | NMT (b) (4) % | Yes. The specification is consistent with ICH Q3B(R2). The degradant is derived from naloxone (MDD of naloxone is 40 mg). |
| | NMT (b) (4) % | Yes. The specification is consistent with ICH Q3B(R2). The degradant is derived from naloxone (MDD of naloxone is 40 mg). |
| | NMT (b) (4) % | Yes. The specification is consistent with ICH Q3B(R2). This is derived from oxycodone and the proposed specification (b) (4) approved specification in OxyContin. |
| Each Individual Unknown | NMT (b) (4) % | Yes |

The specification for the impurities/degradants is listed in the table above. As per ICH Q3B(R2) the qualification threshold for a drug product with a maximum daily dose between 10 and 100 mg is NMT (b) (4) TDI, whichever is lower. For a maximum daily dose of 80 mg oxycodone and 40 mg of naloxone, a specification at release of NMT (b) (4) % for each degradant would result in (b) (4) the degradant in oxycodone and naloxone, respectively. Thus, a specification of NT (b) (4) % will meet ICH Q3B(R2) threshold for drug product degradants of naloxone if the MRDD is 40 mg naloxone hydrochloride. It is noted that the drug substance impurity (b) (4)

(b) (4) was not listed in the drug product specification as a degradant because this drug substance impurity is not expected to be a drug product degradant.

The proposed specification for (b) (4) of NMT (b) (4) % exceeds the ICH Q3B(R2) qualification threshold and therefore must be adequately qualified for safety. According to the Applicant, (b) (4) is a known metabolite of oxycodone and naloxone and significant levels of this metabolite were measured in the 3-month rat combination toxicology study. The Applicant did not provide scientific literature to support the claim that (b) (4) is a metabolite of naloxone, (b) (4)

The proposed specification is acceptable.

2.6 Proposed Clinical Population and Dosing Regimen

Targiniq is proposed for the management of pain severe enough to require daily, around-the-clock, long-term opioid treatment and for which alternative treatment options are inadequate. Targiniq is a combination product containing oxycodone hydrochloride and naloxone hydrochloride in a 2:1 ratio. The naloxone component is intended to deter abuse via the intranasal and intravenous route of administration. Targiniq is formulated as oral extended-release tablet that will be available in three dosage strengths, 10/5 mg, 20/10 mg, and 40/20 mg, and is intended for twice daily dosing (every 12 hours). The Applicant has proposed labeling to recommend that dosing should not exceed 80 mg oxycodone and 40 mg naloxone per day. They provided no clear rationale for this limitation in labeling.

2.7 Regulatory Background

The controlled-release combination of oxycodone and naloxone is approved in Europe under the trade name Targin and is indicated for the management of moderate to severe chronic pain unresponsive to non-narcotic analgesia while counteracting opioid-induced constipation. Targin was first approved in Germany. Since 2006, Targiniq has been approved in 36 European and other countries.

On September 23, 2013, NDA 205777 was submitted via the 505(b)(2) pathway with the referenced drugs Narcan (NDA 16636, Endo Pharmaceuticals Inc) and cross-reference to their OxyContin NDA (NDA 22272, Purdue Pharma LP). Regulatory meetings were held under IND 70851 beginning in 2004 via the preIND process. IND 70851 was not formally submitted until February 26, 2010. Two preIND meetings were held; the first one on May 26, 2005 and the second preIND meeting was held on February 24, 2009. An EOP2 meeting was held on November 18, 2010. On April 7, 2011, the Applicant requested a meeting with the Division to discuss their proposed Phase 3 protocol (ONU3701) and statistical analysis plan intended to support the analgesia indication

(b) (4). The Division provided written response to Purdue on August 19, 2011. A pre-NDA meeting was held on September 13, 2012.

3 Studies Submitted

3.1 Studies Reviewed

| Report № | Study Title | Module/CTD Description |
|----------------------------|--|---|
| Pharmacology | | |
| № OXUPR02-95.0 | Opioid receptor binding and functional profiles for the opioids naltrexone, naloxone, hydrochloride and oxycodone and their metabolites at the human mu, kappa, delta and ORL-1 receptors. | 4.2.1.1/Primary Pharmacodynamics |
| Safety Pharmacology | | |
| № NDSE-585 | K ⁺ channel (HERG) for 29 compounds | 4.2.1.3/Safety Pharmacology |
| № ONU-N-015 | Dose range finding and pulmonary evaluation of orally administered oxycodone hydrochloride in rats, alone and in combination with naloxone | |
| Pharmacokinetics | | |
| № OXN-P-024 | Pharmacokinetics of mixture of oxycodone:naloxone at a 2:1 ratio in male and female rhesus monkey following a single oral or single intravenous administration at two different dose levels. | 4.2.2.2/Absorption |
| № OXN-P-065 | Determination of the pharmacokinetics of naloxone and its metabolites in plasma and CSF after multiple oral doses to rats. | 4.2.2.3/Distribution |
| № OXN-P-065 | In vitro stability of oxycodone and oxycodone N-oxide in human plasma and human liver S9 fractions. | 4.2.2.4/Metabolism |
| № OXN-P-011 | In vitro evaluation of possible metabolic interactions between oxycodone, naltrexone, naloxone and acetaminophen in cryopreserved human hepatocytes | 4.2.2.6/ Pharmacokinetic Drug Interaction |
| № OXUDR02-074 | In vitro evaluation of oxycodone, naloxone and their combinations as inhibitors of human cytochrome P450 and cytosolic enzymes. | |
| Toxicology | | |
| № OXN-N-001 | OXN (oxycodone hydrochloride/naloxone hydrochloride in a 2:1 ratio): An acute and a 7-day repeat dose oral gavage toxicity study in rats. | 4.2.3.1/Single-Dose Toxicity |
| № KPC 24-87 | 52 Week dietary study in the rat. | |
| № N003003D | Nine month oral toxicity study of naloxone HCl | |

| Report № | Study Title | Module/CTD Description |
|-------------------------------------|--|---|
| | in dogs. | |
| Nº NDSE-609 | A 3-month oral (gavage) toxicity study in rats with a 28-day recovery period with either the combination of oxycodone hydrochloride and naloxone hydrochloride [REDACTED] ^{(b) (4)} or naloxone hydrochloride [REDACTED] ^{(b) (4)} alone. | 4.2.3.2/Repeat-Dose Toxicity |
| Nº NDSE-610-GLP | A 3-month oral (gavage) toxicity study in dogs with a 28-day recovery period with either the combination of oxycodone hydrochloride and naloxone hydrochloride [REDACTED] ^{(b) (4)} or naloxone hydrochloride [REDACTED] ^{(b) (4)} alone. | |
| Genetic Toxicology | | |
| Nº 70/8409 | Bacterial mutagenicity tests: Naloxone chlorhydrate. | |
| Nº 778441 | Oxycodone hydrochloride injection 50 mg/mL containing dimer (OH-Dimer) and oxycodone hydrochloride injection mg/mL reference formulation (OH-form): Testing for mutagenic activity with <i>Salmonella typhimurium</i> TA 1535, TA 100, TA 1537 and TA 98 and <i>Escherichia WP2μrA</i> . | |
| Nº 71/8409 | Tests for gene mutation in L5178Y mouse lymphoma cells treated with naloxone. | 4.2.3.3/Genotoxicity |
| Nº 74/8506 | Metaphase analysis of human lymphocytes treated with naloxone. | |
| Nº 778436 | Oxycodone hydrochloride injection 50 mg/mL containing dimer (OH-Dimer) and oxycodone hydrochloride injection 50 mg/mL reference formulation (OH-Form): Chromosomal aberration assay with human peripheral lymphocytes culture in vitro. | |
| Nº N003003A | Bone marrow micronucleus test in mice treated with naloxone HCl. | |
| Carcinogenicity | | |
| Nº ONU-N-009 | Naloxone Hydrochloride: 26-week repeated dose oral carcinogenicity study in Tg.rasH2 mice. | |
| Nº N003003F | 2-Year oral oncogenicity study of naloxone HCl in Sprague-Dawley rats. | 4.2.3.4/Carcinogenicity |
| Reproductive and Development | | |
| Nº KPC/32/86 | Naloxone: Rat fertility and general reproductive performance study. | 4.2.3.5.1/Fertility and Early Embryonic Development |
| Nº KPC/33-85/R | Rat teratology study | 4.2.3.5.2/Embryo-fetal Development |
| Nº KPC/35-85 | Rabbit teratology study | |
| Nº KPC/34/85 | Rat peri- and post natal study | 4.2.3.5.3/Perinatal and Postnatal Development |

3.2 Studies Not Reviewed (Formally)

| Report № | Study Title | Module/CTD Description |
|--------------|---|------------------------------|
| | Toxicology | |
| № KPC/18/PSB | Single dose (oral) limit test in the mouse: Naloxone. | 4.2.3.1/Single-Dose Toxicity |
| № KPC/19/PSB | Single dose (oral) limit test in the rabbit: Naloxone. | |
| № DSE-332 | A single dose range-finding acute toxicity study in rats with oxycodone hydrochloride using oral (gavage) route of administration. | |
| № KPC/17/PSB | Single dose (oral) limit test in the rat: Naloxone. | |
| № NDSE-706 | 2-Week intermittent intravenous infusion toxicity and toxicokinetic study with naloxone HCL in dogs. | |
| № N00300D | Nine month oral toxicity study of naloxone in dogs. | |
| № KPC/28/C | 13 Week oral toxicity study in the dog: naloxone | |
| № NDSE-562 | A 3-month oral (capsule) toxicity study in dogs with a 28-day recovery period with oxycodone hydrochloride. | |
| № NDSE-610 | A 3-month oral (gavage) toxicity study in dogs with a 28-day recovery period with the combination of oxycodone hydrochloride and naloxone hydrochloride (b) (4) or naloxone hydrochloride (b) (4) alone. | |
| № NDSE-496 | A 28-day oral (capsule) toxicity study in dogs with a 28-day recovery period with oxycodone hydrochloride. | |
| № NDSE-498 | A preliminary dose-ranging five-day toxicity study in dogs with oxycodone hydrochloride using the oral (capsule) route of administration. | 4.2.3.2/Repeat-Dose Toxicity |
| № NDSE-591 | A 28-day oral (gavage) dose range-finding study in dogs with either the combination of oxycodone hydrochloride and naloxone hydrochloride (b) (4) or naloxone hydrochloride (b) (4) alone. | |
| № NDSE-595 | Preliminary 2-week oral toxicity study of bulk oxycodone hydrochloride in dogs. | |
| № OXN-N-002 | Escalating dose range-finding (DRF) toxicity study and determination of maximum-tolerated dose (MTD) with OXN via oral (gavage) in dogs. | |
| № OXN-N-004 | 4-week oral gavage toxicity and toxicokinetic study with OXN in dogs. | |
| № N003003E | Three month oral toxicity study of naloxone HCl in mice. | |
| № KPC/21/C | 28-Day dietary range finding study in mouse. | |
| № ONU-N-001 | Naloxone hydrochloride: 28-day repeated dose oral toxicity and toxicokinetic study in Tg.rasH2 mice. | |
| № KPC/25/C | 13-Week oral toxicity study in the rat: Naloxone. | |
| № NDSE-609 | A 3-month oral (gavage) toxicity study in rats with a 28-day recovery period with either the combination of oxycodone hydrochloride and naloxone hydrochloride (b) (4) or naloxone hydrochloride (b) (4) alone. | |
| № KPC/20/C | 28-Day dose range finding study in the rat: Naloxone. | |
| № KPC/22/C | 28-Day oral range finding study in the rat. | |
| № KPC/36/C | 28 Day range finding study in the rat: Naloxone. | |
| № NDSE-495 | A 28-day oral (gavage) toxicity study in rats with a 28-day recovery period with oxycodone hydrochloride. | |

| Report № | Study Title | Module/CTD Description |
|--------------|--|------------------------|
| Nº NDSE-497 | A preliminary range-finding 8-day oral (gavage) toxicity study in rats with oxycodone hydrochloride. | |
| Nº NDSE-590 | A 28-day oral (gavage) dose range-finding study in rats with either the combination of oxycodone hydrochloride and naloxone hydrochloride (b) (4) or naloxone hydrochloride (b) (4) alone. | |
| Nº OXN-N-001 | OXN a combination of oxycodone (OX) and naloxone (N) at a fixed ratio of 2:1 free base, respectively. | |
| Nº OXN-N-003 | OXN (oxycodone hydrochloride/naloxone hydrochloride in a 2:1 ratio): A 4-week oral gavage toxicity study with a 2-week recovery period in rats. | |

The above studies were not reviewed formally for this NDA as they were not either not GLP studies or were shorter term toxicology studies and the pivotal GLP chronic toxicology provided adequate safety characterization of the naloxone or drug product combination.

3.3 Previous Reviews Referenced

Refer to the pharmacology/toxicology review of the OxyContin NDA (Purdue Pharma; NDA 20-553) by BeLinda Hayes, PhD dated August 21, 1995 and pharmacology/toxicology review of the OxyContin reformulation NDA (Purdue Pharma NDA 22-272) by Elizabeth Bolan, PhD dated May 1, 2008.

4 Pharmacology

4.1 Primary Pharmacology

Oxycodone is an opioid agonist with activity at the mu opioid receptor. Activation of the mu opioid receptor is associated with analgesia, respiratory depression, decreased gastrointestinal motility, euphoria, and physical dependence.

Mechanism of action: The in vitro pharmacological study assessing the binding and functional properties provided corollary evidences that oxycodone and naloxone mechanism of action was primarily mediated via the mu opioid receptor. The oxycodone metabolite oxymorphone also displayed binding affinity for the mu opioid receptor. Oxycodone and its metabolites did not display affinity for the kappa or delta receptors.

Study Title: Opioid receptor binding and functional profiles for the opioids naltrexone, naloxone, hydrochloride and oxycodone and their metabolites at the human mu, kappa, delta and ORL-1 receptors (non-GLP).

Study Report №: OXUPR02-95.0

Objective of the study: As part of the OXU and HXA/HCX projects, the in vitro binding and functional profiles for several opioid agonists (hydrocodone and oxycodone) and antagonists (naltrexone and naloxone) and their metabolites at the four classic opioid receptors, mu, kappa, delta, and ORL-1 were evaluated.

Methods. Using a recombinant human opioid receptors expressed in HEK-293 cells radioligand dose-displacement assays were conducted to measure the relative affinities, binding K_i values, at the mu, kappa, delta, and ORL-1 opioid receptors. The potency and efficacy of agonism for the different opioids were evaluated in the functional [^{35}S]GTP γ S binding assays.

Key Study Findings:

Table 7. In vitro binding profile for oxycodone and naloxone and their metabolites

| | | Binding K_i Values (nM) \pm SEM | | | |
|----------------------|------------------------|-------------------------------------|-----------------|----------------|----------------|
| | | Opioid Receptor Types | | | |
| | Compounds | Mu | Kappa | Delta | ORL-1 |
| Controls | Naloxone | 40 \pm 04 | 34 \pm 06 | 538 \pm 78 | >10,000 |
| | DAMGO | 37 \pm 19 | 5539 \pm 1520 | Inactive | Inactive |
| | Nociceptin | Inactive | 7705 \pm 3187 | Inactive | 0.28 \pm 003 |
| | U-69,593 | >10,000 | 3.0 \pm 0.5 | Inactive | >10,000 |
| | Met-Enkephalin | 48 \pm 7 | >10,000 | n.d. | >10,000 |
| Oxycodone | Oxycodone | 61 \pm 16 | 2616 \pm 488 | >10,000 | >10,000 |
| | Oxymorphone | 3.1 \pm 08 | 66 \pm 8 | >10,000 | >10,000 |
| | Noroxycodone | 240 \pm 40 | 2562 \pm 570 | >10,000 | Inactive |
| | 6 α -oxycodol | 821 \pm 148 | 5935 \pm 963 | >10,000 | Inactive |
| | 6 β -oxycodol | 187 \pm 28 | 4605 \pm 959 | >10,000 | Inactive |
| Naloxone | Naloxone Control | 4.0 \pm 04 | 3.4 \pm 06 | 538 \pm 78 | >10,000 |
| | Naloxone 3-glucuronide | 1656 \pm 572 | 2024 \pm 438 | >10,000 | Inactive |
| | 6 α -Naloxol | 7.0 \pm 1.3 | 3.1 \pm 1.3 | 3772 \pm 782 | >10,000 |
| | 6 β -Naloxol | 17 \pm 4 | 2.7 \pm 0.8 | 1651 \pm 336 | >10,000 |
| | Noroxyphrine | 20 \pm 3 | 82 \pm 16 | >10,000 | >10,000 |
| n.d.: Not determined | | | | | |

Results from the in vitro binding assay are presented in the table above.

- Oxycodone and its metabolite oxymorphone showed selective affinity for the mu opioid receptor. Oxymorphone demonstrated 19 times greater affinity than oxycodone at the mu opioid receptor.
- Oxycodone metabolites demonstrated lower affinity than their parent compound at the opioid receptors.

- Oxycodone, naloxone, and their metabolites had no evidence of binding activity at the ORL-1 receptor.
- Similar to the parent compound, 6 α -naloxol and 6 β -naloxol displayed affinity for the mu and kappa opioid receptors.

Table 8. In vitro functional GTP γ S activity for oxycodone and naloxone and their metabolites

| | | Functional GTP γ S Activity | | | | | | | |
|-----------|------------------------|------------------------------------|---------------------|--------------------------|------------------------|--------------------------|-----------------------|--------------------------|--------------------|
| | | Opioid Receptor Types | | | | | | | |
| | | Mu | | Kappa | | Delta | | ORL-1 | |
| | Compounds | EC ₅₀ (nM) | Efficacy % DAMGO | EC ₅₀ (nM) | Efficacy % U-69,593 | EC ₅₀ (nM) | Efficacy % Met-ENK | EC ₅₀ (nM) | Efficacy % Noci |
| Controls | Naloxone | I.A. | 0% | 21 ± 4 | 15 ± 2% | I.A. | 0% | n.d. | |
| | DAMGO | 205 ± 24 | 100% | n.d. | | n.d. | | n.d. | |
| | Nociceptin | n.d. | | n.d. | | n.d. | | 0.45 ± 006 | 100% |
| | U-69,593 | n.d. | | 24 ± 8 | 100% | n.d. | | n.d. | |
| | Met-Enkephalin | 73 ± 10 | 89 ± 1% | n.d. | | 8.2 ± 1.4 | 100% | n.d. | |
| Oxycodone | Oxycodone | 2562 ± 212 | 35 ± 6% | n.d. | | n.d. | | n.d. | |
| | Oxymorphone | 56 ± 17 | 46 ± 2% | 555 ± 88 | 51 ± 8% | n.d. | | n.d. | |
| | Noroxycodone | 7334 ± 1507 | 19 ± 3% | n.d. | | n.d. | | n.d. | |
| | 6 α -oxycodol | >10000 | 13 ± 1% | n.d. | | n.d. | | n.d. | |
| | 6 β -oxycodol | 4624 ± 102 | 29 ± 2% | n.d. | | n.d. | | n.d. | |
| Naloxone | Naloxone | I.A. | 0% | 21 ± 4 | 15 ± 2% | I.A. | 0% | n.d. | |
| | Naloxone 3-glucuronide | I.A. | 0% | n.d. | | n.d. | | n.d. | |
| | 6 α -Naloxol | I.A. | 0% | 32 ± 3 | 36 ± 2% | n.d. | | n.d. | |
| | 6 β -Naloxol | I.A. | 0% | S.A. | 9 ± 2% | n.d. | | n.d. | |
| | Noroxyphorphone | 605 ± 22 | 37 ± 2% | 1197 ± 36 | 68 ± 7% | n.d. | | n.d. | |

n.d.: Not determined
I.A.: Inactive
S.A.: Slightly active

Results from the functional binding assay are presented in the table above.

- Naloxone and its metabolites were inactive in the mu functional assay, indicating antagonist activity.
- Naloxone was inactive in the delta functional assay.
- The metabolite noroxyphorphone was a partial agonist in the mu functional assay compared to DAMGO.
- Oxycodone was a partial agonist in the mu functional assay compared to DAMGO. Oxymorphone was slightly more efficacious than the parent compound in the mu functional assay.

4.2 Secondary Pharmacology

The Applicant conducted a study in C57BL/6 RAG^{-/-} knockout mice to compare oxycodone-induced inhibition of small intestinal transit to that produced by an oxycodone/naloxone combination following repeated oral administration.

Study Title: Effect of multiple oral doses of oxycodone and oxycodone/naloxone combination on small intestinal motility in male C57BL/6 RAG1^{-/-} knockout mice (non-GLP, 2012).

Study Report №: OXN-P-078

Objective of the study: The two primary objectives of the study were: 1) to compare oxycodone-induced constipation to oxycodone/naloxone combination-induced constipation after multiple oral dosing, and 2) to evaluate specified serum, gastrointestinal tract samples, and mesenteric lymph nodes for microbiology testing.

Methods. GI transit time was evaluated in male C57BL/6 RAG1^{-/-} knockout mice (n = 8/group). C57BL/6 RAG1^{-/-} knockout mice received three times daily oral gavage administration of oxycodone alone (10 or 3 mg/kg), oxycodone in combination with naloxone (10 mg/kg oxycodone with 5 mg naloxone; or 3 mg/kg oxycodone with 0.15 mg/kg naloxone), or vehicle control, for seven days; plus one additional administration of the morning of the eighth day.

Intestinal motility was assessed in 5 out of 8 animals per group. Fifteen minutes after the eighth morning dosing, 5% activated charcoal powder in 10% arabic gum in sterile water was delivered in the stomach via gavage. The distance covered by charcoal in the small intestine and intestinal transit (%) in the oxycodone and oxycodone-naloxone combination treated mice was compared to the vehicle and oxycodone treated mice, respectively. Percent inhibition was also calculated.

Key Study Findings:

Results showed that oxycodone induced constipation as evidence by reduction in small intestinal transit rate. The co-administration of naloxone reduced oxycodone-induced constipation.

Table 9. Transit and inhibition rates in male C57bL/6RAG1 knockout mice orally administered oxycodone or oxycodone and naloxone combination.

| Nominal Dose (mg free base/kg) | | Small Intestine Length (cm) (Mean ± SE) | Charcoal Meal Front (cm) (Mean ± SE) | Transit Rate (Mean ± SE) | Inhibition Rate (%) |
|-----------------------------------|----------|--|---|--------------------------------|---------------------------|
| Oxycodone | Naloxone | | | | |
| 0 | 0 | 42.2±0.5 | 26.2±3.0 | 62.0 ±6.7 | - |
| 10 | 0 | 42.3±0.9 | 10.7±1.5 | 25.1±3.1 ^a | 59.5% ^a |
| 10 | 5 | 41.7±0.8 | 22.0±1.9 | 52.9±4.9 ^b | 14.7% ^b |
| 3 | 0 | 39.2±2.5 | 20.4±2.3 | 41.9±5.9 | 32.4% |
| 3 | 1.5 | 41.7±0.4 | 23.2±2.8 | 55.8±7.1 | 10.0% |

a: Statistically significant relative to vehicle control (2-tail Student's t-Test p < 0.01)

b: Statistically significant relative to 10 mg/kg Oxycodone alone (2-tail Student's t-Test p < 0.01)

- Oxycodone at a dose of 10 mg/kg statistically significantly inhibited small intestinal transit by approximately 60% compared to vehicle.
- The co-administration of 5 mg/kg naloxone significantly reduced oxycodone-induced inhibition of small intestinal transit.
- Co-administration of 1.5 mg/kg naloxone reduced oxycodone-induced inhibition of intestinal transit but did not reach statistical significance.

4.3 Safety Pharmacology

Neurological effects: The Applicant did not conduct formal safety pharmacology studies to evaluate potential neurological safety concerns with oxycodone or naloxone administration.

Cardiovascular effects: The cardiovascular effects of oxycodone and naloxone were characterized in one in vitro study. A summary of this study is discussed below. The Applicant did not conduct formal in vivo safety pharmacology studies to evaluate potential cardiovascular safety concerns with oxycodone or naloxone administration.

Study Title: K⁺ channel (HERG) for 29 compounds (non-GLP).

Study Report №: NDSE-585

Objective of the study: To evaluate the effects of 29 opioids, including oxycodone and naloxone, effects on the rapidly activating rectifying potassium (IKr).

Methods: The potential of oxycodone, naloxone and their metabolites to inhibit potassium current in cardiac action potential duration and QT interval was studied electrophysiologically in vitro using human embryonic kidney cell line (HEK293) that stably expressed human-ether-a-go-go-related (hERG) gene encoded potassium channel on hERG-mediated potassium current were evaluated in using a whole-cell patch-clamp technique. All test compounds were evaluated at perfusion concentrations of 250 ng/mL. The positive and negative controls were E-4031 (20 nM) and ciprofloxacin (10 µM), respectively. Each test compound was tested on at least three different cells.

Key Study Findings:

Table 10. Summary of hERG data for selected opioids and other compounds.

| (b) (4) Compound I.D. | Client Compound I.D. | INHIBITION OF TAIL CURRENT (%) MEAN | SEM | Molarity (μ M) | Potency Rankin (including bibliography re) |
|--------------------------|------------------------------|--|-----|------------------------|---|
| Test Concentration | | 250 ng/mL | | | |
| 4920-013 | tramadol HCl | 0.7 | 0.6 | 0.83 | No Effect |
| 4920-003 | hydrocodone bitartrate (BIT) | 4.6 | 1.8 | 0.42 | Low * |
| 4920-001 | oxycodone HCl | 5.5 | 4.4 | 0.71 | Low * |
| 4920-018 | morphine sulfate | 7.5 | 4.5 | 0.37 | Low * |
| 4920-004 | norhydrocodone HCl | 8.0 | 0.5 | 0.78 | Low * |
| 4920-007 | naltrexone HCl | 8.1 | 4.3 | 0.66 | Low * |
| 4920-032 | ketoconazole | 8.1 | 2.2 | 0.47 | Low * |
| 4920-021 | meperidine | 9.9 | 2.8 | 1.01 | Low * |
| 4920-016 | norbuprenorphine | 12.3 | 0.5 | 0.55 | Low * |
| 4920-019 | V11294A HCl | 13.6 | 1.2 | 0.61 | Low * |
| 4920-006 | hydromorphone HCl | 15.0 | 7.2 | 0.78 | Low * |
| 4920-022 | methadone | 17.1 | 7.8 | 0.81 | Low * |
| 4920-024 | sotalol | 17.4 | 1.1 | 0.92 | Low * |
| 4920-009 | naloxone HCl | 17.9 | 1.3 | 0.69 | Low * |
| 4920-002 | noroxycodone HCl | 21.1 | 2.3 | 0.78 | Low * |
| 4920-027 | diphenhydramine HCl | 23.9 | 2.0 | 0.98 | Low * |
| 4920-017 | bupivacaine base | 30.7 | 3.0 | 0.87 | Medium * |
| 4920-028 | pyrilamine maleate | 32.4 | 3.5 | 0.62 | Medium * |
| 4920-014 | O-desmethyltramadol | 35.3 | 3.0 | 1.00 | Medium * |
| 4920-005 | oxymorphone base | 45.0 | 7.4 | 0.83 | Medium * |
| 4920-029 | ofloxacin | 45.1 | 3.2 | 0.69 | Medium * |
| 4920-008 | 6b-naltrexol | 45.2 | 0.8 | 0.70 | Medium * |
| 4920-026 | chlorpheniramine maleate | 46.3 | 2.3 | 0.64 | Medium * |
| 4920-010 | naloxol | 49.0 | 5.0 | 0.73 | Medium * |
| 4920-011 | fentanyl citrate | 53.7 | 5.2 | 0.47 | High * |
| 4920-020 | codeine | 54.7 | 3.3 | 0.84 | High * |
| 4920-030 | astemizole | 78.4 | 1.6 | 0.54 | High * |
| 4920-031 | terfenadine | 85.0 | 3.0 | 0.53 | High * |
| 4920-025 | cisapride | 85.8 | 5.2 | 0.54 | High * |
| 4920-012 | norfentanyl | ND | ND | 0.78 | ND * |
| 4920-015 | buprenorphine | ND | ND | 0.53 | ND * |
| 4920-023 | pentazocine | ND | ND | 0.88 | ND * |

Notes:

1. Potency Ranking:

- High: inhibition is greater than 50%
 Medium: inhibition equals to or is greater than 25% but less than 50%
 Low: inhibition is less than 25%
 No effect: inhibition is less than 1%
 ND: not determined

* No published reports in relation to I_{Kr} or HERG-encoded currents.

- Under the conditions of the study, oxycodone and naloxone were a weak inhibitor of hERG-mediated potassium channel current. Results suggested that oxycodone and naloxone are not a significant direct hERG channel blocker.

Pulmonary effects:

Study Title: Dose range finding and pulmonary evaluation of orally administered oxycodone hydrochloride in rats, alone and in combination with naloxone (GLP).

Study Report №: ONU-N-015

Objective of the study: To determine the maximum tolerated dose and to characterize the respiratory effects of oxycodone alone and in combination with naloxone in rats following oral administration.

Method: For the pulmonary phase, female CD®[Crl:CD®(SD)] rats (n = 6/group) were orally administered vehicle, oxycodone alone (150 mg/kg), naloxone alone (75 mg/kg), or oxycodone/naloxone combination (150/75 and 150/12.5 mg/kg). Another six rats per test article groups were used for toxicokinetic analysis.

The effects of oral doses of the test articles on respiratory parameters using a plethysmograph chamber were investigated in conscious rats. The respiratory parameters assessed were respiratory rate, minute volume, tidal volume, peak inspiratory flow, peak expiratory flow, inspiratory times, expiratory times and enhanced pause. Respiratory parameters were measured before dosing, at 15-minutes, and periodically for up to 4 hours after drug treatment.

Key Study Findings:

Results showed that oxycodone, at a dose of 150 mg/kg, decreased respiratory rate, minute volume, peak inspiratory and expiratory flow, and increased inspiration time. Also, the results showed that naloxone co-administered with oxycodone at a ratio of 2:1 significantly attenuated respiratory effects that were observed after the administration of oxycodone alone. The results are presented in the Applicant's tables for test article effects on respiratory parameters are reproduced below.

Table 11. Summary of respiratory rate data

| Summary of Respiratory Rate Values, breaths/minute - FEMALE | | | | | | | | |
|--|----------------|------------------|---------|-----------|-----------|-----------|--------|-----------|
| Mixed Model Analysis of 0.25 through 4 Hour Time Interval Values (Segment 1) | | | | | | | | |
| Covariate = Average of 1-hour Predose, with ARH(1) Covariance Structure | | | | | | | | |
| Group | Covariate Mean | Statistics | Overall | 0.25 Hour | 0.50 Hour | 0.75 Hour | 1 Hour | 1.25 Hour |
| 0 mg/kg Vehicle | 181.08 | Mean | 134.34 | 193.24 | 224.25 | 126.91 | 112.97 | 114.84 |
| | | N | 3 | 3 | 3 | 3 | 3 | 3 |
| | | LSMean | 133.43 | 192.33 | 223.34 | 126.00 | 112.06 | 113.93 |
| | | LSM s.e. | 9.88 | 27.99 | 28.06 | 17.68 | 11.48 | 13.42 |
| 150 mg/kg Oxycodone | 142.55 | Mean | 111.52 | 149.35 | 95.65 | 95.49 | 96.31 | 104.13 |
| | | N | 6 | 6 | 6 | 6 | 6 | 6 |
| | | LSMean | 112.11 | 149.94 | 96.24 | 96.08 | 96.90 | 104.72 |
| | | LSM s.e. | 6.97 | 19.79 | 19.83 | 12.49 | 8.10 | 9.48 |
| 75 mg/kg Naloxone | 155.76 | Adjusted p-value | 0.223 | 0.329 | 0.004* | 0.426 | 0.533 | 0.872 |
| | | Mean | 134.22 | 239.32 | 116.17 | 118.23 | 112.61 | 114.51 |
| | | N | 6 | 6 | 6 | 6 | 6 | 6 |
| | | LSMean | 134.30 | 239.39 | 116.25 | 118.31 | 112.69 | 114.58 |
| 150 + 75 mg/kg Oxycodone:Naloxone | 180.18 | LSM s.e. | 6.88 | 19.76 | 19.80 | 12.45 | 8.03 | 9.42 |
| | | Adjusted p-value | 0.984 | 0.329 | 0.013* | 0.846 | 0.937 | 0.944 |
| | | Mean | 140.46 | 240.59 | 185.12 | 152.63 | 128.26 | 128.71 |
| | | N | 6 | 6 | 6 | 6 | 6 | 6 |
| 150 + 12.5 mg/kg Oxycodone:Naloxone | 140.94 | LSMean | 139.59 | 239.71 | 184.25 | 151.76 | 127.38 | 127.84 |
| | | LSM s.e. | 7.07 | 19.82 | 19.87 | 12.55 | 8.19 | 9.55 |
| | | Adjusted p-value | 0.882 | 0.329 | 0.270 | 0.460 | 0.533 | 0.706 |
| | | Mean | 130.41 | 160.01 | 124.13 | 134.51 | 119.53 | 116.72 |
| | | N | 6 | 6 | 6 | 6 | 6 | 6 |
| | | LSMean | 131.06 | 160.67 | 124.78 | 135.17 | 120.18 | 117.37 |
| | | LSM s.e. | 6.99 | 19.79 | 19.84 | 12.50 | 8.12 | 9.49 |
| | | Adjusted p-value | 0.984 | 0.333 | 0.016* | 0.846 | 0.707 | 0.937 |

Table 12. Summary of minute volume data

| Summary of Minute Volume Values, mL/minute - FE MALE | | | | | | | | |
|--|----------------|------------------|---------|-----------|-----------|-----------|--------|-----------|
| Mixed Model Analysis of 0.25 through 4 Hour Time Interval Values (Segment 1) | | | | | | | | |
| Covariate = Average of 1-hour Predose, with ARH(1) Covariance Structure | | | | | | | | |
| Group | Covariate Mean | Statistics | Overall | 0.25 Hour | 0.50 Hour | 0.75 Hour | 1 Hour | 1.25 Hour |
| 0 mg/kg Vehicle | 148.80 | Mean | 129.47 | 198.21 | 173.66 | 120.39 | 117.86 | 125.29 |
| | | N | 3 | 3 | 3 | 3 | 3 | 3 |
| | | LSMean | 126.06 | 194.81 | 170.26 | 116.98 | 114.46 | 121.88 |
| | | LSM s.e. | 10.83 | 15.67 | 15.11 | 14.61 | 15.10 | 14.47 |
| 150 mg/kg Oxycodone | 127.20 | Mean | 115.79 | 138.06 | 94.08 | 89.35 | 91.60 | 101.70 |
| | | N | 6 | 6 | 6 | 6 | 6 | 6 |
| | | LSMean | 117.42 | 139.70 | 95.72 | 90.99 | 93.23 | 103.33 |
| | | LSM s.e. | 7.43 | 10.92 | 10.52 | 10.17 | 10.52 | 10.06 |
| 75 mg/kg Naloxone | 136.86 | Adjusted p-value | 0.777 | 0.031* | 0.005* | 0.311 | 0.414 | 0.633 |
| | | Mean | 126.50 | 189.36 | 119.86 | 122.33 | 118.02 | 119.81 |
| | | N | 6 | 6 | 6 | 6 | 6 | 6 |
| | | LSMean | 125.88 | 188.74 | 119.24 | 121.71 | 117.40 | 119.19 |
| 150 + 75 mg/kg Oxycodone:Naloxone | 136.58 | LSM s.e. | 7.26 | 10.81 | 10.40 | 10.04 | 10.40 | 9.94 |
| | | Adjusted p-value | 0.999 | 0.909 | 0.027* | 0.769 | 0.862 | 0.898 |
| | | Mean | 141.14 | 194.13 | 148.31 | 156.61 | 156.15 | 149.57 |
| | | N | 6 | 6 | 6 | 6 | 6 | 6 |
| | | LSMean | 140.58 | 193.57 | 147.75 | 156.05 | 155.60 | 149.02 |
| | | LSM s.e. | 7.25 | 10.81 | 10.40 | 10.04 | 10.39 | 9.93 |
| | | Adjusted p-value | 0.626 | 0.954 | 0.206 | 0.095 | 0.095 | 0.386 |
| Group | Covariate Mean | Statistics | Overall | 0.25 Hour | 0.50 Hour | 0.75 Hour | 1 Hour | 1.25 Hour |
| 150 + 12.5 mg/kg Oxycodone:Naloxone | 128.89 | Mean | 136.61 | 160.90 | 126.94 | 126.42 | 141.44 | 135.14 |
| | | N | 6 | 6 | 6 | 6 | 6 | 6 |
| | | LSMean | 137.85 | 162.14 | 128.18 | 127.66 | 142.88 | 136.38 |
| | | LSM s.e. | 7.35 | 10.87 | 10.46 | 10.10 | 10.46 | 10.00 |
| | | Adjusted p-value | 0.699 | 0.199 | 0.058 | 0.717 | 0.279 | 0.642 |
| Group | Covariate Mean | Statistics | 3 Hour | 3.25 Hour | 3.50 Hour | 3.75 Hour | 4 Hour | |
| 0 mg/kg Vehicle | 148.80 | Mean | 116.47 | 128.89 | 131.90 | 107.46 | 133.64 | |
| | | N | 3 | 3 | 3 | 3 | 3 | |
| | | LSMean | 113.07 | 125.49 | 128.49 | 104.06 | 130.24 | |
| | | LSM s.e. | 14.93 | 15.45 | 24.47 | 22.74 | 20.41 | |
| 150 mg/kg Oxycodone | 127.20 | Mean | 123.24 | 133.33 | 132.06 | 124.37 | 140.78 | |
| | | N | 6 | 6 | 6 | 6 | 6 | |
| | | LSMean | 124.87 | 134.96 | 133.89 | 126.00 | 142.41 | |
| | | LSM s.e. | 10.39 | 10.77 | 17.21 | 15.98 | 14.32 | |
| 75 mg/kg Naloxone | 136.86 | Adjusted p-value | 0.855 | 0.891 | 0.982 | 0.424 | 0.729 | |
| | | Mean | 119.68 | 123.67 | 135.71 | 124.58 | 122.86 | |
| | | N | 6 | 6 | 6 | 6 | 6 | |
| | | LSMean | 119.06 | 123.05 | 135.09 | 123.96 | 122.24 | |
| 150 + 75 mg/kg Oxycodone:Naloxone | 136.58 | LSM s.e. | 10.27 | 10.65 | 17.13 | 15.90 | 14.23 | |
| | | Adjusted p-value | 0.855 | 0.946 | 0.982 | 0.424 | 0.729 | |
| | | Mean | 124.59 | 121.14 | 131.43 | 156.16 | 155.08 | |
| | | N | 6 | 6 | 6 | 6 | 6 | |
| | | LSMean | 124.04 | 120.59 | 130.87 | 155.60 | 154.53 | |
| | | LSM s.e. | 10.27 | 10.65 | 17.13 | 15.89 | 14.23 | |
| | | Adjusted p-value | 0.855 | 0.946 | 0.982 | 0.037* | 0.411 | |
| Group | Covariate Mean | Statistics | 3 Hour | 3.25 Hour | 3.50 Hour | 3.75 Hour | 4 Hour | |
| 150 + 12.5 mg/kg Oxycodone:Naloxone | 128.89 | Mean | 139.44 | 140.91 | 153.82 | 160.65 | 160.85 | |
| | | N | 6 | 6 | 6 | 6 | 6 | |
| | | LSMean | 140.68 | 142.15 | 155.06 | 161.89 | 162.09 | |
| | | LSM s.e. | 10.34 | 10.71 | 17.17 | 15.94 | 14.27 | |
| | | Adjusted p-value | 0.426 | 0.714 | 0.585 | 0.028* | 0.285 | |

Table 13. Summary of peak expiratory flow data

| Summary of Peak Expiratory Flow Values, mL/s - FEMALE | | | | | | | | |
|--|----------------|------------------|---------|-----------|-----------|-----------|--------|-----------|
| Mixed Model Analysis of 0.25 through 4 Hour Time Interval Values (Segment 1) | | | | | | | | |
| Covariate = Average of 1-hour Predose, with ARH(1) Covariance Structure | | | | | | | | |
| Group | Covariate Mean | Statistics | Overall | 0.25 Hour | 0.50 Hour | 0.75 Hour | 1 Hour | 1.25 Hour |
| 0 mg/kg Vehicle | 8.52 | Mean | 6.96 | 9.78 | 9.58 | 6.72 | 6.35 | 6.46 |
| | | N | 3 | 3 | 3 | 3 | 3 | 3 |
| | | LSMean | 6.88 | 9.70 | 9.50 | 6.64 | 6.28 | 6.38 |
| | | LSM s.e. | 0.53 | 1.01 | 0.93 | 0.75 | 0.63 | 0.62 |
| 150 mg/kg Oxycodone | 6.94 | Mean | 6.69 | 8.32 | 5.77 | 5.72 | 5.81 | 6.24 |
| | | N | 6 | 6 | 6 | 6 | 6 | 6 |
| | | LSMean | 6.75 | 8.38 | 5.83 | 5.78 | 5.87 | 6.30 |
| | | LSM s.e. | 0.38 | 0.72 | 0.66 | 0.53 | 0.45 | 0.44 |
| | | Adjusted p-value | 0.969 | 0.617 | 0.011* | 0.630 | 0.808 | 0.992 |
| 75 mg/kg Naloxone | 7.78 | Mean | 6.90 | 10.47 | 6.41 | 6.51 | 6.32 | 6.41 |
| | | N | 6 | 6 | 6 | 6 | 6 | 6 |
| | | LSMean | 6.89 | 10.46 | 6.40 | 6.49 | 6.31 | 6.40 |
| | | LSM s.e. | 0.36 | 0.71 | 0.65 | 0.52 | 0.44 | 0.43 |
| 150 + 75 mg/kg Oxycodone:Naloxone | 8.12 | Mean | 8.20 | 10.70 | 8.39 | 8.83 | 8.76 | 8.63 |
| | | N | 6 | 6 | 6 | 6 | 6 | 6 |
| | | LSMean | 8.15 | 10.66 | 8.35 | 8.79 | 8.71 | 8.59 |
| | | LSM s.e. | 0.37 | 0.72 | 0.66 | 0.53 | 0.44 | 0.44 |
| | | Adjusted p-value | 0.144 | 0.735 | 0.318 | 0.068 | 0.008* | 0.018* |

- Respiratory rates were statistically decreased in the 150 mg/kg oxycodone, 75 mg/kg naloxone, and the 150/1.5 oxycodone/naloxone groups at 0.5 hours post-dosing.
- Minute volume was statistically decreased in the 150 mg/kg oxycodone group at 0.25 and 0.5 hours post-dosing.
- Minute volume was statistically decreased in the 75 mg/kg naloxone group at 0.5 hours post-dosing.
- Following the oral administration of 150/12.5 mg/kg oxycodone/ naloxone, minute volume was increased up to 4 hours after dosing, reaching statistical significance at 3.75 hours post-dosing.
- Peak inspiratory flow was statistically decreased in the 150 mg/kg oxycodone group at 0.25 and 0.5 hours post-dosing, with a non-statistical trend noted through 2.25 hours post-dosing.
- Peak inspiratory flow was statistically decreased in the 75 mg/kg naloxone group at 0.5 hours post-dosing.
- Peak inspiratory flow was statistically decreased in the 150/12.5 mg/kg oxycodone/naloxone group at 0.25 and 0.5 hours post-dosing.
- Peak expiratory flow was statistically decreased in the 150 mg/kg oxycodone group at 0.5 hours post-dosing, with a non-statistical trend noted through 2.25 hours post-dosing.
- Peak expiratory flow was statistically decreased in the 75 mg/kg naloxone group at 0.5 hours post-dosing.
- Peak expiratory flow was statistically increased in the 150/75 mg/kg oxycodone/naloxone group at 1.0 and 1.25 hours post-dosing.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

5.1.1. Absorption

Study Title: Pharmacokinetics of mixture of oxycodone:naloxone at a 2:1 ratio in male and female rhesus monkey following a single oral or single intravenous administration at two different dose levels. (GLP)

Study №: OXN-P-024

Study Objective: To determine the pharmacokinetic profiles of a mixture of oxycodone:naloxone at a 2:1 ratio in male and female rhesus monkeys following oral or intravenous administration and to identify differences.

This GLP study was a nonrandomized crossover design conducted in female and male non-naive rhesus monkeys. Oxycodone:naloxone mixture was administered by oral gavage ($n = 3/\text{sex}$) and intravenously (females: $n = 2$, males: $n = 1$). The experimental design for this study is shown in the table (reproduced from Applicant's submission) below. Prior to dosing, the animals were fasted overnight. After the collection of blood at the 1-hour time point, food was re-introduced to the animals.

Table 14. Study experimental design

| Study Phase | Dose frequency | OXY/NAL Dose levels | OXY/NAL Dose concentration | Dose volume | Route | N=/sex/dose group |
|--------------------|----------------|---------------------|----------------------------|-------------|-------|---|
| First Dose | | | | | | |
| 1 | Once | 1/0.5 mg/kg | 0.2/0.1 mg/mL | 5 mL/kg | P.O. | 3 males (M1334,M1337 and M999) and 3 females (M1227, M1257 and M1341) |
| Second Dose | | | | | | |
| 2 | Once | 2/1 mg/kg | 0.4/0.2 mg/mL | 5 mL/kg | P.O. | 3 males (M1334,M1337 and M999) and 3 females (M1227, M1257 and M1341) |
| Third dose | | | | | | |
| 3 | Once | 1/0.5 mg/kg | 0.893/0.488 mg/mL | 0.5 mL/kg | I.V. | 2 females (M1257 and M1341) and 1 male (M1291) |

Following oral administration, blood samples (approximately 1 mL) were taken by venipuncture of the saphenous, femoral, or cephalic vein at predose, 0.5, 1, 2, 4, 6, 8, and 24 hours post-administration. Blood samples (approximately 1 mL) were obtained at predose, 5 min, 1 h, 2 h, 4 h, and 6 h post-dosing after intravenous administration.

Quantification of oxycodone, oxymorphone, noroxymorphone, noroxycodone, naloxone, 6 β -naloxol and naloxone-3 β -glucuronide plasma concentration was determined using a liquid chromatography with tandem mass spectrometry (LC-MS/MS) methodology. Lower limit of quantification (LLOQ) for naloxone and its metabolites was 0.040 ng/mL and for oxycodone and its metabolites was 0.40 ng/mL.

Key study findings:

| | Route | Oxycodone: naloxone Dose (mg/kg) | T _{max} (h) | C _{max} (ng/mL) | AUC _{t-last} (ng·h/mL) | F % |
|--------------------------------|-------|----------------------------------|----------------------|--------------------------|---------------------------------|---------|
| Oxycodone | Oral | 1.0:0.5 | M: 1.0 ± 0.866 | M: 33.8 ± 10.9 | M: 83.5 ± 18.7 | M: 18.7 |
| | | | F: 13.33 ± 1.15 | F: 15.4 ± 4.16 | F: 55.9 ± 4.39 | F: 8.0 |
| | Oral | 2.0:1.0 | M: 0.5 ± 0.0 | M: 92.0 ± 33.0 | M: 190.0 ± 116 | |
| | | | F: 2.33 ± 1.53 | F: 30.1 ± 1.75 | F: 112.0 ± 12.3 | |
| Oxymorphone | Oral | 1.0:0.5 | M: 1.0 ± 0.866 | M: 0.948 ± 0.593 | M: 0.648 ± 0.464 | |
| | | | F: 4.0* | F: 0.807* | F: 2.09* | |
| | Oral | 2.0:1.0 | M: 0.5 ± 0.0 | M: 2.75 ± 0.811 | M: 4.39 ± 2.68 | |
| | | | F: 4.67 ± 3.06 | F: 0.910 ± 0.262 | F: 256.0 ± 1.83 | |
| Noroxymorphone | Oral | 1.0:0.5 | M: 0.833 ± 0.289 | M: 25.8 ± 13.5 | M: 106.0 ± 93.7 | |
| | | | F: 4.67 ± 3.06 | F: 10.9 ± 5.57 | F: 85.0 ± 21.6 | |
| | Oral | 2.0:1.0 | M: 0.50 ± 0.0 | M: 88.0 ± 23.6 | M: 256.0 ± 164.0 | |
| | | | F: 1.67 ± 0.577 | F: 19.4 ± 10.3 | F: 172.0 ± 57.6 | |
| Noroxycodone | Oral | 1.0:0.5 | M: 1.0 ± 0.866 | M: 24.0 ± 6.41 | M: 256.0 ± 164.0 | |
| | | | F: 3.33 ± 1.15 | F: 10.8 ± 9.54 | F: 172.0 ± 57.6 | |
| | Oral | 2.0:1.0 | M: 0.5 ± 0.0 | M: 79.4 ± 30.4 | M: 138.0 ± 73.0 | |
| | | | F: 1.67 ± 0.577 | F: 19.4 ± 14.3 | F: 99.2 ± 96.3 | |
| Naloxone | Oral | 1.0:0.5 | M: 3.5 ± 2.78 | M: 108.0 ± 17.7 | M: 519.0 ± 144.0 | |
| | | | F: 2.33 ± 1.53 | F: 470.0 ± 606.0 | F: 1464.0 ± 1589.0 | |
| | Oral | 2.0:1.0 | M: 2.17 ± 1.76 | M: 295.0 ± 93.5 | M: 1445.0 ± 385.0 | |
| | | | F: 2.33 ± 1.53 | F: 736.0 ± 853.0 | F: 3232.0 ± 2209.0 | |
| Naloxone 3 β glucuronide | Oral | 1.0:0.5 | M: 0.50 ± 0.0 | M: 250.0 ± 135.0 | M: 809.0 ± 260.0 | |
| | | | F: 2.67 ± 1.15 | F: 142.0 ± 110.0 | F: 692.0 ± 193.0 | |
| | Oral | 2.0:1.0 | M: 0.667 ± 0.289 | M: 555.0 ± 186.0 | M: 1725.0 ± 530.0 | |
| | | | F: 1.67 ± 0.557 | F: 198.0 ± 83.0 | F: 1320.0 ± 397.0 | |

| | Route | Oxycodone: naloxone Dose (mg/kg) | T _{max} (h) | C _{max} (ng/mL) | AUC _{t-last} (ng·h/mL) | F % |
|------------|-------|-------------------------------------|----------------------|-----------------------------|------------------------------------|-----|
| 6β-naloxol | Oral | 1.0:0.5 | M: 1.67 ± 2.02 | M: 67.6 ± 23.4 | M: 247.0 ± 205.0 | |
| | | | F: 3.33 ± 1.15 | F: 294.0 ± 351.0 | F: 1018.0 ± 1125 | |
| | | 2.0:1.0 | M: 2.0 ± 1.73 | M: 149.0 ± 42.6 | M: 1469.0 ± 1299.0 | |
| | | | F: 4.67 ± 3.06 | F: 429.0 ± 421.0 | F: 2789.0 ± 1802.0 | |

*: n = 1

Pharmacokinetic data following oral administration of a oxycodone:naloxone mixture in a 2:1 ratio are presented in the above table.

- In males following oral administration, oxycodone was rapidly absorbed; C_{max} was obtained at 0.5 h after both doses of the oxycodone:naloxone mixture.
- C_{max} and AUC increased in a dose-proportional manner.
- Noroxymorphone and noroxycodone were the major metabolites of oxycodone. Metabolites to parent ratios ranged from 0.349 to 2.01 and from 0.889 to 2.22 for noroxycodone and noroxymorphone, respectively.
- Naloxone was slowly absorbed in both males and females; T_{max} ranged from approximately 2 to 3.5 hours.
- Naloxone was extensively metabolized; naloxone-3β-glucuronide was the major metabolite. Metabolite to parent ratio was greater than 200:1.

5.1.2. Distribution

The Applicant did not conduct any distribution studies with an oxycodone:naloxone mixture at a 2:1 ratio as for the drug product. A distribution study was conducted to characterize the pharmacokinetic profile of naloxone and its metabolites in blood and cerebral spinal fluid following single and multiple oral doses of naloxone to male rats.

Study Title: Determination of the pharmacokinetics of naloxone and its metabolites in plasma and CSF after multiple oral doses to rats. (GLP compliance)

Study №: OXN-P-065

Method: Male rats were dosed in accordance to the study design below. Animals in Groups 2, 3, and 4 were orally (gavage) administered naloxone hydrochloride for 5 consecutive days at dose levels of 5, 10, and 20 mg/kg/day, respectively. All animals were fasted at least 6 hours prior to dosing through approximately 4 hours post-dosing on Study Days 1 and 5.

| Group | Number of Males | Test Article | Dose Route | Target Dose Level (mg/kg) | Target Dose Concentration (mg/mL) | Target Dose Volume (mL/kg) |
|--------------------------|-----------------|--------------|------------|---------------------------|-----------------------------------|----------------------------|
| <u>Feasibility Phase</u> | | | | | | |
| 1 | 6 | Water | Oral | NA | NA | 5 |
| <u>Definitive Phase</u> | | | | | | |
| 2 | 5 | Naloxone | Oral | 5 | 1 | 5 |
| 3 | 5 | Naloxone | Oral | 10 | 2 | 5 |
| 4 | 5 | Naloxone | Oral | 20 | 4 | 5 |

NA Not applicable; animals were administered water.

For the feasibility and definitive phases, blood samples (approximately 0.4 mL) were collected from a jugular-vein cannula at predose, 0.25, 0.5, 1, 2, 4, and 8 hours post-administration on Study Days 1 and 5. Blood samples collected during the feasibility phase were discarded and not processed to plasma. Cerebral spinal fluid (approximately 15 mcL for the feasibility and 10 mcL for the definitive phase) was collected from each animal via the intracisternal cannula at predose, 0.5, 1, 2, 4, and 8 hours post-administration on Study Days 1 and 5. Cerebral spinal fluid samples collected during the feasibility phase were discarded and not processed.

All animals were observed twice daily for mortality and overt clinical signs. Cageside observations for general health and appearance were performed daily.

Quantification of naloxone, 6 β -naloxol, and naloxone-3 β -glucuronide plasma and CSF concentrations were determined using a liquid chromatography with mass spectrometry (LC-MS) methodology. Pharmacokinetic analysis of plasma and CSF included C_{max} , T_{max} , $t_{1/2}$, and AUC. Lower limit of quantification (LLOQ) for naloxone, 6 β -naloxol, and naloxone-3 β -glucuronide was 0.20, 0.40, and 0.50 ng/mL in plasma, respectively, and 0.60, 1.2, and 1.5 ng/mL for CSF, respectively.

Deviation from the protocol.

- On Study Day 5, blood samples were not collected from Animal № B26364 (Group 1) at 1, 2, 4 and 8 hours post-dosing.
- Blood sample at 0.25 hours post-dosing collected from Animal № B26373 was collected one minute outside of the acceptable time range.
- On Study Day 5, 13 mcL of CSF was collected from animal № B26372 (Group 4) on Study Day 5.

Key Study Findings:

Study results were consistent with results in the published literature; naloxone rapidly diffuses across the blood brain barrier. Its metabolite naloxone-3 β -glucuronide also readily crossed the blood brain barrier.

Plasma pharmacokinetic analysis for naloxone and its metabolite naloxone-3 β -glucuronide is presented in the table below.

Table 15. Summary of mean pharmacokinetic metrics of naloxone and naloxone-3 β -glucuronide in rat plasma.

| | | Naloxone Dose (mg/kg) | | |
|---|------------------------------|---------------------------------------|---|--------------------------------------|
| | | 5 | 10 | 20 |
| | | No of animals → | 5 | 5 |
| Plasma: Naloxone | C _{max} (ng/mL) | D1: 2.67 ± 0.909 D5: 2.88 ± 0.381 | D1: 6.23 ± 2.866 D5: 6.22 ± 3.609 | D1: 11.1 ± 2.73 D5: 11.4 ± 3.42 |
| | T _{max} | D1: 0.3 ± 0.0 D5: 0.3 ± 0.0 | D1: 0.3 ± 0.0 D5: 0.3 ± 0.0 | D1: 0.3 ± 0.11 D5: 0.3 ± 0.11 |
| | AUC ₀₋₈ (ng·h/mL) | D1: 3.21 ± 0.680 D5: 5.41 ± 1.669 | D1: 6.79 ± 2.0 D5: 12.8 ± 4.833 | D1: 14.1 ± 3.678 D5: 22.6 ± 4.15 |
| | t _{1/2} (h) | D1: 0.7 ± 0.10 D5: 1.2 ± NA | D1: 0.8 ± 0.29 D5: NA | D1: 0.7 ± 0.05 D5: NA |
| | C _{max} (ng/mL) | D1: 499.0 ± 155.2 D5: 1130 ± 220.6 | D1: 878.0 ± 473.8 D5: 1880.0 ± 613.0 | D1: 993 ± 370.4 D5: 2310 ± 719.9 |
| | T _{max} | D1: 0.3 ± 0.0 D5: 0.3 ± 0.0 | D1: 0.3 ± 0.11 D5: 0.3 ± 0.11 | D1: 0.4 ± 0.14 D5: 0.3 ± 0.0 |
| Plasma: Naloxone-3 β -glucuronide | AUC ₀₋₈ (ng·h/mL) | D1: 377.0 ± 74.5 D5: 1010 ± 341.2 | D1: 681.0 ± 322.1 D5: 2050.0 ± 551.0 | D1: 1090 ± 276.2 D5: 2960 ± 943.6 |
| | t _{1/2} (h) | D1: 1.7 ± 0.64 D5: NA | D1: 1.0** ± 0.08 D5: NA | D1: 1.3 ± 0.29 D5: 2.3** ± NA |
| | M/P AUC ₀₋₈ ratio | D1: 119.0 ± 20.9 D5: 187 ± 26.2 | D1: 109.0 ± 61.6 D5: 169 ± 53.9 | D1: 6.79 ± 1.887 D5: 135.0 ± 55.0 |
| | NA: Not Applicable | | | |
| | **: n = 2 | | | |

- Naloxone plasma level increased in a dose-dependent manner.
- Naloxone was readily absorbed following oral administration; mean T_{max} was 0.3 at all dose levels on Days 1 and 5.
- Increase in C_{max} and AUC for naloxone was generally dose proportional.
- No accumulation of naloxone occurred after multiple dosing.
- Exposure to naloxone-3 β -glucuronide in plasma increased with the increase in naloxone dose levels.
- Naloxone-3 β -glucuronide readily appeared in plasma; mean T_{max} ranged from 0.3 to 0.4 hours on Day 1 and was 0.3 hours on Day 5 for all doses.
- Accumulation of naloxone-3 β -glucuronide occurred after repeated dosing.
- Naloxone was extensively metabolized to naloxone-3 β -glucuronide following oral administration; the AUC₀₋₈ metabolite to parent ratios ranged from 135 to 187 on Day 5.
- Increases in C_{max} and AUC₀₋₈ were generally less than dose proportional.

Plasma pharmacokinetic analysis for 6 β -naloxol is presented in the Applicant's table below.

Table 16. Summary of mean pharmacokinetic metrics of 6 β -naloxol in rat plasma.

| Interval | Metric | 10 mg/kg/day | | | 20 mg/kg/day | | |
|----------|--|--------------|-------|----|--------------|--------|---------|
| | | N | Mean | SD | N | Mean | SD |
| Day 1 | C _{max} (ng/mL) | 1 | 0.469 | NA | 5 | 0.672 | 0.1737 |
| | DN C _{max} [(ng/mL)/(mg/kg/day)] | 1 | 0.047 | NA | 5 | 0.034 | 0.0087 |
| | T _{max} (h) | 1 | 0.3 | NA | 5 | 0.3 | 0 |
| | AUC _{0-t} (ng·h/mL) | 0 | NA | NA | 1 | 0.61 | NA |
| | AUC ₀₋₈ (ng·h/mL) | 0 | NA | NA | 1 | 0.82 | NA |
| | DN AUC ₀₋₈ [(ng·h/mL)/(mg/kg/day)] | NA | NA | NA | 1 | 0.041 | NA |
| | AUC _{0-∞} (ng·h/mL) | 0 | NA | NA | 0 | NA | NA |
| | t _{1/2} (h) | 0 | NA | NA | 0 | NA | NA |
| | M/P Ratio (AUC ₀₋₈) | NA | NA | NA | NA | NA | NA |
| | | | | | | | |
| Day 5 | C _{max} (ng/mL) | 2 | 0.772 | NA | 5 | 1.17 | 0.518 |
| | DN C _{max} [(ng/mL)/(mg/kg/day)] | 2 | 0.077 | NA | 5 | 0.059 | 0.0259 |
| | T _{max} (h) | 2 | 0.3 | NA | 5 | 0.3 | 0.11 |
| | AUC _{0-t} (ng·h/mL) | 0 | NA | NA | 3 | 1.20 | 0.347 |
| | AUC ₀₋₈ (ng·h/mL) | 0 | NA | NA | 3 | 1.79 | 0.860 |
| | DN AUC ₀₋₈ [(ng·h/mL)/(mg/kg/day)] | NA | NA | NA | 3 | 0.090 | 0.0430 |
| | t _{1/2} (h) | 0 | NA | NA | 0 | NA | NA |
| | M/P Ratio (AUC ₀₋₈) | NA | NA | NA | 3 | 0.0707 | 0.03310 |
| | | | | | | | |

NA: Not applicable.

Note: No PK parameters were calculated for Group 2 (5 mg/kg/day), due to the lack of quantifiable concentrations.

- Due to limited data above the limit of quantification for 6 β -naloxol, pharmacokinetic analysis was limited. However, exposure to 6 β -naloxol in plasma increased with increasing doses of naloxone.
- Naloxone was not extensively metabolized to 6 β -naloxol; AUC₀₋₈ metabolite to parent ratios was 0.0707 following the oral administration of 20 mg/kg of naloxone on Day 5.

CSF pharmacokinetic analysis for naloxone and its metabolite naloxone-3 β -glucuronide is presented in the table below.

Table 17. Summary of mean pharmacokinetic metrics of naloxone and naloxone-3 β -glucuronide in rat CSF.

| | | Naloxone Dose (mg/kg) | | |
|------------------|---------------------------------|-------------------------------------|--------------------------------------|--------------------------------------|
| | | 5 | 10 | 20 |
| No of animals→ | 5 | 5 | 5 | 5 |
| | C _{max} (ng/mL) | D1: 2.40 ± 3.018 D5: 1.2 ± 0.224 | D1: 2.58 ± 0.886 D5: 2.85 ± 2.337 | D1: 5.51 ± 2.292 D5: 4.34 ± 1.825 |
| T _{max} | D1: 0.4 ± 0.22 D5: 0.5 ± 0.0 | D1: 0.6 ± 0.22 D5: 0.5 ± 0.0 | D1: 0.5 ± 0.0 D5: 0.5 ± 0.0 | |

| | | | | |
|-------------------------------------|---|-------------------|------------------|------------------|
| CSF: Naloxone | AUC₀₋₈ (ng·h/mL) | D1: NA | D1: 4.85 ± NA | D1: 6.73 ± 2.059 |
| | | D5: NA | D5: 6.93 ± 2.782 | D5: 10.9 ± 3.89 |
| | t_{1/2} (h) | D1: NA | D1: NA | D1: NA |
| | | D5: NA | D5: NA | D5: 3.5 ± NA |
| | CSF/Plasma C_{max} (%) | D1: 120.0 ± 188.2 | D1: 44.0 ± 13.2 | D1: 49.0 ± 15.7 |
| | | D5: 42.0 ± 5.3 | D5: 67.0 ± NA | D5: 38.0 ± 10.0 |
| CSF: Naloxone-3β-glucuronide | CSF/Plasma AUC₀₋₈ (%) | D1: NA | D1: 42.0 ± 8.8 | D1: 47.5 ± 10.5 |
| | | D5: NA | D5: 49.0 ± 1.2 | D5: 47.0 ± 10.2 |
| | C_{max} (ng/mL) | D1: 7.82 ± 2.698 | D1: 21.0 ± 11.06 | D1: 22.8 ± 8.88 |
| | | D5: 14.2 ± 1.57 | D5: 24.6 ± 7.66 | D5: 28.0 ± 7.37 |
| | T_{max} | D1: 0.6 ± 0.22 | D1: 1.0 ± 0.61 | D1: 1.0 ± 0.61 |
| | | D5: 0.6 ± 0.22 | D5: 0.7 ± 0.27 | D5: 0.7 ± 0.27 |
| CSF: Naloxone-3β-glucuronide | AUC₀₋₈ (ng·h/mL) | D1: 21.4 ± 8.03 | D1: 31.2 ± 10.27 | D1: 43.3 ± 7.43 |
| | | D5: 28.2 ± 9.56 | D5: 54.6 ± 19.6 | D5: 74.5 ± 22.15 |
| | t_{1/2} (h) | D1: NA | D1: 0.9 ± NA | D1: NA |
| | | D5: 3.0 ± NA | D5: 3.2 ± NA | D5: 2.8 ± NA |
| | M/P AUC₀₋₈ ratio | D1: NA | D1: 8.17 ± NA | D1: 6.79 ± 1.887 |
| | | D5: NA | D5: 10.1 ± 5.16 | D5: 7.64 ± 4.338 |
| CSF: Naloxone-3β-glucuronide | CSF/Plasma C_{max} (%) | D1: 1.6 ± 0.43 | D1: 4.7 ± 6.83 | D1: 2.3 ± 0.47 |
| | | D5: 1.3 ± 0.29 | D5: 1.3 ± 0.22 | D5: 1.2 ± 0.17 |
| | CSF/Plasma AUC₀₋₈ (%) | D1: 5.8 ± 2.65 | D1: 6.0 ± 4.34 | D1: 4.1 ± 0.97 |
| | | D5: 2.9 ± 1.17 | D5: 2.7 ± 0.5 | D5: 2.6 ± 0.42 |

- Naloxone and naloxone-3β-glucuronide highly crossed the blood brain barrier and readily appeared in the CSF following oral administration of naloxone.
- In the CSF, exposure to naloxone and naloxone-3β-glucuronide increased with increasing doses of naloxone.
- Increases in mean C_{max} and AUC₀₋₈ for naloxone and naloxone-3β-glucuronide were generally less than dose proportional
- CSF levels of naloxone were approximately 47 to 67% of those observed in the plasma.
- CSF levels of naloxone-3β-glucuronide were approximately 1.2 to 4.7% of those observed in plasma.
- CSF levels of 6β-naloxol were below the lower limit of quantification (1.2 ng/mL) following single and multiple doses of naloxone.

5.1.3. Metabolism

Study Title: In vitro stability of oxycodone and oxycodone N-oxide in human plasma and human liver S9 fractions (non-GLP).

Study №: № OXY-P-027

Study Objective. To evaluate the stability of oxycodone and its N-oxide in human plasma and human liver S9 fraction; and evaluate the interconversion between oxycodone and its N-oxide in human plasma and human liver S9 fraction.

Method: Oxycodone or oxycodone N-oxide, at a nominal concentration of 10 mcM, were incubated in human liver S9 fractions (contained drug-metabolizing enzymes including cytochromes P450, flavin monooxygenases, and UDP glucuronyl transferase) and human plasma collected from mixed gender human subjects (n = 6). Human liver S9 fractions and plasma were incubated for up to 2 hours at 37°C. Incubation media were analyzed for oxycodone and oxycodone N-oxide levels with LC/MS methodology. The lower limit for quantification of oxycodone and oxycodone N-oxide was 0.2 mcM.

Key Study Findings:

- Oxycodone did not show conversion to oxycodone N-oxide in human liver S9 fractions in the presence of NADPH or NADH.

Table 18. Summary of concentration (mCM) of oxycodone and its conversion product oxycodone N-oxide in incubations with human liver S9 fractions

| Incubation Conditions | Time-point (hr) | Test Article | Conversion Product |
|-----------------------|-----------------|------------------------|--------------------|
| | | Oxycodone ^a | Oxycodone N-Oxide |
| NADPH | 0 | 10.0 | ND |
| | 0.5 | 8.28 | ND |
| | 1 | 8.43 | ND |
| | 2 | 7.09 | ND |
| NADH | 0 | 10.0 | ND |
| | 0.5 | 9.14 | ND |
| | 1 | 8.56 | ND |
| | 2 | 8.43 | ND |

ND = Not detectable (<0.02 µM). LOQ = 6.62 ng/mL

^aThe concentration of oxycodone in the incubation was calculated using the oxycodone concentration at the 0-hr incubation (10 µM).

- Oxycodone N-oxide showed spontaneous conversion to oxycodone in human liver S9 fractions in the presence of NADPH or NADH. Within 2 hours, oxycodone N-oxide had completely converted to oxycodone (98%).

Table 19. Summary of concentration (mCM) of oxycodone N-oxide and its conversion product oxycodone in incubations with human liver S9 fractions

| Incubation Conditions | Time-point (hr) | Test Article | Conversion Product |
|-----------------------|-----------------|--------------------------------|------------------------|
| | | Oxycodone N-Oxide ^a | Oxycodone ^b |
| NADPH | 0 | 4.16 | 5.84 |
| | 0.5 | 0.80 | 8.78 |
| | 1 | 0.61 | 7.85 |
| | 2 | 0.24 | 7.62 |
| NADH | 0 | 2.50 | 7.50 |
| | 0.5 | 0.27 | 10.5 |
| | 1 | 0.41 | 9.72 |
| | 2 | 0.30 | 8.78 |

^aThe concentration of oxycodone N-oxide in the 0-hr sample was calculated by subtracting the oxycodone concentration in the 0-hr sample from the target concentration (10 µM) assuming that oxycodone is the only conversion product. The concentration in the incubated samples were calculated using the oxycodone N-oxide concentration at 0-hr incubation (4.16 µM for NADPH or 2.50 µM for NADH).^bThe concentration of oxycodone (appearance) in the incubations was calculated using the oxycodone standard curves. Separate curves were prepared for NADPH and NADH incubation conditions.

- Oxycodone N-oxide was not detected in the oxycodone incubations in human plasma. This finding may be due to oxycodone N-oxide being metabolically unstable and its metabolic transition back to oxycodone. Oxycodone N-oxide showed a spontaneous conversion to oxycodone in human plasma,

Table 20. Interconversion of oxycodone N-oxide and oxycodone in human plasma

| Test Article | Conversion Product | Test Article | Conversion Product |
|-----------------|-----------------------------|-------------------------------------|-------------------------------------|
| Time-point (hr) | Oxycodone (μM) | Oxycodone N-Oxide (μM) | Oxycodone N-Oxide (μM) |
| 0 | 10 | ND | 8.31 |
| 0.5 | 9.75 | ND | 8.53 |
| 1 | 9.73 | ND | 8.90 |
| 2 | 10.2 | ND | 7.97 |

5.1.4. Excretion

The Applicant did not conduct formal excretion studies to with oxycodone:naloxone combinations. The Applicant submitted three study reports from the published literature. The primary findings of these studies indicated that oxycodone and naloxone and their respective metabolites were primarily excreted in the urine (Ishida, et al., 1982).

5.1.5. Pharmacokinetic drug interactions

Study Title: In vitro evaluation of possible metabolic interactions between oxycodone, naltrexone, naloxone, and acetaminophen in cryopreserved human hepatocytes (non-GLP, 2005).

Study №: OXN-P-011

Study Objective. To evaluate the potential metabolic interactions in cryopreserved human hepatocytes between oxycodone and naloxone administered alone or in combination with drugs such as naltrexone, acetaminophen (APAP), and aspirin (ASA) that are likely to be co-administered in cryopreserved.

Method. Test articles were incubated at 0, 0.1, 1.0, 100, 500, and 1000 mcM with pooled cryopreserved human hepatocytes for 15 to 60 min at 37°C. The reactions were terminated with the addition of 2% formic acid in acetonitrile and then the samples were centrifuged. The clear supernatants were analyzed by LC-MS/MS for noroxycodone, oxymorphone, 6 β -naloxol, naloxone-3 β -glucuronide, 6 β -naltrexol, naltrexone-3 β -glucuronide, and acetaminophen (APAP) glucuronide. “Interactions with oxycodone were evaluated by co-incubating oxycodone (15 mcM) with naloxone, naltrexone, or

APAP (0.1 to 1000 mcM). Interactions with naloxone were evaluated by co-incubating naloxone (10 M) with naltrexone or APAP (0.1 to 1000 mcM). Interactions with the oxycodone:naloxone combination were evaluated by co-incubating the combination at 15/7.5, 30/15, and 60/30 mcM with naltrexone, APAP or ASA (0.1 to 1000 mcM)."

Key Study Findings:

Based on the results presented below, the study suggested that at therapeutic concentrations that the likelihood for clinical drug-drug interactions of naltrexone, acetaminophen, or acetylsalicylic acid with oxycodone and naloxone may be minimal.

Table 21. Effects of naltrexone, acetaminophen on oxycodone and naloxone metabolism in cryopreserved human hepatocytes.

| Inhibitor | Naloxone | | Oxycodone | |
|---------------|--|---|--|--|
| | Ketoreduction IC ₅₀ (μM) | 3β-Glucuronidation IC ₅₀ (μM) | N-Demethylation IC ₅₀ (μM) | O-Demethylation IC ₅₀ (μM) |
| Naltrexone | 14.7 ± 1.1 | 327 ± 65 | > 1000 | > 1000 ^a |
| Acetaminophen | 556 ± 150 | > 1000 | > 1000 | > 1000 |
| Naloxone | NA | NA | 660 ± 230 | 9.98 ± 2.22 |

^a Activation observed at 500 and 1000 μM

- Results from the table above indicated that naloxone was a weak inhibitor of oxycodone (at 15 mcM) metabolism.
 - IC₅₀ values for noroxycodone and oxymorphone formation was 660 ± 230 mcM, and 9.98 ± 2.2 mcM, respectively.
- Results from the table above indicated that naltrexone and APAP were weak inhibitors of naloxone (at 10 mcM) metabolism.
 - Naltrexone inhibited 6β-naloxol (keto reduction) formation and naloxone-3β-glucuronidation.
 - APAP inhibited the formation of 6β-naloxol from naloxone and did not significantly inhibit the formation of naloxone-3β-glucuronidation from naloxone.
 - Naltrexone and APAP were poor inhibitors of oxycodone; they did not significantly inhibit the metabolism of oxycodone.

Table 22. Effects of naltrexone, acetaminophen, and acetylsalicylic acid on the metabolism of the combination of oxycodone and naloxone.

| OXY:NLX ^a | Inhibitor | Naloxone | | Oxycodone | |
|----------------------|---------------|--|---|--|--|
| | | Ketoreduction IC ₅₀ (μM) | 3β-Glucuronidation IC ₅₀ (μM) | N-Demethylation IC ₅₀ (μM) | O-Demethylation IC ₅₀ (μM) |
| 15 μM: 7.5 μM | Naltrexone | 12.1 ± 2.26 | 283 ± 102 | >1000 | > 1000 ^b |
| | Acetaminophen | 936 ± 392 | > 1000 | >1000 | > 1000 |
| | ASA | 158 ± 28 | > 1000 ^b | >1000 | > 1000 |
| 30 μM: 15 μM | Naltrexone | 21.2 ± 8.1 | 517 ± 65 | >1000 | > 1000 ^b |
| | Acetaminophen | 442 ± 313 | > 1000 | >1000 | > 1000 |
| | ASA | 101 ± 21 | > 1000 ^b | >1000 | > 1000 |
| 60 μM: 30 μM | Naltrexone | 13.9 ± 8.8 | 499 ± 189 | >1000 | > 1000 ^b |
| | Acetaminophen | 396 ± 184 | >1000 | >1000 | > 1000 |
| | ASA | 66.8 ± 11 | > 1000 ^b | >1000 | > 1000 |

^a Concentration ratio of oxycodone (OXY) to naloxone (NLX)^b Activation observed at 500 and 1000 μM

- At all three combinations of oxycodone and naloxone, a moderate inhibition of 6β-naloxol was observed in the presence of naltrexone.
 - The IC₅₀ values ranging from 12.1 to 21.21 μM.
 - The IC₅₀ values for naloxone-β3-glucuronide formation ranged from 283 to 517 μM.
- Acetaminophen and acetylsalicylic acid were weak inhibitors of 6β-naloxol formation.

Study Title: In vitro evaluation of oxycodone, naloxone, and their combinations as inhibitors of human cytochrome P450 and cytosolic enzymes (non-GLP).

Study №: OXUDR02-074

Study Objectives. The objective of the study was “to evaluate the metabolism-dependent inhibition of oxycodone and naloxone. Also to determine the drug-drug interaction potential of oxycodone, naloxone, and their combinations with drugs known to be metabolized by major human CYP isoforms.”

Method. The potential effects of oxycodone on naloxone metabolism were investigated in pooled human hepatic microsomes that were monitored for various CYP marker activities. Human hepatic cytosolic fraction (1 mg/mL) was incubated with oxycodone (at a final concentration of 0.0 (methanol), 0.014, 0.028, 0.07, 0.14, 0.28, 0.42, 0.7, and 11.4 μM) followed by the addition of naloxone (at a final concentration of 125.0, 250.0, and 500.0 μM).

The potential effects of naloxone on oxycodone metabolism were also investigated in pooled human hepatic microsomes that were monitored for various CYP marker activities. Human hepatic cytosolic fraction (1 mg/mL) was incubated with naloxone (at a final concentration of 0.0 (methanol), 0.27, 1.35, 2.7, 13.5, 27.0, 54.0, and 81.0 nM) followed by the addition of oxycodone (at a final concentration of 50.0, 100.0, and 200.0 μM).

For the oxycodone and naloxone studies, “the reaction mixture also contained 50 mM potassium phosphate buffer (pH 7.4), MgCl₂ (5 mM), EDTA (1 mM), and NADPH (0.2 mM), as final concentration in a total volume of 0.2 mL. The reaction was initiated by the addition of NADPH and the incubations were carried out in duplicate at 37°C for 0 and 60 minutes. Incubation of liver cytosol with naloxone in the absence of NADPH for 0 and 60 minutes served as a negative control.”

Effects of Oxycodone, Naloxone, and their Combinations on Human

Cytochrome P450 (CYP) Marker Activities. The effects of oxycodone, naloxone, and their combinations on various CYP marker activities were evaluated at the following concentrations:

Oxycodone: 0, 0.014, 0.028, 0.07, 0.14, 0.28, 0.42, 0.7, and 1.4 mcM

Naloxone: 0, 0.27, 1.35, 2.7, 13.5, 27, 54, and 81 nM

Oxycodone:Naloxone Combination (mcM:nM): 0.014:0.27, 0.14:13.5, and 1.4:81.0

The potential of effects of oxycodone, naloxone, and their combinations to interact with the cytochrome P450 isoforms CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 were investigated using specific probe substrates for the CYP450 isoforms from pooled human hepatic microsomes. The following probe substrates were used in this in vitro study: CYP1A2-dependent 7-ethoxyresorufin O-dealkylase (10 mcM, EROD), CYP2A6-dependent coumarin hydroxylase (10 mcM), CYP2C9-dependent tolbutamide hydroxylase (150 mcM), CYP2C19-dependent S-mephenytoin 4'-hydroxylase (100 mcM), CYP2D6-dependent bufuralol 1'-hydroxylase (50 mcM), CYP2E1-dependent chlorzoxazone hydroxylase (250 mcM), and CYP3A4-dependent testosterone 6β-hydroxylase (200 mcM) activities.

“The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.4), MgCl₂ (5 mM), EDTA (1 mM), and NADPH (0.2 mM), as final concentration in a total volume of 200 mcL. The reaction was initiated by the addition of NADPH (1mM, final concentration).” Incubations were carried out in duplicate at 37°C for 45 minutes. The reaction was stopped by the addition of 4 mL dichloromethane. [REDACTED] (b) (4)

Key Study Findings:

- As depicted in the table below, there were no metabolic interactions between oxycodone and naloxone under the conditions of the study.
 - Oxycodone displayed no inhibition on the rate of formation of 6 β -naloxone over a concentration range up to 500 ng/mL.
 - Naloxone in the concentration range up to 200 nM did not alter the oxidative metabolism of oxycodone under the condition of the study; the formation of noroxycodone and oxymorphone was not altered.

Table 23. IC₅₀ values for naloxone effects on oxycodone N- and O-demethylation and oxycodone effects on naloxone keto-reduction.

| Inhibitor | Naloxone keto-reduction | Oxycodone N-demethylation | Oxycodone O-demethylation |
|-----------|------------------------------|------------------------------|------------------------------|
| Naloxone | | >81(nM) ^a | >81(nM) ^a |
| Oxycodone | >1.4 (μ M) ^b | | |

^a No inhibition with 50, 100 and 200 μ M oxycodone

^b No inhibition with 125, 250 and 500 μ M naloxone

- As depicted in the table below, at therapeutic concentrations, oxycodone, naloxone and oxycodone:naloxone combinations did not inhibit CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4. Thus suggesting that one would not expect a potential clinical drug-drug interaction to occur with known co-administered drugs metabolized by these CYP isoforms.

Table 24. Effects of oxycodone, naloxone and oxycodone:naloxone on CYP marker activities.

| CYP-Isoforms | Marker Activity | Oxycodone (0-1.4 μ M) | Naloxone (0-81 nM) | Oxycodone:Naloxone | | |
|--------------|-------------------------------------|------------------------------|-----------------------|--------------------|-----|------|
| | | | | Low | Mid | High |
| CYP1A2 | 7-EthoxresorufinO-dealkylase | NI | NI | NI | NI | NI |
| CYP2A6 | Coumarin 7-hydroxylase | NI | NI | NI | NI | NI |
| CYP2C9 | Tolbutamide hydroxylase | NI | NI | NI | NI | NI |
| CYP2C19 | S-Mephenytoin 4'-hydroxylase | NI | NI | NI | NI | NI |
| CYP2D6 | Bufluralol 1'-hydroxylase | NI | NI | NI | NI | NI |
| CYP2E1 | Chlorzoxazone 6-hydroxylase | NI | NI | NI | NI | NI |
| CYP3A4 | Testosterone 6 β -hydroxylase | NI | NI | NI | NI | NI |

NI - No Inhibition

5.1.6. Other Pharmacokinetic Studies

None

5.2 Toxicokinetics

Toxicokinetic data are included in the toxicity studies.

6 General Toxicology

6.1 Single-Dose Toxicity

Single-dose toxicology studies were conducted in rats (OXN-N-001) and dogs (OXN-N-002) following oral administration. These studies were not considered pivotal in the safety assessment of the oxycodone/naloxone combination product at a 2:1 ratio. A brief summary of these studies are presented in the table below.

| Study № | Species/strain Number/group Route of administration Dose volume/Excipient | Oxycodone/Naloxone Dose (mg/kg/mg/kg) Oxycodone (mg/kg) | Endpoints | Key Findings |
|-----------|--|--|---|--|
| OXN-N-001 | Rats/ <i>Rattus norvegicus</i> Phase 1 (Single dose): 12/sex/group Phase 3 (7 consecutive days): 9/sex/group Oral gavage 10 mL/kg | Phase 1: Oxycodone/Naloxone - 25/12.5, 40/20, 100/50, 150/75, and 200/100 mg/kg/day Oxycodone - 25 and 100 mg/kg/day Phase 2: Oxycodone/Naloxone - 50/25, 100/50 and 200/100 mg/kg/day | Mortality, clinical observations, body weight, food consumption, gross and histopathology, toxicokinetic analysis | <u>Clinical Observations:</u> ↓activity, protruding eyeballs, ↑ muscle tone and/or self-biting (at ≥ 70/35 mg/kg), stereotypic behavior. Incidence and duration increased in a dose-dependent manner. <u>Mortality:</u> None <u>Body weight:</u> No effects <u>Food consumption:</u> No effects <u>Gross Pathology:</u> No effects <u>Histopathology:</u> No effects <u>MTD:</u> ≥ 200/100 mg/kg |
| OXN-N-002 | Dogs/Beagles 1/sex/group Oral gavage | 8/4, 10/5, 16/8, 20/10, 24/12, 28/14, 32/16, 36/18, and 40/20 8 and 10 | Mortality, clinical observations, body weight, food consumption, gross and histopathology, | <u>Clinical Observations:</u> excessive salivation and hypoactivity Clinical signs were |

| Study № | Species/strain Number/group Route of administration Dose volume/Excipient | Oxycodone/Naloxone Dose (mg/kg/mg/kg) Oxycodone (mg/kg) | Endpoints | Key Findings |
|---------|--|---|------------------------|--|
| | | | toxicokinetic analysis | <p>more prolonged in the oxycodone/naloxone group compared to the oxycodone only group.</p> <p>Mortality: None</p> <p>Food consumption: On day of dosing, food consumption in oxycodone/naloxone or oxycodone treatment groups showed a markedly drop in food consumption. Decrease in food consumption was resolved within 24 to 48 hours.</p> <p>MTD: ≥ 40/20 mg/kg</p> |

6.2 Repeat-Dose Toxicity

Study title: 52 Week dietary study in the rat

Study no.: KPC 24-87
 Study report location: EDR
 Conducting laboratory and location: (b) (4)

Date of study initiation: September 11, 1985
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: Naloxone chlorhydrate, Lot № 5 ARE 807 and Lot № 6 ARE 802, Purity: not indicated in report

Key Study Findings

Naloxone hydrochloride (0, 25, 75, and 225 mg/kg/day) was orally administered via the diet to rats for 52 weeks with the following results:

| Key Study Findings | |
|---------------------------|---|
| Mortality/Survival | Five unscheduled deaths occurred. One control male, one LD animal, two MD animals, and one HD animal. The deaths were not attributed to the naloxone treatment. |
| Clinical Signs | No treatment-related clinical signs were noted by the Applicant. |
| Body Weight | A dose-dependent reduction in body weight gain was observed at the end of the treatment period in both sexes. Over the 52-week dosing period, a reduction of approximately 10%, 20% and 40% was observed following the LD, MD, and HD of naloxone. |
| Hematology | No remarkable changes in hematology parameters during Weeks 5, 13, 25, and 51. |
| Histopathology | No treatment-related findings. |
| Summary | The NOAEL was identified at < 25 mg/kg/day (in agreement with the Applicant) based on the drug-related reduction in body weight following the oral administration of naloxone at all doses of naloxone. Toxicokinetic data were not obtained in this study. |

Methods

Doses: 0, 25, 75 and 225 mg/kg/day
 Frequency of dosing: Continuously for 52 weeks
 Route of administration: Dietary admixture
 Dose volume: N/A
 Formulation/Vehicle: Drug was mixed with commercially available rodent diet, S.D.S. R and M No. 1. Vehicle was described as powdered rodent diet
 Species/Strain: Rats/Crl:CD(SD)BR
 Number/Sex/Group:

| | | Number of animals and gender | |
|--------------|-------------------------|-------------------------------------|------------------------|
| Group | Dose (mg/kg/day) | Main Toxicity Groups | Satellite Group |
| 1 | 0 (vehicle control) | 25 M and 25 F | 10 M and 10 F |
| 2 | 25 | 25 M and 25 F | 10 M and 10 F |
| 3 | 75 | 25 M and 25 F | 10 M and 10 F |
| 4 | 225 | 25 M and 25 F | 10 M and 10 F |

Age: 3 to 4 weeks of age (on day of arrival)
 Weight: At start of treatment
Males (range): 116 g to 178 g
Females (range): 107 g to 156 g
 Satellite groups: Satellite group were included for clinical pathology examination.

 Clinical pathology examinations were performed on the satellite group. Clinical pathology examinations were performed during Weeks 5, 13, and 25 in 10 animals/sex/group. During Week 52, clinical pathology examinations were performed on 3 animals/sex/group. Blood samples were collected around a mean time of 1000 hours.
 Unique study design: None
 Deviation from study protocol: Deviations are described under the appropriate observation(s).

Observations and Results

Toxicokinetics

Not conducted.

Mortality

All animals were observed daily for morbidity and mortality throughout the duration of the study.

Table 25. Summary of unscheduled deaths

| Sex/Animal No | Group/Dose | Week of unscheduled death & clinical symptoms | Microscopic Finding(s) | Conclusion |
|------------------|-------------------|---|--|--|
| M/3 | Toxicity/ 0 mg/kg | Week 51: died at blood sampling; no clinical signs observed | Microscopic Findings: findings in the kidneys, thymus, lungs, pancreas, liver, adrenals, mammary glands and brain were minimal; reviewer concluded that they were unremarkable and not considered to be toxicologically significant | Reviewer concluded that the death was not related to naloxone. |
| M/47 | Toxicity/25 | Week 46: Found | Not performed | Reviewer |

| Sex/Animal No | Group/Dose | Week of unscheduled death & clinical symptoms | Microscopic Finding(s) | Conclusion |
|--------------------------|--------------------|--|---|--|
| | mg/kg | dead.; no clinical signs observed | | concluded that the death was not related to naloxone. |
| M/53 | Toxicity/75 mg/kg | Week 52: euthanized in extremis; no clinical signs observed | Not performed | Reviewer concluded that the death was not related to naloxone. |
| F/135 | Toxicity/25 mg/kg | Week 45: euthanized in extremis; no clinical signs observed | | Reviewer concluded that the death was not likely related to naloxone due to the lack of a clear dose-dependency. |
| F/183 | Toxicity/225 mg/kg | Week 30: euthanized in extremis; no clinical signs observed | Microscopic Findings: findings in the lungs, spleen, liver, adrenals, mammary glands, ovaries, thyroid, uterus, and mesenteric lymph nodes were minimal; reviewer concluded that they were unremarkable and not considered to be toxicologically significant | Reviewer concluded that the death was not related to naloxone. |

Five animals died during the study. Summary of the deaths are presented in the table above. These deaths were not attributed to the naloxone treatment.

Clinical Signs

Clinical observations were performed daily throughout the duration of the study.

No data were recorded, but the Applicant claimed none occurred at any dose in any group during the treatment.

Body Weights

Body weights were recorded at the start of the study and weekly thereafter.

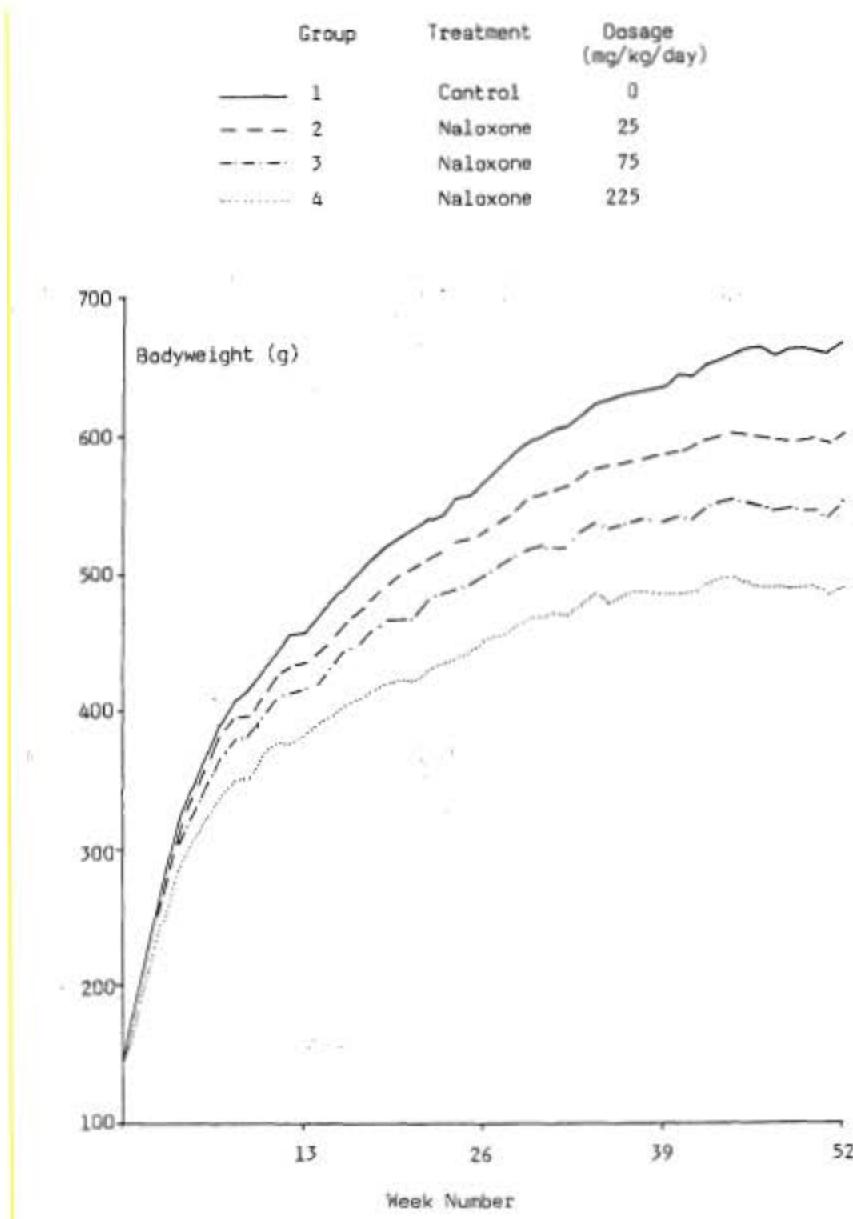
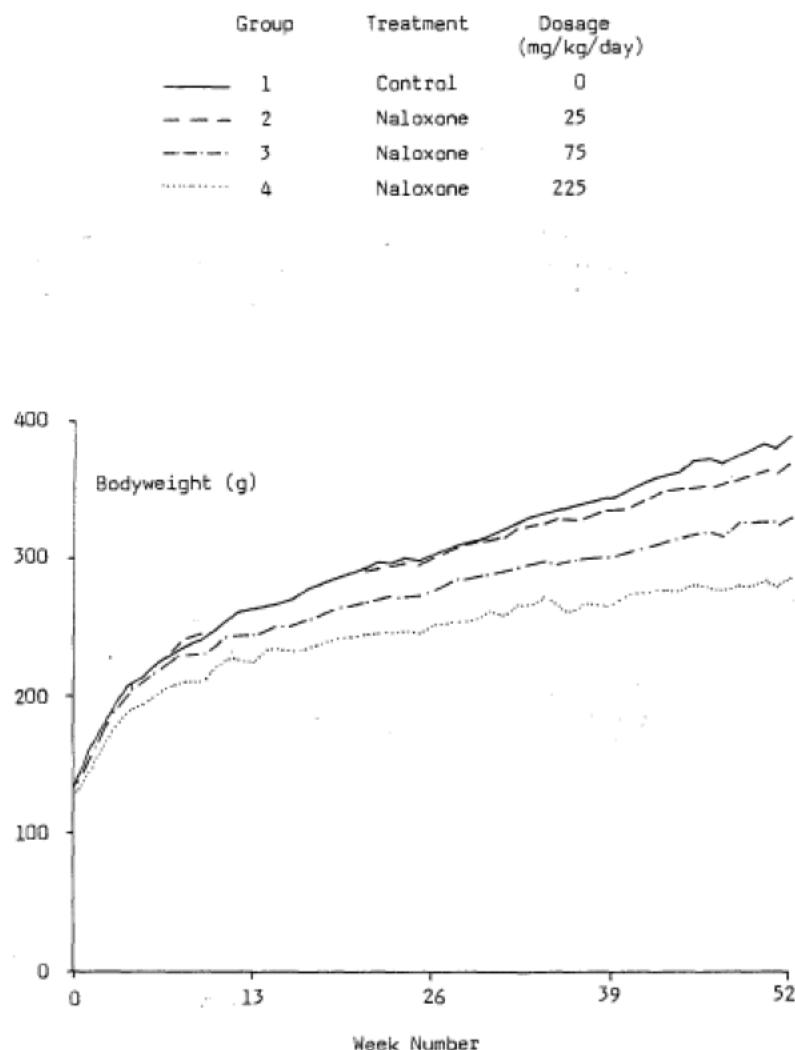
Figure 1. Mean body weight – males

Figure 2. Mean body weight – females**Table 26. Effect of naloxone on body weight gain vs. control animals**

| | Mean Body Weight Gain (g) from Weeks 0- 52 (% of control) | | | |
|---------|---|------------|------------|------------|
| | Naloxone dose (mg/kg/day) | | | |
| | Control | 25 | 75 | 225 |
| Males | 521 | 455 (-12%) | 406 (-22%) | 344 (-34%) |
| Females | 260 | 235 (-10%) | 199 (-23%) | 157 (-40%) |

Treatment-related effects on mean absolute body weight and body weight gain were observed. As depicted in the Applicant's figures above, a dose-dependent reduction in mean body weight was observed in both genders. Also, as depicted in the above table, a dose- and gender-dependent reduction in body weight gain was observed at the end of the treatment period. Over the 52-week dosing period, a reduction of approximately 10%, 20% and 40% was observed following the LD, MD, and HD of naloxone,

respectively. Females appeared more sensitive to naloxone-induced reduction in body weight gain. Decrease in body weight was not correlated with a change in food consumption.

Food Consumption

Food consumption per cage of rats was recorded. Group mean weekly intake was calculated (g/rat/week).

Female rats did not demonstrate any alteration in food consumption at doses up to 225 mg/kg/day. Males dosed with 225 mg/kg/day exhibited a slight reduction in food consumption; relative to control approximately 5%, 9%, and 7% decrease in food consumption was exhibited during Weeks 1 thru 26, Weeks 27 thru 52, and over the 52-week study period, respectively.

Ophthalmoscopy

Using a direct ophthalmoscope, the eyes of all animals (control and high dose groups only) were examined prior to the start of treatment and during treatment Weeks 9, 25, and 51. Thirty minutes prior to the examination, the eyes were dilated using the mydriatic agent homatropine hydrobromide (2% w/v).

No data were recorded, but the Applicant claimed no treatment-related ocular changes occurred.

ECG

Not performed.

Hematology

Hematology examinations were performed on the satellite group prior to treatment initiation. During the treatment period, blood was collected from the first surviving 10 male and 10 female rats in each group during Weeks 5, 13, 25, and 51 of treatment. Blood was collected via the retro-orbital sinus from animals under light ether anesthesia before dosing. The following hematology parameters were examined.

| Hematology | | |
|--|----------|--------------|
| Parameter | Examined | Not Examined |
| White Blood Cell Parameters | | |
| Total White Blood cell count (WBC) | X | |
| Differential leukocyte count (%: Neut, Lymph, Mono, Eosin) | X | |
| Leukocyte morphology | | X |
| Red Blood Cell Parameter | | |
| Red Blood cell count (RBC) | X | |
| Reticulocyte Count | | X |

| Hematology | | |
|--|----------|--------------|
| Parameter | Examined | Not Examined |
| Hemoglobin (Hb) | X | |
| Hematocrit (PCV) | X | |
| Platelet count (PLT) | | X |
| Mean platelet volume (MPV) | | X |
| Mean cell volume (MCV) | X | |
| Mean cell hemoglobin concentration (MCHC) | X | |
| Mean corpuscular hemoglobin concentration (MCHC) | X | |
| Red cell distribution width (RDW) | | X |
| Hemoglobin distribution width (HDW) | | X |
| Cytologic morphology | | X |
| Platelet morphology | | X |

There were no remarkable changes in hematology parameters during Weeks 5, 13, 25, and 51. There were several statistically differences during the treatment period, but they did not occur in a dose-dependent manner. Though statistically significant, the Applicant reported that the changes were within the laboratory normal range; therefore, the reviewer does not consider these changes to be toxicologically significant or adverse.

Coagulation: The following coagulation parameters were examined:

| Coagulation Parameters |
|-----------------------------------|
| Partial thromboplastin Time (PTT) |
| Prothrombin Time (PT) |

There were no remarkable changes in coagulation parameters during Weeks 5, 13, 25, and 51. There were several statistically significant differences during the treatment period, including increased partial thromboplastin time in Group 3 males during Weeks 5 and 51, decreased prothrombin time in Group 3 females during Week 13, decreased prothrombin time in Groups 2, 3, and 4 males during Weeks 13, 25 and in Group 2 male during Weeks 25. These differences did not occur in a dose-dependent manner. Though statistically significant, the Applicant reported that the changes were within the laboratory normal range; therefore, the reviewer does not consider these changes to be toxicologic significant or adverse.

Clinical Chemistry

The following clinical chemistry parameters were examined from the first surviving 10 male and 10 female rats in each group during Weeks 5, 13, 25, and 51 of treatment:

| Clinical Chemistry | | |
|----------------------------------|----------|--------------|
| Parameter | Examined | Not Examined |
| Aspartate aminotransferase (AST) | | X |
| Alanine aminotransferase (ALT) | | X |
| Alkaline phosphatase (A. Phos) | X | |

| Clinical Chemistry | | |
|---|---|---|
| Blood urea nitrogen (BUN) | X | |
| Creatinine | | X |
| Glucose (Glu) | X | |
| Cholesterol | | X |
| Triglycerides | | X |
| Total protein (TP) | X | |
| Albumin | X | |
| Globulin (Alpha 1, Alpha 2, Beta) and Gamma | X | |
| Albumin/globulin | | X |
| Total bilirubin | | X |
| Sodium (Na) | X | |
| Potassium (K) | X | |
| Chloride | | X |
| Calcium | | X |
| Phosphorous | | X |
| Gamma-glutamyl transferase | | X |
| Gamma-oxaloacetate transaminase (GOT) | X | |
| Glutamate-pyruvate transaminase (GPT) | X | |

Reviewer Comment: The Applicant did not perform the complete standard clinical chemistry examination.

Several statistically significant differences were noted during the treatment periods, including decreased % alpha 2 globulin during in LD (-20%) and MD (-205) females and decreased % gamma globulin in HD (-7%) males during Week 25; increased % gamma globulin levels in LD (+50%), MD (+50%) and HD (+50%) females, increased % Alpha 2 globulin levels in MD (+67%) and HD (+33%) females, and increased glutamate-pyruvate transaminase in MD (30%) females during Week 13; gamma-oxaloacetate transaminase in LD (+14%) females during Week 5. Reviewer does not consider these changes to be toxicologically significant or adverse.

Urinalysis

The urine was collected overnight (17 hours) in food and water fasted animals. The following urine parameters were examined from the first surviving 10 male and 10 female rats in each group during Weeks 5, 13, 25, and 51 of treatment:

| Urine Parameters | |
|---|--|
| Urinalysis | |
| Bilirubin | |
| Blood | |
| Glucose | |
| Ketones | |
| Microscopic examination: Erythrocytes (E), Leucocytes (L), Crystals (X), Debris (D) and Casts (C) | |

| Urine Parameters | |
|------------------|--|
| Urinalysis | |
| pH | |
| Protein | |
| Specific gravity | |
| Volume | |

No data were recorded, but the Applicant reported no treatment-related effects on the composition and cellularity of urine.

Gross Pathology

All surviving (fasted overnight prior to the scheduled sacrificed) animals were

| | | Percent Control Changes in Organ Weight Males | | | |
|----------|-----------------------------------|--|---------------------|---------------------|---------------------|
| | | 0 | 25 | 75 | 225 |
| Organ | Parameter | | | | |
| Brain | Absolute wt. (g) | 2.20 ± 0.115 | -0.45% | +0.9% | -1.4% |
| | Relative-to body weight (ratio %) | 0.34 ± 0.044 | +8.8% ^a | +12.0% ^c | +32.4% ^c |
| Heart | Absolute wt. (g) | 1.68 ± 0.183 | -2.4% | -7.7% ^a | -10.1% ^a |
| | Relative-to body weight (ratio %) | 0.26 ± 0.028 | +7.7% ^a | +11.5% ^c | +19.2% ^b |
| Liver | Absolute wt. (g) | 21.98 ± 4.671 | +8.8% | -7.7% | -15.2% ^c |
| | Relative-to body weight (ratio %) | 3.33 ± 0.366 | +21.3% ^c | +12.0% ^b | +15.3% ^c |
| Kidneys | Absolute wt. (g) | 4.15 ± 0.381 | -1.4% | -8.9% ^b | -14.9% ^c |
| | Relative-to body weight (ratio %) | 0.64 ± 0.072 | +7.8% ^a | +9.3% ^b | +14.1% ^c |
| Spleen | Absolute wt. (g) | 1.04 ± 0.194 | -2.9% | -12.5% ^b | -16.3% ^c |
| | Relative-to body weight (ratio %) | 0.16 ± 0.029 | +6.3% | +6.3% | +12.5% |
| Prostate | Absolute wt. (g) | 0.8. ± 0.193 | -13.6% | -11.1% | -27.2% ^c |
| | Relative-to body weight (ratio %) | 0.13 ± 0.036 | -7.7% | NC | -7.7% |
| Lungs | Absolute wt. (g) | 2.12 ± 0.196 | -4.7% | -2.8% | -9.4% |
| | Relative-to body weight (ratio %) | 0.33 ± 0.040 | +3.0% | +15.2% ^c | +21.2% ^c |

a: Statistically significant when compared to control at $p \leq 0.05$

b: Statistically significant when compared to control at $p \leq 0.01$

c: Statistically significant when compared to control at $p \leq 0.001$

treatment-related macroscopic findings observations.

Organ Weights

Absolute and relative organ-to-body weights were calculated for the following organs for all the animals sacrificed at the scheduled necropsy:

anesthetized by carbon dioxide asphyxiation after 52 weeks of treatm ent. Macroscopic evalua tion was perfor med. There were no notabl e

| | |
|--|--------------------------|
| Adrenal glands (paired) | Pituitary gland |
| Brain | Spleen |
| Heart | Prostate |
| Kidneys (paired) | Spleen |
| Liver | Testes (paired) |
| Lungs | Thyroid and parathyroid* |
| Ovaries (paired) | Uterus |
| Paired organs were weighed together. | |
| *: after a minimum of 24 hours after fixing in 10% buffered formalin | |

Table 27. Significant organ weight changes in males administered naloxone.

| | | Percent Control Changes in Organ Weight | | | |
|----------|-----------------------------------|---|---------------------|---------------------|---------------------|
| | | Males | | | |
| | Dose mg/kg/day → | 0 | 25 | 75 | 225 |
| Organ | Parameter | | | | |
| Brain | Absolute wt. (g) | 2.20 ± 0.115 | -0.45% | +0.9% | -1.4% |
| | Relative-to body weight (ratio %) | 0.34 ± 0.044 | +8.8% ^a | +12.0% ^c | +32.4% ^c |
| Heart | Absolute wt. (g) | 1.68 ± 0.183 | -2.4% | -7.7% ^a | -10.1% ^a |
| | Relative-to body weight (ratio %) | 0.26 ± 0.028 | +7.7% ^a | +11.5% ^c | +19.2% ^b |
| Liver | Absolute wt. (g) | 21.98 ± 4.671 | +8.8% | -7.7% | -15.2% ^c |
| | Relative-to body weight (ratio %) | 3.33 ± 0.366 | +21.3% ^c | +12.0% ^b | +15.3% ^c |
| Kidneys | Absolute wt. (g) | 4.15 ± 0.381 | -1.4% | -8.9% ^b | -14.9% ^c |
| | Relative-to body weight (ratio %) | 0.64 ± 0.072 | +7.8% ^a | +9.3% ^b | +14.1% ^c |
| Spleen | Absolute wt. (g) | 1.04 ± 0.194 | -2.9% | -12.5% ^b | -16.3% ^c |
| | Relative-to body weight (ratio %) | 0.16 ± 0.029 | +6.3% | +6.3% | +12.5% |
| Prostate | Absolute wt. (g) | 0.8. ± 0.193 | -13.6% | -11.1% | -27.2% ^c |
| | Relative-to body weight (ratio %) | 0.13 ± 0.036 | -7.7% | NC | -7.7% |
| Lungs | Absolute wt. (g) | 2.12 ± 0.196 | -4.7% | -2.8% | -9.4% |
| | Relative-to body weight (ratio %) | 0.33 ± 0.040 | +3.0% | +15.2% ^c | +21.2% ^c |

a: Statistically significant when compared to control at $p \leq 0.05$
 b: Statistically significant when compared to control at $p \leq 0.01$
 c: Statistically significant when compared to control at $p \leq 0.001$

Table 28. Significant organ weight changes in females administered naloxone.

| | | Percent Control Changes in Organ Weight | | | |
|-------|------------------|---|-------|-------|---------------------|
| | | Females | | | |
| | Dose mg/kg/day → | 0 | 25 | 75 | 225 |
| Organ | Parameter | | | | |
| Heart | Absolute wt. (g) | 1.16 ± 0.197 | -1.7% | -7.8% | -10.1% ^a |
| | Relative-to body | 0.31 ± 0.057 | +3.2% | +6.5% | -17.2% ^c |

| | | Percent Control Changes in Organ Weight | | | |
|---|-----------------------------------|---|---------------------|---------------------|---------------------|
| | | Females | | | |
| | Dose mg/kg/day → | 0 | 25 | 75 | 225 |
| Organ | Parameter | | -0.45% | +0.9% | -1.4% |
| Liver | weight (ratio %) | | | | |
| | Absolute wt. (g) | 12.87 ± 1.948 | +6.5% | -6.5% | -18.6% ^c |
| Kidneys | Relative-to body weight (ratio %) | 3.37 ± 0.341 | +12.8% ^c | +9.5% ^b | +10.4% ^b |
| | Absolute wt. (g) | 2.47 ± 0.344 | +0.8% | -3.6% | -10.1% ^b |
| Spleen | Relative-to body weight (ratio %) | 0.65 ± 0.078 | +7.7% | +12.3% ^c | +21.5% ^c |
| | Absolute wt. (g) | 0.55± 0.082 | -1.8% | +1.8% | -5.5% |
| Lungs | Relative-to body weight (ratio %) | 0.15 ± 0.030 | NC | +13.3% ^a | +11.9% ^c |
| | Absolute wt. (g) | 1.51± 0.140 | +5.3% ^a | -2.0% | -2.0% |
| a: Statistically significant when compared to control at p≤ 0.05 b: Statistically significant when compared to control at p≤ 0.01 c: Statistically significant when compared to control at p≤ 0.001 | | | | | |

Notable statistically significant changes in organ weights are presented in the above tables. Treatment-related relative organ weight was noted in the liver and kidney of LD, MD, and HD males. In females the relative liver weight of all females and the relative kidney of the MD and HD groups were treatment-related. The relative lung weight of the MD and HD males and females was increased compared to the control animals. Due to a lack of a dose-dependent relationship, the other statistically significant changes are considered incidental/not toxicologically significant.

Reviewer Comment: The reviewer does not consider the organ weight change in the kidney, liver, and lung to be toxicological significant because no microscopic changes or hematological changes were associated with the organ weight change.

Histopathology

Adequate Battery: Yes

Peer Review: No, a signed pathology report was not submitted.

The following tissues were collected from all animals. Histological examination was conducted on control group and HD group animals. All tissues were fixed in 10% buffered formalin with the exception of the eyes and optic nerve, which were fixed in Davidson's solution. Tissue selected for microscopic analysis were processed and stained with hematoxylin and eosin stain and examined by light microscopy.

| Tissue | Collected & Preserved | Microscopic Examination | | | |
|-------------------|-----------------------|-------------------------|---------|---------|---------|
| | | Group 1 | Group 2 | Group 3 | Group 4 |
| Adrenal gland (2) | X | X | | | X |

| Tissue | Collected & Preserved | Microscopic Examination | | | |
|------------------------------------|-----------------------|-------------------------|---------|---------|---------|
| | | Group 1 | Group 2 | Group 3 | Group 4 |
| Aorta | X | | | | |
| Aortic arch | X | X | | | X |
| Bone | X | | | | |
| Bone marrow section – sternum, rib | X | | | | |
| Brain | X | X | | | X |
| Clitoral gland | | | | | |
| Coagulating gland | | | | | |
| Epididymis (2) | X | X | | | X |
| Esophagus | X | | | | |
| Eyes | X | X | | | X |
| Gallbladder | | | | | |
| Harderian gland | | | | | |
| Heart | X | X | | | X |
| Kidney | X | X | | | X |
| Lacrimal gland | | | | | |
| Large intestine, cecum | X | X | | | X |
| Large intestine, colon | X | X | | | X |
| Large intestine, rectum | | | | | |
| Larynx | | | | | |
| Liver | X | X | | | X |
| Lungs | X | X | | | X |
| Lymph node, cervical | X | X | | | X |
| Lymph node, mandibular | | | | | |
| Lymph node, mesenteric | X | X | | | X |
| Mammary gland (cranial, caudal) | X | X | | | X |
| Nerve, sciatic | X | X | | | X |
| Ovaries | X | X | | | X |
| Oviduct | | | | | |
| Pancreas | X | X | | | X |
| Parathyroid gland | X | X | | | X |
| Peyer's Patch | | | | | |
| Pharynx | | | | | |
| Pituitary | X | X | | | X |
| Preputial gland | X | X | | | X |
| Prostate | X | X | | | X |
| Salivary gland | X | X | | | X |
| Seminal vesicle | X | | | | |
| Skeletal muscle, biceps femoris | X | | | | X |
| Skin | X | | | | |
| Small intestine, duodenum | X | X | | | X |
| Small intestine, ileum | X | X | | | X |
| Small intestine, jejunum | X | X | | | X |
| Spinal cord | X | | | | |
| Spleen | X | X | | | X |
| Stomach | X | X | | | X |
| Target Organ | | | | | |
| Testis | X | X | | | X |
| Thymus | X | X | | | X |
| Thyroid | X | X | | | X |
| Tongue | X | | | | X |

| Tissue | Collected & Preserved | Microscopic Examination | | | |
|---|-----------------------|-------------------------|---------|---------|---------|
| | | Group 1 | Group 2 | Group 3 | Group 4 |
| Trachea | X | | | | |
| Ureter | X | | | | |
| Urinary Bladder | X | X | | | X |
| Uterus | X | X | | | X |
| Vagina | | | | | |
| Zymbal's gland (auditory sebaceous gland) | | | | | |
| Abnormalities/Gross lesions | X | X | | | |

Histological Findings

There were no notable treatment-related microscopic observations.

Special Evaluation

None

Dosing Solution Analysis

Formulation analysis demonstrated that naloxone admixtures designed to deliver doses of 25, 75, and 225 mg/kg were maintained within 10% of nominal values throughout the study period. "Analytical results indicate the prepared admixtures were generally of satisfactory test article concentration and homogeneity."

Study title: Nine month oral toxicity study of naloxone HCl in dogs

| | |
|-------------------------------------|---|
| Study no.: | N003003D |
| Study report location: | EDR |
| Conducting laboratory and location: | (b) (4) [redacted] |
| Date of study initiation: | June 8, 1998 (in life portion of the study) |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity: | Naloxone hydrochloride, Lot № 1492V06666 and Lot № X03717, Purity: not reported |

Key Study Findings

Naloxone hydrochloride (0, 5, 25, and 125/75 mg/kg/day) was orally administered to dogs for 9 months with the following results:

| | Key Study Findings |
|---------------------------|---|
| Mortality/Survival | One HD female (№ 412) was humanely euthanized on Day 3 due to treatment-related clinical signs. |
| Clinical Signs | Primary treatment-related clinical signs observed in the HD animals orally administered 125 mg/kg of naloxone were ataxia, convulsions, disorientation, tremors and cyanosis, These clinical signs were observed from Day 1 to Day 3. |
| Hematology | No apparent treatment-related alterations in the hematologic parameters were observed at 5, 25, or 125/75 mg/kg/day. |
| Histopathology | Acute hemorrhage in the brain, eyes (iris), heart (valve), pituitary gland, and thymus was the primary microscopic finding noted in the unscheduled death in the one HD female (№ 412) following the oral administration of 125 mg/kg/day naloxone. |
| Toxicokinetic | There were adequate dose-related exposure in all dose groups; exposure to naloxone increased with increasing dose. Naloxone was rapidly absorbed; mean peak plasma was obtained within 0.5 to 2 hours. |
| Summary | <p>The NOAEL was identified as 75 mg/kg/day (in agreement with the Applicant) based on the drug-related clinical signs observed following the oral administration of naloxone at a dose of 125 mg/kg/day. This NOAEL corresponds to AUC values of 274.51 and 296.29 ng·h/mL on Day 274 in males and females, respectively after repeated daily dosing for 9 months. C_{max} values at this dose on Day 274 were 84.7 (males) and 53.8 (females) ng/mL. NOAEL corresponds to an human equivalent dose (HED) of 41.7 mg/kg (2500 mg naloxone/60 kg person) which provides a safety margin of approximately 62.5 based on body surface area. The convulsive dose of 125 mg/kg in the dog corresponds to a HED of 4167 mg (104-times the MRDD of naloxone via this drug product).</p> <p>In terms of C_{max}, the NOAEL in the female dogs resulted in a C_{max} of 53.8 ng/mL, which is 248-times higher than the C_{max} in humans following multiple doses of 40/20 BID (0.217 ng/mL) as per the product labeling.</p> |

Methods**Doses:**

| Dose (mg/kg/day) | | | |
|--|----------|-----------|----------------|
| 0 (control) | 5 | 25 | 125/75* |
| 4/sex | 4/sex | 4/sex | 4/sex |
| *: On Day 4 (females) and on Day 5 (males), the 125 mg/kg dose was decreased to 75 mg/kg | | | |

Frequency of dosing: Daily for 274 consecutive days

Route of administration: Oral

Dose volume: N/A

Formulation/Vehicle: Capsule/gelatin capsule

Species/Strain: Dogs/Beagle Dogs

Number/Sex/Group: 4/sex/group

Age: Approx. 5 to 7 months old (at initiation of study)

Weight: Approx. 6.92 to 9.94 kg (at initiation of study)

Satellite groups: None

Unique study design: None

Deviation from study protocol: Deviations are described under the appropriate observation(s).

Observations and Results**Toxicokinetics**

Blood samples were collected for toxicokinetic evaluation prior to dosing (except on Day 1), at, 0.5, 1, 2, 4, 8, 12, and 24 hours after dosing on the Days 1 (Week 1), Days 28 (Week 4) and Day 274 (Week 39) from 4 animals/time point/treatment group. Plasma concentrations of unconjugated naloxone were determined by using a HPLC/MS method. The lower limit of quantification was 0.5 ng/mL.

Table 29. Mean toxicokinetic parameters after oral administration of naloxone HCl during study Weeks 1, 4, and 39.

| Study Days | Dose (mg/kg/day) | Sex | T_{max} (h) | C_{max} (ng/mL) | AUC_t (ng.h/mL) |
|-------------------|-------------------------|------------|----------------------------|--------------------------------|----------------------------------|
| 1 | 5 | M | 0.5 | 7.38 | ND |
| | 5 | F | 0.75 | 2.55 | 8.28 |
| | | | | | |
| | 25 | M | 0.76 | 97.99 | 217.22 [‡] |
| | 25 | F | 0.76 | 28.12 [*] | 9217.69 |
| | | | | | |
| | 125 | M | 0.83 | 2202 | 2787.30 |
| | 125 | F | 1.63 | 1124 | 1811.90 |

| Study Days | Dose (mg/kg/day) | Sex | T _{max} (h) | C _{max} (ng/mL) | AUC _t (ng.h/mL) |
|------------|------------------|-----|----------------------|--------------------------|----------------------------|
| 28 | 5 | M | 0.75 | 9.33 | NA |
| | 5 | F | 3.75 | 3.65 | NA |
| | | | | | |
| | 25 | M | 0.75 | 16.34 | 87.16* |
| | 25 | F | 1.0 | 22.59 | 109.59* |
| | | | | | |
| | 75 | M | 0.88 | 80.75* | 416.57 |
| 274 | 75 | F | 1.67 | 240.87 | 1643.99 |
| | | | | | |
| | 5 | M | 0.50 | 3.55 | NA |
| | 5 | F | 1.5 | 3.08 | 11.32* |
| | | | | | |
| | 25 | M | 1.76 | 22.80 | 85.75 ⁺ |
| | 25 | F | 1.0 | 24.8 | 78.62* |
| | | | | | |
| | 75 | M | 2.0 | 84.7 | 274.51 ⁺ |
| | 75 | F | 1.67 | 53.8 | 296.29 ⁺ |
| | | | | | |

‡: AUC estimated at 12 hours after dosing
 +: AUC estimated at 24 hours after dosing
 *: Mean based on 3 animals

Toxicokinetic data (reproduced from Applicant's data) presented in table above. Absorption of naloxone was dose-dependent. Naloxone was rapidly absorbed on study Days 1, 28 and 274; mean peak plasma was obtained within 0.5 to 2 hours and 0.75 to 3.75 hours in males and females, respectively following the oral administration of naloxone at doses of 5, 25, and 125/75 mg/kg/day. Systemic exposure, as assessed with the C_{max} and AUC_t, was apparent at all dose levels; exposure increased in a dose-dependent manner. Accumulation of naloxone with repeated dosing was not observed nor was an effect of gender on exposure to drug.

Mortality

All animals were observed twice daily (at least 6 hours apart) for morbidity and mortality throughout the duration of the study.

Table 30. Summary of clinical signs and macroscopic finding in animal № 412.

| Group | Animal № | Day of sacrifice | Observed clinical signs | Macroscopic Findings |
|-----------|----------|------------------|--|---|
| High Dose | 412 | 3 | <ul style="list-style-type: none"> • tremors • disorientation • ataxia • convulsions • hyperthermic • cyanotic • unresponsive | <ul style="list-style-type: none"> • Lungs: discoloration • Heart: fluid in the pericardial sac |

| Group | Animal № | Day of sacrifice | Observed clinical signs | Macroscopic Findings |
|-------|----------|------------------|-------------------------|----------------------|
| | | | • laterally recumbent | |

There was one unscheduled death. Female dog № 412 was humanely euthanized on Day 3 after displaying the clinical signs listed in the table above.

Clinical Signs

Clinical observations for evidence of toxicity were performed daily.

Table 31. Selective clinical signs observed in dogs orally administered naloxone for 9 months.

| Dose (mg/kg/day) | Observation | No of Animals Affected | Mean First Day of Observation | Mean Last Day of Observation | Total Observation |
|---------------------|--------------------|---------------------------|----------------------------------|---------------------------------|----------------------|
| Males | | | | | |
| 0 | Diarrhea | 2 | 5 | 170 | 20 |
| 5 | | 1 | 63 | 160 | 2 |
| 25 | | 3 | 28 | 174 | 27 |
| 75 ^a | | 3 | 22 | 73 | 8 |
| 0 | Emesis | 4 | 24 | 205 | 34 |
| 5 | | 4 | 8 | 249 | 41 |
| 25 | | 4 | 33 | 229 | 34 |
| 125/75 ^a | | 4 | 1 | 253 | 53 |
| 125/75 ^a | Cyanotic | 1 | 1 | 1 | 1 |
| 125/75 ^a | Ataxic | 1 | 1 | 1 | 1 |
| 125/75 ^a | Convulsive | 1 | 1 | 1 | 1 |
| 125/75 ^a | Disoriented | 1 | 1 | 1 | 1 |
| Females | | | | | |
| 0 | Diarrhea | 3 | 47 | 95 | 6 |
| 5 | | 3 | 80 | 168 | 16 |
| 25 | | 2 | 73 | 73 | 2 |
| 125/75 ^a | | 1 | 112 | 112 | 1 |
| 0 | Emesis | 4 | 84 | 240 | 19 |
| 5 | | 4 | 43 | 240 | 54 |
| 25 | | 4 | 105 | 251 | 37 |
| 125/75 ^a | | 4 | 1 | 186 | 42 |
| 25 | Salivation | 2 | 76 | 275 | 312 |
| 125/75 ^a | | 3 | 2 | 275 | 689 |
| 125/75 ^a | Cyanotic | 1 | 3 | 3 | 1 |

| Dose (mg/kg/day) | Observation | No of Animals Affected | Mean First Day of Observation | Mean Last Day of Observation | Total Observation |
|---------------------|--------------------------|------------------------|-------------------------------|------------------------------|-------------------|
| 125/75 ^a | Ataxic | 2 | 3 | 3 | 4 |
| | | | | | |
| 125/75 ^a | Convulsive | 2 | 3 | 3 | 4 |
| | | | | | |
| 125/75 ^a | Disoriented | 2 | 3 | 3 | 4 |
| | | | | | |
| 125/75 ^a | Tremors | 1 | 3 | 3 | 2 |
| | | | | | |
| 125/75 ^a | Rapid respiration | 1 | 2 | 2 | 1 |

^a The dose of 125 mg/kg was reduced to 75 mg/kg on Day 4 in females and Day 5 in males.

Selective clinical signs observed in male and female dogs are presented in the table above. In the one HD female premature decedents following the oral administration of 125 mg/kg of naloxone HCl, clinical signs included tremors, disorientation ataxia, convulsions ,hyperthermia, cyanotic, unresponsiveness and lateral recumbent. One HD male (№ 404) displayed disorientation, ataxia and convulsions following the oral administration of 125 mg/kg of naloxone HCl (HED 4167 mg).

The primary clinical signs observed in the HD animals orally administered 125 mg/kg of naloxone were ataxia, convulsions, disorientation, tremors and cyanosis. These clinical signs were observed from Day 1 to Day 3. Subsequently, the 125 mg/kg dose was lowered to 75 mg/kg. Convulsions were not noted in animals dosed with 75 mg/kg. Sporadic incidences of the digestive clinical signs and emesis were observed in all dose levels. All other findings were either not dose-related or were in a comparable number of control animals, and were thus not considered to be drug-related.

Body Weights

Body weights were recorded weekly and on the day of necropsy. On Day 1, bodyweight was recorded prior to dosing.

No treatment-related changes in body weights were observed.

Food Consumption

Food consumption was recorded daily (except during periods of fasting) beginning at Week 1. Food consumption was recorded as all, ¾, ½, ¼, or none.

No treatment-related changes in food consumption were observed.

Ophthalmoscopy

Ophthalmologic examination was performed by a qualified veterinarian pre-dosing period and during Week 39. Ophthalmologic examination included the anterior and posterior chambers and adnexal structures of the eye. Anterior examination included the cornea, sclera, iris, pupil, lens, aqueous, and anterior chamber. Posterior structures examined included the vitreous, retina, and optic disk. Adnexal structures examined included the conjunctiva, eyelids, and eyelashes. Prior to the examination, the eyes were dilated using the mydriatic agent tropicamide hydrochloride (Mydriacyl®).

No treatment-related ophthalmic changes were observed.

ECG

Electrocardiography recordings were performed once during the predosing phase. On Weeks 4 and 39 of the treatment period, electrocardiography recordings were performed on all animals at approximately 30 minutes post-dosing. ECG was collected using V1252 multipurpose preamplifier and a V1203 three channel isolated amplifier leads (I, aVf, and V₁₀). ECG measurements were qualitatively evaluated for rhythm and morphology by a board certified veterinary cardiologist. The following parameters were also evaluated: blood pressure and heart rate data.

No apparent treatment-related changes were observed.

Hematology

Blood (approximately 5-7 mL) was collected from each animal (fasted for at least 12 hours prior to sampling) during the predosing phase (Day -6 prior to dose initiation) and during Weeks 4 (Day 22), 12 (Day 81), and prior (Day 275) to the scheduled euthanasia. The following hematology parameters were examined:

| Hematology | | |
|--|----------|--------------|
| Parameter | Examined | Not Examined |
| White Blood Cell Parameters | | |
| White Blood cell count (WBC) | X | |
| Differential leukocyte count (% and Absolute) | X | |
| Leukocyte morphology | | X |
| Red Blood Cell Parameter | | |
| Red Blood cell count (RBC) | X | |
| Reticulocyte Count (RET) | X | |
| Hemoglobin (HGB) | X | |
| Hematocrit (HCT) | X | |
| Platelet count (PLT) | X | |
| Mean platelet volume (MPV) | | X |
| Mean corpuscular volume (MCV) | X | |
| Mean corpuscular hemoglobin (MCH) | X | |
| Mean corpuscular hemoglobin concentration (MCHC) | X | |

| Hematology | | |
|-------------------------------------|----------|--------------|
| Parameter | Examined | Not Examined |
| White Blood Cell Parameters | | |
| Red cell distribution width (RDW) | | X |
| Hemoglobin distribution width (HDW) | | X |
| Cytologic morphology | X | |
| Platelet morphology | | X |

No apparent treatment-related changes were observed.

Coagulation: The following coagulation parameters were examined:

| Coagulation Parameters |
|--|
| Activated Partial Thromboplastin Time (APTT) |
| Prothrombin Time (PT) |

No treatment-related effects were observed.

Clinical Chemistry

Blood (approximately 5-7 mL) was collected from each animals (fasted for at least 12 hours prior to sampling) during the predosing phase (Day -6 prior to dose initiation) and during Weeks 4 (Day 22), 12 (Day 81), and prior (Day 275) to the scheduled euthanasia. The following clinical chemistry parameters were examined:

| Clinical Chemistry | | |
|----------------------------------|----------|--------------|
| Parameter | Examined | Not Examined |
| Aspartate aminotransferase (AST) | X | |
| Alanine aminotransferase (ALT) | X | |
| Alkaline phosphatase (ALP) | X | |
| Blood urea nitrogen (BUN) | X | |
| Creatinine | X | |
| Glucose (GLU) | X | |
| Cholesterol (CHOL) | X | |
| Triglycerides (TRIG) | X | |
| Total protein (TP) | X | |
| Albumin (ALB) | X | |
| Globulin (GLOB) | X | |
| Albumin/globulin ratio (A/G) | X | |
| Total bilirubin (TB) | X | |
| Sodium (NA) | X | |
| Potassium (K) | X | |
| Chloride (CL) | X | |
| Calcium (CA) | X | |
| Phosphorous (PHOS) | X | |
| Gamma-glutamyl transferase (GGT) | X | |

No apparent treatment-related effects were observed in blood chemistry.

Urinalysis

The following parameters were collected the night before each scheduled clinical pathology blood sampling over an approximate 16-hour interval.

| Urine Parameters |
|-------------------------------------|
| Urinalysis |
| Appearance (clarity and color) |
| Bilirubin |
| Glucose |
| Ketones |
| Microscopic examination of sediment |
| Occult blood |
| pH |
| Protein |
| Specific gravity |
| Urobilinogen |
| Volume |

Unremarkable, no naloxone-related findings were observed on urine chemistry parameters.

Gross Pathology

At the scheduled necropsy during Week 40 (Day 275), all dogs were anesthetized with a barbiturate overdose and exsanguinated. Macroscopic evaluation was performed on all orifices, external surface of the body, and cranial, thoracic, abdominal, and pelvic cavities with their contents.

There were no notable treatment-related macroscopic observations.

Organ Weights

Absolute organ weight, relative organ-to-body weight and organ-to brain weight were calculated as organ weight ratio as percentage of body weight and brain weight for all study animals sacrificed at the scheduled necropsy. Organs weighed are tabulated below:

| | |
|--------------------------------------|------------------|
| Adrenal glands (paired) | Ovaries (paired) |
| Brain | Pituitary gland |
| Heart | Spleen |
| Kidneys (paired) | Testes (paired) |
| Liver | |
| Paired organs were weighed together. | |

Table 32. Significant changes in mean organ weight in male dogs following oral administration of naloxone HCl.

| Organ | Parameter | Percent Control Changes in Organ Weight Males | | | |
|--------|------------------------------------|--|---------|---------|---------|
| | | Dose mg/kg/day → | 0 | 5 | 25 |
| | | 75 ^a | | | |
| Brain | Absolute wt. (g) | 92.18 ± 7.58 | -7.9% | -14.6%* | -10.9% |
| | Relative-to body weight (ratio %) | 0.73 ± 0.07 | -9.6% | -9.6% | -9.6% |
| Spleen | Absolute wt. (g) | 62.93 ± 6.25 | +71.3%* | +15.9% | +37.6 |
| | Relative-to body weight (ratio %) | 0.50 ± 0.04 | +66.0%* | +22.0% | +38.0%* |
| | Relative-to-brain weight (ratio %) | 68.3 ± 4.4 | +85.9%* | +35.4%* | +54.6%* |
| Testis | Absolute wt. (g) | 13.92 ± 2.58 | +38.8%* | +2.9% | +16.7% |
| | Relative-to body weight (ratio %) | 0.11 ± 0.02 | +36.4%* | +9.1% | +18.2% |
| | Relative-to-brain weight (ratio %) | 15.3 ± 3.5 | +51.6%* | +19.0% | +30.0% |

*: Statistically significant when compared to control at p≤ 0.05

There were no treatment-related changes in organ weight in both sexes. As depicted in the table above, the spleen and testis were statistically significantly increased when adjusted for brain weight and body weight in male dogs, but demonstrated no dose-dependency. These changes are considered incidental findings by the reviewer.

Histopathology

Adequate Battery: Yes

Peer Review: It was stated that a certified veterinarian analyzed the slides; however a signed pathology report was not submitted.

The following tissues were collected from all animals. Histological examination was conducted on all naloxone-treated dogs, control group, and unscheduled death animals. All tissues were fixed in 10% neutral buffered formalin with the exception of the eyes that were fixed in 3% glutaraldehyde. Bone marrow for cytology was fixed in methanol. Tissue selected for microscopic analysis were processed and stained with hematoxylin and eosin stain and examined by light microscopy.

| Tissue | Collected & Preserved | Microscopic Examination | | | |
|------------------------------------|-----------------------|-------------------------|---------|---------|---------|
| | | Group 1 | Group 2 | Group 3 | Group 4 |
| Adrenal gland (2) | X | X | X | X | X |
| Aorta – thoracic | X | X | X | X | X |
| Bone – femur, sternum | X | X | X | X | X |
| Bone marrow section – sternum, rib | X | X | X | X | X |

| Tissue | Collected & Preserved | Microscopic Examination | | | |
|--|-----------------------|-------------------------|---------|---------|---------|
| | | Group 1 | Group 2 | Group 3 | Group 4 |
| Bone marrow for cytology – sternum, rib ^a | X | X | X | X | X |
| Brain (forebrain, midbrain, hindbrain) | X | X | X | X | X |
| Clitoral gland | | | | | |
| Coagulating gland | | | | | |
| Epididymis (2) | X | X | X | X | X |
| Esophagus | X | X | X | X | X |
| Eyes | X | X | X | X | X |
| Gallbladder | X | X | X | X | X |
| Harderian gland | | | | | |
| Heart | X | X | X | X | X |
| Kidney | X | X | X | X | X |
| Lacrimal gland | X | X | X | X | X |
| Large intestine, cecum | X | X | X | X | X |
| Large intestine, colon | X | X | X | X | X |
| Large intestine, rectum | | | | | |
| Larynx | | | | | X |
| Liver | X | X | X | X | X |
| Lungs/bronchi (left apical, right diaphragmatic) | X | X | X | X | X |
| Lymph node, mandibular | X | X | X | X | X |
| Lymph node, mesenteric | X | X | X | X | X |
| Mammary gland | X | X | X | X | X |
| Nerve, sciatic | X | X | X | X | X |
| Ovaries | X | X | X | X | X |
| Oviduct | | | | | |
| Pancreas | X | X | X | X | X |
| Parathyroid gland | X | X | X | X | X |
| Peyer's Patch | | | | | |
| Pharynx | | | | | |
| Pituitary | X | X | X | X | X |
| Preputial gland | | | | | |
| Prostate | X | X | X | X | X |
| Salivary gland, mandibular | X | X | X | X | X |
| Salivary gland, parotid | | | | | |
| Salivary gland, sublingual | | | | | |
| Seminal vesicle | | | | | |
| Skeletal muscle, biceps femoris | X | X | X | X | X |
| Skin | X | X | X | X | X |
| Small intestine, duodenum | X | X | X | X | X |
| Small intestine, ileum | X | X | X | X | X |
| Small intestine, jejunum | X | X | X | X | X |
| Spinal cord, cervical | | | | | |
| Spinal cord, lumbar | X | X | X | X | X |
| Spinal cord, thoracic | X | X | X | X | X |
| Spleen | X | X | X | X | X |
| Stomach cardiac, fundic, pyloric) | X | X | X | X | X |
| Target Organ | | | | | |
| Testis | X | X | X | X | X |
| Thymus | X | X | X | X | X |

| Tissue | Collected & Preserved | Microscopic Examination | | | |
|---|-----------------------|-------------------------|---------|---------|---------|
| | | Group 1 | Group 2 | Group 3 | Group 4 |
| Thyroid | X | X | X | X | |
| Tongue | X | X | X | X | X |
| Trachea | X | X | X | X | X |
| Ureter | | | | | |
| Urinary Bladder | X | X | X | X | X |
| Uterus with cervix | X | X | X | X | X |
| Vagina | X | X | X | X | X |
| Zymbal's gland (auditory sebaceous gland) | | | | | |
| Gross lesions | X | X | X | X | X |

Histological Findings

Table 33. Histopathological finding in unscheduled death

| | Incidence of Microscopic Findings | | | | |
|--|-----------------------------------|---|---|----|-----|
| | Dose (mg/kg/day) | 0 | 5 | 25 | 125 |
| No examined → | 4 | 4 | 4 | 4 | |
| Brain | | | | | |
| Hemorrhage | 0 | 0 | 0 | 1 | |
| Eye | | | | | |
| Nictitans Gland, hyperplasia | 0 | 1 | 0 | 1 | |
| Iris, hemorrhage | 0 | 0 | 0 | 1 | |
| Heart | | | | | |
| Epicardial, hyperplasia | 0 | 0 | 0 | 0 | |
| Lymphocytic infiltrate | 0 | 0 | 0 | 0 | |
| Intramural coronary artery, medial hypertrophy | 0 | 1 | 1 | 0 | |
| Hemorrhage | 0 | 0 | 0 | 1 | |
| Pituitary gland | | | | | |
| Cyst(s) | 1 | 1 | 0 | 0 | |
| Hemorrhage | 0 | 0 | 0 | 1 | |
| Thymus | | | | | |
| Involution | 4 | 4 | 4 | 4 | |
| Hemorrhage | 0 | 0 | 0 | 1 | |

The above table details relevant naloxone-related microscopic findings observed to occur in the one unscheduled death in the one HD female (No 412). Histopathological changes were observed were acute hemorrhage in the brain, eyes (iris), heart (valve), pituitary gland, and thymus. The Applicant stated that the "pattern of hemorrhage was particularly severe in the cerebellum, and in this organ, it was presumed to have contributed to death." The hemorrhage was peracute; no inflammatory response was associated with the hemorrhage.

No treatment-related microscopic findings were noted in dogs from the final sacrifice. Also, no hemorrhage was observed in any organs.

Special Evaluation

None

Dosing Solution Analysis

Analysis was not provided.

Study title: A 3-month oral (gavage) toxicity study in rats with a 28-day recovery period with either the combination of oxycodone hydrochloride and naloxone hydrochloride [REDACTED] (b) (4) or naloxone hydrochloride [REDACTED] (b) (4) alone.

Study no.: NDSE-609

Study report location: EDR

Conducting laboratory and location: [REDACTED] (b) (4)

Date of study initiation:

Main Phase: March 12, 2002 (initiation of dosing)

Toxicokinetic Phase: March 5, 2002 (administration of first dose)

Yes, signed July 28, 2004

Yes, signed July 26, 2004

GLP compliance:
QA statement:

Oxycodone hydrochloride, Lot №
0010032, 93.7% (base is 90% of the salt)
purity

Drug, lot #, and % purity:

Naloxone hydrochloride [REDACTED] (b) (4) Lot №
C06698, 100% (base is 82% of the salt)
purity

Key Study Findings

Oxycodone/naloxone (0.0/0.0, 4.0/0.34, 10.0/0.85, 25.0/2.13 mg/kg/day or naloxone hydrochloride only (0.0/2.13 mg/kg/day) was orally administered to rats for 3 months with the following results:

| | Key Study Findings |
|--------------------|---|
| Mortality/Survival | No mortality occurred during the conduct of this study prior to terminal sacrifice. |

| | |
|-------------------------|---|
| Clinical Signs | Primary clinical signs observed in the MD and HD animals orally administered oxycodone/naloxone were biting/chewing on the cage, self-mutilation, and hyper-reactivity to handling. Females were more affected than the males. The gender difference is attributable to the higher systemic exposure to oxycodone in female animals. After 3-months of treatment and a 28-day recovery period, clinical signs of withdrawal were not observed. |
| Body Weight | Dose-dependent decrease in body weight was observed in the oxycodone/naloxone treatment groups. Decrease in body weight was correlated with reduction in food consumption. |
| Food Consumption | Dose-dependent reduction in food consumption was observed in the oxycodone/naloxone treatment groups. |
| Organ Weight | Statistically significantly decreased liver weights were noted for males in LD, MD, and HD dose oxycodone/naloxone groups. The liver weight change was not correlated with structural changes or liver function as assessed by serum chemistry (i.e., increased ALT, AST, bilirubin) and histopathological examinations, respectively. |
| Histopathology | No treatment-related histopathologic findings were apparent. Minimal or mild granulomatous inflammation were observed in the lungs of the MD & HD oxycodone/naloxone animals but are considered as an indirect effect of the treatment. |
| Toxicokinetic | There were adequate dose-related exposure in all dose groups; exposure to oxycodone and naloxone increased with increasing dose. Oxycodone and naloxone were rapidly absorbed; mean peak plasma was obtained at 0.5 hours post-dosing. Oxycodone metabolites noroxycodone, noroxymorphone, and oxymorphone for oxycodone and parent oxycodone were identified in the plasma after 3-months of oral dosing with oxycodone/naloxone. After 3-months of dosing with oxycodone/naloxone or naloxone, the metabolites naloxone-3-glucuronide, and 6-β-naloxol and parent naloxone were detected. |
| Summary | The administration of oxycodone/naloxone at a ratio of 12:1 elicited oxycodone-related toxicity; including overt clinical signs, decreased body weight, decreased food consumption. No apparent toxicity was noted in the naloxone treatment group only. Withdrawal signs were not observed after 3 months of dosing with oxycodone/naloxone. |

| | |
|--|---|
| | The NOAEL was identified as 25/2.13 mg/kg/day (in agreement with the Applicant) based on the reversibility of the drug-related decrease in body weight and food consumption. This NOAEL corresponds to oxycodone's AUC values of 96 and 1301ng/mL on Day 89 in males and females, respectively after repeated daily dosing for 3 months. Oxycodone's C_{max} values at this dose on Day 89 was 275 (males) and 550 (females) ng/mL. NOAEL corresponds to an HED of 24.3 mg/kg. This NOAEL corresponds to naloxone's AUC values of 2356 and 3030ng/mL on Day 89 in males and females, respectively after repeated daily dosing for 3 months. Naloxone's C_{max} values at this dose on Day 89 was 1143 (males) and 2049 (females) ng/mL. |
|--|---|

Methods

Doses:

| Group | No. of Animals | | Dose of Oxycodone (mg/kg/day) | Conc. of Oxycodone (mg/mL) | Dose of Naloxone (mg/kg/day) | Conc. of Naloxone (mg/mL) | Dosage Volume (mL/kg) |
|-------|----------------|--------|-------------------------------------|----------------------------------|------------------------------------|---------------------------------|-----------------------------|
| | Male | Female | | | | | |
| 1 | 15 (5) | 15 (5) | 0 | 0 | 0 | 0 | 10 |
| 2 | 15 | 15 | 4.0 | 0.4 | 0.34 | 0.034 | 10 |
| 3 | 15 | 15 | 10.0 | 1.0 | 0.85 | 0.085 | 10 |
| 4 | 15 (5) | 15 (5) | 25.0 | 2.5 | 2.13 | 0.213 | 10 |
| 5 | 15 (5) | 15 (5) | 0 | 0 | 2.13 | 0.213 | 10 |

Note: The first day of dosing was defined as Study Day 0. All dose levels are expressed in terms of the base. The test articles were not adjusted for moisture content. Animals in parentheses were allowed a 28-day recovery period at the end of three months of dosing.

Frequency of dosing: Once daily for 3-months
 Route of administration: Oral gavage
 Dose volume: 10 mL/kg
 Formulation/Vehicle: Solution/ Deionized distilled water
 Species/Strain: Rats/Sprague Dawley Crl:CD®(SD)IGS BR
 Number/Sex/Group:
 Main Study: 15/sex/group
 Recovery Group: 5/sex/group (Groups 1, 4, 5)
 Age: Approximately eight to nine weeks old at randomization
 Weight: Males: 294 to 351 grams at randomization
 Females: 195 to 225 grams at randomization
 Satellite groups: Satellite group were included for toxicokinetic analysis.

| Group | Oxycodone/naloxone Dose (mg/kg/day) | No of animals and gender |
|--------------|--|---------------------------------|
| | | Toxicokinetic Group |
| 1 | 0/0 (vehicle control) | 5 M and 5 F |
| 2 | 4.0/0.34 | 0 M and 0 F |
| 3 | 10.0/0.85 | 0 M and 00 F |
| 4 | 25.0/2.13 | 5 M and 5 F |
| 5 | 0.0/2.13 | 5 M and 5 F |

Unique study design: None
 Deviation from study protocol: Deviations are described under the appropriate protocol: observation(s).

Observations and Results

Toxicokinetics

Blood samples were collected from the vena cava (approx. 6 mL) for toxicokinetic evaluation prior to dosing, and at, 0.5, 1, 4, 8, 12, and 24 hours after dosing on the Days 0, 27, and 89 from 3 animals/sex/time point. Plasma concentrations of oxycodone, noroxymorphone, oxymorphone, noroxycodone, naloxone, naloxone-3-glucuronide, and 6β-naloxol were determined by using a HPLC/MS method. The lower limit of quantification was 0.5 ng/mL.

Following the oral administration of an oxycodone/naloxone mixture for 89 days, the oxycodone metabolites noroxycodone, noroxymorphone, and oxymorphone were detected in the plasma. Naloxone-3-glucuronide, naloxone, and 6-β-naloxol were also detected. The major circulating metabolites were noroxycodone and naloxone-3-glucuronide. Noroxymorphone was the only metabolite that was detected in the drug product. The Applicant summary of the toxicokinetic data for each of these compounds are summarized below.

Oxycodone

Table 34. Mean toxicokinetic data for oxycodone in rats orally administered oxycodone/naloxone for 3 months

| Dose (oxy/nal) mg/kg/day | Day | Gender | t _{max} (h) | C _{max} (ng/mL) | N C _{max} | AUC ₀₋₁ (ng•h/mL) | N AUC | R* |
|--------------------------------|-----|--------|-------------------------|-----------------------------|--------------------|---------------------------------|-------|-----|
| 4/0.34 | 0 | M | 0.5 | 10.8 | 2.70 | 30.2 | 7.56 | NA |
| | | F | 0.5 | 46.0 | 11.5 | 120 | 29.9 | NA |
| | 27 | M | 0.5 | 28.2 | 7.05 | 61.9 | 15.5 | 2.0 |
| | | F | 0.5 | 93.5 | 23.4 | 187 | 46.6 | 1.6 |
| | 89 | M | 0.5 | 33.8 | 8.45 | 44.9 | 11.2 | 1.5 |
| | | F | 0.5 | 88.0 | 22.0 | 180 | 45.1 | 1.5 |
| 10/0.85 | 0 | M | 0.5 | 22.7 | 2.27 | 62.0 | 6.20 | NA |
| | | F | 0.5 | 70.1 | 7.01 | 440 | 44.0 | NA |
| | 27 | M | 0.5 | 108 | 10.8 | 180 | 18.0 | 2.9 |
| | | F | 0.5 | 374 | 37.4 | 644 | 64.4 | 1.5 |
| | 89 | M | 0.5 | 70.0 | 7.00 | 193 | 19.3 | 3.1 |
| | | F | 0.5 | 249 | 24.9 | 544 | 54.4 | 1.2 |
| 25/2.13 | 0 | M | 0.5 | 53.6 | 2.14 | 254 | 10.2 | NA |
| | | F | 0.5 | 150 | 6.00 | 993 | 39.7 | NA |
| | 27 | M | 0.5 | 227 | 9.08 | 552 | 22.1 | 2.2 |
| | | F | 0.5 | 1040 | 41.6 | 1886 | 75.4 | 1.9 |
| | 89 | M | 0.5 | 275 | 11.0 | 496 | 19.8 | 2.0 |
| | | F | 0.5 | 550 | 22.0 | 1301 | 52.0 | 1.3 |

* R: Accumulation factor = AUC_{day D}/AUC_{day 0}

- On Days 0, 27, and 89, C_{max} was reached at 0.5 h after dosing.
- C_{max} and AUC increased in a dose-proportional manner.
- Some accumulation occurred in both males and females after repeat-dosing.

Noroxymorphone

Table 35. Mean toxicokinetic data for noroxymorphone in rats orally administered oxycodone/naloxone for 3 months

| Dose (oxy/nal) mg/kg/day | Day | Gender | t _{max} (h) | C _{max} (ng/mL) | N C _{max} | AUC ₀₋₁ (ng·h/mL) | N AUC | R* |
|--------------------------------|-----|--------|-------------------------|-----------------------------|--------------------|---------------------------------|-------|-----|
| 4/0.34 | 0 | M | 0.5 | 34.2 | 8.55 | 139 | 34.7 | NA |
| | | F | 0.5 | 4.60 | 1.15 | 22.2 | 5.55 | NA |
| | 27 | M | 0.5 | 45.4 | 11.4 | 136 | 34.1 | 1.0 |
| | | F | 0.5 | 13.4 | 3.35 | 29.4 | 7.35 | 1.3 |
| | 89 | M | 0.5 | 60.9 | 15.2 | 113 | 28.3 | 0.8 |
| | | F | 1 | 12.1 | 3.03 | 31.3 | 7.83 | 1.4 |
| 10/0.85 | 0 | M | 0.5 | 135 | 13.5 | 280 | 28.0 | NA |
| | | F | 0.5 | 13.8 | 1.38 | 55.2 | 5.52 | NA |
| | 27 | M | 0.5 | 120 | 12.0 | 349 | 34.9 | 1.2 |
| | | F | 0.5 | 28.5 | 2.85 | 75.1 | 7.51 | 1.4 |
| | 89 | M | 0.5 | 158 | 15.8 | 431 | 43.1 | 1.5 |
| | | F | 0.5 | 50.5 | 5.05 | 87.6 | 8.76 | 1.6 |
| 25/2.13 | 0 | M | 0.5 | 139 | 5.56 | 999 | 40.0 | NA |
| | | F | 1 | 15.4 | 0.616 | 137 | 5.47 | NA |
| | 27 | M | 0.5 | 525 | 21.0 | 1227 | 49.1 | 1.2 |
| | | F | 0.5 | 81.2 | 3.25 | 250 | 10.0 | 1.8 |
| | 89 | M | 0.5 | 420 | 16.8 | 1074 | 43.0 | 1.1 |
| | | F | 0.5 | 65.3 | 2.61 | 239 | 9.55 | 1.7 |
| 0/2.13 | 0 | M | 0.5 | 11.4 | 5.35 | 37.9 | 17.8 | NA |
| | | F | 1 | 6.84 | 3.21 | 23.1 | 10.9 | NA |
| | 27 | M | 0.5 | 12.2 | 5.73 | 49.3 | 23.2 | 1.3 |
| | | F | 0.5 | 7.74 | 3.63 | 27.1 | 12.7 | 1.2 |
| | 89 | M | 0.5 | 17.0 | 7.98 | 51.5 | 24.2 | 1.4 |
| | | F | 0.5 | 12.4 | 5.82 | 34.6 | 16.3 | 1.5 |

* R: Accumulation factor = AUC_{day 0}/AUC_{day 1}

- Noroxymorphone is a known metabolite of naloxone and oxycodone. It was present in the plasma in all oxycodone/naloxone treatment groups and naloxone only group.
- C_{max} was reached at 0.5 h after dosing on Days 0, 27, and 89.

Noroxycodone**Table 36. Mean toxicokinetic data for noroxycodone in rats orally administered oxycodone/naloxone for 3 months**

| Dose (oxy/nal) mg/kg/day | Day | Gender | t _{max} (h) | C _{max} (ng/mL) | N C _{max} | AUC _{0-t} (ng•h/mL) | N AUC | R* |
|--------------------------------|-----|--------|-------------------------|-----------------------------|--------------------|---------------------------------|-------|-----|
| 4/0.34 | 0 | M | 0.5 | 108 | 27.0 | 429 | 107 | NA |
| | | F | 0.5 | 48.2 | 12.1 | 150 | 37.5 | NA |
| | 27 | M | 0.5 | 191 | 47.8 | 489 | 122 | 1.1 |
| | | F | 0.5 | 118 | 29.5 | 274 | 68.5 | 1.8 |
| 10/0.85 | 89 | M | 0.5 | 257 | 64.3 | 382 | 95.5 | 0.9 |
| | | F | 0.5 | 99.6 | 24.9 | 230 | 57.5 | 1.5 |
| | 0 | M | 0.5 | 263 | 26.3 | 846 | 84.6 | NA |
| | | F | 0.5 | 87.0 | 8.70 | 450 | 45.0 | NA |
| 25/2.13 | 27 | M | 0.5 | 751 | 75.1 | 1681 | 168 | 2.0 |
| | | F | 0.5 | 373 | 37.3 | 950 | 95.0 | 2.1 |
| | 89 | M | 0.5 | 698 | 69.8 | 1750 | 175 | 2.1 |
| | | F | 0.5 | 299 | 29.9 | 663 | 66.3 | 1.5 |
| | 0 | M | 0.5 | 412 | 16.5 | 2564 | 103 | NA |
| | | F | 1 | 153 | 6.12 | 1156 | 46.2 | NA |
| | 27 | M | 0.5 | 2000 | 80.0 | 5632 | 225 | 2.2 |
| | | F | 0.5 | 955 | 38.2 | 2491 | 99.6 | 2.2 |
| | 89 | M | 0.5 | 1257 | 50.3 | 4005 | 160 | 1.6 |
| | | F | 0.5 | 593 | 23.7 | 2028 | 81.1 | 1.8 |

* R: Accumulation factor= AUC_{day D}/AUC_{day 0}

- C_{max} was reached at 0.5 h after dosing on Days 0, 27 and 89.

Naloxone**Table 37. Mean toxicokinetic data for naloxone in rats orally administered oxycodone/naloxone for 3 months**

| Dose (oxynal) mg/kg/day | Day | Gender | t _{max} (h) | C _{max} (μ g/mL) | N C _{max} | AUC _{0-t} (μ g·h/mL) | N AUC | R* |
|-------------------------------|-----|--------|-------------------------|-----------------------------------|--------------------|---------------------------------------|-------|-----|
| 4/0.34 | 0 | M | 0.5 | 74.1 | 218 | 228 | 671 | NA |
| | | F | 1 | 168 | 494 | 525 | 1543 | NA |
| | 27 | M | 0.5 | 141 | 415 | 518 | 1522 | 2.3 |
| | | F | 0.5 | 544 | 1600 | 947 | 2784 | 1.8 |
| 10/0.85 | 89 | M | 0.5 | 180 | 529 | 418 | 1229 | 1.8 |
| | | F | 0.5 | 351 | 1032 | 521 | 1532 | 1.0 |
| | 0 | M | 0.5 | 193 | 227 | 804 | 946 | NA |
| | | F | 1 | 266 | 313 | 1199 | 1411 | NA |
| 25/2.13 | 27 | M | 0.5 | 606 | 713 | 1544 | 1817 | 1.9 |
| | | F | 0.5 | 755 | 888 | 1855 | 2183 | 1.5 |
| | 89 | M | 0.5 | 327 | 385 | 1185 | 1394 | 1.5 |
| | | F | 0.5 | 640 | 753 | 1423 | 1675 | 1.2 |
| 0/2.13 | 0 | M | 0.5 | 245 | 115 | 1809 | 849 | NA |
| | | F | 1 | 1854 | 870 | 4996 | 2346 | NA |
| | 27 | M | 0.5 | 1441 | 677 | 3205 | 1505 | 1.8 |
| | | F | 0.5 | 4852 | 2278 | 6696 | 3144 | 1.3 |
| 89 | M | 0.5 | 1522 | 715 | 3261 | 1531 | 1.8 | |
| | | F | 0.5 | 2112 | 992 | 2946 | 1383 | 0.6 |
| | 0 | M | 0.5 | 2090 | 981 | 2403 | 1128 | NA |
| | | F | 0.5 | 2186 | 1026 | 3186 | 1496 | NA |
| 0/2.13 | 27 | M | 0.5 | 830 | 390 | 2425 | 1139 | 1.0 |
| | | F | 0.5 | 2983 | 1400 | 4667 | 2191 | 1.5 |
| | 89 | M | 0.5 | 1143 | 537 | 2356 | 1106 | 1.0 |
| | | F | 0.5 | 2049 | 962 | 3030 | 1422 | 1.0 |

* R: Accumulation factor = AUC_{day 89} / AUC_{day 0}

Naloxone-3-glucuronide**Table 38. Mean toxicokinetic data for naloxone-3-glucuronide in rats orally administered oxycodone/naloxone for 3 months**

| Dose (oxynal) mg/kg/day | Day | Gender | t _{max} (h) | C _{max} (pg/mL) | N C _{max} | AUC ₀₋₁ (pg•h/mL) | N AUC | R* |
|-------------------------------|-----|--------|-------------------------|-----------------------------|--------------------|---------------------------------|--------|-----|
| 4/0.34 | 0 | M | 0.5 | 15795 | 46456 | 51595 | 151750 | NA |
| | | F | 0.5 | 19595 | 57632 | 53613 | 157685 | NA |
| | 27 | M | 0.5 | 19943 | 58656 | 48398 | 142348 | 0.9 |
| | | F | 1 | 29220 | 85941 | 87460 | 257235 | 1.6 |
| | 89 | M | 0.5 | 28138 | 82759 | 44207 | 130021 | 0.9 |
| | | F | 0.5 | 27556 | 81047 | 46812 | 137682 | 0.9 |
| 10/0.85 | 0 | M | 0.5 | 48375 | 56912 | 87268 | 102668 | NA |
| | | F | 0.5 | 27016 | 31784 | 116453 | 137004 | NA |
| | 27 | M | 0.5 | 69082 | 81273 | 155457 | 182890 | 1.8 |
| | | F | 0.5 | 103418 | 121668 | 139448 | 164056 | 1.2 |
| | 89 | M | 0.5 | 52173 | 61380 | 136752 | 160884 | 1.6 |
| | | F | 0.5 | 121458 | 142892 | 184679 | 217269 | 1.6 |
| 25/2.13 | 0 | M | 0.5 | 71807 | 33712 | 284019 | 133342 | NA |
| | | F | 0.5 | 60674 | 28485 | 280573 | 131724 | NA |
| | 27 | M | 0.5 | 94211 | 44231 | 221085 | 103796 | 0.8 |
| | | F | 0.5 | 236796 | 111172 | 341962 | 160546 | 1.2 |
| | 89 | M | 0.5 | 146474 | 68767 | 312858 | 146882 | 1.1 |
| | | F | 0.5 | 148299 | 69624 | 306568 | 143929 | 1.1 |
| 0/2.13 | 0 | M | 0.5 | 252025 | 118322 | 280294 | 131593 | NA |
| | | F | 0.5 | 242419 | 113812 | 339261 | 159277 | NA |
| | 27 | M | 0.5 | 176519 | 82873 | 327768 | 153881 | 1.2 |
| | | F | 0.5 | 255402 | 119907 | 485950 | 228145 | 1.4 |
| | 89 | M | 0.5 | 301391 | 141498 | 490293 | 230184 | 1.7 |
| | | F | 0.5 | 383187 | 179900 | 456169 | 214164 | 1.3 |

* R: Accumulation factor = AUC_{day 0}/AUC_{day 89}

- C_{max} was reached at 0.5 h after dosing on Days 0, 27 and 89.

6 β -Naloxol**Table 39. Mean toxicokinetic data for 6 β -naloxol in rats orally administered oxycodone/naloxone for 3 months**

| Dose (oxy/nal) mg/kg/day | Day | Gender | t _{max} (h) | C _{max} (μ g/mL) | N C _{max} | AUC _{0-t} (μ g•h/mL) | N AUC | R* |
|--------------------------------|-----|--------|-------------------------|-----------------------------------|--------------------|---------------------------------------|-------|-----|
| 4/0.34 | 0 | M | NC | NC | NC | NC | NC | NC |
| | | F | 0.5 | 18.0 | 52.9 | 109 | 319 | NA |
| | 27 | M | NC | NC | NC | NC | NC | NC |
| | | F | 0.5 | 58.2 | 171 | 224 | 658 | 2.1 |
| 10/0.85 | 89 | M | NC | NC | NC | NC | NC | NC |
| | | F | 0.5 | 34.3 | 101 | 219 | 643 | 2.0 |
| | 0 | M | 0.5 | 10.5 | 12.4 | NR | NR | NA |
| | | F | 0.5 | 37.6 | 44.2 | 461 | 543 | NA |
| 25/2.13 | 27 | M | 0.5 | 46.2 | 54.4 | 241 | 283 | NC |
| | | F | 0.5 | 162 | 191 | 634 | 746 | 1.4 |
| | 89 | M | 0.5 | 25.2 | 29.6 | 213 | 250 | NC |
| | | F | 0.5 | 209 | 246 | 520 | 611 | 1.1 |
| 0/2.13 | 0 | M | 0.5 | 14.7 | 6.90 | NR | NR | NA |
| | | F | 0.5 | 101 | 47.4 | 882 | 414 | NA |
| | 27 | M | 1 | 106 | 49.8 | 409 | 192 | NC |
| | | F | 1 | 514 | 241 | 2256 | 1059 | 2.6 |
| 89 | M | 0.5 | 150 | 70.4 | 465 | 218 | NC | |
| | | F | 0.5 | 414 | 194 | 1927 | 905 | 2.2 |
| | 0 | M | 0.5 | 49.2 | 23.1 | 302 | 142 | NA |
| | | F | 1 | 195 | 91.5 | 1644 | 772 | NA |
| 0/2.13 | 27 | M | 0.5 | 35.4 | 16.6 | 315 | 148 | 1.0 |
| | | F | 0.5 | 190 | 89.2 | 2115 | 993 | 1.3 |
| | 89 | M | 0.5 | 41.9 | 19.7 | 213 | 99.8 | 0.7 |
| | | F | 0.5 | 273 | 128 | 2798 | 1313 | 1.7 |

* R: Accumulation factor = AUC_{day D}/AUC_{day 0}

- “There was a gender difference in the exposure of 6 β -Naloxol, with female rats showing exposure values higher than in male rats.”
- 6 β -Naloxol was not present in the plasma of males in the LD group. It was present, but at very low levels following all doses of oxycodone/naloxone.

Mortality

All animals were observed twice daily (morning and afternoon) for morbidity and mortality.

No deaths occurred. All animals survived to scheduled euthanasia at the end of the treatment period and recovery period.

Clinical Signs

Detailed cageside clinical observations were performed weekly for signs of toxicity prior to dosing. Cageside observations were performed daily for signs of overt toxic effects prior to dosing and between 0.5- and 2-hours after dosing. "On Study Day 0 only, each animal was examined (cage-side) prior to dosing and at approximately 15 minutes, 30 minutes, 1 hour, 2 hours, and 4 hours after dosing." During the recovery period, naloxone cageside observations were performed daily. On the scheduled day of euthanasia at the end of the treatment or recovery period, a detailed clinical observation was performed.

Table 40. Clinical signs observed in rats orally administered oxycodone/naloxone or naloxone for 3-months.

| Clinical Observation | Dose (mg/kg/day) | | | | | |
|------------------------------|----------------------|------|------|------|-------|------|
| | Oxycodone | 0.0 | 4.0 | 10.0 | 25.0 | 0 |
| | Naloxone | 0 | 0.34 | 0.85 | 2.13 | 2.13 |
| No in Group | | 15 | 15 | 15 | 15 | 15 |
| Males (frequency/animals) | | | | | | |
| Biting/Chewing on Cage | Post-dose: 0.5 – 2 h | 0/0 | 0/0 | 1/1 | 13/10 | 0/0 |
| Increased Activity | Post-dose: 0.5 – 2 h | 0/0 | 0/0 | 0/0 | 1/1 | 0/0 |
| Vocalization | | 0/0 | 0/0 | 1/1 | 3/3 | 0/0 |
| Hyper-reactivity to handling | | 0/0 | 0/0 | 1/1 | 1/1 | 0/0 |
| Hair loss | | 21/4 | 42/4 | 43/6 | 95/12 | 26/7 |
| Scabs | | 4/1 | 15/5 | 5/3 | 3/1 | 2/2 |
| Self-Mutilation | Post-dose: 0.5 – 2 h | 0/0 | 0/0 | 0/0 | 2/2 | 0/0 |
| Females | | | | | | |
| Vocalization | | 0/0 | 0/0 | 0/0 | 6/5 | 0/0 |
| Hyper-reactivity to handling | | 0/0 | 0/0 | 0/0 | 4/4 | 0/0 |
| Hair loss | | 29/4 | 32/5 | 73/7 | 4/3 | 26/7 |
| Scabs | | 0/0 | 1/1 | 8/3 | 35/7 | 0/0 |
| Decreased Activity | Post-dose: 0.5 – 2 h | 0/0 | 0/0 | 0/0 | 7/5 | 0/0 |
| Self-Mutilation | Post-dose: 0.5 – 2 h | 0/0 | 0/0 | 1/1 | 23/8 | 0/0 |
| Biting/Chewing on Cage | Post-dose: 0.5 – 2 h | 0/0 | 0/0 | 3/2 | 6/5 | 0/0 |

Clinical signs observed in the treatment groups are presented in the table above. The observed clinical signs in both sexes were primarily seen in the oxycodone/naloxone treatment groups and not in the naloxone groups. These clinical signs were observed in the MD and HD groups and consisted primarily of biting/chewing on the cage, self-mutilation, and hyper-reactivity to handling. Females were more affected than males, which is attributable to the higher systemic exposure to oxycodone in females. No apparent clinical signs were observed in the naloxone only group. The reviewer does not consider these clinical signs to have clinical relevance.

At the end of the recovery period, these clinical signs had resolved in the HD group. Also, no withdrawal signs were observed following the repeated dosing of oxycodone/naloxone or naloxone.

Body Weights

Body weights were recorded weekly during the predose phase prior to randomization on Day -4 and Day 0. During the dosing and recovery phases, body weight was recorded weekly. During the recovery phase, individual body weights were recorded for the first five days of the recovery phase and weekly thereafter. A final fasted body weight was obtained for the main study animals on the day of scheduled euthanasia at the end of the treatment (Day 91, 92, or 93) or recovery (Day 123) period.

Table 41. Oxycodone/naloxone and naloxone effects on body weight in male rats.

| Percent Control Changes in Mean Body Weight | | | | | |
|---|--------------------------------|----------|----------------|-----------------|----------|
| | Oxycodone/Naloxone (mg/kg/day) | | | | |
| | 0.0/0/0 | 4.0/0.34 | 10.0/0.85 | 25.0/2.13 | 0.0/2.13 |
| Males | | | | | |
| No of animals examined → | 20 | 15 | 15 | 20 | 20 |
| Dosing Day | Dosing Phase | | | | |
| 0 | 349 ± 18.0 | -1.1% | -1.1% | +0.29% | -1.1% |
| 7 | 376 ± 21.8 | -2.7% | -4.0% | -4.3%** | -2.1% |
| 14 | 400 ± 23.9 | -2.25% | -4.0% | -11.8%** | -2.5% |
| 21 | 427 ± 26.1 | -2.8% | -5.6%* | -12.4%** | -1.9% |
| 28 | 451 ± 28.9 | -4.0% | -7.1%** | -15.1% | -3.3% |
| 35 | 468 ± 30.9 | -2.5% | -6.8%** | -15.4%** | -2.1% |
| 42 | 481 ± 32.8 | -3.1% | -7.9%** | -16.8%** | -2.1% |
| 49 | 497 ± 35.5 | -3.2% | -7.5%** | -17.3% | -2.0% |
| 56 | 504 ± 37.9 | -2.4% | -7.1%* | -16.5%** | -0.8% |
| 63 | 511 ± 37.0 | -2.2% | -7.6%** | -16.6%** | -0.78% |
| 70 | 519 ± 39.4 | -1.0% | -7.7%** | -15.8%** | -0.4% |
| 77 | 531 ± 44.1 | -2.6% | 8.9%** | -16.9%** | -0.9% |
| 84 | 538 ± 44.0 | -2.6% | -8.9%** | -17.8%** | -1.1% |
| 90 | 547 ± 46.2 | -2.6% | -9.0%** | -17.6%** | -0.18% |
| Recovery Phase | | | | | |
| Recovery Day | Recovery Phase | | | | |
| No of animals examined → | 5 | | | 5 | 5 |
| Dosing Day 94 | 555 ± 67.2 | | | -18.9%** | -4.7% |

| | Percent Control Changes in Mean Body Weight | | | | |
|-----|---|----------|-----------|-----------|----------|
| | Oxycodone/Naloxone (mg/kg/day) | | | | |
| | 0.0/0.0 | 4.0/0.34 | 10.0/0.85 | 25.0/2.13 | 0.0/2.13 |
| 95 | 553 ± 70.1 | | | -21.1%** | -3.8% |
| 96 | 554 ± 68.6 | | | -18.4%* | -3.4% |
| 97 | 558 ± 71.4 | | | -16.1%* | -3.9% |
| 98 | 555 ± 71.0 | | | -14.4% | -3.8% |
| 100 | 558 ± 68.7 | | | -13.1% | -4.1% |
| 107 | 572 ± 71.7 | | | -10.5% | -4.4% |
| 114 | 575 ± 73.3 | | | -7.8% | -3.5% |
| 121 | 584 ± 75.7 | | | -6.8% | -3.1% |

*: Statistically significant when compared to control at $p \leq 0.05$ (ANOVA and Dunnett's)
**: Statistically significant when compared to control at $p \leq 0.01$ (ANOVA and Dunnett's)

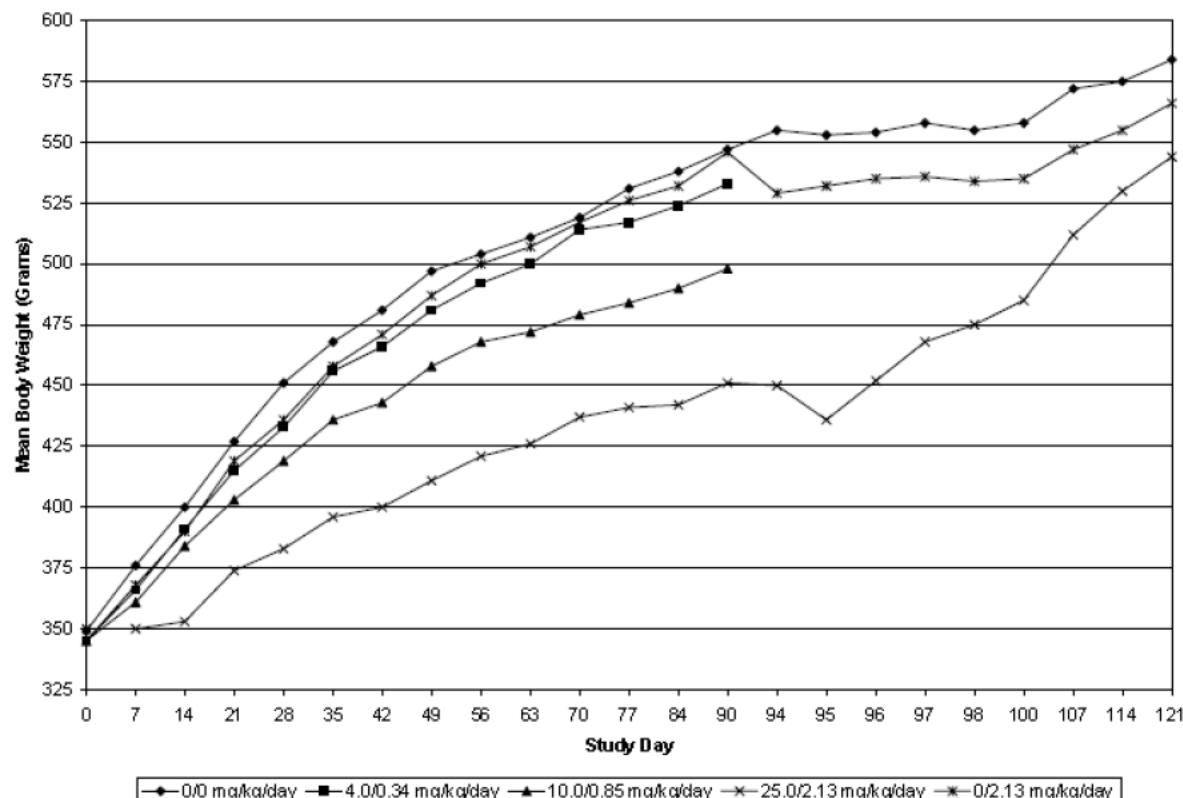
Figure 3. Graph of mean body weight values - male rats.

Table 42. Oxycodone/naloxone and naloxone effects on body weight gain in male rats.

| | Percent Control Changes in Mean Body Weight Gain | | | | |
|---------------------------------|--|----------------|-----------------|------------------|-----------------|
| | Oxycodone/Naloxone: (mg/kg/day) | | | | |
| | 0.0/0/0 | 4.0/0.34 | 10.0/0.85 | 25.0/2.13 | 0.0/2.13 |
| Nº of animals examined → | 20 | 15 | 15 | 20 | 20 |
| Days | | | Males | | |
| 0 - 7 | 27 ± 5.9 ^k | -18.5% | -40.7% | -100.0%** | -14.8% |
| 7 - 14 | 25 ± 4.9 ^k | NC | -12.0% | -84.0%** | -12.0% |
| 14 - 21 | 27 ± 4.2 ^k | -14.8% | -25.9%** | -22.2% | +7.4% |
| 21 - 28 | 24 ± 5.8 ^d | -25.0%* | -33.3%** | -62.5%** | -50.0%** |
| 28 - 35 | 17 ± 10.3 ^k | +35.3% | NC | -29.4%* | +29.4% |
| 35 - 42 | 12 ± 4.6 ^d | -16.7% | -41.7%** | -66.7%** | +16.7% |
| 42 - 49 | 16 ± 6.4 ^d | -6.3% | -6.3% | -31.3% | NC |
| 49 - 56 | 7 ± 6.3 ^k | +57.1% | +28.6% | +57.1% | +71.4 |
| 56 - 63 | 7 ± 9.2 ^d | +14.2% | -42.9% | -28.6% | +14.2% |
| 63 - 70 | 8 ± 6.4 ^k | +75% | -12.5% | +37.5% | +25.0% |
| 70 - 73 | 12 ± 7.6 ^k | -75.0%* | -58.3% | -66.7%* | -25.0% |
| 77 - 84 | 7 ± 5.2 ^k | NC | -14.3% | -85.7%** | -14.3% |
| 84 - 90 | 9 ± 5.5 ^k | NC | -11.1% | NC | +44.4% |
| | | | | | |
| | Recovery Phase | | | | |
| Nº of animals examined → | 5 | | | 5 | 5 |
| Recovery Day | | | | | |
| 90 - 94 | 2 ± 2.3 ^k | | | -900%* | -300% |
| 96 - 97 | 4 ± 3.3 ^d | | | +300%* | 50.0% |

*: Statistically significant when compared to control at p≤ 0.05 (ANOVA and Dunnett's (d))
**: Statistically significant when compared to control at p≤ 0.01 (ANOVA and Dunnett's or Kruskal-Wallis/Dunn's (k))

Males. Body weight data are presented in the tables and figure above. Dose-dependent decreases in body weight were observed in the oxycodone/naloxone treatment groups. No treatment-related changes in body weights were observed in the LD oxycodone/naloxone group and naloxone only group. Relative to control, males in the MD and HD oxycodone/naloxone groups showed a statistically significant decrease in mean body weight starting at Day 28 and Day 7 respectively. The reduction in body weight was 8 to 9% and 4 to 18% lower in the MD and HD oxycodone/naloxone groups, respectively. During the recovery period, the mean body weight of the HD oxycodone/naloxone group displayed a pattern of rebounding. At the end of the recovery period, the mean body weight was within 7% of the control group.

During Days 0-7, 14-28, and 35-42, the mean body weight gain of MD oxycodone/naloxone males was statistically significantly lower than controls. Mean body weight gain was statistically significantly lower than controls for males in the HD oxycodone/naloxone groups during Days -14, 21-42, and 70-84.

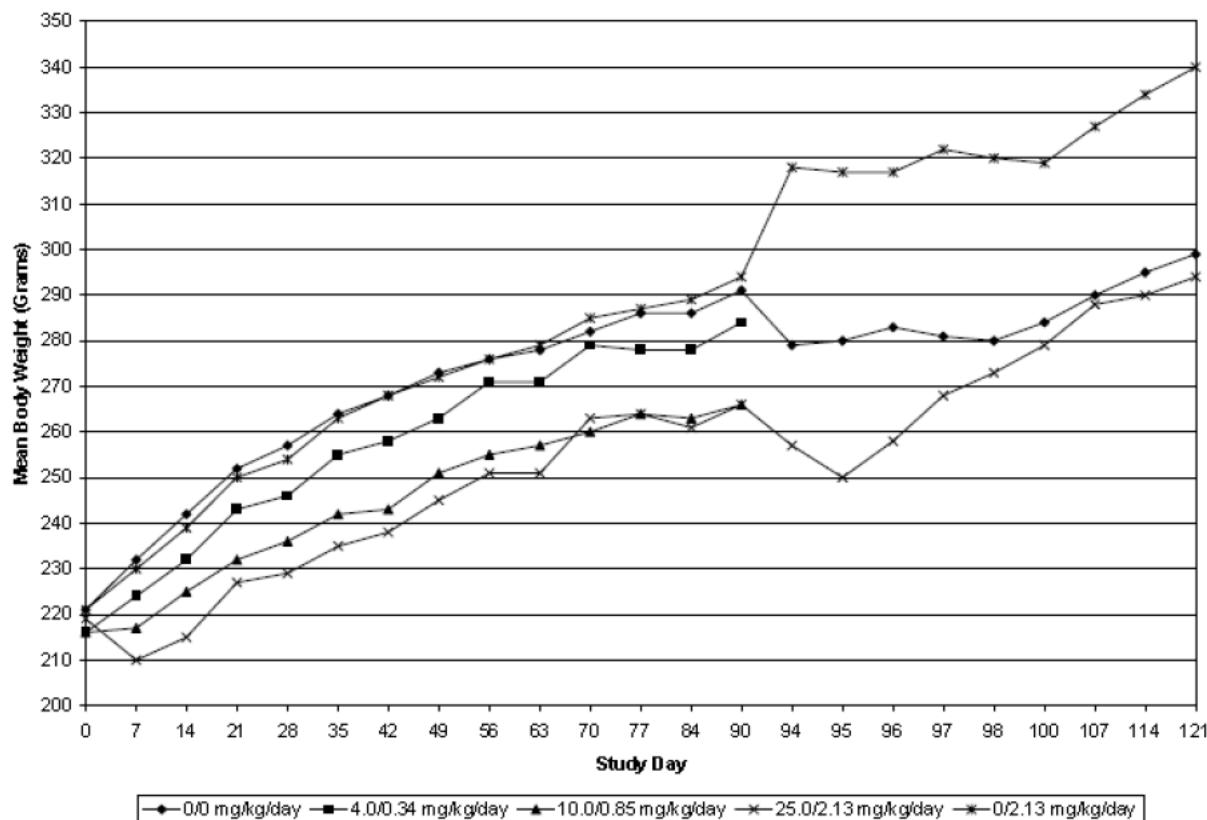
Naloxone had no effect on body weights.

Table 43. Oxycodone/naloxone and naloxone effects on body weight in female rats.

| Percent Control Changes in Mean Body Weight | | | | | |
|---|---------------------------------|--------------|-----------|-----------|----------|
| | Oxycodone/Naloxone: (mg/kg/day) | | | | |
| | 0.0/0/0 | 4.0/0.34 | 10.0/0.85 | 25.0/2.13 | 0.0/2.13 |
| Nº of animals examined → | 20 | 15 | 15 | 20 | 20 |
| | | Dosing Phase | | | |
| 0 | 221 ± 10.8 | -2.3% | -2.3% | -0.9% | -2.3% |
| 7 | 232 ± 14.7 | -3.4% | -6.5%** | -9.5%** | -0.86% |
| 14 | 242 ± 11.4 | -4.1%* | -7.0%** | -11.1%** | -1.2% |
| 21 | 252 ± 11.5 | -3.6% | -7.9%** | -9.9%** | 0.8% |
| 28 | 257 ± 12.1 | -4.3% | -8.2%** | -10.9%** | -1.2% |
| 35 | 264 ± 15.2 | -3.4% | -8.3%** | -11.0%** | -0.38% |
| 42 | 268 ± 14.9 | -3.7% | -9.3%** | -11.2%** | NC |
| 49 | 273 ± 14.7 | -3.2% | -8.1%** | -10.2%** | -0.37% |
| 56 | 276 ± 16.1 | -1.8% | -7.6%** | -9.1%** | NC |
| 63 | 278 ± 16.8 | -2.5% | -7.6%** | -9.7%** | -0.36% |
| 70 | 282 ± 15.3 ^k | -1.1% | -7.8%** | -6.7%** | +1.1% |
| 77 | 286 ± 44.1 ^k | -2.8% | -7.7%** | -7.7%** | +0.35% |
| 84 | 286 ± 16.0 ^k | -2.8% | -8.0%** | -8.7%** | -1.0% |
| 90 | 291 ± 17.6 ^k | -2.4% | -8.6%** | -8.6%** | +1.0% |
| | | | | | |
| Recovery Day | Recovery Phase | | | | |
| Nº of animals examined → | 5 | | | 5 | 5 |
| Dosing Day 94 | 279 ± 14.1 | | | -10.7% | +14.0%* |
| 95 | 280 ± 13.7 | | | -10.7% | -13.2%* |
| 96 | 283 ± 12.3 | | | -8.8% | +12.0% |
| 97 | 281 ± 13.8 ^k | | | -4.6%* | +14.6% |
| 98 | 280 ± 16.7 ^k | | | -2.5% | -14.2% |
| 100 | 284 ± 14.6 ^k | | | -1.7% | +12.3% |
| 107 | 290 ± 16.4 ^k | | | -0.69 | +12.8% |
| 114 | 295 ± 18.8 ^k | | | -1.7% | +13.2% |
| 121 | 299 ± 20.5 ^k | | | -1.6% | +13.7% |

*: Statistically significant when compared to control at p≤ 0.05 (ANOVA and Dunnett's or Kruskal-Wallis/Dunn's (k))

**: Statistically significant when compared to control at p≤ 0.01 (ANOVA and Dunnett's)

Figure 4. Graph of mean body weight values - female rats.**Table 44. Oxycodone/naloxone and naloxone effects on body weight gain in female rats.**

| | Percent Control Changes in Mean Body Weight Gain | | | | |
|-------------------------------|--|----------|------------|------------|------------|
| | Oxycodone/Naloxone: (mg/kg/day) | | | | |
| | 0.0/0/0 | 4.0/0.34 | 10.0/0.85 | 25.0/2.13 | 0.0/2.13 |
| No of animals examined | 20 | 15 | 15 | 20 | 20 |
| → | | | | | |
| Days | | | | | |
| 0 - 7 | 10 ± 8.2 ^d | -20.0% | -100.0%** | -190.0%** | -10.0% |
| 7 - 14 | 11 ± 6.3 ^k | -36.3% | -27.2% | -54.5%** | -18.2% |
| 14 - 21 | 10 ± 4.2 ^d | +10.0% | -20.0% | +20.0% | +10.0% |
| 21 - 28 | 6 ± 4.1 ^d | -33.3% | -33.3% | -50.0% | -16.7% |
| 28 - 35 | 7 ± 6.9 ^d | +28.6% | -14.3% | -14.3% | +14.3% |
| 35 - 42 | 4 ± 5.4 ^d | -50.0% | -75.0% | -25.0% | +50.0% |
| 42 - 49 | 6 ± 4.1 ^d | -16.7% | +33.3% | +16.7% | -33.3% |
| 49 - 56 | 2 ± 5.7 ^k | +350% | +100.0% | +200.0% | +100.0% |
| 56 - 63 | 2 ± 6.1 ^d | -100.0% | -50.0% | -150.0% | +50.0% |
| 63 - 70 | 5± 3.5 ^k | +60.0% | -40.0% | +140.0%** | +20.0% |
| 70 - 77 | 4 ± 4.3 ^d | -125.0% | NC | -75.0% | -50.0% |
| 77 - 84 | 0 ± 5.5 ^d | NC | -1.0 ± 4.8 | -3.0 ± 7.4 | 2.0 ± 4.9% |
| 84 - 90 | 5 ± 5.1 ^d | +20.0% | -40.0% | NC | NC |

| Percent Control Changes in Mean Body Weight Gain | | | | | |
|--|---------------------------------|----------|-----------|------------|-----------|
| | Oxycodone/Naloxone: (mg/kg/day) | | | | |
| | 0.0/0/0 | 4.0/0.34 | 10.0/0.85 | 25.0/2.13 | 0.0/2.13 |
| Nº of animals examined → | Recovery Phase 5 | | | 5 | 5 |
| 96– 97 | -2 ± 1.9 ^d | | | 10 ± 2.6** | 5 ± 2.2** |

**: Statistically significant when compared to control at $p \leq 0.01$ (ANOVA and Dunnett's(d) or Kruskal-Wallis/Dunn's (k))

Females. Body weight data are presented in the tables and figure above. Mean body weights of females in the high-dose naloxone alone group were comparable to controls throughout the treatment period. Dose-dependent decrease in body weight was observed in the oxycodone/naloxone treatment groups. Mean body weights of females in the LD oxycodone/naloxone group was statistically significant lower than the controls on Day 14; however this difference was not considered to be toxicologically significant since the mean body weights remained within approximately 4% of controls throughout the dosing period. No treatment-related changes in body weights were observed in the LD oxycodone/naloxone group and naloxone only group. Relative to control, females in the MD and HD oxycodone/naloxone groups showed a statistically significant decrease in mean body weight from Study Days 7 to 90. The reduction in body weight was 7 to 9% and 7 to 11% lower in the MD and HD oxycodone/naloxone groups, respectively. During the recovery period, the mean body weight of the HD oxycodone/naloxone group remained slightly but not statistically lower than the controls during the first four days of the recovery period. However, at the end of the recovery period, relative to the control group, the mean body weight of the HD females was within 2% of the control animals.

Mean body weight gain was statistically higher than controls for females in the high-dose naloxone alone group during Day 96-97. Mean body weight gain was statistically lower than controls for females in the mid-dose oxycodone/naloxone group during Days 0-7 and for females in the high-dose oxycodone/naloxone group during Days 0-14. In contrast to these changes, mean body weight gain of females in the high-dose oxycodone/naloxone group was statistically higher than controls during Days 63-70. Slight but not statistically significant body weight loss occurred for females in the high-dose oxycodone/naloxone group during Days 90-94 and 94-95. However, for the remainder of the recovery period, mean body weight gain of females in the high-dose oxycodone/naloxone group was comparable to or exceeded controls. Mean body weight gain of HD group was statistically higher than controls during Day 96-97. No statistically significant differences in mean body weight gain were noted for females in the low-dose oxycodone/naloxone group during the treatment period. Also, mean body weight gain of females in the high-dose naloxone alone group was comparable to controls throughout the treatment period.

No statistically significant differences in body weight gain were observed in females in the HLD oxycodone/naloxone group. During Days 0-7 and Days 0-14, the mean body weight gain of females in the MD and HD oxycodone/naloxone was statistically significantly lower than controls, respectively. Mean body weight gain of females in the HD oxycodone/naloxone group was statistically higher than controls during Days 63-70.

"However, for the remainder of the recovery period, mean body weight gain of females in the high-dose oxycodone/naloxone group was comparable to or exceeded controls. *The observed oxycodone/naloxone-related reduction in mean body weight and mean body weight gain was correlated to the observed reduction in food consumption. The reviewer does not consider the effects on body weight gain to be of toxicological significance since it was reversible.*

Food Consumption

Food consumption was recorded weekly during the pre-dosing, dosing, and recovery phases. Total food consumption was calculated as g/animals/day. Food equivalent and food efficiency was also calculated.

Naloxone had no effect on food consumption. Significant reduction in mean food consumption (grams/animal/day) was observed in both female and male rats in the low-mid- and high-dose oxycodone/naloxone groups throughout the dosing period. Relative to control, the reduction in mean food consumption was 7 to 11%, 7 to 19%, and 12-24% lower in the LD, MD, and HD oxycodone/naloxone groups, respectively. In females, the reduction in mean food consumption was 8 to 9%, 9 to 14%, and 8 to 20% lower than controls in the LD, MD, and HD oxycodone/naloxone groups, respectively.

During the recovery period, mean food consumption in males and females in the HD oxycodone/naloxone, mean food consumption was statistically lower during Days 90-96 and Days 90-94, respectively. Subsequently, at the end of the recovery period, the mean food consumption of all male and female rats dosed with HD oxycodone/naloxone was comparable to control values.

Ophthalmoscopy

Ophthalmologic examination was performed on all main study rats by a board-certified veterinary ophthalmologist on pre-dosing period (Day -20) and near the end of the dosing period (Day 90). Ophthalmologic examination included a biomicroscopic and indirect ophthalmoscopic examination. Prior to the examination, the eyes were dilated using the mydriatic agent Mydriacyl® (0.5%).

No treatment-related ophthalmic changes were observed.

ECG

Not performed.

Hematology

Blood was collected via the ocular orbit plexus from the main study rats (fasted overnight prior to sampling) prior to the scheduled euthanasia at the end of the

treatment period (Days 91, 92, or 93) and the recovery rats at the end of the recovery period (Day 122). The following hematology parameters were examined:

| Hematology | | |
|--|----------|--------------|
| Parameter | Examined | Not Examined |
| White Blood Cell Parameters | | |
| White blood cell count (WBC) | X | |
| Differential leukocyte count (% and Absolute) | X | |
| Leukocyte morphology | | X |
| Red Blood Cell Parameter | | |
| Red Blood cell count (RBC) | X | |
| Reticulocyte count (RET) | X | |
| Hemoglobin (HGB) | X | |
| Hematocrit (HCT) | X | |
| Platelet count (PLT) | X | |
| Mean platelet volume (MPV) | | X |
| Mean corpuscular volume (MCV) | X | |
| Mean corpuscular hemoglobin (MCH) | X | |
| Mean corpuscular hemoglobin concentration (MCHC) | X | |
| Red cell distribution width (RDW) | | X |
| Hemoglobin distribution width (HDW) | | X |
| Cytologic morphology | X | |
| Platelet morphology | | X |

Table 45. Incidence of notable hematological parameters in males

| Parameter | Study Day | Control | Dose (mg/kg/day) | | | |
|------------------------------|-----------|--------------|-------------------------------|-----------|-----------|---------|
| | | | 4.0/0.34 | 10.0/0.85 | 25.0/2.13 | 0/2.13 |
| Males | | | | | | |
| | | | RBC-related parameters | | | |
| HGB | 91-93 | 16.1 ± 0.46 | +3.7% | +5.0%* | +6.8%** | +3.1% |
| | 122 | 16.4 ± 0.40 | | | +0.6% | +3.0% |
| HCT | 91-93 | 41.1 ± 2.49 | +4.4% | +2.2% | +9.0%** | 2.7% |
| | 122 | 39.7 ± 1.26 | | | +1.3% | +3.8% |
| MCV | 91-93 | 49.8 ± 2.44 | +2.2% | +2.4% | +8.8%** | NC |
| | 122 | 46.8 ± 1.79 | | | +6.8%* | 3.8% |
| MCH | 91-93 | 19.6 ± 0.95 | +1.5% | +2.6% | +6.6%** | -0.5% |
| | 122 | 19.3 ± 0.76 | | | +6.2% | 3.1% |
| WBC-related parameter | | | | | | |
| Segmented Neutrophils | 91-93 | 2.53 ± 0.372 | -0.4% | +12.3% | +9.1% | +28.9%* |
| +14.0% | 122 | 2.93 ± 1.046 | | | +18.8% | +2.45% |

Table 46. Incidence of notable hematological parameters in females

| Parameter | Study Day | Control | Dose (mg/kg/day) | | | |
|----------------|-----------|---------|-------------------------------|-----------|-----------|--------|
| | | | 4.0/0.34 | 10.0/0.85 | 25.0/2.13 | 0/2.13 |
| Females | | | | | | |
| | | | RBC-related parameters | | | |

| | | | Dose (mg/kg/day) | | | |
|------------------------------|-------|--------------|---------------------|-----------|-----------|--------|
| Parameter | | Control | 4.0/0.34 | 10.0/0.85 | 25.0/2.13 | 0/2.13 |
| HGB | 91-93 | 15.2 ± 0.39 | NC | +1.3% | +5.3%* | +3.9% |
| | 122 | 15.3 ± 0.60 | | | -2.6% | -2.0% |
| HCT | 91-93 | 39.2 ± 1.60 | +3.3% | +5.4% | +11.2%** | +7.7%* |
| | 122 | 38.5 ± 1.60 | | | +2.1% | -0.8% |
| MCV | 91-93 | 52.3 ± 2.48 | +3.1% | +5.4% | +7.3%** | +1.9% |
| | 122 | 51.5 ± 1.05 | | | +0.6% | -2.5% |
| MCHC | 91-93 | 38.9 ± 1.38 | -3.3%* | -4.1%** | -5.7%** | -3.6%* |
| | 122 | 39.9 ± 2.17 | | | -5.0% | -1.5% |
| WBC-related parameter | | | | | | |
| Leukocytes | 91-93 | 5.56 ± 1.212 | +21.0% | +38.1% | +43.2%* | +19.6% |
| | 122 | 5.42 ± 1.590 | | | +3.7% | +2.6% |

Males. At the end of the treatment period, a few notable red blood cell-related parameters reached statistical significance (see reviewer-generated table, above). An increased in mean hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin was noted. At the end of the recovery period, MCV was still statistically increased compared to control.

Females. At the end of the treatment period, a few notable red blood cell-related parameters reached statistical significant (see reviewer-generated table, above). An increased in mean hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin and decrease in mean corpuscular hemoglobin concentration was noted. The effect on red-blood cells was reversible.

Reviewer comment. The mean and individual values for these red blood cells-related parameters were within the range of the SLI historical control data; hence the reviewer does not consider these changes to be toxicologically significant.

Coagulation: The following coagulation parameters were examined:

| Coagulation Parameters |
|--|
| Activated Partial Thromboplastin Time (APTT) |
| Prothrombin Time (PT) |

No treatment-related effects were observed.

Clinical Chemistry

Blood was collected were collected via the ocular orbit plexus from the main study rats (fasted overnight prior to sampling) prior to the scheduled euthanasia at the end of the treatment period (Days 91, 92, or 93) and the recovery rats at the end of the recovery period (Day 122). The following clinical chemistry parameters were examined:

| Clinical Chemistry | | |
|----------------------------------|----------|--------------|
| Parameter | Examined | Not Examined |
| Aspartate aminotransferase (AST) | X | |
| Alanine aminotransferase (ALT) | X | |
| Alkaline phosphatase (ALP) | X | |
| Blood urea nitrogen (BUN) | X | |
| Creatinine | X | |
| Glucose (GLU) | X | |
| Cholesterol (CHOL) | X | |
| Triglycerides (TRIG) | X | |
| Total protein (TP) | X | |
| Albumin (ALB) | X | |
| Globulin (GLOB) | X | |
| Albumin/globulin ratio (A/G) | X | |
| Total bilirubin (TB) | X | |
| Sodium (NA) | X | |
| Potassium (K) | X | |
| Chloride (CL) | X | |
| Calcium (CA) | X | |
| Phosphorous (PHOS) | X | |
| Gamma-glutamyl transferase (GGT) | X | |

No apparent treatment-related effects were observed in blood chemistry.

Urinalysis

The following parameters were collected the night before the initiation of blood collection for clinical pathology analysis over an approximate 16-hour interval.

| Urine Parameters |
|-------------------------------------|
| Urinalysis |
| Appearance (clarity and color) |
| Bilirubin |
| Glucose |
| Gross appearance |
| Ketones |
| Leukocytes |
| Microscopic examination of sediment |
| Occult blood |
| pH |
| Protein |
| Specific gravity |
| Urobilinogen |
| Volume |

Unremarkable, no oxycodone/naloxone or naloxone-related findings were observed on urine chemistry parameters.

Gross Pathology

All animals (fasted overnight prior to the scheduled sacrificed) were euthanized by carbon dioxide inhalation and exsanguinated and necropsied at the end of the dosing period (Day 91, 92 or 93) and recovery period (Day 122). Macroscopic evaluation was performed on all orifices, external surface of the body, and cranial, thoracic, abdominal, and pelvic cavities with their contents. "A board-certified veterinary pathologist, Dr. J. Dale Thurman, was present at the scheduled necropsies."

There were no notable treatment-related macroscopic observations.

Organ Weights

Absolute, relative organ-to-body weight and organ-to-brain weight were calculated for all the animals sacrificed at the scheduled necropsy:

| | |
|--------------------------------------|--------------------------|
| Adrenal glands (paired) | Prostate |
| Brain | Salivary gland |
| Heart | Spleen |
| Kidneys (paired) | Testes (paired) |
| Liver | Thyroid with parathyroid |
| Lung with bronchi | |
| Ovaries (paired) | |
| Pituitary gland | |
| Paired organs were weighed together. | |

Males. Statistically significant differences were observed in several organs. Most notable observations were in the thyroid gland, heart, lungs, liver, kidney, and prostate following oral administration of oxycodone/naloxone in males.

- As depicted in the table below (copied from Applicant's submission), a dose-dependent significant decrease in liver absolute weight, weight relative-to-body weight in LD, MD, and HD males compared to control treated animals and relative-to-brain weight was noted. Also, a dose-dependent significant decrease in kidney weight relative-to brain weight was observed in MD and HD males compared to control animals. A statistically significant decrease in liver weight relative-to-body weight was noted in the naloxone males compared to control treated animals. At the end of the recovery period, the organ weights of the oxycodone/naloxone and naloxone treated males were comparable to the control animals. The liver weight changes were not correlated with any microscopic changes in the liver or clinical chemistry changes indicative of liver function.

| Male Liver Weight Data | | | | | |
|--------------------------------------|--------|---------|----------|----------|---------|
| Group: | 1 | 2 | 3 | 4 | 5 |
| Oxycodone Level (mg/kg/day): | 0 | 4.0 | 10.0 | 25.0 | 0 |
| Naloxone Level (mg/kg/day): | 0 | 0.34 | 0.85 | 2.13 | 2.13 |
| Absolute Liver Weight (Grams) | | | | | |
| Study Day 91/92/93 | 14.79 | 13.33* | 12.08** | 10.21** | 13.67 |
| Study Day 122 (Recovery) | 14.54 | N/A | N/A | 12.84 | 13.77 |
| Liver to Body Weight Ratios | | | | | |
| Study Day 91/92/93 | 2.865 | 2.592** | 2.485** | 2.338** | 2.627** |
| Study Day 122 (Recovery) | 2.621 | N/A | N/A | 2.543 | 2.570 |
| Liver to Brain Weight Ratios | | | | | |
| Study Day 91/92/93 | 653.94 | 595.32 | 545.62** | 462.27** | 601.68 |
| Study Day 122 (Recovery) | 633.81 | N/A | N/A | 564.06 | 603.31 |

- A dose-dependent significant decrease in kidney weight, both absolute and relative-to-body weight was observed in LD, MD, and HD males compared to control treated animals. Also, a dose-dependent significant decrease in kidney weight relative-to-brain weight was observed in MD and HD males compared to control animals. At the end of the recovery period, the organ weight of the oxycodone/naloxone and naloxone treated males were comparable to the control animals.
- A significant decrease in thyroid gland weight, both absolute and relative-to-brain weight, was observed in HD males compared to control treated animals. At the end of the recovery period, the organ weight of the oxycodone/naloxone and naloxone treated males were comparable to the control animals.
- A significant decrease in heart weight, both absolute and relative-to-brain weight, was observed in HD males compared to control treated animals. At the end of the recovery period, the organ weight of the oxycodone/naloxone and naloxone treated males were comparable to the control animals.
- A significant decrease in prostate weight, both absolute and relative-to-brain weight, was observed in HD males compared to control treated animals. At the end of the recovery period, the organ weight of the oxycodone/naloxone and naloxone treated males were comparable to the control animals.
- A significant decrease in absolute lung weight was observed in HD males compared to control treated animals. At the end of the recovery period, the organ weight of the oxycodone/naloxone and naloxone treated males were comparable to the control animals.
- A statistically significant decrease in kidney weight relative-to-body weight and relative-to-brain weight was noted in the naloxone males compared to control treated animals. At the end of the recovery period, the organ weight of the naloxone treated males was comparable to the control animals.

Females. Statistically significant differences were observed in several organs. Most notable observations were in the brain, thyroid gland, heart, lungs, salivary gland, and spleen following oral administration of oxycodone/naloxone in females. At the end of the treatment period, a significant decrease in kidney weight relative-to-brain weight was observed in MD and HD females compared to controls. A significant increase in brain, lungs, and salivary gland weight relative-to-body weight was observed in MD and HD females compared to controls. A significant decrease in heart weight and weight relative-to-brain weight was observed in MD and HD females and MD females, respectively, compared to controls. A significant decrease in spleen weight relative-to-brain was noted in HD females compared to control treated animals. At the end of the recovery period, organ weight comparable to the control animals.

Reviewer comment. These statistically significant weight changes are considered incidental/not toxicologically significant.

Histopathology

Adequate Battery: Yes

Peer Review: Formal peer review was conducted by [REDACTED] (b) (4)
[REDACTED] (January 30, 2003).

The following tissues were collected from all animals. Histological examination was conducted on all naloxone-treated dogs, control group, and unscheduled death animals. All tissues were fixed in 10% neutral buffered formalin with the exception of bone marrow smears. Bone marrow smears were stained with Wright-Giemsa stain. Tissue selected for microscopic analysis were processed and stained with hematoxylin and eosin stain and examined by light microscopy.

| Tissue | Collected & Preserved | Microscopic Examination | | | |
|---|-----------------------|-------------------------|---------|---------|-------------|
| | | Group 1 | Group 2 | Group 3 | Group 4 & 5 |
| Adrenal gland (2) | X | X | X | X | X |
| Aorta – thoracic | X | X | X | X | X |
| Bone – femur, sternum | X | X | X | X | X |
| Bone marrow for cytology | X | X | X | X | X |
| Brain (medulla/ppons, cerebellum, cerebrum) | X | X | X | X | X |
| Clitoral gland | | | | | |
| Coagulating gland | | | | | |
| Epididymis (2) | X | X | X | X | X |
| Esophagus | X | X | X | X | X |
| Eyes | X | X | X | X | X |
| Femur (including articular surface) and bone marrow | X | X | X | X | X |
| Gallbladder | | | | | |
| Harderian gland | | | | | |

| Tissue | Collected & Preserved | Microscopic Examination | | | |
|---|-----------------------|-------------------------|---------|---------|-------------|
| | | Group 1 | Group 2 | Group 3 | Group 4 & 5 |
| Heart | X | X | X | X | X |
| Kidney | X | X | X | X | X |
| Exorbital Lacrimal gland | X | X | X | X | X |
| Large intestine, cecum | X | X | X | X | X |
| Large intestine, colon | X | X | X | X | X |
| Large intestine, rectum | | | | | |
| Larynx | | | | | X |
| Liver (3 sections collected) | X | X | X | X | X |
| Lungs (infused with formalin) with bronchi) | X | X | X | X | X |
| Lymph node, submandibular | X | X | X | X | X |
| Lymph node, mediastinal | X | X | X | X | X |
| Lymph node, mesenteric | X | X | X | X | X |
| Mammary gland | X | X | X | X | X |
| Nerve, sciatic | X | X | X | X | X |
| Ovaries | X | X | X | X | X |
| Oviduct | | | | | |
| Pancreas | X | X | X | X | X |
| Parathyroid gland | X | X | X | X | X |
| Peyer's Patch | | | | | |
| Pharynx | | | | | |
| Pituitary | X | X | X | X | X |
| Preputial gland | | | | | |
| Prostate | X | X | X | X | X |
| Salivary gland, mandibular | | | | | |
| Salivary gland, parotid | | | | | |
| Salivary gland, sublingual | | | | | |
| Salivary gland, submaxillary | X | X | X | X | X |
| Seminal vesicle | X | X | X | X | X |
| Skeletal muscle, thigh | X | X | X | X | X |
| Skin | X | X | X | X | X |
| Small intestine, duodenum | X | X | X | X | X |
| Small intestine, ileum | X | X | X | X | X |
| Small intestine, jejunum | X | X | X | X | X |
| Spinal cord, cervical | | | | | |
| Spinal cord, lumbar | X | X | X | X | X |
| Spinal cord, midthoracic | X | X | X | X | X |
| Spleen | X | X | X | X | X |
| Stomach (glandular and nonglandular) | X | X | X | X | X |
| Target Organ | | | | | |
| Testis | X | X | X | X | X |
| Thymus | X | X | X | X | X |
| Thyroid | X | X | X | X | |
| Tongue | X | X | X | X | X |
| Trachea | X | X | X | X | X |
| Ureter | | | | | |
| Urinary Bladder | X | X | X | X | X |
| Uterus | X | X | X | X | X |
| Vagina | X | X | X | X | X |
| Zymbal's gland (auditory sebaceous | | | | | |

| Tissue | Collected & Preserved | Microscopic Examination | | | |
|---------------|-----------------------|-------------------------|---------|---------|-------------|
| | | Group 1 | Group 2 | Group 3 | Group 4 & 5 |
| gland) | | | | | |
| Gross lesions | X | X | X | X | X |

Histological Findings

Table 47. Incidences of histopathologic findings - males

| Organ /Finding | Incidences of Histopathologic Findings (treatment period) - Males | | | | |
|------------------------------------|---|----------|-----------|-----------|----------|
| | Oxycodone/naloxone (mg/kg/day) | | | | |
| Nº examined → | 0/0 | 4.0/0.34 | 10.0/0.85 | 25.0/2.13 | 0.0/2.13 |
| Lung | | | | | |
| Inflammation | 2 | 1 | 5 | 9 | 3 |
| Acute, mild | 0 | 0 | 0 | 1 | 0 |
| Chronic, minimal | 1 | 1 | 3 | 0 | 3 |
| Chronic, mild | 0 | 0 | 0 | 0 | 0 |
| Chronic/active, minimal | 1 | 0 | 0 | 0 | 0 |
| Chronic/active, mild | 0 | 0 | 1 | 2 | 0 |
| Granulomatous, minimal | 0 | 0 | 1 | 3 | 0 |
| Granulomatous, mild | 0 | 0 | 0 | 3 | 0 |
| Multinucleated giant cells present | 0 | 0 | 2 | 8 | 0 |
| Within normal limits | 14 | 10 | 5 | 9 | 15 |

Table 48. Incidences of histopathologic findings - females

| Organ /Finding | Incidences of Histopathologic Findings (treatment period) - Females | | | | |
|------------------------------------|---|----------|-----------|-----------|----------|
| | Oxycodone/naloxone (mg/kg/day) | | | | |
| Nº examined → | 0/0 | 4.0/0.34 | 10.0/0.85 | 25.0/2.13 | 0.0/2.13 |
| Lung | | | | | |
| Inflammation | 2 | 2 | 6 | 12 | 0 |
| Acute, mild | 0 | 0 | 0 | 0 | 0 |
| Chronic, minimal | 1 | 1 | 1 | 2 | 0 |
| Chronic, mild | 0 | 0 | 1 | 1 | 0 |
| Chronic/active, minimal | 0 | 1 | 0 | 1 | 0 |
| Chronic/active, mild | 0 | 0 | 1 | 2 | 0 |
| Granulomatous, minimal | 1 | 0 | 0 | 3 | 0 |
| Granulomatous, mild | 0 | 0 | 4 | 4 | 0 |
| Multinucleated giant cells present | 1 | 1 | 4 | 9 | 0 |
| Within normal limits | 17 | 9 | 7 | 5 | 13 |

No treatment-related histopathologic findings were observed. However, as noted in the tables above, there was an increase incidence of inflammation in the lungs in both

sexes. The Applicant reported that the granulomatous inflammation had a multifocal distribution in the lungs of all treatment groups. But there was a significant increase in the frequency and severity of granulomatous inflammation of the lungs in the MD and HD oxycodone/naloxone groups.

Reviewer comment. *The granulomatous inflammation in the lung is considered to be an indirect effect of the treatment. It can be due to the gavage procedure or by the animals inhaling a foreign body.*

Special Evaluation

None

Dosing Solution Analysis

“Homogeneity analyses revealed that the average results of the oxycodone samples were within 5.1% of the nominal concentrations for the study.” For naloxone samples, the results revealed that the samples were within 5.9% of the nominal concentrations for the study.

Stability analyses revealed that the average results of the oxycodone and naloxone dose formulations were 6.8% and 10.4% of the dose concentration for the study respectively.

Concentration verification analyses revealed that the average results of the oxycodone and naloxone dose formulations were within 6.8% and 10.4% of the nominal concentrations for this study, respectively.

Study title: A 3-month oral (gavage) toxicity study in dogs with a 28-day recovery period with either the combination of oxycodone hydrochloride and naloxone hydrochloride [REDACTED] (b) (4) or naloxone hydrochloride [REDACTED] (b) (4) alone

Study no.: NDSE-610-GLP

Study report location: EDR

Conducting laboratory and location: [REDACTED] (b) (4)

Date of study initiation:

Males: March 5, 2003 (initiation of dosing)

Females: March 7, 2002 (initiation of dosing)

GLP compliance: Yes, signed on November 15, 2004

QA statement: Yes, signed on November 14, 2004

Drug, lot #, and % purity: Oxycodone hydrochloride, Lot № 0010032, 93.7% (base is 90% of the salt) purity

Naloxone hydrochloride [REDACTED] (b) (4) Lot № C06698, 100% (base is 82% of the salt) purity

Key Study Findings

Oxycodone/naloxone (0.0/0.0, 0.3/0.026, 4.0/0.34, 8.0/0.68 mg/kg/day or naloxone hydrochloride only (0.0/0.68 mg/kg/day) was orally administered to Beagle dogs for 3 months with the following results:

| | Key Study Findings |
|---------------------------|--|
| Mortality/Survival | No mortality occurred during the conduct of this study prior to terminal sacrifice. |
| Clinical Signs | Primary clinical signs observed in HD males and females orally administered oxycodone/naloxone were salivation, vomitus and no feces. Decrease activity was also observed in males. After 3-months of treatment and a 28-day recovery period, clinical signs of withdrawal was not observed |
| Body Weight | Significant reduction in body weight was observed during Weeks 1 and 2 in the MD and HD males and Week 1 in the MD and HD females. |
| Food Consumption | No meaningful toxicological effect was observed in food consumption in both sexes following the oral administration of oxycodone/naloxone or naloxone alone. |

| | Key Study Findings |
|-----------------------|---|
| Organ Weight | No treatment-related effects. |
| Histopathology | No treatment-related histopathologic findings were apparent. |
| Toxicokinetic | <p>There were adequate dose-related exposure in all dose groups; exposure to oxycodone and naloxone increased with increasing dose. Oxycodone and naloxone was rapidly absorbed.</p> <p>Oxycodone metabolites noroxycodone, noroxymorphone, and oxymorphone for oxycodone and parent oxycodone were identified in the plasma after 3-months of oral dosing with oxycodone/naloxone. After 3-months of dosing with oxycodone/naloxone or naloxone, the metabolites naloxone-3-glucuronide, and 6-β-naloxol and parent naloxone were detected.</p> |
| Summary | <p>The administration of oxycodone/naloxone at a ratio of 12:1 resulted in minimal systemic toxicity. Withdrawal signs were not observed after 3 months of dosing with oxycodone/naloxone.</p> <p>The NOAEL was identified as 8/0.68 mg/kg/day (not in agreement with the Applicant; Applicant identified the NOAEL as 0.3/0.026) based on the reversibility of the drug-related decrease in body weight and food consumption. This NOAEL corresponds to oxycodone's AUC values of 3568 and 2728 ng/mL on Day 89 in males and females, respectively after repeated daily dosing for 3 months. Oxycodone's C_{max} values at this dose on Day 89 was 987 (males) and 1017 (females) ng/mL.</p> <p>This NOAEL corresponds to naloxone's AUC values of 3310 and 4915 ng/mL on Day 89 in males and females, respectively after repeated daily dosing for 3 months. Naloxone's C_{max} values at this dose on Day 89 was 1079 (males) and 975 (females) ng/mL.</p> |

Methods**Doses:**

| Group | Dose of Oxycodone (mg/kg/day) | Dose of Naloxone (mg/kg/day) |
|---------------------|--|---|
| 1 (vehicle control) | 0.0 | 0.0 |
| 2 | 0.3 | 0.026 |
| 3 | 4.0 | 0.34 |
| 4 | 8.0 | 0.68 |
| 5 | 0 | 0.68 |

All doses are expressed in terms of base.

Frequency of dosing: Once daily for 90 days

Route of administration: Oral (gavage)

Dose volume: 1.0 mL/kg

Formulation/Vehicle: Solution/deionized distilled water (adjustment of pH to approx. 3.4-3.6 with 1 N HCl)

Species/Strain: Dogs/Beagle Dogs

Number/Sex/Group: 4/sex/group

Age: Approx. 5 to 6 months old (at in-life initiation of study)

Weight: Males: Approx. 7.2 to 8.9 kg (at in-life initiation of study)

Females: Approx. 5.9 to 7.8 kg (at in-life initiation of study)

Satellite groups: Satellite group were included for toxicokinetic analysis.

| Group | Oxycodone/naloxone Dose (mg/kg/day) | No of animals and gender |
|--------------|--|---------------------------------|
| | | Toxicokinetic Group |
| 1 | 0/0 (vehicle control) | 3 M and 3 F |
| 2 | 0.3/0.026 | 0 M and 0 F |
| 3 | 4.0/0.34 | 0 M and 0 F |
| 4 | 8.0/0.68 | 3 M and 3 F |
| 5 | 0.0/0.68 | 3 M and 3 F |

Unique study design: None

Deviation from study protocol: Deviations are described under the appropriate observation(s).

Observations and Results**Toxicokinetics**

Blood samples were collected from the jugular vein (approx. 5 mL) for toxicokinetic evaluation prior to dosing (0 hour), and at 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours after dosing on the Days 0, 22, and 89 from 4 animals/sex/time point. Plasma concentrations of oxycodone and its metabolites noroxymorphone, oxymorphone, noroxycodone were determined by using liquid chromatography in tandem with mass spectroscopy (LC-MS/MS) with a lower limit of quantification (LLOQ) of 0.500 ng/mL for all analytes.

Plasma concentration of naloxone and its metabolites, naloxone-3-glucuronide, and 6 β -naloxol were determined also using the LC-MS/MS method with a LLOQ of 10 pg/mL (naloxone, 6 β -naloxol) and 100.0 pg/mL (naloxone-3-glucuronide).

Following the oral administration of oxycodone/naloxone mixture or a naloxone only for 89 days, the metabolic profile in dogs was qualitatively similar to that observed in rats.

Oxycodone. Oxycodone was rapidly metabolized to oxymorphone, noroxycodone, and noroxymorphone. Oxycodone metabolites were detected as early as 0.3 hour after dosing. Parent and metabolites increased in a dose-dependent manner. No gender differences were noted in the levels of the metabolites. Noroxycodone was the major metabolite of oxycodone. Toxicokinetic data for oxycodone/naloxone following oral administration of 8.0/0.68 mg/kg/day at Day 89 is presented in the table below.

Accumulation of oxycodone and its metabolite was low in the HD group (oxycodone/naloxone 8.0/0.68 mg/kg/day). Accumulation factor ranged from 1.2 to 1.6 and 1.1 to 1.2 in male and female dogs, respectively. Accumulation factor for the major metabolite noroxycodone ranged from 1.2 to 1.5 and 1.2 to 1.4 in male and female dogs, respectively.

Table 49. Mean toxicokinetic data for oxycodone and its metabolites in beagle dogs in the high dose group of oxycodone/naloxone.

| Compound | Day | Gender | t _{max} (h) | C _{max} (ng/mL) | AUC _{0-t} (ng·h/mL) |
|----------------|-----|--------|----------------------|--------------------------|------------------------------|
| Oxycodone | 0 | M | 0.3 | 1312 | 2525 |
| | | F | 0.3 | 1094 | 2389 |
| | 22 | M | 0.6 | 869 | 2641 |
| | | F | 0.5 | 794 | 2604 |
| | 89 | M | 1.3 | 987 | 3568 |
| | | F | 0.4 | 1017 | 2728 |
| Noroxymorphone | 0 | M | 0.5 | 67.6 | 411 |
| | | F | 0.4 | 58.5 | 502 |
| | 22 | M | 1.0 | 43.2 | 466 |
| | | F | 1.4 | 39.4 | 486 |
| | 89 | M | 1.2 | 41.7 | 509 |
| | | F | 2.3 | 34.4 | 383 |
| Oxymorphone | 0 | M | 0.4 | 13.1 | 20.4 |
| | | F | 0.4 | 10.7 | 20.7 |
| | 22 | M | 0.7 | 7.17 | 19.4 |
| | | F | 0.5 | 6.84 | 20.3 |
| | 89 | M | 0.5 | 8.55 | 23.9 |
| | | F | 0.6 | 8.66 | 19.9 |
| Noroxycodone | 0 | M | 0.4 | 3177 | 11492 |
| | | F | 0.4 | 2364 | 10939 |
| | 22 | M | 1.1 | 1856 | 12348 |
| | | F | 1.8 | 1903 | 13645 |
| | 89 | M | 1.6 | 2168 | 15341 |
| | | F | 0.8 | 2227 | 14433 |

Naloxone. Naloxone was rapidly metabolized to naloxone-3-glucuronide, being detected between 0.3 to 9.7 hours after dosing. The metabolite 6β-naloxol was not detected in all of the treated animals. Parent and metabolites increased in a dose-dependent manner. No gender differences were noted in the levels of the metabolites. Toxicokinetic data for naloxone oxycodone/naloxone following oral administration of oxycodone/naloxone (8.0/0.68 mg/kg/day) at Day 89 is presented in the table below. Accumulation of naloxone and its metabolite was low in the HD group (oxycodone/naloxone 8.0/0.68 mg/kg/day) or naloxone only group.

Table 50. Mean toxicokinetic data for naloxone and its metabolites in beagle dogs in the high dose group of oxycodone/naloxone or naloxone only.

| Analyte | Oxy/Nal m g/kg/day | Day | Gender | t _{max} (h) | C _{max} (pg/m L) | AUC _{0-t} (pg·h/m L) |
|------------------------|-----------------------|-----|--------|-------------------------|------------------------------|----------------------------------|
| Naloxone | 8/0.68 | 0 | M | 0.3 | 1583 | 2768 |
| | | | F | 0.3 | 1301 | 2407 |
| | | 89 | M | 0.4 | 1079 | 4146 |
| | | | F | 0.3 | 1246 | 2822 |
| | 0/0.68 | 0 | M | 0.3 | 1721 | 3656 |
| | | | F | 0.3 | 1834 | 3988 |
| | | 89 | M | 0.3 | 1079 | 3310 |
| | | | F | 0.3 | 975 | 4915 |
| Naloxone 3-glucuronide | 8/0.68 | 0 | M | 0.3 | 577263 | 1049745 |
| | | | F | 0.3 | 460189 | 968377 |
| | | 89 | M | 0.5 | 346801 | 1642970 |
| | | | F | 0.3 | 485547 | 1006298 |
| | 0/0.68 | 0 | M | 0.6 | 382242 | 1384548 |
| | | | F | 0.5 | 481930 | 1337316 |
| | | 89 | M | 0.3 | 502465 | 1291279 |
| | | | F | 0.7 | 348155 | 1826306 |
| 6 β -Naloxol | 8/0.68 | 0 | M | NA | <10.0 | <10.0 |
| | | | F | NA | <10.0 | <10.0 |
| | | 89 | M | NA | <10.0 | <10.0 |
| | | | F | NA | <10.0 | <10.0 |
| | 0/0.68 | 0 | M | NA | <10.0 | <10.0 |
| | | | F | NA | <10.0 | <10.0 |
| | | 89 | M | NA | <10.0 | <10.0 |
| | | | F | NA | <10.0 | <10.0 |

Mortality

All animals were observed twice daily (morning and afternoon) for morbidity and mortality.

No deaths occurred. All animals survived to scheduled euthanasia at the end of the treatment period and recovery period.

Clinical Signs

Detailed (out of cage) clinical observations were performed weekly for signs of toxicity prior to dosing. Cageside observations were performed daily for signs of overt toxic

effects prior to dosing and between 0.5- and 2-hours after dosing. On Study Day 0 only, each animal was examined (cage-side) prior to dosing and at approximately 15 minutes, 30 minutes, 1 hour, 2 hours, and 4 hours after dosing. Detailed (out of cage) clinical observations were performed weekly thereafter. During the recovery period, cageside observations were performed daily. On the scheduled day of euthanasia at the end of the treatment or recovery period, a detailed clinical observation was performed.

Table 51. Clinical signs observed in dogs orally administered oxycodone/naloxone or naloxone for 3-months.

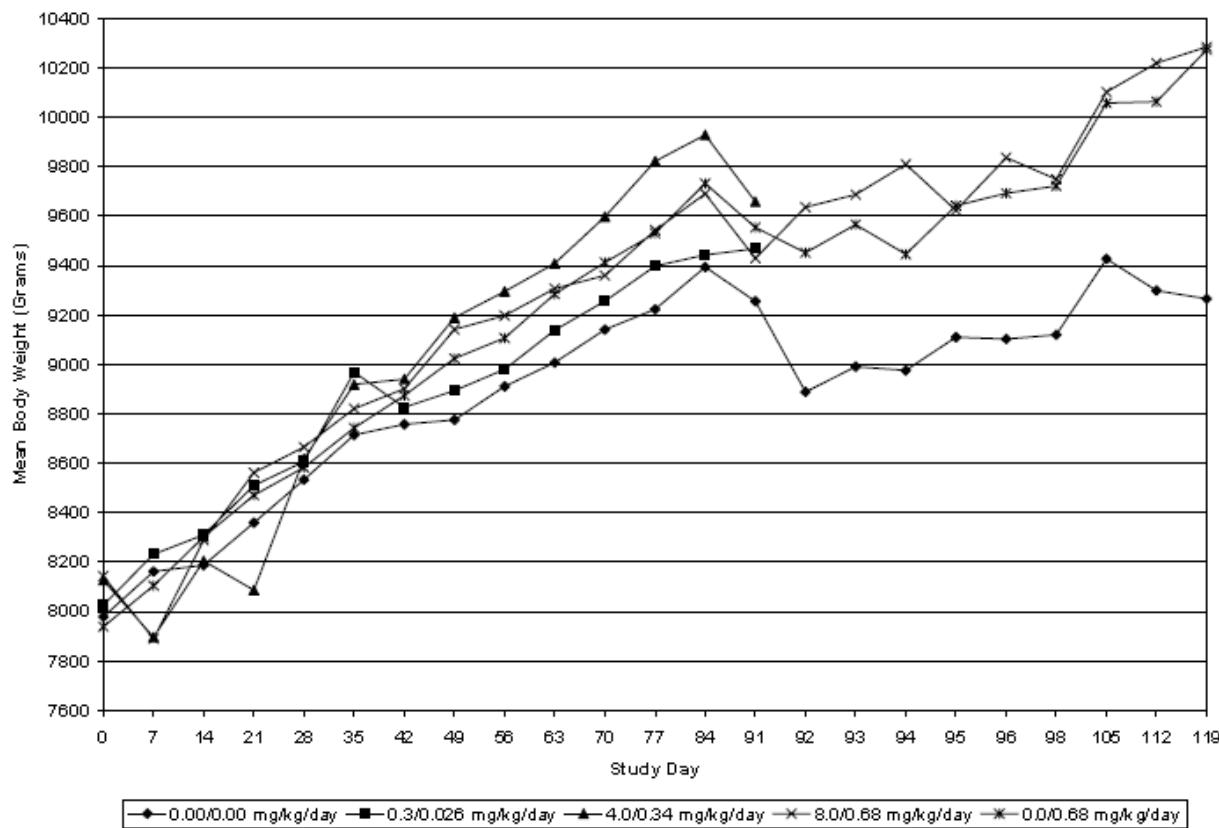
| Clinical Observation | Dose (mg/kg/day) | | | | |
|---|----------------------|-----|-------|------|-------|
| | Oxycodone | 0.0 | 0.3 | 4.0 | 8.0 |
| | Naloxone | 0.0 | 0.026 | 0.34 | 0.68 |
| № in Group | | 7 | 4 | 4 | 7 |
| Males: Clinical Observations (frequency/animals) | | | | | |
| Salivation | Pre-dose | 0/0 | 0/0 | 18/1 | 30/3 |
| Salivation | Post-dose: 0.5 – 2 h | 1/1 | 0/0 | 92/2 | 157/7 |
| Vomitus | Post-dosing | 0/0 | 0/0 | 5/3 | 16/2 |
| No feces | | 0/0 | 0/0 | 2/2 | 3/3 |
| Females: Clinical Observations (frequency/animals) | | | | | |
| Salivation | Pre-dose | 0/0 | 0/0 | 0/0 | 6/1 |
| Salivation | Post-dose: 0.5 – 2 h | 0/0 | 0/0 | 15/4 | 62/2 |
| Vomitus | Post-dose: 0.5 – 2 h | 0/0 | 1/1 | 11/2 | 2/2 |
| No Feces | | 0/0 | 0/0 | 2/2 | 3/3 |

Treatment-related clinical signs are presented in the table above. Overt clinical signs were observed in the both sexes in the MD and HD oxycodone/naloxone groups during the treatment period. Salivation, vomitus, and no feces were observed in both males and females. Decrease activity was also observed in males.

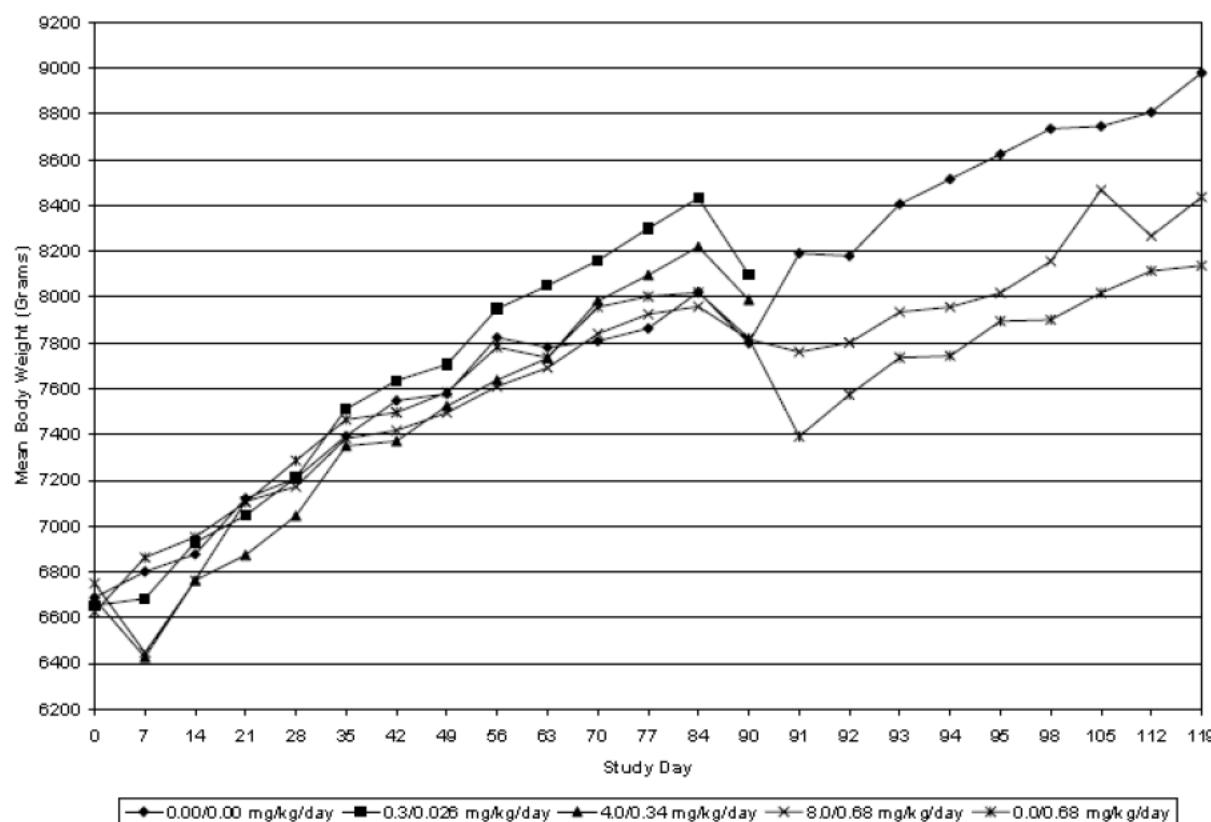
No remarkable clinical signs were observed in males or females in the HD naloxone alone group during the treatment or recovery period. Also, no withdrawal signs were observed during the recovery period.

Body Weights

Body weights were recorded on Days -8, -1 and (pretreatment) and weekly thereafter during the treatment period). During the recovery phase, individual body weights were recorded for the first five days of the recovery phase and weekly thereafter. “A final fasted body weight was recorded on the day of scheduled euthanasia at the end of the treatment or recovery period.”

Table 52. Graph of mean body weight values - male dogs.

Males. Body weight data are presented in the figure above. Compared to controls, a statistically significant decrease in mean body weight gain was observed in males at 4.0/0.34 and 8.0/0.68 mg/kg/day oxycodone/naloxone during the Weeks 1 (Days 0-7) and 2 (Days 7-14) of treatment. Naloxone had no effect on body weights.

Table 53. Graph of mean body weight values - female dogs.

Female. Body weight data are presented in the figure above. Compared to controls, a statistically significant decrease in mean body weight gain was observed in females at 4.0/0.34 and 8.0/0.68 mg/kg/day oxycodone/naloxone during the first week of dosing. Naloxone had no effect on body weights.

Food Consumption

Food consumption was recorded daily from Days -8 to -1 and from Day 0 to the day prior to scheduled euthanasia at the end of the treatment or recovery period. Food equivalent and food efficiency was also calculated.

No meaningful toxicological effect was observed in food consumption in both sexes following the oral administration of oxycodone/naloxone or naloxone alone. Significant reduction in mean food consumption (grams/animal/day) was observed in MD and HD male and HD female dogs in oxycodone/naloxone groups during the first week of dosing compared to controls. Also, during Days 32-33, 39-40, and 82-84, mean food consumption of males in the high-dose oxycodone/naloxone group was significantly reduced. Mean food consumption of males and females in the high-dose oxycodone/naloxone group was comparable to controls throughout the remaining of the treatment period and recovery period. Naloxone had no significant effect on mean food

consumption except on Days 83-83; a significant reduction in mean food consumption was observed in HD males.

Ophthalmoscopy

Ophthalmologic examination was performed on all dogs by a board-certified veterinary ophthalmologist once prior to in-life initiation (Days -19/-8 for males and on Days -21/-10 for females) pre-dosing period (Day -20), at approximately Weeks 4 and 8 and near the end of the dosing period (Day 90 for males and Day 88 for females) and recovery period (Day 118 for males and Day 116 for females). Ophthalmologic examination included a biomicroscopic and indirect ophthalmoscopic examination. Prior to the examination, the eyes were dilated using the mydriatic agent Mydriacyl® (0.5%).

No treatment-related ophthalmic changes.

ECG

Electrocardiography recordings were performed twice during the predosing phase, prior to dosing (Day 0). On Days 0, 13, 29, 55, 87 (males), and 88 (females) of the treatment period, electrocardiography recordings were performed at 0 (prior to dosing), 0.5, 2, and 24 hours after dosing and near the conclusion of the recovery period. ECG was collected using standard bipolar leads I, II, III and augmented unipolar leads aV_R, aV_L, and aV_F. Traces were analyzed for heart rate and intervals (RR, PR, QRS, and QT). QTc intervals were also measured (QTc Frederica's).

No apparent treatment-related changes were observed in PR interval, QT interval, RR interval, QT_c interval electrocardiographic complexes, QRS duration, or heart rate in either males or females orally administered oxycodone/naloxone at all dose levels.

One dog in the naloxone only group showed a rare left ventricular premature depolarization on Day 13 (at 24 h post-dosing) and Day 29 at 0.5 and 24 hours posting-dosing). All other parameters measured were unremarkable. Due to occurring in one dog, naloxone is considered to not induce ECG changes in dogs.

Hematology

Blood was collected were collected via the jugular vein from all animals (fasted overnight prior to sampling) twice prior to in-life initiation, on Day 6, Day 55, and prior to the scheduled euthanasia at the end of the treatment period (Days 91 for females and Day 92 for males) and at the end of the recovery period (Day 120). The following hematology parameters were examined:

| Hematology | | |
|--|----------|--------------|
| Parameter | Examined | Not Examined |
| White Blood Cell Parameters | | |
| White Blood cell count (WBC) | | X |
| Differential leukocyte count (% and Absolute) | X | |
| Leukocyte morphology | | X |
| Red Blood Cell Parameter | | |
| Red Blood cell count (RBC) | X | |
| Reticulocyte Count (RET) | X | |
| Hemoglobin (HGB) | X | |
| Hematocrit (HCT) | X | |
| Platelet count (PLT) | X | |
| Mean platelet volume (MPV) | | X |
| Mean corpuscular volume (MCV) | X | |
| Mean corpuscular hemoglobin (MCH) | X | |
| Mean corpuscular hemoglobin concentration (MCHC) | X | |
| Red cell distribution width (RDW) | | X |
| Hemoglobin distribution width (HDW) | | X |
| Cytologic morphology | X | |
| Platelet morphology | | X |

No treatment-related effects were noted on hematologic parameters.

Coagulation: The following coagulation parameters were examined:

| Coagulation Parameters |
|--|
| Activated Partial Thromboplastin Time (APTT) |
| Prothrombin Time (PT) |

No treatment-related effects were observed. However several statistically significant effects were noted in males and females dogs, these red blood cells-related parameters were within the range of the SLI historical control data and were not correlated with any histopathologic changes. Hence, the reviewer does not consider these changes to be toxicologically significant.

Clinical Chemistry

Blood was collected were collected via the jugular vein from all animals (fasted overnight prior to sampling) twice prior to in-life initiation, on Day 6, Day 55, and prior to the scheduled euthanasia at the end of the treatment period (Days 91 for females and Day 92 for males) and at the end of the recovery period (Day 120). The following hematology parameters were examined:

| Clinical Chemistry | | |
|----------------------------------|----------|--------------|
| Parameter | Examined | Not Examined |
| Aspartate aminotransferase (AST) | X | |
| Alanine aminotransferase (ALT) | X | |
| Alkaline phosphatase (ALP) | X | |
| Blood urea nitrogen (BUN) | X | |
| Creatinine | X | |
| Creatine phosphokinase | X | |
| Glucose (GLU) | X | |
| Cholesterol (CHOL) | X | |
| Triglycerides (TRIG) | X | |
| Total protein (TP) | X | |
| Albumin (ALB) | X | |
| Globulin (GLOB) | X | |
| Albumin/globulin ratio (A/G) | X | |
| Total bilirubin (TB) | X | |
| Sodium (NA) | X | |
| Potassium (K) | X | |
| Chloride (CL) | X | |
| Calcium (CA) | X | |
| Phosphorous (PHOS) | X | |
| Gamma-glutamyl transferase (GGT) | | X |

No apparent treatment-related effects were observed in blood chemistry.

Urinalysis

The following parameters were collected overnight from all (fasted) once prior to in-life the initiation (males on Day -12 and females on Day -13), at the end of the treatment period (males on Day 91 and females on Day 92) and at the end of the recovery period (Day 120).

| Urine Parameters |
|-------------------------------------|
| Urinalysis |
| Appearance (clarity and color) |
| Bilirubin |
| Glucose |
| Gross appearance |
| Ketones |
| Microscopic examination of sediment |
| Occult blood |
| pH |
| Protein |
| Specific gravity |
| Volume |

Unremarkable, no oxycodone/naloxone or naloxone-related findings were observed on urine chemistry parameters.

Gross Pathology

All animals (fasted overnight prior to the scheduled sacrificed) were euthanized by intravenous injection of sodium pentobarbital and exsanguinated and necropsied at the end of the dosing period (Day 91 or 92) and recovery period (Day 120). Macroscopic evaluation was performed on all orifices, external surface of the body, and cranial, thoracic, abdominal, and pelvic cavities with their contents. "A board-certified veterinary pathologist, Dr. [REDACTED]^{(b) (4)}, was present at the scheduled necropsies."

There were no notable treatment-related macroscopic observations.

Organ Weights

Absolute, relative organ-to-body weight and organ-to-brain weight were calculated for all the animals sacrificed at the scheduled necropsy:

| | |
|--|----------------------------|
| Adrenal glands (paired) | Prostate |
| Brain | Salivary gland, mandibular |
| Heart | Spleen |
| Kidneys (paired: Formal peer review was conducted by [REDACTED] ^{(b) (4)} [REDACTED] (January 30, 2003). | Testes (paired) |
| Liver with gallbladder | Thyroid with parathyroid |
| Lung with bronchi | |
| Ovaries (paired) | |
| Pituitary gland | |
| Paired organs were weighed together. | |

No treatment-related effects on organ weights were noted.

Histopathology

Adequate Battery

Peer Review: Formal peer review was conducted by [REDACTED]^{(b) (4)}
[REDACTED] (Purdue Pharma L.P., January 10, 2003).

The following tissues were collected from all animals. Histological examination was conducted on all naloxone-treated dogs, control group, and unscheduled death animals.

All tissues were fixed in 10% neutral buffered formalin with the exception of bone marrow smears. Bone marrow smears were stained with Wright-Giemsa stain. Tissue selected for microscopic analysis were processed and stained with hematoxylin and eosin stain and examined by light microscopy.

| Tissue | Collected & Preserved | Microscopic Examination | | | |
|---|-----------------------|-------------------------|---------|---------|-------------|
| | | Group 1 | Group 2 | Group 3 | Group 4 & 5 |
| Adrenal gland (2) | X | X | X | X | X |
| Aorta | X | X | X | X | X |
| Bone – femur, sternum | X | X | X | X | X |
| Bone marrow, rib | X | X | X | X | X |
| Brain (medulla/pons, cerebellum, cerebrum) | X | X | X | X | X |
| Clitoral gland | | | | | |
| Coagulating gland | | | | | |
| Ear | X | X | X | X | X |
| Epididymis (2) | X | X | X | X | X |
| Esophagus | X | X | X | X | X |
| Eyes | X | X | X | X | X |
| Femur (including articular surface) and bone marrow | X | X | X | X | X |
| Gallbladder | X | X | X | X | X |
| Harderian gland | | | | | |
| Heart | X | X | X | X | X |
| Kidney | X | X | X | X | X |
| Exorbital Lacrimal gland | X | X | X | X | X |
| Large intestine, cecum | X | X | X | X | X |
| Large intestine, colon | X | X | X | X | X |
| Large intestine, rectum | X | X | X | X | X |
| Larynx | | | | | X |
| Liver (3 sections collected) | X | X | X | X | X |
| Lungs (infused with formalin) with bronchi) | X | X | X | X | X |
| Lymph node, submandibular | X | X | X | X | X |
| Lymph node, mediastinal | X | X | X | X | X |
| Lymph node, mesenteric | X | X | X | X | X |
| Mammary gland | X | X | X | X | X |
| Nerve, sciatic | X | X | X | X | X |
| Ovaries | X | X | X | X | X |
| Oviduct | | | | | |
| Pancreas | X | X | X | X | X |
| Parathyroid gland | X | X | X | X | X |
| Peyers Patch | | | | | |
| Pharynx | | | | | |
| Pituitary | X | X | X | X | X |
| Preputial gland | | | | | |
| Prostate | X | X | X | X | X |
| Salivary gland, mandibular | X | X | X | X | X |
| Salivary gland, parotid | | | | | |
| Salivary gland, sublingual | | | | | |
| Salivary gland, submaxillary | X | X | X | X | X |
| Seminal vesicle | X | X | X | X | X |

| Tissue | Collected & Preserved | Microscopic Examination | | | |
|---|-----------------------|-------------------------|---------|---------|-------------|
| | | Group 1 | Group 2 | Group 3 | Group 4 & 5 |
| Skeletal muscle, thigh | X | X | X | X | X |
| Skin | X | X | X | X | X |
| Small intestine, duodenum | X | X | X | X | X |
| Small intestine, ileum | X | X | X | X | X |
| Small intestine, jejunum | X | X | X | X | X |
| Spinal cord, cervical | X | X | X | X | X |
| Spinal cord, lumbar | X | X | X | X | X |
| Spinal cord, midthoracic | X | X | X | X | X |
| Spleen | X | X | X | X | X |
| Stomach (glandular and nonglandular) | X | X | X | X | X |
| Target Organ | | | | | |
| Testis | X | X | X | X | X |
| Thymus | X | X | X | X | X |
| Thyroid | X | X | X | X | |
| Tongue | X | X | X | X | X |
| Trachea | X | X | X | X | X |
| Ureter | | | | | |
| Urinary Bladder | X | X | X | X | X |
| Uterus | X | X | X | X | X |
| Vagina | X | X | X | X | X |
| Zymbal's gland (auditory sebaceous gland) | | | | | |
| Gross lesions | X | X | X | X | X |

Histological Findings

No treatment-related microscopic findings were noted.

Special Evaluation

None

Toxicokinetics

Dosing Solution Analysis

The results for the analysis are the same as for the rat 3-month toxicity study with oxycodone/naloxone or naloxone only since the same batches were used in the dog study

Homogeneity Analyses. "Homogeneity analyses revealed that the average results of the oxycodone samples were within 5.1% of the nominal concentrations for the study." For naloxone samples, the results revealed that the samples were within 5.9% of the nominal concentrations for the study.

Stability Analyses. "The stock and diluted formulations of both oxycodone and naloxone were stable for both 24 hours (after room temperature storage) and 10 days (after refrigerated storage)."

Concentration Verification Analyses. Results revealed that the average results of the oxycodone and naloxone dose formulations were within 6.9% and 14.1% of the nominal concentrations for this study, respectively.

7 Genetic Toxicology

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

Study title: Bacterial mutagenicity tests: Naloxone chlorhydrate

| | |
|-------------------------------------|---|
| Study no.: | 70/8409 |
| Study report location: | EDR, 4.2.3.3.1 |
| Conducting laboratory and location: | (b) (4) [redacted] |
| Date of study initiation: | September 9, 1984 |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity: | Naloxone chlorhydrate, Lot № XY802, % purity not indicated |

Key Study Findings

Under the conditions of the study, naloxone chlorhydrate negative in the bacterial reverse mutation assay. Naloxone chlorhydrate did not cause a positive increase in the mean revertants with any of the tester strains either in the presence or absence of metabolic activation.

Methods

Strains: *Salmonella typhimurium* TA100, TA1535, TA98, TA1538, and TA1537

Concentrations in definitive study: Without S9 mix and with S9 mix:
All tester strains: 0, 1.6, 8, 40, 200, 1000, and 5000 mcg/plate

Basis of concentration selection: The Applicant conducted a pilot Ames test. Naloxone chlorhydrate was tested in the tested in *S. typhimurium* strain TA100 in the presence and absence of S9 mix at concentrations of 0, 1.6, 8, 40, 200, 1000, and 5000 mcg/plate. There no signs of cytotoxicity, precipitation, and no mutagenicity observed. Therefore, the Applicant chose the OECD protocol guideline recommended maximal test concentration (5000 mcg/plate) as the highest concentration for the definitive test.

Negative control: Distilled water

Positive control: Without S9 mix:
9-aminoacridine (9AA, 50.0 mcg/plate) for TA1537
2-nitrofluorene (2NF, 0.5 mcg/plate) for TA98 and TA1538
sodium azide (SA, 1.0 mcg/plate) for TA100 and TA1535

With S9 mix:
2-aminoanthracene (2-AA, 2.0 mcg/plate) for TA98, TA100, TA1535, TA1537, and TA1538

Formulation/Vehicle: Solution/Distilled water

Incubation & sampling time: The test was carried out in triplicate, with and without S9-mix. Before the experiment, 15 mL of nutrient broth were inoculated from single colonies of each of the *S. typhimurium* strain, and incubated at 37°C in a stationary culture.

The mutagenicity test was performed according to the plate-incorporation procedures. The following components were added sequentially to 2 mL of top agar: 0.1 mL of a dilution of naloxone chlorhydrate (in triplicate), 0.1 mL of distilled water (negative control), 0.1 mL

overnight bacterial culture (approximately 10^8 organism) and 0.5 mL (10% S-9 mix (where appropriate). These ingredients were rapidly mixed on a Whirlimixer than poured on to prepared minimal agar plates and allowed to set. All plates were then inverted and allowed to incubate at 37°C for 48 hours.

Study Validity

The study appears to be valid for the following reasons: 1) the appropriate strains were tested, 2) the appropriate controls were used, and 3) the positive control substances produced reliable results. The assay methods, positive and negative controls, and the concentrations of drug used for the definitive study were adequate.

Results

Under the conditions of the study, naloxone chlorhydrate was negative in the bacterial reverse mutation assay.

Naloxone chlorhydrate was evaluated at the following concentrations: of 0, 1.6, 8, 40, 200, 1000, and 5000 mcg/plate. Cyotoxic effect was not observed at concentrations up to 5000 mcg/plate. No precipitation was observed in any of the strains. In the mutagenicity assay, the positive controls induced mutation frequencies as expected; the mean revertants per plate were within expected ranges. Naloxone chlorhydrate did not induce mutation frequencies in any of the *Salmonella typhimurium* strains (TA987, TA100, TA1535, TA1537, or TA1538) in the presence or absence of the metabolic activator; no increase in revertants was observed at any dose.

Study title: Oxycodone hydrochloride injection 50 mg/mL containing dimer (OH-Dimer) and oxycodone hydrochloride injection mg/mL reference formulation (OH-form): Testing for mutagenic activity with *Salmonella typhimurium* TA1535, TA100, TA1537 and TA98 and *Escherichia WP2μrA*.

Study no.: 778441
Study report location: EDR, 4.2.3.3.1
Conducting laboratory and location: [REDACTED] (b) (4)

Date of study initiation: January 26, 2007
GLP compliance: Yes, signed August 22, 2007
QA statement: Yes, signed August 17, 2007
Drug, lot #, and % purity: Oxycodone hydrochloride (OH-Dimer),
Lot № PN2969, 99.8% purity

Key Study Findings

Under the conditions of the study, oxycodone hydrochloride dimer (OH-dimer) was not mutagenic in the bacterial reverse mutation assay.

Methods

- Strains: *Salmonella typhimurium* TA100, TA1535, TA98, and TA1537
Escherichia coli WP2uvrA
- Concentrations in definitive study: 17, 50, 167, 500, 1667, and 5000 mcg/plate
- Basis of concentration selection: A dose range-finding study in the absence and presence of S9-mix using Strain TA100 was executed by the Applicant to assess the bacterial killing activity of the compound. Triplicates of Strains TA100 were evaluated. Concentrations of the oxycodone hydrochloride injection 50 mg/mL containing dimer (OH-dimer) and oxycodone hydrochloride injection 50 mg/mL reference formulation (OH-form) used were 17, 50, 167, 500, 1667, and 5000 mcg per plate.
- There were no signs of cytotoxicity or precipitation observed. Therefore, the sponsor chose the OECD recommended maximal test concentration (5000 mcg/plate) as the highest concentration to test.
- Negative control: Citric acid monohydrate, sodium citrate, sodium chloride in WFI, pH 5.0.
- Positive control: Without S9 mix:
N-Ethyl-N-Nitro-N-nitrosoguanadine (ENNG, 2.0 mcg/plate) for WP2uvrA
2-nitrofluorene (2NF, 1.0 mcg/plate) for TA98
sodium azide (NaN_3 , 1.0 mcg/plate) for TA100 and TA1535
9-aminoacridine (9AA, 80.0 mcg/plate) for TA1537
- With S9 mix:
2-aminoanthracene (2AAN, 2.0 mcg/plate) for TA1535 and TA1537
2AAN (0.5 mcg/plate) for TA98 and TA100
2AAN (20.0 mcg/plate) for WP2uvrA
- Formulation/Vehicle: Solution/Citric acid monohydrate, sodium citrate, sodium chloride in WFI, pH 5.0.
- Incubation & sampling time: Two independent tests were conducted; plate incorporation test and preincubation test. The tests were carried out in triplicate,

with and without S9-mix.

Preincubation Test. The test substance solutions, negative control or positive control were mixed. After pre-incubation, the mixture was overlaid onto soft agar plates. The agar plate was incubated at 37°C for 2 or 3 days for the mutation test. After the incubation period, the number of colonies was counted using an automatic colony counter. The plates were also examined for microcolonies.

Plate Incorporation Test. Soft agar (2 mL) was dispensed into small sterile tubes. S9-mix or 0.05 M phosphate buffer, pH 7.4 was added to the tubes, followed by 0.1 mL of bacteria. The solvent or test article (100 mcL) was added last. The tube contents were poured onto minimal medium plates which contained 20 mL of 1.5% purified agar, in Vogel-Bonner Medium E with 2% glucose. After the soft agar had set, the plates were inverted and incubated at 37°C for 2 or 3 days.

Criteria for a positive response:

According to the Applicant, a test compound was considered positive (mutagenic) for each strain if the following criteria were met:

1. The bacteria demonstrated their typical responses to crystal violet, ampicillin and u.v. light.
2. At least 2 of the vehicle control plates were within the following ranges: TA1535, 4-30; TA1537, 1-30; TA98, 10-60; TA100, 60-200; and *E. coli* WP2uvrA, 1-60.
3. On at least 2 of the positive control plates there were at least x 2 the mean vehicle control mutant numbers per plate, or in the case of TA 100, at least x 1.5 the mean vehicle control mutant numbers per plate.
4. No toxicity or contamination was observed in at least 4 dose levels.
5. In cases where a mutagenic response was observed, no more than one dose level was discarded before the dose that gave the highest significant mean colony number.

Study Validity

The study appears to be valid for the following reasons: 1) the appropriate strains were tested, 2) the appropriate controls were used, and 3) the positive control substances produced reliable results. The assay methods, positive and negative controls, and the concentrations of drug used for the definitive study were adequate.

Results

Under the conditions of the study, oxycodone hydrochloride dimer (OH-dimer) was negative in the bacterial reverse mutation assay.

| Metabolic Activation | Test Article | Concentration (µg/plate) TA 1535 | Revertants Colonies (Mean ± SD) | | | WP2uvrA | |
|----------------------|--------------------------------------|-------------------------------------|---------------------------------|------------|-----------|----------|--------|
| | | | TA 1537 | TA 98 | TA 100 | | |
| Without Activation | Vehicle | 100 µL/plate | 16 ± 3 | 14 ± 5 | 29 ± 11 | 96 ± 12 | 10 ± 7 |
| | Oxycodone Hydrochloride Injection | 17 | 8 ± 2 | 16 ± 6 | 30 ± 4 | 97 ± 12 | 14 ± 2 |
| | 50 mg/ml Containing Dimer (OH-Dimer) | 50 | 12 ± 5 | 17 ± 3 | 27 ± 6 | 118 ± 7 | 11 ± 4 |
| | | 167 | 17 ± 8 | 14 ± 10 | 24 ± 6 | 89 ± 11 | 13 ± 1 |
| | | 500 | 18 ± 5 | 14 ± 3 | 31 ± 9 | 61 ± 16 | 9 ± 1 |
| | | 1667 | 15 ± 9 | 12 ± 2 | 27 ± 5 | 84 ± 17 | 9 ± 5 |
| | | 5000 | 14 ± 3 | 6 ± 3 | 23 ± 2 | 88 ± 11 | 11 ± 3 |
| | NaN ₃ | 1 | 293 ± 16 | | | 947 ± 58 | |
| | 9AA | 80 | | 4439 ± 459 | | | |
| | 2NF | 1 | | | 1057 ± 56 | | |
| | ENNG | 2 | | | | 287 ± 32 | |
| With Activation | Vehicle | 100 µL/plate | 12 ± 3 | 10 ± 3 | 27 ± 12 | 85 ± 3 | 11 ± 5 |
| | Oxycodone Hydrochloride Injection | 17 | 12 ± 2 | 10 ± 4 | 21 ± 8 | 81 ± 3 | 15 ± 6 |
| | 50 mg/ml Containing Dimer (OH-Dimer) | 50 | 12 ± 6 | 6 ± 3 | 25 ± 1 | 101 ± 9 | 16 ± 6 |
| | | 167 | 10 ± 2 | 8 ± 5 | 20 ± 10 | 100 ± 4 | 15 ± 6 |
| | | 500 | 14 ± 4 | 12 ± 5 | 31 ± 6 | 97 ± 10 | 12 ± 5 |
| | | 1667 | 14 ± 5 | 12 ± 5 | 24 ± 6 | 98 ± 5 | 12 ± 2 |
| | | 5000 | 15 ± 3 | 9 ± 5 | 26 ± 5 | 96 ± 15 | 12 ± 2 |
| | 2AAN | 2 | | | 368 ± 45 | 526 ± 53 | |
| | | 0.5 | 321 ± 22 | 162 ± 10 | | | |
| | | 20 | | | | 630 ± 77 | |
| | NaN ₃ | Sodium Azide | | | | | |
| | 9AA | 9-Aminocridine | | | | | |
| | 2NF | 2-Nitrofluorene | | | | | |
| | ENNG | N-Ethyl-N-Nitro-N-nitrosoguanidine | | | | | |
| | 2AAN | 2-Aminoanthracene | | | | | |

Applicant's result from the plate incorporation test is reproduced in the table above. In the plate incorporation mutagenicity assay; the negative control induced mutation frequencies as expected; the mean revertants per plate were within historical control data for the laboratory. The positive controls induced mutation frequencies as expected in the bacteria strains. Compared to the vehicle control groups, oxycodone hydrochloride dimer did not increase the number of revertants for the *Salmonella typhimurium* strains (TA98, TA100, TA1535, or TA1537) or the *Escherichia coli* test strain (WP2uvrA) in the presence or absence of the metabolic activator.

| Metabolic Activation | Test Article | | Concentration ($\mu\text{g}/\text{plate}$) | | Revertant Colonies (Mean \pm SD) | | |
|----------------------|--|------------------------------------|---|----------------|------------------------------------|--------------|-------------|
| | | | TA 1535 | TA 1537 | TA 98 | TA 100 | WP2uvrA |
| Without Activation | Vehicle | 100 $\mu\text{L}/\text{plate}$ | 9 \pm 2 | 10 \pm 2 | 21 \pm 3 | 86 \pm 5 | 3 \pm 2 |
| | Oxycodone Hydrochloride Injection 50 mg/ml Containing Dimer (OH-Dimer) | 17 | 15 \pm 5 | 16 \pm 1 | 20 \pm 2 | 85 \pm 5 | 3 \pm 3 |
| | | 50 | 10 \pm 3 | 10 \pm 5 | 19 \pm 4 | 81 \pm 9 | 7 \pm 1 |
| | | 167 | 9 \pm 3 | 8 \pm 4 | 18 \pm 7 | 84 \pm 7 | 8 \pm 3 |
| | | 500 | 9 \pm 1 | 10 \pm 3 | 22 \pm 10 | 84 \pm 14 | 6 \pm 3 |
| | | 1667 | 9 \pm 3 | 14 \pm 3 | 25 \pm 3 | 86 \pm 10 | 8 \pm 4 |
| | | 5000 | 9 \pm 3 | 9 \pm 5 | 23 \pm 10 | 80 \pm 16 | 6 \pm 2 |
| | NaN ₃ | 1 | 465 \pm 26 | | | 997 \pm 27 | |
| | 9AA | 80 | | 5550 \pm 419 | | | |
| | 2NF | 1 | | | 682 \pm 59 | | |
| With Activation | ENNG | 2 | | | | 264 \pm 35 | |
| | Vehicle | 100 $\mu\text{L}/\text{plate}$ | 14 \pm 2 | 10 \pm 4 | 31 \pm 7 | 83 \pm 9 | 6 \pm 2 |
| | Oxycodone Hydrochloride Injection 50 mg/ml Containing Dimer (OH-Dimer) | 17 | 17 \pm 3 | 15 \pm 4 | 36 \pm 6 | 77 \pm 7 | 7 \pm 5 |
| | | 50 | 16 \pm 2 | 14 \pm 5 | 33 \pm 7 | 87 \pm 2 | 8 \pm 3 |
| | | 167 | 12 \pm 2 | 17 \pm 2 | 33 \pm 8 | 94 \pm 9 | 7 \pm 4 |
| | | 500 | 17 \pm 2 | 12 \pm 6 | 30 \pm 6 | 91 \pm 10 | 6 \pm 3 |
| | | 1667 | 14 \pm 2 | 19 \pm 2 | 27 \pm 7 | 94 \pm 9 | 6 \pm 3 |
| | | 5000 | 15 \pm 4 | 9 \pm 1 | 29 \pm 3 | 115 \pm 21 | 10 \pm 3 |
| | 2AAN | 2 | | | 648 \pm 66 | 610 \pm 54 | |
| | | 0.5 | | | | | 306 \pm 7 |
| | | 20 | 307 \pm 20 | 340 \pm 53 | | | |
| NaN ₃ | | Sodium Azide | | | | | |
| 9AA | | 9-Aminoacridine | | | | | |
| 2NF | | 2-Nitrofluorene | | | | | |
| ENNG | | N-Ethyl-N-Nitro-N-nitrosoguanidine | | | | | |
| 2AAN | | 2-Aminanthracene | | | | | |

Applicant results from the preincubation test are reproduced in the table above. Consistent with the results observed in the plate incorporation test, oxycodone hydrochloride dimer was not mutagenic; the number of revertants for the *Salmonella typhimurium* strains (TA98, TA100, TA1535, or TA1537) or the *Escherichia coli* test strain (WP2uvrA) was not increased in the presence or absence of the metabolic activator relative to the vehicle control. Also, the negative control induced mutation frequencies as expected; the mean revertants per plate were within historical control data for the laboratory. The positive controls effect induced mutation frequencies as expected in the bacteria strains.

7.2 *In Vitro Assays in Mammalian Cells*

Study title: Tests for gene mutation in L5178Y mouse lymphoma cells treated with naloxone

Study no.: 71/8409
Study report location: EDR, 4.2.3.3.1
Conducting laboratory and location: [REDACTED] (b) (4)

Date of study initiation: Not indicated (Final report dated August 1986)
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Naloxone chlorhydrate, Lot № XY802, purity not specified in final study report.

Key Study Findings

Naloxone is mutagenic, causing mutants resistant to both ouabain and 6-thioguanine in L5178Y cells. Under the conditions of the study, naloxone chlorhydrate induced a positive mutagenic response in the presence or absence of metabolic activation.

Methods

Cell line: L5178Y mouse lymphoma cells
Concentrations in definitive study: + S-9: 3.125, 12.5, and 50 mcg/mL
-S-9: 25, 100, and 400 mcg/mL
Basis of concentration selection: The Applicant conducted a dose-range finding cytotoxicity study. Naloxone chlorhydrate was tested at concentrations of 25, 100, and 400 mcg/mL in the absence of S9-mix; and at 3.125, 12.5, and 50 mcg/mL in the presence of S9-mix.

After a 2-hour exposure period, there were signs of cytotoxicity. Naloxone at a concentration of 400 mcg/mL and 50 mcg/mL reduced the relative survival, compared to controls, to 29% in the absence of S-9 mix and 23% in the presence of S-9 mix, respectively. Therefore, the Applicant chose these concentrations as the maximum concentration for the definitive study.
Negative control: Dimethylsulphoxide (DMSO)
Positive control: Ethylmethanesulphonate (EMS, final concentration of 8 mM) and benz(a)pyrene

Formulation/Vehicle: (BP, final concentration of 2 mcg/mL) Culture medium/DMSO at 100x the required final concentration

Incubation & sampling time: Two definitive assays were conducted the presence and absence of S9-mix. L5178Y cells (10 mL) in growth phase were incubated with DMSO (0.1 mL) or naloxone dissolved in DMSO giving the final concentration of 25, 100, and 400 mcg/mL in the absence of S9-mix; and at 3.125, 12.5, and 50 mcg/mL in the presence of S9-mix. Cells were incubated with drug for 2-hours. The cells were then washed twice with phosphate buffered saline, resuspended in their original volumes of fresh R20 medium and incubated for one hour at 37°C to recover. Subsequently, the cells were counted, diluted to 1×10^5 cells/mL in R10 medium and incubated overnight.

Plating. On each day that the cells were plated to look for ouabain or 6-thioguanine resistant mutants, cells were also plated for survival on non-selective media.

Ouabain Selection. Experiment 1 – the cells were plated for assessment of resistance of ouabain on Days 5 and 6. Experiment 2 – cells were plated for assessment of resistance of ouabain on Days 6 and 7.

6-Thioguanine Selection. Experiments 1 and 2 – the cells were plated for assessment of resistance of 6-thioguanine on Days 8 and 9.

Study Validity

The applicant did not specially describe the criteria for study validity.

The reviewer considers the criteria for a positive response were as follows:

1. Mutation frequency statistically significantly higher than that for the controls
2. Concentration-dependent increased mutation frequency

3. An increase in the mutation frequency of small colonies indicates clastogenicity

Results

Study 1.

Table 54. Induced mutant frequencies per 10⁶ survivors – Experiment 1.

| Test Article | Assay 1 | | | | | | | | | |
|---|-----------------------------|-----------------|--|----------|----------|----------|--|----------|---------|----------|
| | Dose Level (μ g/mL) | | Induced mutant frequency/10 ⁶ survivors for Oubain | | | | Induced mutant frequency/10 ⁶ survivors for 6- Thioguanine | | | |
| | -S9 | +S9 | Day 5 | | Day 6 | | Day 8 | | Day 9 | |
| Vehicle Control | - | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Positive Control | 8 mM EMS | 2 μ g/ml BP | 9.35 | 4.44 | 12.23 | 5.02 | 438.1 | 52.7 | 63.5 | 125.9 |
| Naloxone HCl | 25 | 3.125 | 2.19 | 0 | 0.50 | 0 | 104.1 | 286.7 | 41.5 | 12.3 |
| | 100 | 12.5 | 2.61 | 0 | 6.74 | 0 | 55.1 | 212.7 | 19.5 | 89.9 |
| | 400 | 50 | 0.59 | 0 | 1.16 | 0 | 239.1 | 42.7 | 47.5 | 0 |
| Values of chi-squared found from numbers of empty and full wells counted | | | | | | | | | | |
| Positive Control | 8 mM EMS | 2 μ g/ml BP | 34.26* ** | 28.21*** | 34.87*** | 17.80*** | 18.38*** | 20.16*** | 7.05** | 13.96*** |
| Naloxone HCl | 25 | 3.125 | 6.89** | NS | NS | NS | 9.55* | 9.04** | 7.37** | NS |
| | 100 | 12.5 | 8.82** | 4.5* | 20.88*** | NS | NS | 12.52*** | NS | NS |
| | 400 | 50 | NS | NS | NS | NS | NS | NS | 10.17** | NS |

* p<0.05; ** p<0.01; *** p<0.001; NS = not significant

Naloxone was tested from 25 to 400 mcg/mL and 3.125 to 50 mcg/mL in the absence and presence of S9-mix, respectively, in the definitive assay. Results from the definitive test are presented in the Applicant's table reproduced above. In the assay, the positive control produced the expected results; EMS and BPD statistically significantly increased the mutant frequencies for both selective agents (oubain and 6-thioguanine). Exposure of the L5178Y mouse lymphoma cells to naloxone resulted in significant increases in mutant resistant to oubain and 6-thioguanine in the presence and absence of S-9 mix.

Study 2.

Table 55. Induced mutant frequencies per 10⁶ survivors – Experiment 2.

| Test Article | Assay 2 | | | | | | | | | | | |
|--|-----------------------------|-----------------|---|---------|----------|-------|--|----------|----------|----------|----|----|
| | Dose Level (μ g/ml) | | Induced mutant frequency/10 ⁶ survivors for Ouabain | | | | Induced mutant frequency/10 ⁶ survivors for 6- Thioguanine | | | | | |
| | -S9 | +S9 | Day 5 | | Day 6 | | Day 8 | | Day 9 | | | |
| Vehicle Control | - | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| Positive Control | 8 mM EMS | 2 μ g/ml BP | 5.78 | 9.76 | 8.98 | 0.68 | 441.2 | 174.0 | 1778.8 | 266.5 | | |
| Naloxone HCl | 25 | 3.125 | 0 | 2.48 | 6.42 | 2.28 | 32.0 | 0 | 0 | 0 | | |
| | 100 | 12.5 | 3.86 | 0.57 | 11.75 | 0 | 67.53 | 0 | 281.8 | 0 | | |
| | 400 | 50 | 0.27 | 0.56 | 0 | 0 | 1.26 | 0 | 29.1 | 0 | | |
| Values of chi-squared found from numbers of empty and full wells counted | | | | | | | | | | | | |
| Positive Control | 8 mM EMS | 2 μ g/ml BP | 20.08*** | 10.32** | 1.12NS | 6.62* | 103.55*** | 61.55*** | 86.40*** | 37.01*** | | |
| Naloxone HCl | 25 | 3.125 | NS | NS | 15.25*** | NS | NS | NS | NS | NS | NS | NS |
| | 100 | 12.5 | 15.02** | NS | 18.68*** | NS | 31.65*** | NS | 15.19*** | 23.04*** | | |
| | 400 | 50 | ** | NS | NS | 4.90* | 4.25* | NS | NS | 7.20** | | |
| | | | NS | | | | | | | | | |

* p<0.05; ** p<0.01; *** p<0.001; NS = not significant

Results from the definitive assay 2 are presented in the Applicant's table reproduced above. Consistent with the results from Assay 1, the positive control produced the expected results; EMS and BP statistically significantly increased the mutant frequencies for both selective agents (oubain and 6-thioguanine). Exposure of the L5178Y mouse lymphoma cells to naloxone resulted in significant increases in mutant resistant to oubain and 6-thioguanine in the presence and absence of S-9 mix.

Study title: Metaphase analysis of human lymphocytes treated with naloxone

| | |
|-------------------------------------|--|
| Study no.: | 74/8506 |
| Study report location: | EDR, 4.2.3.3.1 |
| Conducting laboratory and location: | (b) (4) |
| Date of study initiation: | Not indicated (Final report dated April 1986) |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity: | Naloxone chlorhydrate, Lot № XY802, purity not indicated in study report |

Key Study Findings

Under the conditions of this assay, naloxone was a potential clastogenic in the human lymphocyte cultures in vitro in the presence of metabolic activation.

Methods

Cell line: Human peripheral blood lymphocytes obtained from 2 healthy volunteers with no history of chromosome fragility, no recent x-ray exposure and no recent virus history

Concentrations in definitive study: 0.375, 0.75, 1.5, and 3.0 mg/mL were used both in the presence and absence of S9 metabolism.

Basis of concentration selection: Dose selection was based on the results from a dose-range cytotoxicity assay. Naloxone chlorhydrate was tested in blood from Donor A at concentrations of 16 mcg/mL, 80 mcg/mL, 400 mcg/mL, 2 mg/mL and 10 mg/mL in the presence and absence of S9 mix.

There was little effect of naloxone on mitotic index up to 2.0 mg/mL in either the presence or absence of S9-mix. Mitotic index at 10 mg/mL was uncountable. Therefore, the Applicant selected 3 mg/mL as the highest concentration for the definitive test.

Negative control: Water

Positive control: With S9-mix: cyclophosphamide (CPA, 25 mcg/mL)
Without S9-mix: methylmethane sulphonate (MMS, 20 mcg/mL)

Formulation/Vehicle: Serum free medium (RPMI 1640)

Incubation & sampling time: Twelve cultures were established from each donor and incubated at 37°C for 48 hours. Cells cultures were exposed to naloxone for 3 hours in the absence and presence of metabolic activation. After treatment, cells were washed and resuspended in fresh medium and reincubated for another 25 hours.

Approximately 21 hours after treatment initiation, demecolcine (0.15 mcg/mL) was added to the culture for 4 hours to initiate mitotic arrest (i.e., arrest the cells in a metaphase-like stage of mitosis). After exposure to demecolcine, metaphase cells were harvested by centrifugation. Cells were collected by centrifugation, fixed, stained and the metaphase were analyzed

for chromosomal aberrations. A total of 100 cells/culture/plate were analyzed for structural and numerical aberrations. Two set of slides per donor was made.

Study Validity

The study appears valid for the following reasons:

- The appropriate positive controls were employed according to OECD guidelines.
- The appropriate number of cells was evaluated.
- The conditions of the assay are appropriate based upon OECD guidelines.
- The dose selection based upon cytotoxicity (i.e., reduction of mitotic index) was acceptable.

Results

Table 56. Results from chromosomal aberration assay.

| Metabolic Activation | Test Article | Dose Level (mg/ml) | Mitotic Index | Aberrations/100 cells | | | | | Number of cells with aberrations/100 cells | |
|----------------------|--------------------------|--------------------|---------------|-----------------------|-----------------|----------------|---------------|-------|--|-------|
| | | | | gaps | Chromosome del. | Chromatid del. | Isolocus Del. | Other | -gaps | +gaps |
| Without Activation | Solvent control | - | 5.65 | 4.5 | 0.5 | 2 | 0 | 0.5 | 3 | 7.5 |
| | Naloxone HCl | 0.375 | 7.87 | 3.5 | 0 | 0 | 0 | 2 | 2 | 5.5 |
| | | 0.75 | 5.18 | 2.5 | 0 | 0.5 | 0 | 1 | 1.5 | 4 |
| | | 1.5 | 3.08 | 3.5 | 0 | 0.5 | 1.5 | 1 | 3 | 6.5 |
| | | 3.0 ¹³ | 5.62 | - | - | - | - | - | - | - |
| With Activation | Methylmethane sulphonate | 0.02 | Not given | 0 | 0 | 12 | 0 | 4 | 22* | 22 |
| | Solvent control | - | 2.77 | 0.5 | 0 | 0 | 2.5 | 0 | 0 | 0.5 |
| | Naloxone HCl | 0.375 | 2.6 | 6.5 | 0 | 8 | 0 | 0.5 | 11* | 17.5 |
| | | 0.75 | 4.77 | 2.5 | 0 | 1.5 | 2 | 0.5 | 2 | 5 |
| | | 1.5 | 4.62 | 1 | 1 | 3 | 0 | 1.5 | 7.5* | 8.5 |
| | | 3 | 2.5 | 8 | 0 | 3 | 14 | 0 | 3 | 11 |
| | Cyclophosamide | 0.025 | Not given | 18 | 2 | 22 | 0 | 6 | 46* | 64 |

¹⁴ One replicate; two donors

¹⁵ This dose level couldn't be scored because the spreads obtained were so poor. With S9 only one donor could be scored

Results from the definitive chromosomal aberration tests are presented in the Applicant's table reproduced above. Results from the positive controls confirmed the ability to detect chromosomal aberrations under the conditions of the assay. The positive controls methylmethane sulphonate and cyclophosamide induced statistically significant increases in chromosomal aberrations over the vehicle control in the absence and presence of S-9 mix, respectively.

Reduction in mitotic index was not observed with naloxone at doses up to 3 mg/mL in the presence and absence of S9 mix. There was no evidence of chromosomal aberration of naloxone in the absence of S9 mix. However in the presence of S9 mix, a non dose-related increase in chromosomal aberrations was statistically significant at concentrations of 0.375 and 1.5 mg/mL.

Study title: Oxycodone hydrochloride injection 50 mg/mL containing dimer (OH-Dimer) and oxycodone hydrochloride injection 50 mg/mL reference formulation (OH-Form): Chromosomal aberration assay with human peripheral lymphocytes culture in vitro.

Study no.: 778436
Study report location: EDR, 4.2.3.3.1
Conducting laboratory and location: [REDACTED] (b) (4)

Date of study initiation: January 24, 2007
GLP compliance: Yes, signed September 3, 2007
QA statement: Yes, signed August 31, 2007
Drug, lot #, and % purity: Oxycodone hydrochloride (OH-Dimer),
Lot № PN2969, 99.8% purity

Key Study Findings

Methods

Cell line: Human peripheral blood lymphocytes obtained from 1 healthy volunteer.
Concentrations in definitive study: 313, 625, 1250, and 2500 mcg/mL
Basis of concentration selection: Dose selection was based on the results from a dose-range cytotoxicity assay. The dose levels of oxycodone hydrochloride dimer tested were:

- + S9 mix: 625, 1250, 2500, 3750, and 5000 mcg/mL
- -S9 mix: 156, 313, 625, 1250, 2500, 3750, and 5000 mcg/mL

In the presence of S9 mix, oxycodone dimer reduced mitotic indices at 2500 mcg/mL and there were insufficient metaphase for assessment at 5000 mcg/mL in Assay 1. In Assay 2, there were insufficient metaphase for assessment at 3750 and 5000 mcg/mL. In the absence of S9 mix, oxycodone dimer reduced mitotic index at 3750 mcg/mL in Assay 2 (25 h treatment and harvested at 29 h). At 5000 mcg/mL, there were not sufficient metaphases for analysis.

Therefore, the Applicant chose 2500 mcg/mL as the highest dose level for the definitive assay.

Negative control: Citric acid monohydrate, sodium citrate, sodium chloride in WFI, pH 5.0.

Positive control: + S9: cyclophosphamide (CPH) at 10-40 mcg/mL
-S9: mitomycin C (MMC) at 0.1-0.8 mcg/mL

- Formulation/Vehicle:** Citric acid monohydrate, sodium citrate, sodium chloride in WFI, pH 5.0.
- Incubation & sampling time:** The test was carried out in duplicate (positive control tested using single culture) in the presence and the absence of S9-mix.

Treatment schedule is presented in the table below:

| S9 Mix | Cultures Established | Test | Treatment Period (Includes 1 h wash) | Recovery Period | Colcemid | Harvest |
|--------------------|-------------------------|---------------|--------------------------------------|-----------------|----------|---------|
| Presence of S9 mix | ca 48 h before exposure | Tests 1 and 2 | 0-5 h | 5-26 h | 26-29 h | 29 h |
| Absence of S9 mix | | Test 1 | 0-5 h | 5-26 h | 26-29 h | 29 h |
| | | Test 2 | 0-25 h | 25-26 h | 26-29 h | 29 h |
| | | | | 25-50 h | 50-53 h | 53 h |

Treatment of with the test articles was as following:

| S9 Mix | Test | Growth Medium | S9 Mix | Dosing Solution | Final Volume (approx) |
|--------------------|---------------|---------------|--------|-----------------|-----------------------|
| Presence of S9 mix | Tests 1 and 2 | 3.6 mL | 0.9 mL | 0.5 mL | 5 mL |
| Absence of S9 mix | | 4.5 mL | - | 0.5 mL | 5 mL |
| | | 9 mL | - | 1 mL | 10 mL |

"After treatment, cells were washed twice with serum free medium then full growth medium added, for the recovery period and colcemid treatment. Cells were sedimented by centrifugation (approximately 500 g) prior to each wash. Final culture volumes after washing were approximately 5 mL or 10 mL (Test 2, absence of S9 mix).

In Test 2 (absence of S9 mix), after washing, the cultures were divided into 2 by pipetting 5 mL of the 10 mL final volume into fresh culture tubes. One culture for harvesting at 29 h, the other at 53 h."

Cells were harvested at 29 h or 53 h from the initiation of treatment. Cell division was arrested by addition of the spindle inhibitor Colcemid® at a final concentration of 0.075 mcg/mL. After the 3 hour exposure to Colcemid®, metaphase cells were harvested by centrifugation. Thereafter, "cultures were harvested by sedimenting the cells with centrifugation (approximately 500 g) then treatment with hypotonic solution (0.56% KCl) for 30 min at 37°C." The cells were fixed by adding freshly prepared fixative (methanol:glacial acetic acid 3:1, v/v). Fixed cells were dropped on clean, grease-free slides and coverslip mounted.

Study Validity

The study appears valid for the following reasons:

- The appropriate positive controls were employed according to OECD guidelines.
- An acceptable number of mitotic cells were evaluated (1000 cells per culture).
- The conditions of the assay are appropriate based upon OECD guidelines.
- The frequency of cells with structural chromosome aberrations in the vehicle controls was within the historical control range for the vehicle controls.
- The dose selection based upon cytotoxicity (i.e., reduction of mitotic index) was acceptable.

Results

There was no evidence of chromosomal aberrations in oxycodone hydrochloride OH-dimer treated human peripheral blood lymphocyte. There were no meaningful differences in structural aberrations between the OH-form and OH-dimer of oxycodone.

Table 57. Test 1 chromosomal aberration assay with S9 mix, 5 h treatment, 29 h harvest

| Treatment Group | Conc. (µg/mL) | Decoded Culture No. | No. of Cells Scored | Structural Aberrations | | | | | | Aberration Frequency | Aberrant Cell Frequency | | | Numerical Aberrations % Aneuploid Cells | |
|-----------------|---------------|---------------------|---------------------|------------------------|---|---|------------|---|---|----------------------|-------------------------|----------------|---------|--|---|
| | | | | Chromatid | | | Chromosome | | | | Including Gaps | Excluding Gaps | | | |
| | | | | G | B | F | G | B | F | | Judge | % Judge | % Judge | | |
| Vehicle | 10% | 1 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 |
| | | 2 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 1 |
| OH-Form | 625 | 13 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 |
| | | 14 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 |
| | 1250 | 15 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 |
| | | 16 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 |
| | 2500 | 17 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 1 |
| | | 18 | 100 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0.01 | - | 1 | - | 0 |
| | 5000 | 19 | 100 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0.01 | - | 1 | - | 0 |
| | | 20 | 90a | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 1 |

Culture 15: 1 polyploid cell – no damage
 Culture 19: 1 polyploid cell – no damage
 Culture 20: 1 polyploid cell – no damage

| Treatment Group | Conc. (µg/mL) | Decoded Culture No. | No. of Cells Scored | Structural Aberrations | | | | | | Aberration Frequency Lesions/Cell Judge | Aberrant Cell Frequency | | Numerical Aberrations % Aneuploid Cells | | | |
|-----------------|------------------|---------------------|---------------------|------------------------|---|---|------------|---|---|---|---------------------------|---------------------------|--|----|---|----|
| | | | | Chromatid | | | Chromosome | | | | Including Gaps % Judge | Excluding Gaps % Judge | | | | |
| | | | | G | B | F | G | B | F | | Judge | % Judge | | | | |
| OH-Dimer | 313 | 29 | 100 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0.01 | - | 1 | - | 1 | |
| | | 30 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 | |
| | 625 | 31 | 100 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0.01 | - | 1 | - | 1 | |
| | | 32 | 100 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0.01 | - | 1 | - | 0 | |
| | 1250 | 33 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 1 | |
| | | 34 | 100 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0.01 | - | 1 | - | 2 | |
| | 2500 | 35 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 1 | |
| | | 36 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 | |
| | Cyclophosphamide | 10 | 39 | 100 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0.04 | - | 4 | + | 1 |
| | | 20 | 40 | 71a | 0 | 5 | 1 | 0 | 0 | 0 | 1 | 0.11 | + | 10 | + | 10 |
| Untreated | - | 43 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 | |
| | | 44 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 | |

Table 58. Test 1 chromosomal aberration assay without S9 mix, 5 h treatment, 29 h harvest

| Treatment Group | Conc. (µg/mL) | Decoded Culture No. | No. of Cells Scored | Structural Aberrations | | | | | | Aberration Frequency Lesions/Cell Judge | Aberrant Cell Frequency | | Numerical Aberrations % Aneuploid Cells | | |
|-----------------|---------------|---------------------|---------------------|------------------------|---|---|------------|---|---|---|---------------------------|---------------------------|--|---|---|
| | | | | Chromatid | | | Chromosome | | | | Including Gaps % Judge | Excluding Gaps % Judge | | | |
| | | | | G | B | F | G | B | F | | Judge | % Judge | | | |
| Vehicle | 10% | 117 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 |
| | | 118 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 |
| | 625 | 123 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 |
| | | 124 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 |
| | 1250 | 125 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 |
| | | 126 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 |
| | 2500 | 127 | 100 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0.01 | - | 1 | - | 0 |
| | | 128 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 |
| | 3750 | 129 | 91 a | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 |
| | | 130 | 53 a | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 |

| Treatment Group | Conc. (µg/mL) | Decoded Culture No. | No. of Cells Scored | Structural Aberrations | | | | | | Aberration Frequency Lesions/Cell | Aberrant Cell Frequency | | % Aneuploid Cells |
|-----------------|---------------|---------------------|---------------------|------------------------|---|---|------------|---|---|--------------------------------------|---------------------------|---------------------------|--------------------|
| | | | | Chromatid | | | Chromosome | | | | Including Gaps Judge % | Excluding Gaps Judge % | |
| | | | | G | B | F | G | B | F | E | D | R | |
| OH-Dimer | 625 | 75 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 - 0 - 0 - 1 |
| | | 76 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 - 0 - 0 - 1 |
| | 1250 | 77 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0.02 - 1 - 1 - 1 |
| | | 78 | 100 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.01 - 1 - 0 - 0 |
| | 2500 | 79 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 - 0 - 0 - 2 |
| | | 80 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 - 0 - 0 - 4 |
| | 5000 | 81 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 - 0 - 0 - 4 |
| | | 82 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 - 0 - 0 - 1 |
| | Mitomycin C | 0.7 | 85 | 100 | 0 | 5 | 3 | 0 | 0 | 0 | 2 | 0 | 0 0.12 + 7 + 7 + 7 |
| | | 0.8 | 86 | 100 | 0 | 6 | 0 | 0 | 0 | 0 | 1 | 0 | 0 0.08 + 5 + 5 + 5 |
| Untreated | - | 87 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 - 0 - 0 - 1 |
| | | 88 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 - 0 - 0 - 1 |

Culture 75: 1 polyploid cell and 1 endoreduplicated cell – no damage

Culture 79: 1 polyploid cell – no damage

Culture 80: 1 polyploid cell – no damage

Culture 85: 1 polyploid cell – no damage

Culture 86: 2 polyploid cells – no damage

Results from the chromosomal aberration Assay 1 are presented in the tables above. No differences were observed between oxycodone hydrochloride OH-dimer (OH-dimer) and oxycodone hydrochloride reference form (OH-form) following a 5-hour treatment period and 29 hour harvest time. Oxycodone hydrochloride OH-dimer (OH-dimer) and oxycodone hydrochloride reference form (OH-form) did not induce structural chromosomal aberrations in the absence and presence of metabolic activation. However, an increase level of numerical aberrations was noted in cultures treated with both OH-form and OH-dimer at concentrations of 2500 and 3750 mcg/mL.

Results from the positive controls confirmed the ability to detect chromosomal aberrations under the conditions of the assay. The positive controls Mitomycin C and cyclophosphamide increased structural aberrations over the vehicle control in the absence and presence of S9 mix. For the negative and positive controls, the frequencies of chromosomal aberration formations were within the historical control range.

Table 59. Test 2 chromosomal aberration assay with S9 mix, 5 h treatment, 29 h harvest

| Treatment Group | Conc. (µg/mL) | Decoded Culture No. | No. of Cells Scored | Structural Aberrations | | | | | | Aberration Frequency | Aberrant Cell Frequency | | | Numerical Aberrations % Aneuploid Cells | | |
|-----------------|---------------|---------------------|---------------------|------------------------|---|---|------------|---|---|----------------------|-------------------------|---------|----------------|--|---|---|
| | | | | Chromatid | | | Chromosome | | | | Lesions/Cell | | Including Gaps | | | |
| | | | | G | B | F | G | B | F | | Judge | % Judge | % Judge | % Judge | | |
| Vehicle | 10% | 89 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 1 | |
| | | 90 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | - | 0 | |
| OH-Form | 1250 | 93 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 |
| | | 94 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 1 |
| | 3750 | 97 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 |
| | | 98 | 100 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.01 | - | 1 | - | 0 |
| | 5000 | 99 | 95a | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 |
| | | 100 | 100 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0.01 | - | 1 | - | 1 |

| Treatment Group | Conc. (µg/mL) | Decoded Culture No. | No. of Cells Scored | Structural Aberrations | | | | | | Aberration Frequency | Aberrant Cell Frequency | | | Numerical Aberrations % Aneuploid Cells | | | | | |
|------------------|---------------|---------------------|---------------------|------------------------|---|---|------------|---|---|----------------------|-------------------------|---------|----------------|--|----|---|---|---|---|
| | | | | Chromatid | | | Chromosome | | | | Lesions/Cell | | Including Gaps | | | | | | |
| | | | | G | B | F | G | B | F | | Judge | % Judge | % Judge | % Judge | | | | | |
| OH-Dimer | 625 | 101 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 1 | | | |
| | | 102 | 100 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.01 | - | 1 | - | 0 | | | |
| | 1250 | 103 | 100 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.01 | - | 1 | - | 0 | | | |
| | | 104 | 100 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0.01 | - | 1 | - | 0 | | | |
| | 2500 | 105 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 | | | |
| | | 106 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 | | | |
| Cyclophosphamide | 10 | 111 | 100 | 5 | 2 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0.11 | + | 10 | + | 5 | + | 0 |
| | 20 | 112 | 100 | 4 | 7 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0.14 | + | 9 | + | 8 | + | 0 |
| Untreated | - | 115 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 | - | 1 | |
| | | 116 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 | - | 0 | |

Culture 101: 1 endoreduplicated cell – no damage

Culture 102: 1 endoreduplicated cell – no damage

Culture 103: 1 endoreduplicated cell – no damage

Culture 115: 1 endoreduplicated cell – no damage

Table 60. Test 2 chromosomal aberration assay without S9 mix, 25 h treatment, 29 h harvest

| Treatment Group | Conc. (µg/mL) | Decoded Culture No. | No. of Cells Scored | Structural Aberrations | | | | | | | | Aberration Frequency | Aberrant Cell Frequency | Numerical Aberrations | | | | | | |
|-----------------|---------------|---------------------|---------------------|------------------------|---|---|------------|---|---|---------|---|----------------------|-------------------------|-----------------------|---|---|---|---|---|---|
| | | | | Chromatid | | | Chromosome | | | Complex | | Multiple | Other | | | | | | | |
| | | | | G | B | F | G | B | F | E | D | R | | | | | | | | |
| Vehicle | 10% | 117 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 | - | 0 |
| | | 118 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 | - | 0 |
| OH-Form | 625 | 123 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 | - | 0 |
| | | 124 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 | - | 0 |
| | 1250 | 125 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 | - | 0 |
| | | 126 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 | - | 0 |
| | 2500 | 127 | 100 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.01 | - | 1 | - | 0 | - | 0 |
| | | 128 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 | - | 0 |
| | 3750 | 129 | 91 a | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 | - | 0 |
| | | 130 | 53 a | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 | - | 0 |

| Treatment Group | Conc. (µg/mL) | Decoded Culture No. | No. of Cells Scored | Structural Aberrations | | | | | | | | Aberration Frequency | Aberrant Cell Frequency | Numerical Aberrations | | | | | | | |
|-----------------|---------------|---------------------|---------------------|------------------------|---|---|------------|---|---|---------|---|----------------------|-------------------------|-----------------------|------|---|---|----|---|----|---|
| | | | | Chromatid | | | Chromosome | | | Complex | | Multiple | Other | | | | | | | | |
| | | | | G | B | F | G | B | F | E | D | R | | | | | | | | | |
| OH-Dimer | 1250 | 139 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 | - | 0 | |
| | | 140 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 | - | 0 | |
| | 2500 | 141 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 | - | 0 | |
| | | 142 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 | - | 0 | |
| | 3750 | 143 | 100 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.01 | - | 1 | - | 1 | - | 0 | |
| | | 144 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 | - | 0 | |
| | 0.15 | 148 | 100 | 0 | 3 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0.07 | + | 4 | +- | 4 | +- | 0 |
| | 0.5 | 150 | 100 | 0 | 6 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0.14 | + | 8 | + | 8 | + | 0 |
| | Untreated | 151 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 | - | 0 | |
| | | 152 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 | - | 0 | - | 0 | - | 0 | |

Results from the chromosomal aberration Assay 2 are presented in the tables above (reproduced from the Applicant's submission). Consistent with the results from Assay 1, no differences were observed between oxycodone hydrochloride OH-dimer (OH-dimer) and oxycodone hydrochloride reference form (OH-form) following a 5-hour treatment period and 29-hour harvest time. Oxycodone hydrochloride OH-dimer (OH-dimer) and oxycodone hydrochloride reference form (OH-form) did not induce structural chromosomal aberrations in the absence and presence of metabolic activation.

7.3 In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Bone marrow micronucleus test in mice treated with naloxone HCl

Study no: N003003A
Study report location: EDR, 4.2.3.3.2
Conducting laboratory and location: (b) (4)

Date of study initiation: November 7, 1997
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Naloxone HCl, Lot № V06666, purity not indicated in final study report.

Key Study Findings

Under the conditions of the assay, naloxone hydrochloride was negative for the induction of micronucleus polychromatic erythrocytes in the bone marrow of mice and there was no evidence the drug was clastogenic or damaged the mitotic apparatus.

Methods

Doses in definitive study: 0, 20, 100, and 500 mg/kg
Frequency of dosing: Once
Route of administration: Oral (gavage)
Dose volume: Naloxone Groups: 20 mL/kg; Mitomycin C: 40 mL/kg
Formulation/Vehicle: Solution/deionized water
Species/Strain: Mice/CD-1®
Number/Sex/Group: 15/sex/group
24 hour Sampling Time: 5/sex/group
48 hour Sampling Time: 5/sex/group
72 hour Sampling Time: 5/sex/group
Satellite groups: None
Basis of dose selection: In a dose-range finding study, 36 animals (3/sex/group) received a single oral gavage dose of naloxone HCl (0, 100, 250, 500, 1000, and 2000 mg/kg) and were sacrificed 72 hours after treatment. Mortality was noted in all males and females at 2000 mg/kg dose and in 2 out of 3 females at the 1000 mg/kg dose. Convulsions, tremors, lethargy, and vocalization were observed at doses \geq 1000 mg/kg.
Negative control: Deionized water
Positive control: Mitomycin C (1 mg/kg)

Study Validity

Based on the review of the study methodology including the doses selected for female and male mice, the exposure time, the use of positive and negative controls, and the outcome of these controls (negative control (vehicle) and positive control treated mice produced results, the study was considered valid.

Criteria for a positive response: The following criteria were established for a positive response: *test article* caused a statistically significant dose-related increase in the number of micronucleated polychromatic erythrocytes, or if at least one dose exhibited a significant reproducible increase over its concurrent negative control article.

Results

Naloxone hydrochloride did not induced micronucleus formation under these study conditions.

Table 61. Summary of abnormal clinical observations

| Dose Group (mg/kg) | Clinical Sign | No. of Animals Affected | Mean First Day Observed | Mean Last Day Observed | Total No. of Observations |
|-----------------------|---------------|----------------------------|----------------------------|---------------------------|------------------------------|
| Males | | | | | |
| 500 | LETHARGIC | 4 | 1 | 1 | 4 |
| 500 | CONVULSIVE | 4 | 1 | 1 | 4 |
| 500 | TREMORS | 4 | 1 | 1 | 4 |
| 500 | VOCALIZING | 4 | 1 | 1 | 4 |
| Females | | | | | |
| 500 | LETHARGIC | 6 | 1 | 1 | 6 |
| 500 | CONVULSIVE | 3 | 1 | 1 | 3 |
| 500 | TREMORS | 6 | 1 | 1 | 6 |
| 500 | VOCALIZING | 2 | 1 | 1 | 2 |

No naloxone-related mortalities were observed; all mice survived to the scheduled necropsy. Clinical signs of toxicity were observed following the oral administration of naloxone. Clinical signs observations are presented in the table above (reproduced from the Applicant's submission). Convulsions, tremors, lethargy, and vocalization were was the primary treatment-related clinical sign observed Study Day 1 following the oral administration of 500 mg/kg of naloxone hydrochloride.

Analysis of the bone marrow cells from the tibias collected at 24, 48, and 72 hours after treatment is presented in the tables below (reproduced from the Applicant's submission) below. As expected, mitomycin C at a dose of 1 mg/kg significantly increased the frequency of micronucleated polychromatic erythrocyte in both male and female mice compared to the vehicle control; mitomycin C produced a 3.8- and 4.5-fold increase in micronucleated polychromatic erythrocytes in males and females,

respectively. , The 1 mg/kg dose group represents the data from the positive control mitomycin C-treated animals.

Table 62. Summary of group mean micronucleus evaluation in males.

| Dose Group (mg/kg) | % PCE | | | % Micronucleated PCE | | | % NCE | | | Ratio % PCE : % NCE [†] | | |
|--------------------|-------|------|---|----------------------|-----|---|-------|------|---|----------------------------------|------|---|
| | Mean | SD | N | Mean | SD | N | Mean | SD | N | Mean | SD | N |
| Day 2 | | | | | | | | | | | | |
| 0 | 30.8 | 3.3 | 5 | 0.9 | 0.2 | 5 | 69.2 | 3.3 | 5 | 0.45 | 0.07 | 5 |
| 20 | 33.6 | 7.0 | 5 | 0.5* | 0.2 | 5 | 66.4 | 7.0 | 5 | 0.52 | 0.17 | 5 |
| 100 | 28.8 | 3.6 | 5 | 0.8 | 0.3 | 5 | 71.2 | 3.6 | 5 | 0.41 | 0.07 | 5 |
| 500 | 35.8 | 7.0 | 5 | 0.7 | 0.4 | 5 | 64.2 | 7.0 | 5 | 0.57 | 0.18 | 5 |
| 1 | 29.4 | 5.0 | 5 | 3.4* | 1.4 | 5 | 70.6 | 5.0 | 5 | 0.42 | 0.10 | 5 |
| Day 3 | | | | | | | | | | | | |
| 0 | 29.4 | 5.3 | 5 | 0.5 | 0.2 | 5 | 70.6 | 5.3 | 5 | 0.42 | 0.10 | 5 |
| 20 | 22.2 | 4.1 | 5 | 0.6 | 0.3 | 5 | 77.8 | 4.1 | 5 | 0.29 | 0.07 | 5 |
| 100 | 21.0* | 3.2 | 5 | 0.7 | 0.4 | 5 | 79.0* | 3.2 | 5 | 0.27 | 0.05 | 5 |
| 500 | 16.4* | 6.8 | 5 | 0.6 | 0.3 | 5 | 83.6* | 6.8 | 5 | 0.20 | 0.10 | 5 |
| Day 4 | | | | | | | | | | | | |
| 0 | 36.6 | 9.7 | 5 | 0.5 | 0.5 | 5 | 63.4 | 9.7 | 5 | 0.61 | 0.27 | 5 |
| 20 | 44.8 | 4.8 | 5 | 0.3 | 0.2 | 4 | 55.2 | 4.8 | 5 | 0.82 | 0.16 | 5 |
| 100 | 35.2 | 21.1 | 5 | 0.4 | 0.2 | 5 | 64.8 | 21.1 | 5 | 0.80 | 0.99 | 5 |
| 500 | 39.0 | 7.0 | 5 | 0.6 | 0.4 | 5 | 61.0 | 7.0 | 5 | 0.66 | 0.19 | 5 |

*Statistically significant at p≤0.05.

PCE = Polychromatic erythrocytes

NCE = Normochromic erythrocytes

† = Mean values of %PCE and %NCE used to determine ratio.

Table 63. Summary of group mean micronucleus evaluation in females

| Dose Group (mg/kg) | % PCE | | | % Micronucleated PCE | | | % NCE | | | Ratio % PCE : % NCE [†] | | |
|--------------------|-------|------|---|----------------------|-----|---|-------|------|---|----------------------------------|------|---|
| | Mean | SD | N | Mean | SD | N | Mean | SD | N | Mean | SD | N |
| Day 2 | | | | | | | | | | | | |
| 0 | 32.0 | 8.5 | 5 | 0.6 | 0.4 | 5 | 68.0 | 8.5 | 5 | 0.49 | 0.21 | 5 |
| 20 | 31.2 | 9.2 | 5 | 0.6 | 0.2 | 5 | 68.8 | 9.2 | 5 | 0.48 | 0.22 | 5 |
| 100 | 28.8 | 5.0 | 5 | 0.7 | 0.5 | 5 | 71.2 | 5.0 | 5 | 0.41 | 0.09 | 5 |
| 500 | 35.0 | 6.9 | 5 | 0.6 | 0.3 | 5 | 65.0 | 6.9 | 5 | 0.55 | 0.19 | 5 |
| 1 | 39.2 | 17.9 | 5 | 2.7* | 1.0 | 5 | 60.8 | 17.9 | 5 | 0.84 | 0.84 | 5 |
| Day 3 | | | | | | | | | | | | |
| 0 | 27.2 | 5.5 | 5 | 0.8 | 0.4 | 5 | 72.8 | 5.5 | 5 | 0.38 | 0.10 | 5 |
| 20 | 30.2 | 2.4 | 5 | 0.6 | 0.2 | 5 | 69.8 | 2.4 | 5 | 0.43 | 0.05 | 5 |
| 100 | 26.0 | 9.1 | 5 | 0.9 | 0.4 | 5 | 74.0 | 9.1 | 5 | 0.37 | 0.18 | 5 |
| 500 | 27.0 | 5.2 | 5 | 1.0 | 0.5 | 5 | 73.0 | 5.2 | 5 | 0.38 | 0.10 | 5 |
| Day 4 | | | | | | | | | | | | |
| 0 | 33.2 | 5.5 | 5 | 0.6 | 0.4 | 5 | 66.8 | 5.5 | 5 | 0.50 | 0.12 | 5 |
| 20 | 24.2 | 9.1 | 5 | 0.5 | 0.3 | 5 | 75.8 | 9.1 | 5 | 0.33 | 0.16 | 5 |
| 100 | 43.0 | 18.1 | 5 | 0.7 | 0.3 | 5 | 57.0 | 18.1 | 5 | 1.03 | 0.10 | 5 |
| 500 | 46.6 | 20.9 | 5 | 0.7 | 0.2 | 5 | 53.4 | 20.9 | 5 | 1.23 | 1.22 | 5 |

*Statistically significant at p≤0.05.

PCE = Polychromatic erythrocytes

NCE = Normochromic erythrocytes

† = Mean values of %PCE and %NCE used to determine ratio.

There was no treatment-related increase in the percent micronucleated polychromatic erythrocyte at the three target timepoints which demonstrated that naloxone did not induce micronuclei in the bone marrow cells of male and female mice. However, naloxone was cytotoxic to the bone marrow at the mid- and high-doses evaluated. At the MD (100 mg/kg) and HD (500 mg/kg), a statistical significant decrease in polychromatic erythrocytes was observed in males at the 48-hour timepoint. Relative to vehicle control, a 29% and 44% decrease in the percentage of polychromatic erythrocytes was observed in the MD and HD males, respectively. Thus, suggesting a depression of bone marrow proliferation occurring in males.

7.4 Other Genetic Toxicity Studies

None

8 Carcinogenicity

Study title: Naloxone Hydrochloride: 26-week repeated dose oral carcinogenicity study in Tg.rasH2 mice

Study no.: ONU-N-009
Study report location: EDR, 4.2.3.4
Conducting laboratory and location: (b) (4)

Date of study initiation: February 8, 2011
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Naloxone hydrochloride (b) (4) Lot C06698, Purity: 91.4%
CAC concurrence: Yes. An Executive CAC (eCAC) meeting was held on January 18, 2010. The Applicant proposed a 26-week study with doses of 0, 30, 100, and 300 mg/kg/day for both male and female Tg.rasH2 mice, primarily based on mortality. The eCAC recommended doses of 0, 25, 75, and 200 mg/kg/day based on a MTD defined by increased mortality at 600 and 800 mg/kg/day and potentially dose-limiting clinical signs (e.g., ataxia, hunched posture, labored breathing) at the dose of 400 mg/kg/day. The Applicant accepted eCAC recommendations and the 26-

week study were conducted as recommended by eCAC.

Key Study Findings

| | Key Study Findings |
|--------------------------------|---|
| Survival | There were no survival-related issues due to naloxone administration. There were no statistically significant differences in survival rates in naloxone-treated animals compared to control. Therefore, mortality did not confound tumor data and statistical adjustment for survival was not necessary. |
| Clinical Signs | No treatment-related clinical signs were observed during the study. |
| Body Weight | Treatment-related effects on mean absolute body weight were observed in both HD males and females. A statistically significant increase in body weight gain was noted in both sexes in the HD group. |
| Non-neoplastic findings | An increase incidence and severity of hyperplasia of squamous epithelium lining in the non-glandular stomach occurred in both sexes in the MD and HD groups. However, this histological finding was not associated with an increased incidence of stomach tumors. |
| Tumorigenicity | There was no statistically significant increase in neoplastic finding in this study. Incidence of pulmonary neoplasms, hemangiomas and hemangiosarcomas and other neoplasms in multiple organs were observed in the vehicle control and naloxone-treated animals. These tumors occurred with a low incidence and were within the historical control range. The positive control, urethane, produced the types and frequencies of tumors expected for this model. |
| Summary | There was no evidence for naloxone-related oncogenic potential in rasH2 (hemizygous) mice that received naloxone via oral gavage for 26 weeks at dose level of 25, 75, and 200 mg/kg/day. |

| Key Study Findings | |
|---------------------------|---|
| | NOAEL for stomach lesions was 25 mg/kg/day. |

Adequacy of Carcinogenicity Study

The study design of the carcinogenic 26-week transgenic mouse model was adequate.

Appropriateness of Test Models

The transgenic Tg.rasH2 mouse model is an acceptable alternative model for assessing the carcinogenic potential of nongenotoxic and genotoxic compounds. Pritchard and colleagues (2003) reported that analysis of several transgenic mouse models as a predictor of human carcinogenicity susceptibility has indicated that the Tg.rasH2 model offered 81% correct determination for predicting carcinogenicity (1% false positive, 0% false negative) of known carcinogens versus non-carcinogens.

Evaluation of Tumor Findings

Naloxone hydrochloride was found to be negative.

Methods

Doses: 0, 25, 75, 200 mg/kg/day
 Frequency of dosing: Once daily
 Dose volume: 10 mL/kg
 Route of administration: Oral (gavage)
 Formulation/Vehicle: Solution/Sterile water for injection, USP
 Basis of dose selection: MTD (mortality).

A 13-week oral toxicity study (Study ONU-N-001), with naloxone hydrochloride [REDACTED]^{(b)(4)} using Tg.rasH2 mice at doses of 200, 400, 600, and 800 mg/kg/day (free base). Mortality was the limiting toxicity. Mortality occurred at doses of 600 and 800 mg/kg/day in both males and females. Therefore, doses < 600 mg/kg/day was considered the MTD.

Species/Strain: Mice/CByB6F1-Tg(HRAS)2Jic (+/- hemizygous c-Hras)

Number/Sex/Group:

| Group | Dose (mg/kg/day) | Number of animals/sex Main Toxicity Groups |
|-----------------------|------------------|---|
| 1 (vehicle control) | 0 | 30 |
| 2 (positive control)* | 1000 | 15 |
| 3 | 25 | 25 |
| 4 | 75 | 25 |
| 5 | 200 | 30 |

*: The positive control animals were administered a total of 3 intraperitoneal injections of urethane, one each on Study Days 1, 3 and 5

Age: Approximately 7 to 8 weeks of age (at initiation of dosing)
 Animal housing: Individually housed
 Paradigm for dietary restriction: None
 Dual control employed: Yes; a negative (vehicle) and positive (urethane) control group
 Interim sacrifice: None
 Satellite groups: Toxicokinetic Group:

| Group | Dose (mg/kg/day) | Number of animals/sex | |
|-----------------------|------------------|-----------------------|--|
| | | TK Group** | |
| 1 (vehicle control) | 0 | 8 | |
| 2 (positive control)* | 1000 | - | |
| 3 | 25 | 40 | |
| 4 | 75 | 40 | |
| 5 | 200 | 40 | |

*: The positive control animals were administered a total of 3 intraperitoneal injections of urethane, one each on study Days 1, 3 and 5.
 **: "Extra TK animals (2/sex in the control and 4/sex in each test article group) were included to ensure adequate animals for TK bleeding."

Deviation from study protocol: Deviations are described under the appropriate observation(s)

Observations and Results

Mortality

All main and toxicokinetic animals were observed for morbidity, and mortality twice daily (at least 6 hours apart) throughout the duration of the study.

Deviation from the protocol. "On March 05, 2011, the mortality check was not documented for some animal; however, stock and TK animals were alive and healthy on the day before and the day after." The reviewer does not feel that this affected the integrity or interpretation of the study.

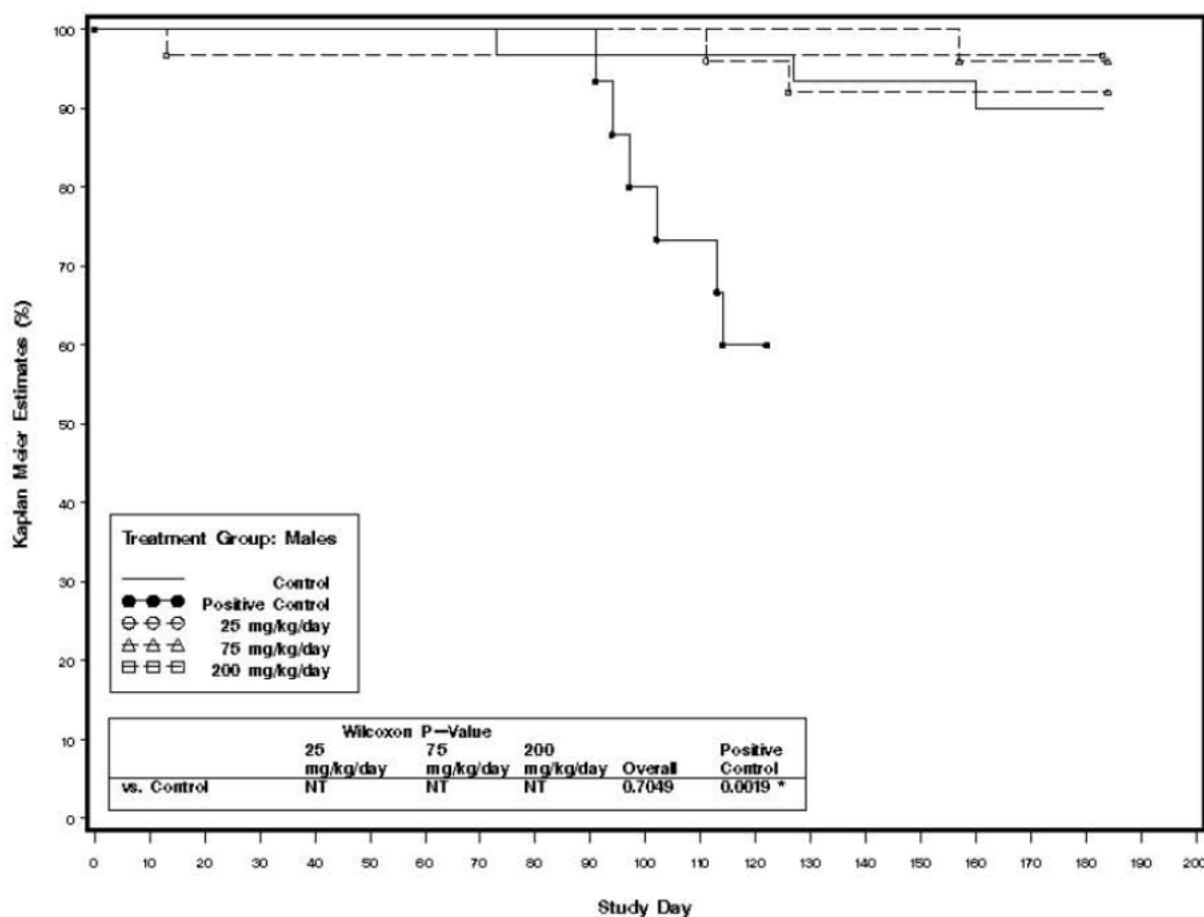
Table 64. Mortality and survivor data for 26-week carcinogenicity study in main study Tg.rasH2 mice

| Dose (mg/kg/day) | 0 | 0 | 25 | 75 | 200 |
|--|-------------|----------------|-------------|-------------|---------------|
| No of animals → | 30 | 15 | 25 | 25 | 30 |
| Males | | | | | |
| Accidental deaths | 0 | 0 | 0 | 0 | 0 |
| Euthanized <i>in extremis</i> (Moribund sacrifice) | 2 | 1 | 2 | 1 | 0 |
| Found dead | 1 | 5 | 0 | 0 | 1 |
| Total Unscheduled Deaths | 3 | 6 ^a | 2 | 1 | 1 |
| Survivors to study scheduled termination | 27 (90%) | 0 ^b | 23 (92%) | 24 (96%) | 29 (96.7%) |

| Dose (mg/kg/day) | 0 | 0 | 25 | 75 | 200 |
|--|--------------|----------------|-------------|--------------|--------------|
| No of animals → | 30 | 15 | 25 | 25 | 30 |
| (% Survival) | | | | | |
| Female | | | | | |
| Accidental deaths | 0 | 0 | 0 | 0 | 0 |
| Euthanized <i>in extremis</i> (<i>Moribund sacrifice</i>) | 0 | 4 | 0 | 0 | 0 |
| Found dead | 0 | 1 | 1 | 0 | 0 |
| Total Unscheduled Deaths | 0 | 5 ^a | 1 | 0 | 0 |
| Survivors to study scheduled termination (% Survival) | 30 (100%) | 0 ^b | 24 (96%) | 25 (100%) | 30 (100%) |

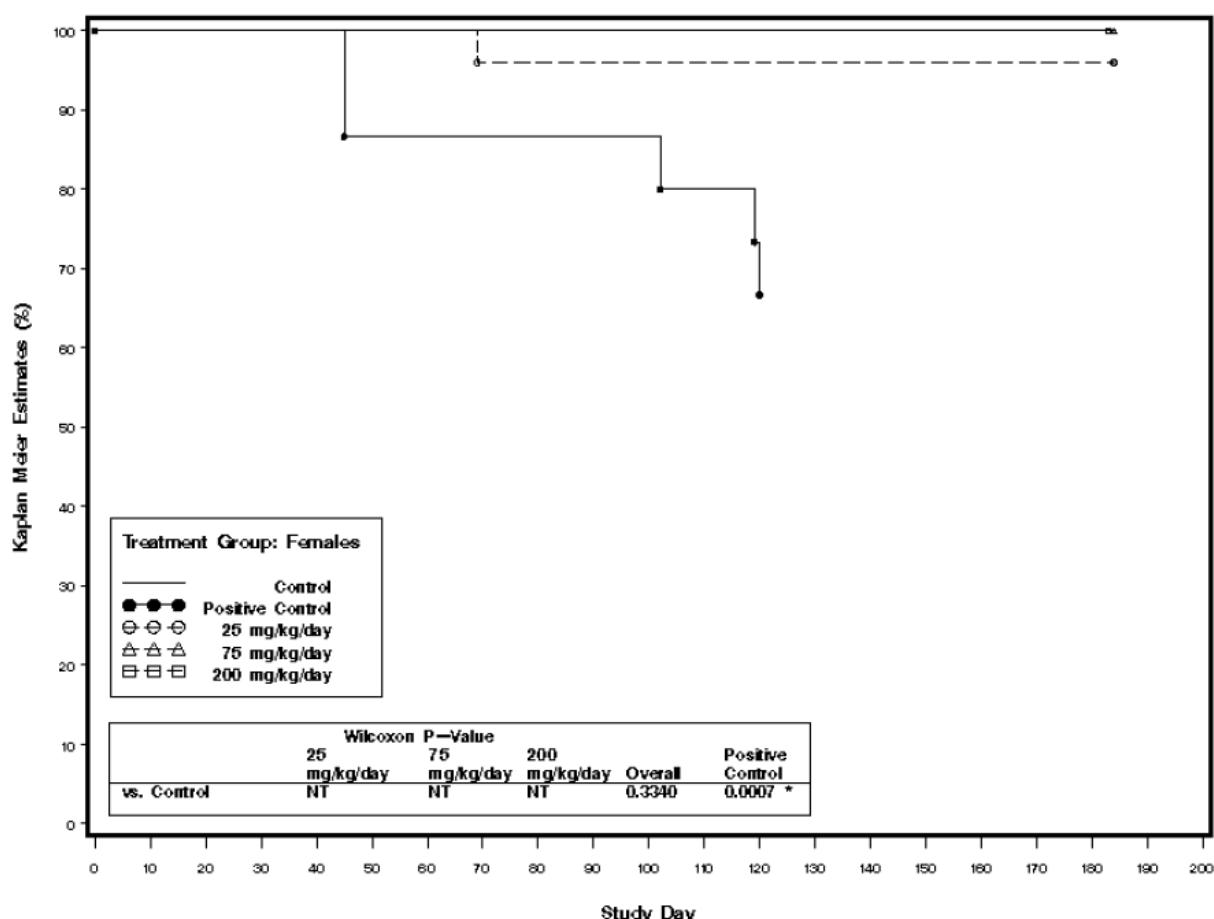
a: Significantly different from control ($p < 0.05$, Fischer's Exact Test)
 b: Surviving positive control animals were sacrificed on Day 120 (females) and Day 122 (males) due to high incidence of mortality

Figure 5. Kaplan-Meier estimates of survival estimates for male Tg.rasHan2 mice in the 26-week carcinogenicity study



* - statistically significant. NT – Not tested due to non-significant overall comparison across all groups.

Figure 6. Kaplan-Meier estimates of survival estimates for female Tg.rasHan2 mice in the 26-week carcinogenicity study



* - statistically significant. NT – Not tested due to non-significant overall comparison across all groups.

Mortality and survivor data are presented in the tables and figures above. In the main study groups, a slight increase in early mortality (found dead or sacrificed moribund) was observed in a few control and naloxone-treated animals. There was 3/30, 2/25, 1/25, and 1/30 death in males in Groups 1, 3, 4, and 5, respectively. In the main study females, there was 1 death in Group 3. In the toxicokinetic groups, 2 of 40 males in Group 5, 1 of 40 females in Group 3, and 3 of 40 females in Group 5 died early (found dead or moribund sacrifice).

There were no survival-related issues due to naloxone administration. There were no statistically significant differences in survival rates in naloxone-treated animals compared to control. Overall survival rate ranged from 90 to 96.7% and 96 to 100% in naloxone-treated males and females, respectively. Therefore, mortality did not confound tumor data. However, there was a significant effect of the positive control urethane, on survival rate. Compared to control animals, Fisher's Exact Test revealed a statistically significant increase in mortality of both sexes in the positive control animals. The Applicant reported that "due to high incidence of mortality and clinical signs of

toxicity, all surviving positive control animals were sacrificed as a group on Day 120 (females) or Day 122 (males)."

Table 65. Summary of mortality in main study Tg.rasH2 mice

| Group | Sex | Mode of Death | Animal Number | Day of Removal | Cause of Death |
|-------|-----|---------------|---------------|----------------|-------------------------------------|
| 1 | M | ND | 8212 | 73 | Undetermined |
| 1 | M | MS | 8214 | 127 | Multicentric Lymphoma |
| 1 | M | MS | 8227 | 160 | Stomach, squamous cell carcinoma |
| 3 | M | MS | 8258 | 126 | Cranial and splenic hemangiosarcoma |
| 3 | M | MS | 8261 | 111 | Skin, hemangiosarcoma |
| 4 | M | MS | 8276 | 157 | Multi centric Mesothelioma |
| 5 | M | ND | 8319 | 13 | Undetermined |
| 3 | F | ND | 8395 | 69 | Multi centric Mesothelioma |

M = Male; F = Female

ND = Natural Death

MS = Moribund Sacrifice

Group 1 = 0 mg/kg/day (Vehicle Control)

Group 4 = 75 mg/kg/day

Group 3 = 25 mg/kg/day

Group 5 = 200 mg/kg/day

Mortality and survivor data are presented in the tables and figures above.

Clinical Signs

All main study animals were observed cage side for clinical signs of toxicity once daily, within 2 hours after dosing. Detailed hands-on clinical examinations were performed on Study Day 1 and weekly thereafter when the main study animals were weighed.

Deviation from the protocol. "On March 27, 2011, June 03, 211, June 24, 2011, and July 23, 2011, cage side observations were not performed within the 2 hour time limit; observation was performed from "11 minutes to 2 hours and 21 minutes outside of the two-hour time specifications". The mortality check was not documented for some animal; however, stock and TK animals were alive and healthy on the day before and the day after." The reviewer does not feel that this affected the integrity or interpretation of the study.

Table 66. Summary of cage side clinical observation - Males

| | | Dose (mg/kg/day) | | | | |
|----------------------|-------------------|------------------|------|----|----|-----|
| | | 0 | 1000 | 25 | 75 | 200 |
| Clinical Observation | | | | | | |
| Ataxic | No of Observation | - | 32 | - | - | - |

| | | | | | | |
|------------------|-------------------|-----------|-------|-----------|-----------|---|
| | Nº of Animals | - | 11* | - | - | - |
| | Day from – to | - | 1 – 5 | - | - | - |
| ↓ motor activity | Nº of Observation | 2 | 45 | - | 1 | - |
| | Nº of Animals | 1 | 15 | - | | |
| | Day from – to | 158 – 159 | 1 – 5 | 157 – 157 | - | |
| Prostrate | Nº of Observation | - | 21 | - | - | - |
| | Nº of Animals | - | 7* | - | - | - |
| | Day from – to | - | 1 – 5 | - | - | - |
| Rapid & Shallow | Nº of Observation | 2 | 30 | - | 4 | - |
| | Nº of Animals | 1 | 15* | - | 1 | - |
| | Day from – to | 158 – 159 | 3 – 5 | - | 154 – 157 | - |

*: Significantly different from control ($p<0.05$, Fischer's Exact Test)

-: Not applicable

Table 67. Summary of cage side clinical observation - Females

| | | Dose (mg/kg/day) | | | | |
|-----------------------------|-------------------|------------------|-------|----|---------|---------|
| | | 0 | 1000 | 25 | 75 | 200 |
| Clinical Observation | | | | | | |
| Ataxic | Nº of Observation | - | 38 | - | - | - |
| | Nº of Animals | - | 13* | - | - | - |
| | Day from – to | - | 1 – 5 | - | - | - |
| ↓ motor activity | Nº of Observation | - | 38 | - | - | - |
| | Nº of Animals | - | 15* | - | - | - |
| | Day from – to | - | 1 – 5 | - | - | - |
| Rapid & Shallow | Nº of Observation | - | 48 | - | - | - |
| | Nº of Animals | - | 15* | - | - | - |
| | Day from – to | - | 1 – 5 | - | - | - |
| Hunched | Nº of Observation | - | - | - | 3 | 5 |
| | Nº of Animals | - | - | - | 2 | 4 |
| | Day from – to | - | - | - | 32 – 94 | 32 – 45 |

*: Significantly different from control ($p<0.05$, Fischer's Exact Test)

-: Not applicable

In the positive control animals, there was an increase incidence of ataxic, decrease motor activity and rapid/shallow breathing noted during cageside observation compared to the positive control.

Table 68. Summary of hands on clinical observation - Males

| | | Dose (mg/kg/day) | | | | |
|-----------------------------|-------------------|------------------|----------|-----------|-----------|----------|
| | | 0 | 1000 | 25 | 75 | 200 |
| Clinical Observation | | | | | | |
| Hunched | Nº of Observation | 2 | 6 | 6 | 4 | 4 |
| | Nº of Animals → | 2 | 4 | 2 | 4 | 2 |
| | Day from – to | 127 – 156 | 92 – 120 | 92 – 120 | 106 – 183 | 29 - 169 |
| Rapid & Shallow | Nº of Observation | 1 | 8 | 3 | - | - |
| | Nº of Animals | 1 | 5* | 1 | - | - |
| | Day from – to | 127 – 127 | 106- 120 | 106 – 120 | - | - |

| | | Dose (mg/kg/day) | | | | |
|----------------------|-------------------|------------------|----------|----------|----------|----------|
| | | 0 | 1000 | 25 | 75 | 200 |
| Clinical Observation | | | | | | |
| Thin | Nº of Observation | 2 | 6 | - | 5 | 9 |
| | Nº of Animals | 2 | 4 | - | 3 | 7 |
| | Day from – to | 99 – 127 | 8 -120 | - | 85 – 169 | 29 - 169 |
| Mass | Nº of Observation | 31 | 10 | - | - | 26 |
| | Nº of Animals | 2 | 2 | - | - | 1 |
| | Day from – to | 64 – 183 | 57 – 120 | - | - | 8 -183 |
| Nodule | Nº of Observation | 18 | 12 | 10 | - | - |
| | Nº of Animals | 3 | 3 | 2 | - | - |
| | Day from – to | 43 – 169 | 78 – 120 | 71 – 183 | - | - |

*: Significantly different from control (p<0.05, Fischer's Exact Test)
-: Not applicable

Table 69. Summary of hands on clinical observation - Females

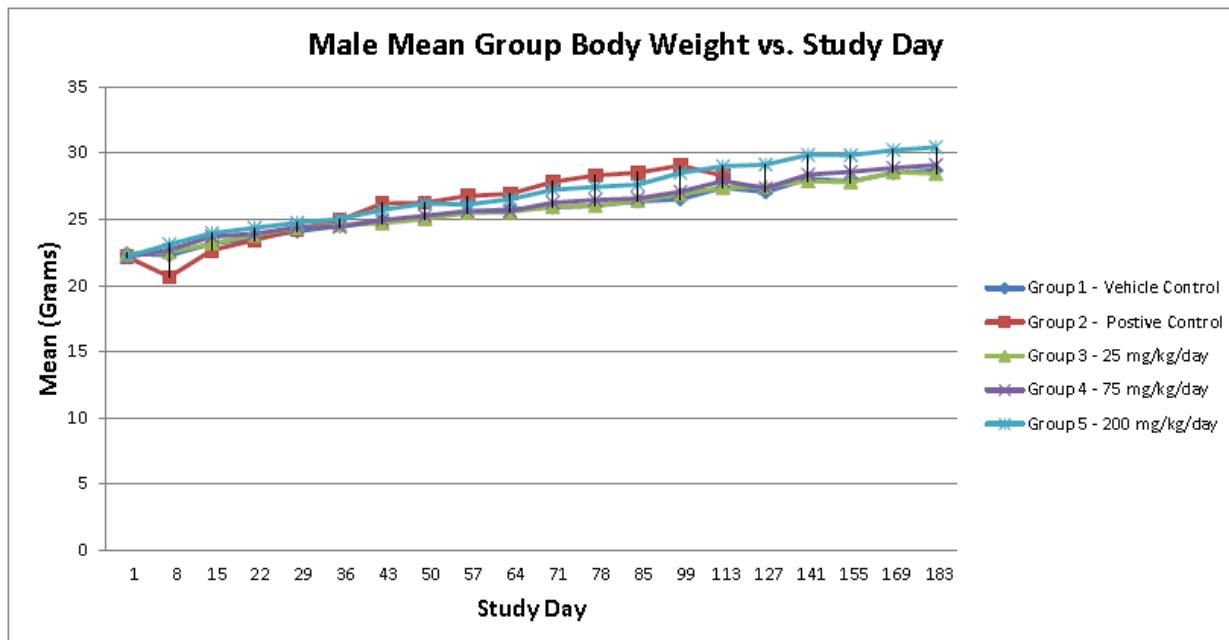
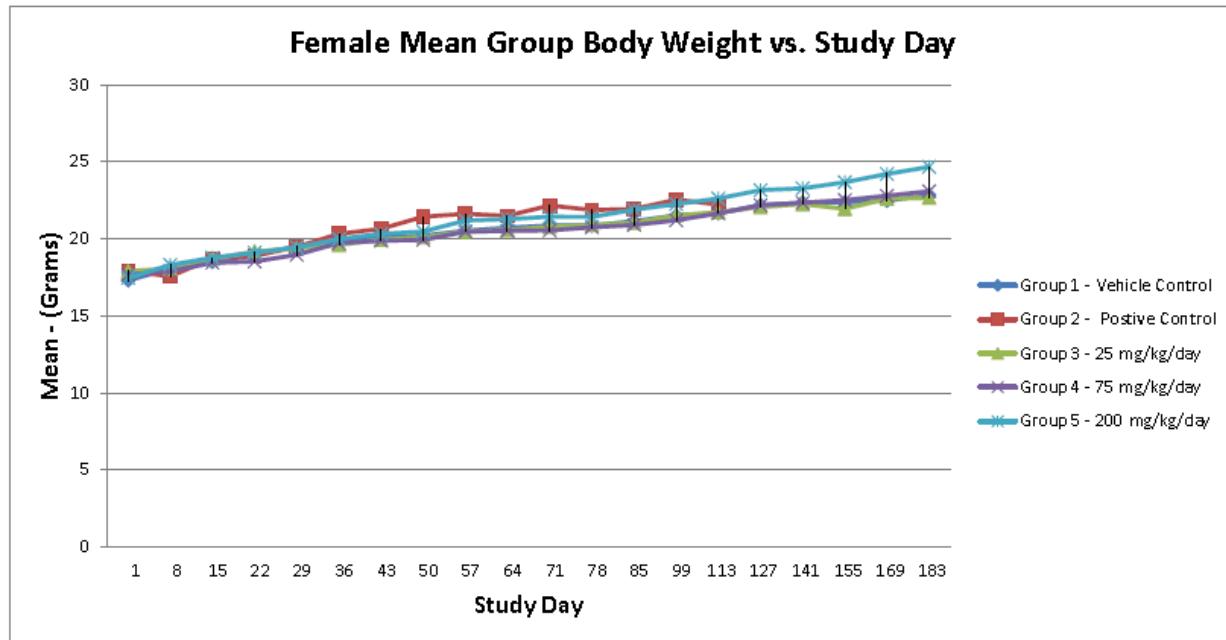
| | | Dose (mg/kg/day) | | | | |
|----------------------|-------------------|------------------|-----------|----------|----------|----------|
| | | 0 | 1000 | 25 | 75 | 200 |
| Clinical Observation | | | | | | |
| Hunched | Nº of Observation | 1 | 5 | 3 | 5 | 1 |
| | Nº of Animals → | 1 | 4* | 3 | 5 | 1 |
| | Day from – to | 127 – 127 | 29 – 120 | 99 – 169 | 99 – 99 | 29 - 29 |
| Thin | Nº of Observation | 8 | 7 | 10 | 15 | 3 |
| | Nº of Animals | 4 | 5 | 9* | 9* | 3 |
| | Day from – to | 8 – 127 | 103 -120 | 8 – 183 | 15 – 140 | 29 – 127 |
| Nodule | Nº of Observation | - | 1 | - | - | 18 |
| | Nº of Animals | - | 1 | - | - | 18 |
| | Day from – to | - | 120 – 120 | - | - | 29 - 148 |

*: Significantly different from control (p<0.05, Fischer's Exact Test)
-: Not applicable

The primary clinical signs observed during the hands on observation included an increase incidence of mass (males), nodules (females) and thinness in naloxone-treated Tg.rasH2 mice compared to the positive control.

Body Weights

Body weights of main and toxicokinetic study animals were recorded once weekly beginning on Study Day 1 through Week 13 and biweekly thereafter.

Figure 7. Mean body weights values in males**Figure 8. Mean body weights values in females**

Treatment-related effects on mean absolute body weight were observed in both HD males and females. Applicant's figures for weekly mean absolute body weight are presented above. Weekly mean absolute body weight in the positive control and HD naloxone-treated groups in both sexes was sporadically statistically significantly higher than the vehicle control group. Compared to vehicle control, mean absolute body weight increase were statistically significantly higher in HD naloxone-treated males on

Study Days 8, 15, 50, 71, 78, 99, 127, 141, and 155. Mean absolute body weights in the HD naloxone-treated females was significantly higher on Study Days 57, 85, 99, 113, 127, 141, 155, 169, and 183. and females. Group mean absolute weight increase ranged from 3.4% to 7.7% higher than the vehicle control in HD naloxone-treated males and from 3.2% to 8.0% higher than the vehicle control in the HD naloxone-treated females.

Compared to vehicle control, mean body weight increase were statistically significantly higher in positive control males from Study Days 71 to 99. Mean body weights in the positive control treated females were significantly higher than vehicle control females from Study Days 50 to 78 and on Study Day 99. Group mean body weight increase ranged from 7.4% to 9.4% higher than the vehicle control in the positive control males and was 5.0% higher than the vehicle control in the positive control females.

Table 70. Mean body weight gain in Tg.rasH2 mice from Day 1 to Day 183

| Naloxone Dose (mg/kg/day) | Absolute Body Weight Gain | | Percent Body Weight Gain | |
|--------------------------------------|--------------------------------------|---------------------|---------------------------------|---------------|
| | M | F | M | F |
| 0 | 6.26 ± 2.21 | 5.32 ± 1.58 | 27.99 ± 9.92 | 30.71 ± 10.53 |
| 25 | 5.83 ± 2.22 | 4.74 ± 1.02 | 25.59 ± 8.49 | 26.53 ± 6.07 |
| 75 | 6.83 ± 3.21 | 5.41 ± 1.690 | 30.57 ± 13.90 | 30.60 ± 8.99 |
| 200 | 8.27* ± 2.30 | 6.98* ± 1.82 | 37.38 ± 10.55 | 39.72 ± 11.43 |

*: Statistically significant when compared to control at p≤ 0.05 (Way Analysis of Variance)

Mean body weight gain data from Study Days 1 to 183 are presented in the table above. Treatment-related effects on body weight gain were observed in both males than females. Mean body weight gain increased in a non-dose-dependent manner.

Food Consumption

Food consumption for main study animals was recorded through the duration of the study. No food consumption observations were collected from the toxicokinetic animals.

Weekly mean food consumption in the positive control (males and females), LD (females) MD (males and females), and HD (males and females) naloxone-treated groups was sporadically statistically significantly higher than the vehicle control group. However, the observed increase in food consumption was not correlated with the increase in body weight.

Gross Pathology

All surviving main study animals and positive control animals were euthanized by carbon dioxide asphyxiation on Study Days 183 or 184 and Study Days 120 (females) and 122 (males), respectively . Necropsy examinations were also performed on main

study animals that were euthanized in extremis or found dead. Macroscopic evaluation was performed.

Macroscopic observations

Table 71. Summary of Macroscopic Observations - All male Tg.rasH2 mice

| Male Tg.rasH2 Mice | | | | | | |
|---------------------------|-------------------------------|-------------------------|-----------------|-----------|-----------|------------|
| | | Dose (mg/kg/day) | | | | |
| | | Urethane | Naloxone | 25 | 75 | 200 |
| Organ | No of animals examined | 30 | 15 | 25 | 25 | 30 |
| Lungs with bronchi | No visible lesion | | | | | |
| | Nodule; white; all lobes | 0 | 11 | 0 | 0 | 0 |
| Spleen | No visible lesion | 25 | 0 | 25 | 24 | 30 |
| | Nodules | 0 | 10 | 0 | 1 | 0 |
| | Mass | 0 | 3 | 0 | 0 | 0 |
| | Mass, tan | 0 | 1 | 0 | 0 | 0 |
| | Focus | 0 | 1 | 0 | 0 | 0 |

Table 72. Summary of Macroscopic Observations - All female Tg.rasH2 mice

| Female Tg.rasH2 Mice | | | | | | |
|-----------------------------|-------------------------------|-------------------------|-----------------|-----------|-----------|------------|
| | | Dose (mg/kg/day) | | | | |
| | | Urethane | Naloxone | 25 | 75 | 200 |
| Organ | No of animals examined | 30 | 15 | 25 | 25 | 30 |
| Lungs with bronchi | No visible lesion | 28 | 2 | 25 | 25 | 28 |
| | Nodule; white; all lobes | 0 | 12 | 0 | 0 | 0 |
| Spleen | No visible lesion | 29 | 1 | 25 | 24 | 29 |
| | Nodules | 0 | 4 | 0 | 0 | 0 |
| | Mass | 0 | 1 | 0 | 0 | 0 |
| | Mass, dark | 0 | 1 | 0 | 0 | 0 |
| | Focus | 0 | 2 | 0 | 0 | 0 |

In the naloxone-treated groups, no treatment-related macroscopic observations were noted in males or females. Treatment-related macroscopic pulmonary and splenic lesions were noted in the urethane (1000 mg/kg/day) positive control animals. These lesions are expected from urethane.

Organ weights

Statistical significant organ weight changes in the liver, spleen, heart, and adrenal gland were reported by the Applicant.

Liver: Relative to vehicle control, the weight relative-to-body-weight in the LD, and HD males were significantly decreased by 5.5% and 9.8%, respectively. In females, liver

weight relative-to-body-weight in the MD and HD groups were significantly decreased by 5.1 and 6.6% compared to vehicle control, respectively.

Spleen: Relative to vehicle control, the weight relative-to-body-weight in the LD, and HD males were significantly decreased by 10.5% and 10.6%, respectively. In females, the absolute spleen weight and weight relative-to-body-weight in the MD groups were significantly decreased by 11.1% and 14.2% compared to vehicle control, respectively. Relative to control, spleen weight relative-to-body weight in HD females was significantly decreased by 14.0%.

Adrenal Glands: Relative to control, absolute weight of HD females was significantly increased by 13.5%.

Heart: Relative to control, absolute weight of LD and HD females was significantly increased by 7.6% and 7.7%, respectively.

Reviewer comments. The reviewer does not consider these organ weight changes to be toxicological or biological relevance because no histological changes were correlated with the organ weight changes.

Histopathology

Peer Review: Yes: The peer review pathology report was submitted and signed by [REDACTED] ^{(b) (4)}.

The standard/routine organ and tissues were collected from all animals Group 1 (control) and Groups 3 thru 5 (naloxone treatment groups). The identified target organ (skin) was microscopically examined in the MD animals. Also, all gross lesions from all the animals were examined microscopically. All tissues were fixed in 10% neutral buffered formalin. Tissue selected for microscopic analysis were processed and stained with hematoxylin eosin and examined by light microscopy.

| Tissue | Organ Weight | Collected and Preserved | Microscopic Examination | | |
|---------------------------------|--------------|-------------------------|-------------------------|--------------------|-----------------------|
| | | | Group 1 (Vehicle) | Group 2 (Urethane) | Groups 3-5 (Naloxone) |
| Adrenal gland | X | X | X | | X |
| Aorta | | X | X | | X |
| Bone marrow smear | | X | | | |
| Bone (femur and sternum) | | X | X | | X |
| Bone marrow (femur and sternum) | | X | X | | X |
| Brain | X | X | X | | X |
| Clitoral gland | | | | | |

| Tissue | Organ Weight | Collected and Preserved | Microscopic Examination | | |
|------------------------------|--------------|-------------------------|-------------------------|--------------------|-----------------------|
| | | | Group 1 (Vehicle) | Group 2 (Urethane) | Groups 3-5 (Naloxone) |
| Coagulating gland | | | | | |
| Epididymis | | X | X | | X |
| Esophagus | | X | X | | X |
| Eye | | X | X | | X |
| Gallbladder | | X | X | | X |
| Harderian gland | | X | X | | X |
| Heart | X | X | X | | X |
| Joint, tibiofemoral; | | | | | |
| Kidney | X | X | X | | X |
| Lacrimal gland, exorbital | | | | | |
| Large intestine, cecum | | X | X | | X |
| Large intestine, colon | | X | X | | X |
| Large intestine, rectum | | X | X | | X |
| Larynx | | X | X | | |
| Liver | X | X | X | | X |
| Lung | | X | X | X | X |
| Lymph node, mandibular | | X | X | | X |
| Lymph node, mediastinal | | X | X | | X |
| Lymph node, mesenteric | | X | X | | X |
| Mammary gland (females only) | | X | X | | X |
| Nasal cavity | | X | X | | X |
| Ovaries | X | X | X | | X |
| Oviduct | | X | X | | |
| Pancreas | | X | X | | X |
| Parathyroid glands | | X | X | | X |
| Peyers Patch | | | | | |
| Pharynx | | | | | |
| Pituitary gland | | X | X | | X |
| Preputial gland | | | | | |
| Prostate gland | | X | X | | X |
| Salivary gland | | X | X | | X |
| Salivary gland, parotid | | X | X | | |
| Salivary gland, sublingual | | X | X | | |
| Sciatic nerve | | X | X | | X |
| Seminal vesicle | | X | X | | X |
| Skeletal muscle, (thigh) | | X | X | | X |
| Skin, mammary area | | X | X | | X |

| Tissue | Organ Weight | Collected and Preserved | Microscopic Examination | | |
|---|--------------|-------------------------|-------------------------|--------------------|-----------------------|
| | | | Group 1 (Vehicle) | Group 2 (Urethane) | Groups 3-5 (Naloxone) |
| 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, lumbar | | X | X | | X |
| Spinal cord, thoracic | | X | X | | X |
| Spleen | X | X | X | X | X |
| Stomach | | X | X | | X |
| Testis | X | X | X | | X |
| Thymus | | X | X | | |
| Thyroid gland | | X | X | | X |
| Tongue | | | | | |
| Trachea | | X | X | | X |
| Ureter | | | | | |
| Urinary Bladder | | X | X | | X |
| Uterus | | X | X | | X |
| Vagina | | X | X | | X |
| Zymbal's gland | | | | | |
| Gross lesions, including mass | | X | X | | X |
| Group 1: vehicle control | | | | | |
| Group 2: positive control (1000 mg/kg/day urethane) | | | | | |
| Group 3: 25 mg/kg/day naloxone | | | | | |
| Group 4: 75 mg/kg/day naloxone | | | | | |
| Group 5: 200 mg/kg/day naloxone | | | | | |

Neoplastic Microscopic Findings

Incidence of pulmonary neoplasms, hemangiomas, and hemangiosarcoma and other neoplasms in multiple organs were observed in the vehicle control and naloxone-treated animals. These tumors occurred with a low incidence and were within the historical control range (HCR). These neoplasms are discussed below.

Pulmonary Neoplasms

Table 73. Incidence of pulmonary neoplasms in Tg.rasH2 mice

| | No of animals with neoplasm | | | | | HCR |
|--------------------|-----------------------------|----------|----------|----|----|-----|
| No Tg.rasH2 mice → | 30 | 15 | 25 | 25 | 30 | |
| Group → | 1 | 2 | 3 | 4 | 5 | |
| Treatment → | Control | Urethane | Naloxone | | | |

| Dose (mg/kg/day) | 0 | 1000 (urethane) | 25 | 75 | 200 | |
|-----------------------|---|--------------------|----|----|-----|-----|
| Males | | | | | | |
| Adenoma, single | 3 | 0 | 2 | 1 | 4 | 0-6 |
| Adenoma, multiple | 1 | 15* | 1 | 0 | 0 | 0-1 |
| Carcinoma | 0 | 6† | 1 | 1 | 0 | 0-2 |
| Adenoma and carcinoma | 4 | 15† | 4 | 2 | 4 | 0-7 |
| Female | | | | | | |
| Adenoma, single | 0 | 0 | 2 | 1 | 1 | 0-6 |
| Adenoma, multiple | 0 | 15* | 0 | 0 | 0 | 0-1 |
| Carcinoma | 1 | 8† | 0 | 0 | 0 | 0-1 |
| Adenoma and carcinoma | 1 | 15† | 2 | 1 | 1 | 0-6 |

†: Statistically significant compared to Control Group 1 ($p<0.01$, Fisher's Exact Test)

*: Statistically significant incidence of single and multiple adenomas compared to control Group 1 ($p<0.01$, Fisher's Exact Test)

HCR: ^{(b) (4)} Historical Control Range

There was no naloxone-related effect on the incidences of pulmonary neoplasms in either sex. Compared to the vehicle control animals, no statistically significant increase or dose-response for pulmonary neoplasms or neoplasm combination were observed in any of the naloxone-treated groups; and the frequency were similar to concurrent historical controls.

The positive control animals (Group 2) had a statistically (Fisher's p-value <0.05) higher incidence of pulmonary neoplasms than the vehicle control. The observed pulmonary neoplasms in urethane-treated mice included multiple adenomas, carcinoma, and adenoma/carcinoma combination in both sexes. Pulmonary neoplasms are spontaneous neoplasms in Tg.rasH2 mice (Morton, et al., 2002; Paranjpe, et al., 2013).

Splenic Neoplasms

Table 74. Incidence of splenic neoplasms in Tg.rasH2 mice

| | No of animals with neoplasm | | | | | HCR |
|--------------------|-----------------------------|----------|----------|----|-----|-----|
| No Tg.rasH2 mice → | 30 | 15 | 25 | 25 | 30 | |
| Group → | 1 | 2 | 3 | 4 | 5 | |
| Treatment → | Control | Urethane | Naloxone | | | |
| Dose (mg/kg/day) | 0 | 1000 | 25 | 75 | 200 | |
| Male | | | | | | |
| Hemangiosarcoma | 1 | 14† | 1 | 1 | 1 | 0-4 |
| Female | | | | | | |
| Hemangiosarcoma | 1 | 13† | 1 | 1 | 1 | 0-4 |

†: Statistically significant compared to Control Group 1 ($p<0.05$, Fisher's Exact Test)

HCR: ^{(b) (4)} Historical Control Range

Splenic hemangiosarcomas were observed in a few animals in the naloxone-treated animals. The incidence of splenic hemangiosarcomas was comparable to the vehicle control group and no statistically significant increase or dose-response in any of the naloxone-treated groups was observed. Also, the incidence of hemangiosarcoma in the naloxone-treated animals was present at similar frequency to concurrent historical controls, and therefore not considered drug-related.

Urethane-related effect on the incidence of splenic neoplasms was observed in both sexes. The Applicant's statistical analysis revealed a significant (Fisher's p-value <0.05) increase in the incidence of splenic hemangiosarcoma in the positive control Tg.rasH2 mice (Group 2) compared to vehicle control. Splenic neoplasms are spontaneous neoplasms are spontaneous in Tg.rasH2 mice (Gulezian, et al., 2000).

Hemangiosarcomas

Table 75. Incidence of multiple organ hemangiosarcomas and in Tg.rasH2 mice

| | № of animals with neoplasm | | | | | HCR |
|------------------------------|----------------------------|-----------------|----------------|----|-----|-----|
| № Tg.rasH2 mice → | 30 | 15 | 25 | 25 | 30 | |
| Group → | 1 | 2 | 3 | 4 | 5 | |
| Treatment → | Control | Urethane | Naloxone | | | |
| Dose (mg/kg/day) | 0 | 1000 | 25 | 75 | 200 | |
| | Male | | | | | |
| Spleen | 1 | 14 [†] | 1 | 1 | 1 | 0-4 |
| Testes | 1 | | 0 | 0 | 0 | 0-1 |
| Cranium | 0 | | 1 | 0 | 0 | NR |
| Skin | 0 | | 1 | 0 | 0 | 0-1 |
| Combined incidence | 2 | | 2 ^a | 1 | 1 | 0-4 |
| | Female | | | | | |
| Spleen | 1 | 13 [†] | 1 | 1 | 1 | 0-4 |
| Lung | 0 | | 0 | 1 | 0 | 0-1 |
| Uterus | 1 | | 0 | 0 | 1 | 0-2 |
| Vagina | 0 | | 0 | 0 | 1 | 0-1 |
| Mediastinum ^b | 0 | | 0 | 1 | 0 | NR |
| Urinary bladder ^b | 0 | | 0 | 0 | 0 | NR |
| Combined incidence | 2 | | 1 | 4 | 3 | 0-3 |

†: Statistically significant compared to Control Group 1 (p<0.05, Fisher's Exact Test)
a: Primary hemangiosarcoma diagnosed in one animal in the cranium and spleen.
b: Hemangioma diagnosed for this organ
HCR: █^{(b) (4)} Historical Control Range
NR: Not recorded in █^{(b) (4)} Historical Control Range

Hemangiosarcomas and hemangioma were observed in a few animals in the naloxone-treated animals. The observed neoplasm was spread across various organs; these incidences of hemangiosarcomas and hemangioma were not considered drug-related because they were present at similar frequency to concurrent historical controls. The

Applicant's statistical analysis revealed no significant increase in incidence in the naloxone-treated groups compared to vehicle control.

Multiple Organ Neoplasms

Table 76. Incidence of multiple organ neoplasms in naloxone-treated animals compared to control animals

| | No of animals with neoplasm | | | | HCR |
|----------------------------------|--|----|----|-----|-----|
| No Tg.rasH2 mice → | 30 | 25 | 25 | 30 | |
| Group → | 1 | 3 | 4 | 5 | |
| Dose (mg/kg/day) | 0 | 25 | 75 | 200 | |
| Male | | | | | |
| Lymphoma multicentric | 1 | 0 | 0 | 0 | 0-1 |
| Mesothelioma multicenteric | 0 | 0 | 1 | 0 | 0-1 |
| Harderian gland, adenoma | 0 | 0 | 0 | 1 | 0-2 |
| Stomach, papilloma | 1 | 0 | 0 | 1 | 0-1 |
| Stomach, squamous cell carcinoma | 1 | 1 | 0 | 0 | 0-1 |
| Liver, adenoma | 0 | 0 | 0 | 1 | 0-1 |
| Female | | | | | |
| Mesothelioma multicenteric | 0 | 1 | 0 | 0 | 0-1 |
| Nasal cavity, carcinoma | 0 | 1 | 0 | 0 | 0-1 |
| Stomach, papilloma | 0 | 1 | 0 | 0 | 0-1 |
| Harderian gland, adenoma | 0 | 2 | 0 | 2 | 0-4 |
| Harderian gland, carcinoma | 0 | 0 | 0 | 1 | 0-2 |
| Urinary bladder leiomyoma | 0 | 1 | 0 | 0 | NR |
| HCR: | (b) (4) Historical Control Range | | | | |
| NR: | Not recorded in (b) (4) Historical Control Range | | | | |

The neoplasms observed were identified in the stomach, liver, harderian gland, nasal cavity, and urinary bladder. The Applicant's statistical analysis revealed no significant increase in incidence in the naloxone-treated groups compared to vehicle control. These neoplasms occurred with a low incidence and were within the historical control range.

FDA Statistical Analysis. FDA statistic reviewer (Mohammad Atiar Rahman, PhD) agrees with the Applicant that there was no treatment-relates neoplastic findings in the naloxone-treated animals. As stated in the review, "this reviewer's analysis did not show statistically significant dose response relationship across the negative control and treated groups in any of the observed tumor types. The pairwise comparison also did not show statistically significant increased incidence of any of the observed tumor types in the treated groups compared to the negative control in either sex." However, as expected, the statistic reviewer's pairwise comparison showed a statistically significant increased incidence of alveolar-bronchiolar adenoma, alveolar-bronchiolar carcinoma, hemangiosarcoma in lungs with bronchiole, and hemangiosarcoma in spleen in positive control compared to the negative control in both sexes.

Non Neoplastic Microscopic Findings

Table 77. Microscopic non-neoplastic findings in Tg.rasH2 mice administered naloxone

| Non-glandular stomach microscopic findings | | | | | | | | | |
|--|----------|------|----|----|-----|--------|----|----|-----|
| Sex → | Severity | Male | | | | Female | | | |
| Dose (mg/kg/day) | | 0 | 25 | 75 | 200 | 0 | 25 | 75 | 200 |
| No of Animal Examined → | | 30 | 25 | 25 | 30 | 30 | 25 | 25 | 30 |
| Non glandular stomach | | | | | | | | | |
| Hyperplasia | | 0 | 0 | 3 | 11 | 0 | 0 | 3 | 8 |
| | Minimal | 0 | 0 | 1 | 4 | 0 | 0 | 0 | 6 |
| | Mild | 0 | 0 | 1 | 6 | 0 | 0 | 3 | 1 |
| | Moderate | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 |
| Skin | | | | | | | | | |
| Inflammation, chronic-active | | 14 | 18 | 22 | 19 | 7 | 3 | 14 | 17 |
| | Mild | 3 | 0 | 1 | 1 | 0 | 0 | 1 | 2 |
| | Moderate | 11 | 18 | 20 | 18 | 7 | 3 | 13 | 14 |
| | Severe | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |

The incidences and severities of the non-neoplastic lesions are presented in the table above. A dose-dependent increase in the incidence and severity of hyperplasia of the squamous epithelium lining in the non-glandular stomach in female and male Tg.rasH2 mice were observed. Relative to control, the incidence of hyperplasia in the non-glandular stomach was more prominent in HD males and HD females. An increase incidence of neoplasm in the non-glandular stomach was not correlated with this microscopic finding.

Toxicokinetics

Blood samples were collected for toxicokinetic evaluation after at least 27 days of treatment (Days 28-9) and after at least 25 weeks of treatment (Day 175-176.). Blood were not collected from positive control animals (Group 2) for toxicokinetic evaluation. Blood was collected at predose, 0.25, 1, 2, 6, and 24 hours after dosing from 3 animals/sex/group/timepoint and at approximately 0.25 postdosing from Group 1 animals. Plasma concentrations of naloxone, 6β-naloxol, and naloxone-3β-D-glucuronide were determined by using liquid chromatography with tandem mass spectrometric (LC-MS/MS). The lower limit of quantification was 0.200 ng/mL for naloxone and 0.400 ng/mL for 6β-naloxol.

Toxicokinetic analysis at Day 27 and during Week 26 of the 26-week study demonstrated that Tg.rasH2 mice orally treated with naloxone was exposed to naloxone and its metabolites 6β-naloxol and 3β-D-glucuronide.

Table 78. Toxicokinetic parameters for naloxone in Tg.rash2 mice plasma

| Interval | Dose Group | Dose Level (mg/kg/day) | Sex | C _{max} (ng/mL) | DN C _{max} [(ng/mL)/(mg/kg/day)] | T _{max} (hr) | AUC ₀₋₁ (ng·hr/mL) | AUC ₀₋₂₄ (ng·hr/mL) | DN AUC ₀₋₂₄ [(ng·hr/mL)/(mg/kg/day)] | AR AUC ₀₋₂₄ |
|----------|------------|------------------------|-----|--------------------------|---|-----------------------|-------------------------------|--------------------------------|---|------------------------|
| Day 28 | 3 | 25 | M | 57.7 | 2.31 | 0.250 | 94.6 | 94.6 | 3.78 | NA |
| | | | F | 140 | 5.61 | 0.250 | 107 | 107 | 4.28 | NA |
| | 4 | 75 | M | 416 | 5.54 | 0.250 | 378 | 378 | 5.04 | NA |
| | | | F | 501 | 6.68 | 0.250 | 401 | 401 | 5.34 | NA |
| | 5 | 200 | M | 915 | 4.58 | 0.250 | 1058 | 1058 | 5.29 | NA |
| | | | F | 1253 | 6.27 | 0.250 | 1116 | 1116 | 5.58 | NA |
| | Day 175 | 3 | M | 95.4 | 3.82 | 0.250 | 94.3 | 113 | 4.50 | 1.19 |
| | | | F | 102 | 4.06 | 0.250 | 84.0 | 89.3 | 3.57 | 0.835 |
| | | 4 | M | 727 | 9.69 | 1.00 | 985 | 985 | 13.1 | 2.61 |
| | | | F | 676 | 9.02 | 0.250 | 475 | 475 | 6.34 | 1.19 |
| | 5 | 200 | M | 2700 | 13.5 | 0.250 | 2218 | 2218 | 11.1 | 2.10 |
| | | | F | 1813 | 9.07 | 0.250 | 1337 | 1337 | 6.68 | 1.20 |

NA Not applicable.

Naloxone. Toxicokinetic parameters for naloxone is presented in the table above (reproduced from NDA 205777 submission). Systemic exposure to naloxone HCl increased in a dose-dependent manner. Naloxone was rapidly absorbed with a T_{max} of 0.25 h on Day 25 and Day 175 with the exception of Group 4 males on Day 175 where T_{max} was observed at 1 hour. The increase in C_{max} and AUC_{0-24h} were generally greater than dose proportional in the MD and HD groups on Day 25 and Day 175. Exposure was higher on Day 175 than on Day 28 for both sexes, suggesting drug accumulation after multiple dosing. A gender difference in exposure was observed; females had a lightly higher systemic exposure than males.

Table 79. Toxicokinetic parameters for 6 β - naloxol in Tg.rasH2 mice plasma

| Interval | Dose Group | Dose Level (mg/kg/day) | Sex | C _{max} (ng/mL) | DN C _{max} [(ng/mL)/(mg/kg/day)] | T _{max} (hr) | AUC ₀₋₄ (ng·hr/mL) | AUC ₀₋₂₄ (ng·hr/mL) | DN AUC ₀₋₂₄ [(ng·hr/mL)/(mg/kg/day)] | M:P Ratio AUC ₀₋₂₄ | AR AUC ₀₋₂₄ |
|----------|------------|------------------------|-----|--------------------------|---|-----------------------|-------------------------------|--------------------------------|---|-------------------------------|------------------------|
| Day 28 | 3 | 25 | M | 1.87 | 0.0749 | 0.250 | 2.67 | 4.08 | 0.163 | 0.0431 | NA |
| | | | F | 10.9 | 0.435 | 0.250 | NR | NR | NA | NA | NA |
| | 4 | 75 | M | 31.4 | 0.418 | 0.250 | 21.1 | 25.1 | 0.335 | 0.0665 | NA |
| | | | F | 42.9 | 0.572 | 0.250 | 26.6 | 29.2 | 0.390 | 0.0729 | NA |
| | 5 | 200 | M | 81.9 | 0.410 | 0.250 | 60.6 | 69.3 | 0.347 | 0.0656 | NA |
| | | | F | 82.4 | 0.412 | 0.250 | 61.1 | 65.3 | 0.326 | 0.0585 | NA |
| Day 175 | 3 | 25 | M | 2.96 | 0.118 | 0.250 | 5.16 | 7.69 | 0.307 | 0.0683 | 1.88 |
| | | | F | 3.30 | 0.132 | 0.250 | NR | NR | NA | NA | NA |
| | 4 | 75 | M | 18.2 | 0.243 | 1.00 | 28.4 | 36.1 | 0.481 | 0.0367 | 1.44 |
| | | | F | 22.6 | 0.302 | 0.250 | 17.6 | 19.0 | 0.253 | 0.0399 | 0.649 |
| | 5 | 200 | M | 131 | 0.657 | 0.250 | 102 | 112 | 0.560 | 0.0505 | 1.62 |
| | | | F | 153 | 0.767 | 0.250 | 92.4 | 95.3 | 0.476 | 0.0713 | 1.46 |

NA Not applicable.

NR Not reported.

6 β -naloxol. Toxicokinetic parameters for 6 β -naloxol is presented in the table above (reproduced from NDA 205777 submission). Systemic exposure to 6 β -naloxol increased with increasing doses of naloxone HCl ^{(b) (4)} in a dose-dependent manner. 6 β -Naloxol readily appeared in the plasma following the oral administration of naloxone. Similar to naloxone, a T_{max} of 0.25 h on Day 25 and Day 175 was observed with the exception of Group 4 males on Day 175 where T_{max} was observed at 1 hour. The AUC₀₋₂₄ metabolite to parent ratios ranged from 0.0367 to 0.0729, suggesting that naloxone was not extensively converted to 6 β -naloxol in Tg.rasH2 mice following oral administration of naloxone HCl ^{(b) (4)}

Table 80. Toxicokinetic parameters for naloxone-3 β -D-glucuronide in Tg.rasH2 mice plasma

| Interval | Dose Group | Dose Level (mg/kg/day) | Sex | C _{max} (ng/mL) | DN C _{max} [(ng/mL)/(mg/kg/day)] | T _{max} (hr) | AUC _{0-t} (ng·hr/mL) | AUC ₀₋₂₄ (ng·hr/mL) | DN AUC ₀₋₂₄ [(ng·hr/mL)/(mg/kg/day)] | M:P Ratio AUC ₀₋₂₄ | AR AUC ₀₋₂₄ |
|----------|------------|------------------------|-----|--------------------------|---|-----------------------|-------------------------------|--------------------------------|---|-------------------------------|------------------------|
| Day 28 | 3 | 25 | M | 15933 | 637 | 0.250 | 14232 | 14232 | 569 | 150 | NA |
| | | | F | 9573 | 383 | 0.250 | 8018 | 8018 | 321 | 75.0 | NA |
| | 4 | 75 | M | 29800 | 397 | 0.250 | 34785 | 34785 | 464 | 92.1 | NA |
| | | | F | 21833 | 291 | 0.250 | 29688 | 29688 | 396 | 74.1 | NA |
| | 5 | 200 | M | 57500 | 288 | 0.250 | 114741 | 114741 | 574 | 108 | NA |
| | | | F | 34333 | 172 | 0.250 | 89455 | 89455 | 447 | 80.1 | NA |
| | Day 175 | 3 | M | 16700 | 668 | 0.250 | 13508 | 13508 | 540 | 120 | 0.949 |
| | | | F | 13433 | 537 | 0.250 | 11148 | 11148 | 446 | 125 | 1.39 |
| | | 4 | M | 30533 | 407 | 0.250 | 40438 | 40438 | 539 | 41.1 | 1.16 |
| | | | F | 39067 | 521 | 0.250 | 37283 | 37283 | 497 | 78.4 | 1.26 |
| | 5 | 200 | M | 58067 | 290 | 1.00 | 135582 | 135582 | 678 | 61.1 | 1.18 |
| | | | F | 64200 | 321 | 1.00 | 103151 | 103151 | 516 | 77.2 | 1.15 |

NA Not applicable.

Naloxone- 3 β -D-glucuronide. Toxicokinetic parameters for naloxone- 3 β -D-glucuronide in Tg.rasH2 plasma is presented in the table above (reproduced from NDA 205777 submission). Systemic exposure to naloxone- 3 β -D-glucuronide increased with increasing doses of naloxone HCl ^{(b)(4)} in a dose-dependent manner. Naloxone- 3 β -D-glucuronide readily appeared in the plasma following the oral administration of naloxone. Similar to naloxone, a T_{max} of 0.25 h on Day 25 and Day 175 was observed with the exception of Group 4 males on Day 175 where T_{max} was observed at 1 hour. The AUC₀₋₂₄ metabolite to parent ratios ranged from 41.1 to 150, suggesting that naloxone was extensively converted to naloxone- 3 β -D-glucuronide in Tg.rasH2 mice following oral administration of naloxone HCl ^{(b)(4)}

Dosing Solution Analysis

All formulations met the acceptance criteria of 90 – 110% of the target concentration with \leq 5% relative standard deviation, and were determined of have been accurately prepared.

Stability. Stability of naloxone hydrochloride ^{(b)(4)} in sterile water was previously established by the Applicant at 20 and 80 mg/mL for a period of at least 10 days when stored at 2-8°C. The stability of naloxone hydrochloride ^{(b)(4)} in sterile water at 2.5, 7.5 and 20 mg/mL was evaluated at room temperature and reanalyzed after 8 and 22 hours at 2-8°C. Stability testing at 2.5 and 20 mg/mL was conducted after 8 and 14 days. Concentrations at each stability timepoint were within 90-110% of the original concentration determined at timepoint 0. “Naloxone hydrochloride ^{(b)(4)} in sterile water, at concentrations of 2.55, 7.43, and 20.5 mg/mL is stable for at least 22 hours at

room temperature. Naloxone hydrochloride [REDACTED] ^{(b) (4)} in sterile water, at concentrations of 2.55 & 20.5 mg/mL is stable for at least 14 days at 2-8°C."

Concentrations of the dosing solutions were verified. The mean concentration results for all samples analyzed at dose formulations of 2.5, 7.5, and 20 mg/mL, from the first formulation prep, first interim formulation prep, second interim formulation prep, and last formulation prep varied from 90% to 105% of the respective theoretical values. Each formulation analyzed met the acceptance criteria of 90-110% of the target concentration.

Study title: 2-Year oral oncogenicity study of naloxone HCl in Sprague-Dawley rats

Study no.: N003003F
Study report location: EDR, 4.2.3.4
Conducting laboratory and location: [REDACTED]

Date of study initiation: February 15, 1999 (treatment initiation)
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Naloxone hydrochloride [REDACTED] ^{(b) (4)} Lot C06698, Purity: 91.4%
CAC concurrence: No

Key Study Findings

| | Key Study Findings |
|-----------------------|--|
| Survival | Naloxone HCl administered at a dose of 100 mg/kg/day produced excessive mortality with survival rate in males beginning to decline. Presumed cause of deaths for the early decedents in the control and high-dosage animals were due primarily to pituitary adenomas in both sexes and mammary tumors in female rats. |
| Clinical Signs | No treatment-related clinical signs were observed during the study. |
| Body Weight | A dose-dependent decrease in body weight was observed. A statistically significant decrease in body weight was noted in both sexes at doses \geq 20 mg/kg/day. |

| | Key Study Findings |
|--------------------------------|---|
| Non-neoplastic findings | An increase incidence in stomach ulceration was observed in the HD males. An increase incidence of cystic endometrial hyperplasia of the uterus was observed in the HD females. |
| Tumorigenicity | Overall, rats dietary administered naloxone HCl showed no evidence of carcinogenic potential. No significant increases in neoplastic lesions were observed. |
| Summary | Naloxone HCl administered at oral doses of 4, 20, or 100 mg/kg/day was not tumorigenic in Sprague-Dawley rats. |

Adequacy of Carcinogenicity Study

This 2-year rat carcinogenicity study was adequate because the study duration was appropriate for a valid carcinogenicity assessment (104-weeks), the doses tested were adequate and the standard observation parameters were measured. The doses evaluated were judged to have reached MTD as evidence by findings of dose-related decrease of body weight which reached greater than 10% in the HD groups when compared to control groups. Survival was adequate at the end of the dosing period for statistical evaluation of the parameters examined.

Appropriateness of Test Models

This test model is appropriate because the rat is a universal model routinely used for evaluating the carcinogenicity potential of variety of chemicals.

Evaluation of Tumor Findings

Methods

Doses: 0 (Control 1), 0 (Control 2), 4, 20, 100 mg/kg
 Frequency of dosing: Daily
 Dose volume: Non-applicable
 Route of administration: Oral (dietary)
 Formulation/Vehicle: Premix feed/premix diet
 Basis of dose selection: The Applicant did not provide information on the criteria of the basis of doses section.
 Species/Strain: Rats/Sprague-Dawley

Number/Sex/Group:

| Group | Dose (mg/kg) | No of animal/sex |
|-------|--------------|------------------|
| 1 | 0 (control) | 60 |
| 2 | 0 (control) | 60 |
| 3 | 4 | 60 |
| 4 | 20 | 60 |
| 5 | 100 | 60 |

Age: 6 to 8 weeks of age (at initiation of dosing)
 Animal housing: Individually housed
 Paradigm for dietary restriction: None
 Dual control employed: Yes
 Interim sacrifice: No
 Satellite groups: Toxicokinetic Group

| Group | Dose (mg/kg) | Number of animals and gender | |
|-------|--------------|------------------------------|---------------------|
| | | Main Toxicity Groups | Toxicokinetic Group |
| 1 | 0 (control) | 60 M and 60 F | 10 M and 10 F |
| 2 | 0 (control) | 60 M and 60 F | 0 |
| 3 | 4 | 60 M and 60 F | 10 M and 10 F |
| 4 | 20 | 60 M and 60 F | 10 M and 10 F |
| 5 | 100 | 60 M and 60 F | 10 M and 10 F |

Deviation from study protocol: Deviations are described under the appropriate observation(s). None of the deviations appear to compromise the study results.

Observations and Results**Mortality**

All main and toxicokinetic animals were observed for morbidity, and mortality twice daily (once in the morning and once in the afternoon, at least 6 hours apart) throughout the duration of the study.

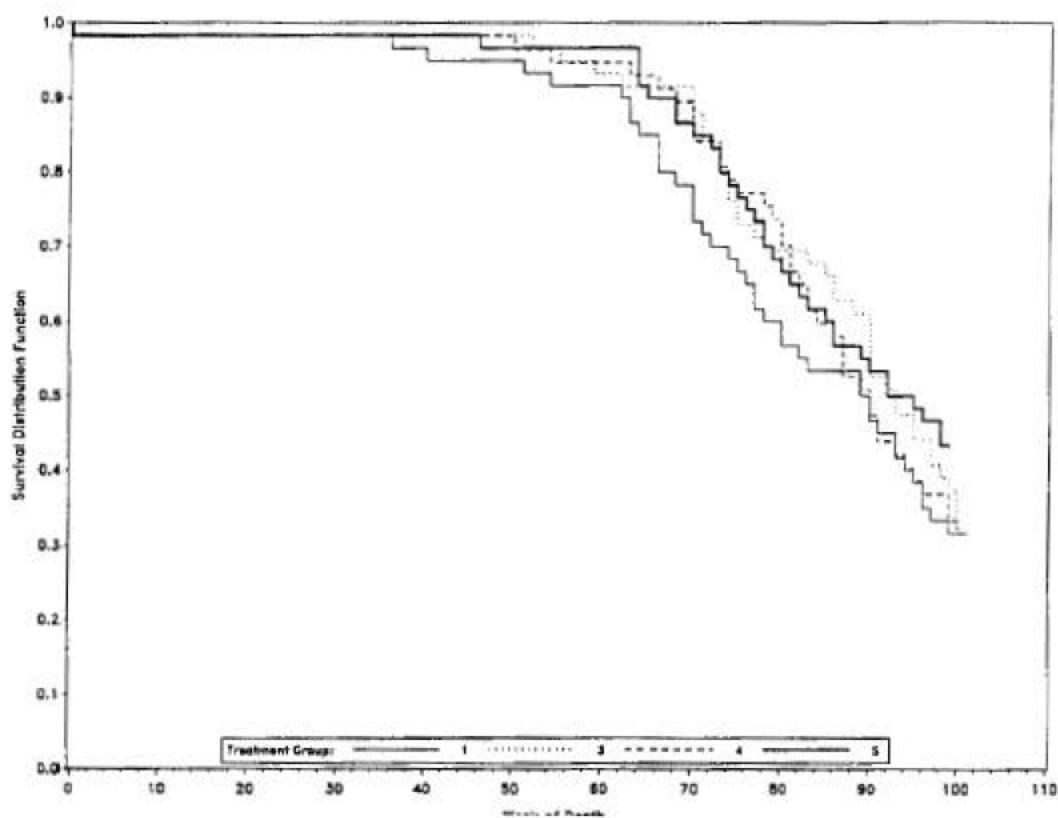
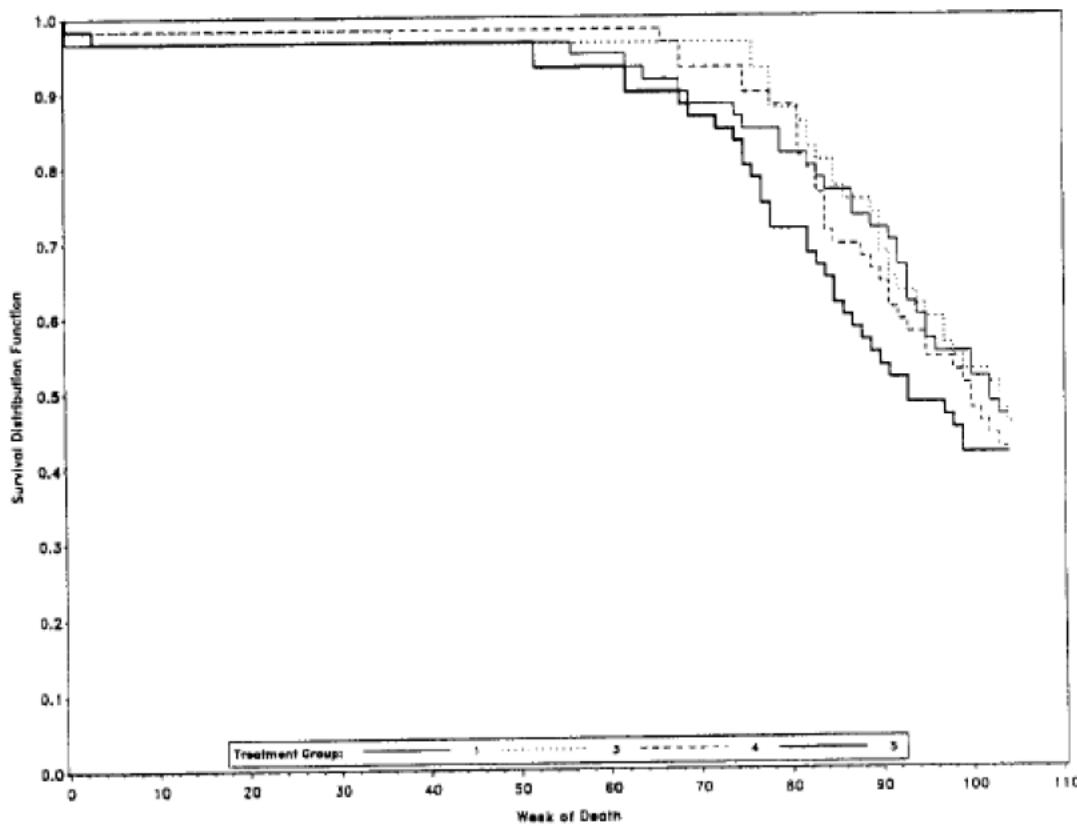
Table 81. Kaplan-Meier estimates of survival estimates for male rats in the 2-year carcinogenicity study

Table 82. Kaplan-Meier estimates of survival estimates for female rats in the 2-year carcinogenicity study**Table 83. Mortality and survivor data for 2-year carcinogenicity study in main study rats**

| Dose (mg/kg/day) | 0 | 4 | 20 | 100 |
|--|-------------|-------------|-------------|-------------|
| No of animals → | 60 | 60 | 60 | 60 |
| | Males | | | |
| Natural deaths | 21 | 17 | 21 | 10 |
| Euthanized <i>in extremis</i> (<i>Moribund sacrifice</i>) | 20 | 23 | 18 | 24 |
| Total Unscheduled Deaths | 41 | 40 | 39 | 34 |
| Survivors to study scheduled termination (% Survival) [†] | 19 (32%) | 20 (32%) | 21 (32%) | 26 (43%) |
| Mean Survival* (Weeks) | 85 | 89 | 88 | 88 |
| | Females | | | |
| Natural deaths | 9 | 8 | 6 | 8 |
| Euthanized <i>in extremis</i> (<i>Moribund sacrifice</i>) | 23 | 23 | 28 | 27 |
| Total Unscheduled | 32 | 31 | 34 | 35 |

| Dose (mg/kg/day) | 0 | 4 | 20 | 100 |
|---|-------------|-------------|-------------|-------------|
| No of animals → | 60 | 60 | 60 | 60 |
| Deaths | | | | |
| Survivors to study scheduled termination (% Survival) | 28 (47%) | 29 (46%) | 25 (42%) | 25 (42%) |
| Mean Survival* (Weeks) | 94 | 96 | 95 | 90 |

‡: "Kaplan-Meier estimated probability of survival at time of the terminal sacrifice."

*: "Mean of all death times, the reported value underestimates the true mean survival time because some observations were censored."

Mortality within the treatment groups is presented in table and figures above. The highest survival was observed in the HD and the lowest survival was in the vehicle control groups. The overall incidence of survival to Week 105 was comparable to vehicle control for males in the LD (4.0 mg/kg) and in the MD (100 mg/kg) groups. All naloxone-treated male rats were terminated early during Week 101 when the animal in the control group (# 1) survivor reached 20 surviving animals. Compared to the vehicle control females, mortality incidence was comparable.

Table 84. Statistical analysis of survival data

| Test | Heterogeneity | Pairwise Comparisons to Vehicle Control | | |
|---------------------------|---------------|---|----------|-----------|
| | | Low Dose | Mid Dose | High Dose |
| Males | | | | |
| Log-Rank ¹ | 0.5360 | 0.4852 | 0.6856 | 0.1618 |
| Wilcoxon ² | 0.4696 | 0.2484 | 0.4016 | 0.1437 |
| Tarone Trend ³ | | 0.8606 | | |
| Females | | | | |
| Log-Rank ¹ | 0.7101 | 0.9878 | 0.6452 | 0.3571 |
| Wilcoxon ² | 0.4227 | 0.8792 | 0.7010 | 0.2372 |
| Tarone Trend ³ | | 0.1317 | | |

1. Statistical tests of survival based on Cox's log-rank test.
2. Statistical tests of survival based on generalized Wilcoxon test.
3. Statistical tests of linear dependency of survivorship on the dose-response based on Tarone's trend test.

Statistical analysis by the Applicant showed no differences among the groups (heterogeneity) or between the groups (pairwise comparison). Also, there was no dose-response trend in survival (Tarone trend) for either males or females.

Microscopic examination of tissues suggested that the majority of early deaths were due to pituitary adenomas in both sexes (66 females and 64 males) and mammary tumors (fibroadenomas and adenocarcinoma) in females (32 rats).

FDA Statistical Analysis. The FDA statistics reviewer's analysis concurred with the Applicant's analysis. The pairwise comparison analysis did not show statistically significant dose response relationship in mortality across vehicle control and treated groups in either sex. "The pairwise comparisons also did not show statistically significant increased mortality in any of the treated groups compared to the vehicle control in either sex."

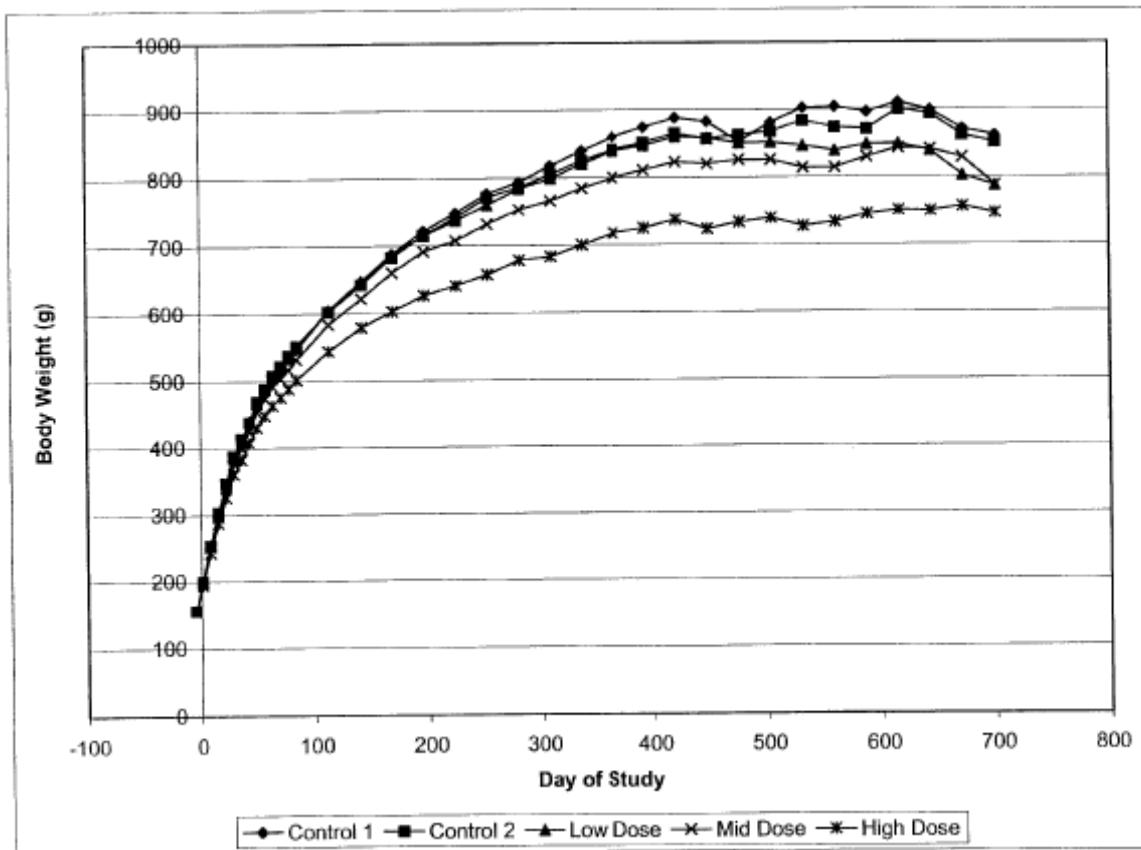
Clinical Signs

All main study animals were observed for clinical signs of toxicity weekly. Beginning at Week 52, as part of the schedule weekly clinical observations, all toxicity animals were palpitated for the presence of tumor masses.

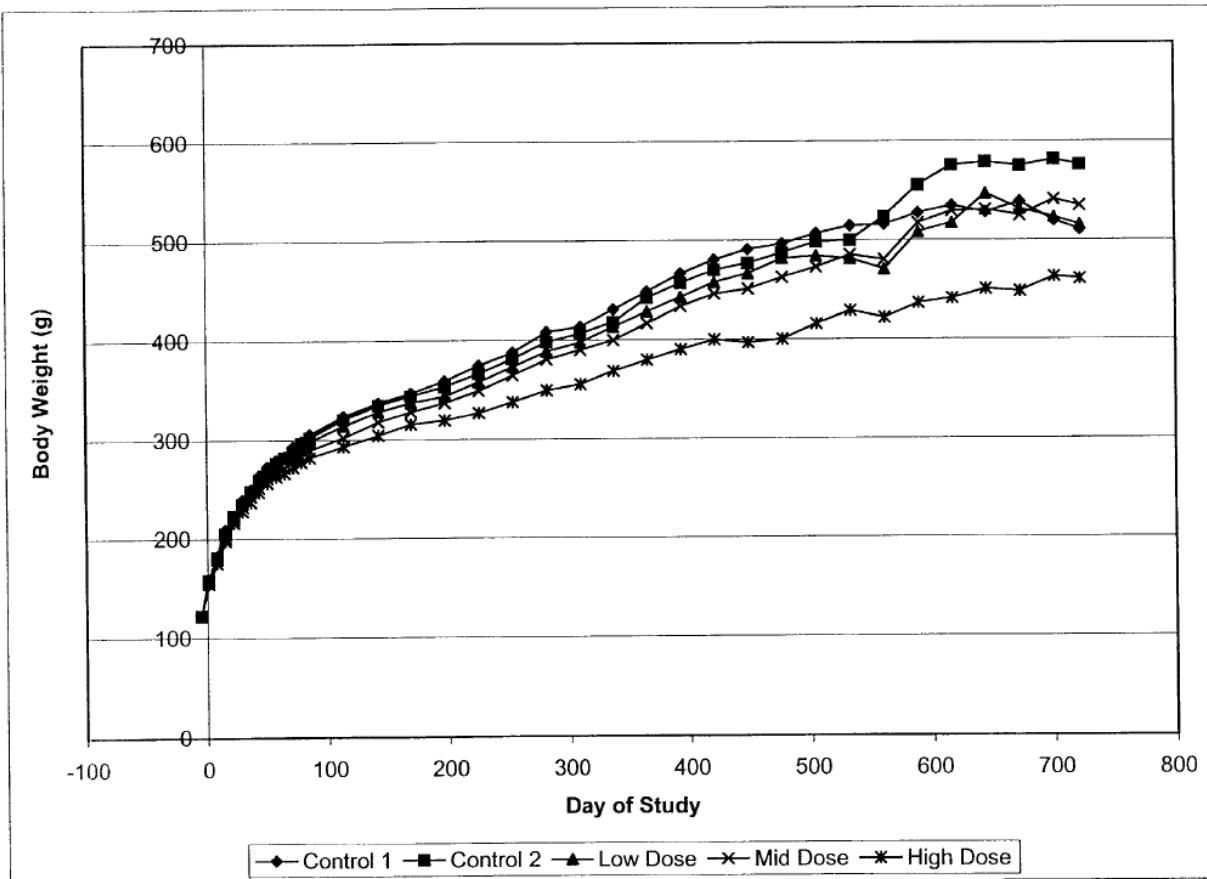
No treatment-related clinical signs were observed during the study.

Body Weights

Body weights of main toxicity animals were recorded prestudy (at group assignment), Day 1, weekly through Week 13, and then every fourth week thereafter. Body weight was recorded prior to necropsy.

Table 85. Growth curve for males

The weekly mean body weights of the high dose males were statistically significantly decreased compared to the control group between Study Days 8 and 703-705 (decreased ranged between 3.7% - 19.4%). Relative to the control males, the weekly mean body weights of the mid dose males were statistically significantly decreased on Day 36 (-3.1%) and from Days 113 to 617 (ranged between -3.4% and -10.0%).

Table 86. Growth curve for females

Naloxone treated females showed a similar pattern in growth rate as observed in naloxone-treated male rats. The weekly mean body weights of the high dose females were statistically significantly decreased when compared to the control group between at all but one weight collection (Day 22) between Study Days 8 and 673. These significant ranged between 4.1 – 19.4%. Relative to the control males, the weekly mean body weights of the mid dose males were statistically significantly decreased on Day 15 (-3.9%) and from Days 43 to 449 (ranged between -4.8% and -8.10%).

Food Consumption

Food consumption (grams/day) for main toxicity animals was recorded weekly through Week 13, and then every fourth week thereafter until study termination.

Compared to the Group 1 control males, there was a statistically significant decrease in food consumption beginning on Day 29 in the HD males. Food consumption was sporadically significant different from Group 1 controls from males in the LD and MD groups. In HD females, there was a statistically significant decrease in mean food consumption beginning on Day 8 compared to the control Group 1 females. Food consumption was sporadically significant different from Group 1 controls from females and males in the LD and MD groups.

The decrease food consumption in the high dose group was correlated with the decrease in body weight. The reviewer considers the decrease in food consumption and bodyweight gain to be treatment-related and adverse. It is unclear if the decreased food consumption was due to palatability issues or an actual pharmacologic effect of naloxone on taste perception.

Gross Pathology

After 100 weeks and 104 week, necropsy was performed on all surviving male and female toxicity animals, respectively. The animals were humanely euthanized by carbon dioxide overdose and exsanguinated and necropsied in the presence of a veterinary pathologist. Complete necropsies were also performed on all toxicity animals that died during the study or were euthanized for moribund or humane reasons. Macroscopic evaluation was performed.

Macroscopic observations

No significant naloxone-related macroscopic findings were noted. The incidence of the gross findings between treated and control animals were comparable.

Histopathology

Peer Review: Formal peer review was conducted by [REDACTED]^{(b) (4)},

The following tissues were collected from all animals. Histological examination was conducted on the high-dose, and, control group # 1 toxicity group animals. Complete histopathological evaluations were performed on all toxicity animals that died during the study or were euthanized for moribund conditions. Tissue selected for microscopic analysis were processed and stained with hematoxylin and eosin stain and examined by light microscopy.

| Tissue | Organ Weight | Collected and Preserved | Microscopic Examination | | | |
|--|--------------|-------------------------|-------------------------|---------|---------|---------|
| | | | Group 1 | Group 3 | Group 4 | Group 5 |
| Adrenal gland | | X | X | | | X |
| Aortic – Thoracic | | X | X | | | X |
| Bone marrow smear | | | | | | |
| Bone with marrow, femur | | X | X | | | X |
| Bone with bone marrow ^a | | X | X | | | X |
| Brain (forebrain, midbrain, hindbrain) | X | X | X | | | X |
| Clitoral gland | | | | | | |
| Coagulating gland | | | | | | |
| Epididymis | | X | X | | | X |
| Esophagus | | X | X | | | X |
| Eye (with optic nerve) | | X | X | | | X |

| Tissue | Organ Weight | Collected and Preserved | Microscopic Examination | | | |
|---|--------------|-------------------------|-------------------------|---------|---------|---------|
| | | | Group 1 | Group 3 | Group 4 | Group 5 |
| Gallbladder | | | | | | |
| Harderian glands | | X | X | | | X |
| Heart | X | X | X | | | X |
| Joint, tibiofemoral; | | | | | | |
| Kidneys | X | X | X | | | X |
| Lacrimal gland, exorbital | | | | | | |
| Large intestine, cecum | | X | X | | | X |
| Large intestine, colon | | X | X | | | X |
| Large intestine, rectum | | X | X | | | X |
| Larynx | | | | | | |
| Liver | X | X | X | | | X |
| Lungs with Bronchi | X | X | X | | | X |
| Lymph node, mandibular | | X | X | | | X |
| Lymph node, mesenteric | | X | X | | | X |
| Mammary gland ^b | | X | X | | | X |
| Nose (4 sections) | | | | | | |
| Nerve, sciatic | | X | X | | | X |
| Ovaries | X | X | X | | | X |
| Oviduct | | | | | | |
| Pancreas | | X | X | | | X |
| Parathyroid gland | | X | | | | |
| Peyers Patch | | | | | | |
| Pharynx | | | | | | |
| Pituitary | | X | X | | | X |
| Preputial gland | | | | | | |
| Prostate | | X | X | | | X |
| Salivary gland, mandibular | | X | X | | | X |
| Salivary gland, parotid | | | | | | |
| Salivary gland, sublingual | | | | | | |
| Seminal vesicle | | X | X | | | X |
| Skeletal muscle, biceps femoris | | X | X | | | X |
| Skin | | X | X | | | X |
| Small intestine, duodenum | | X | X | | | X |
| Small intestine, ileum | | X | X | | | X |
| Small intestine, jejunum | | X | | | | |
| Spinal cord, cervical | | | | | | |
| Spinal cord, thoracolumbar | | X | X | | | X |
| Spleen | X | X | X | | | X |
| Stomach, glandular | | X | X | | | X |
| Stomach, nonglandular | | X | X | | | X |
| Target Organ | | X | X | X | X | X |
| Testis | X | X | X | | | X |
| Thymus | | X | X | | | X |
| Thyroid | | X | X | | | X |
| Tongue | | | | | | |
| Trachea | | X | X | | | X |
| Ureter | | | | | | |
| Urinary Bladder | | X | X | | | X |
| Uterus with cervix | X | X | X | | | X |
| Vagina | | X | X | | | X |
| Zymbal's gland (auditory sebaceous gland) | | | | | | |
| Gross lesions | | | | | | |
| Tissue masses with regional lymph node | | | | | | |

Note: Tissues from Group 2 vehicle-control animals were retained in case there were insufficient numbers of surviving

| Tissue | Organ Weight | Collected and Preserved | Microscopic Examination | | | |
|--|--------------|-------------------------|-------------------------|---------|---------|---------|
| | | | Group 1 | Group 3 | Group 4 | Group 5 |
| animals from Group 1 control animals to make a meaningful comparison against treated groups. These tissues were not trimmed nor processed. | | | | | | |

Neoplastic**Neoplastic Microscopic Findings**

Table 87. Summary table of combined neoplastic findings.

| Tissue | Tumor Name | Type | Trend Test ¹ | | Tumor Occurrence (Percentage) | | | |
|-----------------|--|------------|-------------------------|---------------|-------------------------------|-----|-----|------|
| | | | Asymptotic p-value | Exact p-value | Control #1 | Low | Mid | High |
| Males | | | | | | | | |
| Adrenal Gland | Benign Pheochromocytoma Single | Incidental | 0.6956 | 0.7587 | 12% | 8% | 7% | 10% |
| | | Fatal | 0.4977 | 0.5022 | | | | |
| | | Combined | 0.5994 | 0.6195 | | | | |
| | Benign Pheochromocytoma Multiple | Incidental | 0.9693 | 1.0000 | 5% | 5% | 2% | 7% |
| | | Fatal | 0.9831 | 1.0000 | | | | |
| | | Combined | 0.9732 | 1.0000 | | | | |
| | Benign Pheochromocytoma Single or Multiple | Incidental | 0.7232 | 0.7316 | 17% | 14% | 9% | 17% |
| | | Fatal | 0.5676 | 0.5817 | | | | |
| | | Combined | 0.7123 | 0.7420 | | | | |
| Mammary Gland | Benign Fibroadenoma Single | Incidental | 0.4640 | 0.6196 | 2% | 2% | 2% | 0% |
| | | Fatal | 0.3886 | 0.4622 | | | | |
| | | Combined | 0.4392 | 0.6166 | | | | |
| | Mammary Tumor ² Any | Incidental | 0.4640 | 0.6196 | 2% | 2% | 2% | 0% |
| | | Fatal | 0.3886 | 0.4622 | | | | |
| | | Combined | 0.4392 | 0.6166 | | | | |
| Pituitary Gland | Benign Adenoma Par Distalis | Incidental | 0.8989 | 0.9058 | 48% | 44% | 49% | 47% |
| | | Fatal | 0.5640 | 0.5909 | | | | |
| | | Combined | 0.7296 | 0.7487 | | | | |
| Females | | | | | | | | |
| Adrenal Gland | Benign Pheochromocytoma Single | Incidental | 0.2229 | 0.2676 | 5% | 0% | 5% | 8% |
| | Benign Pheochromocytoma Single or Multiple | Incidental | 0.2229 | 0.2676 | 5% | 0% | 5% | 8% |
| Mammary Gland | Benign Adenolipoma | Incidental | 0.8723 | 1.0000 | 0% | 2% | 2% | 0% |
| | Benign Adenoma Single | Incidental | 0.3387 | 0.3903 | 7% | 14% | 8% | 13% |
| | | Fatal | 0.2754 | 0.3172 | | | | |
| | | Combined | 0.3341 | 0.3489 | | | | |
| | Benign Adenoma Multiple | Incidental | 0.9009 | 1.0000 | 5% | 2% | 8% | 2% |
| | Benign Fibroadenoma Single | Incidental | 0.5761 | 0.6239 | 23% | 28% | 24% | 25% |
| | | Fatal | 0.6128 | 0.6579 | | | | |
| | | Combined | 0.5014 | 0.5352 | | | | |
| | Benign Fibroadenoma Multiple | Incidental | 0.1439 | 0.1652 | 15% | 18% | 17% | 5% |
| | | Fatal | 0.2256 | 0.2452 | | | | |
| | | Combined | 0.1748 | 0.2021 | | | | |
| | Malignant Adenocarcinoma Single | Incidental | 0.3590 | 0.3972 | 17% | 12% | 22% | 20% |
| | | Fatal | 0.2293 | 0.2538 | | | | |
| | | Combined | 0.2509 | 0.2707 | | | | |
| | Malignant Adenocarcinoma Multiple | Incidental | 0.5539 | 0.5703 | 3% | 7% | 5% | 2% |
| | | Fatal | 0.6655 | 0.6761 | | | | |
| | | Combined | 0.6118 | 0.6722 | | | | |
| | Mammary Tumor ² Any | Incidental | 0.4257 | 0.4336 | 48% | 63% | 63% | 52% |
| | | Fatal | 0.3081 | 0.3119 | | | | |
| | | Combined | 0.2400 | 0.2522 | | | | |
| Pituitary Gland | Benign Adenoma Par Distalis | Incidental | 0.6699 | 0.7151 | 65% | 68% | 63% | 62% |
| | | Fatal | 0.6934 | 0.7062 | | | | |
| | | Combined | 0.7483 | 0.7568 | | | | |

1. The following methods were used in the trend test and comparisons: Incidental tumors: Cochran - Armitage trend test, using Mantel-Haenzel method to pool over discrete time intervals; Fatal tumors: Tarone's trend test; Combined incidental and fatal tumors: Peto's trend test. Both asymptotic and exact permutation versions of these tests were applied. The exact versions of these tests are more appropriate when fewer than 10 tumors were observed over the study groups.

2. Animal with at least one tumor of Mammary gland – Benign Adenolipoma, Benign Adenoma, Benign Fibroadenoma, or Malignant Adenocarcinoma.

As depicted in the Applicant's table above, there were no statistically significant, dose-related trend in neoplastic findings at p<0.005 level (for common neoplasms) or the

p<0.025 level (for rare neoplasms) was observed. Also, there was no significant increase in neoplastic findings for either male or female animals level (for rare neoplasms) was observed.

Unscheduled Necropsy.

Table 88. Microscopic neoplastic findings in unscheduled deaths (Males)

| Incidence (percentage) of microscopic findings of unscheduled deaths | | | | | | |
|--|--------------------------------|------------------|---------------------------|--------|--------|-----|
| Males | | | | | | |
| | | | Naloxone Dose (mg/kg/day) | | | |
| Tissue | Finding | Nº Examined → | 0 | 4 | 20 | 100 |
| Adrenal Gland | Pheochromocytoma, single | 41 | 40 | 38 | 34 | |
| | Pheochromocytoma, bilateral | 4 (10%) | 4 (10%) | 3 (8%) | 2 (6%) | |
| | | 1 (2%) | 2 (5%) | 1 (3%) | 3 (9%) | |
| Mammary Gland | | Nº Examined → | 38 | 39 | 39 | 34 |
| | Adenoma, single | 0 | 0 | 0 | 0 | |
| | Adenoma, multiple | 0 | 0 | 0 | 0 | |
| | Fibroadenoma, single | 0 | 1 (3%) | 1 (3%) | 0 | |
| | Fibroadenoma, multiple | 0 | 0 | 0 | 0 | |
| | Adenocarcinoma, single | 0 | 0 | 0 | 0 | |
| | Adenocarcinoma, multiple | 0 | 0 | 0 | 0 | |
| Pituitary Gland | Any mammary tumor ¹ | 0 | 1 (3%) | 1 (3%) | 0 | |
| | Adenoma, pars distalis | 41 | 39 | 39 | 34 | |
| | | (41%) | (59%) | (56%) | (59%) | |

1: "Any mammary tumor was calculated by adding the individual animals in each group that had one of the following diagnoses: adenoma (single or multiple), adenolipoma, fibroadenoma (single or multiple), or adenocarcinoma (single or multiple)."

Table 89. Microscopic neoplastic findings in unscheduled deaths (Females)

| Incidence (percentage) of microscopic findings of unscheduled deaths | | | | | | |
|--|---------|--|---------------------------|---|----|-----|
| Females | | | | | | |
| | | | Naloxone Dose (mg/kg/day) | | | |
| Tissue | Finding | | 0 | 4 | 20 | 100 |
| | | | | | | |

| Incidence (percentage) of microscopic findings of unscheduled deaths | | | | | | |
|--|--------------------------------|---------------|---------------------------|----------|----------|-----|
| Females | | | | | | |
| | | | Naloxone Dose (mg/kg/day) | | | |
| Tissue | Finding | No Examined → | 0 | 4 | 20 | 100 |
| Adrenal Gland | Pheochromocytoma, single | 32 | 31 | 34 | 35 | |
| | Pheochromocytoma, bilateral | 1 (3%) | 0 | 0 | 3 (9%) | |
| | | 0 | 0 | 0 | 0 | |
| Mammary Gland | No Examined → | 32 | 31 | 34 | 35 | |
| | Adenoma, single | 3 (9%) | 4 (13%) | 5 (15%) | 4 (11%) | |
| | Adenoma, multiple | 0 | 1 (3%) | 2 (6%) | 0 | |
| | Fibroadenoma, single | 9 (28%) | 8 (26%) | 8 (24%) | 4 (11%) | |
| | Fibroadenoma, multiple | 4 (13%) | 2 (6%) | 5 (15%) | 2 (6%) | |
| | Adenocarcinoma, single | 6 (19%) | 3 (10%) | 9 (26%) | 9 (26%) | |
| | Adenocarcinoma, multiple | 1 (3%) | 3 (10%) | 3 (9%) | 1 (3%) | |
| | Any mammary tumor ¹ | 16 (50%) | 15 (48%) | 24 (71%) | 17 (49%) | |
| Pituitary Gland | No Examined → | 32 | 31 | 34 | 35 | |
| | Adenoma, pars distalis | 22 (69%) | 24 (77%) | 22 (65%) | 24 (69%) | |

1: "Any mammary tumor was calculated by adding the individual animals in each group that had one of the following diagnoses: adenoma (single or multiple), adenolipoma, fibroadenoma (single or multiple), or adenocarcinoma (single or multiple)."

The incidences of the neoplastic lesions in early deaths are presented in the tables above. In the early deaths, the majority of these deaths were due to pituitary adenomas in females ($n = 66$) and males ($n = 64$) and mammary tumors in females. Compared to control, the observed neoplastic findings in the treated animals were not statistically significant for either males or female animals.

FDA Statistical Analysis. The FDA statistic reviewer (Mohammad Atiar Rahman, PhD) reported a statistically significant increase in the incidence of benign basal cell adenoma in skin that reached statistical significance for trend ($p \leq 0.05$) in male rats. However, pairwise comparison did not show a statistically significant increase incidence in any of the observed tumor types in treatment groups compared to vehicle control in either sex (Table below reproduced from the statistical review).

Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pairwise Comparisons of Treated Groups and Control in Rats

| Sex | Organ Name | Tumor Name | P_Value | | | | | | | | |
|--------|---------------|-------------------------------------|---------|------|-----|-----|------|-----------|---------|---------|---------|
| | | | Veh | Cont | Low | Med | High | Dose Resp | C vs. L | C vs. M | C vs. H |
| Male | Skin | Benign basal cell adenoma | 0 | 0 | 1 | 3 | | 0.0209* | . | 0.5301 | 0.1441 |
| Female | Brain | Malignant Pituitary adenocarcinoma, | 2 | 0 | 1 | 5 | | 0.0138 | 1.0000 | 0.8790 | 0.1908 |
| | Adrenal gland | Benign pheochromocytoma | 3 | 0 | 3 | 5 | | 0.0418 | 1.0000 | 0.6615 | 0.3203 |

*Statistically significant

Non Neoplastic Microscopic Findings

Scheduled Necropsy.

Table 90. Summary of non-neoplastic findings in all naloxone-treated animals

| ORGAN/DIAGNOSIS | dose (mg/kg) | Males | | Females | |
|-----------------------------------|--------------------|-------------|-------------|-------------|-------------|
| | | 0 | 100 | 0 | 100 |
| Liver | number examined | 60 | 60 | 60 | 60 |
| fatty change | (average severity) | 14 (0.3) | 14 (0.5) | 26 (0.7) | 13 (0.3) |
| Stomach | number examined | 60 | 60 | 60 | 60 |
| ulceration | (average severity) | 0 (0.0) | 6 (0.1) | 1 (0.0) | 0 (0.0) |
| Uterus | number examined | | | 60 | 60 |
| cystic endometrial hyperplasia | (average severity) | | | 22 (0.9) | 33 (1.1) |
| Testes | number examined | 60 | 60 | | |
| degeneration, germinal epithelium | (average severity) | 14 (0.6) | 6 (0.4) | | |

The incidences and severities of the non-neoplastic lesions for all naloxone-treated animals are presented in the table above (copied from the Applicant's submission). Compared to control males, an increase incidence in stomach ulceration was observed in the HD males. An increase incidence of cystic endometrial hyperplasia of the uterus was observed in the naloxone-treated high dose females compared to the vehicle control.

Unscheduled/Early Necropsy.**Table 91. Summary of non-neoplastic findings in early deaths (males)**

| Incidence of non-neoplastic findings of unscheduled deaths | | | | | |
|--|-------------------------------------|------------------|---------------------------|-----|-----|
| Males | | | | | |
| | | | Naloxone Dose (mg/kg/day) | | |
| | | No Examined → | 0 | 4 | 20 |
| Tissue | Finding | | 39 | 39 | 100 |
| Heart | Cardiomyopathy | Total | 35 | 39 | 36 |
| | | Average Severity | 1.4 | 1.8 | 1.7 |
| Kidney | Nephropathy | Total | 40 | 39 | 37 |
| | | Average Severity | 1.6 | 2.5 | 2.1 |
| Liver | Biliary hyperplasia and fibrosis | Total | 27 | 31 | 32 |
| | | Average Severity | 0.9 | 1.2 | 1.1 |
| | Fatty changes | Total | 11 | 15 | 12 |
| | | Average Severity | 0.4 | 0.6 | 0.5 |
| Pancreas | Inflammation | Total | 13 | 9 | 8 |
| | | Average Severity | 0.4 | 0.3 | 0.2 |
| Pituitary gland | Hyperplasia, pars distalis | Total | 10 | 10 | 6 |
| | | Average Severity | 0.5 | 0.5 | 0.4 |
| Sciatic nerve | Axonal degeneration | Total | 6 | 13 | 8 |
| | | Average Severity | 0.1 | 0.3 | 0.4 |
| Spleen | Extramedullary hematopoiesis | Total | 7 | 6 | 9 |
| | | Average Severity | 0.3 | 0.4 | 0.3 |
| | Hemosiderosis | Total | 9 | 13 | 9 |
| | | Average Severity | 0.2 | 0.3 | 0.3 |
| Stomach | Hyperplasia, forestomach epithelium | Total | 13 | 9 | 8 |
| | | Average Severity | 0.6 | 0.5 | 0.6 |
| | Ulceration, forestomach | Total | 0 | 3 | 5 |
| | | Average Severity | 0.0 | 0.1 | 0.3 |
| Testes | Degeneration, germinal epithelium | Total | 12 | 3 | 5 |
| | | Average Severity | 0.7 | 0.2 | 0.4 |

Severity Scores

- 1: Minimal, ¼ of the organ was affected
- 2: Mild, ¼ to ½ of the organ was affected
- 3: Moderate
- 4: Marked

Males. Non-neoplastic findings in early deaths in males are presented in the table above. Compared to control, the observed non-neoplastic findings in the treated males were not statistically significant for. Among males that were found dead or moribund, there was an increase incidence of forestomach ulcers and hyperplasia of the epithelium in the high-dose males compared to the control. According to the Applicant, the incidence of forestomach ulcers is comparable to historical control values for control animals (0-8% of all dead, ^{(b) (4)}). All other findings were either not considered dose-related or were observed with equal or greater frequency in vehicle controls, and were therefore not considered drug-related.

Table 92. Summary of non-neoplastic findings in early deaths (females)

| Incidence of non-neoplastic findings of unscheduled deaths | | | | | | | |
|--|--|----------------------|---------------------------|-----------|-----------|-----------|--|
| Females | | | | | | | |
| | | | Naloxone Dose (mg/kg/day) | | | | |
| | | | 0 | 4 | 20 | 100 | |
| Tissue | Finding | No Examined → | 32 | 31 | 34 | 35 | |
| Heart | Cardiomyopathy | Total | 22 | 29 | 25 | 26 | |
| | | Average Severity | 1.1 | 1.3 | 0.9 | 1.1 | |
| Kidney | Nephropathy | Total | 22 | 26 | 30 | 27 | |
| | | Average Severity | 0.9 | 1.1 | 1.0 | 1.0 | |
| Liver | Biliary hyperplasia and fibrosis | Total | 21 | 27 | 29 | 28 | |
| | | Average Severity | 1.0 | 1.2 | 1.0 | 1.2 | |
| Liver | Fatty changes | Total | 17 | 11 | 7 | 7 | |
| | | Average Severity | 1.0 | 0.7 | 0.3 | 0.3 | |
| Pancreas | Inflammation | Total | 2 | 11 | 2 | 6 | |
| | | Average Severity | 0.1 | 0.4 | 0.1 | 0.2 | |
| Pituitary gland | Hyperplasia, pars distalis | Total | 3 | 3 | 7 | 5 | |
| | | Average Severity | 0.2 | 0.4 | 0.4 | 0.3 | |
| Sciatic nerve | Axonal degeneration | Total | 4 | 5 | 6 | 3 | |
| | | Average Severity | 0.1 | 0.2 | 0.2 | 0.1 | |
| Spleen | Extramedullary hematopoiesis | Total | 11 | 12 | 15 | 13 | |
| | | Average Severity | 0.9 | 0.8 | 0.9 | 0.9 | |
| Spleen | Hemosiderosis | | 19 | 18 | 11 | 16 | |
| | | Average Severity | 0.7 | 0.6 | 0.4 | 0.5 | |
| Stomach | Hyperplasia, forestomach epithelium | Total | 6 | 3 | 5 | 7 | |
| | | Average Severity | 0.3 | 0.3 | 0.4 | 0.3 | |
| Stomach | Ulceration, forestomach | Total | 1 | 3 | 0 | 0 | |
| | | Average Severity | 0.1 | 0.2 | 0.0 | 0.00 | |
| Uterus | Cystic endometrial hyperplasia | Total | 11 | 10 | 12 | 16 | |
| | | Average Severity | 0.9 | 0.7 | 0.8 | 0.8 | |
| Severity Scores | | | | | | | |
| 1: Minimal, ¼ of the organ was affected | | | | | | | |
| 2: Mild, ¼ to ½ of the organ was affected | | | | | | | |
| 3: Moderate | | | | | | | |
| 4: Marked | | | | | | | |

Females. Non-neoplastic findings in early deaths in females are presented in the table above. Compared to control, the observed non-neoplastic findings in the treated females were not statistically significant for. Among females that were found dead or moribund, there was an increase incidence of forestomach hyperplasia of the epithelium and cystic endometrial hyperplasia of the uterus in the high-dose females compared to the control. According to the Applicant, the incidence of forestomach ulcers is comparable to historical control values for control animals (0-8% of all dead, (b) (4)). All other findings were either not considered dose-related or were observed with equal or greater frequency in vehicle controls, and were therefore not considered drug-related.

Toxicokinetics

Blood samples (approximately 3.0 mL in females) were collected via orbital puncture after CO₂/O₂ anesthesia for toxicokinetic evaluation from all surviving rats during Weeks 1, 13 and 37. During Week 52, approximately 5.0 mL and 4.0 mL of whole blood was collected from male and female rats, respectively. An additional plasma analysis was collected during Week 58 from the animals. Plasma concentrations of naloxone HCl (conjugated and/or unconjugated) were determined by mass spectrometry.

Table 93. Group mean plasma of naloxone HCl in male and female rats orally administered naloxone for 2-years.

| Dose Group | Target Concentration (mg/kg) | | Naloxone (ng/mL) | | | | |
|----------------|------------------------------|------|------------------|-----------|-----------|-----------|-----------|
| | | | Week 1 | Week 13 | Week 37 | Week 52 | Week 58 |
| Males | | | | | | | |
| Control #1 | 0 | Mean | BLOQ | 3.481E-01 | 8.292E-01 | BLOQ | BLOQ |
| | | SD | -- | -- | -- | -- | -- |
| | | N | 10 | 1a | 1a | 9 | 9 |
| Low Dose | 4 | Mean | 5.882E-01 | 1.111E-00 | 9.028E-01 | 1.927E-00 | 1.203E+01 |
| | | SD | -- | -- | 3.714E-01 | 3.531E-00 | 2.977E-01 |
| | | N | 1a | 1a | 5a | 9a | 2a |
| Mid Dose | 20 | Mean | 2.455E-00 | 3.439E-00 | 5.452E-00 | 4.758E-00 | 2.466E-00 |
| | | SD | 8.837E-01 | 3.239E-00 | 3.046E-00 | 1.549E-00 | 7.273E-01 |
| | | N | 10 | 10 | 10 | 10 | 10 |
| High Dose | 100 | Mean | 1.257E+01 | 1.711E+01 | 2.527E+01 | 2.688E+01 | 2.097E+01 |
| | | SD | 2.905E-00 | 2.732E-00 | 6.372E-00 | 7.328E-00 | 1.163E+01 |
| | | N | 10 | 8 | 10 | 9 | 9 |
| Females | | | | | | | |
| Control #1 | 0 | Mean | BLOQ | 2.894E-00 | 9.513E-01 | BLOQ | BLOQ |
| | | SD | -- | -- | -- | -- | -- |
| | | N | 10 | 2a | 2a | 10 | 10 |
| Low Dose | 4 | Mean | 6.276E-01 | BLOQ | 9.457E-01 | 5.964E-01 | BLOQ |
| | | SD | 6.990E-02 | -- | 2.510E-01 | 8.483E-02 | -- |
| | | N | 3a | 10 | 6a | 4a | 10 |
| Mid Dose | 20 | Mean | 3.714E-00 | 1.853E-00 | 4.018E-00 | 4.249E-00 | 1.766E-00 |
| | | SD | 4.484E-00 | 1.293E-00 | 2.655E-00 | 1.847E-00 | 7.606E-01 |
| | | N | 10 | 7b | 9 | 9 | 9 |
| High Dose | 100 | Mean | 1.435E+01 | 1.944E+01 | 2.937E+01 | 3.051E+01 | 2.044E+01 |
| | | SD | 8.513E-00 | 1.046E+01 | 1.372E+01 | 9.040E-00 | 8.720E-00 |
| | | N | 10 | 10 | 10 | 9 | 9 |

a. N=10. Remaining samples were BLOQ.

b. N=9. Remaining samples were BLOQ.

Plasma concentration data is presented in the table above (copied from Applicant's submission). Systemic exposure to naloxone HCl increased in a dose-dependent manner.

Dosing Solution Analysis

For stability, dose analysis and homogeneity studies, the Applicant reported the following results.

Stability: "The formulation stability study indicated that the formulation stored protected from light at room temperature ($\approx 25^{\circ}\text{C}$) in sealed plastic bags was stable for at least 35 days."

Data Analysis: The 0.0 mg/m: formulations contained no quantifiable naloxone HCl. The average concentration of all other formulation was within 15% of target concentrations, the acceptance limit specified by the sponsor."

Homogeneity: "... naloxone hydrochloride was uniformly distributed in the feed."

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Naloxone: Rat fertility and general reproductive performance study

Study no.: KPC/32/86
Study report location: EDR, 4.2.3.5.1
Conducting laboratory and location: [REDACTED] (b) (4)

Date of study initiation: May 31, 1985 (first day of male dosing)
July 16, 1985 (first day of female dosing)

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: Naloxone chlorhydrate, Lots ARE 802
(used May 30 1985 to October 3, 1985;
Weeks 1 to 19) and ARE 807 (used
October 3, 1985 to October 31, 1985;
Weeks 19 to 23), Purity: not provided

Key Study Findings

Female rats treated with naloxone (0, 50, 200, and 800 mg/kg/day) during the precohabitation period and through weaning in a definitive Segment I study with the following findings:

- Fertility and early embryonic development NOAEL was 800 mg/kg due to a lack of remarkable toxicity at the high dose of 800 mg/kg dose of naloxone.
- Maternal NOAEL was 800 mg/kg, due to an absence of any remarkable toxicity at the 800 mg/kg dose of naloxone.

- Male NOAEL was 200 mg/kg due to significant decrease in body weight at the high dose of naloxone.
- Naloxone treatment had no effects on the F₁ generation; no adverse effects on litter size, sex ratio, or developmental landmarks were noted. At the high dose of 800 mg/kg dose of naloxone, there was an increased incidence of pups with iritis. The NOAEL for the F₁ generation was 200 mg/kg based on the observation of iritis at 800 mg/kg/
- Naloxone treatment had no effects on F₂ generation; no adverse effect on litter size, pup survival, incidence of malformations, developmental landmarks, or body weight were note. NOAEL was 800 mg/kg, due to an absence of any remarkable toxicity at the 800 mg/kg dose of naloxone.

Methods

Doses: 0, 50, 200, and 800 mg/kg/day
 Frequency of dosing: Once daily
 Dose volume: 10 mL/kg
 Route of administration: Oral gavage
 Formulation/Vehicle: solution/distilled water
 Species/Strain: Rats/Sprague Dawley
 Number/Sex/Group:

| Group | Dose (mg/kg/day) | No of F0 generation animals/sex |
|---------------------|------------------|---------------------------------|
| 1 (vehicle control) | 0 | 30 |
| 2 | 50 | 30 |
| 3 | 200 | 30 |
| 4 | 800 | 30 |

Satellite groups: Not performed
 Study design: Daily naloxone administration for males (60 days prior to mating, throughout mating period) and females (14 days prior to mating, through weaning), Segment I.
 Deviation from study protocol: Deviations are described under the appropriate observation(s).

Observations and Results

F₀ Dams

Mortality

Animals were examined daily for mortality.

There was no mortality in males at doses up to 800 mg/kg/day. However, one male in the 50 mg/kg/day (LD) group was sacrificed in extremis after the first mating period (Day

75). Kidney and bladder stones were observed in this animal at necropsy examination. There was no mortality in the females.

Clinical Signs

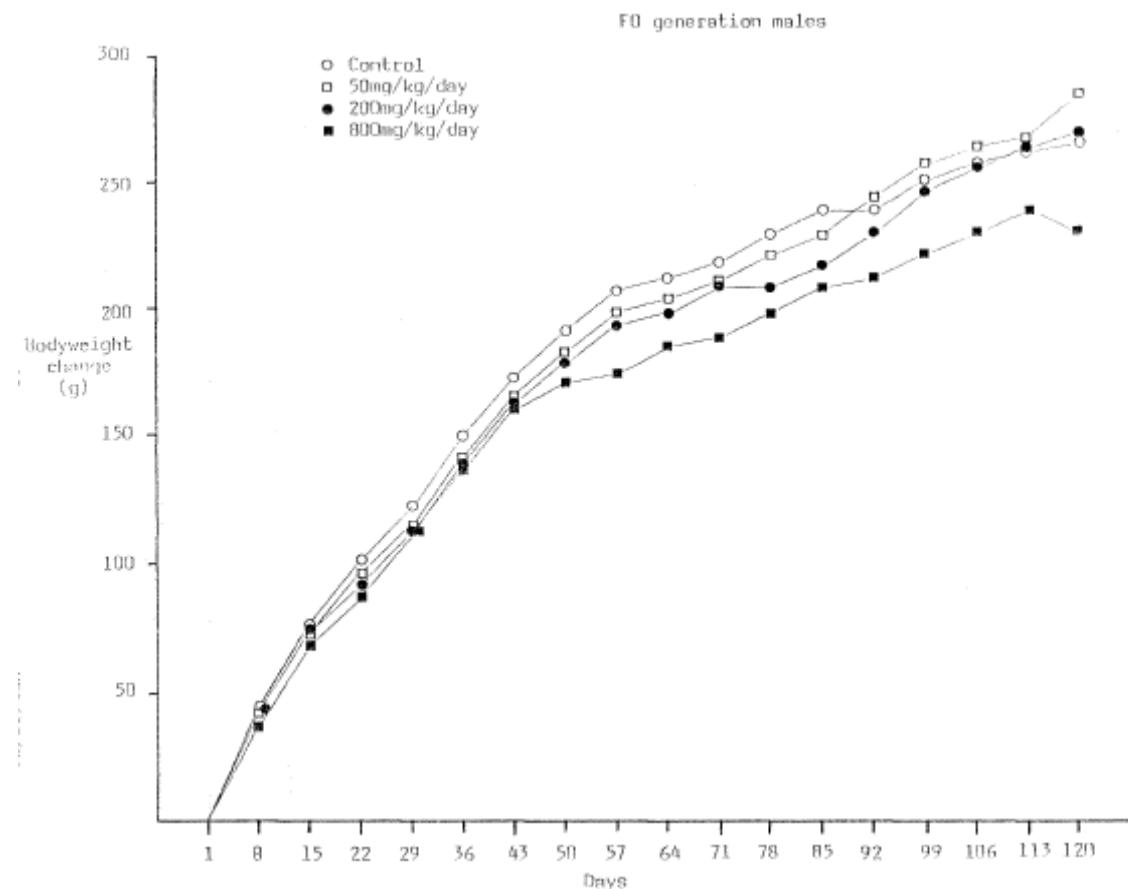
Animals were examined daily for clinical signs of toxicity.

Tremors were the primary naloxone treatment-related clinical signs observed in three females (№ 216, № 217, and № 224) in the HD group. The observed tremor in rat № 216 developed into convulsions which persisted for 3 minutes and recovered 3 minutes later. Female № 217 exhibited tremors after dosing on Day 12 of pregnancy. Female № 224 exhibited tremors and convulsions after dosing on Day 11 of pregnancy

Body Weight

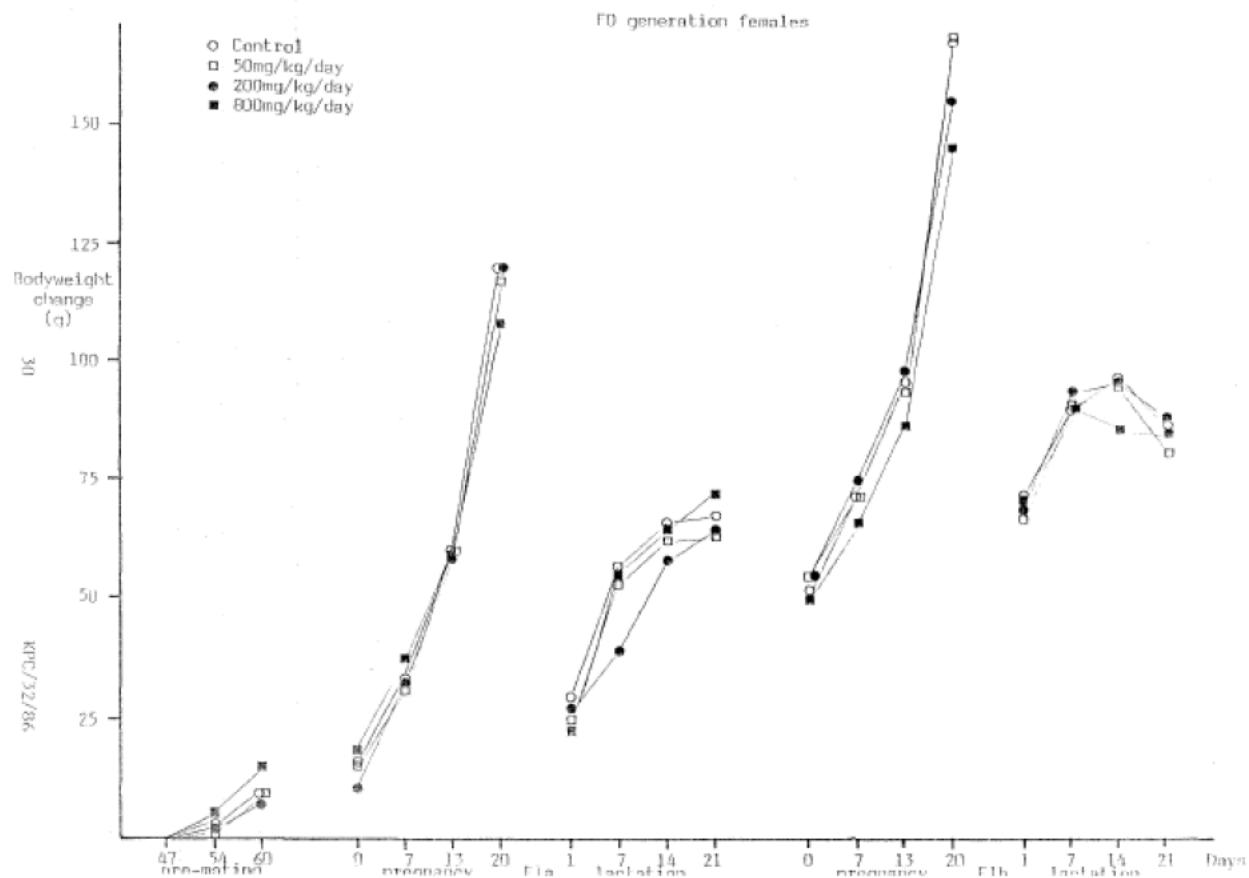
Body weights of the males were recorded weekly. Body weight of F₀ females were recorded weekly during the pre-mating period and daily thereafter.

Figure 9. Group mean body weight change data - males



Male body weight data are presented in the figure above (copied from Applicant's submission, p. 29 of the report). Male body weight gains were decreased from between Days 50 – 120. Mean body weight in the MD males was statistically significantly ($p<0.05$) decreased by 4.4% and 4.3% on Day 78 and Day 85, respectively. Relative to control, the mean body weight in the HD males was statistically significantly ($p<0.05$, $p<0.01$ or $p<0.001$, depending on the day) decreased by 6.9%, 6.0%, 6.5%, 6.5%, and 6.4% on Days 57, 64, 71, 78, and 85, respectively.

Figure 10. Group mean body weight change data - females



Female body weight data is presented in the figure above (copied from Applicant's submission, p 30 of the report). Body weights in females were not remarkably different between dose groups and controls, both during administration prior to mating, during gestational period, and during lactation.

Food Consumption

Food consumption was recorded weekly for the male during the pre-mating dosing period. Food consumption was recorded weekly in females during the pre-mating dosing period, pregnancy period (Days 0-7, 7-13, and 13-20), and lactation period (Days 1-7, 7-14, and 14-21).

Table 94. Summary of food consumption - male

| Dose Level (mg/kg/day) | Days | | | | | | | | |
|------------------------------|---------------|------------|------------|------------|------------|------------|------------|--------------|--------------|
| | 1 to 8 | 8 to 15 | 15 to 22 | 22 to 29 | 29 to 36 | 36 to 43 | 43 to 50 | 50 to 57 | 57 to 60 |
| Control | 26.7 ± 0.5 | 26.8 ± 0.8 | 25.7 ± 0.9 | 26.3 ± 1.1 | 26.4 ± 2.0 | 27.7 ± 0.7 | 26.6 ± 1.2 | 25.0 ± 1.0 | 26.9 ± 0.9 |
| 50 | 26.2 ± 0.6 | 26.6 ± 0.2 | 25.1 ± 0.5 | 26.0 ± 0.5 | 26.9 ± 0.3 | 27.0 ± 0.2 | 26.0 ± 0.7 | 23.5 ± 0.8* | 26.9 ± 0.9 |
| 200 | 26.7 ± 1.1 | 27.1 ± 0.9 | 24.7 ± 1.2 | 26.2 ± 1.5 | 27.9 ± 1.5 | 27.6 ± 0.9 | 26.6 ± 1.2 | 24.7 ± 1.1 | 29.0 ± 1.3** |
| 800 | 24.7 ± 0.8*** | 26.8 ± 1.0 | 25.5 ± 1.2 | 26.0 ± 0.5 | 27.7 ± 0.4 | 27.0 ± 1.0 | 25.5 ± 0.8 | 23.0 ± 0.9** | 26.6 ± 1.0 |

| Analysis of variance | p < 0.001 | NS | NS | NS | NS | NS | NS | p < 0.01 | p < 0.01 |
|-------------------------|-----------|----|----|----|----|----|----|----------|----------|
| | | | | | | | | | |

* = significantly different from control, p < 0.01, Student's t test.

** = significantly different from control, p < 0.05, Student's t test.

*** = significantly different from control, p < 0.001, Student's t test.

As depicted in the table above, reduction in food consumption was sporadic in males during the 8-weeks pre-mating dosing period. Food consumption was significantly reduced in the HD males during the first and eighth week of dosing. These reduction in food intake were likely responsible for the decreased weight gains measured around the same time periods in the 800 mg/kg/day dose group.

All food consumption data for females were unremarkable.

Toxicokinetics

Not performed

Dosing Solution Analysis

Table 95. Analysis of formulations

| Week of study | Preparation date | Theoretical concentration mg/ml | Assayed concentration mg/ml | Percentage of theoretical |
|---------------------|---------------------|---------------------------------------|-----------------------------------|---------------------------------|
| 1 | 30.5.85 | 0 | 0 | - |
| | | 5 | 5.27 | 105 |
| | | 20 | 20.0 | 100 |
| | | 80 | 116.2 | 145 |
| | | 80a | 123.9 | 155 |
| | | 80a | 111.6 | 140 |
| | 31.5.85 | 80a | 76.5 | 96 |
| | | 80b | 80.7 | 101 |
| 5 | 27.6.85 | 0 | 0 | - |
| | | 5 | 4.93 | 99 |
| | | 20 | 19.1 | 96 |
| | | 80 | 86.8 | 109 |
| 9 | 25.7.85 | 0 | 0 | - |
| | | 5 | 5.05 | 101 |
| | | 20 | 20.7 | 104 |
| | | 80 | 74.2 | 93 |
| 13 | 22.8.85 | 0 | 0 | - |
| | | 5 | 4.83 | 97 |
| | | 20 | 21.2 | 106 |
| | | 80 | 79.4 | 99 |
| 17 | 19.9.85 | 0 | 0 | - |
| | | 5 | 5.00 | 100 |
| | | 20 | 19.92 | 100 |
| | | 80 | 73.26 | 92 |

a = re-assay

b = re-formulation

All formulations analysed were prepared using Naloxone from batch 5 ARE 802.

Data for analysis of the naloxone formulation are presented in the table above. Formulation analysis demonstrated that prepared formulations of naloxone were within the acceptable range (92-109% of the theoretical) except for the formulation prepared for the HD group for the first week of dosing. The HD group first formulation was discarded and a second formulation was prepared. Analysis of the second formulation for use in the HD group was within the acceptable limits.

Necropsy

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

Half ($n = 15$) of the F_0 female rats were euthanized on Gestation Day 13 and the remaining half (F_0 litter group) on Gestation Day 21 by CO₂ asphyxiation. Cesarean-sectioned and gross necropsy of the thoracic, abdominal and pelvic viscera was performed (litter group F_0 females). Their major organs were examined macroscopically. Any unusual gross findings were recorded and gross lesion(s) was fixed in neutral buffered 10% formalin. The ovaries and uterus were examined to determine the number of corpora lutea, number and distribution of dead and live fetuses, number and distribution of early and late resorptions.

Examination of Embryo and Fetuses:

Cesarean Section: The number of corpora lutea, number and distribution of resorptions, implants, early and late resorptions, and dead and live fetuses were recorded. Each placenta was examined for size, color, and shape.

Fetal Observations: Each fetus was weighed, sexed, and examined for external alterations.

Visceral Examination: Visceral exams were performed on 50% of the fetus from each dam. These fetuses were examined for soft tissue alterations. The fetuses were fixed in Bouin's solution and the heads were examined. Head sections were retained in alcohol.

Skeletal Examination: Skeletal examination was performed on the remaining 50% of the fetus from each dam. Skeletal malformations and variations were assessed. The fetuses were placed in alcohol and processed (i.e., eviscerated, cleared and stained with alizarin red S) for skeletal examination.

Estrous Cycling, Mating and Fertility Data.

Table 96. Group mean fertility and mating data for F₀ (F1a) generation

| F0 generation (F1a) | | | | | | | | |
|-----------------------|------------------------|------------|-----------|-----------------------------|----------------------|------------------|--------------|-----------------|
| Sex | Dose level (mg/kg/day) | No. paired | No. mated | No. oestrus cycles required | No. pregnant/fertile | Copulation index | Mating index | Fertility index |
| F | Control | 30 | 29 | 30 | 28 | 96.7 | 96.7 | 96.6 |
| F | 50 | 30 | 30 | 33 | 29 | 100.0 | 90.9 | 96.7 |
| F | 200 | 30 | 30 | 32 | 26 | 100.0 | 93.8 | 86.7 |
| F | 800 | 30 | 28 | 31 | 26 | 93.3 | 90.3 | 92.9 |
| Chi ² test | | | | | | NS | NS | NS |
| M | Control | 30 | 28 | - | 27 | 93.3 | - | 96.4 |
| M | 50 | 30 | 29 | - | 28 | 96.7 | - | 96.6 |
| M | 200 | 30 | 28 | - | 25 | 93.3 | - | 89.3 |
| M | 800 | 30 | 26 | - | 24 | 86.7 | - | 92.3 |
| Chi ² test | | | | | | NS | NS | NS |

Table 97. Group mean fertility and mating data for F₀ (F1b) generation

| F0 generation (F1b) | | | | | | | | |
|-----------------------|------------------------|------------|-----------|-----------------------------|----------------------|------------------|--------------|-----------------|
| Sex | Dose level (mg/kg/day) | No. paired | No. mated | No. oestrus cycles required | No. pregnant/fertile | Copulation index | Mating index | Fertility index |
| F | Control | 15 | 15 | 15 | 13 | 100.0 | 100.0 | 86.7 |
| F | 50 | 15 | 15 | 16 | 15 | 100.0 | 93.8 | 100.0 |
| F | 200 | 15 | 15 | 15 | 15 | 100.0 | 100.0 | 100.0 |
| F | 800 | 15 | 15 | 15 | 15 | 100.0 | 100.0 | 100.0 |
| Chi ² test | | | | | | NS | NS | NS |
| M | Control | 15 | 15 | - | 13 | 100.0 | - | 86.7 |
| M | 50 | 15 | 15 | - | 15 | 100.0 | - | 100.0 |
| M | 200 | 15 | 15 | - | 15 | 100.0 | - | 100.0 |
| M | 800 | 15 | 15 | - | 15 | 100.0 | - | 100.0 |
| Chi ² test | | | | | | NS | NS | NS |

Results for the F₀ (F1a) and F₀ (F1b) generation is presented in table above (copied from Sponsor's submission). Female estrous parameters were not different between control and dose groups; at doses up to 800 mg/kg/day, naloxone had no effects on estrous cycling (i.e., vaginal smear pattern) during the precohabitation phase. All mating parameters, including the number of males and females that successfully mated and the number of males and females which were impregnated in the first and second mating periods, were unremarkable. Compared to the vehicle treated rats, no treatment-related effects were observed on mating index (time course of mating), fertility index (overall pregnancy rate), and number of males and females mating (copulation index) in either the F₀-F1a and F₀-F1b pairing.

Gestation Day 13 Laparohysterectomy Data.

Table 98. Summary of pregnancy and litter data from F0 female sacrificed on Gestation Day 13

| F0 generation | | | | | | | | | |
|------------------------|-----------------|-----------------------------------|-----------------------------------|----------------------------|------------------------------------|-------------------------------|-------------------------------|-----------------------------|--|
| Dose level (mg/kg/day) | Number pregnant | Number of corpora lutea + S.D. | Number of implantations + S.D. | Mean pre-implantation loss | No. of early resorptions + S.D. | No. of dead embryos + S.D. | No. of live embryos + S.D. | Mean post-implantation loss | |
| control | 13 | 17.4 + 1.9 | 14.2 + 2.9 | 17.1 | 0.6 + 0.8 | 0.5 + 0.8 | 13.2 + 2.6 | 7.0 | |
| 50 | 14 | 17.3 + 2.2 | 16.1 + 1.7 | 6.3 | 0.6 + 0.6 | 0.3 + 0.5 | 15.2 + 1.5 | 5.6 | |
| 200 | 12 | 17.6 + 2.4 | 15.3 + 1.7 | 12.2 | 0.7 + 0.7 | 0.3 + 0.7 | 14.4 + 2.2 | 7.5 | |
| 800 | 12 | 18.1 + 2.3 | 16.1 + 2.0 | 10.6 | 1.2 + 2.6 | 0.9 + 1.2 | 14.0 + 2.1 | 12.3 | |
| <hr/> | | | | | | | | | |
| Analysis of variance | | NS | NS | | NS | NS | NS | | |
| <hr/> | | | | | | | | | |
| Kruskal-Wallis test | | | | | | | | | |
| <hr/> | | | | | | | | | |

Data from Cesarean section are presented in the tables above (copied from Applicant's submission, p. 41 of the report). Differences in Cesarean data and birth indices were unremarkable between dose groups of naloxone and vehicle control. While there were no statistically significant differences between controls and naloxone-treated groups, preimplantation losses in the treated groups were lower than the control groups. Also, there was a slightly higher mean number of post-implantation losses in the HD group. The increase mean number of post-implantation losses was primarily due to one female (No 214) who had a large litter in which half of the embryos were dead.

F₁ Generation (F_{1a} and F_{1b})

Survival: Checked daily for pups, prior to weaning

Clinical signs: Pups were examined daily for any abnormalities.

Body weight: **Litter:** The total bodyweight of the male pups and female pups in each litter was recorded as soon as possible after birth and on postpartum Days 4 and 14. Mean pup weights were calculated.

F₁ Males: Body weights of the male selected for rearing were recorded weekly after weaning until necropsy.

F₁ Females: Bodyweights of female pups selected for rearing were recorded weekly after weaning until mating was confirmed. Bodyweights were also recorded on Gestational Days 0, 7, 14, and postpartum Days 0, 7, 14, 21.

Food consumption: Not evaluated

Litter Data **Litter Size:** total litter size was recorded as soon as possible after birth and daily thereafter

Sex: pups sex was recorded as soon as possible after birth and daily thereafter

Live Birth Index: (№ pups born alive/total № pups born) x 100

Viability Index: (№ live pups on Day 4 postpartum/№ live pups born) x 100

Lactation Index: № of pups alive on Day 21 postpartum/№ pups present after culling) x 100

Cumulative Survival Index: (№ of pups alive on Day 21 postpartum/№ pups present after culling x № live pups on Day 4 postpartum)/ total № pups born x 100

Sex Ratio: № female pups/ № male pups

Physical development: The development of the following characteristics were recorded for each pup in each litter:

Ears open: Examined daily until occurrence. Reported as the percentage of pups in each litter with ears open on Day 3 post partum.

Static righting reflex: Examined on Day 5 post partum.

Eyes open: Examined daily until occurrence. Reported as the percentage of pups in each litter with eyes open on Day 15 post partum.

F₁ Generation (F_{1a} and F_{1b})

Startle response: Examined on Day 15 post partum.

F₁ Generation (F_{1a} and F_{1b})

Neurological assessment: Not evaluated

Behavioral assessment Open Field: Between postpartum Days 33 and 37, the 15 males and 15 females selected for rearing were tested in open field test.

Learning assessment **E-maze test:** Between postpartum Days 26 and 34, the 15 males and 15 females selected for rearing were tested in the water maze. Each pup was given six runs at 30 minute intervals.

Reproduction At approximately 9 days old, selected F₁ females were paired with a F₁ male from the same group. Pairing continued for a maximum of ten days.

Necropsy Macroscopic necropsy was performed on all pups (except F_{1a} pups retained for rearing), including those culled or found dead.

Culled pups: Euthanized by an intracardiac injection of sodium pentobarbital on Day 4 post partum.

F1a pups: Weaned pups were euthanized by CO₂ asphyxiation after completion of the learning and function tests. Necropsy was performed on unscheduled dams on day of death. Macroscopic examination of the major organs in the thoracic and abdominal cavities was performed. Any unusual gross findings were recorded and tissues showing macroscopic lesions were removed and fixed in buffered 10% formalin.

F1b pups: Weaned pups were euthanized on Postpartum Day 21.

Selected F1 males: Euthanized by CO₂ asphyxiation at approximately 4-weeks after the end of the mating period.

Macroscopic examination of the major organs in the thoracic and abdominal cavities was performed. Any unusual gross findings were recorded and tissues showing macroscopic lesions were removed and fixed in buffered 10% formalin.

Post weaning: **Eye Examination:** Eyes were examined using a direct ophthalmoscope 30 minutes post administration of one drop of homatropine hydrobromide in each eye.

F₁ Generation (F_{1a} and F_{1b})

Auditory Function: Auditory function was assessed in all F_{1a} pups by observation of the Preyer's reflex (acoustic startle)

Observations during lactation

Table 99. Group mean data for pregnancy and litter data from F₀ generation and F_{1a} litter

| F ₀ generation females + F _{1a} generation litters | | | | | | | | | |
|--|-----------------|--|----------------------------------|--------------------------|-----------------------|----------------------|----------------------|--------------------------------|--|
| Dose level (mg/kg/day) | Number pregnant | Mean duration of gestation (days) \pm S.D. | Mean no. of pups born \pm S.D. | Sex ratio at birth M : F | Mean live birth index | Mean viability index | Mean lactation index | Mean cumulative survival index | |
| Control | 15 | 21.9 \pm 0.4 | 13.1 \pm 1.8 | 1 : 0.97 | 95.9 | 85.9 | 88.9 | 68.4 | |
| 50 | 15 | 21.9 \pm 0.4 | 13.5 \pm 2.7 | 1 : 1.13 | 89.4 | 58.6 | 92.0 | 53.0 | |
| 200 | 14 | 21.9 \pm 0.4 | 14.8 \pm 1.8 | 1 : 1.05 | 89.0 | 79.5 | 93.8 | 65.9 | |
| 800 | 14 | 21.7 \pm 0.5 | 13.9 \pm 3.0 | 1 : 0.94 | 98.8 | 83.6 | 95.2 | 78.7 | |

| | | | | | | |
|-----------------------|----|----|----|----|----|----|
| Analysis of variance | NS | NS | NS | NS | NS | NS |
| Kruskal-Wallis test | | | NS | NS | NS | NS |
| Chi ² test | | NS | | | | |

Table 100. Group mean data for pregnancy and litter data from F₀ generation and F_{1b} litter

| F ₀ generation females + F _{1b} generation litters | | | | | | | | | |
|--|-----------------|--|----------------------------------|--------------------------|-----------------------|----------------------|----------------------|--------------------------------|--|
| Dose level (mg/kg/day) | Number pregnant | Mean duration of gestation (days) \pm S.D. | Mean no. of pups born \pm S.D. | Sex ratio at birth M : F | Mean live birth index | Mean viability index | Mean lactation index | Mean cumulative survival index | |
| Control | 13 | 21.9 \pm 0.3 | 14.7 \pm 2.5 | 1 : 0.85 | 99.2 | 97.7 | 49.0 | 48.0 | |
| 50 | 15 | 21.8 \pm 0.4 | 15.6 \pm 2.3 | 1 : 0.84 | 92.2 | 86.5 | 51.8 | 40.5 | |
| 200 | 15 | 22.0 \pm 0.4 | 14.1 \pm 2.4 | 1 : 1.13 | 98.5 | 92.3 | 60.3 | 54.4 | |
| 800 | 15 | 22.0 \pm 0.0 | 14.0 \pm 3.0 | 1 : 1.00 | 96.6 | 93.4 | 47.3 | 42.3 | |

| | | | | | | |
|-----------------------|----|----|----|----|----|----|
| Analysis of variance | NS | NS | NS | NS | NS | NS |
| Kruskal-Wallis test | | | NS | NS | NS | NS |
| Chi ² test | | NS | | | | |

Data are presented in the tables above (copied from Applicant's submission).

Numbers of pups born and pup sexes. Unremarkable

Pup Survival. Compared to control, survival in the LD and MD groups of the F_{1a} was lower. The Applicant noted that this was due to a higher incidence of mortalities in these groups between birth and postpartum Day 7. Survival rate was lower in the MD and HD groups in the F_{1b} litter compared to controls. The lower survival rate was contributed to a higher incidence of mortalities occurring late during lactation (between Days 10 and 14 post partum).

Body weights.**Table 101. Group mean pup bodyweights (g) ± SD during lactation - F_{1a}**

| F _{1a} generation | | | | | |
|----------------------------|-----|-----------|-----------|------------|------------|
| Dose level (mg/kg/day) | Sex | 0 | 4 | 14 | 21 |
| Control | M | 5.7 ± 0.5 | 8.7 ± 1.3 | 30.3 ± 5.7 | 50.4 ± 6.8 |
| 50 | M | 5.6 ± 0.6 | 9.0 ± 1.1 | 31.4 ± 2.5 | 49.9 ± 3.5 |
| 200 | M | 5.4 ± 0.5 | 8.2 ± 0.7 | 30.6 ± 4.3 | 48.8 ± 6.8 |
| 800 | M | 5.5 ± 0.6 | 8.2 ± 1.2 | 29.4 ± 3.1 | 47.6 ± 6.6 |
| Analysis of variance | | | | | |
| | F | NS | NS | NS | NS |
| Control | F | 5.5 ± 0.5 | 8.4 ± 1.3 | 30.6 ± 4.6 | 49.8 ± 6.8 |
| 50 | F | 5.3 ± 0.6 | 8.6 ± 1.4 | 30.6 ± 2.6 | 47.8 ± 5.0 |
| 200 | F | 5.1 ± 0.5 | 7.9 ± 0.8 | 30.1 ± 2.1 | 47.3 ± 4.0 |
| 800 | F | 5.3 ± 0.5 | 7.9 ± 1.1 | 27.6 ± 5.4 | 45.5 ± 7.4 |
| Analysis of variance | | | | | |
| | | NS | NS | NS | NS |

Table 102. Group mean pup bodyweights (g) \pm SD during lactation - F_{1b}

| | | F _{1b} generation | | | |
|---------------------------|-----|----------------------------|---------------|----------------|-----------------|
| Dose level (mg/kg/day) | Sex | 0 | 7 | 14 | 21 |
| Control | M | 5.9 \pm 0.4 | 8.8 \pm 1.3 | 29.1 \pm 5.6 | 50.1 \pm 10.6 |
| 50 | M | 5.6 \pm 0.5 | 8.3 \pm 1.2 | 28.8 \pm 6.2 | 49.8 \pm 8.2 |
| 200 | M | 5.7 \pm 0.5 | 8.4 \pm 1.0 | 30.7 \pm 8.6 | 54.4 \pm 6.8 |
| 800 | M | 6.0 \pm 0.4 | 9.0 \pm 1.4 | 27.8 \pm 8.2 | 47.4 \pm 11.3 |
| Analysis of variance | | NS | NS | NS | NS |
| Control | F | 5.5 \pm 0.4 | 8.3 \pm 1.2 | 26.8 \pm 7.9 | 48.5 \pm 6.9 |
| 50 | F | 5.3 \pm 0.6 | 8.0 \pm 1.3 | 25.3 \pm 9.2 | 47.1 \pm 10.6 |
| 200 | F | 5.5 \pm 0.4 | 8.0 \pm 0.9 | 29.3 \pm 4.9 | 48.6 \pm 8.2 |
| 800 | F | 5.5 \pm 0.3 | 8.3 \pm 1.5 | 30.6 \pm 2.4 | 49.9 \pm 3.4 |
| Analysis of variance | | NS | NS | NS | NS |

Data are presented in the tables above (copied from Sponsor's submission). Body weights of pups in the F_{1a} litters were decreased at 800 mg/kg/day compared to control; but this effect did not reach statistical significance. Mean body weight of the pups in the F_{1b} litters was comparable to the controls throughout lactation.

F₁ postnatal development

F_{1a} pups

Table 103. Summary of physical development during lactation - F_{1a}

| F1a generation | | | | |
|------------------------|---------------------------------|-------------------------------|---------------------------------|--------------------------|
| Dose level (mg/kg/day) | Mean percent of pups \pm S.D. | | | |
| | with ears open on day 3 | with righting reflex on day 5 | with startle response on day 15 | with eyes open on day 15 |
| Control | 63.4 \pm 36.7 | 92.1 \pm 22.8 | 66.6 \pm 20.1 | 96.4 \pm 9.1 |
| 50 | 56.6 \pm 41.7 | 84.7 \pm 13.3 | 69.0 \pm 21.6 | 98.7 \pm 4.3 |
| 200 | 42.1 \pm 37.5 | 84.4 \pm 21.4 | 70.5 \pm 28.3 | 96.4 \pm 12.4 |
| 800 | 38.9 \pm 39.7 | 82.7 \pm 20.1 | 64.4 \pm 28.3 | 89.4 \pm 25.4 |

| Analysis of variance | NS | NS | NS | NS |
|----------------------|----|----|----|----|
|----------------------|----|----|----|----|

As depicted in the Applicant's table above, evidence of delayed development included a delay in the time of eye opening, lower percent of pups with ear opening at Day 3 and a reduced acquisition of the righting and startle responses in the 800 mg/kg/day group when compared to control group offspring. However, these effects did not reach statistical significance.

F1b pups

Table 104. Summary of physical development during lactation - F_{1b}

| F1b generation | | | | |
|------------------------|---------------------------------|-------------------------------|---------------------------------|--------------------------|
| Dose level (mg/kg/day) | Mean percent of pups \pm S.D. | | | |
| | with ears open on day 3 | with righting reflex on day 5 | with startle response on day 15 | with eyes open on day 15 |
| Control | 52.6 \pm 38.1 | 69.2 \pm 18.1 | 60.1 \pm 18.0 | 81.5 \pm 29.4 |
| 50 | 28.4 \pm 32.0 | 55.1 \pm 16.0 | 55.7 \pm 25.1 | 70.1 \pm 33.6 |
| 200 | 32.9 \pm 24.6 | 56.0 \pm 16.5 | 74.9 \pm 15.9 | 86.9 \pm 23.6 |
| 800 | 69.9 \pm 35.7 | 59.3 \pm 21.0 | 67.2 \pm 28.0 | 84.4 \pm 30.0 |

| Analysis of variance | p < 0.01 | NS | NS | NS |
|----------------------|----------|----|----|----|
|----------------------|----------|----|----|----|

As depicted in the Applicant's table above, evidence of delayed development included a reduction in the percent of pups with eye opening on Day 15 and percent of pups with ear opening at Day 3 and a reduced acquisition of the righting response in the 50 and 200 mg/kg/day groups when compared to control group offspring. Also, the pups in the 50 mg/kg/day group had a slightly reduced acquisition of the startle response. However, these effects did not reach statistical significance.

Observations during post weaning – F_{1a}

Behavioral evaluation: There were no treatment-related changes observed in the open field test.

E-maze learning test: Data were unremarkable in male and female F_{1a} offspring of dams orally administered naloxone.

Eye examination: Ophthalmoscopy findings are presented in the tables below (copied from Applicant's submission, p. 52 of the study report).

Table 105. Group mean data for ophthalmoscopy findings - F_{1a}

| Findings | F1 generation | | | | | | | |
|-------------------------------|---------------|--------|-------------|--------|--------------|--------|--------------|--------|
| | Control | | 50mg/kg/day | | 200mg/kg/day | | 800mg/kg/day | |
| | No. of pups | Mean % | No. of pups | Mean % | No. of pups | Mean % | No. of pups | Mean % |
| Lens suture lines | 74 | 77.0 | 56 | 78.9 | 63 | 71.0 | 74 | 74.4 |
| Hyoid remnant | 41 | 40.9 | 28 | 37.5 | 23 | 25.5 | 31 | 29.8 |
| Iritis | 1 | 1.2 | 1 | 1.1 | 3 | 3.5 | 11 | 10.6 |
| Keratitis | 3 | 2.9 | 5 | 6.0 | 4 | 4.2 | 7 | 6.7 |
| Persistent pupillary membrane | 3 | 3.6 | 3 | 3.7 | 0 | 0 | 5 | 4.8 |
| Anterior synaechia | 0 | 0 | 1 | 1.1 | 3 | 3.1 | 9 | 8.7 |
| Hypoaemia | 1 | 0.9 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cataract | 0 | 0 | 1 | 1.1 | 0 | 0 | 0 | 0 |
| Complete constriction of iris | 0 | 0 | 1 | 1.5 | 0 | 0 | 0 | 0 |
| Conjunctivitis | 1 | 0.9 | 0 | 0 | 0 | 0 | 0 | 0 |
| Subluxation | 1 | 0.9 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total no. pups examined | 99 | 100.0 | 73 | 100.0 | 83 | 100.0 | 99 | 100.0 |

Increased incidence of iritis and anterior synaechia (adhesion between the iris and the cornea) was observed in the F_{1a} offspring from dams orally administered 800 mg/kg/day naloxone. The observed iritis, that is the persistence of part of the iris over the pupil, was correlated with the delay in eye opening in this group compared to controls.

Auditory function: Data were unremarkable in male and female F_{1a} offspring of dams orally administered naloxone.

F_{1a} reproduction**F₂ Generation**

| | |
|--------------------------|---|
| Survival: | Checked daily for pups. |
| Clinical signs: | Pups were examined daily for any abnormalities. |
| Body weight: | Body weights of the male and female pups in each litter were recorded as soon as possible after birth and on postpartum Days 4, 14 and 21 of post partum. |
| Food consumption: | Not evaluated |
| Litter Data | <p>Live Birth Index: (No pups born alive/total No pups born) x 100</p> <p>Viability Index: (No live pups on Day 4 postpartum/No live pups born) x 100</p> <p>Lactation Index: No of pups alive on Day 21 postpartum/No pups present after culling) x 100</p> <p>Cumulative Survival Index: (No of pups alive on Day 21 postpartum/No pups present after culling x No live pups on Day 4 postpartum)/ total No pups born x 100</p> <p>Sex Ratio: No female pups/ No male pups</p> |
| Physical development: | After weaning the following examinations were performed on all F ₂ pups: |
| | <p>Eye Examination: Each eye was examined using a direct ophthalmoscope 30-minutes after one drop of homatropine hybromide was placed in each eye.</p> <p>Auditory Function: Preyer's reflex was used to assess auditory function.</p> <p>Eyes open: Examined daily until occurrence. Reported as the percentage of pups in each litter with eyes open on Day 15 post partum.</p> <p>Startle response: Examined on Day 15 post partum.</p> |
| Neurological assessment: | Not evaluated |
| Reproduction: | Not evaluated |
| Necropsy | Macroscopic necropsy was performed on all pups, including those culled or found dead. |
| | Culled pups were euthanized by an intracardiac injection of sodium pentobarbital on Day 4 post partum. |
| | Weaned pups were euthanized by CO ₂ asphyxiation on Postpartum Day 21. Necropsy was performed on unscheduled dams on day of death. Macroscopic examination of the major organs in the thoracic and |

F₂ Generation

abdominal cavities was performed. Any unusual gross findings were recorded and tissues showing macroscopic lesions were removed and fixed in buffered 10% formalin.

Other: For F₂ generation, observations including number and sex of pups, pup survival, pup bodyweight, pup development during lactation, clinical observations during lactation, and pup necropsy findings.

Observations during lactation

Pregnancy and litter findings. Summary of findings are presented in the table below (copied from Applicant's submission, p. 57 of the study report).

Table 106. Group mean pregnancy and litter data

F1 generation females + F2 generation litters

| Dose level (mg/kg/day) | Number pregnant | Mean duration of gestation (days) \pm S.D. | Mean no. of pups born \pm S.D. | Sex ratio at birth M : F | Mean live birth index | Mean viability index | Mean lactation index | Mean cumulative survival index |
|------------------------|-----------------|--|----------------------------------|-----------------------------|-----------------------|----------------------|----------------------|--------------------------------|
| Control | 12 | 21.9 \pm 0.3 | 13.7 \pm 2.5 | 1 : 1.34 | 98.1 | 97.7 | 100.0 | 95.8 |
| 50 | 14 | 22.1 \pm 0.3 | 13.3 \pm 2.2 | 1 : 0.96 | 100.0 | 92.4 | 91.1 | 83.8 |
| 200 | 15 | 22.1 \pm 0.3 | 12.6 \pm 1.8 | 1 : 1.11 | 96.6 | 97.9 | 99.2 | 93.8 |
| 800 | 15 | 21.9 \pm 0.3 | 12.5 \pm 3.0 | 1 : 0.83* | 98.1 | 97.2 | 100.0 | 95.4 |

| | | | | | | | |
|-----------------------|----|----|----------|----|----|----|--|
| Analysis of variance | NS | NS | | | | | |
| Kruskal-Wallis test | | | NS | NS | NS | NS | |
| Chi ² test | | | p < 0.05 | | | | |

* = significantly different from control, p < 0.05, Chi² test.

Findings in F₁ dams: Upon mating of F_{1a} dams, the number of pregnant females, and mean duration of gestation were unremarkable.

Numbers of pups born: Mean number of offspring born in the F₂ generation from F₁ dams orally administered naloxone at doses of 200 and 800 mg/kg/day were slightly lower compared to controls.

Sex ratio: Compared to control, mean number of female offspring born in the F₂ generation from F₁ dams orally administered 800 mg/kg/day naloxone was statistically ($p < 0.05$) lower than the controls. Sex ratio of the male offspring in all treatment doses was comparable to control males.

Pup Survival: Survival was not remarkably different from controls.

Body weight: Pup growth during lactation was not remarkably different from controls.

Physical development: Physical development of F₂ pups was not remarkably different from controls.

F₂ necropsy: No remarkable pathology was observed during necropsy evaluation of stillborn or pups died before weaning and pups after weaning.

9.2 Embryonic Fetal Development

Study title: Rat teratology study

| | |
|-------------------------------------|--|
| Study no.: | KPC/33-85/R |
| Study report location: | EDR, 4.2.3.5.2 |
| Conducting laboratory and location: | (b) (4) |
| Date of study initiation: | Not provided (Final study report dated January 1986) |
| GLP compliance: | Yes |
| QA statement: | No – no statement provided |
| Drug, lot #, and % purity: | Naloxone chlorhydrate, Lot 5ARE802, purity was not provided in the final study report. |

Key Study Findings

Female rats treated with naloxone (50, 200, and 800 mg/kg/day) during Gestation Day 6 through Day 15 in a definitive Segment II study with the following findings:

1. There were no clear treatment-related mortalities noted in the study.
2. Tremors and convulsions were the primary clinical signs observed in the female HD group at the initiation of dosing during the gestational period.
3. Minimal maternal toxicity was observed at 800 mg/kg/day represented by minimal decrease in body weight gain (approximately 5%) during the first-week treatment (GD 7-8), and decrease in food consumption (17.4%) during the treatment period (GD 6-11).

4. There were no treatment-related changes in pre- and post-implantation loss, and live fetus numbers in Caesarean analysis with the exception of a slight increase in HD group.
5. No treatment-related effects were observed in the pregnancy parameters following the oral administration of naloxone at doses up to 800 mg/kg/day.
6. There was a slight but non-significant increase in mean combined fetal body weight in the mid-dose group animals (1.0%) compared to the control.
7. There were no significant malformations (external, soft tissue, or skeletal) between treatment groups, indicating that naloxone was not teratogenic under the conditions tested.
8. The results suggest that the NOAEL for maternal toxicity was 200 mg/kg/day based upon decreased body weight gain and decreased food consumption.
9. The NOAEL for developmental toxicity was 800 mg/kg/day based on lack of developmental toxicity.

Methods

Doses: 0, 50, 200, and 800 mg/kg/day
 Frequency of dosing: Once daily on Days 6-15 of gestation (10 doses total)
 Dose volume: 10 mL/kg
 Route of administration: Oral gavage
 Formulation/Vehicle: solution/distilled water
 Species/Strain: Rat/Sprague-Dawley (COBS of the CD strain)
 Number/Sex/Group:

| Group | Dose (mg/kg/day) | No of Females* |
|--|-------------------------|-----------------------|
| 1 (vehicle control) | 0 | 24 |
| 2 | 50 | 24 |
| 3 | 200 | 24 |
| 4 | 800 | 24 |
| +: to allow for 20 pregnant females per group | | |

Satellite groups: None
 Study design: Daily dosing by oral gavage on Gestational Days 6 – 15. Cesarean/necropsy performed on Day 20.
 Breeding: Virgin female rats were acclimated for ten days before pairing with experienced sexually mature male rats. Females were paired with sexually mature males overnight (3F:1M). Female rats were observed for evidence of copulation, which if present was designated Day 0 of gestation.
 Deviation from study protocol: Deviations are described under the appropriate observation(s)

Observations and Results

Mortality

Animals were examined daily for mortality.

One female (№ 90) in the 800 mg/kg/day (HD) group exhibited convulsions prior to death on the first day of dosing (Day 6).

Clinical Signs

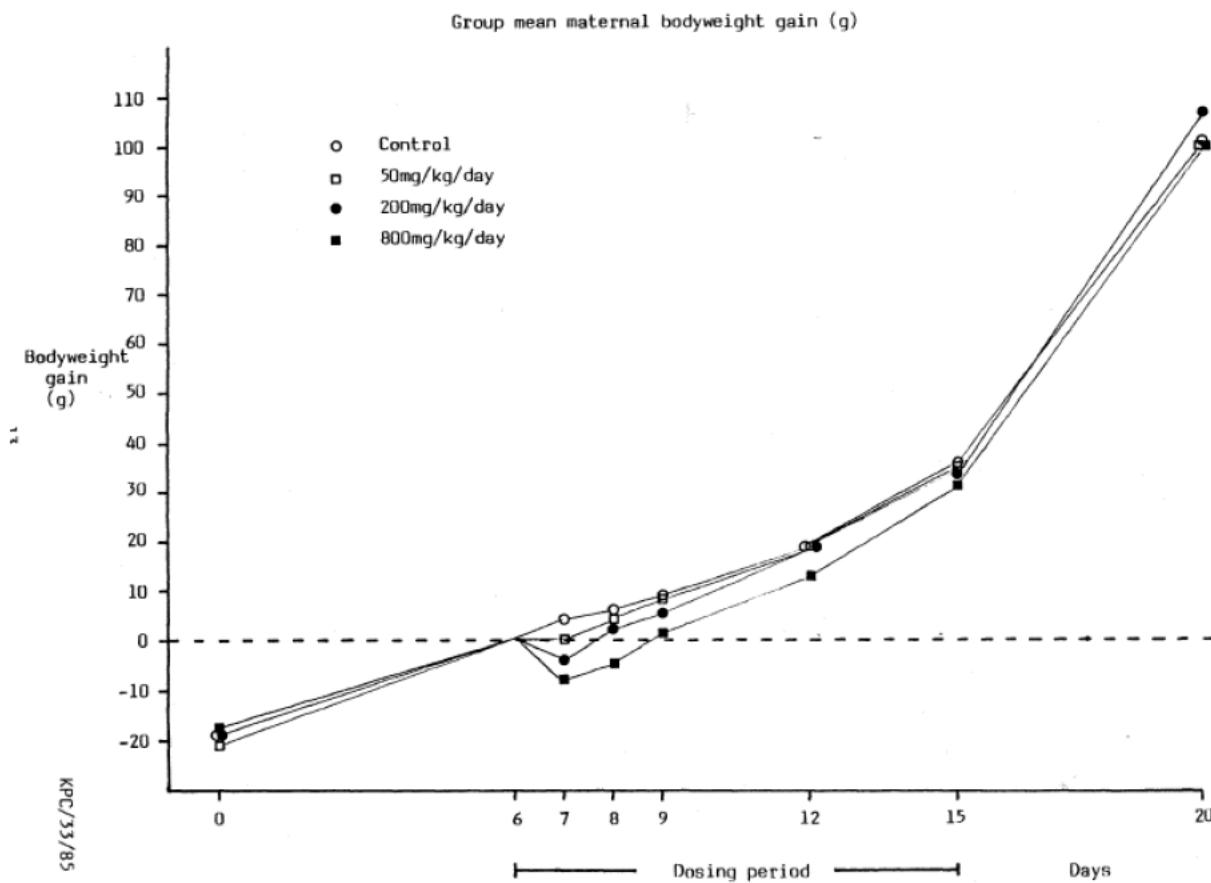
Animals were examined daily for clinical signs of toxicity.

Consistent with the findings in the Segment I conducted in rats, tremors were the primary naloxone treatment-related clinical signs observed in HD females. Tremors and convulsions were observed in five HD females on the first day of dosing (Day 6 of gestation). Tremors only were observed in seven females on Gestation Day 6. Aside from the observed tremors, tremors/convulsions and excessive salivation observed in the MD (Day 6 only) and HD (all dosing days), there were no remarkable findings.

Body Weight

Body weights were recorded on Gestational Day 0, 6-15, and 20.

Figure 11. Group mean body weight gain data in females orally administered naloxone



Female body weight data is presented in the figure above (copied from Applicant's submission, p 38 of the study report). Treatment-related effects on the mean body weight gain were observed in the mid- and high-dose females. As depicted in the figure, the mean body weight gain of the females orally administered naloxone at 50 mg/kg/day during the gestation period was comparable to the vehicle treated females. Compared to vehicle control, mid-dose females exhibited a slight reduction in body weight gain on Gestational Day 7 (-7.6%) and a slight increase in mean bodyweight gain on gestation during Gestation Days 8 through 15 (range from 0.74% - 3.3%). The slight increase in body weight in the MD dams was correlated with an increase in mean food consumption and fetus weight during this period. Relative to control, the mean body weight gain was statistically significantly ($p<0.01$ or $p<0.05$) decreased by 4.9% and 4.8% and on Day 7 and Day 8, respectively. Bodyweight gain remained slightly reduced in HD females throughout the remainder of the dosing period compared to the vehicle control. *The observed decrease in mean body weight in the high dose dams was correlated with a reduction in food consumption during the gestation period.*

Food Consumption

Food consumption was measured over the following periods: Gestational Days 0-6,

6 – 11, 11 – 15, and 15 – 20.

Table 107. Group mean maternal food consumption during pregnancy

| Gestational Days Period | Mean maternal food consumption (g/rat/day) ± SD | | | |
|--------------------------------|--|------------|-------------------------------|--------------------------------|
| | Dose (mg/kg/day) | | | |
| | 0 | 50 | 200 | 800 |
| 0-6 | 21.3 ± 2.8 | 20.7 ± 1.5 | 21.4 ± 2.1 | 20.7 ± 2.1 |
| 6-11 | 21.8 ± 2.7 | 21.6 ± 1.7 | 21.2 ± 2.3 | 18.0 ± 2.1* (-17.4%) |
| 11-15 | 26.3 ± 3.3 | 25.3 ± 2.4 | 26.6 ± 3.0 | 25.8 ± 3.0 |
| 15-20 | 27.2 ± 2.7 | 26.7 ± 1.8 | 28.8 ± 2.5† (-5.9%) | 27.9 ± 2.8 |

+: Statistically significant when compared to control at $p \leq 0.05$, Student's t test
*: Statistically significant when compared to control at $p \leq 0.01$, Student's t test

As depicted in the table above, a minimal decrease in food consumption was observed during the treatment period (Gestational Days 6-11 and 11-15) at 800 mg/kg/day as compared to control. Food consumption was statistically significantly ($p < 0.01$) reduced in the HD females during Gestational Days 6-11. Food consumption was statistically significantly ($p < 0.05$) higher in the MD females from Gestational Days 15-20. This increase in food intake were likely related to the slight increase in maternal bodyweight and the greater mean fetal body weight observed in this group.

Toxicokinetics

Not performed

Dosing Solution Analysis

Table 108. Analysis of formulation

| Preparation date | Theoretical concentration mg/ml | Assayed concentration mg/ml | Percentage of theoretical |
|------------------|---------------------------------|-----------------------------|---------------------------|
| 5.7.85 | 0 | 0 | - |
| | 5 | 4.70 | 94 |
| | 20 | 21.0 | 105 |
| | 80 | 86.2 | 108 |
| 12.7.85 | 0 | 0 | - |
| | 5 | 5.13 | 103 |
| | 20 | 19.1 | 96 |
| | 80 | 81.2 | 102 |
| 19.7.85 | 0 | 0 | - |
| | 5 | 4.59 | 92 |
| | 20 | 18.5 | 93 |
| | 80 | 71.9 | 90 |

As depicted in the table above, the concentration of naloxone was within the acceptable range (90-108% of theoretical range) at all formulation concentration.

Necropsy

All surviving female rats were euthanized by CO₂ asphyxiation on Gestation Day 20. Gross necropsy of the thoracic and abdominal cavities was performed. Their major organs were examined macroscopically. Tissues with gross lesion(s) were fixed in neutral buffered 10% formalin.

Examination of Embryo and Fetuses:

Cesarean Section: The number of corpora lutea, number and distribution of implantation sites, early and late resorptions, and dead and live fetuses were recorded. Each placenta was examined for size, color, and shape.

Fetal Observations: Each fetus was weighed, sexed, and examined for gross external malformations.

Visceral Examination: Visceral exams were performed on all fetuses.

These fetuses were examined for soft tissue alterations. All fetuses were eviscerated, cleared, and stained with Alizarin Red S.

Skeletal Examination: Skeletal examination was performed on all fetuses. Skeletal malformations and variations were assessed. The fetuses were placed in potassium hydroxide solution and processed (i.e., eviscerated, cleared and stained with Alizarin Red S) for skeletal examination. Skeletal

preparations were retained in aqueous glycerol for preservation and storage.

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

Table 109. Summary of Cesarean section data on Gestation Day 20

| Dose level (mg/kg/day) | Number pregnant/ mated | Mean no. of corpora lutea ± S.D. | Mean total no. of implantation sites ± S.D. | Mean no. of live foetuses ± S.D. | Mean pre- implantation loss (%) | Mean post- implantation loss (%) | Sex ratio M : F |
|---------------------------|---------------------------|--|---|--|---------------------------------------|--|--------------------|
| Control | 23/24 | 17.0 ± 3.2 | 13.3 ± 4.8 | 12.3 ± 4.7 | 22.7 | 7.4 | 1 : 1.00 |
| 50 | 22/24 | 16.9 ± 2.7 | 13.4 ± 3.0 | 12.2 ± 3.3 | 20.2 | 9.4 | 1 : 1.03 |
| 200 | 21/24 | 17.5 ± 3.2 | 13.1 ± 4.4 | 12.4 ± 4.3 | 25.1 | 5.8 | 1 : 1.05 |
| 800 | 24/24a | 17.8 ± 3.4 | 13.2 ± 3.8 | 11.9 ± 3.6 | 24.6 | 10.1 | 1 : 0.91 |
| Analysis of variance | | NS | NS | NS | | | |
| Kruskal-Wallis test | | | | NS | NS | | |
| Chi ² test | | | | | | NS | |

a = includes one animal that died.

Pregnancy data. Data from Cesarean section are presented in the table above. There were no treatment-related effects on the number of females that were pregnant. No treatment-related effects were observed in the pregnancy parameters following the oral administration of naloxone at doses of 50, 200, and 800 mg/kg/day; the mean number of total implantations, mean number of corpora lutea, mean number of live fetuses and sex ratio were comparable to control values. However in the high dose dams, a slight non statistical increase in mean pre-implantation loss and post-implantation loss was noted compared to the vehicle control.

Table 110. Summary of fetus mean weight on Gestation Day 20

| Dose level (mg/kg/day) | Males | Females | Combined |
|---------------------------|-------------|-------------|-------------|
| Control | 3.27 ± 0.35 | 3.19 ± 0.35 | 3.25 ± 0.35 |
| 50 | 3.34 ± 0.20 | 3.17 ± 0.24 | 3.24 ± 0.20 |
| 200 | 3.39 ± 0.28 | 3.24 ± 0.32 | 3.32 ± 0.28 |
| 800 | 3.38 ± 0.25 | 3.07 ± 0.49 | 3.22 ± 0.31 |
| Analysis of variance | NS | NS | NS |

Fetal Data. As depicted in tables above, no significant treatment-related effects were observed in mean number of male fetuses and mean number of female fetuses at doses up to 800 mg/kg/day. Mean fetal weight of the low- and high-dose groups was also comparable to the control group. However, in the mid-dose group, the mean fetal weight was slightly higher (1.0%) compared to the control values.

Offspring (Malformations, Variations, etc.)

Table 111. Summary of significant major abnormalities and variants

| Dose level mg/kg/day | Litter/foetal number | Abnormalities |
|-------------------------|-------------------------|---|
| Control | 11/L3 | Duplication of azygous vein. |
| 50 | 29/R3 | Bilateral anophthalmia, umbilicus oedematous. |
| 200 | 63/R2 | Unilateral microphthalmia. |
| 200 | 66/L9 | Thoracic centra 13 and thoracic neural arch R13 absent. Two ribs arising from neural arch R12. |
| 800 | 77/R6 | Bilateral microphthalmia ; hydrocephalus. |
| 800 | 75/R2 | Bilateral anophthalmia, agnathia, abnormally shaped brain, nasal passages constricted; nasopharyngeal canal absent. |

There was no clear treatment-related external and skeletal malformation and variation observed in drug-treated group as compared to control. However, as depicted in the table above (copied from Applicant's submission, p. 25 of the study report), there were a few skeletal malformations observed in fetuses from dams orally administered naloxone. The study report notes that the eye defects are not uncommon in this strain of rat and were considered spontaneous abnormalities even though they only occurred in the treatment arms. As there are no clear dose-dependency to these findings, and were not noted in Study KPC-32-86, they are not likely treatment-related.

Study title: Rabbit teratology study

Study no.: KPC/35-85
 Study report location: EDR, 4.2.3.5.2
 Conducting laboratory and location: [REDACTED] (b) (4)

Date of study initiation: Exact date not provided (Final study report dated October 1985)

GLP compliance: Yes

QA statement: No – no statement provided

Drug, lot #, and % purity: Naloxone chlorhydrate, Lot XY804 and Lot XY5, purity not provided in the study report

Key Study Findings

Female rabbits treated with naloxone (20, 100, and 400 mg/kg/day) during gestation Day 6 through Day 18 in a definitive Segment II study with the following findings:

1. There were three treatment-related mortalities noted in the study.
2. Hyperpnea, ataxia, lethargy, and convulsions were the primary clinical signs observed in the female HD group during the gestational period.
3. There was a slight but non-significant decrease in implantation loss, mean number of females per litter and number of live fetus in the HD groups.
4. There were no significant malformations (external, soft tissue or skeletal) between treatment groups, indicating that naloxone was not teratogenic under the conditions tested.
5. The results suggest that the NOAEL for maternal toxicity was 100 mg/kg/day based upon a slight non statistical decrease in implantation rate and mean number of live fetuses per dams.
6. The NOAEL for developmental toxicity was > 400 mg/kg/day based on lack of developmental toxicity.

Methods

Doses: 0, 20, 100, and 400 mg/kg/day
 Frequency of dosing: Once daily on Days 6 – 18 of gestation
 Dose volume: 5 mL/kg
 Route of administration: Oral gavage
 Formulation/Vehicle: solution/distilled water
 Species/Strain: Rabbit/Dutch

Number/Sex/Group:

| Group | Dose (mg/kg/day) | No of Females |
|---------------------|------------------|-----------------|
| 1 (vehicle control) | 0 | 19 ⁺ |
| 2 | 20 | 15 |
| 3 | 100 | 15 |
| 4 | 400 | 19 ⁺ |

+: An additional 4 females were mated because of potential mortality and abortion in Group 4.

Satellite groups: None

Study design: Test article orally administered during the organogenesis from Day 6 to Day 18 *post-coitum* inclusive. Dams were sacrificed on Day 28 *post-coitum*.

Breeding: Each virgin female rabbit was mated with two different males and then intravenously administered 50 IU chorionic gonadotrophin. Female rabbits were observed for evidence of copulation, which if present was designated Day 0 of gestation.

Deviation from study protocol: Deviations are described under the appropriate observation(s)

Observations and Results

Mortality

Animals were examined daily for mortality.

Table 112. Unscheduled deaths in females orally administered naloxone during Gestation Days 6 through 18

| Group/Animal № | Day of unscheduled death | Clinical Signs | Macroscopic/Microscopic Findings | Reviewer's Conclusion |
|----------------|--------------------------------|--|---|---|
| Group 4/№ 149 | Gestation Day 9 | - convulsions - lethargy - hyperpnea - slight ataxia | - Lungs: scattered pale foci - Fur: around mouth - wet | The observed clinical signs were attributed to naloxone. |
| Group 4/№ 150 | Euthanized on Gestation Day 23 | - aborted | Necropsy not performed | Abortion was attributable to treatment. |
| Group 4/№ 156 | Found dead on Gestation Day 24 | - No feces in tray - On GD 9 – 18, increased rate of respiration observed | - Stomach: non-glandular mucosa – red - Stomach: glandular mucosa – hemorrhagic areas - Spleen: dark, soft - Liver: dark - Abdominal cavity: fluid present, distended with gas | The observed clinical sign was attributed to naloxone. Microscopic findings unlikely attributed to naloxone. |

Three unscheduled deaths occurred in the high dose group. Unscheduled mortality data are summarized in table above.

Clinical Signs

Clinical observations were performed daily during the dosing period and until the scheduled necropsy.

Table 113. Summary of clinical signs in HD females orally administered naloxone during Gestation Days 16 through 18

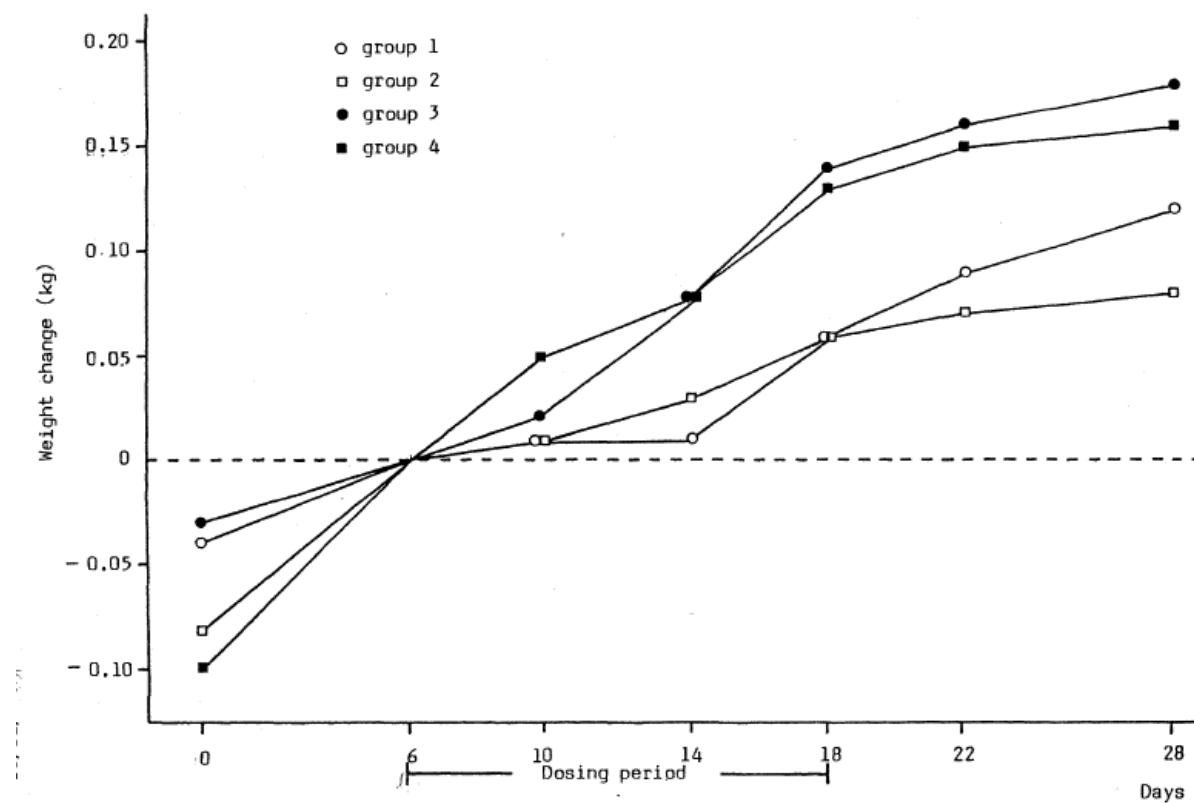
| Clinical signs observed in HD group | | |
|-------------------------------------|---------------------|---|
| Animal № | Day(s) of gestation | Clinical Observation |
| 146 | 11-18 | ↑ rate of respiration |
| 147 | 11-18 | ↑ rate of respiration |
| 148 | 11-18 | ↑ rate of respiration |
| 149 | 9 | - Convulsions - Lethargy - Hyperpnea - Slight ataxia - Died |

| Clinical signs observed in HD group | | |
|-------------------------------------|----------------|---|
| 150 | 9 | - Convulsions - Lethargy - Hyperpnea - Slight ataxia |
| | 10-18 | ↑ rate of respiration |
| | 16-18 | Ataxia |
| | 23 | - Aborted - Humanely euthanized |
| 151 | 10-18 | ↑ rate of respiration |
| 152 | 10 | Lethargy |
| | 10-18 | ↑ rate of respiration |
| 153 | 9 | Lethargy |
| | 9-18 | ↑ rate of respiration |
| 154 | 8-18 | ↑ rate of respiration |
| 155 | 8-18 | ↑ rate of respiration |
| 156 | 9-18 | ↑ rate of respiration |
| | 24 | Found dead |
| 157 | 8-18 | ↑ rate of respiration |
| 158 | 8-18 | ↑ rate of respiration |
| 159 | 8 | Lethargy |
| | 8-18 | ↑ rate of respiration |
| 160 | 8-18 | ↑ rate of respiration |
| 165 | 7-9, 13, 15-18 | ↑ rate of respiration |
| 166 | 7-9, 13-14 | ↑ rate of respiration |
| | 14, 17 | Lethargy |
| 167 | 7-9, 11, 13-18 | ↑ rate of respiration |
| | 11 | Lethargy |
| 168 | 7-9, 11, 13-18 | ↑ rate of respiration |
| | 11 | Lethargy |

Naloxone treatment-related clinical signs were observed in HD females. As depicted in the table above, the primary clinical signs observed in the HD females included: hyperpnea, ataxia, lethargy, and convulsions. There were no remarkable findings in the LD and MD groups.

Body Weight

Body weights were recorded on Gestational Days 0, 3, 6 – 18, 22, 25 and 28.

Table 114. Group mean body weight gain data in females orally administered naloxone

Female body weight data are presented in the figure above (copied from Applicant's submission, p28 of the study report). As depicted in the figure, compared to vehicle control, the mean body weight gain of females orally administered naloxone at 100 and 400 mg/kg/day was increased during the dosing and post-dosing periods. The Applicant attributed the increase bodyweight gain the MD and HD groups to "an unusually low food consumption and bodyweight gain of the control animals rather than an increase associated with naloxone administration." The reviewer concurs with the Applicant. *The observed increase in mean body weight gain in the mid and high dose dams was correlated with an increase in food consumption during the gestation period.*

Food Consumption

Food consumption was measured every two days throughout gestation.

Table 115. Group mean maternal food consumption during pregnancy

| Gestational Days Period | Mean maternal food consumption (g/rat/day) ± S.D. (% change of control) | | | |
|-------------------------|---|-------------|-------------|-------------|
| | Dose (mg/kg/day) | | | |
| | 0 | 20 | 100 | 400 |
| 0-6 | 85.8 ± 24.8 | 92.0 ± 30.1 | 90.7 ± 34.4 | 93.1 ± 17.3 |
| 6-10 | 88.9 ± 20.7 | 92.9 ± 23.6 | 93.8 ± 32.7 | 92.3 ± 20.0 |

| Mean maternal food consumption (g/rat/day) ± S.D. (% change of control) | | | | |
|--|-------------------------|-------------|---------------------------|--------------------------|
| Gestational Days Period | Dose (mg/kg/day) | | | |
| | 0 | 20 | 100 | 400 |
| 10-14 | 78.9 ± 21.3 | 82.9 ± 19.4 | 100.8 ± 25.3* (+27.8%) | 102.8 ± 9.0* (+30.3%) |
| 14-18 | 67.7 ± 23.5 | 72.7 ± 17.9 | 92.3 ± 19.4* (+36.3%) | 97.4 ± 14.4* (+43.9%) |
| 18-24 | 80.3 ± 21.8 | 76.8 ± 15.5 | 88.8 ± 33.5 | 84.1 ± 18.2 |
| 24-28 | 63.3 ± 24.1 | 58.4 ± 31.1 | 65.6 ± 36.3 | 58.3 ± 17.0 |

*: Statistically significant when compared to control at p≤ 0.01, Student's t test

As depicted in the table above, increase in food consumption was observed during the treatment period (Gestational Days 6-18) at all doses of naloxone as compared to control. Food consumption was statistically significantly ($p<0.01$) increased in the MD and HD females during Gestational Days 10-14 and 14-18.

Toxicokinetics

Not performed

Dosing Solution Analysis

Table 116. Analysis of formulation

| Preparation date | Theoretical concentration mg/ml | Actual concentration mg/ml | Percentage of theoretical |
|-------------------------|--|-----------------------------------|----------------------------------|
| 3.3.85 | 4 | 2.99 | 75 |
| | 20 | 18.25 | 91 |
| | 80 | 73.49 | 92 |
| 11.3.85 | 4 | 4.4 | 110 |
| | 20 | 19.3 | 97 |
| | 80 | 76.3 | 95 |
| 18.3.85 | 4 | 3.2 | 80 |
| | 20 | 17.2 | 86 |
| | 80 | 69.9 | 87 |
| 2.4.85 | 80 | 78.2 | 98 |

As depicted in the table above, the concentration of the low-dose formulation (4 mg/mL) of naloxone was variable, ranging from 75 to 110% of theoretical range. The concentrations of the mid dose (20 mg/mL) and high (80 mg/mL) formulations of

naloxone were within the acceptable range (86 – 98% of theoretical range). Results from a stability study showed that solutions of the low and high concentration formulations were stable for 1 day when stored at 4°C.

Necropsy

All surviving female rabbits were euthanized by intravenous administration of sodium pentobarbital on Gestation Day 28. Animals that died before term were also necropsied. Cesarean-sectioned and gross necropsy of the thoracic and abdominal cavities was performed. Any unusual gross findings were recorded and tissues showing macroscopic lesions were removed and fixed in buffered 10% formalin.

Examination of Embryo and Fetuses:

Cesarean Section: Pregnancy status, number of corpora lutea, number and distribution of implantation in uterine horns, early and late resorptions, number of dead and live fetuses were recorded.

Fetal Observations: Live fetuses were weighed, and fetuses were examined for external malformation.

Visceral Examination: Visceral exams were performed on two thirds of the live fetuses; fetuses were eviscerated, cleared and stained with Alizarin Red S. These fetuses were examined for soft tissue alterations.

Skeletal Examination: Skeletal examination was performed on all fetuses. Skeletal malformations and variations were assessed. The remaining fetuses were placed in potassium hydroxide solution and processed (i.e., eviscerated, cleared and stained with Alizarin Red S) for skeletal examination. Skeletal preparations were retained in aqueous glycerol for preservation and storage.

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

Table 117. Summary of Cesarean section data on Gestation Day 28

| Dose Level (mg/kg/day) | Number pregnant | Mean no. of corpora lutea ± S.D. | Mean no. of live fetuses ± S.D. | Mean total no. of implantation sites ± S.D. | Mean pre-implantation loss (%) | Mean post-implantation loss (%) | Mean foetal weight (g) ± S.D. | | | Sex Ratio |
|---------------------------|-----------------|-------------------------------------|------------------------------------|---|--------------------------------|---------------------------------|-------------------------------|------------|------------|-----------|
| | | | | | | | Males | Females | Combined | |
| Control | 16/19 | 9.4 ± 1.5 | 6.8 ± 2.5 | 7.2 ± 2.4 | 21.7 | 8.4 | 31.0 ± 4.9 | 30.4 ± 3.3 | 31.3 ± 4.6 | 1.00 |
| 20 | 15a/15 | 9.3 ± 2.3 | 7.0 ± 2.1 | 7.2 ± 2.0 | 22.6 | 3.2 | 29.3 ± 4.8 | 28.9 ± 5.8 | 29.2 ± 5.1 | 1.18 |
| 100 | 13/15 | 8.7 ± 1.5 | 6.5 ± 2.2 | 7.1 ± 2.3 | 19.1 | 9.0 | 32.6 ± 5.4 | 31.9 ± 5.6 | 32.4 ± 5.0 | 0.84 |
| 400 | 15b/19 | 8.9 ± 2.0 | 5.9 ± 2.0 | 6.4 ± 2.0 | 26.6 | 7.3 | 32.8 ± 3.9 | 32.8 ± 3.4 | 32.6 ± 3.5 | 1.29 |
| Analysis of variance | | NS | NS | NS | | NS | NS | NS | | |
| Kruskal-Wallis test | | | | | NS | NS | | | | |
| Chi ² | | | | | | | | NS | | |

a = includes one female that aborted

b = includes two females that died and one that aborted

Pregnancy data. Data from Cesarean section are presented in the table above. There were no treatment-related effects on the number of females that were pregnant. No treatment-related effects were observed in the pregnancy parameters following the oral administration of naloxone at doses of 20 and 100 mg/kg/day; these parameters were comparable to control values. However in the high dose dams, a slight non statistical decrease in implantation rate and mean number of live fetuses per dams was noted compared to the vehicle control.

Fetal Data. As depicted in tables above, no significant treatment-related effects were observed at doses up to 400 mg/kg/day as compared to the control group. However, in the high-dose group, the mean number of females per litter and number of live fetus were slightly lower compared to the control values.

Offspring (Malformations, Variations, etc.)

Table 118. Summary of major and minor malformations in fetus

| Findings | External and visceral examination of foetuses | | | | | | | |
|---|---|--------|-----------------|--------|-----------------|--------|-----------------|--------|
| | Summary of results | | | | | | | |
| | Control | | 20mg/kg/day | | 100mg/kg/day | | 400mg/kg/day | |
| Findings | No. of foetuses | Mean % | No. of foetuses | Mean % | No. of foetuses | Mean % | No. of foetuses | Mean % |
| Malformations | | | | | | | | |
| Arthrogryposis - unilateral | 1 | 0.9 | 1 | 0.9 | | | | |
| Ectopic (inguinal) and enlarged kidney - unilateral | | | | | | | 1 | 1.0 |
| Exencephaly, cleft palate, open eye - unilateral (seen in a late resorption) | | | | | 1a | - | | |
| Minor anomalies | | | | | | | | |
| Albinism - no skin pigment | | | 1 | 1.2 | | | | |
| Slight displacement of kidney - unilateral | | | | | | | | |
| Spleen reduced | | | | | | | | |
| Gall bladder reduced | | | | | | | | |
| Lateral ventricles slightly enlarged | 1 | 2.1 | 1 | 2.4 | | | 1 | 4.2 |
| Kidney dark - unilateral | | | | | | | 1 | 1.2 |
| Increased renal cavitation - bilateral | 4 | 4.5 | 1 | 0.6 | | | 3 | 6.1 |
| Increased renal cavitation - unilateral | 2 | 1.7 | 4 | 3.3 | 1 | 1.0 | 3 | 5.2 |
| Abdominal haemorrhage | | | | | | | 1 | 1.0 |
| No. of foetuses examined by Wilson technique | 37 | | 32 | | 27 | | 23 | |
| Total no. of foetuses examined | 108 | | 98 | | 84 | | 71 | |

KPC/735/85

a = malformation observed in a late resorption, mean not calculated.
No statistically significant differences found by Kruskall-Wallis test.

Summary data for external, visceral, and skeletal examinations are presented in table above (copied from the Applicant's submission, p. 34 of the study report). No treatment-related major abnormalities, minor abnormalities, or variants were observed.

9.3 Prenatal and Postnatal Development

Study title: Rat peri- and post natal study

| | |
|-------------------------------------|---|
| Study no.: | KPC/34/85 |
| Study report location: | EDR |
| Conducting laboratory and location: | (b) (4) |
| Date of study initiation: | Exact date not provided (Final Report dated January 1986) |
| GLP compliance: | Yes |
| QA statement: | No statement provided |
| Drug, lot #, and % purity: | Naloxone chlorhydrate, Lot ARE 802 and Lot ARE 807, purity not provided in study report |

Key Study Findings

Female rats treated with naloxone (50, 200, and 800 mg/kg/day) during Gestation Day 15 through Postpartum Day 20 in a definitive Segment III study with the following findings:

F₀ Dams

1. There were six treatment-related mortalities in the HD group noted in the study.
2. Convulsions were the primary clinical signs observed in the female HD group during the gestational period.
3. Reduced body weight and body weight gains were observed during the gestation and lactation period with a concomitant reduction in food consumption.
4. Mating and fertility (such as number of pregnancy, mean duration of gestation, mean duration of parturition and gestation index) of F₀ female was not affected when compared to vehicle-treated dams.
5. NOAEL was 50 mg/kg/day due to deaths during the oral administration of 800 mg/kg/day during gestation and decrease body weight of dams administered 200 mg/kg during lactation.

F₁ Pups

1. Fewer newborns per litter in dams orally administered 800 mg/kg/day naloxone was observed.
2. Viability index of newborns for dams orally administered 800 mg/kg/day naloxone was lower compared to control dams.
3. F₁ pups, for all naloxone dose groups, meeting developmental benchmarks was comparable to control dams.
4. NOAEL was 200 mg/kg/day based on reduced viability index and newborns per litter from dams orally administered 800 mg/kg/day naloxone.

Methods

Doses: 0, 50, 200, and 800 mg/kg/day
 Frequency of dosing: Once daily on Day 15 of pregnancy (GD15) to Day 20 post partum (PND20)
 Dose volume: 10 mL/kg
 Route of administration: Oral gavage
 Formulation/Vehicle: solution/distilled water
 Species/Strain: Rat/Sprague-Dawley (COBS of the CD strain)
 Number/Sex/Group:

| Group | Dose (mg/kg/day) | No of Females |
|----------------------------|-------------------------|----------------------|
| 1 (vehicle control) | 0 | 20 |
| 2 | 50 | 20 |
| 3 | 200 | 20 |
| 4 | 800 | 20 |

Satellite groups: None

Study design: Virgin female rats were acclimated for at least seven days before pairing with experienced sexually mature male rats. Virgin female rats were paired with sexually mature males overnight (3F:1M). Female rats were observed for evidence of copulation, which if present was designated Day 0 of gestation. Daily dosing by oral gavage from Gestational Day 15 to postpartum Day 20.

On Postpartum Day 4, all litters containing more than 8 pups, were culled to 8. Culling was performed randomly within sexes, such that the adjusted litters have a sex ratio of 4 males and 4 females.

Necropsy of F₀ dams and F₁ offspring were performed on Postpartum Day 21.

Deviation from study protocol: Deviations are described under the appropriate observation(s)

Observations and Results**F₀ Dams: Observations**

F₀ Dams: Observations

Survival: Animals were examined daily for mortality.

Clinical signs: Clinical observations were performed daily.

Body weight: Body weights were recorded on Gestational Days 0, and daily from Gestational Day 15 to Postpartum Day 21. Body weight was reported for gestational Days 0, 15, 21, and Postpartum Days 1, 7, 14, 21.

Food consumption: Food consumption was measured throughout Gestational Days 15 to 21 and Postpartum Days 14 to 21.

Uterine content: Not evaluated

Necropsy observation: All surviving female rats were euthanized by CO₂ asphyxiation on Postpartum Day 21. Necropsy was performed on unscheduled dams on day of death. Macroscopic examination of the thoracic and abdominal cavities was performed. Any unusual gross findings were recorded and tissues showing macroscopic lesions were removed and fixed in buffered 10% formalin.

Toxicokinetics: Not evaluated

Dosing Solution Analysis

Other: **Parturition:** "The females were observed at 30 minute intervals from 07.00 hours on Day 21 of pregnancy to 17.00 hours on Day 22 of pregnancy. The time at which commencement and completion of parturition occurred was recorded." Duration of parturition was also recorded.
Duration of gestation: Defined as the time elapsing between 0.0 hours on the day sperm was observed in the vaginal smear to the commencement of parturition.

F₀ Dams: Results**Survival****Table 119. Unscheduled deaths in females orally administered naloxone during Gestation Days 15 through Postpartum Day 20**

| Group/Animal № | Day of unscheduled death | Clinical Signs | Macroscopic/Microscopic Findings | Reviewer's Conclusion |
|----------------|--------------------------|--|---|--|
| Group 4/Nº 62 | Postpartum Day 0 | - convulsions observed within 5 to 10 mins after dosing - death followed within 20 mins | - Intestines: contents dark in color | The observed clinical signs were attributed to naloxone. |

| Group/Animal № | Day of unscheduled death | Clinical Signs | Macroscopic/Microscopic Findings | Reviewer's Conclusion |
|----------------|--------------------------|---|---|---|
| | | after the onset of convulsions | | |
| Group 4/Nº 69 | Gestational Day 22 | - convulsions observed within 5 to 10 mins after dosing - death followed within 20 mins after the onset of convulsions | - No abnormalities detected | Abortion was attributable to treatment. |
| Group 4/Nº 70 | Gestational Day 22 | - convulsions observed within 5 to 10 mins after dosing - death followed within 20 mins after the onset of convulsions | - No abnormalities detected | The observed clinical sign was attributed to naloxone. Microscopic findings unlikely attributed to naloxone. |
| Group 4/Nº 73 | Gestational Day 21 | - convulsions observed within 5 to 10 mins after dosing - death followed within 20 mins after the onset of convulsions | - No abnormalities detected | |
| Group 4/Nº 75 | Gestational Day 16 | - No observed clinical signs | - Nose and mouth: fur staining | |
| Group 4/Nº 80 | Gestational Day 17 | - No observed clinical signs | - Lungs and liver: dark in color | |

Six unscheduled deaths occurred in the high dose group. Unscheduled mortality data are summarized in table above. No unscheduled deaths occurred in the LD and MD dams.

Clinical Signs

Table 120. Summary of clinical signs in maternal females orally administered naloxone during Gestation Day 15 through Postpartum Day 20

| Clinical observation | Dose level (mg/kg/day) | Animal number(s) | Day(s) of pregnancy (P)/post partum (pp) |
|-----------------------------------|---------------------------|------------------|---|
| Alopecia | Control | 5 | 20P - 21pp |
| | | 6 | 2pp - 21pp |
| | | 14 | 11pp - 21pp |
| | | 50 | 9pp - 20pp |
| | | 200 | 17pp - 20pp |
| | | 800 | 16P - 20pp |
| | | 68 | 19pp - 20pp |
| Fur staining | Control | 19 | 16pp |
| Peri-oral and nasal staining | 800 | 64 | 16P - 17P |
| Excess salivation after dosing | 50 | 35 | 15pp |
| | 200 | All | throughout dosing |
| | 800 | All | throughout dosing |
| Resistance to dose administration | 800 | All | from approximately day 9pp to end of dosing |
| Convulsions with recovery (a) | 800 | 74 | 21P |
| | | 77 | 20P |
| | | 62 | 0pp |
| | | 69 | 22P |
| Convulsions followed by death (a) | 800 | 70 | 22P |
| | | 73 | 21P |
| | | | |
| Died with no clinical signs | 800 | 75 | 16P |
| | | 80 | 17P |

All other animals - no clinical observations.

(a) = Convulsions occurred within 5 to 10 minutes of dosing. Death followed within 20 minutes of the onset of the convulsions. Recovery, for two animals, was observed 30 minutes after the onset of the convulsions.

Naloxone treatment-related clinical signs were observed in HD females. As depicted in the table above (copied from Applicant's submission, p 28 of the study report), the primary treatment-related clinical sign observed in the HD females included convulsions. Excessive salivation was also observed in all dams in the MD and HD groups throughout the dosing period. There were no remarkable findings in the LD group.

Body Weight

Table 121. Group mean body weight gain data during gestation and lactation periods in females orally administered naloxone

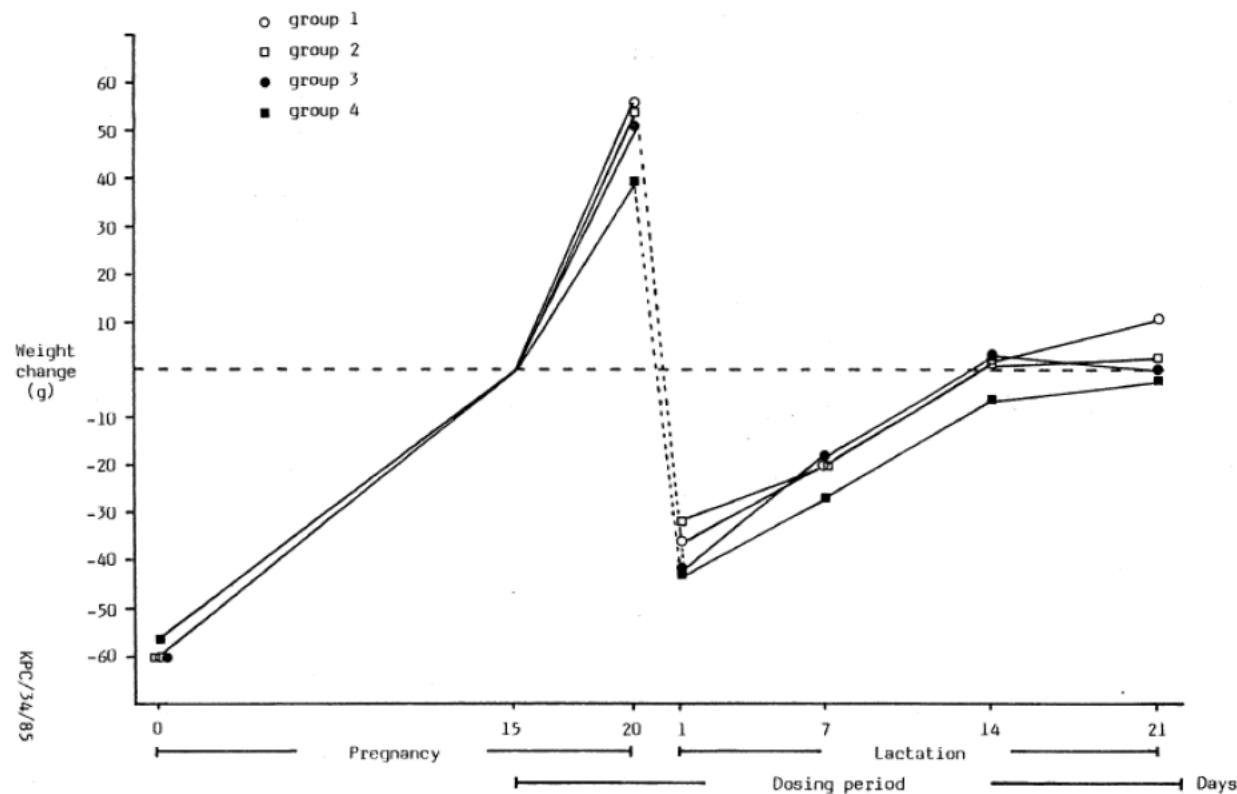


Table 122. Group mean maternal body weight during gestation and lactation

| Mean maternal body weight (g) \pm SD (% change of control) | | | | |
|--|------------------|--------------|--------------------------------------|--------------------------------------|
| | Dose (mg/kg/day) | | | |
| | 0 | 50 | 200 | 800 |
| Gestational Days Period | | | | |
| 0 | 240 \pm 9 | 242 \pm 10 | 237 \pm 11 | 238 \pm 11 |
| 15 | 300 \pm 16 | 302 \pm 20 | 297 \pm 16 | 294 \pm 19 |
| 20 | 356 \pm 20 | 356 \pm 23 | 348 \pm 20 | 333 \pm 26 ^b (-6.4%) |
| Post partum Days Period | | | | |
| 1 | 264 \pm 22 | 270 \pm 27 | 255 \pm 19 | 251 \pm 22 |
| 7 | 280 \pm 20 | 288 \pm 26 | 279 \pm 20 | 267 \pm 15 |
| 14 | 302 \pm 19 | 303 \pm 21 | 300 \pm 19 | 288 \pm 16 |
| 21 | 311 \pm 16 | 304 \pm 20 | 297 \pm 16 ^a (-4.5%) | 292 \pm 22 ^b (-6.1%) |

a: Statistically significant when compared to control at $p \leq 0.05$, Student's t test

b: Statistically significant when compared to control at $p \leq 0.01$, Student's t test

Female body weight data is presented in the figure (copied from Applicant's submission, p 13) and table above. As depicted in the figure, compared to vehicle control, mean body weight gain at 800 mg/kg/day was lower than the controls throughout the dosing and lactation periods. Mean bodyweight gain in the HD dams was significantly lower on Gestational Day 20 ($p < 0.01$) and Postpartum Day 21 ($p < 0.01$). Mean bodyweight gain in the MD dams was significantly lower on Postpartum Day 21 ($p < 0.05$). *The observed decrease in mean body weight gain in the mid and high dose dams was correlated with a decrease in food consumption during the same periods.*

Food Consumption

Table 123. Group mean maternal food consumption during gestation and lactation

| Mean maternal food consumption (g/rat/day) \pm SD (% change of control) | | | | |
|---|------------------|-----------------|----------------|---|
| | Dose (mg/kg/day) | | | |
| | 0 | 50 | 200 | 800 |
| Gestational Days Period | | | | |
| 15 to 21 | 25.8 \pm 5.3 | 25.0 \pm 2.6 | 24.3 \pm 4.5 | 18.4 \pm 3.8 ^c (-28.7%) |
| Post partum Days Period | | | | |
| 1 to 7 | 34.7 \pm 4.3 | 36.8 \pm 7.1 | 38.1 \pm 6.1 | 32.9 \pm 7.5 |
| 7 to 14 | 55.7 \pm 7.7 | 54.5 \pm 7.9 | 54.5 \pm 6.2 | 48.7 \pm 12.2 |
| 14 to 21 | 64.2 \pm 9.8 | 61.4 \pm 10.7 | 60.8 \pm 9.0 | 52.7 \pm 16.3 |

c: Statistically significant when compared to control at $p \leq 0.001$, Student's t test

As depicted in the table above, compared to vehicle control, mean food consumption at 800 mg/kg/day was lower than the controls throughout the dosing and lactation periods. Food consumption was statistically significantly ($p < 0.001$) decrease during Gestational Days 15 -21; 28.7% lower compared to controls. Food consumption in the LD dams was comparable to control dams.

F₀ Necropsy during lactation

There were no remarkable findings in the F₀ dams.

Reproductive findings in F₀ dams

Table 124. Group mean pregnancy data

| Dose level (mg/kg/day) | Number pregnant | Mean duration of gestation (days) ± S.D. | Mean duration of parturition (hours) ± S.D. | Gestation index |
|---------------------------|-----------------|---|--|-----------------|
| Control | 19 | 22.0 ± 0.4 | 2.3 ± 0.6 | 94.7 |
| 50 | 20 | 22.0 ± 0.3 | 2.3 ± 0.6 | 100.0 |
| 200 | 20 | 22.1 ± 0.3 | 2.8 ± 2.2 2.3 ± 0.8d | 100.0 |
| 800 | 20e | 22.3 ± 0.3 | 2.3 ± 0.8 | 100.0f |
| Analysis of variance | | NS | NS | |
| Chi ² test | | | NS | |

d = excluding animal 51 with unusually long parturition.

e = including 6 animals that died.

f = excluding animals that died.

Pregnancy data is presented in the table above (copied from the Applicant's submission, p 16 of the study report). As depicted in the table, pregnancy indices were similar between 0, 50, 200, and 800 mg/kg/day groups with an increase in mean parturition duration in the MD group. The observed long mean parturition in the MD group was due to one Dam (№ 51) with an unusually long parturition.

Dosing Solution Analysis

Table 125. Analysis of formulation

| Preparation date | Theoretical concentration mg/ml | Assayed concentration mg/ml | Percentage of theoretical |
|------------------|---------------------------------|-----------------------------|---------------------------|
| 3.9.85 | 0 | 0 | - |
| | 5 | 5.04 | 101 |
| | 20 | 18.9 | 95 |
| | 80 | 82.8 | 104 |
| 17.9.85 | 0 | 0 | - |
| | 5 | 4.52 | 90 |
| | 20 | 19.14 | 96 |
| | 80 | 78.92 | 99 |
| 1.10.85 | 0 | 0 | - |
| | 5 | 4.58 | 92 |
| | 20 | 19.77 | 99 |
| | 80 | 74.82 | 94 |
| 11.9.85a | 80 | 93.3 | 117 |
| | 80 R1 | 90.3 | 113 |
| | 80 R1 | 88.6 | 111 |
| | 80 R1 | 80.0 | 100 |
| | 80 R2 | 72.2 | 90 |
| | 80 R2 | 83.9 | 105 |
| | 80 R2 | 73.1 | 91 |

a - additional analysis.

R1 - sample checked for homogeneity since original analysis was outside normal limits.

R2 - sample re-analysed with freshly prepared solvent as first analysis were conducted using solvent from week 1 (3/9/85) analyses.

As depicted in the table above, the concentration of naloxone was within an acceptable range of the theoretical range (90-104%).

F₁ Generation

| | |
|--------------------------|--|
| Survival: | Checked daily for pups, prior to weaning |
| Clinical signs: | Pups were examined daily for any abnormalities. |
| Body weight: | Total body weights of the male pups and the total body weights of the female pups in each litter was recorded as soon as possible after birth On Day 21 post partum, pups were weighed individually. |
| Food consumption: | Not evaluated |
| Litter Data | <p>Live Birth Index: (№ pups born alive/total № pups born) x 100</p> <p>Viability Index: (№ live pups on Day 4 postpartum/№ live pups born) x 100</p> <p>Lactation Index: № of pups alive on Day 21 postpartum/№ pups present after culling) x 100</p> <p>Cumulative Survival Index: (№ of pups alive on Day 21 postpartum/№ pups present after culling x № live pups on Day 4 postpartum)/ total № pups born x 100</p> <p>Sex Ratio: № female pups/№ male pups</p> |
| Physical development: | The development of the following characteristics were recorded for each pup in each litter: |
| | <p>Ears open: Examined daily until occurrence. Reported as the percentage of pups in each litter with ears open on Day 3 post partum.</p> <p>Static righting reflex: Examined on Day 5 post partum.</p> <p>Eyes open: Examined daily until occurrence. Reported as the percentage of pups in each litter with eyes open on Day 15 post partum.</p> <p>Startle response: Examined on Day 15 post partum.</p> |
| Neurological assessment: | Not evaluated |
| Reproduction: | Not evaluated |
| Necropsy | <p>Macroscopic necropsy was performed on all pups, including those culled or found dead.</p> <p>Culled pups were euthanized by an intracardiac injection of sodium pentobarbital on Day 4 post partum.</p> <p>Weaned pups were euthanized by CO₂ asphyxiation on Postpartum Day 21. Necropsy was performed on unscheduled dams on day of death. Macroscopic examination of the major organs in the thoracic and abdominal cavities was performed. Any unusual</p> |

F₁ Generation

gross findings were recorded and tissues showing macroscopic lesions were removed and fixed in buffered 10% formalin.

Other:

Body Weight**Table 126. F₁ generation - Group mean body weights (g) ± SD**

| Dose level (mg/kg/day) | Sex | Day post partum | | | |
|---------------------------|-----|-----------------|-----------|------------|------------|
| | | 0 | 4 | 14 | 21 |
| Control | M | 5.8 ± 0.4 | 7.7 ± 1.2 | 28.9 ± 3.3 | 46.8 ± 4.8 |
| 50 | M | 5.9 ± 0.4 | 8.2 ± 1.3 | 27.9 ± 4.2 | 48.0 ± 7.2 |
| 200 | M | 5.9 ± 0.7 | 8.2 ± 1.9 | 27.3 ± 4.1 | 47.3 ± 5.4 |
| 800 | M | 5.8 ± 0.6 | 7.7 ± 1.7 | 27.0 ± 3.6 | 42.4 ± 6.2 |
| Analysis of variance | | NS | NS | NS | NS |
| Control | F | 5.4 ± 0.4 | 7.2 ± 1.1 | 27.8 ± 2.7 | 45.8 ± 3.6 |
| 50 | F | 5.5 ± 0.4 | 7.8 ± 1.2 | 26.9 ± 4.2 | 46.2 ± 6.2 |
| 200 | F | 5.7 ± 1.0 | 7.9 ± 1.8 | 28.0 ± 3.3 | 46.5 ± 3.6 |
| 800 | F | 5.4 ± 0.8 | 7.6 ± 2.1 | 26.6 ± 3.6 | 43.0 ± 6.0 |
| Analysis of variance | | NS | NS | NS | NS |

Pups body weight data are presented in the table above (copied from Applicant's submission, p 18 of the study report). Bodyweights of newborns in the LD and MD groups were comparable to controls. Compared to vehicle control, mean bodyweight was slightly lower in both HD groups male and female pups on Days 14 (M: -6.6% and F: -4.3%) and 21 (M: -9.4% and F: -6.1%) post partum; but this effect did not reach statistical significance.

Newborn (F₁) Observations

Table 127. F₁ generation – group mean litter data

| Dose level (mg/kg/day) | Mean no. of pups born ± S.D. | Sex ratio at birth M : F | Mean live birth index | Mean viability index | Mean lactation index | Mean cumulative survival index |
|---------------------------|---------------------------------|-----------------------------|-----------------------|----------------------|----------------------|--------------------------------|
| Control | 13.6 ± 1.6 | 1 : 0.91 | 93.7 | 81.1 | 86.9 | 70.3 |
| 50 | 12.8 ± 2.6 | 1 : 1.15 | 98.6 | 94.9 | 83.6 | 78.0 |
| 200 | 13.9 ± 3.2 | 1 : 0.90 | 97.6 | 81.6 | 84.2 | 67.8 |
| 800 | 11.8 ± 4.8 | 1 : 1.00 | 93.8 | 71.1 | 85.4 | 60.8 |

| | |
|-----------------------|----|
| Analysis of variance | NS |
| chi ² test | NS |
| Kruskal-Wallis test | NS |

Litter Size Data. As depicted in table above, the mean number of pups in the MD group was comparable to the control group. The data for the number of newborns per litter showed a trend for fewer newborns per litter in dams orally administered 800 mg/kg/day of naloxone; this effect is contributed to a few dams having an unusually small litter size compared to control dams. This effect did not reach statistical significance.

Sex Ratio. As depicted in table above, the sex ratio data were unremarkable.

Live Birth Index. As depicted in table above, live birth index was unremarkable.

Viability Index. Treatment-related effects were observed in the HD group. Compared to control group, viability index was 12.3% lower. This effect did not reach statistical significance. The lower viability index is contributed mainly to three females that had total litter loss.

Lactation Index. Unremarkable

Survival Index. Compared to control group, survival index was 13.5% lower in the HD group. This effect did not reach statistical significance. The lower viability index is contributed mainly to three females that had total litter loss. The Applicant speculated that the mortalities of the HD pups may be contributed to maternal and pups having an infection, possibly viral. However, the exact cause of the mortalities was not ascertained. The Applicant did not submit any data to support this speculation.

F₁ Postnatal Development

Table 128. F₁ generation - developmental landmarks during lactation

| Dose level (mg/kg/day) | Mean percent of pups with ears open on day 3 ± S.D. | Mean percent of pups with righting reflex on day 5 ± S.D. | Mean percent of pups with eyes open on day 15 ± S.D. | Mean percent of pups with startle response on day 15 ± S.D. |
|----------------------------|--|--|---|--|
| Control | 25.1 ± 32.1 | 71.2 ± 21.1 | 68.1 ± 26.1 | 57.4 ± 21.8 |
| 50 | 32.4 ± 40.4 | 71.6 ± 22.6 | 77.1 ± 30.1 | 53.7 ± 23.3 |
| 200 | 30.4 ± 36.8 | 63.9 ± 17.7 | 76.3 ± 26.8 | 54.8 ± 19.2 |
| 800 | 37.3 ± 44.3 | 75.9 ± 15.4 | 75.5 ± 28.4 | 49.1 ± 17.8 |
| Analysis of variance | NS | NS | NS | NS |

Developmental landmark data are presented in the table above (copied from the Applicant's submission, p. 19 of the study report). No treatment-related effects were observed with dosing ≥ 50 mg/kg/day.

F₁ gross observations

There were no remarkable necropsy findings in the F₁ pups.

F₂ Generation

Survival: Not evaluated
 Body weight: Not evaluated
 External evaluation: Not evaluated
 Male/Female ratio: Not evaluated
 Other: Not evaluated

10 Special Toxicology Studies

11 Integrated Summary and Safety Evaluation

To support the NDA for TARGINIQ ER, the Applicant has submitted toxicology studies to support the safety of the naloxone component of TARGINIQ ER. The Applicant has evaluated the repeat-dose toxicity, genetic toxicology, reproductive toxicology, and carcinogenicity of naloxone. The repeat-dose toxicity studies were conducted in the late 80s or early 90s and do not include the level of detail we expect based on current standards for general toxicology studies, thus raising some concerns about the adequacy of these studies. Specifically the purity of the naloxone was frequently not noted in the study report and data on batch comparisons was not provided in the CMC sections of the NDA. However, based on the results, the reviewer feels that the study

reports provide an adequate characterization of the safety of the drug product even without the naloxone purity data as there were clearly treatment-related effects and the studies demonstrated sufficiently large safety margins to support the dose of naloxone proposed even if the purity were extremely low. These studies need not be repeated, particularly given the extensive history of naloxone use at lower levels and the low level of endogenous opioid tone in the body. The genetic toxicology studies were also conductive in the late 80s; however the study design was deemed adequate. Genetic toxicology of the OH-dimer of oxycodone was also assessed. The OH-dimer of oxycodone was not an impurity or a metabolite of oxycodone.

Repeat-dose toxicity studies of 52-weeks and 9-months duration were conducted in rats and dogs, respectively. No organ of toxicity was identified in these studies. In the 52-weeks repeat-dose toxicity study in rats, naloxone hydrochloride was orally administered via the diet at dose levels of 25, 75, and 225 mg/kg/day. Naloxone was well tolerated at doses up to 225 mg/kg/day; there were no treatment-related findings for clinical signs or clinical pathology. A dose-dependent reduction in body weight was observed at the end of the treatment-period in both sexes; reduction of approximately 10%, 20%, and 40% was observed following the LD, MD, and HD of naloxone, respectively. A NOAEL of < 25 mg/kg/day was established based on body weight changes which suggest an exposure margin of < 6-fold on a body surface basis when compared to a daily dose of 40 mg/day in the human. The top dose, which only produced body weight reductions provides an exposure margin of 54-time the MRDD, based on a body surface area comparison.

In the 9-month repeat-dose toxicity study in dogs, naloxone hydrochloride was orally administered at doses of 5, 25, and 125/75. Treatment-related clinical signs observed in the HD animals orally administered 125 mg/kg/day included ataxia, convulsions, disorientation, tremors and cyanosis. As a result of these clinical signs, one HF female was humanely euthanized. The NOAEL of 75 mg/kg/day was established which is 62.5 times the dose of 40 mg of a 60 kg human based on body surface area comparison.

A 3-month repeat-dose toxicity study was conducted in rats with either oxycodone hydrochloride/naloxone hydrochloride combination at ratios of 2:1 and 12:1 or naloxone hydrochloride alone. Oxycodone/naloxone was orally administered at doses of 4.0/0.34, 10/0.85 or 25/2.13 mg/kg day; naloxone only was orally administered at a dose of 0.0/2.13 mg/kg/day. Oxycodone/naloxone was well tolerated at all doses; all animals survived to the termination of the study. Treatment-related clinical signs observed in the MD and HD animals orally administered oxycodone/naloxone included biting/chewing on the cage, self-mutilation, and hyper-reactivity to handling. These effects were attributed to the oxycodone component. Females were more affected than the males. Females had a higher systemic exposure to oxycodone than the males. No apparent toxicity was observed in the animals orally administered naloxone only. Relevant to the clinical indication of TARGINIQ ER, naloxone did not induce opioid-like withdrawal signs in rats chronically administered oxycodone at a dose of 25 mg/day. The NOAEL of 25 mg oxycodone/2.13 mg naloxone was established which is 10-

fold/0.5-fold the dose maximum dose of 80 mg oxycodone/40 mg naloxone of a 60 kg human based on body surface area comparison.

Two MFs are referenced for the naloxone drug substance ([REDACTED] (b) (4) and [REDACTED] (b) (4)). The drug substance impurity [REDACTED] (b) (4) contains a structural alert for mutagenicity. This drug substance impurity has not been adequately qualified for genotoxic potential. ONDQA has deemed the [REDACTED] (b) (4) MF unacceptable for other reasons (see review by Eugenia Nashed, PhD). The drug product contains 6 degradation products. The specifications of NMT [REDACTED] (b) (4)% for these drug product impurities are acceptable if the MDD is 80 mg oxycodone/40 mg naloxone.

The standard ICH battery of genetic toxicology studies was conducted for naloxone. Naloxone hydrochloride tested negative in the in vitro bacterial reverse mutation assay and in the in vivo mouse micronucleus assay in the presence and absence of metabolic activation. Naloxone hydrochloride tested positive in the L51787 mutation assay in the presence and absence of metabolic activation. Naloxone hydrochloride tested positive for clastogenic activity in the in vitro chromosome aberration assay in the presence of metabolic activation. Collectively, these data suggest that naloxone hydrochloride has clastogenic potential.

Carcinogenicity studies have been conducted with naloxone hydrochloride, specifically the Applicant completed a 6-month study in Tg.rash2 mice and a 2-year study in rats. These carcinogenicity assessments suggest that naloxone has no tumorigenic potential.

A full battery of developmental and reproductive toxicology studies has been conducted with naloxone hydrochloride. Embryo-fetal development studies were conducted in pregnant rats treated with 50, 200, and 800 mg/kg/day by oral gavage organogenesis. The maternal NOAEL was 800 mg/kg/day based on no evidence of treatment-related maternal toxicity. No effects on embryofetal development were seen in rat at doses up to 800 mg/kg/day. However, delayed development was suggested that included delay in the time of eye and ear opening in the 800 mg/kg/day groups. Increase incidence of iritis was correlated with the delay in eye opening in the HD group. The observed developmental eyes findings are not considered to be clinically relevant because the observed iritis was not reproducible in the other reproductive toxicology study. Developmental NOAEL was established at 200 mg/kg/day based on iritis observed in the F₀ generation. This dose yields an exposure margin of 48.6 on a mg/m² basis when compared to a daily dose of 40 mg naloxone in humans.

Embryofetal developmental studies were conducted in pregnant rabbits treated with 20, 100, and 400 mg/kg/day naloxone hydrochloride by oral gavage during organogenesis. No effects on implantation, post-implantation losses, fetal weight, or sex ratio were noted. Incidences of fetal malformations were comparable in naloxone groups and vehicle control group. The NOAEL for developmental toxicity in this study was established at > 400 mg/kg/day. This dose yields an exposure margin >97 on a mg/m² basis when compared to a daily dose of 40 mg naloxone. The NOAEL for maternal toxicity was established at 200 mg/kg/day based on treatment-related clinical signs.

A dedicated pre- and post-natal development study was conducted in rats treated with doses of 50, 200, and 800 mg/kg/day naloxone hydrochloride by oral gavage. Naloxone did not affect pre- and postnatal development in rats at doses that produced maternal toxicity with the exception of fewer newborns per litter and a lower viability index in dams orally administered 800 mg/kg/day of naloxone. However, consistent with the findings in the embryofetal development study, naloxone did not affect development in the surviving pups.

Developmental and reproductive toxicology studies have been conducted with oxycodone hydrochloride and previously submitted under NDA 20-553 (Segment II) and NDA 22-272 (Segment I and III) and have been reviewed by Dr. BeLinda Hayes, PhD and Elizabeth Bolan, PhD, respectively. Overall, these reproductive studies indicated that oxycodone had no effect on fertility reproduction or on the early embryonic development of the rat. Oxycodone did not affect pre- and postnatal development in rats at doses that produced maternal toxicity.

A Pregnancy Category C is recommended for this product primarily based on the lack of combination toxicology data; the relevant results will be described in the product label. Exposure comparisons for the label will be based on body surface area because animal AUC values are not available. The MRDD of oxycodone and naloxone will be 40 mg and 20 mg BID, respectively; therefore, the human dose of 80 mg oxycodone and 40 mg naloxone will be used as the exposure comparison for the nonclinical studies.

Approximate exposure margins for the human relative to the maximum recommended daily dose of 80 mg oxycodone and 40 mg naloxone in patients approximately 60 kg in weight are presented in the following table.

| Study Type | Species | Toxicity | NOAEL (mg/kg) | Safety Margin Based on body surface |
|-----------------------------|----------------|---|-----------------------------|--|
| 52-wks toxicity study | Rat | Reduction in body weight | < 25 mg/kg/day | < 6-fold |
| 9-month toxicity study | Dog | Clinical signs: ataxia, convulsions, disorientation, tremors and cyanosis | 75 | 60-fold |
| 3-month toxicity study | Rat | Opioid-related clinical signs | 25 oxycodone/2.13 naloxone | 10-fold/0.5-fold |
| Embryonic fetal development | Rats | Maternal: None Fetal: delayed eye opening and iritis | Maternal: 800 Fetal: 200 | Maternal: 192-fold Fetal: 48.6-fold |
| Embryonic fetal development | Rabbits | No developmental toxicity | >400 | > 97-fold |
| Peri- and postnatal study | Rats | Maternal: Mortalities and reduction in body weight during lactation | 50 | 12-fold |

12 Appendix/Attachments

Reference List

Baldacci A, Caslavská J, Wey AB and Thormann W (2004) Identification of new oxycodone metabolites in human urine by capillary electrophoresis-multiple-stage ion-trap mass spectrometry. *J Chromatogr A* **1051**:273-282.

Cone EJ, Darwin WD, Buchwald WF and Gorodetzky CW. Comparative metabolism and excretion of oxycodone in man and laboratory animals. Fed Proceedings 43(1), 65. 1984.

Ref Type: Abstract

Gulezian D, Jacobson-Kram D, McCullough CB, Olson H, Recio L, Robinson D, Storer R, Tennant R, Ward JM and Neumann DA (2000) Use of transgenic animals for carcinogenicity testing: considerations and implications for risk assessment. *Toxicol Pathol* **28**:482-499.

Ishida T, Oguri K and Yoshimura H (1982) Determination of oxycodone metabolites in urines and feces of several mammalian species. *J Pharmacobiodyn* **5**:521-525.

Lalovic B, Kharasch E, Hoffer C, Risler L, Liu-Chen LY and Shen DD (2006) Pharmacokinetics and pharmacodynamics of oral oxycodone in healthy human subjects: role of circulating active metabolites. *Clin Pharmacol Ther* **79**:461-479.

Morton D, Alden CL, Roth AJ and Usui T (2002) The Tg rasH2 mouse in cancer hazard identification. *Toxicol Pathol* **30**:139-146.

Paranjpe MG, Elbekaei RH, Shah SA, Hickman M, Wenk ML and Zahalka EA (2013) Historical control data of spontaneous tumors in transgenic CByB6F1-Tg(HRAS)2Jic (Tg.rasH2) mice. *Int J Toxicol* **32**:48-57.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

BELINDA A HAYES
06/23/2014
Correct version of PharmTox review of NDA 205777,

RICHARD D MELLON
06/24/2014
I concur with Dr. Hayes that, from a nonclinical pharmacology toxicology perspective, NDA 205777 may be approved with the recommended PMR.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 205-777 Applicant: Purdue Pharma L.P. Stamp Date: September 23, 2013

Drug Name: Oxycodone NDA/BLA Type: NDA, 505(b)(2)
HCl/Naloxone HCl controlled-release tablet (Targiniq)

On initial overview of the NDA/BLA application for filing:

| | Content Parameter | Yes | No | Comment |
|---|--|-----|----|--|
| 1 | Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin? | X | | |
| 2 | Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin? | X | | |
| 3 | Is the pharmacology/toxicology section legible so that substantive review can begin? | X | | |
| 4 | Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)? | X | | |
| 5 | If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA). | X | | |
| 6 | Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route? | X | | |
| 7 | Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations? | X | | GLP statement was included in the pivotal study reports. |

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement**

| | Content Parameter | Yes | No | Comment |
|----|---|------------|-----------|---|
| 8 | Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions? | | X | The Application did not contain adequate safety qualification data for (b) (4). The Sponsor claims that the specification is as low as technically feasible at this time. This will require review and input from our CMC colleagues. Therefore, this cannot be deemed a filing issue. |
| 9 | Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57? | | X | We will fix this during the review. We do not consider this a filing issue. The exposure margins are actually present in the referenced product labeling and will depend, in part, on the final dosing regimen. |
| 10 | Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.) | | X | The Applicant did address the oxycodone genotoxic impurities (b) (4), (b) (4) and the genotoxic impurity (b) (4). At the proposed MDD of 80 mg/day oxycodone, for the potential (b) (4) impurities of oxycodone, the maximum daily intake will be (b) (4), which is lower than the 1.5 mcg/day limit for a genotoxic potential. At the proposed MDD of 40 mg/day naloxone, at a level of (b) (4), the maximum daily intake of the clastogenic (b) (4) will be (b) (4) which exceeds the 1.5 mcg/day limit. If technically feasible, the limit should be reduced to (b) (4) in order to meet the 1.5 mcg/day acceptable limit, if we agree with the MDD of 80:40 mg oxycodone:naloxone. At the MTDD of 0.75 grams per day naloxone, the daily exposure would be (b) (4). This cannot be deemed a RTF issue, as the clinical data must be reviewed to determine if the proposed MDD will be accepted. |
| 11 | Has the applicant addressed any abuse potential issues in the submission? | | X | Two nonclinical study reports were submitted to evaluate the withdrawal and physical dependence of oxycodone/naloxone. Also, a summary of nonclinical data in the published literature relevant to the abuse potential of oxycodone and naloxone was submitted. Three clinical abuse liability studies (ONU103, ONU1004, and ONU9001) were |

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement
010908

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement**

| | Content Parameter | Yes | No | Comment |
|----|---|------------|-----------|--|
| | | | | conducted in recreational opioid users (ONU1003, ONU1007 and ONDU9001) and opioid dependent subjects (ONU1004, ONU1008) to characterize the abuse potential of the clinical formulation. Also, several in vitro studies were conducted to address the physical manipulation and chemical extraction potential of the to-be-marketed formulation. |
| 12 | If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted? | | | N/A |

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

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

During the course of development, the Division informed the Sponsor that the maximum theoretical daily dose (MTDD) of oxycodone in controlled-release drug products intended for chronic use is 1.5 grams per day. Via this formulation, that would result in 750 mg of naloxone. The proposed drug product labeling; however, states that the maximum daily dose (MDD) should not exceed 80 mg of oxycodone. This appears to be based on adverse GI effects of the drug with increasing total daily dose of naloxone. The MDD will have to be determined upon review of the clinical data. As such, the drug substance and drug product specifications and safety of excipients must also be deemed to be review issues.

We note that the maximum daily intake of the clastogenic [REDACTED] (b)(4) exceeded the 1.5 mcg/day limit; at a level of [REDACTED] (b)(4) the maximum daily intake will be [REDACTED] (b)(4) at the MTDD. The Applicant did state that the current specification is the technological limit at this time. They will work with the supplier to conform with the FDA's guidance impurity intake limit. Ultimately, this will be a review issue and cannot be deemed a filing issue.

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

Upon review of your proposed drug substance specification for [REDACTED] (b)(4) we note that you have proposed a maximum daily dose (MDD) of NMT 40 mg of naloxone via this drug product. We will have to review your justification for this proposed MDD, as the maximum theoretical daily dose for oxycodone via this drug product would result in 750 mg of naloxone. This clearly has an impact on the drug substance (DS) and drug product (DP) specifications. Agreement on the final DS and DP specifications will require review of your clinical justification. Further, we note that you have concluded that based on current manufacturing capability a specification of NMT [REDACTED] (b)(4) is the lowest feasible level. This will also require review. If we do not agree with your analysis of the data, further discussions will be required to determine the final

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

specifications, the need to tighten the proposed specification, or the need for post-marketing
^{(b) (4)} studies to adequately qualify the genotoxic potential of

Reviewing Pharmacologist

Date

Team Leader/Supervisor

Date

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

BELINDA A HAYES
10/29/2013
PharmTox filing checklist for NDA 205-777.

RICHARD D MELLON
10/29/2013