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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

NDA 20-459

APR 17 1995

Ohmeda, Incorporated
Pharmaceutical Products Division
P.O. Box 804
Liberty Corner, New Jersey 07938-0804

Attention: Mr. Robert I. Outwater
Senior Director
Worldwide Regulatory Affairs

Dear Mr. Outwater:

Please refer to your April 27, 1994, new drug application submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Revex (nalmefene hydrochloride injection) in 1 mL ampuls (100 µg/mL) and 2 mL ampuls (1 mg/mL).

We acknowledge receipt of your amendments dated July 29, 1994; August 4, 1994; September 26 and 27, 1994; October 5, 1994; December 1 and 22, 1994; and February 23 and March 9, 13 and 17, 1995.

This new drug application provides for Revex to be used for the reversal of opioid drug effects, including respiratory depression, induced by either natural or synthetic opioids and for the management of known or suspected opioid overdose.

We have completed the review of this application as amended and have concluded that adequate information has been presented to demonstrate that the drug product is safe and effective for use as recommended in the enclosed draft labeling. Accordingly, this application is approved effective on the date of this letter.

The final printed labeling (FPL) must be identical to the enclosed draft labeling. Marketing the product with FPL that is not identical to this draft labeling may render the product misbranded and an unapproved new drug.

Please submit 15 copies of the FPL as soon as it is available,

in no case more than 30 days after it is printed. Ten copies should be individually mounted on heavy-weight paper or similar material. For administrative purposes, this submission should be designated "FINAL PRINTED LABELING" for approved NDA 20-459. Approval of this labeling by FDA is not required before it is used.

Should additional information relating to the safety and effectiveness of the drug become available, revision of that labeling may be required.

Validation of the regulatory methods has not been completed. At the present time, it is the policy of the Center not to withhold approval because the methods are being validated. Nevertheless, we expect your continued cooperation to resolve any deficiencies that may occur.

We also acknowledge your submissions of March 9 and 13, 1995, committing to performing the following Phase 4 studies. Please submit protocols for these studies as soon as possible. The original copy of the Phase 4 study protocols and reports should be submitted to this Division, with a copy to the Division of Drug Information Resources, HFD-80. Since that Division is responsible for tracking Phase 4 studies, a copy of all future communications regarding Phase 4 studies should also be sent to them.

1. To conduct an adequate study, by means of experiment, observational study, or case registries, to establish whether nalmefene may be safely used in the newborn.

2. To conduct observational or experimental studies of the drug's kinetics and the safety of its use in pediatric populations.

3. To examine the role of entero-hepatic recirculation in the duration of action of nalmefene by studying the experimental pharmacokinetics of the parent drug and its glucuronide in animals and/or man.

4. To complete the ongoing P450 metabolic probe analysis of the drug and to submit the study report within six months of the date of this letter.

5. To examine the methodology of Graham, Shimizu et al. (Brain Research, 1993, 632(1-2): 346-50) and to conduct a study sufficient to determine whether the relative enantiomeric composition of nalmefene may vary in the final drug product. And, if so, to examine the enantiomers for biological activity and pharmacokinetic differences.

Please submit one market package of the drug product when it is available.

Under section 736(a)(1)(B)(ii) of the Prescription Drug User Fee Act of 1992, this letter triggers the remaining 50% of the fee assessed for application. You will receive an invoice for the amount due within the next month. Payment will be due within 30 days of the date of the invoice.

We remind you that you must comply with the requirements for an approved NDA set forth under 21 CFR 314.80 and 314.81.

Sincerely yours,

Pilot Drug Evaluation Staff
Drug Review Team
Office of Drug Evaluation II
Center for Drug Evaluation and Research

Curtis Wright, M.D., M.P.H.
Medical Officer

Pramod Maturu, Ph.D., M.B.A.
Chemist

Ruth Stevens, Ph.D.
Pharmacokineticist

BeLinda A. Hayes, Ph.D.
Pharmacologist

Thomas Permutt, Ph.D.
Statistician

Bonnie McNeal
Consumer Safety Officer

Robert F. Bedford, M.D.
Acting Director

Enclosure: Draft Labeling

Final Version of 3/17/95

APR 17 1995

REVEX™ (nalmefene hydrochloride injection).

DESCRIPTION

REVEX™ (nalmefene hydrochloride injection), an opioid antagonist, is a 6-methylene analogue of naltrexone. The chemical structure is shown below:

(Structure)

Molecular Formula: $C_{21}H_{25}NO_3 \bullet HCl$
Molecular Weight: 375.9, CAS # 58895-64-0
Chemical name: 17-(Cyclopropylmethyl)-4,5a-epoxy-6-methylenemorphinan-3,14-diol, hydrochloride salt.

Nalmefene hydrochloride is a white to off-white crystalline powder which is freely soluble in water up to 130 mg/mL and slightly soluble in chloroform up to 0.13 mg/mL, with a pK_a of 7.6.

REVEX™ is available as a sterile solution for intravenous, intramuscular, and subcutaneous administration in two concentrations, containing 100 µg or 1.0 mg of nalmefene free base per mL. The 100 µg/mL concentration contains 110.8 µg of nalmefene hydrochloride and the 1.0 mg/mL concentration contains 1.108 mg of nalmefene hydrochloride per mL. Both concentrations contain 9.0 mg of sodium chloride per mL and the pH is adjusted to 3.9 with hydrochloric acid.

Concentrations and dosages of REVEX™ are expressed as the free base equivalent of nalmefene.

CLINICAL PHARMACOLOGY

Pharmacodynamics

REVEX™ prevents or reverses the effects of opioids, including respiratory depression, sedation, and hypotension. Pharmacodynamic studies have shown that REVEX™ has a longer duration of action than naloxone at fully reversing doses. REVEX™ has no opioid agonist activity.

REVEX™ is not known to produce respiratory depression, psychotomimetic effects, or pupillary constriction. No pharmacological activity was observed when REVEX™ was administered in the absence of opioid agonists.

REVEX™ has not been shown to produce tolerance, physical dependence, or abuse potential.

REVEX™ can produce acute withdrawal symptoms in individuals who are opioid dependent.

Pharmacokinetics

Nalmefene exhibited dose proportional pharmacokinetics following intravenous administration of 0.5 mg to

2.0 mg. Pharmacokinetic parameters for nalmefene after a
1 mg intravenous administration in adult male volunteers are listed in Table 1.

Table 1: Mean (CV%) Nalmefene Pharmacokinetic Parameters
In Adult Males Following a 1 mg Intravenous Dose

Parameter	Young, n=18	Elderly, n=11
Age	19-32	62-80
C_p at 5 minutes (ng/mL)	3.7 (29)	5.8 (38)
V_{dss} (L/kg)	8.6 (19)	8.6 (29)
V_c (L/kg)	3.9 (29)	2.8 (41)
AUC_{0-inf} (ng-hr/mL)	16.6 (27)	17.3 (14)
Terminal $T_{1/2}$ (hr)	10.8 (48)	9.4 (49)
Cl_{plasma} (L/hr/kg)	0.8 (23)	0.8 (18)

Absorption
Nalmefene was completely bioavailable following intramuscular or subcutaneous administration in 12 male volunteers relative to intravenous nalmefene. The relative bioavailabilities of intramuscular and subcutaneous routes of administration were 101.5%±8.1% (Mean ± SD) and 99.7%±6.9%, respectively. Nalmefene will be administered primarily as an intravenous bolus, however, nalmefene can be given intramuscularly (IM) or subcutaneously (SC) if venous access cannot be established. While the time to maximum plasma nalmefene concentration was 2.3±1.1 hours following intramuscular and 1.5±1.2 hours following subcutaneous administrations, therapeutic plasma concentrations are likely to be reached within 5-15 minutes after a 1 mg dose in an emergency. Because of the variability in the speed of absorption for IM & SC dosing , and the inability to titrate to effect, great care should be taken if repeated doses must be given by these routes.

Distribution
Following a 1 mg parenteral dose, nalmefene was rapidly distributed. In a study of brain receptor occupancy, a 1 mg dose of nalmefene blocked over 80% of brain opioid receptors within 5 minutes after administration. The apparent volumes of distribution centrally (V_c) and at steady-state (V_{dss}) are 3.9 ± 1.1 L/kg and 8.6 ± 1.7 L/kg, respectively. Ultrafiltration studies of nalmefene have demonstrated that 45% (CV 4.1%) is bound to plasma proteins over a concentration range of 0.1 to 2 µg/mL. An *in vitro* determination of the distribution of nalmefene in human blood demonstrated that nalmefene distributed 67% (CV 8.7%) into red blood cells and 39% (CV 6.4%) into plasma. The whole blood to plasma ratio was 1.3 (CV 6.6%) over the nominal concentration range in whole blood from 0.376 to 30 ng/mL.

Metabolism
Nalmefene is metabolized by the liver, primarily by glucuronide conjugation, and excreted in the urine. Nalmefene is also metabolized to trace amounts of an N-dealkylated metabolite. Nalmefene glucuronide is inactive and the N-dealkylated metabolite has minimal pharmacological activity. Less than 5% of nalmefene is excreted in the urine unchanged. Seventeen percent (17%) of the nalmefene dose is excreted in the feces. The plasma concentration-time profile in some subjects suggests that nalmefene undergoes enterohepatic recycling.

Elimination
After intravenous administration of 1 mg REVEX™ to normal males (ages 19-32), plasma concentrations declined biexponentially with a redistribution and a terminal elimination half-life of 41 ± 34 minutes and 10.8 ± 5.2 hours, respectively. The systemic clearance of nalmefene is 0.8 ± 0.2 L/hr/kg and the renal clearance is 0.08 ± 0.04 L/hr/kg.

Special Populations

Elderly
Dose proportionality was observed in nalmefene AUC_{0-inf} following 0.5 to 2 mg intravenous administration to elderly male subjects. Following a 1 mg intravenous nalmefene dose, there were no significant differences between young (19-32 years) and elderly (62-80 years) adult male subjects with respect to plasma clearance, steady-state volume of distribution, or half-life. There was an apparent age-related decrease in the central volume of distribution (young: 3.9 ± 1.1 L/kg, elderly: 2.8 ± 1.1 L/kg) that resulted in a greater initial nalmefene concentration in the elderly group. While initial nalmefene plasma concentrations were transiently higher in the elderly, it would not be anticipated that this population would require dosing adjustment. No clinical adverse events were noted in the elderly following the 1 mg intravenous nalmefene dose.

Patients with Hepatic Impairment
Subjects with hepatic disease, when compared to matched normal controls, had a 28.3% decrease in plasma clearance of nalmefene (0.56 ± 0.21 L/hr/kg versus 0.78 ± 0.24 L/hr/kg, respectively). Elimination half-life increased from 10.2 ± 2.2 hours to 11.9 ± 2.0 hours in the hepatically impaired. No dosage adjustment is recommended since nalmefene will be administered as an acute course of therapy.

Patients with Renal Impairment
There was a statistically significant 27% decrease in plasma clearance of nalmefene in the end-stage renal disease (ESRD) population during interdialysis (0.57 ± 0.20 L/hr/kg) and a 25% decreased plasma clearance in the ESRD population during intradialysis (0.59 ± 0.18 L/hr/kg) compared to normals (0.79 ± 0.24 L/hr/kg). The elimination half-life was prolonged in ESRD patients from 10.2 ± 2.2 hours in normals to 26.1 ± 9.9 hours (see DOSAGE AND ADMINISTRATION).

Gender Differences
There has not been sufficient pharmacokinetic study to make a definitive statement as to whether the pharmacokinetics of nalmefene differs between the genders.

CLINICAL TRIALS

REVEX™ has been administered to reverse the effects of opioids after general anesthesia and in the treatment of overdose. It has also been used to reverse the systemic effects of intrathecal opioids.

Reversal of Postoperative Opioid Depression
REVEX™ (N=326) was studied in 5 controlled trials in patients who had received morphine or fentanyl intraoperatively. The primary efficacy criterion was the reversal of respiratory depression. A positive reversal was defined as both an increase in respiratory rate by 5 breaths per minute and a minimum respiratory rate of 12 breaths per minute. Five minutes after administration, initial single REVEX™ doses of

0.1, 0.25, 0.5, or 1.0 µg/kg had effectively reversed respiratory depression in a dose-dependent manner. Twenty minutes after initial administration, respiratory depression had been effectively reversed in most patients receiving cumulative doses within the recommended range (0.1 to 1.0 µg/kg). Total doses of REVEX™ above 1.0 µg/kg did not increase the therapeutic response. The postoperative administration of REVEX™ at the recommended doses did not prevent the analgesic response to subsequently administered opioids.

Reversal Of The Effect Of Intrathecally Administered Opioids

Intravenous REVEX™ at doses of 0.5 and 1.0 µg/kg was administered to 47 patients given intrathecal morphine. One to 2 doses of 0.5 and 1.0 µg/kg REVEX™ reversed respiratory depression in most patients. The administration of REVEX™ at the recommended doses did not prevent the analgesic response to subsequently administered opioids.

Management Of Known Or Suspected Opioid Overdose

REVEX™ (N=284) at doses of 0.5 mg to 2.0 mg was studied in 4 trials of patients who were presumed to have taken an opioid overdose. REVEX™ doses of 0.5 mg to 1.0 mg effectively reversed respiratory depression within 2 to 5 minutes in most patients subsequently confirmed to have opioid overdose. A total dose greater than 1.5 mg did not increase the therapeutic response.

INDICATIONS AND USAGE

REVEX™ is indicated for the complete or partial reversal of opioid drug effects, including respiratory depression, induced by either natural or synthetic opioids.

REVEX™ is indicated in the management of known or suspected opioid overdose.

CONTRAINDICATIONS

REVEX™ is contraindicated in patients with a known hypersensitivity to the product.

WARNINGS

Use of REVEX™ in Emergencies

REVEX™, like all drugs in this class, is not the primary treatment for ventilatory failure. In most emergency settings, treatment with REVEX™ should follow, not precede, the establishment of a patent airway, ventilatory assistance, administration of oxygen, and establishment of circulatory access.

Risk of Recurrent Respiratory Depression

Accidental overdose with long acting opioids [such as methadone and *levo*-alpha- acetylmethadol (LAAM)] may result in prolonged respiratory depression. Respiratory depression in both the post-operative and overdose setting may be complex and involve the effects of anesthetic agents, neuromuscular blockers, and other drugs. While REVEX™ has a longer duration of action than naloxone in fully reversing doses, the physician should be aware that a recurrence of respiratory depression is possible, even after an apparently adequate initial response to REVEX™ treatment.

Patients treated with REVEX™ should be observed until, in the opinion of the physician, there is no reasonable risk of recurrent respiratory depression.

PRECAUTIONS

General

Cardiovascular Risks with Narcotic Antagonists
Pulmonary edema, cardiovascular instability, hypotension, hypertension, ventricular tachycardia, and ventricular fibrillation have been reported in connection with opioid reversal in both post-operative and emergency department settings. In many cases, these effects appear to be the result of abrupt reversal of opioid effects.

Although REVEX™ has been used safely in patients with pre-existing cardiac disease, all drugs of this class should be used with caution in patients at high cardiovascular risk or who have received potentially cardiotoxic drugs. (See DOSAGE AND ADMINISTRATION.)

Risk Of Precipitated Withdrawal
REVEX™, like other opioid antagonists, is known to produce acute withdrawal symptoms and, therefore, should be used with extreme caution in patients with known physical dependence on opioids or following surgery involving high doses of opioids. Imprudent use or excessive doses of opioid antagonists in the postoperative setting has been associated with hypertension, tachycardia, and excessive mortality in patients at high risk for cardiovascular complications. (See PRECAUTIONS.)

Incomplete Reversal Of Buprenorphine
Preclinical studies have shown that nalmefene at doses up to 10 mg/kg (437 times the maximum recommended human dose) produced incomplete reversal of buprenorphine-induced analgesia in animal models. This appears to be a consequence of a high affinity and slow displacement of buprenorphine from the opioid receptors. Hence, REVEX™ may not completely reverse buprenorphine-induced respiratory depression.

Drug Interactions
REVEX™ has been administered after benzodiazepines, inhalational anesthetics, muscle relaxants, and muscle relaxant antagonists administered in conjunction with general anesthesia. It also has been administered in outpatient settings, both in trials in conscious sedation and in the emergency management of overdose following a wide variety of agents. No deleterious interactions have been observed.

Preclinical studies have shown that both flumazenil and nalmefene can induce seizures in animals. The coadministration of both flumazenil and nalmefene produced fewer seizures than expected in a study in rodents, based on the expected effects of each drug alone. Based on these data, an adverse interaction from the coadministration of the two drugs is not expected, but physicians should remain aware of the potential risk of seizures from agents in these classes.

Carcinogenesis, Mutagenesis, Impairment of Fertility
Nalmefene did not have mutagenic activity in the Ames test with five bacterial strains or the mouse lymphoma assay. Clastogenic activity was not observed in the mouse micronucleus test or in the cytogenic

bone marrow assay in rats. However, nalmefene did exhibit a weak but significant clastogenic activity in the human lymphocyte metaphase assay in the absence but not in the presence of exogenous metabolic activation. Oral administration of nalmefene up to 1200 mg/m^2/day did not affect fertility, reproductive performance, and offspring survival in rats.

Use in Pregnancy

PREGNANCY CATEGORY B

Reproduction studies have been performed in rats (up to 1200 mg/m^2/day) and rabbits (up to 2400 mg/m^2/day) by oral administration of nalmefene and in rabbits by intravenous administration up to 96 mg/m^2/day (114 times the human dose). There was no evidence of impaired fertility or harm to the fetus. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers

Nalmefene and its metabolites were secreted into rat milk, reaching concentrations approximately three times those in plasma at one hour and decreasing to about half the corresponding plasma concentrations by 24 hours following bolus administration. As no clinical information is available, caution should be exercised when REVEX™ is administered to a nursing woman.

Use in Children

Safety and effectiveness of REVEX™ in children have not been established.

Use in Neonates

The safety and effectiveness of REVEX™ in neonates have not been established in clinical studies. In a preclinical study, nalmefene was administered by subcutaneous injection to rat pups at doses up to 205 mg/m^2/day throughout maternal lactation without producing adverse effects. A preclinical study evaluating the irritancy of the dosage form following arterial and venous administration in animals showed no vascular irritancy.

REVEX™ should only be used in the resuscitation of the newborn when, in the opinion of the treating physician, the expected benefits outweigh the risks.

ADVERSE REACTIONS

Adverse event information was obtained following administration of REVEX™ to 152 normal volunteers and in controlled clinical trials to 1127 patients for the treatment of opioid overdose or for postoperative opioid reversal.

Nalmefene was well tolerated and showed no serious toxicity during experimental administration to healthy individuals, even when given at 15 times the highest recommended dose. In a small number of subjects, at doses exceeding the recommended REVEX™ dose, nalmefene produced symptoms suggestive of reversal of endogenous opioids, such as have been reported for other narcotic antagonist drugs. These symptoms (nausea, chills, myalgia, dysphoria, abdominal cramps, and joint pain) were usually transient and occurred at

very low frequency.

Such symptoms of precipitated opioid withdrawal at the recommended clinical doses were seen in both postoperative and overdose patients who were later found to have had histories of covert opioid use. Symptoms of precipitated withdrawal were similar to those seen with other opioid antagonists, were transient following the lower doses used in the postoperative setting, and more prolonged following the administration of the larger doses used in the treatment of overdose.

Tachycardia and nausea following the use of nalmefene in the postoperative setting were reported at the same frequencies as for naloxone at equivalent doses. The risk of both these adverse events was low at doses giving partial opioid reversal and increased with increases in dose. Thus, total doses larger than 1.0 µg/kg in the postoperative setting and 1.5 mg/70 kg in the treatment of overdose are not recommended.

Relative Frequencies of Common Adverse Reactions
with an incidence greater than 1%. (all patients, all clinical settings)

Adverse Event	Nalmefene N=1127	Naloxone N=369	Placebo N=77
Nausea	18%	18%	6%
Vomiting	9%	7%	4%
Tachycardia	5%	8%	-
Hypertension	5%	7%	-
Postoperative pain	4%	4%	N/A
Fever	3%	4%	-
Dizziness	3%	4%	1%
Headache	1%	1%	4%
Chills	1%	1%	-
Hypotension	1%	1%	-
Vasodilatation	1%	1%	-

Incidence less than 1%

Cardiovascular: Bradycardia, arrhythmia
Digestive: Diarrhea, Dry mouth
Nervous System: Somnolence, depression, agitation, nervousness, tremor, confusion, withdrawal syndrome, myoclonus
Respiratory: Pharyngitis
Skin: Pruritus
Urogenital: Urinary retention

The incidence of adverse events was highest in patients who received more than the recommended dose of REVEX™.

Laboratory findings: Transient increases in CPK were reported as adverse events in 0.5% of the postoperative patients studied. These increases were believed to be related to surgery and not believed to be related to the administration of REVEX™. Increases in AST were reported as adverse events in 0.3% of the

patients receiving either nalmefene or naloxone. The clinical significance of this finding is unknown. No cases of hepatitis or hepatic injury due to either nalmefene or naloxone were observed in the clinical trials.

DRUG ABUSE AND DEPENDENCE

REVEX™ is an opioid antagonist with no agonist activity. It has no demonstrated abuse potential, is not addictive, and is not a controlled substance.

OVERDOSAGE

Intravenous doses of up to 24 mg of nalmefene, administered to healthy volunteers in the absence of opioid agonists, produced no serious adverse reactions, severe signs or symptoms, or clinically significant laboratory abnormalities. As with all opioid antagonists, use in patients physically dependent on opioids can result in precipitated withdrawal reactions that may result in symptoms that require medical attention. Treatment of such cases should be symptomatic and supportive. Administration of large amounts of opioids to patients receiving opioid antagonists in an attempt to overcome a full blockade has resulted in adverse respiratory and circulatory reactions.

DOSAGE AND ADMINISTRATION

IMPORTANT INFORMATION - DOSAGE FORMS

REVEX™ is supplied in two concentrations which are packaged in ampules of different appearance: an ampul with a blue label containing ONE (1) mL at a concentration suitable for postoperative use (100 µg/mL) and an ampul with a green label containing TWO (2) mL suitable for the management of overdose (1 mg/mL, 10 times as concentrated, 20 times as much drug). Proper steps should be taken to prevent use of the incorrect dosage form.

General Principles

REVEX™ should be titrated to reverse the undesired effects of opioids. Once adequate reversal has been established, additional administration is not required and may actually be harmful due to unwanted reversal of analgesia or precipitated withdrawal.

Duration of Action

The duration of action of REVEX™ is as long as most opioid analgesics. The apparent duration of action of REVEX will vary, however, depending on the half life and plasma concentration of the narcotic being reversed, the presence or absence of other drugs affecting the brain or muscles of respiration, and the dose of REVEX administered. Partially reversing doses of REVEX™ (1 µg/kg) lose their effect as the drug is redistributed through the body, and the effects of these low doses may not last more than 30-60 minutes in the presence of persistent opioid effects. Fully reversing doses (1 mg/70 kg) have been shown to last many hours in both experimental and clinical studies, but may complicate the management of patients who are in pain, at high cardiovascular risk, or who are physically dependent on opioids.

The recommended doses represent a compromise between a desirable controlled reversal and the need for prompt response and adequate duration of action. Using higher dosages or shorter intervals between incremental doses is likely to increase the incidence and severity of symptoms related to acute withdrawal

such as nausea, vomiting, elevated blood pressure, and anxiety.

Patients Tolerant To Or Physically Dependent On Opioids

REVEX™ may cause acute withdrawal symptoms in individuals who have some degree of tolerance to and dependence on opioids. These patients should be closely observed for symptoms of withdrawal following administration of the initial and subsequent injections of REVEX™. Subsequent doses should be administered with intervals of at least 2-5 minutes between doses to allow the full effect of each incremental dose of REVEX™ to be reached.

Recommended Doses for Reversal of Postoperative Opioid Depression:

Use 100µg/mL dosage strength (blue label) and see Table 2 for initial doses.

The goal of treatment with REVEX™ in the postoperative setting is to achieve reversal of excessive opioid effects without inducing a complete reversal and acute pain. This is best accomplished with an initial dose of 0.25 µg/kg followed by 0.25 µg/kg incremental doses at 2-5 minute intervals, stopping as soon as the desired degree of opioid reversal is obtained. A cumulative total dose above 1.0 µg/kg does not provide additional therapeutic effect.

Table 2: Reversal of Post Operative Opioid Depression

Body Weight	mL of REVEX™ 100µg/mL Solution
50 kg	0.125
60 kg	0.150
70 kg	0.175
80 kg	0.200
90 kg	0.225
100 kg	0.250

In cases where the patient is known to be at increased cardiovascular risk, it may be desirable to dilute REVEX™ 1:1 with saline or sterile water and use smaller initial and incremental doses of 0.1µg/kg.

Management of Known or Suspected Opioid Overdose

Use 1.0 mg/mL dosage strength (green label).

The recommended initial dose of REVEX™ for non-opioid dependent patients is 0.5 mg/70 kg. If needed, this may be followed by a second dose of 1.0 mg/70 kg, 2-5 minutes later. If a total dose of 1.5 mg /70 kg has been administered without clinical response, additional REVEX™ is unlikely to have an effect. Patients should not be given more REVEX™ than is required to restore the respiratory rate to normal, thus minimizing the likelihood of cardiovascular stress and precipitated withdrawal syndrome.

If there is a reasonable suspicion of opioid dependency, a challenge dose of REVEX™ 0.1 mg/70 kg should be administered initially. If there is no evidence of withdrawal in 2 minutes, the recommended dosing should be followed.

REVEX™ had no effect in cases where opioids were not responsible for sedation and hypoventilation. Therefore, patients should only be treated with REVEX™ when the likelihood of an opioid overdose is high, based on a history of opioid overdose or the clinical presentation of respiratory depression with concurrent pupillary constriction.

Repeated Dosing

REVEX™ is the longest acting of the currently available parenteral opioid antagonists. If recurrence of respiratory depression does occur, the dose should again be titrated to clinical effect using incremental doses to avoid over-reversal.

Hepatic and Renal disease.

Hepatic disease and renal failure substantially reduce the clearance of nalmefene (see PHARMACOKINETICS). For single episodes of opioid antagonism, adjustment of REVEX™ dosage is not required. However, in patients with renal failure, the incremental doses should be delivered slowly (over 60 seconds) to minimize the hypertension and dizziness reported following the abrupt administration of nalmefene to such patients.

Loss of Intravenous Access

Should intravenous access be lost or not readily obtainable, a pharmacokinetic study has shown that a single dose of REVEX™ should be effective within 5-15 minutes after intramuscular or subcutaneous doses of 1.0 mg (see PHARMACOKINETICS).

SAFETY AND HANDLING

REVEX™ is distributed in sealed ampuls and represents no known risk to health care workers. As with all parenterals, care should be taken to prevent the generation and inhalation of aerosols during preparation and use. Dermal absorption of spilled REVEX™ should be prevented by prompt removal of contaminated clothing and rinsing the skin thoroughly with cool water.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.

HOW SUPPLIED

REVEX™ (nalmefene hydrochloride injection) is available in the following presentations:

An ampul containing 1 mL of 100 µg/mL nalmefene base (Blue Label)
(NDC 10019-315-21)

An ampul containing 2 mL of 1 mg/mL nalmefene base (Green Label)
(NDC 10019-311-22)

Store at controlled room temperature.
Manufactured for
Ohmeda Pharmaceutical Products Division Inc.,
Liberty Corner, NJ 07938

Manufactured by:
Akorn Manufacturing Co.,
Decatur, IL 62525

U.S. Pats 4,535,157 and 3,896,226

(FDA note: Cleared all disciplines 3/12/95)
(Cleared office 4/17/95)

OHMEDA
THE BOC GROUP

Pharmaceutical Products Division

Ohmeda Inc
110 Allen Road PO Box 804
Liberty Corner NJ 07938 0804
908 647 9200

March 13, 1995

Food and Drug Administration
Center for Drug Evaluation & Research
Office of Drug Evaluation II (HFD-007)
Document Control Room 9B-23
5600 Fishers Lane
Rockville MD 20857

Attention: Robert Bedford, MD - Acting Director
Pilot Drug Evaluation Staff

re: Revex™ - (nalmefene hydrochloride) Injection
NDA 20-459

Dear Dr. Bedford:

Reference is made to the Final Agency Label Draft of 3/10/95 for Revex™ (nalmefene hydrochloride) Injection.

As discussed with Dr. Curtis Wright in a teleconference this morning (March 3, 1995), we accept the labeling with the inclusion of the three following minor changes:

Line 77	Change with to within (editorial)
Line 303	Change 350 to 349 (as per FDA request)
Line 444	Change µg to µg/mL (for technical correctness)

We have made these changes to the Ohmeda Label Draft of 3/13/95 (Version 16) which is enclosed as Attachment 1.

Regarding the Phase IV Recommendations:

As per discussions with Dr. Curtis Wright, we agree to examine the role of entero-hepatic recirculation in the duration of action of nalmefene (Recommendation #3).

We have previously agreed to Recommendations 1, 2, 4 and 5 in our correspondence dated March 9, 1995; however, for completeness, all five Phase IV recommendations and our responses are restated as follows:

Recommendation #1 - Adequate study, by means of experiment, observational study, or case registries, to establish if nalmefene may be safely used in the newborn.

We agree.

Recommendation #2: - To conduct observational or experimental studies in pediatric populations looking at the drug's kinetics and the safety of its use in pediatric populations.

Protocol 20, "An Open-Label Pharmacokinetic Study of Nalmefene Use in Prophylaxis of Untoward Side Effects Induced by Epidural Fentanyl in the Post-Operative, Pediatric Patient", which would address this recommendation is currently ongoing; however, we believe further protocol discussions with the Agency may be necessary as we have so far been unsuccessful in enrolling patients.

Recommendation #3: - To establish the role of entero-hepatic recirculation in the duration of action of nalmefene by study of the experimental pharmacokinetics of the parent drug and its glucuronide in animals and/or man.

We agree to examine the role of entero-hepatic recirculation in the duration of action of nalmefene. Further discussion with the agency may be necessary during development of a protocol

Recommendation #4: - Complete the P450 metabolic probe analysis and report the results by six months after approval.

We agree.

Recommendation #5: - Examine the methodology of Graham, Shimizu et al (Brain Research, 1993, 632 (1-2): 346-50) and conduct sufficient study to determine if the relative entiomeric composition of nalmefene may vary in the final drug product. If so, then examine the enantiomers for biological activity and pharmacokinetic differences.

We agree to examine the above-referenced methodology.

With this submission, we have addressed all outstanding issues and look forward to recieivng an approval letter in the near future.

If you have any questions concerning this submission, please call Ms Ingrid E. Smith, Director - Domestic Regulatory Affairs, at 908/604-7701.

Sincerely yours,

Ingrid E Smith for

Robert I. Outwater
Senior Director - Worldwide Regulatory Affairs

enc: Form FDA 356h
Ohmeda Label Draft of 3/13/95 (Version 16)

OHMEDA
A BOC GROUP

Pharmaceutical Products Division

Ohmeda Inc
110 Allen Road PO Box 804
Liberty Corner NJ 07938 0804
908 647 9200

March 9, 1995

Food and Drug Administration
Center for Drug Evaluation & Research
Office of Drug Evaluation II (HFD-007)
Document Control Room 9B-23
5600 Fishers Lane
Rockville MD 20857

Attention: Robert Bedford, MD - Acting Director
Pilot Drug Evaluation Staff

re: Revex™ - (nalmefene hydrochloride) Injection
NDA 20-459

Dear Dr. Bedford:

Reference is made to the Final Agency Label Draft of 2/27/95 for Revex™ (nalmefene hydrochloride) Injection.

We have reviewed the labeling and have made revisions as noted. Most revisions were minor. However, we have made the following major changes:

1. The **"DOSAGE & ADMINISTRATION"** section has been revised to be more in line with the dosing actually carried out in our clinical trials and for which we have justification.

2. The labeling of the 2 different ampuls has been color-coded and as such, we believe, a warning regarding their possible mix-up is no longer warranted.

3. The sections **"Cardiovascular Risks with Narcotic Antagonists"**, **"Risk of Recurrent Respiratory Depression"**, and **"Use of Revex™ in Emergencies"** have been moved to **"PRECAUTIONS"** as we believe the statements relate to special care to be exercised by the practitioner rather than to evidence of an association of a serious hazard with Revex™.

Dr Robert Bedford, HFD-007
March 9, 1995

4. Most of the wording in "Use of Revex™ in Emergencies" had also been included in the general "PRECAUTIONS" section. As such, the heading was moved to that section.

5. We have retained the message that patients should be observed until, in the opinion of the physician, there is no reasonable risk of recurrent respiratory depression; however, we do not believe the drug requires that this care be translated into a bolded warning.

6. The "HOW SUPPLIED" section has been revised to clarify the contents of the ampuls and the new color-coding of the 2 concentrations.

In support, the following attachments are provided:

- Attachment 3: Document identifying the various changes and providing a rationale.

Dr Robert Bedford, HFD-007
March 9, 1995

We also make reference to the Memorandum dated 3/x/95 by Dr Robert Bedford, and have the following comments/suggestions:

1. On page 1, last paragraph, line 1, may we suggest that the sentence be revised to read "Nalmefene is closely related to naltrexone, substituting a methylene group for the keto group."

2. On page 2, last paragraph, last sentence of the "Efficacy Summary" section, we would very much appreciate a clarification of the statement: "Nalmefene solution was not irritating in the animal model, was tolerated by rat pups, and showed a longer duration of effect than naloxone in dogs, although the doses of both opioid and reversal agent had a much greater influence on the duration of respiratory depression than which reversal agent was used."

3. On page 3, last line of the discussion entitled "Safety", please revise the therapeutic index ratio of 100:1 to 24:1.

Regarding the Phase IV Recommendations:

Recommendation #1 - Adequate study, by means of experiment, observational study, or case registries, to establish if nalmefene may be safely used in the newborn.

We agree.

Recommendation #2: - To conduct observational or experimental studies in pediatric populations looking at the drug's kinetics and the safety of its use in pediatric populations.

Protocol 20, "An Open-Label Pharmokinetic Study of Nalmefene Use in Prophylaxis of Untoward Side Effects Induced by Epidural Fentanyl in the Post-Operative, Pediatric Patient", which would address this recommendation is currently ongoing; however, we believe further protocol discussions with the Agency may be necessary as we have so far been unsuccessful in enrolling patients.

Recommendation #3: - To establish the role of entero-hepatic recirculation in the duration of action of nalmefene by study of the experimental pharmacokinetics of the parent drug and its glucuronide in animals and/or man.

Dr Robert Bedford, HFD-007
March 9, 1995

We believe this recommendation requires further clarification and discussion regarding rationale, parameters to be measured, and the ultimate impact on dosing.

Recommendation #4: - Complete the P450 metabolic probe analysis and report the results by six months after approval.

We agree.

Recommendation #5: - Examine the methodology of Graham, Shimizu et al (Brain Research, 1993, 632 (1-2): 346-50) and conduct sufficient study to determine if the relative antiomeric composition of nalmefene may vary in the final drug product. If so, then examine the enantiomers for biological activity and pharmacokinetic differences.

We agree to examine the above-referenced methodology.

We look forward to hearing from you and working with you in finalizing the Revex™ labeling. If you have any questions concerning this submission, please call Ms Ingrid E. Smith, Director - Domestic Regulatory Affairs, at 908/604-7701.

Sincerely yours,

Ingrid E Smith for

Robert I. Outwater
Senior Director - Worldwide Regulatory Affairs

enc: Form FDA 356h
Attachments 1 - 8

Attachm 3

MAR 09 '95 03:08PM

REVEX™ (nalmefene hydrochloride)Injection
NDA 20-459
Revised Package Insert Rationale

Line #	Change From	Change To	Rationale
Global	ug/kg, mg/ml , Kg and REVEX	µg/kg, mg/mL, kg and REVEX™	
12	Molecular Formula: C21H25NO3•HCl	Molecular Formula: $C_{21}H_{25}NO_3$•HCl	Incorporate sub and super scripts
14	17-(Cyclopropylmethyl)-4,5 alpha epoxy-6-methylenemorphinan-3,14-diol, hydrochloride salt	17-(Cyclopropylmethyl)-4,5α-epoxy-6-methylenemorphinan-3,14-diol hydrochloride salt.	Chemical name as stated in USAN
33		Pharmacodynamics	Added subheading for clarification
35		Pharmacodynamic studies have shown that REVEX™ has a longer duration of action than naloxone at fully reversing doses	Added statement regarding duration of action since information is based on results of Pharmacodynamic studies (same statement as proposed by FDA on lines 203-204).
45	REVEX will produce acute withdrawal symptoms in individuals who are opioid dependent	REVEX™ can produce acute withdrawal symptoms in individuals who are opioid dependent.	Changed "will" to "can". REVEX™ can produce these symptoms if given in high doses, but if the recommended dosing is followed, REVEX™ will probably not produce them (symptoms)

Line #	Change From	Change To	Rationale
48-54	Nalmefene is metabolized to nalmefene glucuronide and to trace amounts of a dealkylated metabolite, nornalmefene. Nornalmefene has been shown to have antinociceptive activity in animals following intra-thecal administration and to be active in the guinea pig ileum model. Nalmefene glucuronide is inactive, although there is some evidence that it may be subject to enterohepatic recirculation and consequent reabsorption as nalmefene from the colon.	DELETED from this section	The human information was incorporated into the METABOLISM section and reworded for clarity. The nornalmefene animal data was modified to reflect metabolite activity after systemic administration.
59	..during acute care procedures	DELETED	Not all operations are acute care
67-69	The time to maximum plasma nalmefene concentration was 2.3±1.1 hours following intramuscular and 1.5±1.2 hours following subcutaneous administrations.	While the time to maximum plasma nalmefene concentration was 2.3±1.1 hours following intramuscular and 1.5±1.2 hours following subcutaneous administrations, therapeutic plasma concentrations were reached in 5 to 15 minutes.	Added phrase to indicate time when plasma levels were ≥ 0.5 ng/mL the level associated with measurable activity in pharmacodynamic and efficacy studies (13, JF110).
69-78	Nalmefene exhibited dose proportional pharmacokinetics from 0.5 mg to 2.0 mg following intravenous administration. The following pharmacokinetic parameters for nalmefene after a 1 mg intravenous administration in 18 male volunteers were derived and are listed in Table 1.	Nalmefene exhibited dose proportional pharmacokinetics following intravenous administration of 0.5 mg to 2.0 mg. Pharmacokinetic parameters for nalmefene after a 1 mg intravenous administration in adult male volunteers are listed in Table 1.	Moved statement and corresponding table from Absorption to Pharmacokinetics because of IV administration. Also clarified status of volunteers. Minor rewording.
75-78		Information on the elderly was incorporated into the table.	As requested by FDA
77	C_{max}	C_p	Clarification
85	3.85, 8.5	3.9, 8.6	Numbers rounded to nearest tenth.
87-88	concentration range of 0.1 to 2 mg/mL	concentration range of 0.1 to 2 µg/mL	Corrected mg to µg

Line #	Change From	Change To	Rationale
88	An *in vitro* determination of the distribution of nalmefene in red blood cells...	An *in vitro* evaluation of the distribution of nalmefene in human red blood cells...	Added "human" for clarification.
91-92		The whole blood to plasma ratio was 1.3(CV 6.6%).	This information was requested by FDA.
98-100	Nalmefene is metabolized by the liver, primarily by glucuronide conjugation, and excreted in the urine. Less than 5% of nalmefene is excreted in the urine unchanged. Trace amounts of N-dealkylated nalmefene have been detected in the urine. Seventeen percent (17%) of the nalmefene dose is excreted in the feces	Nalmefene is metabolized by the liver, primarily by glucuronide conjugation, and excreted in the urine. Nalmefene is also metabolized to trace amounts of an N-dealkylated metabolite. Nalmefene glucuronide is inactive and the N-dealkylated metabolite has minimal pharmacological activity. Less than 5% of nalmefene is excreted in the urine unchanged. Seventeen percent (17%) of the nalmefene dose is excreted in the feces. The plasma concentration-time profile in some subjects suggests that nalmefene undergoes enterohepatic recycling.	Reworded to include information from FDA lines 48-54 and for clarification
103	nalmefene	REVEX™	Editorial
103		to normal males (ages 19-32)	Added for clarification
106	0.79 ± 0.24 L/hr/Kg	0.8 ± 0.2 L/hr/kg	Rounded numbers to nearest tenth
107	0.078 ± 0.042 L/hr/kg	0.08 ± 0.04	Rounded numbers to nearest hundredth
111-112	Dose proportionality was observed in AUC_{0-Inf} from 0.5 to 2 mg iv nalmefene in elderly male subjects (65-80 years)	Dose proportionality was observed in nalmefene AUC_{0-Inf} following 0.5 to 2 mg intravenous administration to elderly male subjects (62-80 years).	Corrected age of elderly patients and reworded for clarity
113	iv	intravenous	Editorial
113	meaningful	significant	Requested by FDA (Dr. Stevens)
113-114	adult and elderly male	young and elderly adult male	Reworded for clarification

Line #	Change From:	Change To:	Rationale
114	...to clearance...	...to plasma clearance...	Added "plasma" for clarification
116	adult	young	Changed for consistency
123	Hepatic	Patients with Hepatic Impairment	Clarified heading
123	liver	hepatic	Changed liver to hepatic for consistency
130	Renal	Patients with Renal Impairment	Changed for clarification
134	The half-life...	The elimination half-life...	Added "elimination" for clarification
171	(per 70kg)	DELETED	Deleted to state actual conditions of use.
172	Five minutes after administration,...	Two to five minutes after administration, . .	Nalmefene Study 201 contains data demonstrating 2 minute onset of effective reversal of respiratory depression. NDA Volume 44
194-199	<u>Use of Revex in Emergencies</u> REVEX, like all drugs in this class, is not the primary treatment for ventilatory failure. In most emergency settings, treatment with REVEX should follow, not precede, the establishment of a patent airway, ventilatory assistance, administration of oxygen, and establishment of circulatory access.		Statement moved from Warnings to Precautions.. This is a general precaution outlining special care to be exercised by the practitioner in a given clinical setting and not a specific safety concern of REVEX™ treatment.

Line #	Change From	Change To	Rationale
201-219	Risk of Recurrent Respiratory Depression Cases of accidental or intentional overdose may involve long acting opioids such as methadone or LAAM. While REVEX has a longer duration of action than naloxone in fully reversing doses, it should not be relied upon to maintain reversal after administration of long acting opioids or in cases of mixed drug or drug and alcohol overdose. Respiratory depression in the postoperative setting is complex and may involve the interactions of opioids with anesthetic agents and neuromuscular blocking drugs not affected by the use of REVEX. Physicians should be aware that a recurrence of respiratory depression is possible, even after an apparently adequate initial response to treatment with REVEX. PATIENTS TREATED WITH REVEX MUST NOT BE RELEASED FROM OBSERVATION UNTIL, IN THE OPINION OF THE RESPONSIBLE TREATING PHYSICIAN, THERE IS NO REASONABLE RISK OF RECURRENCE OF RESPIRATORY DEPRESSION.	**Risk of Recurrent Respiratory Depression** Accidental overdose with long acting opioids [such as methadone and *levo*-alpha- acetylmethadol (LAAM)] may result in prolonged respiratory depression. Respiratory depression in both the post-operative and overdose setting may be complex and involve the effects of anesthetic agents, neuromuscular blockers and other drugs. While REVEX™ has a longer duration of action than naloxone in fully reversing doses, the physician should be aware that a recurrence of respiratory depression is possible, even after an apparently adequate initial response to REVEX™ treatment. In such cases, patients treated with REVEX™ should be observed until, in the opinion of the physician, there is no reasonable risk of recurrent respiratory depression.	Section moved from Warnings to Precautions because no renarcotization was observed in the 106 opioid positive overdose patients in the NDA (04A 1.1 05,08,201) indicating that renarcotization is uncommon. In Study 13, reversal of balanced anesthesia with morphine, there were no instances of serious respiratory depression (RR <8). Additional nalmefene was administered in some cases and some patients were given Narcan without improvement indicating that their conditions was not due to opioid, but to other factors. Appropriate post-operative observation should be a standard precaution and not a warning specific to Nalmefene.

Line #	Change From	Change To	Rationale
221-235	<u>Cardiovascular Risks with Narcotic Antagonists</u> Cardiovascular instability, hypotension, hypertension, ventricular tachycardia, ventricular fibrillation and pulmonary edema have been reported in connection with opioid reversal in both post-operative and emergency department settings. In many cases, these effects appear to be the result of abrupt reversal of opioid effects, resulting in a combination of precipitated withdrawal and/or the abrupt return of pain with consequent tachycardia, increased circulating catecholamines, and cardiovascular stress. Although REVEX has been used safely in patients with pre-existing cardiac disease, all drugs of this class should be used with caution in patients at high cardiovascular risk or who have received potentially cardiotoxic drugs.	**Cardiovascular Risks with Narcotic Antagonists** Pulmonary edema, cardiovascular instability, hypotension, hypertension, ventricular tachycardia and ventricular fibrillation have been reported in connection with opioid reversal in both post-operative and emergency department settings. In many cases, these effects appear to be the result of abrupt reversal of opioid effects. Although REVEX™ has been used safely in patients with pre-existing cardiac disease, all drugs of this class should be used with caution in patients at high cardiovascular risk or who have received potentially cardiotoxic drugs. (See DOSAGE AND ADMINISTRATION)	This section was moved to Precautions. If the recommended dosing regimen is followed, the cardiovascular risks will be minimized. "See DOSAGE AND ADMINISTRATION" has been added to direct the practitioner to the correct dosing regimen for cardiac risk patients. Pulmonary edema was moved from last to first in the listing of cardiovascular events in recognition of FDA concerns. "Results of abrupt withdrawal"has been deleted. We do not have data to support the mechanism and therefore prefer to leave it out In addition, the new color coded concentrations should avoid the risk of confusion and should lessen the possibility of precipitous reversal of both respiratory depression and analgesia in a high-risk cardiac patient
238-239	10 mg/kg (10 times the human dose)	10 mg/kg (437 times the maximum recommended human dose)	"10 times" was changed to "437 times" - correction as maximum human dose is 1.6 mg/70kg
239	ineffective in reversing buprenorphine	incomplete reversal of buprenorphine	"Ineffective" changed to "incomplete" as we saw partial reversal in these studies.

Line #	Change From	Change To	Rationale
242	Revex™ may be ineffective in reversing buprenorphine-induced respiratory depression.	REVEX™ may not completely reverse buprenorphine-induced respiratory depression.	"Ineffective" changed to "not completely reverse" as we saw partial reversal in these studies.
253	...as described above.	(see PRECAUTIONS)	Deleted...as describe above and replaced with (see PRECATIONS) as the cardiovascular statement has been moved to precautions
255-260	Pulmonary Edema A few cases of abrupt-onset pulmonary edema in previously healthy individuals have been reported following the administration of drugs of this class. No treatment has been uniformly effective, but at least one report has suggested intravenous phentolamine may be useful in managing this rare adverse event.	DELETED	Please see Attachment 5 for rationale
265-269	Successful use of REVEX in the management of overdose has depended on the concurrent use of proper resuscitative measures such as; maintenance of a patent airway, artificial ventilation, administration of oxygen, cardiac massage, and vasopressor agents, all used as necessary to counteract acute opioid overdose.	DELETED	Deleted, as this statement is almost identical to statement under "Use of REVEX™ in Emergencies" which is now in PRECAUTIONS.
289	...four bacterial strains	...five bacterial strains	Correction in number of bacterial strains
303	8000 times the human dose	DELETED	This calculation can not be made as it is a comparison between oral and intravenous doses.
305	The results did not reveal evidence...	There was no evidence...	Editorial

Line #	Change From	Change To	Rationale
316	Caution should be exercised...	As no clinical information is available, caution should be exercised ...	Phrase added for clarification
329	Rre-clinical study of the irritancy of the dosage form in both arterial and venous administration in animals showed no vascular irritancy	A preclinical study evaluating the irritancy of the dosage form following arterial and venous administration in animals showed no vascular irritancy.	Editorial
333	over	DELETE	Editorial
342-344	Nalmefene was well tolerated in experimental administration to healthy individuals, showing no serious toxicity, even when given at 24 times the recommended dose	Nalmefene was well tolerated and showed no serious toxicity during experimental administration to healthy, even when given at 15 times the highest recommended dose	Reworded for clarification and changed 24 times to 15 times based on a dose of 1.6 mg/kg
350	usual	recommended	Editorial
361	...increased with frequency with increases in dose.	...increased with increases in dose.	Editorial
362-364	It is for this reason that single doses larger than 1.0 mcg/kg in the postoperative setting and 1.0 mg/70 kg in the treatment of overdose are not recommended.	Thus, total doses larger than 1.0 µg/kg in the postoperative setting and 1.6 mg/70 kg in the treatment of overdose are not recommended.	Editorial; change in numbers to reflect revised dosing in DOSAGE AND ADMINISTRATION.
391	diarrhea	Diarrhea	Editorial
392	agitation	Agitation	Editorial
397	bradycardia	Bradycardia	Editorial
398	dry mouth	Dry mouth	Editorial
399	somnolence	Somnolence	Editorial
400	pharyngitis	Pharyngitis	Editorial
401	pruritus	Pruritus	Editorial
402	urinary	Urinary	Editorial
411	naloxone or nalmefene	nalmefene or naloxone	Reversed order
418	REVEX is a pure opioid antagonist drug.	REVEX™ is an opioid antagonist with no agonist activity.	Agrees with wording in DESCRIPTION and Pharmacodynamic sections.
419	in animals and man	DELETED	Extraneous
424	REVEX	nalmefene	Clarification

Line #	Change From	Change To	Rationale
428	will	can	Changed will to can. REVEX™ will not necessarily precipitate withdrawal if given in small doses.
430-431	but should not include administration of additional opioids to try to overcome the blockade.	DELETED	We feel this is redundant as the risk is sufficiently addressed in the following sentence.
444-458	WARNING - POSSIBLE CONFUSION OF DOSAGE FORMS REVEX is supplied in two distinct concentrations, an ampul containing ONE (1) mL at a concentration suitable for postoperative use (100 µg/mL) and an ampule containing TWO (2) mLs suitable for the management of overdose (1000 µg/mL, TEN times as concentrated). These dosage forms have been chosen so that either is likaly to be effective and tolerated if mistaken for the other in an emergency situation, provided they are given by the recommended incremental dosage protocol. Even though this safety measure has been built into the medication delivery system, routine use of the overdose strength (1000 µg/mL, 2 mL ampule) in the postoperative period is not recommended, as it is too concentrated to allow incremental titration. It is suggested that anesthesiologists and hospital pharmacists take steps suitable to their facility to prevent confusion between the two dosage forms.	IMPORTANT INFORMATION - DOSAGE FORMS REVEX™ is supplied in two concentrations, an ampul containing ONE (1) mL at a concentration suitable for postoperative use (100 µg/mL - blue label) and an ampul containing TWO (2) mL suitable for the management of overdose (1 mg/mL - green label, TEN times as concentrated). **Use of the overdose strength in the postoperative period is not recommended, as it is too concentrated to allow incremental titration**. Proper steps should be taken to prevent use of the incorrect dosage form.	We have differentiated the labels and cartons of the 2 distinct concentrations by using different colored bands (see Attachment 5) We feel it is potentially confusing to have a "warning" outside the "Warning" section and believe that "Important Information" will provide the desired level of attention. Also, because of its importance, we believe this information should be presented as the first statement under DOSAGE AND ADMINISTRATION. The text has been changed to incorporate the label differentiation and the revised Ohmeda recommended dosing. Additionally, the statement re use of the overdose strength in the postoperative period has been bolded.

Line #	Change From	Change To	Rationale
460	Dose Dependent Duration of Action	Duration of Action	"Dose dependent" has been deleted as the duration of action is not solely dependent on the dose administered.
462	The exact duration	The apparent duration	Changed exact to apparent. It is not the duration of REVEX™ that changes, but the patient's clinical condition. Other factors such as the degree of stimulation can also affect respiration
463	dose	plasma concentration	Plasma concentration takes into account both the dose and time that the narcotic was taken
469	1mg/70kg	1.6 mg/70kg	Changed to reflect revised dosing.
481-509	<u>Recommended Doses for Reversal of Postoperative Opioid Depression</u> - Entire section changed SEE REVISED PACKAGE INSERT FOR TEXT Ohmeda's recommended post-operative dosing is based on providing a clearer statment of the desired therapeutic effect and our experience in clinical trials. For "Recommended Doses for Reversal of Post-operative Opioid Depression" - ". .. .. giving the drug in 0.25µg/kg incremental doses at 2 - 5 minute intervals, stopping as soon as the desired effect is obtained ....." - If the wrong ampul is used, the dose will be 2.5µg/kg or .175 mg for a 70 kg man. This dose is above the 0.1 mg challenge dose, but well below the 0.5 mg which was well tolerated even in addicts with stigmata of heavy opioid abuse. The most likely consequence of such an overdose would be precipitation of severe pain which could be reversed by administration of additional opioid analgesia.		
511-517	<u>Patients tolerant to or physically dependent on opioids</u>	<u>Patients Tolerant To Or Physically Dependent On Opioids</u>	This section was moved to precede the dosing information as it pertains to physically dependent patients in either setting (indication).
516	...of at least 5 minutes...	...of 2-5 minutes...	Changed to be consistent with recommended dosing

Line #	Change From	Change To	Rationale
519-530	<u>The Management of Known or Suspected Opioid Overdose</u> - Entire section changed SEE REVISED PACKAGE INSERT FOR TEXT We have experienced difficulties explaining to consultant emergency room doctors why we recommend a dose for which there is no supportive data. In the NDA there were no cases of serious withdrawal precipitated by the 0.5 mg dose. At least 4 patients with probable dependency received that dose. Patients at risk of dependence on opioids should probably receive a low challenge dose - similar to what is required for ReVia™. However, we have no data on which to base the recommendation of a 0.1 mg challenge dose. We would very much appreciate receiving references or information so that we shall be able to respond to queries about our recommendation. For overdose patients, the only risk in using the wrong concentration is an apparent lack of efficacy. Please note that the labels for each concentration will have different color bands, so once familiar with a dose, practitioners should also be visually aware if the wrong ampul is obtained/used.		
538	<u>Repeated Dosing due to Resedation</u>	<u>Repeated Dosing</u>	Changed heading since dosing may also need to be repeated due to respiratory depression
541-544	but repeated doses may be required, especially when patients have received the lowest incremental doses. In such cases, the progressive incremental dosing strategy initially used should be repeated to avoid over-reversal and consequent loss of analgesia	if recurrence of respiratory depression does occur, the dose should again be titrated to clinical effect using incremental doses to avoid over-reversal and consequent loss of analgesia.	Revised to reflect recommended dosing schedule and minor editorial changes.
546-547	Liver	Hepatic	Changed for consistency
576-577	2 mL ampule, containing 2 mg/mL nalmefene base (NDC 10019-311-22)1 mL ampule, containing 100 µg/mL nalmefene base (NDC 10019-315-21)	An ampule containing 2 mL of 1 mg/mL nalmefene base (Green Label) (NDC 10019-311-22) An ampule containing 1 mL of 100 µg/mL nalmefene base (Blue Label) (NDC 10019-315-21)	The wording was changed to emphasize the difference in amount of fill in each ampul and to indicate the different colored labels.

Line #	Change From	Change To	Rationale
585	Taylor Pharmaceutical Co.,	Akorn Manufacturing Co.,	Taylor Pharmacal Co. changed their name (see Attachment 6)

h:\regulate\nalmefene\nda\PIRAT.doc

OHMEDA

THE BOC GROUP

Pharmaceutical Products Division

Ohmeda Inc
110 Allen Road PO Box 804
Liberty Corner NJ 07938 0804
908 647 9200

13 December 1993

Robert Outwater
Senior Director, Worldwide Regulatory Affairs
Ohmeda Pharmaceutical Products Division Inc.
110 Allen Road
Liberty Corner, NJ 07938

Re: Patent Certification-REVEX™ (nalmefene injection)

Dear Mr. Outwater:

This is to certify that Anaquest, Inc. is the exclusive licensee for the rights to the parenteral administration of nalmefene, i.e. 17-(cyclopropylmethyl)-4,5α-epoxy-6-methylenemorphinan-3, 14-diol, as a narcotic antagonist. These rights were acquired form IVAX Pharmaceutical, Inc. now Baker Cummins Pharmaceutical, of Miami, Florida. Anaquest, Inc., by legal change of name, is now Ohmeda Pharmaceutical Products Division Inc. The rights are granted under the following U.S. Patents:

U.S. Patent No. 3,814,768, which claims Nalmefene as a chemical entity. This patent expired on June 4, 1991; and

U.S. Patent No. 3,896,226, which claims a method of antagonizing narcotics by the administration of Nalmefene. This patent, which also claims pharmaceutical compositions containing Nalmefene expired on July 22, 1992.

If you require any additional information with regard to the patents discussed herein, please so inform me.

Very truly yours,

R. Hain Swope
Patent Counsel

0009

Formerly Anaquest Inc

EXCLUSIVITY SUMMARY FOR NDA # 20-459 SUPPL # —

Trade Name Rejex Generic Name Valmefene Hydrochloride

Applicant Name Ohmeda PPD, Inc HFD # 007

Approval Date If Known _____

PART I IS AN EXCLUSIVITY DETERMINATION NEEDED?

1. An exclusivity determination will be made for all original applications, but only for certain supplements. Complete PARTS II and III of this Exclusivity Summary only if you answer "yes" to one or more of the following question about the submission.

a) Is it an original NDA?

YES /✓/ NO /___/

b) Is it an effectiveness supplement?

YES /___/ NO /✓/

If yes, what type? (SE1, SE2, etc.) _____

c) Did it require the review of clinical data other than to support a safety claim or change in labeling related to safety? (If it required review only of bioavailability or bioequivalence data, answer "no.")

YES /✓/ NO /___/

If your answer is "no" because you believe the study is a bioavailability study and, therefore, not eligible for exclusivity, EXPLAIN why it is a bioavailability study, including your reasons for disagreeing with any arguments made by the applicant that the study was not simply a bioavailability study.

_____

_____

If it is a supplement requiring the review of clinical data but it is not an effectiveness supplement, describe the change or claim that is supported by the clinical data:

_____

_____

Form OGD-011347 Revised 7-90

cc: Original NDA Division File HFD-85 Mary Ann ~~Ward~~ Holovac

(d) Did the applicant request exclusivity?

YES /✓/ NO /___/

If the answer to (d) is "yes," how many years of exclusivity did the applicant request?

3 years

IF YOU HAVE ANSWERED "NO" TO ALL OF THE ABOVE QUESTIONS, GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

2. Has a product with the same active ingredient(s), dosage form, strength, route of administration, and dosing schedule, previously been approved by FDA for the same use?

YES /___/ NO /✓/

If yes, NDA #_____. Drug Name _____.

IF THE ANSWER TO QUESTION 2 IS "YES," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

3. Is this drug product or indication a DESI upgrade?

YES /___/ NO /✓/

IF THE ANSWER TO QUESTION 3 IS "YES," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8 (even if a study was required for the upgrade).

PART II FIVE-YEAR EXCLUSIVITY FOR NEW CHEMICAL ENTITIES

(Answer either #1 or #2 as appropriate)

1. Single active ingredient product.

Has FDA previously approved under section 505 of the Act any drug product containing the same active moiety as the drug under consideration? Answer "yes" if the active moiety (including other esterified forms, salts, complexes, chelates or clathrates) has been previously approved, but this particular form of the active moiety, e.g., this particular ester or salt (including salts with hydrogen or coordination bonding) or other non-covalent derivative (such as a complex, chelate, or clathrate) has not been approved. Answer "no" if the compound requires metabolic conversion (other than deesterification of an esterified form of the drug) to produce an already approved active moiety.

YES /___/ NO /✓/

If "yes," identify the approved drug product(s) containing the active moiety, and, if known, the NDA #(s).

NDA# _____ _____

NDA# _____ _____

NDA# _____ _____

2. Combination product.

If the product contains more than one active moiety(as defined in Part II, #1), has FDA previously approved an application under section 505 containing any one of the active moieties in the drug product? If, for example, the combination contains one never-before-approved active moiety and one previously approved active moiety, answer "yes." (An active moiety that is marketed under an OTC monograph, but that was never approved under an NDA, is considered not previously approved.)

NA YES /___/ NO /___/

If "yes," identify the approved drug product(s) containing the active moiety, and, if known, the NDA #(s).

NDA# _____ _____

NDA# _____ _____

NDA# _____ _____

IF THE ANSWER TO QUESTION 1 OR 2 UNDER PART II IS "NO," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8. IF "YES" GO TO PART III.

PART III THREE-YEAR EXCLUSIVITY FOR NDA'S AND SUPPLEMENTS

To qualify for three years of exclusivity, an application or supplement must contain "reports of new clinical investigations (other than bioavailability studies) essential to the approval of the application and conducted or sponsored by the applicant." This section should be completed only if the answer to PART II, Question 1 or 2 was "yes."

1. Does the application contain reports of clinical investigations? (The Agency interprets "clinical investigations" to mean investigations conducted on humans other than bioavailability studies.) If the application contains clinical investigations only by virtue of a right of reference to clinical investigations in another application, answer "yes," then skip to question 3(a). If the answer to 3(a) is "yes" for any investigation referred to in another application, do not complete remainder of summary for that investigation.

YES /___/ NO /___/

IF "NO," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

2. A clinical investigation is "essential to the approval" if the Agency could not have approved the application or supplement without relying on that investigation. Thus, the investigation is not essential to the approval if 1) no clinical investigation is necessary to support the supplement or application in light of previously approved applications (i.e., information other than clinical trials, such as bioavailability data, would be sufficient to provide a basis for approval as an ANDA or 505(b)(2) application because of what is already known about a previously approved product), or 2) there are published reports of studies (other than those conducted or sponsored by the applicant) or other publicly available data that independently would have been sufficient to support approval of the application, without reference to the clinical investigation submitted in the application.

(a) In light of previously approved applications, is a clinical investigation (either conducted by the applicant or available from some other source, including the published literature) necessary to support approval of the application or supplement?

YES /___/ NO /___/

If "no," state the basis for your conclusion that a clinical trial is not necessary for approval AND GO DIRECTLY TO SIGNATURE BLOCK ON PAGE 8:

_____

_____

(b) Did the applicant submit a list of published studies relevant to the safety and effectiveness of this drug product and a statement that the publicly available data would not independently support approval of the application?

YES /___/ NO /___/

(1) If the answer to 2(b) is "yes," do you personally know of any reason to disagree with the applicant's conclusion?

YES /___/ NO /___/

If yes, explain: _____

_____

(2) If the answer to 2(b) is "no," are you aware of published studies not conducted or sponsored by the applicant or other publicly available data that could independently demonstrate the safety and effectiveness of this drug product?

YES /___/ NO /___/

If yes, explain: _____

_____

(c) If the answers to (b)(1) and (b)(2) were both "no," identify the clinical investigations submitted in the application that are essential to the approval:

_____

_____

Studies comparing two products with the same ingredient(s) are considered to be bioavailability studies for the purpose of this section.

3. In addition to being essential, investigations must be "new" to support exclusivity. The agency interprets "new clinical investigation" to mean an investigation that 1) has not been relied on by the agency to demonstrate the effectiveness of a previously approved drug for any indication and 2) does not duplicate the results of another investigation that was relied on by the agency to demonstrate the effectiveness of a previously approved drug product, i.e., does not redemonstrate something the agency considers to have been demonstrated in an already approved application.

a) For each investigation identified as "essential to the approval," has the investigation been relied on by the agency to demonstrate the effectiveness of a previously approved drug product? (If the investigation was relied on only to support the safety of a previously approved drug, answer "no.")

Investigation #1 YES /___/ NO /___/

Investigation #2 YES /___/ NO /___/

If you have answered "yes" for one or more investigations, identify each such investigation and the NDA in which each was relied upon:

_____ _____

_____ _____

b) For each investigation identified as "essential to the approval", does the investigation duplicate the results of another investigation that was relied on by the agency to support the effectiveness of a previously approved drug product?

Investigation #1 YES /___/ NO /___/

Investigation #2 YES /___/ NO /___/

If you have answered "yes" for one or more investigation, identify the NDA in which a similar investigation was relied on:

_____ _____

_____ _____

c) If the answers to 3(a) and 3(b) are no, identify each "new" investigation in the application or supplement that is essential to the approval (i.e., the investigations listed in #2(c), less any that are not "new"):

_____ _____

_____ _____

4. To be eligible for exclusivity, a new investigation that is essential to approval must also have been conducted or sponsored by the applicant. An investigation was "conducted or sponsored by" the applicant if, before or during the conduct of the investigation, 1) the applicant was the sponsor of the IND named in the form FDA 1571 filed with the Agency, or 2) the applicant (or its predecessor in interest) provided substantial support for the study. Ordinarily, substantial support will mean providing 50 percent or more of the cost of the study.

a) For each investigation identified in response to question 3(c): if the investigation was carried out under an IND, was the applicant identified on the FDA 1571 as the sponsor?

Investigation #1	
IND # _____ YES /___/	NO /___/ Explain: _____
	_____
Investigation #2	
IND # _____ YES /___/	NO /___/ Explain: _____
	_____

(b) For each investigation not carried out under an IND or for which the applicant was not identified as the sponsor, did the applicant certify that it or the applicant's predecessor in interest provided substantial support for the study?

Investigation #1	
YES /___/ Explain _____	NO /___/ Explain _____
_____	_____
_____	_____
Investigation #2	
YES /___/ Explain _____	NO /___/ Explain _____
_____	_____
_____	_____

(c) Notwithstanding an answer of "yes" to (a) or (b), are there other reasons to believe that the applicant should not be credited with having "conducted or sponsored" the study? (Purchased studies may not be used as the basis for exclusivity. However, if all rights to the drug are purchased (not just studies on the drug), the applicant may be considered to have sponsored or conducted the studies sponsored or conducted by its predecessor in interest.)

YES /___/ NO /___/

If yes, explain: _____

_____

Bonnie McNeal
Signature
Title: Consumer Safety Officer

March 15, 1995
Date

John E Hyde M.D.
Signature of Office/
Division Director

March 15, 1995
Date

cc: Original NDA Division File HFD-85 Mary Ann ~~Ward~~ Holovac

WRIGHT

DEC 5 1994

Statistical Review and Evaluation

NDA 20-459
Name of drug: Revex (nalmefene hydrochloride injection)
Applicant: Ohmeda
Indication: Reversal of opioid anesthesia or overdose

Documents reviewed:
Volume 1, 27 April 1994
Medical officer review, 31 July 1994

Reviewer: Thomas Permutt
Date of review: 5 December 1994

Introduction

This is a secondary review to the Medical Officer Review of 31 July 94 by Dr. Curtis Wright. Dr. Wright's clinical evaluation makes appropriate use of the statistical data, and I support his conclusions. Accordingly, this review will simply clarify a few points from a statistician's point of view.

Nalmefene is an opioid antagonist analogous to naloxone but with a longer half-life. The proposed labeling is for reversal of effects of narcotics in two settings, overdose and anesthesia. In both cases the primary measure of efficacy is reversal of the slow or stopped breathing associated with the opioid exposure. In both cases naloxone is accepted as a safe and effective treatment for a life-threatening or serious condition. Accordingly there were no placebo-controlled studies. Nalmefene in various doses was compared to naloxone in randomized trials.

The patients in the clinical trials were fairly representative of the target population. There were substantial numbers of blacks and whites, men and women, but few Asians or older patients.

Overdose

There were three controlled triáls of nalmefene in narcotic overdose patients. One was characterized by the sponsor and the medical officer as inconclusive due to inadequate enrollment. Of the other two, one (05) was characterized as "substantial"

Appendix I: Formulation and Biopharmaceutics Study Summary and Cross-Study Comparison of Nalmefene Pharmacokinetic Parameters.

2. Drug Product

A. COMPOSITION
Complete statement of quantitative composition of the drug product:

Nalmefene Injection, 1.0 mg/mL formulation

Components	Quantity (mg/mL)
Nalmefene HCl	1.108*

* Equivalent to 1.0 mg nalmefene free base

Nalmefene Injection, 0.1 mg/mL Formulation

Components	Quantity (mg/mL)
Nalmefene HCl	0.1108*

* Equivalent to 0.10 mg nalmefene free base

B. Manufacturer information:

Table 1
Biopharmaceutic Study Summary

Study No.	Dose Form Route of administration	Study Design	Dose	Batch No. Plant Date of Mfr	No. of Subjects	Date of Submission	Previous Agency Correspondence	Applicant's Comments
R7	solution i.v., p.o.	ascending dose	0.5, 1.0, 2.0 mg i.v., 2, 4, 8, 16, 32, 64 mg p.o.	laboratory batch	8 healthy male volunteers	Conducted in Ireland		
JF-1-137	solution, ^{14}C i.v., p.o.	cross-over	4.92 mg i.v. 198.7 mg p.o.	Midwest Research Institute	4 healthy male volunteers	Conducted in the U.K.		Nalmefene had high systemic clearance, underwent extensive metabolism to glucuronide, and may have been subject to enterohepatic circulation.
JF-1-101+101A	solution 10 mg/Ml i.v., i.m.	randomized, parallel	2, 6, 12, 24 mg i.v. and 24 mg i.m.	laboratory batch	16 healthy male volunteers (+8 placebo)	PK report submitted 2/27/85		2 mg curve below assay sensitivity 6 -24 mg dose proportional, $T_{1/2}b$ 8.6 hrs, 5% excreted in urine unchanged, 60 % as glucuronide. 24 mg i.m. not different from 24 mg i.v.
JF-1-143	solution i.v.	randomized, 3-way cross-over	0.5, 1.0, 2.0 mg i.v.	[illegible]3 1.0 mg free base/ml	12 healthy male volunteers	submitted 8/25/86	7/22/85 Comment that 12 hr f.u. not long enough	No evidence that nalmefene deviated from dose proportionality over the 0.5-2.0 mg dose range.
	solution, 1 mg/ml i.m., i.v., s.c.	cross-over	1.0 mg i.m., i.v., s.c.	3495-045	12 healthy male volunteers	Submitted 9/7/90	FDA questions 10/4/90 Response 2/19/91	Relative bioavailabilities were 101.5±8.1% and 99.7±6.9% for i.m. and s.c. respectively. Both gave plasma concentrations of 0.5 ng/ml within 15 minutes.
	solution i.v.	parallel, ascending dose	0.5, 1.0, 2.0 mg i.v.	3495-045	18 healthy male volunteers	submitted 3/5/90		With outlier, subject 18, removed the AUC_{∞} were dose proportional.
	solution i.v.	crossover	1.0 m/kg, 7.5 m/kg, 15 m/kg	3495-045	12 healthy male volunteers			Peak plasma concentrations were contaminated by drawing from the same port as drug administered.
	solution i.v.	parallel	0.5 mg, 1.0 mg, 2.0 mg	3495-087	36 elderly volunteers			AUC_{∞}, clearance, and elimination half life were not different from healthy volunteers.
	solution i.v.	open-label	1.8-3.6 m/kg q 6 hr	3495-087	12 pediatric patients (2-7 yrs)			Study ongoing.
	solution i.v.	open-label	1.0 mg	3495-087	12 liver disease patients, 12 controls			Liver disease reduced the clearance of free nalmefene by 28%.
	solution i.v.	open-label	1.0 mg		8 ESRD patients,			

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Table 2
Nalmefene Pharmacokinetic Parameters

Study No.	Dose Form Route of Admin.	Dose mg	C_{peak} ng/Ml	T_{peak} min	V_d L	AUC_{0-t} ng*hr/Ml	$AUC_{0-\infty}$ ng*hr/Ml	$T_{1/2b}$ hrs	Urinary Excr. % dose	Cl_p L/hr	Cl_r L/hr	K_{el}	Comments
R7	solution i.v.	0.5 1.0 2.0	4.3-5.6	10-45									
JF-1-137	solution, ^{14}C, i.v.	4.92 i.v. }99 p.o.		60-120 p.o.				(^{14}C) 16 i.v. 12p.o.	70 total 3-6 unchg.				45% protein bound, glucuronide the main metabolite, oral bioavailability 30-4-%.
JF-1-101 + 101A	solution, i.v.	2 6 12 24			- 485 481 515	16.5 110 189 369	16.9 109 191 385	- 8.2 8.6 8.9	3.8 5.5 5.7 5.0	- 60 65 63	- 3.0 3.6 3.1	- 0.0849 0.0804 0.0776	
JF-1-143	solution i.v.	0.5 1.0 2.0					3.77 7.61 14.7	2.30 2.80 2.94					Normalized AUCs were 7.53, 7.61, and 7.34. Plasma levels were not detectable at 12 hrs after 0.5 mg.
09	solution i.v.	0.5 1.0 2.0	(%CV) 1.77 (42.5) 3.16 (10.9) 5.75 (11.4)	(%CV) 5.00 (0) 11.67 (44.3) 12.00 (91.3)	(%CV) 622.7 (16.8) 501.4 (17.6) 628.2 (20.6)	(%CV) 6.09 (16.4) 16.24 (24.3) 32.94 (20.6)	(%CV) 6.91 (15.3) 17.03 (23.3) 34.00 (20.5)	(%CV) 8.19 (16.8) 7.92 (33.3) 9.66 (17.0)		(%CV) 0.93 (11.0) 0.91 (21.7) 0.76 (20.2)	(%CV)	(%CV) 0.087 (16.3) 0.095 (29.3) 0.073 (17.5)	Excluding outlier number 18 in the 2.0 mg group
10	solution i.m.,	1.0	1.47 (24.2)	137.5 (48.8)	L/kg 9.0 (19.9)	14.94 (17.0)	16.98 (17.8)	9.65 (39.2)	Total 36.2 (20.0)	L/hr/kg 0.76 (19.9)	L/hr/kg 0.01 (41.7)	0.081 (32.7)	Relative Bioavailability i.m. 101.5 (27.7)
	i.v.,	1.0	5.87 (58.4)	5.42 (86.8)	8.0 (15.2)	15.89 (25.6)	17.61 (26.4)	11.11 (38.6)	39.6 (22.8)	0.75 (22.0)	0.01 (58.1)	0.071 (36.9)	
	s.c.	1.0	1.32 (20.0)	88.75 (86.3)	10.2 (17.3)	15.04 (24.4)	17.15 (24.9)	10.77 (38.1)	39.0 (25.4)	0.77 (24.0)	0.01 (68.8)	0.075 (44.9)	s.c. 99.7 (23.9)

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↑ Clrenal values are not correctly reported here.

Ruth E. Stevens 2-13-95

Study No.	Dose Form Route of Admin.	Dose mg	C_{peak} ng/Ml	T_{peak} min	V_d L	AUC_{0-t} g*hr/Ml	$AUC_{0-\infty}$ ng*hr/Ml	$T_{1/2b}$ hrs	Urinary Excr. % dose	Cl_p L/hr	Cl_r L/hr	K_{el}	Comments
19	solution i.v.	0.5	3.71 (172.9)	6.0 (58.4)	L 609.4 (41.4)	7.40 (43.6)	8.16 (41.5)	7.52 (35.8)		l/hr/kg 0.97 51.2		0.102 (31.1)	
		1.0	6.33 (31.8)	5.0 (0)	695.3 (48.1)	16.1 (19.8)	18.65 (17.1)	11.72 (64.4)		0.77 (21.6)		0.075 (40.2)	
		2.0	17.49 (42.7)	5.0 (0)	691.4 (24.6)	31.58 (19.2)	32.93 (18.2)	10.26 (18.5)		0.85 (18.9)		0.070 (18.0)	
21	solution i.v.	2.0 cntrl	62.9 (66.5)	1.8 (49.3)	Vss L 912.5 (32.9)	32.39 (35.2)	34.31 (33.0)	10.17 (21.8)		l/hr/kg 0.786 (41.2)		0.072 (24.4)	
		2.0 pat	50.2 (51.6)	2.2 (58.5)	799.6 (23.9)	42.62 (29.5)	45.18 (31.3)	11.88 (17.3)		0.564 (36.6)		0.060 (17.8)	
22	solution i.v.	1.0 cn'rl	31.5 (66.5)	1.8 (49.3)	912.5 (32.9)	16.19 (35.2)	17.15 (33.0)	10.17 (21.8)		0.786 (41.2)		0.072 (24.4)	Symptoms of dizziness and hypertension caused drug to be given over 60 sec. in ESRD patients.
		1.0 inter	6.8 (33.8)	5.0 (0)	1374.3 (38.5)	21.7 (21.2)	28.37 (31.7)	26.09 (37.9)		0.574 (34.8)		0.029 (42.7)	
		1.0 intra	15.2 (139.7)	5.0 (0)	1387.6 (57.7)	22.2 (32.4)	27.48 (31.2)	25.67 (67.2)		0.589 (30.9)		0.033 (35.5)	
Overall Summary of Compart-mental Analysis	solution i.v. Adult male	1.0	3.72 (29.0)		8.55 (19.4)		16.64 (26.8)	10.77 (48.1)		l/hr 63.58 (23.1)		0.079 (41.8)	18 males from Studies 09 and 10 11 elderly males from Study 19
	Elderly male	1.0	5.83 (38.1)		8.59 (29.0)		17.31 (14.1)	9.38 (49.0)		58.7 (13.5)		0.087 (34.5)	
Pooled Non-compart-mental analysis	solution i.v.	1.0	5.0 (58.0)		597 (22.4)	16.0 (24.4)	17.4 (24.7)	10.0 (40.0)		l/hr 60.3 (21.7)		0.079 (38.0)	

Appendix II: Comparison of Analytical Methodology Used to Quantify Nalmefene And Analytical Methods Used in Clinical Pharmacokinetic Studies.

2 pages
PURGED

APPENDIX III

Study Details of Study R7. Tolerance and Bioavailability (Supportive Study).

Study Details of Study JF-137. Disposition And Metabolism of ^{14}C-Nalmefene Following Intravenous And Oral Administration to Man (Pivotal Study).

Study Details of Study 10. A Bioavailability Study of Nalmefene Given Via Intravenous, Intramuscular, and Subcutaneous Routes in Healthy Volunteers (Pivotal Study).

Study Details of Study 101/101A. Pharmacokinetics of Single Ascending Intravenous and Intramuscular Doses (6, 12, 25 mg) of the Narcotic Antagonist, Nalmefene in Man (Supportive Study).

Study Details of Study JF-143. Dose Proportionality of Nalmefene Following Intravenous Doses of 0.5, 1.0 and 2.0 mg (Supportive Study- Last sampling times were taken from 0-12 hours only).

Study Details of Study 9. A Pharmacokinetic Study of Ascending Doses (0.5, 1 and 2 mg) of Nalmefene in Healthy Volunteers (Pivotal Study- Last sampling times were taken from 0-48 hours).

Study Details of Study 19. A Pharmacokinetic Study of Ascending Doses (0.5, 1, and 2 mg) of Nalmefene in Elderly, Healthy Volunteers (Pivotal Study).

Study Details of Study 21. A Pharmacokinetic Study of Nalmefene in Normal Subjects and Subjects with Mild, Moderate, and Severe Hepatic Dysfunction (Pivotal Study).

Study Details of Study 22. A Study to Determine The Safety and Pharmacokinetics of Nalmefene IV in Renally Impaired Subjects (Pivotal Study).

Study Details of Study 24. An Open Label Study to Compare the Duration of Occupancy of Opiate Receptors by Nalmefene vs. Naloxone Using Dual Probe Detection of Positron Emissions in Normal Subjects.

Study Details of Nalmefene Red Blood Cell Partion.

Study #: R7 Vol 1.24

Study Type: Tolerance and Bioavailabilty

Investigator: Austin Darragh, MD

Study Site: Insitute for Clinical Pharmacology
Dublin Ireland

Single Dose: XX Multiple Dose:
Subjects: Normal XX Patients Young Elderly
Impaired: Renal Hepatic

Cross-over: Parallel:XX N=8 M=8 F=

Subject Type: Group 1 (0.5, 2 mg IV; 2, 8, 32 mg oral; placebo)

Weight: Mean= 71.95 Range=

Age: Mean= 29.5 Range=

Subject Type: Group 2 (1, 2 mg IV; 4, 16, 64 mg oral; placebo)

Weight: Mean= 68.4 Range=

Age: Mean= 23.2 Range=

Treatment	Subj.#	Dose	Dosage Form	Strength	Lot#	Lot Size
Treatment	1-4	0.5, 1.0, 2.0 mg	Intravenous	1.0 mg/ml 0.1 mg/ml	MD-100-10 MD-100-06	unknown
Treatment	1-4	2.0, 4.0, 6.0, 16.0, 32.0 64.0 mg	Oral			
Treatment	1-4	Placebo	IV/Oral			

Fasted Yes; 8 hrs. prior to dosing and 2 hrs. post dosing

Nonfast: No Food Study: No FDA High Fat Breakfast: No

Samples: Plasma 10ml 30 minutes; 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24 hours
Urine
Feces

Assay Methoc
Assay Sensitivity: Unknown

Labeling Claims from Study: Nalmefene was well tolerated up to an intravenous dose of 2.0 mg and an oral dose of 64.0 mg.

and one (JF-201) as "supportive" by the sponsor, but the medical officer found substantial evidence of efficacy in both.

Study 05

This was a randomized trial of nalmefene and naloxone. Dose was titrated to effect, but there were two nalmefene groups, one with increments of 1 mg and one of 2 mg; the incremental dose of naloxone was 2 mg. Patients with apparent overdoses were treated before the results of toxicological testing for opioids were known. The patients therefore included some who were eventually found not to be under the influence of opioids after all, in whom nalmefene or naloxone would not be expected to be effective.

In the opioid-positive subset, naloxone and both doses of nalmefene reversed respiratory depression. The mean respiratory rate changed from 7 breaths per minute in each group at baseline to 12, 17 and 13 after 5 minutes in the low-dose nalmefene, high-dose nalmefene and naloxone groups, respectively. The changes from baseline were statistically significant (paired t-test) in each case. Because of the lack of a placebo control, this statistical significance is of relatively little importance. What is important here is the medical officer's judgment that the effect is clinically meaningful, is comparable to that of naloxone, and is unlikely to be seen without effective treatment. Nalmefene and naloxone also reduced sedation as measured by the Glasgow Coma Scale.

The opioid-negative subset for the most part did not have the respiratory depression whose reversal was the primary criterion of effectiveness. Inclusion of these patients in the analysis or comparison of the results for opioid-positive patients to those in this group would therefore be equally fruitless. There is no evidence that the treatment did these patients harm.

Study JF-201

This was another randomized study of nalmefene (1 mg) and naloxone (2 mg). Again, both restored depressed respiration to normal. Changes from baseline were again statistically significant in both groups: for the opioid-positive patients, 10 breaths per minute with nalmefene and 14 breaths per minute with naloxone at 2 min. The difference for naloxone was statistically more than that for nalmefene at one time point in the opioid-positive group. This effect resulted partly from overcorrection for baseline by subtraction rather than by analysis of covariance. In any case Dr. Wright concludes that it is of no medical significance. Furthermore,

when the analysis was further narrowed from all opioid-positive patients to those showing more definite signs of opioid intoxication (depressed respiration and pinpoint pupils) the difference was in the opposite direction.

Anesthesia

As with overdose, the primary measure of effectiveness was reversal of respiratory depression. As with overdose, the degree of respiratory depression if any at baseline varied widely across patients, and patients without the symptom were not expected to respond to the treatment. A further complication in anesthesia was that slow breathing might have resulted from an abnormally low concentration of carbon dioxide in the blood (hypocarbia), an aftereffect of ventilation during surgery, rather than from the narcotic anesthetic. In this case, again, the narcotic antagonist would not be expected (nor, perhaps, desired) to be effective.

Dr. Wright found substantial evidence of efficacy in three studies (13, 15, 02) and characterized several other, mainly smaller trials as supportive. With respect to study 15 the finding of substantial evidence relies only on the patients in the U.S. and Canada.

Study 13

This was a multicenter randomized trial of nalmefene at one of four initial doses (0.1, 0.25, 0.5 or 1 μg/kg) or naloxone (1 μg/kg) in patients who had morphine as part of anesthesia for abdominal or orthopedic surgery. The dose was titrated to effect, but with respect to the first evaluation (5 min) it is a controlled trial with five arms.

The change in breathing rate from baseline to 5 min was slightly negatively correlated with the baseline score; i.e., patients who had less depressed respiration to begin with had less increase. The sponsor therefore analyzed the change in breathing rate by analysis of covariance, with the baseline rate as covariate. This is perhaps an unnecessarily elaborate model. It is also implausible in the sense that the supposed linear relation does not account for the true nature of the anticipated effect of baseline, which is that nalmefene or naloxone can only be expected to reverse respiratory depression to the extent that there is respiratory depression. In view of the low correlation, however, these methodological considerations are of small consequence. There was a clear increase in respiratory rate, ranging at 5 min from 7 breaths per minute in the lowest-dose nalmefene group to 11 breaths per minute in the highest. The effect of nalmefene increased with dose in this range. The estimated

effects of nalmefene at 0.5 and 1 μg/kg span that of naloxone at 1 μg/kg, suggesting, though with considerable uncertainty, that the nalmefene dose equivalent to 1 μg/kg of naloxone is between 0.5 and 1 μg/kg.

Study 15 U.S./Canada

These are the U.S. and Canadian patients from an international, multicenter, randomized comparison of nalmefene at two doses (0.25 and 0.5 μg/kg) and naloxone (0.5 μg/kg) in patients who had had fentanyl as part of their anesthesia for abdominal surgery. About a quarter of these patients had abnormally low levels of end-expiratory carbon dioxide from ventilation during surgery. To the extent that their respiratory depression resulted from this hypocarbia rather than from the narcotic anesthetic, they would not be expected to respond to nalmefene or naloxone but would be expected to recover spontaneously. For all patients, the mean respiratory rate at 5 min rose significantly, by 7, 9 and 7 breaths per minute in the lower- and higher-dose naltrexone groups and the naloxone group, respectively (difference from baseline, adjusted for baseline by analysis of covariance). Considering only nonhypocarbic patients, the changes were 6, 12 and 13 breaths per minute, showing a dose effect with nalmefene.

Study 02

This trial studied the effects of nalmefene (0.5 or 1 μg/kg) or naloxone (1 μg/kg) in patients who had had intrathecal morphine for abdominal surgery. Again, respiratory rate at 5 min was significantly increased from baseline, by 5, 9 and 6 breaths per minute in the lower- and higher-dose nalmefene groups and the naloxone group, respectively. Both nalmefene groups had fewer doses per patient on average; a proportional-hazards test showed a significant difference among groups in mean time between doses, suggesting a longer duration of action for nalmefene.

Conclusions

Nalmefene, like naloxone, is effective in reversing respiratory depression associated with exposure to opioids, whether in overdose or anesthesia. Safety is discussed in Dr. Wright's review. Approximately 1000 patients were treated with nalmefene, so that a 95 percent upper confidence bound for the frequency of any adverse event not seen is 0.3%.

Thomas Permutt

Thomas Permutt, Ph.D.
Mathematical Statistician

Hoi M. Leung

Hoi M. Leung, Ph.D.
Peer Reviewer

original: NDA 20-459
cc:
HFD-007/McNeal
HFD-007/C. Wright
HFD-007/Permutt
HFD-007/Division file

Nalmefene HCl Injection
Revex[R], 100 ug/mL (1 mL ampule)
1000 ug/mL (2 mL ampule)
NDA No. 20-459
Reviewer: Ruth E. Stevens, Ph.D.
Priority: 1S, New Molecular Entity

Ohmeda Inc.
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Submission Date: 4/27/94

Review of an NDA

SYNOPSIS:
There were a total of 9 studies submitted to the pharmacokinetics section of the NDA. Six of the studies were pivotal (Mass Balance Study, Relative Bioavailability Study, Dose Proportionality Study and Studies in Special Populations (i.e., Elderly, Hepatic Impairment, Renal Impairment and Gender Differences)) and three other studies were supportive (dose proportionality studies). In addition, one study that was submitted to the Clinical Pharmacology Section was reviewed (Duration of Occupancy of Opiate Receptors by Nalmefene). Assay validation was provided for all the studies on the racemic compound, nalmefene. The assays were performed by were specific for intact nalmefene. All pharmacokinetic studies, including clinical, used the 'to-be-marketed' formulation.

NOTE: This 22 page review is abstracted/summarized from the detailed original studies. If further study details or raw data are needed, please refer to the detailed original review of the NDA studies (Appendix I - IV) which is placed in the Drug Files located in the Division of Biopharmaceutics or the PK files located in Pilot Drug Division (HFD-550). This NDA was an interactive IND, and therefore, previous pharmacokinetic reviewers and one medical officer from the agency have been involved with reviewing the pharmacokinetic section of the NDA submission. These reviews are attached in the detailed study summary in Appendix III. Only where there was a difference of opinion in analysis does this reviewer make comments.

RECOMMENDATION:

From a Biopharmaceutics standpoint, the Sponsor has submitted sufficient pharmacokinetic information for the approval of Nalmefene HCl Injection (NDA 20-459) provided the phase IV commitment and labeling issues have been addressed prior to approval.

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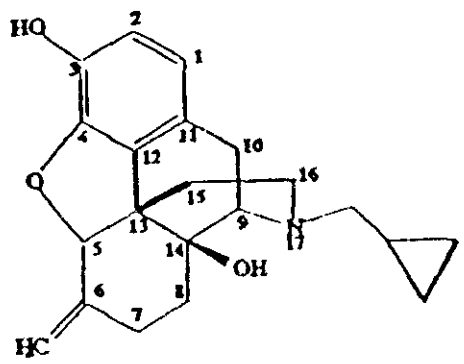
I. BACKGROUND:

Nalmefene hydrochloride injection (Revex®), described herein, has been developed by Ohmeda Inc. (formerly Anaquest), of Liberty Corner, New Jersey and is being manufactured by Taylor Pharmaceutical of Decatur, Illinois. Nalmefene will be indicated for the complete or partial reversal of opioid depression, including respiratory depression, and for the diagnosis and treatment of suspected opioid overdose. Nalmefene will be administered primarily as an intravenous bolus, but may be given intramuscularly or subcutaneously if venous access cannot be established.

Nalmefene is a selective opioid antagonist (μ, κ, and δ opioid binding sites) structurally (Figure 1) and pharmacologically similar to naloxone, although it has a longer duration of action at fully reversing doses. Naloxone is the only opioid antagonist currently available for intravenous clinical use. However, a major disadvantage of naloxone is its short elimination half-life (approximately 1.2 hours) and duration of action relative to the opioid agonists (most are 2-4 hours) that it is used to antagonize. Due to the short duration of action relative to the clinically used opioid agonist, the potential exists for renarcotization. In comparison, nalmefene has a significantly longer elimination half-life (approximately 10.2 hours) and its duration of action has been demonstrated to be longer than naloxone in fully reversing doses (1 mg/70 Kg). The dose of nalmefene that was administered consistently among the pharmacokinetic studies was the 1.0 mg or approximately 14 µg/kg intravenous dose.

Figure 1: The chemical structure of racemic nalmefene.



As stated in the labeling, nalmefene dosing will be titrated to reverse the undesired effects of opioids. The recommended intravenous dosage for the reversal of postoperative opioid depression is 0.1-0.5 µg/kg increments given at 2-5 minute intervals until the desired degree of reversal is achieved. A total dose above 1.0 µg/kg does not provide additional therapeutic effect. For the management of known or suspected opioid overdose the recommended dosage is to titrate to clinical effect using up to three incremental doses. The initial dose would be 0.1 mg iv nalmefene. If needed, 0.5 mg (second dose) and then 1.0 mg (third dose). If a total dose of 1.6 mg has been administered without clinical response, then additional doses of nalmefene are unlikely to have an effect. Once adequate reversal has been established additional administration would not be required and might actually be harmful due to unwanted reversal of analgesia or precipitated withdrawal. The titration of dosing for both postoperative and opoid overdose were based on the following rationale. In the event that the two strengths of nalmefene (100 µg/mL and 1000 µg/mL) get interchanged

by accident in the clinical setting, a postoperative patient would not be overdosed if given the higher strength according to the lower strength dosing paradigm. Alternatively, an opioid overdose patient would not be underdosed if given the lower strength of nalmefene.

With intramuscular or subcutaneous administration, the time to maximum concentration is delayed (after the first dose compared to an intravenous administration (5 minutes). Thus, there is the potential for accumulation of nalmefene if the drug is administered by either intramuscular or subcutaneous routes in quick succession. The safety of the second and third titrated doses has not been established by the intramuscular and subcutaneous route of administration and care should be given not to overdose patients by subsequent doses.

The duration of action of nalmefene is as long as most opioids, minimizing the risk of renarcotization. However, the exact duration of action of nalmefene will vary depending on the half-life and dose of the narcotic being reversed, the presence or absence of other drugs affecting the brain or respiratory muscles, and the dose of nalmefene administered. Partially reversing doses of nalmefene (1 μg/kg) lose their effect as the drug is redistributed through the body, and the effects of these low doses may not last more than 30-60 minutes in the presence of high concentrations of strong opiates. Fully reversing doses (1 mg/70 Kg) have been shown to last many hours in both experimental and clinical studies, but may complicate the management of patients who are in pain, at high cardiovascular risk, or who are physically dependent on opioids.

II. BIOAVAILABILITY:

The relative bioavailabilities of intramuscular and subcutaneous routes of administration were 101.5% (CV 8%) and 99.7% (CV 7%) respectively.

Study 10 was a cross-over study conducted in 12 healthy, caucasian male volunteers. The study determined the bioavailability of nalmefene administered by intramuscular and subcutaneous routes relative to the intravenous route The relative bioavailabilities of intramuscular and subcutaneous routes of administration and pharmacokinetic parameters of nalmefene in plasma after a 1 mg single dose are presented in Table I. The time to maximum concentration (Tmax) was delayed in both the intramuscular and subcutaneous routes of administration when compared to the intravenous route of administration. The high degree of variability associated with intramuscular (CV 48%) and subcutaneous (CV 83%) time to peak make it difficult to predict the appropriate timing for the second and third titration doses to a patient without intravenous access.

Table 1: Nalmefene in plasma after a 1 mg single dose, Mean ± SD, n=12.

Route of Administration	AUC0-inf (ng*hr/mL)	Cmax (ng/mL)	Tmax (hrs)	Bioavailability %
IV	17.61 (4.64)	5.89 (3.22)	0.09 (0.08)	Reference
IM	16.98 (3.02)	1.47 (0.36)	2.29 (1.12)	101.5 (8.1)
SC	17.15 (4.27)	1.32 (0.26)	1.48 (1.23)	99.7 (6.9)

III. PHARMACOKINETICS:

1. **Disposition Study (^{14}C-Nalmefene):**

Note: Inspection of the numbers in Table 2 revealed incorrect summations in this mass balance study. The total radioactivity measured in the urine, following both the oral and iv route of administration, did not equal the sum of the radioactivity measured from the labelled components (unconjugated metabolites and the conjugated metabolites). Although the study is deficient in this regard, useful data can still be extrapolated from this study.

Nalmefene undergoes 30% metabolism to its glucuronide conjugate and less than 5% of intact nalmefene is excreted renally. Seventeen percent (17%) of nalmefene is excreted in the feces. The metabolites have no antagonist or agonist properties.

Study JF-137 was a study of the disposition of ^{14}C-nalmefene. Absorption, distribution, metabolism and excretion were investigated in 4 healthy, male volunteers. Each volunteer received an iv bolus injection of 5 mg ^{14}C-nalmefene HCl followed by an oral dose of 200 mg nalmefene containing 5 mg ^{14}C-nalmefene HCl after a 14 day wash-out period. Blood, urine and feces were collected for 5-7 days post dose. Nalmefene was 50% metabolized, with less than 5% of the dose recovered attributable to intact nalmefene. The major portion of circulating plasma radioactivity was identified as nalmefene glucuronide. Thin-layer chromatography (TLC) analysis indicated that nalmefene accounted for most of the unconjugated metabolites (5%). Trace amounts of N-dealkylated nalmefene were detected. Nalmefene was excreted 17% in the feces. Table 2 contains the results of excretable radioactivity, expressed as a percent of the dose administered. According to the mass balance equation, the maximum amount of nalmefene excreted was approximately 74%, leaving about 26% of nalmefene unaccounted. The metabolites have been shown to have no antagonist or agonist properties.

Ultrafiltration studies of nalmefene have demonstrated that 45% (CV 4.1%) is bound to

plasma proteins over a concentration range of 0.1 to 2 μg/mL. An *in vitro* determination of the distribution of nalmefene in whole blood demonstrated that nalmefene distributed 67% (CV 8.7%) into red blood cells and 39% (CV 6.4%) into plasma over the nominal concentration range in whole blood from 0.376 to 30 ng/mL.

Table 2: Excretion and Distribution of radioactivity in urine and feces following iv, 5 mg, ^{14}C-nalmefene HCl and oral, 200 mg, ^{14}C-nalmefene HCl. Mean ± SD, n=4. Duration of urine collection was from 0-120 hours and for feces, 0-160 hours.

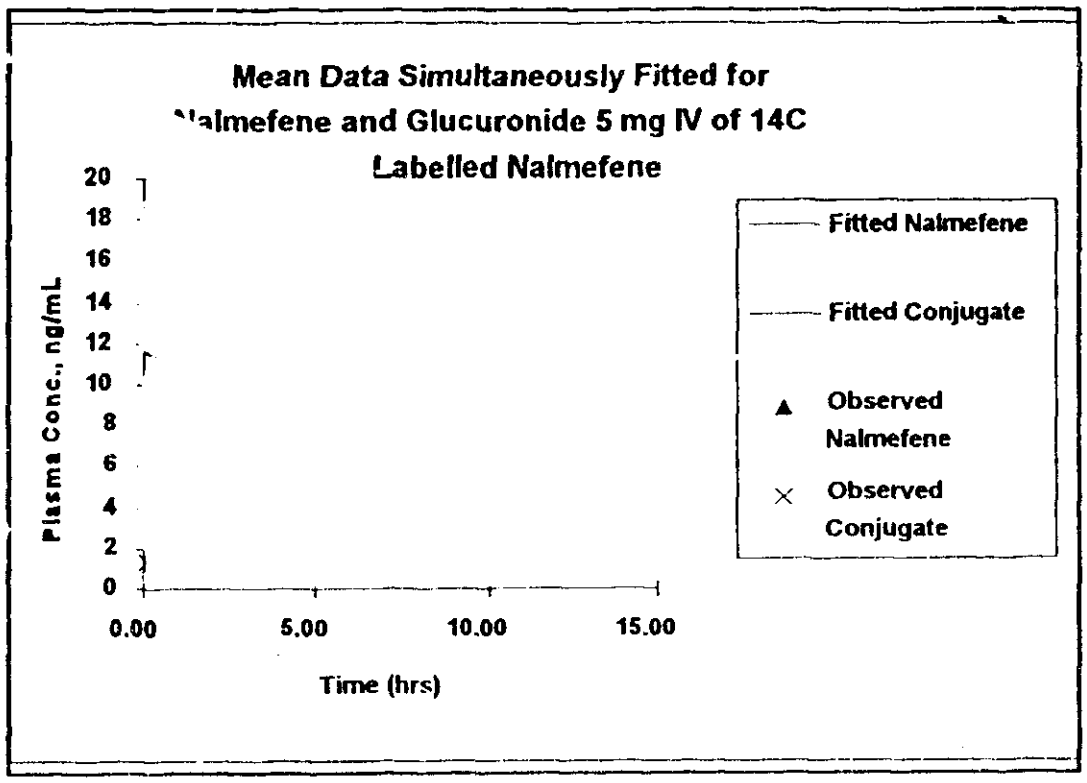
Route of Administration	Urine Total ^{14}C (Unconjugated and Conjugated) (% of dose)	Urine Unconjugated (i.e., Nalmefene) (% of dose)	Urine Conjugated (i.e., Glucuronide) (% of dose)	Feces ^{14}C-nalmefene (% of dose)
IV	49.4 ± 15.2	5.2 ± 1.6	33.2 ± 2.2	17.4 ± 0.7
Oral	50.0 ± 12.6	4.0 ± 1.5	19.6 ± 8.6	24.8 ± 3.6

2. **Pharmacokinetic Modeling**: The majority of nalmefene clearance from the blood appears to be by glucuronidation followed by renal excretion. Individual fits of plasma nalmefene and the glucuronide demonstrated that the best fit (based on Akaike Information Criterion) was a 2 compartment open pharmacokinetic model for each. Therefore, the following model (Figure 2) was evaluated for plasma nalmefene and glucuronide concentrations fits with respect to the 5 mg iv ^{14}C-nalmefene administration. The assay was specific for intact nalmefene in plasma.

Figure 2: Pharmacokinetic Model which describes the plasma elimination of nalmefene from man.

This model fitted the data well as shown in Figure 3 and predicts an effective half-life of approximately 4.3 hours due to both the formation of the glucuronide and to a lesser extent the excretion of nalmefene from the central compartment.

Figure 3: Mean plasma data simultaneously fitted for nalmefene and glucuronide concentrations after iv administration of 5 mg ^{14}c-nalmefene, n=4.



3. **Study Comparison, 1 mg iv nalmefene:**

Pharmacokinetic parameters (i.e., Cmax, AUC, CL_{plasma}, etc.) were comparable across studies for the 1 mg nalmefene iv dose.

The dose of nalmefene that was consistently studied in the pharmacokinetic studies was 1 mg. Table 3 compares the pharmacokinetic parameters across two studies in healthy adult male volunteers after receiving 1 mg iv dose of nalmefene. The pharmacokinetic parameters seemed comparable across studies, except possibly Cmax. The slight difference may be due to the experimental differences between the two studies (i.e., sampling time window 4-6 minutes or technique in administering the bolus, etc.) .

Table 3: Comparison of pharmacokinetic parameters after 1 mg iv nalmefene. Mean (CV%).

Study #	Cmax 5 minute (ng/mL)	Vd_{ss} (L/Kg)	$AUC_{0\text{-}inf}$ (ng*hr/mL)	T1/2 β (hrs)	CL_{plasma} (L/hr/Kg)
9, n=6	3.16 (11)	7.4 (16)	17.0 (23)	7.9 (33)	0.94 (22)
10, n=12	5.89 (55)	8.0 (15)	17.6 (26)	11.1 (38)	0.75 (21)

Study 9: Ascending doses (0.5, 1, and 2 mg), Study 10: Cross-over design (iv, im and sc).
The Cmax value reported was the observed plasma nalmefene concentration taken at the first sampling time post dose.

4. **Dose Proportionality:**

Nalmefene exhibited dose proportional pharmacokinetics following intravenous administration from 0.5 mg to 2.0 mg.

Of the five dose proportionality studies submitted in this NDA, only one will be reported in this section, Study 9. The rationale for this choice is that Study R7 and Study 101 /101A used laboratory batches of nalmefene. These studies are considered pilot studies. Study JF-143 took the last blood sample time at 12 hours. This was not long enough to accurately predict the elimination portion of nalmefene or calculate $AUC_{0\text{-}inf}$. Therefore, Study JF-143 was considered supportive. Study 21, an ascending dose escalation study in elderly will be discussed under the Special Populations Section, I. Elderly.

Study 9 was a parallel ascending dose escalation from 0.5 mg to 1.0 mg to 2.0 mg iv nalmefene. There were a total of 18 healthy males aging from 19-35 years enrolled (6 for each dose) in this study. Study 9 claimed that nalmefene exhibited dose independent linear pharmacokinetic profiles in the range from 0.5 mg to 2.0 mg iv. Table 4 lists the pharmacokinetic results from this study. No statistically significant ($p<0.05$) departures from dose-proportionality were found by the sponsor. It can be argued that the wrong statistics were used in this study and that another statistical test (i.e., "power model") would have been appropriate. However, visual inspection and the stability of the AUC and Cmax parameters does not give one concern over this issue. The sponsor reported different Cl (L/hr/kg) values than what this reviewer calculated. The clearance values reported in Table 4 are the values this reviewer calculated, the sponsor reports, 0.5 mg (0.76 L/hr/kg), 1 mg (0.95 L/hr/kg) and 2.0 (0.93 L/hr/kg). The clearance value at the 2.0 mg dose is approximately 18% lower than at the other two doses, but the other parameters all seem to be dose proportional.

Table 4: Mean (CV%) pharmacokinetic parameter values after 0.5, 1.0 and 2.0 mg iv nalmefene, n=6 in each dose group. The study was a parallel design.

Nalmefene Dose (mg)	$AUC_{0\text{-}inf}$ ng*hr/mL	Ref. Dose 2 mg* $AUC_{0\text{-}inf}$ ng*hr/mL	Cmax 5 minute (ng/mL)	Ref. Dose 2 mg* Cmax (ng/mL)	Half-life (hrs)	Clearance (L/hr/Kg)
0.5	6.91 (15)	27.37	1.78 (42)	6.58	8.2 (17)	0.93 (8)
1.0	17.03 (23)	33.28	3.16 (11)	6.29	7.9 (33)	0.91 (1)
2.0	37.06 (26)	33.44	6.13 (18)	5.72	9.4 (17)	0.75 (10)

*Normalized to 2.0 mg dose, Least squares means were used for dose normalized $AUC_{0\text{-}inf}$ and Cmax. The Cmax value reported was the observed plasma nalmefene concentration taken at the first sampling time post dose.

5. **Multiple Dosing:** *No studies were submitted with multiple dosing.* Nalmefene will be administered for acute care procedures.

6. **Pharmacodynamics:**

Nalmefene appears to be more potent and longer lasting than naloxone when given in doses intended for the treatment of opioid overdose (i.e., about 1 mg (potency) to 2 mg (duration)). Nalmefene appears to be more potent than naloxone when given in doses intended for the reversal of induction and maintenance anesthesia, but there is no evidence that it is longer lasting in this dose range. Two subjects who were tested 24 hours after a single dose of 2 mg nalmefene still showed measurable effects (37% blockage).

Note: This study review was done by Dr. Curtis Wright. His review is attached in Appendix III, page 191. This reviewer agrees with the review and the conclusion.

Study 24 delineated the duration of action of naimefene relative to naloxone in humans, using dual probe detection of positron emissions from ^{11}C-carfentanil to evaluate brain opioid receptor occupancy. Eight normal male (n=4) and female (n=4) subjects age 18 to 65 years were studied. Subjects were first treated with the PET ligand ^{11}C-carfentanil and the total (specific and non-specific activity) uptake in brain was determined. The non-specific uptake of ^{11}C-carfentanil was then re-measured in a second session after pre-treatment with a naloxone bolus (10 mg iv) and naloxone infusion (21 µg/kg/hr). The doses completely blocked specific opioid uptake. These two values were then subtracted to determine the non-specific uptake and the specific uptake values (specific uptake = total uptake - non-specific uptake). In a third and fourth session, subjects were then treated with a single iv bolus of naloxone or nalmefene dose. Positron emissions were measured at 5, 120, 240 and 480 minutes with repeated doses of ^{11}C-carfentanil. The objective was to determine how much of the specific uptake was blocked by either nalmefene or naloxone. The data is presented in Figures 3 and 4. The conclusions of this study are stated in the first paragraph under this section (Pharmacodynamics).

Figure 3: Rate of loss of blockage after a single dose of 2 mg iv naloxone or 1 mg iv nalmefene at time 0.

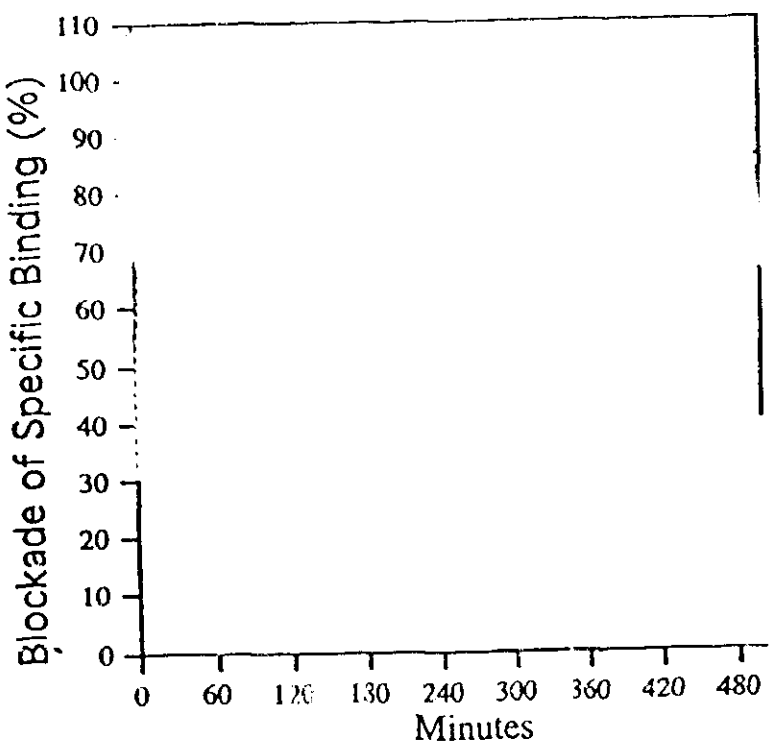
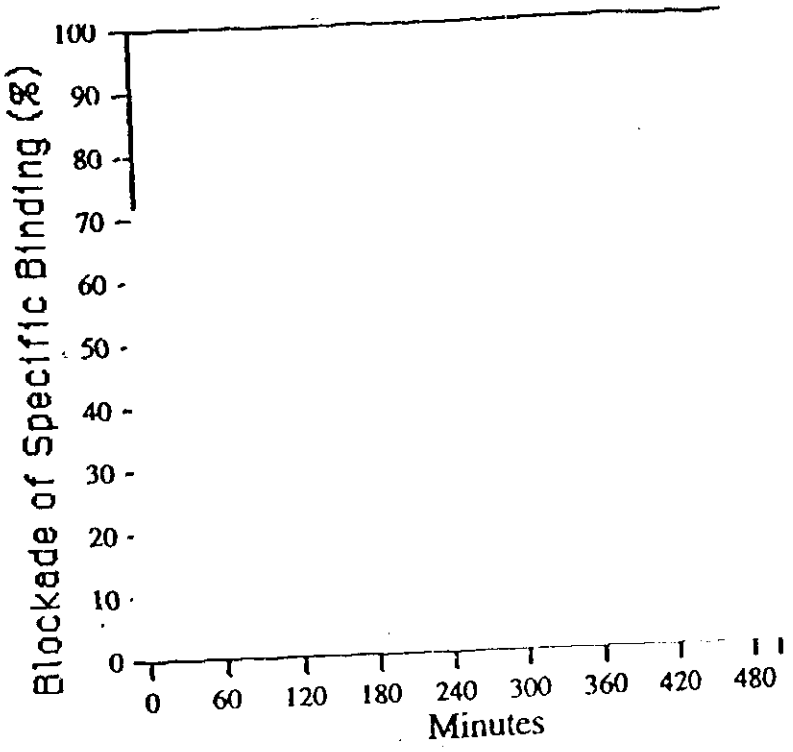


Figure 4: Rate of loss of blockage after a single dose of 2 μg/kg iv naloxone or 1 mg/kg iv nalmefene at time 0.



IV. SPECIAL POPULATIONS:

1. Elderly:

Dose proportionality was observed in AUC_{0-inf} from 0.5 to 2 mg iv nalmefene. Following a 1 mg iv nalmefene dose, there were no significant differences ($p<0.05$) between adult and elderly male subjects with respect to clearance, steady-state volume of distribution or half-life.

1. Study 19 was a parallel, ascending dose escalation study in which doses of 0.5, 1, or 2 mg iv nalmefene were administered to 36 elderly volunteers between the ages of 65 and 80 years. A second analysis compared the pharmacokinetic parameters at the 1 mg iv dose between healthy adult and elderly male volunteers. Three subjects were excluded in the elderly analysis. Two subjects were excluded from the 0.5 mg group because of anomalous plasma nalmefene concentrations. One subject had unusually low plasma concentrations (Cmax was 0.44 ng/mL) and one subject had unusually high (Cmax 23.9 ng/mL) concentrations. The third subject excluded was from the 1 mg group because the terminal slope was essentially flat. An analysis was performed to look at dose proportionality with and without the anomalous data. The outcome of the analysis remained the same in both instances. Table 5 presents the plasma nalmefene pharmacokinetic parameters (excluding the three subjects) obtained from this study. Both ANOVA and regression analysis supported the hypothesis that AUC_{0-inf} was proportional to nalmefene dose. The ANOVA was performed

on log-transformed data and the values for 0.5 mg and 1.0 mg doses were compared to the values for 2.0 mg dose via Student t-test ($p<0.05$) using residual means square error from ANOVA as an estimate of the population variance. The model for the analysis of variance contained treatment (TREATMENT) only. The normalized $AUC_{0\text{-}inf}$ and Cmax were linearly regressed on nalmefene dose and tests for zero-slope and for lack of fit were performed. The plasma elimination half-life was found to significantly shorter in 0.5 mg dose group than in 2.0 mg dose when analyzed by ANOVA, however, regression analysis did not detect any dose effect in the half-life. The normalized Cmax was significantly lower in the 0.5 mg dose group than in 2.0 mg dose when analyzed both by ANOVA and regression.

Table 5: Plasma nalmefene pharmacokinetic parameters in elderly males, Mean (CV%), n=33. This was a parallel study.

PK Parameter	Nalmefene 0.5 mg (n=10)	Nalmefene 1.0 mg (n=11)	Nalmefene 2.0 mg (n=12)
Cmax (ng/mL) 5 minute	2.02 (42)	6.65 (26)	17.49 (43)
T1/2 beta (hr)	7.5 (35)	9.8 (40)	10.3 (19)
MRT (hr)	9.1 (36)	11.0 (30)	11.0 (19)
$AUC_{0\text{-}inf}$ (ng*hr/mL)	7.92 (23)	18.06 (14)	32.93 (18)
Plasma Cl (L/hr/kg)	0.891 (31)	0.797 (13)	0.852 (18)
Vdss (l/Kg)	7.66 (26)	8.57 (30)	9.30 (25)

The Cmax value reported was the observed plasma nalmefene concentration taken at the first sampling time post dose.

2. Comparison of adult male (aging from 19-35 yrs) pharmacokinetic parameters and elderly male pharmacokinetic parameters were conducted by pooling adult males from Studies 9 (n=6) and 10 (n=12) in which a 1 mg iv nalmefene dose was administered to the subjects. In the elderly, following a 1 mg iv dose, blood samples were collected for 48 hours and assayed for nalmefene using radioimmunoassay procedures. The concentration-time profiles were best described using a 2 compartment open pharmacokinetic model in both age groups. The results of the compartmental analysis are described in Table 6. There were no significant differences ($p<0.05$) between the adult and elderly male subjects with respect to clearance, steady-state volume of distribution or half-life. The results demonstrated an age related decrease in the central volume of distribution that resulted in greater initial nalmefene concentration in the elderly group. Several physiological factors that vary with age include vascular volume, cardiac output, organ blood flow, tissue solubility and protein binding. These factors, either individually or cumulatively, may have contributed to the observed difference in the central volume of distribution. While initial nalmefene plasma

concentrations were transiently higher in the elderly, it would not be anticipated that this population would require dosing adjustment. No clinical adverse events were noted during this study.

Table 6: Nalmefene pharmacokinetic parameters (Mean (CV%)) calculated from a 2 compartment open pharmacokinetic model in adult (n=18) and elderly (n=11) male subjects following a 1 mg intravenous bolus dose.

Parameter	Adult Males	Elderly Males	p Value
Age (years)	23.8 (18)	70.7 (8)	-------
Cmax (ng/mL) 5 minute	3.72 (29)	5.8 (38)	0.0046
Vdss (L/kg)	8.55 (19)	8.59 (29)	0.8930
Vc (L/kg)	3.85 (29)	2.82 (41)	0.0151
$AUC_{0\text{-}inf}$ (ng*hr/mL)	16.64 (27)	17.3 (14)	0.4677
T1/2 β (hr)	10.77 (48)	9.38 (49)	0.4866
Cl_{plasma} (L/hr/kg)	0.79 (33)	0.833 (18)	0.4676

The Cmax value reported was the observed plasma nalmefene concentration taken at the first sampling time post dose.

3. Compartmental analysis of nalmefene pharmacokinetics following an iv 1 mg dose in adult and elderly male subjects was performed. Nalmefene pharmacokinetics were determined by weighted nonlinear least squares regression analysis using PC-Nonlin (Ver.4.0). The concentration-time data were fitted by both a two and three compartment model with first-order elimination and iv bolus input. Model selection was based on visual inspection of the data, Akaike information criterion, and the standard error of the parameter estimates. A two-compartment model with weights of $1/Y^2$ was selected as the best model. Thus the data were fit to the following equation: $Cp(t) = Ae^{-\alpha t} + Be^{-\beta t}$. The summary of pharmacokinetic parameter estimates is provided in Table 7 on the next page.

Table 7: Nalmefene pharmacokinetic parameters from a two-compartmental open model fit (Mean ± SD)

Parameter	Adult Males (n=18)	Elderly Males (n=11)	p-Value
A (ng/mL)	2.78 ± 1.14	4.58 ±1.84	0.0085
B (ng/mL)	0.94 ± 0.52	1.25 ± 0.52	0.1624
α (hr^{-1})	1.01 ± 1.21	2.34 ± 2.1	0.0233
β (hr^{-1})	0.079 ± 0.033	0.087 ± 0.030	0.4866

2. **Hepatic:**

Subjects with liver disease, when compared to matched normal controls, had a 28.3% decrease in plasma clearance of nalmefene. Elimination half-life increased from 10.2 hours to 11.9 hours in the hepatically impaired group. No dosage adjustment will be recommended since nalmefene will be administered as an acute course of therapy.

Study 21 enrolled 12 hepatically impaired patients (5 mild, 5 moderate, 3 severe) and 12 normal control subjects. Subjects in the normal group were matched in age, sex, weight and race to a corresponding subject in the hepatically impaired group. Hepatically impaired subjects were classified based on Pugh's Classification. There were 4 women out 24 subjects enrolled in the study; two in the normal group, 1 in the moderate group and 1 subject in the severe group. The pharmacokinetic parameters of nalmefene were determined following intravenous bolus administration of 2.0 mg nalmefene. Table 8 lists the results. Subjects with liver disease had a 28.3% decrease in plasma clearance of nalmefene compared to normal subjects (0.56 L/hr/kg versus 0.78 L/hr/kg, respectively). Elimination half-life in the hepatically moderate and severe patients increased from 10.2 hours to 12.2 hours. One subject (male) in the mild hepatically impaired group was not included in the analysis due to unusually high plasma nalmefene concentrations at the 18 hour (65.8 ng/mL) and 48 hour (0.7 ng/mL) time points. Given the range observed among all other subjects, one subject was considered an outlier by the sponsor. The average nalmefene concentration of the other patients at 18 hours was 1.1 ng/mL and 0.2 ng/mL at 48 hours. It is acknowledged that the value reported for the 1 minute Cmax parameter appears to be very different among all the groups. However, this is considered to a function of study design rather than a physiological phenomenon because by the 5 minute time point all the Cmax values reported seem in agreement among the groups.

A P450 metabolic probe analysis (caffeine, chlorzoxazone, debrisoquine, mepheytoin, dapsone) was done but not completed in time for the nalmefene analysis.

Table 8: Summary of plasma nalmefene pharmacokinetic parameters following a 2 mg iv nalmefene dose. Mean (CV%).

	Cmax* 1 minute 5 minute (ng/mL)	T1/2 β (hrs)	$AUC_{0\text{-}inf}$ ng*hr/mL (2 mg dose)	Cl_{plasma} (L/hr/kg)	Cl_{renal} 0-48 hours (L/hr/kg)
Normal n=12	64.6 (69) 18.2 (25)	10.2 (22)	34.3 (33)		
Hepatic pooled 12	50.2 (52) 16.7 (18)	11.9 (17)	45.2 (31)		
Mild n=4	57.0 (54) 14.5 (22)	11.2 (18)	34.4 (5)		
Moderate n=5	58.3 (42) 17.8 (15)	12.2 (20)	49.8 (25)		
Severe n=3	27.7 (25) 17.9 (17)	12.2 (16)	51.9 (40)		?)

*Cmax is reported for 1 minute and 5 minute sampling times. The 5 minute is included for cross study comparisons.

3. **Renal:**

There was a statistically significant 27% decrease in plasma clearance of nalmefene in the end stage renal disease (ESRD) population during interdialysis and a 25% decreased plasma clearance in the ESRD population during intradialysis compared to normals. The half-life was prolonged in end-state renal disease patients from 10.2 hours in normals to 26.1 hours.

In Study 22, 1.0 mg of iv nalmefene was administered to 8 volunteers (3 female, 5 male) with ESRD on chronic hemodialysis therapy. The twelve normal subjects from Study 21 (Hepatic impairment) were used for control data. During the first interdialysis clinic visit (1 day immediately post-hemodialysis and prior to the next hemodialysis treatment) each subject received 1 mg bolus dose of nalmefene iv. After a 1 to 2 week washout, subjects returned to the dialysis clinic where they received another 1 mg bolus dose of nalmefene iv 4 hours prior to hemodialysis (intradialysis). Table 9 presents the results. With respect to inter- and intradialysis, dialysis did not significantly alter the drug elimination pattern.

Table 9: Plasma nalmefene pharmacokinetic parameters in normal and end-stage renal disease after 1 mg iv nalmefene, Mean (CV%), n=8. Normal subjects are from the Hepatic impaired study in which 2 mg iv nalmefene was administered, n =12.

Subjects	Cmax 5 minute (ng/mL)	T1/2 β (hours)	$AUC_{0\text{-}inf}$ (ng*hr/mL)	Cl_{plasma} (L/hr/kg)	Cl_{renal} 0-48 hours (L/hr/kg)
Interdialysis	6.85 (34)	26.1 (38)	28.3 (32)		
Intradialysis#	15.23 (139)	25.7 (67)	27.5 (31)		
Normals normalized to 1 mg	18.2 (25)	10.2 (22)	34.3 (33)		

* Interdialysis last sample collections were at 44 hours.

Dialysis time 4 -5 hours.

The Cmax value reported was the observed plasma nalmefene concentration taken at the first sampling time post dose.

4. **Gender Analysis:**

No definitive statement can be made as to whether the pharmacokinetics of nalmefene differs between genders due to the small number of women enrolled in the studies.

All the pharmacokinetic studies enrolled men with the exception of the hepatic impairment and renal impairment studies in which men and women were enrolled. Four (4) out 24 subjects enrolled in the hepatic study were females; two were enrolled in the normal group, 1 in the moderate group and 1 in the severe group. There was inadequate power to draw any conclusion regarding whether female subjects had a different pharmacokinetic profile than men following a single 2.0 mg iv nalmefene dose. In the renal study, 3 out of 8 patients enrolled were women. Table 10 lists a comparison of some of the pharmacokinetic parameters after a single 1.0 mg iv nalmefene dose. Based on these small numbers, it appears that women with ESRD may clear nalmefene from the plasma faster than men (36% faster) with ESRD. In fairness to the sponsor, Ohmeda assumed sponsorship (1988) of the NDA from Ivax Baker Cummins who purchased the sponsorship (1987) from Key Pharmaceuticals. Clinical development by Key Pharmaceuticals was initiated in 1983 through 1986, a period of time when women were not enrolled in pharmacokinetic studies.

Table 10: Comparison of pharmacokinetic parameters between genders that have end-stage renal disease. Subjects were administered 1 mg iv nalmefene dose, Mean (CV%).

Study 22	Cmax (ng/mL) 5 minute	T1/2 β (hours)	CL_{plasma} (L*hr/kg)
Women, n=3	7.8 (28)	25.5 (48)	0.42 (33)
Men, n=5	6.3 (38)	26.4 (37)	0.66 (27)

The Cmax value reported was the observed plasma nalmefene concentration taken at the first sampling time post dose.

V. DRUG INTERACTIONS:

No formal drug interaction studies were submitted to this NDA. Apparently in two of the clinical studies in which nalmefene was administered for the reversal of opioid depression postoperatively, nalmefene was found to be safe and effective in the presence of a number of drugs used in surgical procedures. Specifically, premedication with anticholinergic, antihistaminic, antibacterial and antiemetic drugs; operative medication with antihypertensive, inhalational anesthetic and neuromuscular blocking agents; and postoperative medication with antacid, antiemetic and narcotic analgesic agents.

VI. ANALYTICAL:

Nalmefene in plasma and urine was analyzed by a modified nethod . The linear range of this analytical If samples were above the limit of quantitation during the analysis they were diluted with blank control plasma and reassayed (2 to 200 fold dilutions). The lower limit of quantification was 0.056 ng/mL. The mean percent recovery for the plasma extraction was 87.8%. Table 11 summarizes the interday precision and accuracy of the assay in plasma.

Table 11: Interday precision and accuracy in plasma for nalmefene.

Conc. Added (ng/mL)	Mean Measured Conc. (ng/mL)	Number of samples	%CV
0.169	0.155	54	25.8
0.339	0.346	54	14
0.677	0.702	53	15.7

In urine, the standard curve was linear from 0.3125 to 8 ng/mL for total nalmefene in urine (glucuronide metabolites and nalmefene) and from 0.03125-8 ng/mL for free urine (nalmefene). In urine the recovery was 89.7%. Table 12 summarizes the interday precision and accuracy in urine.

Table 12: Interday precision and accuracy in urine for total (glucuronide metabolites and nalmefene) and free (nalmefene).

Conc. Added (ng/mL)	Conc. Found (ng/mL)	Number of samples	%CV
0.677	0.582	58	24.1
1.35	1.23	60	12.8
3.60	3.18	60	14.2

The assay specificity for intact nalmefene in clinical plasma samples was determined by comparing it with an established a high performance liquid chromatography procedure. Over a concentration range of 4-58 ng/mL the correlation coefficient, regression line-slope, and y-intercept were 0.998, 0.996 and 0.38, respectively.

VII. STUDY CONCLUSIONS:

From the studies submitted in support of this NDA, the Sponsor has adequately described the pharmacokinetics of nalmefene after intravenous administration. In particular the Sponsor has demonstrated the following:

1. The relative bioavailability of nalmefene from intramuscular and subcutaneous routes of administration were 101.5% (CV 8%) and 99.7% (CV 7%), respectively, compared to the intravenous route of administration.

2. The pharmacokinetics were adequately described from single dose administration. In addition, nalmefene exhibited dose linear pharmacokinetic profile following intravenous administration from 0.5 mg to 2.0 mg.

3. The pharmacokinetics in elderly males were adequately described following a single 0.5 mg, 1 mg or 2 mg iv nalmefene dose. Dose proportionality was observed in $AUC_{0\text{-}inf}$ from 0.5 to 2 mg iv nalmefene in the elderly. Following a 1 mg iv nalmefene dose, there were no significant differences between adult and elderly male subjects with respect to clearance, steady-state volume of distribution or half-life.

4. **Comments on the NDA Submission:** Ohmeda submitted an excellent pharmacokinetic submission in terms of organization. Most of the information (i.e., formulation, study design, data, etc.) was easily accessible and well documented. However, some of the Sponsor's results require careful interpretation or validation. If someone were to research the original jackets for information, the following might be useful:

1. Study 10: Renal clearance for nalmefene 0-48 hours was incorrectly calculated for the iv, im and sc routes of administration. Recalculated values are attached in Appendix III at the end of Study 10.

2. Volume of distribution parameter values are actually values for volume of distribution at steady-state which were calculated by Clearance*MRT.

3. Study 9: The plasma clearance values (L/hr/kg) reported by the sponsor for the 0.5 mg, 1 mg, and 2 mg doses are in disagreement (See Section Pharmacokinetics, 4: Dose Proportionality).

4. Study 24: Not enough information was provided to validate the % blockage values generated, specifically the baseline mean normalized activity. The information was requested and is placed in Appendix III after Study 24 details.

VIII. LABELLING:

Attached in Appendix IV, page 203, is the first draft of the label submitted by the Sponsor. The Division of Biopharmaceutics has a standardized pharmacokinetic label that groups information systematically. In keeping with this recommendation the following pharmacokinetic section of the label is proposed for nalmefene.

Pharmacokinetics

Nalmefene will be administered primarily as an intravenous bolus during acute care procedures, however, nalmefene can be given intramuscularly or subcutaneously if venous access cannot be established.

Absorption

Nalmefene was completely bioavailable following intramuscular or subcutaneous administration in 12 male volunteers relative to intravenous nalmefene. The relative bioavailabilities of intramuscular and subcutaneous routes of administration were 101.5%$\pm$8.1% (Mean $\pm$ SD) and 99.7%$\pm$6.9%, respectively. The time to maximum plasma nalmefene concentration was 2.3$\pm$1.1 hours following intramuscular and 1.5$\pm$1.2 hours following subcutaneous administrations. Nalmefene exhibited dose proportional

pharmacokinetics from 0.5 mg to 2.0 mg following intravenous administration. The following pharmacokinetic parameters for nalmefene after a 1 mg intravenous administration in 18 adult and 11 elderly male volunteers were derived and are listed in Table 1.

Table 1: Nalmefene pharmacokinetic parameters (Mean (CV%) in 18 adult males, aging from 19-35 years and in 11 elderly males, aging from 65-80 years, following a 1 mg intravenous dose.

Parameter	Cmax (ng/mL) 5 minute*	Vdss (L/kg)	Vc (L/kg)	$AUC_{0\text{-}inf}$ ng*hr/mL	T1/2 β (hr)	Cl_{plasma} (L/hr/kg)
Adult (n=18)	3.72 (29)	8.55 (19)	3.85 (29)	16.64 (27)	10.77 (48)	0.79 (33)
Elderly (n=11)	5.89 (38)	8.59 (29)	2.82 (41)	17.30 (14)	9.38 (49)	0.83 (18)

* The Cmax value reported was the observed plasma nalmefene concentration taken at the first sampling time post dose.

Distribution

Following 1 mg parenteral dose, nalmefene was rapidly distributed. In a study of brain receptor occupancy, a 1.0 mg dose of nalmefene blocked over 80% of brain opioid receptors within 5 minutes after administration. The apparent volume of distribution centrally (Vc) and at steady-state (Vdss) is 3.85 ± 1.1 L/kg and 8.5 ± 1.7 L/kg, respectively. Ultrafiltration studies of nalmefene have demonstrated that 45% (CV 4.1%) is bound to plasma proteins over a concentration range of 0.1 to 2 µg/mL. An *in vitro* determination of the distribution of nalmefene in blood demonstrated that nalmefene distributed 67% (CV 8.7%) into red blood cells and 39% (CV 6.4%) into plasma over the nominal concentration range in whole blood from 0.376 to 30 ng/mL. The whole blood to plasma ratio was 1.29 (CV 6.6%) from 0.376 to 30 ng/mL.

Metabolism

Nalmefene is metabolized by the liver, primarily by glucuronide conjugation, and excreted in the urine. Less than 5% of nalmefene is excreted in the urine unchanged. Trace amounts of N-dealkylated nalmefene have been detected in the urine. Seventeen percent (17%) of nalmefene dose is excreted in the feces.

Elimination

After intravenous administration of 1 mg nalmefene, plasma concentrations declined biexponentially, with a redistribution and a terminal elimination half-life of 41 ± 34 minutes and 10.8 ± 5.2 hours, respectively. The systemic clearance of nalmefene is 0.79 ± 0.24 L/hr/Kg and the renal clearance is 0.078 ± 0.042 L/hr/Kg.

Special Populations

Elderly: Dose proportionality was observed in $AUC_{0\text{-}inf}$ from 0.5 to 2 mg iv nalmefene in elderly male subjects (65-80 years). Following a 1 mg iv nalmefene dose, there were no significant differences ($p<0.05$) between adult and elderly male subjects with respect to clearance, steady-state volume of distribution or half-life. There was an apparent age related decrease in the central volume of distribution (adult; 3.9 $\pm$ 1.1 L/kg, elderly; 2.8 $\pm$ 1.1 L/kg) that resulted in greater initial nalmefene concentration in the elderly group. While initial nalmefene plasma concentrations were transiently higher in the elderly, it would not be anticipated that this population would require dosing adjustment. No clinical adverse events were noted in the elderly following the 1 mg intravenous nalmefene dose.

Hepatic: Subjects with liver disease, when compared to matched normal controls, had a 28.3% decrease in plasma clearance of nalmefene (0.56 $\pm$ 0.21 L/hr/kg versus 0.78 $\pm$ 0.24 L/hr/kg, respectively). Elimination half-life increased from 10.2 $\pm$ 2.2 hours to 11.9 $\pm$ 2.0 hours in the hepatically impaired. No dosage adjustment is recommended since nalmefene will be administered as an acute course of therapy.

Renal: There was a statistically significant 27% decrease in plasma clearance of nalmefene in the end stage renal disease (ESRD) population during interdialysis (0.57 $\pm$ 0.20 L/hr/kg) and a 25% decreased plasma clearance in the ESRD population during intradialysis (0.59 $\pm$ 0.18 L/hr/kg) compared to normals (0.79 $\pm$ 0.24 L/hr/kg). The half-life was prolonged in ESRD patients from 10.2 $\pm$ 2.2 hours in normals to 26.1 $\pm$ 9.9 hours. See Dosage and Administration.

Gender Differences: No definitive statement can be made as to whether the pharmacokinetics of nalmefene differs between genders.

IX. PHASE IV COMMITMENTS:

1. A commitment to continue to recruit pediatric patients into Study

2. To finish the P450 metabolic probe analysis and submit the results to the agency within 6 months.

X. FINAL CONCLUSIONS:

Based on the information presented in this NDA submission, the product that is the subject of NDA 20-459 , Nalmefene HCl Injection, is approvable from a biopharmaceutic standpoint, provided that the Sponsor commits to work with the Agency to address phase IV commitments and labeling changes.

Ruth E. Stevens, Ph.D.

Ruth E. Stevens 3-3-95

Senior Pharmacokineticist
Pilot Drug Evaluation Staff

Peer Reviewer, Peter Lockwood, MSc. P Lockwood 3-6-95

Biopharmaceutics Day (2/24/95), Collins, Ludden, Malinowski, Fleischer, Chen, Hepp, Gillespie, Hussain, Lockwood and Stevens

cc:

HFD 007 original NDA 20-459	(X1)
HFD 007 CSO (McNeal)	(X1)
HFD 007 Stevens, PK files	(X2)
HFD 426 (Fleischer)	(X1)
HFD 427 (Chen)	(X1)
HFD 420 (Chron., Drug, Reviewer)	(X3)
HFD 19 (FOI)	(X1)
HFD 344 (Viswanathan)	(X1)

NALMEFENE NDA PHARMACOKINETICS

Study Type: Disposition and Metabolism Study #: JF137 Vol. 1.24

Investigator: Peter Dewland, MD

Study Site: Simbec Research Ltd.
Merthyr Tydfil, UK

Single Dose: XX Multiple Dose:
Subjects: Normal XX Patients Young Elderly
Impaired: Renal Hepatic

Cross-over: XX Parallel: N= 4 M= 4 F=

Subject Type: Normal Males

Weight: Mean= Unk Range=

Age: Mean= Unk Range=

Treatment	Subj.#	Dose	Dosage Form	Strength	Lot#	Lot Size

Fasted Yes; 10 hrs. prior to dosing and 3 hrs. post dosing

Nonfast: No Food Study: No FDA High Fat Breakfast: No

Samples:	Plasma 10ml	0, 5, 10, 15, 30 minutes; 1, 2, 3, 4, 6, 7, 10, 12, 16, 24, 36, 48 72, 96, 120 hours
	Urine total	0-6, 6-12, 12-24, 24-48, 48-72, 96-120 hours
	Feces total	Collected at every decation

Labeling Claims from Study: Nalmefene had a high systemic clearance and underwent extensive metabolism to a glucuronide.

Disposition and Metabolism of 14C-Nalmefene Following Intravenous and Oral Administration to Man.

STUDY NO. JF-137 VOLUME: 1.24 PAGES: 195-236

The review of study JF-137 has been reviewed in detail by Dr. Curtis Wright (medical officer in HFD-007). There are a few additional points from this study that this reviewer would like to address:

1. Modeling from the IV administration: The model that was proposed by Dr. Curtis Wright (see his full review in Appendix V) was a simplified model and the estimates should be interpreted carefully. His estimates of the pharmacokinetic parameters were derived numerically from a simultaneous fit between nalmefene and the glucuronide. Based on the data, this reviewer proposes the following model for nalmefene and the glucuronide:

The simultaneous equations for nalmefene and the glucuronide were solved explicitly. The model fit the data well (as shown below).

In the model it should be noted th[illegible] can not be distinguished, because we don't know the exact amount that is being eliminated as nalmefene, glucuronide or unconjugated metabolites. The concentrations of nalmefene and the glucuronide were averaged from 4 male volunteers and then fitted to the model proposed on the first page. The mean pharmacokinetic estimates are as follows:

2. Urinay excretion accounted for approximately 50% of the radioactivity, 5% as unconjugated metabolites. TLC analysis inidicated that nalmefene accounted for most of the unconjugated metabolites. Trace amounts of N-dealkylated nalmefene were detected. Nalmefene glucuronide accounted for most of the conjugated metabolites. Table 1 contains the results.

Table 1. Excretion and Distribution of radioactivity in urine and feces following iv, 5 mg, ^{14}C-nalmefene HCl and oral, 200 mg, ^{14}C-nalmefene HCl. Mean $\pm$ Std, n=4. Duration of the urine collection was from 0-120 hours and for feces, 0-160 hours.

Route of Administration	Urine Total ^{14}C (% of dose)	Urine Unconjugated (% of dose)	Urine Conjugated (% of dose)	Feces ^{14}C-nalmefene (% of dose)
IV	49.4 $\pm$ 15.2	5.2 $\pm$ 1.6	33.2 $\pm$ 2.2	17.4 $\pm$ 0.7
Oral	50.0 $\pm$ 12.6	4.0 $\pm$ 1.5	19.6 $\pm$ 8.6	24.8 $\pm$ 3.6

3. Nalmefene exhibited a percent binding to plasma proteins of 45 $\pm$ 1.8% in the concentration range of 0.1 to 2 ug/ml.

NDA #: 20,459.000
Trade and generic name & form: Nalmefene (Revex) Injection
Sponsor: Ohmeda
Letter Date: 4/27/94
Date Completed:

Type of Submission: NDA Submission, New Study
Date Received: 4/29/94
Reviewer: Curtis Wright MD, MPH
Peer Reviewer: Bob Bedford, MD
Security Level: TradeSecret/Confidential (FDA and Special Government Employees)
ACCOUNTING DATA: RESEARCH- 1 hr
WRITING- 30 min

MOR TRIAL JF-137

PROTOCO JF-137

DISPOSITION AND METABOLISM OF ^{14}C-NALMEFENE FOLLOWING INTRAVENOUS AND ORAL ADMINISTRATION TO MAN

Principal Investigator: Peter Dewland, M.D.
Department of Clinical Research
Simbec Research Ltd.
Merthyr Tydfil, U.K.

Study Publication: None
Study Initiation: July 17, 1985
Study Completion: August 19, 1985

Study Objective
The objective of this study was to evaluate the metabolic profile of ^{14}C Nalmefene following administration of single oral and intravenous doses of the radio-labeled drug to male volunteers.

Study Design as planned

Each subject received a pre treatment medical history, physical examination and clinical laboratory evaluations. The subjects had no history of hypersensitivity to drugs and avoided any self-administered medication for 14 days prior to and for the duration of the study.

Following an overnight fast of at least 10 hours each volunteer received treatment A (i.v.dose) and the fast maintained for 3 hours after the dose. After a 14 day wash-out period between the last blood samples collected following treatment A, each subject received treatment B (oral dose) with the same fasting restrictions.

This study was an open labeled study with four subjects each receiving the drug i.v. and orally in solution, so that each subject receives a total radiation dose of 100 µCi, (i.e. 50 µCi from the i.v. dose and 50 µCi from the oral dose).

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Results

IV dosing- Nalmefene appeared rapidly in plasma following IV administration and accounted for the major portion of the total ^{14}C-activity in plasma. Plasma protein binding of nalmefene was 45% and remained constant over a concentration range of 0.1 to 2 mcg/mL. Nalmefene clearance from the blood appears to be by glucronidation followed by renal excretion (see below).

Modeling- The following model was evaluated with respect to the IV administration:

This model fit the data well (as shown), and showed an effective half-life due to formation of the glucuronide of 4 hours, and a peripheral reservoir that maintains a low plasma level for longer.

70 k

TIME (hours)

Enterohepatic recirculation or other hydrolysis of the glucuronide is suggested, but unproved.

Treatment A consisted of an intravenous bolus injection of 6 mg of ^{14}C-nalmefene HCl with an activity of approximately 50 µCi. The 25 mg injection of ^{14}C-nalmefene HCl (SA 8.43 µCi per mg) was dissolved in 25 mls of isotonic saline and 6 mls of the resulting solution was administered to each of the four subjects.

Treatment B consisted of 200 mg of oral nalmefene in which there was 6 mg of ^{14}C nalmefene HCl. The dose was prepared as follows: 30 mg of ^{14}C nalmefene HCl (SA 8.43 µCi per mg) was diluted with 970 mg of unlabelled nalmefene (i.e. cold nalmefene) and the whole 1 g was dissolved in 500 mls of water.

Heparinized blood (10 mL) was obtained immediately before and at 5, 10, 15, 30 min., 1, 2, 3, 4, 6, 7, 10, 12, 16, 24, 36, 48, 72, 96 and 120 hours after Treatment A. The 5 and 10 minute samples were omitted following the oral Treatment B. Plasma was separated and stored frozen in plastic tubes at -20° until assayed.

Urine was collected at 0-6 6-12, 12-24, 24-48, 48-72 and 96-120 hours. The volume of each collection was recorded and a 10 mL aliquot taken and frozen to await analysis. Total fecal collections were obtained at each defecation for a period of 7 days and the time of each collection marked.

Drug Administration as performed

Treatment A consisted of an i.v. bolus injection of 4.92 mg of ^{14}C-nalmefene HCl with a total activity of 41.49 µCi, as a solution in 5 mL of isotonic saline.

Treatment B consisted of an oral dose of 198.7 mg of ^{14}C-nalmefene HCl with a total activity of 43.63 µCi as a solution on 100 mL of water. (The dose was prepared by appropriate dilution of the ^{14}C-nalmefene HCl used in Treatment A with unlabelled drug.) The drinking glass was rinsed twice with 50 mL of water and the contents swallowed.

Oral Dosing
The oral dose was readily absorbed and peak plasma concentrations of nalmefene and its major metabolite, nalmefene glucuronide, were reached at 1-2 hour after dosing.

Mass balance
Up to 70% of the total i.v. ^{14}C-dose was excreted in the urine with 17% in the feces. Of the total ^{14}C oral dose, up to 60% was excreted in the urine with 27% in the feces. Only about 3-6% of the dose was excreted in urine as intact nalmefene following both routes of administration and nalmefene glucuronide was the major metabolite excreted.

Nalmefene has a high systemic clearance and undergoes metabolism to its glucuronide conjugate which may be subject to enterohepatic cycling. N-dealkylation does not play a significant role in the metabolism of the drug.

Conclusions

Nalmefene is a drug which has a high systemic clearance and is metabolized in man almost exclusively to its 3-0-glucuronide conjugate. Following i.v. and p.o. administration of ^{14}C labeled drug, up to 80% of the dose is excreted in the urine and feces over a period of one week. Assay methodology used in study of the drug must be able to differentiate the parent from the glucuronide due to the high glucuronide levels, and conditions affecting glucuronidation are likely to affect clearance of the drug.

CC: Original NDA
HFD-007 Division File
HFD-007 C Wright
HFD-007 CSO Mcneal
HFD-340
R/D & F/T : CWIV
Location: CWIV Archive

C Wright 6/15/94
Curtis Wright
Team Medical Review Officer
PDES (HFD-007)

RF Bedford 6/22/94
Robert Bedford
Senior Medical Officer for Anesthesia

12 pages
PURGED
Patient
info

NALMEFENE NDA PHARMACOKINETICS

Vol. 1.25

Study Type: Bioavailability Study #: 10
Investigator: James Kisicki, MD
Study Site: Harris Laboratories
624 Peach Street
Lincoln, NE 68501

Single Dose: XX Multiple Dose:

Subjects: Normal XX Patients: Young Elderly

Impaired: Renal Hepatic

Cross-over: XX Parallel: N= 12 M= 12 F=

Subject Type: 1.0 mg Normal Males

Weight: Mea =80.4 Rang

Age: Mean=24 Rang

Treatment	Subj.#	Dose	Dosage Form	Strength	Lot#	Lot Size
Treatment	1-12	1.0 mg	Intravenous Subcutaneous Intramuscular	1.0 mg/ml	3495-045	11,950

Fasted Yes; 8 hrs. prior to dosing and 4 hrs. post dosing

Nonfast: No Food Study: No FDA High Fat Breakfast: No

Samples:	Plasma 10ml	0, 2, 5, 10, 15, 30 minutes; 1, 2, 2.5, 3, 4, 6, 8, 10, 12, 18, 24, 48 hours
	Urine 48 Hours	0-4, 4-8, 8-12, 12-16, 16-24, 24-48

Assay Metho
Assay Sensitivity

Labeling Claims from Study: The relative bioavailabilities of intramuscular and subcutaneous routes of administration were 101.5±8.1% and 99.7±6.9%. Both the intramuscular and subcutaneous routes of administration gave ~~therapeutic~~ plasma levels of 0.5 ng/ml in all subjects within 15 minutes.

at least ↑ ↑ at least
nalmefene

Concur with conclusion as modified.
Ruth E. Stevens 2-13-95

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A Bioavailability Study of Nalmefene Given Via Intravenous, Intramuscular, and Subcutaneous Routes in Healthy Volunteers.

STUDY NO. #10 VOLUME: 1.25 PAGES: 1-336

This review is additional results from Study 10 that was not mentioned on the previous study detailed sheet. The assay validation was reviewed by Iftekhar Mahmood, Ph.D. from the Division of Biopharmaceutics, FDA and will not be reviewed again. His findings are attached at the end of this study and are acceptable.

Results from Study 10:

1. The relative bioavailabilities of intramuscular and subcutaneous routes of administration were 101.5 $\pm$ 8.1% and 99.7 $\pm$ 6.9%.

2. Nalmefene in plasma after a 1 mg single dose, Mean $\pm$ Std, n=12.

Route of Administration	AUC0-inf (ng*hr/mL)	Cmax (ng/mL)	Tmax (hrs)	Bioavailability %
IV	17.61 (4.64)	5.89 (3.22)	----	Reference
IM	16.98 (3.02)	1.47 (0.36)	2.29 (1.12)	101.5 (8.1)
SC	17.15 (4.27)	1.32 (0.26)	1.48 (1.23)	99.7 (6.9)

3. Summary of elimination and clearance after administration of 1 mg nalmefene, Mean $\pm$ Std, n=12:

Route of Administration	T1/2β (hrs)	Total Systemic Clearance (L/hr/kg)	Renal Clearance (L/hr/kg) 0-48 hrs
IV	11.11 (4.28)	0.75 (0.16)	0.06 (0.03)
IM	9.65 (3.78)	0.76 (0.15)	0.06 (0.03)
SC	10.77 (4.10)	0.77 (0.19)	0.05 (0.03)

4. Nalmefene in Urine: Summary of amount of nalmefene dose eliminated in the urine and renal clearance after administration of 1 mg nalmefene, Mean ± Std, n = 12:

Route of Administration	Amount of Free and Conjugates (%of Dose)	Free Nalmefene (%of Dose)	Ave. Renal Clearance 0-4 hrs (L/hr/Kg)	Ave. Renal Clearance 12-24 hrs (L/hr/Kg)
IV	40.3 (9.2)	7.5 (3.6)	0.07 (0.06)	0.07 (0.03)
IM	36.2 (7.2)	7.6 (2.5)	0.09 (0.06)	0.05 (0.02)
SC	39.0 (9.9)	6.9 (3.0)	0.08 (0.04)	0.06 (0.03)

Revex™ (nalmefene hydrochloride injection)

Study 10 - *"A Bioavailability Study Of Nalmefene Given Via Intravenous, Intramuscular, And Subcutaneous Routes In Healthy Volunteers"*

A programming error was detected in calculating the total free nalmefene urine concentration levels between 0 and 48 hours (Tables 14, 20, and 26). The amount was erroneously calculated as the sum of free nalmefene urine concentration levels between 12-16 hours and 24-48 hours only. The free nalmefene urine concentration levels inadvertently excluded were 0-4, 4-8, 8-12, and 16-24 hours.

The following summary tables were therefore revised accordingly and are attached:

Table 14 Free Nalmefene Renal Clearance (L/hr/kg) for Intravenous Nalmefene Subjects

Table 20 Free Nalmefene Renal Clearance (L/hr/kg) for Intramuscular Nalmefene Subjects

Table 26 Free Nalmefene Renal Clearance (L/hr/kg) for Subcutaneous Nalmefene Subjects

In reviewing all study reports relating to renal clearance, it was determined that this error related only to Study 10. In Studies 9 and 19, no urine collections were made. In Studies 21 and 22, (Table 9.7.2.1, Volume 30, page 60 and Table 9.6.2.1, Volume 32, page 56, respectively) the appropriate urine concentrations were used. The free nalmefene renal clearance of subjects in those studies was found to be comparable to the revised results for the renal clearance of subjects in Study 10.

TELECOPY/FAX TRANSMISSION

Attn.:	Ms. Bonnie McNeal	From:	Ingrid Smith
At:	FDA - Pilot Drug Evaluation	Dept:	Regulatory Affairs
Fax #:	(301) 443-4935	Tel #:	(908) 604-7701
Date:	February 13, 1995	# pages (total)	5

RE: **Revex™ (nalmefene hydrochloride injection)**
NDA 20-459
Responses to Clinical Pharmacokinetic Reviewer's Questions

Dear Bonnie:

Attached are Ohmeda's responses to Dr. Stevens' questions from Friday, February 10, 1995, concerning the Free Nalmefene Renal Clearance values in Study 10. Please let us know if further clarification is needed.

Sincerely,

Ingrid

Ingrid Smith
Director - Domestic Regulatory Affairs

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P.2

FEB 13 '95 04:55PM

3 pages

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"Patient

Info

NALMEFENE NDA PHARMACOKINETICS

Volume 1.26

Study Type: Pilot Single Application Pharmacokinetics Study #: 101A
Investigator: Donald Weidler, MD PhD
Study Site: University of Miami School of Medicine Division of Clinical Pharmacology
PO Box 015996 (D7-3) Miami, FL 33101

Single Dose: XX Multiple Dose:
Subjects: Normal XX Patients Young Elderly
Impaired: Renal Hepatic

Cross-over: Parallel: XX N= 24 M= 24 F=

Subject Type: 6.0 mg Normal Males

Weight:lbs Mean=160.8 Range:

Age: Mean= Range=

Subject Type: 12.0 mg Normal Males

Weight:lbs Mean=151.3 Range:

Age: Mean= Range=

Subject Type: 24.0 mg Normal Males

Weight:lbs Mean=160.3 Range=

Age: Mean= Range=

Treatment	Subj.#	Dose	Dosage Form	Strength	Lot#	Lot Size
Treatment	7, 8, 10, 12	6.0 mg	Intravenous	1.0 mg/ml	83177-1	Unknown
Treatment	14, 15, 17, 18	12.0 mg	Intravenous	10.0 mg/ml	K-3-11013	Unknown
Treatment	19, 20, 21,24	24.0 mg	Intravenous Intramuscular	10.0 mg/ml	K-3-11013	Unknown
Treatment	9, 11, 13, 16, 22, 23	Placebo	Intravenous		MD-100-12	Unknown

Fasted: No

Nonfast: No Food Study: No FDA High Fat Breakfast: No

Samples: Plasma 7ml 0, 5, 10, 15, 30 minutes; 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 30, 36, 42, 48 hours

Urine Total -12-0, 0-6, 6-12, 12-24, 24-48

Feces

Assay Method.
Assay Sensitivity

Labeling Claims from Study: Intravenous doses of nalmefene up to 24 mg were well tolerated. Nalmefene exhibited a dose independent linear pharmacokinetic profile at doses at or below 24 mg I.V.

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NDA #: 20,459.000
Trade and generic name & form: Nalmefene (Revex) Injection
Sponsor: Ohmeda
Letter Date: 4/27/94
Date Completed:

Type of Submission: NDA Submission, New Study
Date Received: 4/29/94
Reviewer: Curtis Wright MD, MPH
Peer Reviewer: Bob Bedford, MD
Security Level: TradeSecret/Confidential (FDA and Special Government Employees)

ACCOUNTING DATA: RESEARCH- 2 hr
WRITING- 1 hr

MOR TRIAL JF-143

STUDY SUMMARY: PROTOCOL JF-143

DOSE PROPORTIONALITY OF NALMEFENE FOLLOWING INTRAVENOUS DOSES OF 0.5, 1.0 AND 2.0 MG.

Principle Investigator: Albert Cohen, M.D.
Perisular Testing Corp.
Miami, FL 33169

Study Publication: None
Study Initiation: January 29, 1985
Study Completion: April 16, 1985
Study Objective

The objective of this open-label study was to determine the dose proportionality of nalmefene following i.v. doses of 0 5, 1.0 and 2.0 mg by measuring plasma concentrations of intact study drug.

Study Design

Protocol JF-143 was designed as an open-label, randomized, 3-way cross-over study to determine the effects of nalmefene in regard to dose proportionality.

Criteria for Study Inclusion

Study patients were male volunteers between the ages of 24 and 45 and within 10% of their ideal body weight.

Drug Administration

Nalmefene in 0.5, 1.0 or 2.0 mg and isotonic saline were administered intravenously as a single dose injection.

Study Methods

The study was to consist of three individual treatment periods, each lasting twelve (12) hours, with a three day washout between each treatment period. A total of twelve (12) volunteers were to be enrolled.

Following an overnight fast of at least 8 hours, each volunteer received study drug and the fast was maintained for 2 hours after study drug administration.

Heparinized blood (7 mL) was obtained immediately before and at 5, 10, 15, 30, 45 min, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 hours after each study drug administration. Plasma was separated and stored frozen at -17°C pending nalmefene assay.

Each volunteer was to have a complete physical examination and clinical laboratory tests within eight (8) hours after the final dose of study drug.

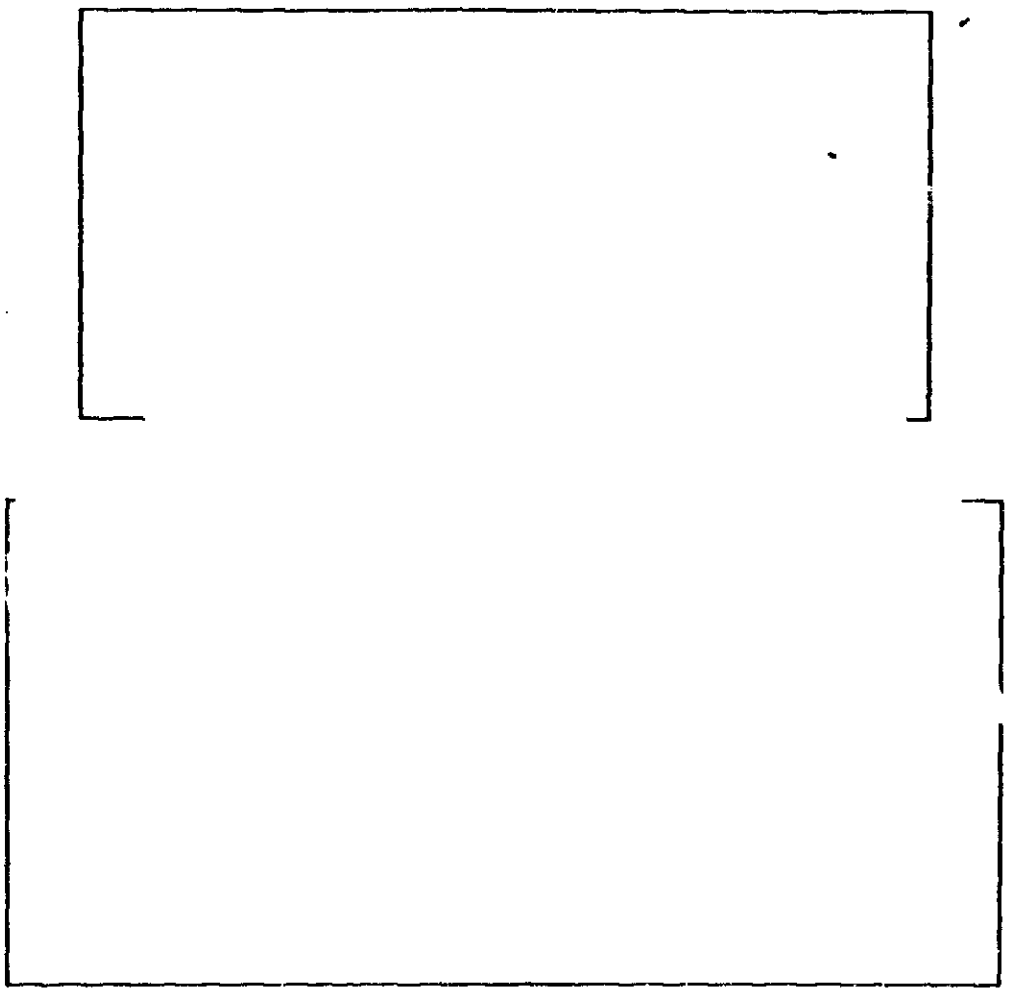
Results

Nalmefene was well tolerated when administered as an i.v. bolus in doses of 0.5, 1.0 and 2.0 mg; only mild and transient adverse effects, such as lightheadedness and nausea, were noted in 5 of the 12 subjects during the course of the present study.

The observed mean plasma levels were: (mean of 12 (SD))

	0.5 mg	**1.0 mg**	**2.0 mg**
5 minutes			
10 minutes			
15 minutes			
30 minutes			
45 minutes			
60 minutes			
90 minutes			
2 hours			
3 hours			
4 hours			
6 hours			
8 hours			
10 hours			
12 hours			

This data was fitted into a 2 compartment open model with a redistributive half life of 13 minutes and an elimination half life of 71 minutes:



As can be seen from the fits, the PK data showed dose proportionality in the range studied, and a reasonable predictive ability from the model.

Dose proportionality was also evaluated by linear regression analysis of the individual AUC_{∞} values versus dose. There was a linear relationship with a correlation coefficient of 0.90. Linear regression analysis of the mean AUC_{∞} values versus dose gave a correlation coefficient of 0.99.

All of the individual plasma concentration-time data obtained for the 2 mg dose in the 12 subjects were also fitted simultaneously with the biexponential equation using the PC-NONLIN program. The individual plasma concentrations for the 1.0 and 0.5 mg doses were then superimposed over the computed line obtained for the 2.0 mg dose after adjusting the values for ordinate axis intercepts, P and A, of the 2.0 mg line by a factor of 1/2 and 1/4, respectively. In most instances, the plasma concentrations are scattered about the line demonstrating identical kinetics and dose proportionality at each dose level.

Adverse Events

The number of patients who experienced an adverse event are as follows:

Adverse Events			
	Nalmefene 0.5 mg	Nalmefene 1.0 mg	Nalmefene 2.0 mg
Numb Mouth	0(0)	0(0)	1(8)
Nausea	0(0)	0(0)	3(25)
Upper Respiratory Infection	0(0)	1(8)	2(17)
Lightheadedness	0(0)	0(0)	2(17)
Fever	0(0)	0(0)	1(8)
Infiltration of Injection Site	1(8)	0(0)	0(0)
Tenderness at Injection Site	0(0)	0(0)	1(8)
Source Data: Case Report Forms			

Conclusions

There was excellent linearity between the administered dose and the area under the plasma concentration-time profile. The results of this study supports previous findings and demonstrates that nalmefene exhibits a dose independent linear kinetic profile following i.v. administration of 0.5 mg to 2.0 mg of the drug.

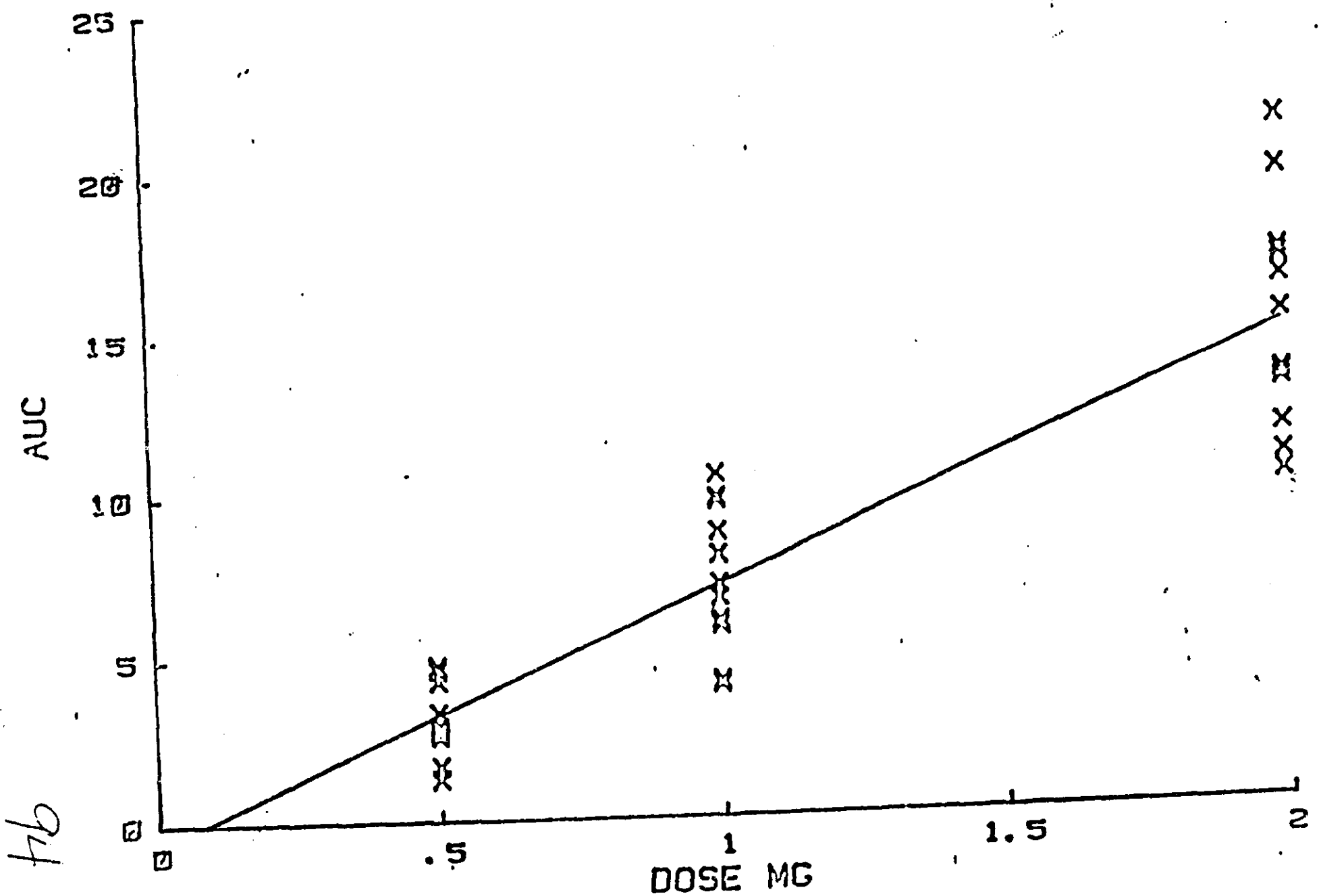
CC: Original NDA
HFD-007 Division File
HFD-007 C Wright
HFD-007 CSO Mcneal
HFD-340
R/D & F/T : CWIV
Location: CWIV Archive

C Wright 6/15/94
Curtis Wright
Team Medical Review Officer
PDES (HFD-007)

R E Bedford 6/22/94
Robert Bedford
Senior Medical Officer for Anesthesia

3 pages
purged
Patient info

Figure 1. Linear regression fit of individual AUC_∞ values versus dose of nalmefene in 12 normal men (correlation coefficient = 0.90).



NALMEFENE NDA PHARMACOKINETICS

Study Type: Pharmacokinetics and Dose Proportionality Study #: 09 Vol. 1.27

Investigator: James Kisicki, MD

Study Site: Harris Laboratories
624 Peach Street
Lincoln, NE 68501

Single Dose: XX Multiple Dose:
Subjects: Normal XX Patients Young Elderly
Impaired: Renal Hepatic

Cross-over: Parallel: XX N= 18 M= 18 F=

Subject Type: 0.5 mg Normal Males

Weight: Mean= 79.4 Range=

Age: Mean= 28 Range=

Subject Type: 1.0 mg Normal Males

Weight: Mean= 67.5 Range:

Age: Mean= 24 Range

Subject Type: 2.0 mg Normal Males

Weight: Mean= 74.8 Range:

Age: Mean= 27 Range:

Treatment	Subj.#	Dose	Dosage Form	Strength	Lot#	Lot Size
Treatment	1-6	0.5 mg	Intravenous	1.0 mg/ml	3495-045	11,950
Treatment	7-12	1.0 mg	Intravenous	1.0 mg/ml	3495-045	11,950
Treatment	13-18	2.0 mg	Intravenous	1.0 mg/ml	3495-045	11,950

Fasted Yes; 8 hrs. prior to dosing and 4 hrs. post dosing

Nonfast: No Food Study: No FDA High Fat Breakfast: No

Samples: Plasma 10ml 0, 5, 10, 15 minutes; 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 22, 24, 30, 40, 48 hours
Urine
Feces

Assay Method:
Assay Sensitivity:

Labeling Claims from Study: Nalmefene exhibited a linear pharmacokinetic profile over the dose range of 0.5 to 2.0 mg given intravenously.

concur with conclusion Ruth Stevens 9-3-95

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NALMEFENE NDA PHARMACOKINETICS

Study Type: Pharmacokinetics - Hepatic Subjects Study #: 21 Volume 1.30
Investigator: Gary R. Matzke, Pharm.D.
Study Site: University of Pittsburgh School of Pharmacy
724 Salk Hall, Pittsburgh PA 15261

Single Dose: XX
Subjects: Normal XX
Impaired: Renal

Multiple Dose:
Patients Young Elderly
Hepatic XX

Cross-over: Parallel: XX N= 24 M= 20 F=4

Subject Type: 2.0 mg Normal Males and Females

Weight: Mean=81.85 Range=

Age: Mean=48.23 Range=

Subject Type: 2.0 mg Hepatic Males and Females

Weight: Mean=89.03 Range=

Age: Mean=47.88 Range=

Treatment	Subj.#	Dose	Dosage Form	Strength	Lot#	Lot Size

Fasted Yes; 8 hrs. prior to dosing and 4 hrs. post dosing

Nonfast: No Food Study: No FDA High Fat Breakfast: No

Samples: Plasma 142ml 0, 1, 3, 5, 10, 15, 30, 45 minutes; 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 18, 24, 36, 48 hours
Additional 1 hr and 24 hr protein binding

Urine Total 0-4, 4-8, 8-12, 12-24, 24-36, 36-48 hrs

Assay Method:
Assay Sensitivity:

Labeling Claims from Study:
There is a 28 % reduction in total body clearance of nalmefene in the hepatically impaired population.
Using ANOVA the difference between normal and hepatic subjects is statistically significant (p=0.02).

	Cmax (ng)	$T_{1/2\beta}$ (hr)	Vd (L)	$AUC_{0-\infty}$ (ng/hr/mL)	Cl (L/hr/kg)
Normal (2.0 mg)	62.9	10.2	913	34.3	0.786
Hepatic (2.0 mg)	50.2	11.9	800	45.2	0.564

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NAME OF COMPANY: OHMEDA PROPRIETARY NAME: REVEX™ ESTABLISHED NAME: nalmefene injection	
Title of the study: A Pharmacokinetic Study of Nalmefene in Normal Subjects and Subjects with Mild, Moderate, and Severe Hepatic Dysfunction	
Investigator: G. Matzke, Pharm.D.	
Study centre: University of Pittsburgh (Pittsburgh, PA)	
Publication (reference): Internal report, 21.	
Studied period (years): 1993	**Clinical Phase:** I
Objectives: To characterize the pharmacokinetic profile of nalmefene in hepatically impaired (mild, moderate, and severe) subjects matched in age, gender, and weight to healthy subjects as controls.	
Methodology: Open-label	
Number of subjects (total and for each treatment): 12 normal subjects + 12 hepatically impaired. Total enrollment: 24. Mean age: 69.9. Sex: male and female	
Diagnosis and criteria for inclusion: Healthy male or female volunteers for the normal subject group, and hepatically impaired males and females. All subjects between 21 and 80 years. Normal subjects were to match hepatically impaired subjects according to the following criteria: ± 5 years or 10% for age, ± 25% for weight, and matched gender and race.	
Test product, dose and mode of administration, batch No.: 1.0 mg/ml nalmefene *(Lot# 3495-087).*	
Duration of treatment: All patients remained in the clinic for fifty-six hours.	
Reference therapy, dose and mode of administration, batch No.: None	
Criteria for evaluation: Safety: Medical histories were compared to subjective findings noted by the patient at the end of the study, physical examinations, clinical and laboratory tests were evaluated and compared between pre- and post-dosing.	
Statistical methods:	
SUMMARY - CONCLUSIONS: Total body clearance of nalmefene was 28% lower in hepatically impaired subjects compared to normal subjects (0.563 L/hr/kg and 0.786 L/hr/kg, respectively). This difference correlates with two useful indices of overall hepatic function as evaluated in the same normal and hepatic subjects: antipyrine as a general marker of hepatic metabolism and galactose as a marker of hepatic blood flow. The difference between normal and hepatically impaired subjects in total body clearance of nalmefene was statistically significant ($p=0.022$). However, the magnitude of the difference is not so great as to necessitate changing the recommended dosage for acute use in hepatically impaired patients. There were no statistically significant differences between the normal and hepatically impaired subject groups with respect to changes in vital signs, incidence of adverse events, or changes in laboratory parameters. The most common adverse events were dizziness, nausea, and headache. All adverse events were mild or moderate in severity and transient in nature. No subject died during the study. Overall, nalmefene was well tolerated.	

NALMEFENE NDA PHARMACOKINETICS

Study Type: Pharmacokinetics - Renal Subjects Study #: 22 Vol. 1.32
Investigator: Gary R. Matzke, Pharm.D.
Study Site: University of Pittsburgh School of Pharmacy
724 Salk Hall, Pittsburgh PA 15261

Single Dose: Multiple Dose: XX
Subjects: Normal Patients Young Elderly
Impaired: Renal XX Hepatic

Cross-over: Parallel: XX N=9 M=5 F=4

Subject Type: 1.0 mg Renal Males and Females

Weight: Mean=67.73 Range=

Age: Mean=46.55 Range:

Treatment	Subj.#	Dose	Dosage Form	Strength	Lot#	Lot Size
Treatment 201-204	1.0 2.0 mg	Intravenous	1.0 mg/ml	3495-087	15,790	

Fasted Yes; 8 hrs. prior to dosing and 4 hrs. post dosing

Nonfast: No Food Study: No FDA High Fat Breakfast: No

Samples: Plasma 253ml Visit 1: 0, 5, 15, 30 minutes; 1, 2, 3, 6, 9, 12, 18, 24, 36, 44 hours
Visit 2: 0, 5, 15, 30 minutes; 1, 2, 3, 4, 5, 6, 6.5, 8, 9, 10, 12, 18, 24, 36, 48 hours
Pre- and post-filter samples at 5, 6.5, and 8 hours.

Urine Total 0-4, 4-8, 8-12, 12-24, 24-36, 36-48 hrs

Dialysate Total 0-0.5, 0.5-1, 1-2, 2-3, 3-4

Assay Method
Assay Sensitivity: Limit of Quantitation

Labeling Claims from Study:
There is a statistically significant 26.9 % decreased plasma clearance of nalmefene in the end stage renal disease (ESRD) population during interdialysis (p=0.038 ANOVA), and a 25.0% decreased plasma clearance in the ESRD population during intradialysis (p=0.052 ANOVA), compared to normal subjects.

	$T_{1/2}$ (hr)	Vd (L)	$AUC_{0-\infty}$ (ng/hr/mL)	Cl (L/hr/kg)	Kel (1/hr)
Interdialysis (1.0 mg)	26.1	1374	28.4	0.574	0.029
Intradialysis (1.0 mg)	25.7	1388	27.5	0.589	0.033
Normal (from study 21 normalized to 1.0 mg)	10.2	913	17.2	0.786	0.072

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NAME OF COMPANY: **OHMEDA** PROPRIETARY NAME: **REVEX™** ESTABLISHED NAME: **nalmefene injection**	
Title of the study: A Study To Determine The Safety and Pharmacokinetics of Nalmefene IV in Renally Impaired Subjects	
Investigator: G. Matzke, Pharm.D.	
Study centre: University of Pittsburgh (Pittsburgh, PA)	
Publication (reference): Internal report	
Studied period (years): 1993	**Clinical Phase:** I
Objectives: The objective of this study was to characterize the safety and pharmacokinetic profile of a single 1.0 mg bolus dose of intravenous nalmefene in subjects with renal impairment as compared to healthy subjects as controls.	
Methodology: Open-label	
Number of subjects (total and for each treatment): 8 renally impaired subjects. Total enrollment: 8. Pharmacokinetic data and results from the 12 normal subjects of Protocol 21 were to be used for comparison. Mean age: 47.5. Sex: male and female	
Diagnosis and criteria for inclusion: Renally impaired volunteers on chronic hemodialysis treatment, males and females. All subjects between 21 and 80 years.	
Test product, dose and mode of administration, batch No.: 1.0 mg/ml nalmefene (Lot# 3495-087).	
Duration of treatment: All patients remained in the clinic for fifty-six hours.	
Reference therapy, dose and mode of administration, batch No.: None	
Criteria for evaluation: Safety: Medical histories were compared to subjective findings noted by the patient at the end of the study, physical examinations, clinical and laboratory tests were evaluated and compared between pre- and post-dosing.	
Statistical methods: Regression, one-way analysis of variance	

Medical Officer Review
Nalmefene Study 24

NDA #: 20,459.000
Trade and generic name & form: Nalmefene (Revex) Injection
Sponsor: Ohmeda
Letter Date: 4/27/94
Date Completed: 6/6/94

Type of Submission: NDA Submission, New Study
Date Received: 4/29/94
Review Completed : 6/6/94
Reviewer: Curtis Wright MD, MPH
Peer Reviewer: Bob Bedford, MD
Date Cleared Peer: 6/8/94
Security Level: TradeSecret/Confidential (FDA and Special Government Employees)

ACCOUNTING DATA: RESEARCH- 4 hr
WRITING- 2 hr

Abstract

This is a clinical pharmacology study looking at the duration of specific and non-specific blockade of carfentanil in the CNS induced by a single administration of naloxone and nalmefene in doses used for treatment of overdose and reversal of anesthesia.

Protocol 24 (Final Report):

An Open Label Study to Compare the Duration of Occupancy of Opiate Receptors by Nalmefene vs. Naloxone using Dual Probe Detection of Positron Emissions in Normal Human Subjects

Principal Investigator: Henry N. Wagner, M.D.
John Hopkins University School
of Hygiene and Public Health
615 N. Wolfe Street
Baltimore, MD 21205

Study Publications: None
Date of Initiation: May 1993
Date of Completion: December 1993

Study Objectives

The objective of this study is to determine the difference in the duration of opiate receptor occupancy by nalmefene at doses of 1.0 mg and 1.0 mcg/kg vs naloxone at doses of 2.0 mg and 2.0 mcg/kg in normal human subjects.

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Study Methods

In a randomized, crossover manner, two groups of four subjects received 1.0 mg of nalmefene and 2.0 mg of nalox e (overdose doses) or 1.0 mcg/kg of nalmefene or 2.0 mcg/kg of naloxone (reversal of an esia doses). Occupancy of brain opioid receptors was studied using [^{11}C] carfentanil as source of positrons. Dual probe detection was used in eight subjects and positron emission tomography (PET) in the ninth subject (to image the uptake of the PET ligand). Venous blood samples and vital signs were obtained before and at 2 hour intervals throughout the 8 hour duration of the study.

Evaluation Groups

Disposition of Subjects			
	mg dose	mcg/kg dose	Total
Subjects enrolled	5	4	9
Subjects randomized	5	4	9
Subjects treated	5	4	9
Subjects completed study	5	4	9
Subjects analyzed for efficacy	5	4	9
Subjects analyzed for safety	5	4	9

Criteria for Study Inclusion

The study was to include healthy male and female subjects, between the ages of 18 and 65. The female subjects must have been using an approved form of birth control prior to admittance.

Drug Administration

Test Formulations: 1.0 mg/ml solution (Lot #3495-087).
naloxone was supplied by the investigator

Criteria for Evaluation

The response to study drug was based on pharmacodynamic evaluation of the receptor site binding, vital signs, laboratory evaluations, medical history/physical examinations and adverse events.

The pharmacodynamic evaluation of brain mu opioid receptor occupancy was performed as follows: A dual probe detection system was used to measure [^{11}C]carfentanil. Occupancy of brain opioid receptors by a competing opioid ligand, nalmefene or the control naloxone, was proportional to the speed with which [^{11}C]carfentanil washes out of the brain, i.e. the slope index and inversely proportional to the clearance half-time.

The percentage blockade was measured by interpolation of the mean brain [^{11}C]carfentanil concentration from 40 to 60 minutes betwe the mean concentration at 0% receptor occupancy determined at baseline and 100% receptor occupancy determined after a 10 mg bolus followed by an infusion of naloxone.

Results

Subjects were first treated with the PET ligand [^{11}C]carfentanil and the total (specific and non-specific activity) uptake determined. The non-specific uptake of [^{11}C]carfentanil was then re-measured in a second session after pre-treatment with a naloxone bolus (10 mg IV) and naloxone infusion (21 mcg/kg/hr) which completely blocks specific opioid uptake. These two values were then subtracted to get the non-specific uptake and the specific uptake values (specific uptake = total uptake - non-specific uptake).

In a third and fourth session, subjects were then treated with a single IV bolus of naloxone or nalmefene, then probed @ 5, 120, 240 & 480 minutes with repeated doses of carfentanil, looking to see how much of the specific uptake was blocked.

The primary results from the study are shown in the following table.

Actual Carfentanil Occupancy Data

Subject	RX	Total Uptake	Non-specific Uptake	Specific Uptake	5 MIN	120 MIN	240 MIN	480 MIN	1440 MIN

The mean data looked like this:

Mean Percent Occupancy (SEM)

	5 MIN	120 MIN	240 MIN	480 MIN
Naloxone 2 mg	75(5)	43(5)	40(8)	14(8)
Naloxone 2 mcg/kg	44(9)	11(7)	29(10)	14(11)
Nalmefene 1 mg	99(9)	90(5)	95(8)	85(8)
Nalmefene 1 mcg/kg	53(12)	34(20)	37(12)	29(17)

Safety Parameters

No abnormalities in physical examinations, vital signs, 12-lead electrocardiogram or clinical laboratory tests were related to nalmefene administration. There were no adverse events reported.

Conclusions

Nalmefene clearly appears to be both more potent and longer lasting than naloxone when given in doses intended for the treatment of overdose (about 1 & 2 mg respectively). It also appears to be more potent than naloxone when given in doses intended for the reversal of anesthesia, but this study provides no evidence that it is longer lasting in this dose range. Two subjects who were tested 24 hours after a single dose of 2 mg nalmefene still showed measurable effects (37% blockade).

Although there is still controversy in the agency about the role of brain imaging studies in the approval process, this study, when taken with the clinical studies in overdose, provides strong evidence that the larger dose of nalmefene is longer lasting than similar doses of naloxone.

CC: Original NDA
HFD-007 Division File
HFD-007 C Wright
HFD-007 CSO Mcneal
HFD-340
R/D & F/T : CWIV
Location: CWIV Archive

Curtis Wright
Team Medical Review Officer
PDES (HFD-007)

R E Bonfiglio
Peer Reviewer
June 8, 1994

Concur with reviewer. Ruth E Stevens
2-13-95

Dear Dr Stevens:

In order to address your concerns regarding the % blockade for nalmefene and naltrexone at 5 mins, 2 hours, 4 hours, and 8 hours, we requested an additional data set (baseline mean normalized activity) from John's Hopkins. Using these data (enclosed herewith), we have recalculated all values for the % blockade and have provided an example of the calculations for subject 102, who received 1 mcg/kg nalmefene at 5 mins. Except for the differences noted below, all values calculated agree with the values stated in the report.

- (nalmefene) - The data set for this subject could not be located by our contact at John's Hopkins. The % blockade for this subject was, therefore, not able to be verified.

- (naloxone) - The % blockade at 5 mins, 2 hours, 4 hours, and 8 hours was calculated to be 39, -11, 12, -28%, respectively. The report lists percentages of 60, 10, 33, -7.

- (naloxone) - The % blockade at 2 hours and 8 hours was calculated to be 64%. The report lists 75%.

Although there is a discrepancy in the values of the % blockade for subjects 106 and 107, the overall conclusion that nalmefene has a longer duration of action is not affected as the actual % blockade for naloxone is lower than the % blockade stated in the report.

I. E. Smith

Ingrid E. Smith
Director, Domestic Regulatory Affairs

196(2)

5 pages
purged

CENTER FOR DRUG EVALUATION AND RESEARCH

PILOT DRUG EVALUATION STAFF

PHARMACOLOGY REVIEW

NDA #: NDA 20-459

SPONSOR: Ohemda PPD Inc
Liberty Corner, NJ

PRODUCT: Nalmetene Hydrochloride Injection (Revex™)

CHEMICAL NAME: 17-(Cyclopropylmethyl)-4,5α-epoxy-6-methylenemorphinan-3,14-diol hydrochloride

DOSAGE FORM: Aqueous solution for parenteral administration

CLINICAL DOSAGE: 2mL ampuls (1 mg/mL); 2mL syringes (1 mg/mL); 1 mL ampuls (100 μg/mL). All concentrations are expressed as the free base.

SUBMISSION DATE: April 27 , 1994
REVIEWER DATE: February 22, 1995

CATEGORY: Opioid Antagonist
INDICATIONS: Complete or partial reversal of opioid depression, including respiratory depression; diagnosis and treatment of suspected opioid overdose.

RELATED INDs:

REVIEWER: BeLinda A. Hayes, Ph.D.

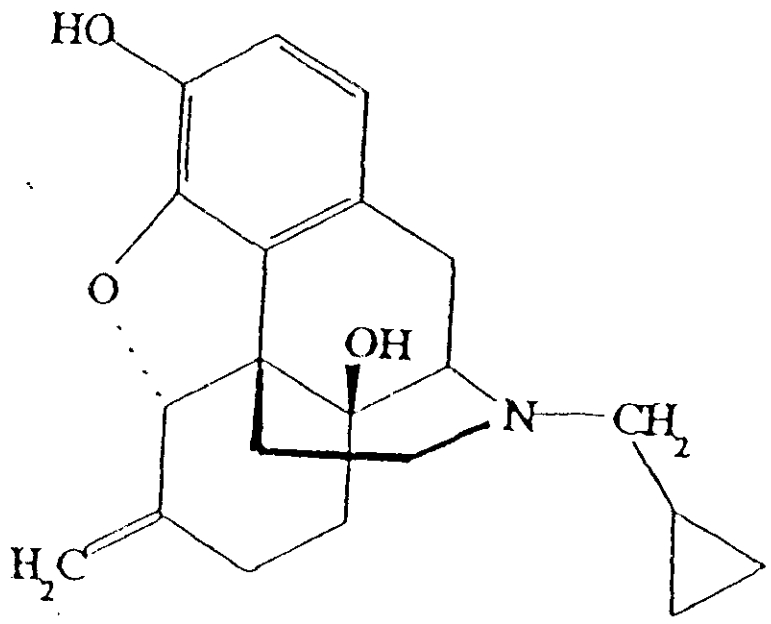
DATE RECEIVED BY REVIEWER: May 4, 1994

CSO: Bonnie McNeal

TABLE OF CONTENTS

DRUG SUBSTANCE.

Nalmefene hydrochloride is a white to off-white crystalline powder. It is freely spluble in water and methanol, soluble in ethanol, slightly soluble in acetonitrile, and very slightly soluble in chloroform.



Empirical Formula: $C_{21}H_{25}NO_3 \cdot HCL$ **Molecular Weight**: 375.9 g/mole

COMPOSITION OF DRUG PRODUCT:

Dosage Composition Of The 1.0 mg/mL Formulation

Composition	Amount (mg/mL)
Nalmefene HCL	1.108 (equivalent to 1.0 mg nalmefene free base)

Dosage Composition Of The 0.1 mg/mL Formulation

Composition	Amount (mg/mL)
Nalmefene HCL	0.1108 (equivalent to 0.10 mg nalmefene free base)

FOREIGN MARKETING HISTORY: Not applicable.

BACKGROUND.

Nalmefene, 17-(cyclopropylmethyl)-4,5α-epoxy-6-methylene morphinan-3,14-diol, is a derivative of naltrexone. It is an opioid antagonist that is structurally and pharmacologically similar to naloxone. Nalmefene proposed indication is for complete or partial reversal of opioid depression, including respiratory depression, and for the diagnosis and treatment of opioid overdose. Parenteral, intravenous and intramuscular, administration is the indicated route of administration.

Currently, naloxone is the only available medication for these indications. However, there are two problems that has been associated with naloxone. Because naloxone has a short half-life following parenteral administration, the potential of renarcotization occurring in patients that has overdosed on a long-acting opioid is high. Additionally, cases of idiosyncratic pulmonary edema has been observed in some patients. Henceforth and then later Ohmeda, seen the need to develop a compound that has the same pharmacological profile as naloxone but a longer duration of action and without the adverse effect that has been associated with naloxone.

Naltrexone, also an opioid antagonist, with a long half-life was evaluated as a potential candidate for these indications. However, problems arose with naltrexone that made it an unsuitable therapeutic agent. It is well known that tolerance develops to the pharmacological actions of morphine. Thus, when an opioid antagonist is administered; a precipitated withdrawal reactions occurs. When naltrexone was administered to overdose patients, the observed precipitated withdrawal symptoms were prolonged and difficult to manage.

Subsequently, nalmefene was developed. In their development program, the sponsor has accumulated both clinical and preclinical data to show that nalmefene is an effective opioid reversal agent, has a long duration of action, and will not produce a severe withdrawal reaction in opiate-dependent patients. The reader is directed to the medical officer's review for the details of the clinical work.

PHARMACOLOGY STUDIES

LIST OF PRECLINICAL PHARMACOLOGY STUDIES: Vol. 6

SUMMARY OF PHARMACOLOGICAL STUDIES WITH NALMEFENE			
TEST TYPE	**MODEL**	**SPECIE(S)**	**ROUTE**
PRIMARY PHARMACOLOGICAL ACTIVITY			
Analgesic Activity	Tail Flick	Rat, Mouse	IV, SC
Analgesic Activity	Writhing-PPQ	Mouse	SC
Analgesic Activity	Writhing-AA	Mouse	SC
Opioid Antagonism	Tail Temp. Response	Rat	SC
ANCILLARY PHARMACOLOGICAL ACTIVITY			
Mechanism of Action	Guinea Pig Ileum	Guinea Pig	In vitro
Mechanism of Action	Receptor Binding	Rat, Guinea Pig	In vitro
Cardiovascular Effects	-	Dogs	IV
Seizure Activity	-	Rats	IV
Opioid Agonistic Activity of Nornalmefene	Tail Flick and Hot Plate	Rats	IV, Intrathecal
ABUSE LIABILITY			
Overt Effects	Drug-free Rhesus Monkeys	Rhesus Monkeys	IV
Reinforcing Efficacy	Self-Administration	Rhesus Monkeys	IV
Physical Dependence Liability	Single Dose Suppression Test	Rhesus Monkeys	SC
Physical Dependence Liability	Precipitated Withdrawal	Rhesus Monkeys	SC
Physical Dependence Liability	Primary Physical Dependence	Rhesus Monkeys	SC

PPQ: Paraphenylquinone
AA: Acetic Acid
SDS: Single Dose Suppression

Nalmefene, 17-(cyclopropylmethyl)-4,5 α-epoxy-6-methylene morphinan-3,14-diol, a derivative of naltrexone is an opiate antagonist. Its pharmacological profile is qualitatively similar to naloxone; but nalmefene appears to be quantitatively more potent and dislay a longer duration of action than naloxone. Nalmefene mediates its pharmacological actions by binding to central opioid receptors. It competitively displaces opioid agonists from μ, δ, κ opioid receptors.

Preclinical studies have shown that nalmefene is a pure opioid antagonist. It is effective and more potent than naloxone in antagonizing morphine-induced analgesic and equipotent as naloxone in antagonizing fentanyl-induced antinociception. Like naloxone, nalmefene was also effective in reversing the analgesia effects of other opioid agonists such as butorphanol, methadone, nalbuphine, and propoxyphene. In comparison to naloxone, nalmefene reversal effects were longer in duration. Both nalmefene and naloxone, at doses up to 10.0 mg/kg (i.v.), were ineffective in reversing the antinociceptive effects of buprenorphine (3.0 mg/kg).

Nalmefene opioid antagonist property was also demonstrated in the isolated guinea pig ileum preparation. Nalmefene alone did not alter the electrically-induced contractions of the isolated guinea pig ileum. But consistent with the action of naloxone and naltrexone in this preparation, nalmefene inhibited morphine, butorphanol, nalbuphine, and methadone depressant effects on these electrically induced contractions.

Nalmefene lacks abuse potential. Monkeys trained to self-administer codeine did not self-administer nalmefene at doses as high as 0.01 mg/kg/injection. Nalmefene, also, does not produce physical dependence in primates or substitute for morphine in morphine-dependent primates. Consistent with its opioid antagonist properties, it does precipitate opioid withdrawal signs in morphine-dependent monkeys and attenuate the reinforcing efficacy of codeine.

I. BINDING PROFILE.

The binding profile of nalmefene has been evaluated in rat and guinea pig brain membrane and the NovaScreen. Results in both species indicate that nalmefene has affinity for the δ, μ, and κ opioid binding site and binds with greater affinity than naloxone to these sites. In the rat brain membrane preparation, nalmefene antagonized the binding of [^{3}H]-dihydromorphine (DHM), [^{3}H]-ethylketocyclazocine (EKC), and [^{3}H]-D-ala-D-leu enkephalin (DADLE) with IC_{50} in the low affinity nanomolar range (Table 1). In comparison to naloxone, nalmefene was 3.5 times, 12 times and 4.0 times more potent than naloxone at the μ, κ, and δ opioid receptors, respectively. Relative to naltrexone, nalmefene was equally potent at the μ receptor, but approximately twice as potent at both the δ and κ opioid receptors.

Table 1 IC_{50} Values (nM) for Nalmefene, Naloxone, and Naltrexone in Rat Brain Membranes.

COMPOUND	[^{3}H]DHM	[^{3}H]EKC	[^{3}H]DADLE
Nalmefene	1.0	5.1	6.1
Naloxone	3.5	60.0	26.0
Naltrexone	0.9	10.0	10.0

Consistent with results obtained in the rat brain preparation, nalmefene showed high affinity for all three opioid receptors in guinea pig brain. It antagonized the binding of [^{3}H]-[D-Ala,N-Me-Phe,Gly-ol]enkephalin (DAGO), [^{3}H]-ethylketocyclazocine (EKC), and [^{3}H]-[D-Pen^2,D-Pen^5]-enkephalin (DPDPE) to the mu, kappa, and delta receptors, respectively, with IC_{50} in the low nanomolar range (Table 2).

The guinea pig brain membrane preparation was also used to evaluate the binding profile of nalmefene metabolites and bisnalmefene. The IC_{50} values are presented in Table 2. Δ^7-Nalmefene binding profile was similar to that observed with nalmefene. In contrast, nornalmefene exhibited relatively high affinity for the μ receptor and much lower affinities for both the κ and δ opioid receptors. The glucuronide metabolite nalmefene glucuronide had low affinities for the μ and κ opioid receptors; it was inactive at the delta receptor. Bisnalmefene, the degradation product of nalmefene, had little affinity if any for the opioid receptors.

Table 2. IC_{50} Values (nM ± SEM) for Nalmefene, Nalmefene Glucuronide, Nornalmefene, Bisnalmefene, and Δ^7 Nalmefene in Guinea Pig Brain Membrane.

COMPOUND	[^{3}H]DAGO	[^{3}H]EKC	[^{3}H]DPDPE
Nalmefene	0.73 ± 0.14	4.7 ± 0.95	9.3 ± 2.46
Nalmefene Glucuronide	810 ± 203	5064 ± 1812	51% ± 19.8% inhibition @ 10μM
Nornalmefene	18.7 ± 2.5	917 ± 1.0	279 ± 151
Bisnalmefene	21% inhibition @ 10μM	Inactive	22% inhibition @ 10μM
Δ^7-Nalmefene	1.12 ± 0.16	5.66 ± 0.49	8.99 ± 0.19

In the NovaScreen, the binding affinities of nalmefene to the following receptor sites: adenosine, dopamine, GABA, histamine, serotonin, muscarinic, excitatory amino acids, glycine, sigma, ion channels, second messengers and DA, NE, 5-HT, GABA, Choline and Adenosine uptake sites, were evaluated. Nalmefene did not bind with any significant affinity; less than 50% inhibition was observed at the 40 binding sites studies

II. Analgesic and Antinociceptive Activities.

The analgesic activity of nalmefene was assessed in mice. The classical analgesic assays used were: 1) tail-flick procedure; 2) acetic acid writhing test; and 3) paraphenylquinone writhing test. Using the rat tail flick and the hot-plate assays, the potential analgesic property of nornalmefene was studied. The antinociceptive activity of nalmefene was evaluated in the mouse and/or rat tail flick model of opioid-induced analgesia. Nalmefene's potency and duration of action against fentanyl-, morphine-, methadone-, morphine-, codeine-, butorphanol-, nalbupine-, and propoxyphone- induced antinociception was compared to naloxone. These assays were conducted according to the standard protocol for these assays and were conducted under GLPs.

Nalmefene is ineffective as an analgesic agent. It was inactive in the mouse tail flick assay at doses up to 30 mg/kg (sc) and in the mouse acetic acid and paraphenylquinone writhing test at doses up to 100 mg/kg (sc) and 10 mg/kg (sc), respectively.

In contrast, the metabolite nornalmefene does possess analgesic activity. Nornalmefene-induced analgesia is route-dependent. Following intrathecal administration, nornalmefene produced a dose-dependent antinociception in the rat tail flick and hot plate assays. In comparison to fentanyl and morphine, nornalmefene was equipotent as fentanyl and was 2.8 times more potent than morphine in the tail flick test; the A_{50} for nornalmefene, fentanyl, and morphine was 0.4 μg (C.L.: 0.2 - 0.8), 0.6 μg (C.L.: 0.3 - 1.2), and 1.1 μg (C.L.:0.6 - 2.1), respectively. Nornalmefene was a weak analgesic in the hot plate assay; it was 10 times, and 3.5 times less potent than fentanyl and morphine, respectively. The A_{50} for nornalmefene, fentanyl, and morphine was 16.0 μg (C.L.: 5.8 - 44.1), 1.6 μg (1.0 - 2.6), and 4.6 μg (4.2 - 4.9), respectively. Nornalmefene elicited dose-dependent clinical signs similar to morphine and fentanyl following intrathecal administration. These clinical signs included: mild exopthalamia, sedation decreased respiration, labored breathing, and rigidity. Nornalmefene lacked analgesic activity following intravenous administration. At doses of 1.17 and 3.51 mg/kg, nornalmefene did not produce significant antinociception in the hot plate test up to 30 minutes post-injection; the Percent Maximal Possible Effect (%MPE) for 1.17 mg/kg in the tail flick and hot plate assays was 3.2 and 1.8%, respectively. At 3. 51 mg/kg, 1.0% MPE was observed in the hot plat test. 9.5% MPE was observed in the tail flick assay following intravenous administration of 3.51 mg/kg nornalmefene; this was statistically significant ($p \leq 0.05$) from control values.

The ability of nalmefene (0.0003 - 0.1 mg/kg, iv) and naloxone (0.001 - 10 mg/kg) to reverse the analgesic effects of fentanyl (0.010 mg/kg (A_{99}), iv) was assessed in male Sprague Dawley rats (5/dose). Nalmefene was equally as potent as naloxone in antagonizing fentanyl-induced antinociception. The dose of nalmefene and naloxone that produced 50% maximal possible effect (AD_{50}) was 0.0012 mg/kg and 0.0061 mg/kg, respectively (Table 3).

Nalmefene had a longer duration of action than naloxone in antagonizing fentanyl-induced analgesia (Table 3). At an equivalent dose (A_{99}), nalmefene (1.4 mg/kg, i.v.) reversed the analgesic effect of fentanyl for 540 minutes (9 hours); whereas naloxone (0.8 mg/kg, i.v.) only reversed fentanyl effects for 150 minutes (2.5 hours).

Nalmefene at a dose of 0 .01 mg/kg (i.v.) was equally as potent as naloxone (0.02 mg/kg, i.v.) in reversing the analgesic effects of fentanyl (0.01 mg/kg, i.v.) in the tail flick test. Both drugs shifted the dose response curve of fentanyl to the right by 11-folds. The A_{50} of fentanyl plus saline was 0.0045 mg/kg. In the presence of nalmefene or naloxone, the A_{50} was 0.051 mg/kg (Table 3).

Similarly, nalmefene was effective in reversing the analgesic effect of morphine. Male Sprague-Dawley rats were implanted with an Alzet osmotic mini-pup containing morphine sulphate (100 mg/ml). One hour after implantation, tail flick latencies were calculated for each rat to assess morphine-induced analgesia. Subsequently the rats were treated with saline, nalmefene (A_{99}: 1.4 mg/kg, i.v.), or naloxone (A_{99}: 0.8 mg/kg, i.v.) and tail flick latencies were recorded 1, 5, 15, 30, and every 30 minutes until latencies were not significantly different from morphine control times. Nalmefene reversal effect was longer in duration in comparison to naloxone. Morphine-induced analgesia was attenuated for 420 and 150 minutes by nalmefene and naloxone, respectively. Complete recovery from morphine-induced antinociception was not observed in the nalmefene-treated rats until 660 min.

At the A_{99} dose, nalmefene significantly ($p \leq 0.05$) attenuated the antinociceptive effects of the A_{99} doses of butorphanol, methadone, nalbuphine and propoxyphene (Table 4). However, nalmefene was ineffective in reversing buprenorphine-induced antinociceptive. At doses up to 10 mg/kg (i.v.), both nalmefene and naloxone failed to reverse the antinociceptive effects of 3.0 mg/kg of buprenorphine. However, when these compounds were given 2 min prior to buprenorphine, a significant ($p \leq 0.05$) reversal of effects was observed. This observation correlates well with the clinical situation in which high doses of naloxone could not reverse the effects of buprenorphine (Heil et a., 1980; Aceto et al., 1992).

Nalmefene antagonist properties were also evaluated in the tail temperature response in morphine-dependent rats. Morphine dependency was produced in CD rats (5/group) by a subcutaneous implantation of 1 pellet containing 75 mg morphine; two days later the rats were implanted with two additional morphine pellets. Tail skin temperature (TST) response was evaluated two days later. TST was determined prior to and following the subcutaneous administration of either naloxone (0, 0.01, 0.1, 0.5 or 1.0 mg/kg); naltrexone (0.001, 0.005, 0.01, 0.02, 0.1 mg/kg); nalmefene (0.001, 0.004, 0.01, 0.02, or 0.1 mg/kg). TST responses were recorded at 5 min intervals for 90 minutes after drug treatment. Nalmefene produced a dose-dependent increase in the magnitude and duration of the TST response. The three highest doses (0.01, 0.02 and 0.1 mg/kg) of nalmefene significantly elevated the TST response within 5 minutes; maximum response was observed at 15 to 20 minutes after nalmefene treatment. Naloxone and naltrexone also produced a dose-dependent increase in the TST response. Consistent with the results from the other studies, nalmefene was more potent than naltrexone or naloxone; the ED_{50} for nalmefene, naltrexone, and naloxone was 5.3, 11.5, and 152.4 μg/kg, respectively.

Table 3. Comparison of Nalmefene and Naloxone Antagonistic Properties Against Fentanyl.

DRUG	AGONIST A_{50} (mg/kg) (95% Conf. Limit)	*ANTAGONIST* A_{50} (mg/kg) (95% Conf. Limit)	*ANTAGONIST* A_{99} (mg/kg)	Duration of Action (min)
Nalmefene	0.051 (0.028-0.093)	0.0012 (0.0001-0.017)	1.4	540
Naloxone	0.051 (0.017-0.154)	0.0061 (0.001-0.037)	0.8	150

Table 4. Opioid Antagonist effects of Nalmefene Against Several Opioids in the Rat Tail Flick Assay.

% MPE[c] ± S.E.			
OPIOID (A_{99}; mg/kg)	OPIOID ALONE	OPIOID + NALMEFENE (1.4 mg/kg, i.v.)[a,b]	
		1 MINUTES	5 MINUTES
Methadone (1.0)	100 ± 0	27.2 ± 15.9*	13.5 ± 7.3*
Morphine (3.76)	100 ± 0	10.6 ± 5.5*	7.2 ± 3.6*
Codeine (9.5)	100 ± 0	18.5 ± 3.5*	14.6 ± 4.1*
Butorphanol (1.87)	100 ± 0	8.7 ± 2.6*	7.5 ± 4.3*
Propoxyphene (3.0)	100 ± 0	14.0 ± 5.6*	5.3 ± 2.2*
Nalbuphine (20.0)	32 ± 0	-3.6 ± 3.4*	-5.9 ± 3.1*
Buprenorphine (0.3)	64 ± 0	98.8 ± 1.2	100 ± 0

a: Nalmefene was administered 1 minute prior to testing. Antinociception was evaluated at 1 and 5 minutes later.

b: Buprenorphine-treated rats received 10.0 mg/kg of nalmefene.

*: Differences significant ($p < 0.05$) from opioid alone (Student's t-test).

c: %MPE = Percent Maximal Possible Effect

III. Agonist and Antagonist Properties of Nalmefene in The Guinea Pig Ileum Preparation.

To further support the classification of nalmefene as a pure narcotic antagonist, nalmefene was evaluated in the isolated guinea-pig ileum preparation. In this preparation it is well documented that opiate agonists such as morphine depresses the contraction induced by electrical stimulation of the guinea pig ileum muscle; and antagonists such as naloxone, alone, have little effect on the muscle. The co-administration of an antagonists with morphine blocks the agonist inhibition of the muscle contraction. The ability of nalmefene to inhibit the twitch response of the isolated guinea pig ileum and to antagonize the action of morphine on the isolated guinea pig ileum was compared to the effects of naloxone and naltrexone.

In agreement with the effects of naltrexone and naloxone, nalmefene alone, at concentrations up to 100 nM, did not depress the contraction induced by electrical stimulation of the guinea pig ileum. However, nalmefene pretreatment was effective in antagonizing morphine-, butorphanol-, nalbuphine-, and methadone-induced inhibition of the electrically induced twitch. Nalmefene and naloxone both produced a parallel and concentration-dependent shift of the dose-response curve to the right. Nalmefene was 12.5 times more potent than naloxone in blocking the depressant effects of morphine.

In another guinea pig ileum study, the ability of nalmefene (3 nM to 10 μM), Δ^7-nalmefene (3 nM to 10 μM), nalmefene glucuronide (3 nM to 30 μM) and nornalmefene to attenuate morphine depressant effects was studied. Morphine produced a dose-dependent inhibition of twitch height, with an EC_{50} of 233 nM (C.L.: 92 - 589 nM). In the presence of nalmefene, morphine EC_{50} was increased in a concentration-dependent fashion (Table 5). Nalmefene produced a statistically significant ($p<0.05$) rise in twitch height of 6.3 ± 2.1% at a concentration of 300 nM. No significant changes in twitch height were observed at the other concentrations. A statistically significant shift in morphine concentration response curve was observed at 3, 20, and 30 nM nalmefene; 100 nM completely inhibited morphine activity at concentrations of morphine ranging from 3 nM to 10 μM.

Δ^7-nalmefene also inhibited morphine's depressant effects in a concentration-dependent manner. Δ^7-nalmefene in the concentration range 3 nM to 10 μM had no statistically significant effect on twitch height. At a concentration of 100 nM, Δ^7-nalmefene shifted the EC_{50} for morphine from 36 nM (C.L.: 27 - 4.3 μM) to 4.3 μM (C.L.: 2.4 - 7.5 μM); this represents a 120-fold rightward shift of the morphine concentration response curve.

Nalmefene glucuronide did not exhibit narcotic antagonist property in the guinea pig ileum preparation. Nalmefene glucuronide, in the concentration range 3 nM to 30 μM, also did not produce a significant effect on twitch height. 100 nM of nalmefene glucuronide failed to inhibit the effects of morphine; in the of 100 nM of nalmefene glucuronide, morphine EC_{50} shifted from 233 nM to 228 nM (C.L.: 161-324 nM).

Nornalmefene exhibited agonist properties in the guinea pig ileum preparation. In the same fashion as other opiate agonist, nalmefene produced a dose-dependent inhibition of twitch. In this preparation, it was 3.2-fold less potent than morphine; Nornalmefene EC_{50} was 739 nM (C.L.: 470 - 1159 nM).

Table 5. Nalmefene effects on morphine's depressant effect in the guinea pig ileum preparation.

NALMEFENE CONC. (nM)	MORPHINE EC_{50} (nM) 95% CONFIDENCE LIMITS	SHIFT OF MORPHINE CURVE
0.1	546 (373 -798) nM	2.3 fold
1.0	628 (311 - 1268) nM	2.7 fold
3.0	869 (374 - 2021) nM	3.7* fold
10.0	1483 (617 - 3565) nM	6.4* fold
30.0	12100 (9390- 15700) nM	52.0* fold

*: Significantly different ($p < 0.05$) from control.

IV. Cardiovascular Effects of Nalmefene.

The cardiovascular effects of nalmefene were evaluated in conscious dogs fitted with indwelling pressure transducer in the left ventricle of the heart. Heart rate, blood pressure, left ventricular dP/dt, and EKG was monitored after the intravenous administration of 0.08 mg/kg nalmefene or 5 ml of saline. Relative to saline, nalmefene had no discernable effects on systolic blood pressure, heart rate, or left ventricle dP/dt. Also the EKG profile was similar following saline or nalmefene treatment.

V. The Effects of Nalmefene, Flumazenil and the Combination of Nalmefene and Flumazenil on the Induction of Seizures in Rats.

The ability of nalmefene and flumazenil alone and the co-administration of the two drugs to induce seizures in rats was evaluated. Male Sprague Dawley rats (N = 6/dose) were administered intravenous bolus injections of nalmefene (6.4-10 mg/kg) or flumazenil (100-200 mg/kg) or a combination of the two drugs in a 23:1 ratio (flumazenil:nalmefene). They were observed for clonic seizure activity for 10 minutes post-injection. The dose that produced clonic seizures in 50% of the subjects (CD_{50}) was calculated.

The results, as copied from the sponsor, are presented in Table 6. Both nalmefene and flumazenil induced clonic seizures in a dose-dependent manner. Nalmefene was more potent (23 times) than flumazenil in producing seizures. The dose of nalmefene and flumazenil that produced seizures in 50% (CD_{50}) of the rats was 7.45 mg/kg and 167.8 mg/kg, respectively.

When nalmefene and flumazenil were co-administered in a 23:1 ratio (flumazenil:nalmefene), clonic seizures were observed. Based on isobolographic analysis, the two agents produced a significant ($p < 0.05$) subadditive interaction.

Table 6. **The effects of either nalmefene or flumazenil or the drugs combined on the induction of seizures in rats.**

COMPOUND	DOSE	% CLONIC SEIZURES
Nalmefene	6.4	0
	6.9	33.3
	7.4	83.3
	10.0	83.3
CD_{50} = 7.45 (6.42 - 8.66) mg/kg		
Flumazenil	100	0
	141.4	16.7
	200.0	83.3
CD_{50} = 167.8 (144.6 - 194.6) mg/kg		
Flumazenil : Nalmefene	94.3 : 4.1	0
	101.2 : 4.4	33.3
	103.5 : 4.5	66.7
	108.1 : 4.7	100.0
CD_{50} = 106.3 (104.1 - 108.5) mg/kg		

VI. Abuse Liability of Nalmefene.

The abuse liability and physical dependence potential of nalmefene have been evaluated in several standard abuse liability tests: drug-free rhesus monkeys, self-administration paradigm, single dose suppression test, precipitated withdrawal, and the primary physical dependence model.

Nalmefene did not maintain self-administration behavior in rhesus monkeys trained to self-inject codeine (0.32 mg/kg/injection) under a fixed-ratio 30 schedule of reinforcement. Nalmefene at doses of 0.00032, 0.001, 0.032, and 0.01 mg/kg/injection failed to maintain responding above saline levels. However, the antagonist actions of nalmefene were observed in this behavioral model. Responding maintained by 0.32 mg/kg/injection codeine was suppressed on the day following substitution of the higher doses of nalmefene.

In the drug-free rhesus monkeys study, six rhesus monkeys (3 males and 3 females) were administered 0.1 mg/kg of nalmefene intravenously. Each monkey was observed immediately after treatment and for the first 6 hours afterward and for 15 minutes at approximately 12, 18, and 24 hours. Nalmefene-induced clinical signs were observed in all subjects immediately after dosing. These clinical signs included: staring, mild CNS depression coupled with mid tremors and occasional myoclonic jerks.

The ability of nalmefene to induce physical dependence was evaluated in three experimentally naive rhesus monkeys (2 male, 1 female). Nalmefene was administered 4 to 6 times per day for 30-45 days. After being on nalmefene for 15-20 days and 30-35 days, the monkeys were placed into abrupt withdrawal. If no withdrawal signs were observed, the monkeys were given a naloxone challenge at the 15 day withdrawal period an at the end of the study.

On day 17 of the study, the monkeys were abruptly withdrawn. Some signs of withdrawal were observed in all three moneys. Thirteen to fourteen hours into abrupt withdrawal, extreme restlessness, wet-dog shakes and vocalization were seen in two of the three monkeys. The next abrupt withdrawal was conducted on day 30 of the study. Severe slowing was observed in 1 monkey, some restlessness was seen in 2 of 3 monkeys, and drowsiness was observed in all three monkeys. No significant withdrawal signs were observed when a naloxone challenge (1.0 mg/kg, s.c.) was given 19 hours into withdrawal. Two male monkeys died following withdrawal; one on day 30 and one on day 31 of the study. Necropsy revealed fluid retention. It was speculated that chronic administration of nalmefene may have caused up-regulation of opioid receptors in the posterior pituitary gland; thus producing an exaggerated block of vasopressin during acute withdrawal in the monkeys. This postulation is in line with the findings that endogenous and exogenous opioid suppress vasopressin release from the posterior pituitary gland. No withdrawal signs were observed in the third monkey.

Consistent with the inability of nalmefene to produce significant opioid physical dependence in rhesus monkeys, nalmefene also failed to substitute for morphine in the single-dose suppression test. In the single-dose suppression test, rhesus monkeys were made dependent on morphine by giving them a subcutaneous injection of 3.0 mg/kg of morphine every 6 hours. For test of substitution, nalmefene (0.001, 0.008, 0.04 mg/kg, s.c.), morphine (3.0 mg/kg, s.c.), or saline (1 ml/kg, s.c.) was given to monkeys who had not received morphine for 14 to 15 hours and showing signs of withdrawal. During a 2.5 hours observation period, the animals were scored for suppression of withdrawal signs.

Nalmefene failed to substitute for morphine at doses up to 0.04 mg/kg. At the two highest doses (0.008 and 0.04 mg/kg) tested, morphine-induced withdrawal signs were exacerbated.

In the precipitated withdrawal test, *morphine*-dependent monkeys were challenged with nalmefene (0.0025, 0.01, 0.004, and 0.16 mg/kg, s.c.) or naloxone (0.05 mg/kg, s.c.) 2 to 3 hours after their last *morphine* injection. Dose-dependent signs of precipitated withdrawal were observed immediately after nalmefene or naloxone treatment. Relative to the positive control (naloxone), nalmefene was 5 to 10 times

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ABSTRACT

The potential toxicity of nalmefene was evaluated in several species following single and repeated administration. The preclinical toxicology studies are located in Vol. 1.7 - 1.12 and Vol. 1.14 - 1.20. All toxicology studies were performed under GLP requirements.

The acute toxicity of nalmefene was assessed in CD-1 mice, CD rats, and Dutch Belted rabbits following oral, intravenous, and subcutaneous administration. Nalmefene was evaluated at the following dose range: Intravenous (15-75 mg/kg in mice; 25-75 mg/kg in rats; 12-24 mg/kg in rabbits); Oral (200-300 mg/kg in mice; 150-620 mg/kg in rats; 225-700 mg/kg in rabbits); and Subcutaneous (250-1000 mg/kg in mice; 560-1800 mg/kg in rats; 125-210 mg/kg in rabbits). Nalmefene-induced overt signs of toxicity were observed in all three species following acute intravenous, subcutaneous, and oral administration. Clinical signs which were common to each species included: convulsions, tremors, unsteady gait and loss of balance/righting reflex. Other overt signs of toxicity observed frequently were labored breathing, prostration and decreased activity. These clinical signs were most evidence on the day of treatment and their severity and duration was dose-dependent. The onset of the observed clinical signs were route-dependent: *intravenous* > *subcutaneous* > oral. Nalmefene-related deaths occurred in all species. Mortality rate was dose-dependent; the highest dose tested resulted in 100% mortality rate in all species. The time of death was route-dependent. Following intravenous administration, nalmefene-induced death occurred within 5 minutes. Mortalities were prolonged following oral or subcutaneous administration; death occurred within 4 hours for each species after these routes. Based on the results from these acute toxicity studies, the sponsor estimated the LD_{50} of nalmefene to be as following for the species:

ROUTE	LD_{50} (CONFIDENCE LIMITS) (MG/KG)					
	SPECIE					
	CD-1 MICE		CD RAT		DUTCH RABBIT	
	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
INTRAVENOUS	41.0 (35.3 -47.6)	50.0 (35.0 - 71.3)	44.0 (40.0 - 48.4)	48.5 (43.6 - 52.8)	19.5 (17.7 - 21.4)	15.0 (13.6 - 16.5)
ORAL	270 (256 - 286)	298 (257 - 346)	484 (401 - 583)	310 (231 - 415)	486 (311 - 757)	488 (369 - 645)
SUBCUTANEOUS	530 (420 -688)	575 (509 - 648)	1080 (866 - 1347)	1150 (1012 - 1306)	163 (134 - 199)	157 (131 - 188)

Some gender differences were observed to the lethal effects of nalmefene. Female rats and rabbits were more sensitive to the lethal effects of nalmefene following oral and intravenous administration, respectively.

Thirty day subacute toxicity studies were conducted in rats and dogs using the intended route of administration, intravenous injections. Male and female Sprague-Dawley rats received daily dosages of 0 (vehicle), 5, 11, and 25 mg/kg/day; 15/sex/group. Nalmefene-induced mortality was observed in subjects treated with 25 mg/kg of nalmefene; five males and one female in this high dose group. Deaths occurred after 6 to 17 days of treatment and occurred within 5 minutes of dosing, except in one subject. Also, these

deaths were observed to follow episodes of convulsions. Four additional deaths occurred as a consequence of the bleeding procedure. A no-effect dose was not achieved in this study; all doses tested elicited overt signs of toxicity. Nalmefene-related clinical signs were observed immediately (within 1 minutes) after injection. These clinical signs included: salivation, unsteady gait, hypoactivity, urination, tremors, and convulsions. The incidence, severity, and duration of these toxic signs were dose-dependent. In the 5 mg/kg group, the majority of the rats exhibited no signs of toxicity, mild salivation, and/or brief tremors. Rats in the mid (11 mg/kg) and high (25 mg/kg) dose groups exhibited mild to severe tremors, clonic convulsions, jumping behavior, loss of righting reflex, loss of balance, leg spraddle, and/or salivation. With the exception of hypoactivity, these clinical signs had a short duration; lasting for five minutes or less. The duration of the convulsive episodes appeared to increase during the course of the study in the mid and high dose groups; most of the rats were experiencing intermittent convulsions over a period lasting 10 minutes or longer toward the end of the study. Hypoactivity persisted for several hours after dosing. The severity of these observed clinical signs was dose-dependent. Relative to controls, a significant increase in bodyweight was observed in the female rats in the 11 and 25 mg/kg dosing group. Consistent with this effect, these rats also showed an increase in food consumption. A significant reduction in body weight gain was seen in male rats in the low dose group (5.0 mg/kg). Food consumed by the male rats in the 25 mg/kg group was increased; an increase in food consumption was observed in the male rats in the 5 and 11 mg/kg group. Selected hematology, serum biochemistry, and urinalysis parameters evaluated were within normal range. There were no nalmefene-related histopathological changes observed during necropsy.

The subacute toxicity of nalmefene associated with intravenous administration was also evaluated in dogs. Male and female Beagle dogs were randomly assigned to the four treatment groups; 3/sex/dose. The treatment groups were: 0 (sterile saline for injection U.S.P.), 3.0, 4.6, and 8.0 mg/kg. The subjects were dosed once daily for 30 to 32 consecutive days. No mortalities occurred during the study. The nalmefene-induced overt signs of toxicity observed were consistent with those observed in the subacute rat study. They were: convulsions, tremors, unsteady gait, salivation, hypoactivity, ataxia, and leg splaying were observed in the dogs following treatment. Unsteady gait, slight ataxia, salivation, vomiting, and hyperactivity occurred at low incidence in individuals animals dosed with 3.0 mg/kg nalmefene. Following dosing with 4.6 mg/g of nalmefene, brief ataxia, loss of balance, hyperactivity, urination, and defecation were observed. At 8.0 mg/kg/day, nalmefene-induced signs included: tonic-extensor convulsions, violent thrashing, vocalization, ataxia, leg splaying, and salivation. With the exception of hypoactivity, these clinical signs were not present 10 minutes after dosing; some incidences of hypoactivity lasted up to 6 hours after treatment. The incidences and severity of nalmefene-induced overt signs decreased during the study in all treatment groups; such that by the fourth week of the study only heavy salivation was observed in the dogs doses with 3.0, 4.6 or 8.0 mg/kg (with the exception of one convulsive episodes).

No drug-induced deaths occurred. Also, no nalmefene-related effects were observed on bodyweight and food consumption.

Select hematology, serum biochemistry, and urinalysis parameters were observed to differ from control at interim sampling on days 7, and 14 or at study termination. A decrease in mean corpuscular volume was noted in nalmefene-treated females on study day 7. A significant increase in alkaline phosphatase levels was observed on 7 and 29, and days 7 and 14 in males dosed with 4.6 mg/kg and one female treated with 3.0 mg/kg, respectively. In addition, males treated with 8.0 mg/kg of nalmefene showed a significant elevation in SGOT levels on day 14 of the study. Urinalysis revealed an increase incidence in granular casts and white blood cells in the urine of all dogs in the 8.0 mg/kg group.

No treatment-related organ weight changes or adverse histopathology findings were observed in the nalmefene-treated dogs.

The toxicity associated with subchronic (13 weeks) exposure to nalmefene was assessed in rats and dogs following oral administration. In the rat study, 15 male and 15 females Sprague-Dawley rats (CD) received nalmefene as a dietary admixture at dose levels of 20, 100, and 500 mg/kg/day. Three deaths were observed during the study. One male and one female in the control group died as a result of blood sampling; another control male died during week 7 of the study. No deaths occurred in the nalmefene treatment groups. There were no clinical signs of toxicity seen throughout the study in the nalmefene-treatment groups. Relative to control group, males in the nalmefene group exhibited a dose-dependent decrease in body weight. A statistically significant decrease in body weight was observed in the mid-dose males (100 mg/kg) from week 3 and in the high-dose males (500 mg/kg) from week 11. Females in the 100 and 500 mg/kg groups, also, showed a statistically significant decrease in body weight from week 9 and week 3 onward, respectively. Dose-dependent decrease in food consumption was also observed in both males and females rats. In comparison to the control, mean overall reductions of 5, 10, and 17% in the low- mid- and high-dose males and 5,and 10% in the mid- and high-dose females were observed, respectively.

Some hematological and serum biochemistry parameters were affected by nalmefene treatment. A slight, but significant, accelerated mean prothrombin times was noted in the high-dose females after week 4 and 12 of treatments and in the mid-dose females after 4 weeks of treatment. Relative to control a slight but statistically significant prolongation of mean partial thromboplastin times was observed in the mid- and high-dose males after 12 weeks of treatment; and a significant accelerated mean partial thromboplastin time was noted in the high-dose females after week 12. Males in the mid-dose group and females in the high-dose group exhibited a significantly reduced blood urea nitrogen after 4 weeks of treatment. After 12 weeks of treatment, males in the high dose group had a reduction in blood urea nitrogen.

Statistically significant differences were evident in absolute organ weight data between the vehicle-treated and nalmefene-treated rats. The weights of the heart, lungs, spleen, and liver, and the heart weight of the males (100 and 500 mg/kg/day) and females (500 mg/kg/day) were lower than the control, respectively. The weight of the thyroid gland of the males (100 mg/kg/day) was lower than the control values. Brain weights were significantly increased in males at 20 and 100 mg/kg/day and in male and females treated with 500 mg/kg.

In the dog subchronic study, 3 male and 3 female Beagle dogs received daily oral doses of nalmefene at dose levels of 0, 4, 20, and 100 mg/kg. There were three deaths during the study; 2 dogs (1 male and 1 female) died in the 100 mg/kg/day group and one male dog in the high-dose group was killed *in extremis* during week 9. In all cases, death was preceded by a convulsive episode. The one male sacrificed during week 9 also exhibited prostration and profuse salivation 24 hours after dosing. The NOEL was the 4 and 20 mg/kg dosing level. Nalmefene-induced toxicity was observed in the dogs in the 100 mg/kg group. The following clinical signs were observed within one hour of dosing: marked salivation, convulsive episodes, opisthotonos, labored breathing, ataxia, and emesis. The dogs recovered from these effects within 12 to 24 hours. No nalmefene-related effects on body weight gain, food consumption, clinical chemistry, histopathology findings, or organ weight changes were observed. Some significant effects on hematology parameters were observed. Four weeks after treatment, the mean corpuscular volume and the mean corpuscular hemoglobin concentration was lower than control values in males and both males and females treated with 100 mg/kg and 4 mg/kg of nalmefene, respectively. The mean corpuscular volume was higher

in the females after 12 weeks of doing with 4.0 mg/kg.

TOXICOLOGY

ACUTE TOXICITY STUDIES.

The following acute toxicity studies were submitted in Volume 1.7:

SUMMARY OF ACUTE TOXICITY STUDIES			
STUDY №	**ROUTE**	**STUDY CATEGORY**	**SPECIE**
KP5/JF1	IV	LD_{50} Determination	Mouse
KP17/JF1	PO		
KP3/JF1	SC		
KP4/JF1	IV	LD_{50} Determination	Rat
KP/18/JF1	PO		
KP2/JF1	SC		
KP6/JF1	IV	LD_{50} Determination	Rabbit
KP21/JF1	PO		
KP7/JF1	SC		

Acute toxicity of nalmefene was evaluated in mice, rats, and rabbits following intravenous, subcutaneous, and oral administration. These toxicity studies were originally submitted under IND on December 12, 1982 and reviewed by HFD 120 on January 27, 1983. IND was under the sponsorship of These studies were conducted at All studies were conducted in compliance with GLP; QUA statement for each study was present and signed.

In all three species evaluated, nalmefene-related clinical signs were observed. The clinical signs observed the most in all three species were of the CNS variety and included: tremors, convulsions, unsteady gait and loss of balance/righting reflex. Labored breathing, prostration and decreased activity were also frequently observed in the subjects. These overt signs of toxicity were most evidence on the day of treatment; no signs of toxicity were observed during the remainder of the fourteen day observation period. The severity and duration of these clinical signs were dose-dependent. The severity of these overt signs of toxicity was mild in the low dose group, and more severe in the high dose group. The onset of these clinical signs was route-dependent; intravenous route of administration having the more rapid onset followed by the

subcutaneous (5 min) and oral (15 min) routes.

Nalmefene-induced deaths occurred in all three species. The number of mortalities were dose-dependent; the highest dose tested resulted in one hundred percent mortality rate in all species. The onset of death was route-dependent. Deaths occurring more rapidly following the intravenous route; occurring within seconds to five minutes after injection. The onset of death following oral or subcutaneous administration was delayed; occurring within 15 minutes after dosing. In most instances, males in all three species were more sensitive to the lethal effects of nalmefene. Female rats were more sensitive following oral administration and female rabbits were more sensitive to the lethal effects of nalmefene following both the intravenous and subcutaneous route of administration. The results from these studies are summarized in table 7.

TABLE 7. Acute toxicity after a single administration of nalmefene to mice, rats and rabbits.

SPECIES (STRAIN): NUMBER OF ANIMALS/SEX/DOSE			
ROUTE	DOSE (mg/kg)	LD_{50} (MG/KG) (95% CONF. LIMITS)	CLINICAL SIGNS (DOSE (mg/kg) OBSERVED)
MICE (CD-1): 8M & 8F			
IV	M: 15, 30, 40, 75 F: 15, 30, 50, 75	M: 41.0 (35.3 - 47.6) F: 50.0 (35.0 - 71.3)	• Tremors: 15, 30, 40, 50 • Convulsions: 30, 40, 50, 75 • ↓ Activity: 15, 30, 40, 50 • Unsteady Gait: 15, 30, 40, 50 • Labored Breathing: 30, 40, 50, 75 • Loss of Balance/Righting Reflex: 30, 50 • Prostration: 40 • Death: 30-75 (M & F)
PO	M: 200, 230, 260, 300 F: 200, 300, 350, 400	M: 270 (256 - 286) F: 298 (257 - 346)	• Tremors: 200, 260, 300 • Unsteady Gait: 200, 230, 260, 300 • ↓ Activity: 200, 230, 260, 300 • Convulsions: 300, 350, 400 • Loss of Balance/Righting Reflex: 300 • Prostration: 260 • Death: 260-300(M); 200-400(F)
SC	M: 250, 355, 500, 700 F: 250, 398, 563, 631, 981, 1000	M: 530 (420 - 668) F: 575 (509 - 648)	• Convulsions: all doses • Loss of Balance/Righting Reflex: all doses • ↓ Activity: 250, 355, 398, 500 • Dermal Lesion: 250, 355, 398, 500, 563, 631, 700 • Unsteady Gait: 355, 398, 500, 631, 700 • Death: 500-700(M); 563-1000(F)
RATS (CD): 8M & 8F			
IV	M: 25, 40, 45, 48, 60, 75 F: 25, 40, 45, 48, 60, 75	M: 44.0 (40.0 - 48.4) F: 48.5 (43.6 - 52.8)	• Convulsions: 25, 40, 45, 48, 60 • Tremors: 25, 48, 75 • ↓ Activity: 25, 48 • Loss of Balance/Righting Reflex: 25, 40, 48 • Labored Breathing: 40, 45, 48, 60 • Prostration: 40, 60 • Unsteady Gait: 40, 45 • Death: 45-75(M&F)
PO	M: 300, 375, 450, 620 F: 150, 250, 450, 620	M: 484 (401 - 583) F: 310 (231 - 415)	• ↓ Activity: all doses • Convulsions: 200, 300, 375, 450, 620 • Tremors: 150, 250, 375, 620 • Unsteady Gait: 150, 300, 375, 620 • Prostration: 250, 300, 375, 450, 620 • Loss of Balance/Righting Reflex: 250, 300, 375, 450, 620 • Labored Breathing: 300 • Death: 300-620(M); 150-620(F)
SC	M: 560, 800, 110, 1600 F: 910, 1239, 1350	M: 1080 (866 - 1347) F: 1150 (1012 - 1306)	• Convulsions: all doses • Labored Breathing: all doses • ↓ Activity: all doses • Ocular Damage: all doses • Prostration: all doses • Tremors: all doses • Unsteady Gait: all doses • Death: 800-1600(M); 910-1800(F)

TABLE 7 (Cont.). Acute toxicity after a single administration of nalmefene to mice, rats and rabbits.

SPECIES (STRAIN): NUMBER OF ANIMALS/SEX/DOSE			
ROUTE	DOSE (mg/kg)	LD_{50} (MG/KG) (95% CONF. LIMITS)	CLINICAL SIGNS (DOSE (mg/kg) OBSERVED)
RABBITS (DUTCH): 5M & 5F			
IV	M: 12, 14, 15, 17, 20, 24 F: 12, 14, 15, 17	M: 19.5 (17.7 - 21.4) F: 15.0 (13.6 - 16.5)	• Convulsions: all doses • Labored Breathing: all doses • Tremors: 12, 14, 15, 17, 20 • Loss of Balance/Righting Reflex: 12, 15, 17 • Prostration: 15, 20, 24 • ↓ Activity: 15, 17 • Unsteady Gait: 14 • Death: 15-24(M); 12-17(F)
PO	M: 225, 330, 420, 500, 700 F: 225, 330, 420, 500, 700	M: 486 (311 - 757) F: 488 (369 - 645)	• Tremors: all doses • Convulsions: all doses • Unsteady Gait: 225, 330, 420, 500 • ↓ Activity: 420, 500 • Loss of Balance/Righting Reflex: 225, 500, 700 • Labored Breathing: 420 • Death: 500-700(M); 330-700(F)
SC	M: 125, 150, 180, 210 F: 125, 150, 180, 210	M: 163 (134 - 199) F: 157 (131 - 188)	• Tremors: all doses • Convulsions: all doses • ↓ Activity: all doses • Labored Breathing: all doses • Unsteady Gait: all doses • Ocular damage: all doses • Prostration: all doses • Loss of Balance/Righting Reflex: 125, 150, 180 • Death: 125-210(M&F)

SUBACUTE TOXICITY STUDIES:

The following subacute toxicity studies were submitted in Volumes 1.10 and 1.14:

SUMMARY OF SUBACUTE TOXICITY STUDIES WITH NALMEFENE					
STUDY Nº	TEST TYPE	STUDY CATEGORY	ROUTE	DOSAGES (MG/KG/DAY)	SPECIE
KP9R/JF-1	7 Day	Dose Range Finding Study	IV	0, 5, 10, 15, and 25	Rat
KP10M/JF-1	30 Day	Subacute Toxicity Study	IV	0, 5, 11, and 25	Rat
KP-1/JF-1	14 Day	Subacute Toxicity Study	SC	0, and 0.8	Rat
KP8R/JF-1	7 Day	Dose Range Finding Study	IV	0, 1.5, 3.0, 6.0, and 8.0	Dog
KP11M/JF-1	30 Day	Subacute Toxicity Study	IV	0, 3, 4.6, and 8.0	Dog

The subacute toxicity of nalmefene was evaluated in rats following intravenous and subcutaneous administration and in beagle dogs following intravenous administration. These toxicity studies were originally submitted under INC n December 12, 1982 and reviewed by HFD 120 on January 27, 1983. It was under the sponsorship of

In rats, nalmefene was administered intravenously or subcutaneously as a single daily dose for 30 or 14 consecutive days, respectively. The subcutaneous toxicity study in dogs was conducted for 30 consecutive days. Prior to conducting each main study, a dose range finding study (Study Nº KP9R/JF-1 and KP8r/JF-1) was conducted to determine the appropriate doses to use in the final study.

<u>Subacute Toxicity of Nalmefene Administered Intravenously in Rats</u>.

period of 2/4/82 to 4/17/82. The study was conducted in compliance with the Good Laboratory Practice regulations.

One hundred and twenty (60 males and 60 females) Sprague-Dawley rats were used as subjects in this study. When dosing began, the rats were 41 to 44 days of age and their mean body weight (± S.E.) were 178.7 g (±2.4) and 126.9 g (± 1.4) in the males and females, respectively. The rats were singly housed and had continuous access to water and food (Purina Certified Laboratory Chow).

The rats were randomly assigned to the following four groups: 0.0, 5.0, 11.0, and 25.0 mg/kg/day. Fifteen male and fifteen female rats were assigned to each group. These dosages were selected based on the results from the seven day dose range finding study (Study Nº KP9R/JF-1). Nalmefene hydrochloride or vehicle (sterile saline (0.9%) for injection, U.S.P.) was administered daily for 29 to 31 consecutive days, depending on the date of necropsy, by the intravenous route at a rate of 10 to 15 seconds per injection. The injections were made into the caudal vein while the subjects were restrained in a restraining cage. Dosing was performed at approximately the same time each day.

Nalmefene hydrochloride was dissolved in sterile water for injections (U.S.P.) to a stock solution of 5 mg/ml. This stock solution was used to dose the high dose group. The lower doses were prepared by diluting the stock solution to the appropriate concentration. The injection volume was 5 ml/kg.

This study included daily observations for changes in appearance and/or behavior for 29 to 31 consecutive days, body weight (weekly and on the day of necropsy), mortality (daily for 29 to 31 days), food consumption (weekly), urinalysis, serum chemistry (performed on 5M and 5F per group only; 16 hrs after first treatment), hematology, organ weight (liver, kidney, heart, adrenals, testes, brain), macroscopic examination and microscopic evaluation (control and high dose groups only).

Results. A summary of the clinical signs resulting from nalmefene treatment for thirty consecutive days are presented in Table 8. Consistent with the overt signs of toxicity in the acute studies, the toxic signs observed were of the CNS type. Tremors, convulsions, unsteady gait, salivation, jumping behavior, loss of balance/righting reflex were the primary drug-induced clinical signs observed in the majority of the subjects. A dose with no-effect was not achieved in this study. The incidence, severity and duration of these overt signs were dose-related.

In the 5.0 mg/kg group, most of the rats exhibited either no overt signs of toxicity, mild salivation, mild convulsions and/or mild tremors. The tremors and convulsions were rapid in onset, occurring within seconds, and of short duration (5 minutes). By the fourth week of the study, the subjects had developed tolerance to the drug-induced tremors and convulsions; most of the subjects appeared normal after each dosing session.

The major overt signs of toxicity observed in the mid dose (11.0 mg/kg) group throughout the study were mild convulsions and/or mild to severe tremors; these symptoms were evident immediately after the injection and had subsided by 5 minutes post-dosing. The severity of the convulsions and tremors was observed to decrease toward the end of the dosing period. Other clinical signs seen frequently were: jumping behavior, leg straddle, unsteady gait, salivation, and loss of balance/righting reflex. By the fourth week of the study most rats were only exhibiting mild tremors, and/or mild to moderate convulsions, of rapid onset and short duration coupled with mild salivation at 5 minutes post-dosing.

Nalmefene-induced toxicity and mortality were observed in the high dose rats. Both male and female rats in the high dose group (25 mg/kg) reacted to nalmefene, during the first week of the study, with mild to moderate convulsions, tremors, jumping behavior, leg straddle, loss of balance/righting reflex, and salivation. There were occasional reports of oral bleeding, decreased activity, unsteady gait, immobilization, and vocalization. Tremors, mild to moderate convulsions, jumping behavior, and loss of balance/righting reflex were still the predominate reactions to nalmefene during the second week of the study. Other signs frequently observed during this week were: salivation, unsteady gait, vocalization, oral bleeding, and leg straddle. During the third week of the study, convulsions frequently coupled with loss of balance/righting reflex, vocalization, jumping behavior, unsteady gait, and leg straddle were evidence after nalmefene treatment. Mild to severe salivations were observed in all rats five minutes after dosing. Labored breathing, tremors, oral bleeding, and decreased activity were seen less frequently. By the fourth week of the study, the observed clinical sign were fairly uniform. Mild to moderate convulsions and salivation, coupled with one or more of the following symptoms, loss of balance/righting reflex, mild tremors, leg straddle, and jumping behavior, were consistently seen after each treatment. All responses except, for salivation, occurred immediately after injection and lasted for 5 minutes or less except for the convulsions which lasted for 10 minutes or longer. The salivations did not occur until 5 minutes after the injection.

Also in the high dose group, there was an observed change in behavior during the course of the study. These rats became increasingly hyperactive and aggressive behavior during the fourth week of the study.

Nalmefene-induced deaths were observed mainly in the male rats in the high dose group. On day 6 of the study, one male rats died within 5 minutes after treatment; prior to death it had a mild convulsive episode and loss of balance/righting reflex. On the eight day of the study, two males died 5 minutes after receiving their nalmefene injection. Prior to death both rats exhibited tremors, convulsion, prostration, and loss of balance/righting reflex immediately after injection; laborious breathing was also observed in one of the rat. Another male rat died 16 hours after receiving his 13th dose of nalmefene. One male and one female rat died immediately after dosing on day 15 and 17, respectively. The female rat exhibited moderate convulsions, mild jumping behavior and loss of balance/righting reflex immediately before dying. The male death was the result of poor injection techniques. One male and one female died on days 30 and 32, respectively, as a result of blood sampling procedures.

There were drug-induced changes in body weight in the female rats treated with nalmefene in comparison to the control rats. Female rats treated with 11.0 mg/kg showed a statistically significant increase in body weight during the 3rd and 4th week of the study. In female rats in the 25.0 mg/kg group, body weight was statistically higher than the control rats throughout the entire study duration. This increase in body weight paralleled the observed increase in food consumption in these female rats.

In comparison to control values, a significant increase in brain weight was observed in the brain of male and female rats treated with 25.0 mg/kg of nalmefene and the liver of female rats treated with 11.0 and 25.0 mg/kg of nalmefene. A dose-related increase in liver to bodyweight ratios were noted in females in the 11.0 and 25.0 mg/kg nalmefene group.

Group mean hematocrit, partial thromboplastin time, hemoglobin concentration, red blood cell count, mean cell hemoglobin concentration and white blood cells counts were statistically ($p=0.05$ to 0.01) different from the control values in some of the male rats in each nalmefene treatment group. Both male and female rats in the three treatment groups had platelet levels were higher than the control; this increase was not statistically significant. A dose-related increase in the hemoglobin levels was observed in female rats treated with 25.0 mg/kg; according to the sponsor calculations this was statistically significant.

No drug related macroscopic or microscopic changes were observed in either the female or male rats. Lung congestion, early chronic murine pneumonia and hyperplasia of the lymph nodes were common to all groups. Myeloid metaplasia of the spleen and atrophy of the Harderian gland were observed often in nalmefene treated rats. According to the sponsor's veterinary pathologist, these histological changes were stress related.

Table 8. Summary of Clinical Signs in Rats treated intravenously with nalmefene for 1 month.

DOSE (MG/KG)	CLINICAL SIGNS			
	WEEK 1	WEEK 2	WEEK 3	WEEK 4
0.0	• Normal	• Normal	• Normal	• Normal
5.0	• Mild Tremors seen on day 1 - Rapid onset & brief (5 min) - In 1/3 rd of the M & 1/2 of the F • Salivation - Occasional occurrence during the week • ↓ Activity: In 1F on days 5 & 6	• Overt signs observed in male rats - No drug-induced effects observed • Overt signs observed in female rats - Mild Convulsions - Mild tremors - Slight ↓ in activity - Salivation - Unsteady Gait	• Mild salivation at 5 min post-treatment • Mild tremors • Mild Convulsions	• Most of the subjects were normal • See some incidences of salivation
11.0	• Major signs seen in majority of M & F - Mild To Severe Tremors: rapid onset and short duration - Mild Convulsions: rapid onset and short duration • Other frequent occurring reactions early in the week - Jumping Behavior - Leg Straddle - Salivation - Unsteady Gait	Male Rats: • Mild Convulsions & Jumping Behavior - seen in most male rats - rapid onset and short duration (5 min) - ↓ in frequency toward end of week • Other frequent clinical signs of toxicity - mild tremors - salivation - leg straddle - unsteady gait - ↓ activity - loss of balance/righting reflex Female Rats: - Mild and brief convulsions - tremors one day only in 2 rats - 3 females were normal	• Mild to moderate tremors or convulsions - Rapid onset (few seconds) - Short duration • Salivation - 5 min observation period after dosing • Unsteady gait • Jumping Behavior	• Most rats displayed - mild tremors and/or - mild to moderate convulsions - salivation was coupled to the tremors and/or convulsions
25.0	• Responses with rapid onset and short duration (5 min) - Mild to moderate convulsions - Tremors - Jumping Behavior - Leg Straddle - Loss of balance/righting reflex • Salivation • Death: 1 male on day 6	Male Rats: • Death - 2M within 5 min after 8th dosing - 1M 16 hours after the 13th dosing • Major Signs seen in both M & F - Mild to moderate convulsions - Tremors - loss of balance/righting reflex - Jumping Behavior • Other frequent signs seen in M & F - Unsteady gait - Salivation - Vocalization - Leg straddle - oral bleeding	• Death - 2 males: after the 17th dose - 2 females: after the 15th dose • Mild to moderate convulsions - frequently coupled with: loss of balance/righting reflex, vocalization, jumping behavior, leg straddle, unsteady gait • Mild to Severe Salivation - Seen in all rats - 5 min post dosing	• Death - 1M (day 30); 1F (day 32) • Major Signs of toxicity - Mild to moderate convulsions - Salivation • Signs coupled with the major response - Loss of balance/righting reflex - Mild tremors - Leg straddle - Jumping Behavior • Behaviors exhibited prior to dosing - Hyperactivity - Aggressive Behavior

Subacute Toxicity of Nalmefene Administered Subcutaneously in Rats (Study Nº KP-1/JF-1).

This toxicity study was conducted at during the period of August 31, to October 16, 1981. A signed Quality Assurance statement was submitted; no GLP statement was included. This study was conducted for the purpose of assessing the toxicity associated with the subcutaneous administration of 0.8 mg/kg nalmefene for 14 consecutive days in male and female rats.

Twenty Sprague-Dawley rats were subjects for this study. At the initiation of the dosing procedure, the rats were 38 to 45 days of age and they weighed between 152 to 200 grams. The rats were singly housed and had continuous access to water and food (Purina Certified Rodent Chow).

The rats were randomly assigned to the following two groups: 0.0 and 0.8 mg/kg/day. Five male and five female rats were assigned to each group. Nalmefene hydrochloride or vehicle (sterile saline for injection (USP)) was administered daily for 14 days. Nalmefene hydrochloride was dissolved in sterile saline to a stock solution of 0.32 mg/ml. The injection volume was 2.5 ml/kg.

This study included daily observations (at 1, 2, 5 and 24 hours after dosing) for overt signs of toxicity and changes in body weight for 14 consecutive days. On day fifteen all subjects were humanely sacrificed and macroscopic and microscopic (ovaries, testes only) examination conducted.

Results. This dose of nalmefene did not elicit any significant overt signs of toxicity in either the male or female rats. There was no significant difference between the treated and control rats mean bodyweight. No drug-induced death or morphological or histological changes occurred.

Subacute Toxicity of Nalmefene Administered Intravenously in Dogs (Study Nº KP11M/JF-1).

A thirty day toxicity study was conducted in beagle dogs at during the period of 1/28/82 to 4/23/82. The study was conducted in compliance with the Good Laboratory Practice regulations; a Quality Assurance statement was present and signed.

Twenty-four beagle dogs, twelve males and twelve females, were subjects for this study. At the initiation of dosing, the dogs were 19 to 20 months old and their mean body weight ranged from 10.6 to 13.6 kg and 7.4 to 10.0 kg for the males and females, respectively. The dogs were singly housed with free access to water and were restricted to 400 g of food (Purina Certified Canine Diet) per day. The dogs were randomly assigned to the following nalmefene groups: 0, 3.0, 4.6, and 8.0 mg/kg/day. Three males and three females were assigned to each dosing group. These doses were selected based on the results from the seven day dose range finding study (KP8R/JF-1). Nalmefene hydrochloride or vehicle (sterile saline for injection) was administered daily for 30 to 32 consecutive days, depending on the day that the necropsy was conducted, by the intravenous route of administration at a rate of 10 to 15 seconds per injection. The injections site was the cephalic vein; the left and right veins were injected on alternate days.

Nalmefene hydrochloride was dissolved in sterile water for injections (U.S.P.) to a stock solution of 80 mg/ml. This stock solution was used to dose the high dose group. The lower doses were prepared by diluting the stock with saline to the appropriate concentration. The injection volume was 0.2 ml/kg. Dosing was conducted at approximately the same time each day.

This study included daily observations for signs of overt toxicity, change in body weight (study days 0, 7, 14, 21, and 28), urinalysis (4 to 6 days prior to dosing, study days 23 to 25), hematology and biochemical analysis (7 days prior to dosing, study days 0, 7, 14, and 29), ophthalmoscopic examination (study day 0 and 30), daily food consumption, organ weight (brain, heart, liver, kidneys, adrenals and gonads), and macroscopic and microscopic evaluatior .

Results. All dogs survived the entire duration of the study. The primary nalmefene-induced overt signs of toxicity observed in the dogs following intravenous administration included: ataxia, unsteady gait, urination, defecation, salivation, tonic extensor convulsion, vocalization and hypoactivity. With the exception of hypoactivity, the overt signs developed immediately after injection and lasted for 2 to 5 minutes. Hypoactivity lasted for four to seven hours post-injection. These signs were most evident during the first week of dosing; by the fourth week of the study, the number of clinical signs and the severity of them had decreased. With a few exceptions in the high dose group, post-injection salivation was observed occasionally in some of the subjects. Nalmefene-induced overt signs of toxicity are summarized in table 9.

A no-effect dose was not achieved in this study; however in the dose range finding study (KP8R/JF-1) 1.5 mg/kg of nalmefene produced no overt signs of toxicity in dogs receiving a daily single dose for seven consecutive days. Salivation (pre- and post-injection) was the most frequently observed effect following treatment with 3.0 mg/kg of nalmefene.

In the mid dose group (4.6 mg/kg/day), ataxia, urination, defection, salivation and hypoactivity was seen the most frequently following injection during the first week of the study. In subsequent weeks, slight to heavy pre- and post-injection salivation was the predominate toxic sign observed in both female and male dogs.

Tonic extensor seizures were observed in 3 males and 1 female dog during the first week of treatment with 8.0 mg/kg of nalmefene. The seizures occurred rapidly and lasted for approximately 10 seconds. In all cases, violent thrashing, salivation, ataxia, leg splaying, vocalization followed these convulsions. All dogs appeared normal within 4 to 6 minutes after dosing. On day 2, one male dog displayed moderate tremors and salivation immediately after the injection; moderately severe tonic convulsions, thrashing, vocalization, vomiting and ataxia followed the tremors 4 to 7 minutes after the injection. During the remainder of the week, frequently reported toxic signs were hindlimb ataxia, muscle tremors, prolonged hypoactivity, and post-injection salivation. After the first week, the convulsions occurred infrequently.

During the second week, one male dog treated with the high dose of nalmefene had tonic extensor seizures immediately after the tenth dosing; thrashing, vocalization, ataxia, leg splaying and laborious breathing followed the seizures. During the second week, hind limb ataxia (3/3) and pre- and post-dosing salivation was occasionally seen in the female dogs.

During the third week of the study, mild episodes of salivation, and ataxia were frequently observed after nalmefene treatment.

During the fourth week of the study, no overt signs of toxicity were observed in the female dogs. One male dog experienced a severe convulsive episode with thrashing, vocalization and salivation, ataxia, mild tremors developing two minutes after the injection. These drug-induced effects was followed by hypoactivity which lasted for four hours.

No apparent drug-related effects on body weight, food consumption, mean absolute organ weight, ophthalmology, histopathology, macroscopic pathology were observed.

Drug-induced hematology changes were noted in the female dogs dosed with 3.0, 4.6, and 8.0 mg/kg. A decrease in mean cell volume was measured on day 7 of the study. According to the sponsor, only the decrease observed in the 3.0 and 8.0 mg/kg group was statistically significant ($p=0.05$ and 0.01, respectively).

Serum biochemistry analyses on study days 7 and 29 showed a significant increase in alkaline phosphatase level in males treated with 4.6 mg/kg/day of nalmefene and on day 3 of the study in females treated with

3.0 mg/kg/day nalmefene. The group mean value of SGOT was significantly increased on study day 14 in males rats dosed with 8.0 mg/kg/day nalmefene.

Urine analysis showed an apparent increase incidence in granular casts and white blood cells in the urine of both males and female dogs dosed with 8.0 mg/kg/day. These observations may be the result of chronic inflammation observed in several tissues in dogs treated with nalmefene.

Table 9. Summary of Clinical Signs in Dogs treated intravenously with nalmefene for 1 month.

DOSE (MG/KG)	CLINICAL SIGNS			
	WEEK 1	WEEK 2	WEEK 3	WEEK 4
0.0	• Normal	• Normal	• Normal	• Normal
3.0	MALES • Day 1 - 2M: without signs of toxicity - 1M: unsteady gait; slight ataxia in the hind limbs (rapid onset, brief duration) • Days 2 - 7 - 1M: heavy salivation (pre- post-dosing), vomiting (2 incidences) - 1M: hyperactivity FEMALES • Day 1: - No observable overt signs of toxicity • Day 2: - 1F: brief, slight trembling in the hind limbs • End of the week: - 2F: slight to heavy salivation	MALES - No drug-induced effects observed FEMALES - No drug-induced effects observed	MALES • Days 15 and 16 - 1M: hyperactive, and vocal • Days 17-21 pre- and post-dosing salivation FEMALES • No overt toxicity observed	MALES • Most of the subjects were normal • See some incidences of salivation FEMALES • Without overt signs of toxicity
4.6	MALES •Day 1 - Ataxia; urination & defecation (rapid onset and brief duration) • Days 2-7: - ataxia (hind and forelimbs); - heavy salivation - brief or prolonged hypoactivity - loss of balance - vocalization FEMALES • 3F: slight hind limb ataxia & muscle tremors • 2F: Pre & post salivation • 1F: hypoactivity, squatting; defecation • 2F: urination	MALES - Pre- and post-dosing salivation (heavy) FEMALES - Pre- and post-dosing salivation (heavy)	MALES • Slight to heavy salivation FEMALES • 1F: Normal • 2F: slight to heavy salivation	MALES • No overt signs of toxicity • Occasional occurrences of salvation FEMALES • No overt signs of toxicity • Occasional incidences of treatment-associated salivation

Table 9 (Cont.). Summary of Clinical Signs in Dogs treated intravenously with nalmefene for 1 month.

DOSE (mg/KG)	CLINICAL SIGNS			
	WEEK 1	WEEK 2	WEEK 3	WEEK 4
8.0	**MALES** •Day 1: - 3M: moderate to severe extensor convulsions (10 sec duration) followed by violent thrashing, ataxia, vocalization, salivation, splay legs • Day 2 - 1M: brief moderate tremors, salivation followed by moderately severe tonic convulsions, thrashing, vocalization, vomiting, ataxia; - 2M: hind limb ataxia, defecation • Days 3-7 - hind limb ataxia - heavy salivation - prolonged hypoactivity - prostration and/or crouching **FEMALES** • DAY 1 - 1F: ataxia, urination & defecation immediately, followed by brief severe tonic extensor convulsions coupled with thrashing, salivation, ataxia with muscle tremors - 2F: ataxia with or without muscle tremors & vocalization • Days 2-7 - slight hind limb ataxia - muscle tremors - prolonged hypoactivity	**MALES** • Day 10 (1M) - tonic extensor convulsions with thrashing, vocalization, ataxia, leg splaying and labored breathing • Occasional Occurrence - hypoactivity (2M) - hind limb ataxia (2M) **FEMALES** • Occasional Occurrence - Hind limb ataxia (3F) - Pre- and pst-dosing salivation	**MALES** • Days 15 - 20 - 3M: without overt signs of toxicity • Day 21 - 1M: ataxia and urination - 2M: no overt signs of toxicity **FEMALES** • Days 15 - 21 - mild episodes of salivation	**MALES** • Day 21 - heavy salivation • Day 22 - severe convulsion s with thrashing - vocalization - salivation - ataxia - muscle tremors - hypoactiivity (4 hrs post-dosing) **FEMALES** • No overt signs of toxicity

SUBCHRONIC TOXICITY STUDIES.

The following subchronic toxicity studies were submitted in Volumes 1.11 and 1.15:

SUMMARY OF MULTIDOSE TOXICITY STUDIES WITH NALMEFENE				
STUDY Nº	**TEST TYPE**	**STUDY CATEGORY**	**ROUTE**	**SPECIE**
KPC/2/84	28 Days	Dose Range Finding Study	PO	Rat
KPC/5/84	13 Week	Oral Toxicity Study	PO	Rat
KPC1/84	28 Days	Dose Range Finding Study	PO	Dog
KPC/4/C	13 Week	Oral Toxicity Study	PO	Dog

Rat - 13 Week Oral Toxicity Study (Study Nº KPC/5/84).

This thirteen week toxicity study was conducted in rats to assess the potential toxicity of nalmefene when administered in their diet. This route of administration was selected because the oral route was the original anticipated therapeutic route in humans wher Both the main and dose range finding studies were originally submitted under IND on January 29, 1985 and reviewed by HFD 120 on February 13, 1985. This study was conducted at the period of April 9, 1984 to August 1984. This study was conducted in compliance with the Good Laboratory Practice Regulations; a signed Quality Assurance Statement was included in the submission.

One hundred male and one hundred female Sprague-Dawley (CD Strain) rats were subjects for the study. The rats were group housed (5/cage) and had continuous access to water and food (Rodent Diet: SQC No.1 Modified Expanded, Ground, Special Diet Services Limited, Witham, Essex, England).

The rats were randomly assigned to the following four treatment group: 0.0, 20.0, 100.0, and 500.0 mg/kg/day. Fifteen males and 15 females were assigned to each group. These doses were selected based on the findings from the 28 day dose range finding study (Study Nº KPC/2/84). In addition to the main study, two other studies were conducted. A satellite study was conducted in 10 male and 10 female rats to collect information on pre-exposure/baseline clinical pathology. A second study was conducted in six rats/sex/group to obtain blood samples to determine the degree of drug absorption.

Nalmefene chlorhydrate was administered for thirteen consecutive weeks by diluting the rodent diet with nalmefene. The dietary mixture was made fresh weekly and the subjects had ad libitum access.

The rats were observed daily for changes in behavior, appearance, and drug-induced clinical signs. Body weight and food consumption were measured weekly. Clinical chemistry analysis, hematology analysis, and urinalysis were conducted on the first 10 rats/sex/group after the fourth and twelfth week of the dosing. Ophthalmoscopic examination was conducted on all subjects before dosing was started and after the 4th and 12th week of the study in the control and high group subjects only. Macroscopic, and microscopic examinations were conducted at the end of the study. At the time of the necropsy, the weight of the adrenals, brain, lung, liver, kidney, heart, thymus, ovaries, uterus, testes, pituitary, prostrate, and thyroids were measured.

Results. There was no drug-related toxicity observed in the rats. With the exception of three rats in the control group, all rats survived the duration of the study. One male and 1 female died at blood sampling and a second male rat was found dead during the seventh week.

Nalmefene-induced changes in body weight were observed. These effects were dose-related. Both males and female rats in the low dose group (20 mg/kg/day) body weight was comparable to the control group. Suppression of body weight gain was observed in both the female and male rats; the effects were more pronounced in the male. Male rats in 100 mg/kg/day group showed a statistically significant decrease in body weight gain from week 3 thru week 13. From the first week to the end of the study, male rats in the 500 mg/kg/day nalmefene group showed a statistically significant decrease in weight gain. The suppression of body weight gain in the female rats had a delayed onset. Female rats in the third group (100 mg/kg/day) mean body was significantly different from control during weeks 9 thru 12. Female rats treated with 500 mg/kg/day of nalmefene mean body weight was significantly different from control from week 3 thru week 13.

A dose-related reduction in food consumption was also seen throughout the study. As seen with change in body weight gain, these effects were more pronounce in the male than the female rats. In comparison to the control group, the mean overall reduction in food consumption was 5, 10 and 17 % for the low-, mid-, and high- dose group, respectively. Only female rats in the mid- and high-dose groups showed a reduction in food consumption. A 5 and 10 % decrease was observed in the mid- and high-dose group, respectively.

No apparent drug-related effects on ophthalmology, urinalysis values, gross pathology or histopathology occurred. Sporadic non dose-related statistically significant changes were observed in some of the hematology and clinical parameters evaluated. These observed statistically significant differences are summarized the table 10.

Absolute heart, lung, and liver weights were significantly reduced in all males in the three nalmefene treatment groups. The absolute spleen weight was reduced in the mid- and high-dose group only.

Bodyweight related organ weights showed significant increase in brain weights in males from the 20 and 100 mg/kg/day treatment group and in both males and females in the 500 mg/kg. A significant increase in bodyweight related lung, and livers were observed in females from the high-dose group. The lungs of females in the mid-dose group also was significantly increased. Bodyweight related kidneys weights were significantly increased in males and females in the mid-dose and high-dose groups and in the males in the low-dose group.

No significant macroscopic pathology was observed in the nalmefene-treated rats.

Table 10. Summary of Nalmefene Effects on Hematology and Clinical Chemistry Parameters in Rats Treated Orally With Nalmefene For thirteen Weeks.

DOSE (MG/KG)	HEMATOLOGY PARAMETERS		CLINICAL CHEMISTRY PARAMETERS	
	AFTER WEEK 4	AFTER WEEK 12	AFTER WEEK 4	AFTER WEEK 12
0.0				
20.0	**MALES** • No effect **FEMALES** • ↓ Mean Corpuscular Hemoglobin	**MALES** • ↑ Mean Corpuscular Hemoglobin Conc. • ↓ Total Leukocytes Counts **FEMALES** • ↓ Mean Corpuscular Hemoglobin Conc.	**MALES** • ↓ Alkaline Phosphatase **FEMALES** • No effect	**MALES** • ↑ Gamma Globulins • ↓ Total Protein **FEMALES** • ↑ Gamma Globulins
100.0	**MALES** • ↑ Hemoglobin • ↓ RBC • ↑ Lymphocytes counts **FEMALES** • ↓ Prothrombin Time	**MALES** • ↑ Partial Thromboplastin Time **FEMALES** • ↓ Mean Corpuscular Volume	**MALES** • ↓ Urea Nitrogen Values **FEMALES** • ↓ Urea Nitrogen Values • ↓ SGPT • ↓ Beta Globulins	**MALES** • ↓ Beta Globulins • ↓ Total Protein **FEMALES** • ↑ Gamma Globulins
500.0	**MALES** • No effect **FEMALES** • ↓ Hemoglobin • ↓ Prothrombin Time	**MALES** • ↑ Partial Thromboplastin Time **FEMALES** • ↓ Mean Corpuscular Volume • ↓ Total Leukocyte Counts • ↓ Prothrombin Time • ↑ Neutrophil Counts • ↓ Lymphocyte Counts • ↑ Partial Thromboplastin Time	**MALES** • ↓ Urea Nitrogen Values • Gamma Globulins • ↓ Potassium **FEMALES** • ↓ Alkaline Phosphatase • ↓ SGOT • ↓ Sodium	**MALES** • ↓ Blood Urea Nitrogen **FEMALES** • ↑ Sodium

13 Week Oral Toxicity Study In The Dog (Study Nº KPC/4/C).

This thirteen week oral toxicity study was conducted in beagle dogs at in the period of December 1983 to March 1984. This route of administration was selected because the oral route was the original anticipated therapeutic route in humans when The main study and dose range finding study conducted in dogs and rats were originally submitted under IND on October 2, 1984 and reviewed by HFD 120 on December 14, 1984. This study was conducted in compliance with the Good Laboratory Practice Regulations; a signed Quality Assurance Statement was included in the submission.

Twelve male and twelve female beagle dogs were used in this study. Upon arrival, the dogs were between the age of 4 and 5 months and they weight were between 3.8 to 6.8 kg. Following a 6 week acclimation period, the dogs were randomized into 4 treatment groups of 3 dogs/sex/group. The four treatment groups were: 0, 4, 20, and 100 mg/kg. These doses were selected as a result of the dose range finding study (KPC1/94). Nalmefene was administered orally in gelatin capsules.

Clinical signs, mortality, food and water consumption, body weight, hematology, clinical chemistry, urinalysis, and ophthalmology were determined. At the end of the study, the dogs were sacrificed, microscopic and macroscopic examinations were performed.

Results. Because this study has been reviewed in detail earlier, the results are briefly summarized in the table below.

Table 11. Summary of the results from the 13 week toxicity study in dogs.

DOSE (MG/KG)	CLINICAL SIGNS	MORTALITY	FOOD & WATER CONSUMPTION	BODY WEIGHT	HEMATOLOGY	CLINICAL CHEMISTRY	GROSS PATHOLOGY	HISTOPATHOLOGY
0.0	-	-	-	-	-	-	-	-
4.0	• No Effect	• No Effect	• No Effect	• No Effect	• Within Normal Range	• Within Normal Limits	• Normal	• Normal
20.0	• No Effect	• No Effect	• No Effect	• No Effect	• Within Normal Range	• Within Normal Limits	• Normal	• Normal
100.0	• Peri-dosing Salivation: - F > M - up to 2 hrs post dosing • Convulsions: - 2M on 4 occasions - 3F on 7 occasions - Occurred within 1 hr of dosing - Coupled with: emesis, ataxia, salivation, opisthotonos, paddling, gasping respiration - Recovery: complete between 2 and 24 hours	• 1M: Week 1 • 1F: Week(W) 11 • 1M: Killed in extremis W9 • Convulsions prior to death	• No Effect	• No Effect	• Within Normal Range	• Males - Within Normal Limits Females • 2F: Within normal limits • 1F: 1 blood urea nitrogen W12	• Normal	• Normal

CHRONIC TOXICITY STUDIES.

The following chronic toxicity studies were submitted in Volumes 1.11 and 1.15:

SUMMARY OF PRECLINICAL SAFETY STUDIES WITH NALMEFENE				
STUDY Nº	TEST TYPE	STUDY CATEGORY	ROUTE	SPECIE(S)
KPC/15/C	52 Week	Dietary Study		Rat
KPC/6/C	52 Week	Oral Toxicity	PO	Dog

52 Week Dietary Study In Rats.

This 52 week toxicity study was conducted at the period of December 11, 1984 to December 12, 1985. The study was conducted in compliance with the Good Laboratory Practice Regulations; a signed Quality Assurance Statement was included in the submission.

One hundred and twenty male and one hundred and twenty female Sprague Dawley (CD Strain) rats were subjects for this study. On arrival to the study site, the rats were 3 to 4 weeks of age and their body weight was within the range of 60 to 70 grams. The rats were grouped housed (5/cages) and had continuous access to water and food (SQC Rat and Mouse Maintenance Diet No. 1 Expanded, Ground Special Diets Services Limited, Witham England) during the acclimation and treatment periods. The rats were acclimated for 12 days before initiating treatment.

The rats were randomly assigned to the following treatment groups:

GROUP	DOSE (MG/KG/DAY)	Nº MALES/FEMALES PER GROUP
1	0	25/25
2	30	25/25
3	100	25/25
4	300	25/25

In addition to the main study, a satellite study was conducted in ten male and ten female rats to provide pre-exposure clinical pathology information.

All rats were examined daily for drug-induced overt signs of toxicity, change in appearance and mortality. Food consumption for each cage was determined weekly and the group mean weekly intake was expressed as g/rat/week. Body weight was determined before the start of the study and at weekly intervals throughout the remaining study duration. Ophthalmoscopic examination was conducted on all subjects before dosing was started and after the 8th, 24th, and 50th week of the study in the control and high dose group only. Hematology and Urinalysis was conducted in the rats in the satellite study after the 4th, 12th, 24th, and 50th week of the study. Macroscopic and microscopic examinations were performed on all survivors at the end of the study. Organ weight was also determined during the necropsy.

Results. No drug-induced clinical signs were observed during the study. Six rats, 2 males and 4 females, died during the course of the study. As copied from the sponsor the details of these deaths are summarized in the following table:

GROUP AND SEX	ANIMAL NUMBER	WEEK OF DEATH	CIRCUMSTANCES
1M	13	44	Legs trapped in cage grid leading to partial paralysis and subsequent loss of condition. Found dead after two weeks
4M	79	13	Found dead 3 days after blood sampling.
1F	105	51	Died during blood sampling.
1F	109	49	Killed *in extremis*. Cranial mass impeding normal feeding.
3F	152	51	Found dead 1 day after blood sampling.
4F	184	51	Found dead 3 days after blood sampling.

None of the mortalities appear to be a result of the treatment. Four (1 male and 3 females) of the reported deaths was a result of the blood sampling procedure.

Consistent with the findings in the 13 week dietary toxicity study in rats, nalmefene retarded the growth of the rats. This decrease in body weight gain was dose-dependent. Both males and females in the low dose (30 mg/kg/day) group body weight gain was comparable to the control group. Nalmefene-induced suppression of body weight gain was observed in both male and female rats; the effects were more pronounced in the male rats. Male rats in the 100 mg/kg group showed a statistically significant decrease in body weight gain during weeks 1-5, week 20, 40 and 52 of the study. At the termination of the study, the group mean body weight was 13 percent lower than the control group mean body weight. Male rats in the high dose group (300 mg/kg) group mean body weight was statistically significant from the control group from week 1 to the end of the study. At the end of the study, the group mean body weight for the rats in the high dose group was 20% lower than the group mean body weight for the control rats.

The growth of the female rats in the low (30 mg/kg) and intermediate (100 mg/kg) nalmefene groups was comparable to the control rats. In contrast to the male rats in the high dose group, the suppression effects of nalmefene on body weight gain had a delayed onset in the high-dose females. The growth of the female rats in the high dose group was comparable to the control rats during the first sixteen weeks of the study. The rate of body weight gain was lower than the control rats during the remaining of the study, but according to the sponsor this decrease in body weight gain was not statistically significant.

Paralleling the suppression of growth, a dose-dependent decrease in food consumption was observed. Food consumption in the low dose male group was comparable to the control group. Males rats in the intermediate and high dose groups showed a 10% and 7% reduction in food consumption throughout the study, respectively. The food consumption of females in the low, intermediate and high dose group 6%, 26%, and 6-9% less than the control throughout the study, respectively.

No apparent nalmefene-induced effects on ophthalmology, hematological parameters, urinalysis values, or gross pathology were observed.

There were observed significant difference in the absolute weight of some of the organs in rats treated with nalmefene. These results are summarized as following:

ORGAN	OBSERVED TREATMENT GROUPS (MG/KG)	COMMENTS
Heart	• **Males:** 100, 300	• slight statistically significant ↑ in organ to body ratio weight
Liver	• **Females:** 30, 100, 300	• slight statistically significant ↑ in organ to body weight ratio
Spleen	• **Females:** 100, 300	• slight statistically significant ↑ in organ to body weight ratio
Brain	• **Males:** 100, 300 • **Females:** 300	• slight statistically significant ↑ in organ to body weight ratio
Kidneys	• **Males:** 100, 300 • **Females:** 300	• slight statistically significant ↑ in organ to body weight ratio • slight statistically significant ↑ in organ to body weight ratio
Adrenals	• **Males:** 300	• slight statistically significant ↑ in organ to body ratio weight
Lungs	• **Males & Females:** 100, 300	• slight statistical significant ↑ in organ to body ratio weight
Thyroids	• **Males & Females:** 30, 100, 300	• statistically significant ↑ in organ to body ratio weight
Testis	• **Males:** 100, 300	• statistically significant ↑ in organ to body ratio weight

There were some observed differences in the histopathology of some selected organs in both males and females treated with nalmefene in comparison to the untreated rats. In four males treated with 300 mg/kg/day of nalmefene, small areas of hemorrhaging was observed in the papilla of only one kidney. The kidneys of the female (7/25) rats in the high dose group had nephrocalcinosis. There was an observed increased incidence of cortical hyperplasia in the adrenals of females in the high dose group (300 mg/kg/day).

SPECIAL TOXICITY STUDIES

SPECIAL TOXICITY STUDIES

ABSTRACT

Several special toxicity studies were conducted with nalmefene. These studies were: 1) an acute perivascular and intravenous irritation study; 2) an intra-arterial injection tolerance study; 3) an intramuscular irritation study; 4) a subcutaneous injections tolerance study; 5) a delayed dermal sensitization study; and 6) an *in vitro* hemolysis study. A seventh special toxicity study, an acute intravenous toxicity study, was conducted with bisnalmefene, a degradation product of nalmefene. All studies were conducted according to GLP guidelines.

The acute toxicity of bisnalmefene succinate (100 - 250 mg/kg) was assessed in Crl:CD-1 mice following intravenous administration. Clinical signs associated with a single intravenous administration of bisnalmefene succinate included tremors, lethargy, and convulsions. The severity and duration of these clinical signs were dose-related. Bisnalmefene succinate-induced deaths were observed in all four treatment groups; death occurred within 15 minutes of dosing. The acute LD_{50} (95 % confidence limit) of bisnalmefene succinate, calculated for males and females combined, was 133.6 (109-163) mg/kg. At necropsy, dark red discoloration of the lung was observed in two mice in the 100 mg/kg group. A white discoloration of the cornea or red discoloration at the injection site was noted in several mice in the 200 mg/kg group. No visible abnormalities were observed in the 150 mg/kg treatment group.

To assess the intravenous irritation potential of nalmefene and naloxone, a perivascular and intravenous study was conducted in male New Zealand White rabbits. Six rabbits/group were injected with vehicle (0.9% sodium chloride injection U.S.P.), nalmefene or naloxone. 1 ml of naloxone or nalmefene was injected intravenously in the right marginal ear vein and perivascularly with 0.25 ml of nalmefene or naloxone in the left marginal ear vein. The control rabbits were injected with vehicle in the same injection sites as the treated rabbits. All rabbits survived to the scheduled termination. No nalmefene- or naloxone-related change in body weights were observed. Treatment-related irritations were observed. Erythema was observed at the test sites in all of the rabbits; and edema was observed in two of three rabbits in each treatment group.

The local irritation and tolerance to subcutaneous injection of nalmefene were assessed in male and female New Zealand White rabbits. Three rabbits/sex/dose level were injected subcutaneously (beneath the dorsal skin of the trunk) with 2 ml of a 1 or 2 mg/ml solution of nalmefene. No clinical signs or changes in body weight were observed following the single subcutaneous administration. No gross changes were observed at the injection site or surrounding tissue in any of the rabbits treated with 1 mg/ml of nalmefene. However, ecchymosis was observed at necropsy in the rabbits receiving the 2 mg/ml solution of nalmefene. These findings suggest that the 1 mg/ml solution of nalmefene is well tolerated by rabbits after subcutaneous injection and that some irritants effects are produced in the rabbits' tissues after a single subcutaneous injection of nalmefene at a concentration of 2 mg/ml.

The potential of nalmefene to cause local irritation following intra-arterial injection was evaluated in female New Zealand White rabbits (3/group). Following the acute administration of 1 ml of a 1 mg/ml solution of nalmefene or 0.9% sodium chloride, the rabbits were observed for seven consecutive days for changes in body weight and irritant affects at the injection site and surrounding tissue. Nalmefene-induced tremors were seen immediately after dosing; recovery occurred within two minutes. In comparison to the control group, no significant irritant affects were observed at the injection site or surrounding tissues. Histological analysis of the injection site and surrounding areas did not reveal any significant irritant effects; only minor changes were noted in the wall of the auricular artery at the injection site in all of the rabbits in the nalmefene group and in 2 of the 3 saline treated rabbits.

The potential of nalmefene to cause muscle irritation was evaluated in male and female New Zealand White rabbits. The spinae erector muscle of 3 rabbits/sex were injected with 1 ml of a 1mg/ml solution of nalmefene. The contralateral spine erector muscle was injected with 1 ml of the positive control solution, formic acid. Four days after treatment, the amount of swelling was quantified. The results indicated that relative to the formic acid treated muscle, no significant swelling occurred in the muscle injected with nalmefene.

The ability of nalmefene to elicit a sensitization response in guinea pigs was assessed using the delayed contact hypersensitivity test of Magnusson and Kligmar. Twenty female guinea pigs were treated by intradermal injection into the shoulder region with nalmefene, Freund's Complete Adjuvant and a mixture of nalmefene and Feund's Complete Adjuvant. Two weeks after the induction phase all rabbits in the nalmefene and control groups were challenged with a 20% and 10% solution of nalmefene. No allergic response was observed in any rabbits in the nalmefene or control groups

The ability of nalmefene to cause *in vitro* hemolysis in dog and rat blood was assessed utilizing CD rat (n=3) and Beagle dogs (n=3). Approximately 1 ml of blood was collected from each dog and rat. 200 µl of blood from each animal was added into three separate test tubes: a positive control, a negative control and a treatment tube. Absorbance was determined spectrophotometrically and the percent hemolysis was calculated. The results showed that nalmefene does not induce hemolysis in rat or dog blood.

The findings of these special toxicity studies have demonstrated that nalmefene does not have irritant potential *in vivo* when injected perivascularly, intraarterially, intramuscularly, intravenously or subcutaneously at a level of 1 mg/ml. However, when 2 mg/ml of nalmefene was injected subcutaneously, some irritant effects were observed. Nalmefene was not allergenic in guinea pigs or produced hemolysis *in vitro*. Bisnalmefene, the degradation byproduct, was shown to be approximately three times less potent than nalmefene following acute intravenous administration.

I. Acute Intravenous Toxicity Study In Mice With Bisnalmefene Succinate (Study Nº 530-035).

The acute intravenous toxicity of bisnalmefene succinate was conducted at International Research and Development Corporation in Mattawan, Michigan during July 26 to August 14, 1993. The study was carried out in accordance to the guidelines and governmental standards of the Good Laboratory Practice.

The acute intravenous toxicity of bisnalmefene succinate was evaluated in 8 week old male and female Charles River Crl:CD-1® (ICR)BR mice. The mice were randomly assigned to four treatment groups (5/sex/group/dose), with the exception of the high dose group, in which only males (n = 3) were used. The mice were treated with a single intravenous dose of 100, 150, 200, and 250 mg/kg. Bisnalmefene succinate was dissolved in deionized water; a constant dose volume of 10 ml/kg was used.

Observations included: 1) Mortality observed at 1, 2, and 4 hours post-dosing on day 1 and twice daily for an additional 15 days; 2) Pharmacotoxic signs were observed at 1, 2, and 4 hours post-dosing and once daily for 15 additional days; 3) Body weight was measured immediately prior to dosing, on day 8, 15 and day 16 or when a mouse was found dead; 4) Necropsy was performed on mice that died and on day 16 when all mice were sacrificed.

Results. Death was observed in all treatment groups. All deaths occurred on the day of dosing. Most mice in the 150 and 200 mg/kg group died during injection or immediately afterward. Only three males were studied at the high dose (250 mg/kg) because testing was stopped prematurely because of the high mortality rate; all three males dosed with 250 mg/kg convulsed and died immediately after dosing. The LD_{50} value with 95% confidence limits for bisnalmefene succinate was 133.6 (109-163) mg/kg in male and female mice combined. The mortality data is summarized below:

Table 12. Mortality after a single administration of bisnalmefene succinate.

DOSE (mg/kg)	DEATHS		DAY OF OCCURRENCE # DIED:DAY	
	# M/total	# F/total	M	F
100	1/5	1/5	1: Day 1	1: Day 1
150	3/5	4/5	3: Day 1	4: Day 1
200	4/5	4/5	4: Day 1	4: day 1
250	3/3	0/0	3: Day 1	-

Lethargy, tremors, and convulsions were the primary clinical signs observed following single treatment with bisnalmefene succinate. Eight out of ten mice in the 100 mg/kg treatment group exhibited lethargy and/or tremors immediately after dosing. In the 150 mg/kg group, 8 out of 10 mice displayed tremors an/or convulsions after the injection. Convulsions were observed in 6 of 10 mice in the 200 mg/kg group immediately after dosing; death occurred within one minute after dosing.

No treated-related changes were observed in the body weights of surviving animals during the 16 day study.

Necropsies performed on animals dying as a result of treatment or surviving subjects revealed that gross changes were dosed related. Dark red discoloration of the lung was observed in two mice that died after being dosed with 100 mg/kg. Mice in this treatment group that survived to the termjnation day of the study were observed with white discoloration of the cornea or red discoloration at the injection site. Red discoloration at the injection site was also observed at necropsy in seven mice (3 males, 4 females) surviving to day 16 of the study. No gross abnormalities were observed in the mice in the 150 mg/kg treatment group.

II. Acute Perivascular and Intravenous Irritation Study In Rabbits (Study Nº 530-036).

This acute toxicity study was conducted fc August 13 to September 20, 1993. It was performed according to GLP.

The local irritant effects of nalmefene and naloxone were evaluated in male New Zealand White rabbits (3 - 4 months of age). Eighteen rabbits were randomly assigned to three treatment groups; six rabbits per group. The rabbits in the drug groups received a single 1 ml intravenous injection of a 1 mg/ml solution of nalmefene or naloxone in the right marginal ear vein and a single perivascular injection of 0.25 ml of a 1 mg/ml solution of nalmefene or naloxone in the left marginal vein ear. The control group was injected with 0.9 % sodium chloride in the same injection sites as the drug-treated groups.

This study includes daily observation for changes in appearance and/or behavior, vascular irritation, mortality, and changes in body weights for the duration (7 days) of the study. Macroscopic and microscopic examinations were conducted at 24 and 72 hours, and at day 7 post-injection; two animals per time point were examined. Macroscopically, the areas around the injection site in both ears were examined for signs of irritations. The region of the ear surrounding and containing the injection site, and three sites distal to the injection were collected and examined microscopically.

Results. No mortality was observed. There were no significant drug-induced clinical signs. Irritation at the injection site was seen in all rabbits. Slight to well defined erythema was observed in one or both ears in all rabbits in each treatment group. Erythema was seen within one hour post-injection and lasting from one to four days after treatment. Edema, very slight, was observed in two to three rabbits from each group. All incidences of edema occurred in the right ear, with one exception in the control group. Most incidences of edema were observed within 4 hours after injection and did not persist for more than fours days after appearance.

No significant differences in body weight were observed between the control and treated subjects.

Macroscopic analysis at the 24 hour sacrifice time point revealed discoloration of the skin at the left injection site (perivascular) in all rabbits from each group. Rabbits from the nalmefene and naloxone treatment groups also showed discoloration of the skin at the right injection site (intravenous).

Discoloration of the skin at the intravenous and perivascular sites was also observed in some of the rabbits sacrificed at the 72 hour interim sacrifice. One rabbit in the control group had discoloration at the intravenous site. One rabbit in the naloxone group showed discoloration of the skin at both the perivascular and intravenous sites. By day 7, the injections sites were within normal limits for all rabbits from each treatment group.

Microscopically, hemorrhaging, inflammation, heterophil infiltration, edema and occasionally necrosis were seen within the subcutis in varying combinations most rabbits from all treatment groups. In all cases, the severity of the microscopic changes ranged from trace or mild, or rarely moderate. These microscopic changes correlated with the discoloration seen macroscopically. In the rabbits of the 24 hour interim sacrifice, the overall incidence of the various inflammatory lesions and hemorrhage was the greatest at the site of injection and at sites distal to the injection sites in the left and right ear. Their inflammatory lesions consisted of focal and multifocal accumulations of lymphocytes, histocytes and heterophils. The incidence of these lesions showed much intergroup variability. They also decreased with time and in most groups, the incidence of the inflammatory lesions decreased progressively at 1.5, 3.0, and 4.5 cm distal to the injections in both ears.

By 72 hours after dosing, the overall incidence of inflammatory lesions and hemorrhaging had decreased considerably at the primary site of injection and three distal sites. The overall incidence of left ear lesions was higher in the naloxone group in comparison to the control and nalmefene groups. In contrast, the nalmefene group displayed a slightly higher incidence of right ear lesions. No left ear lesions were observed in the nalmefene treated group at 72 hours post-injection.

In comparison to the 24 and 72 hour interim sacrifice, the overall incidence of microscopic lesions at the left and right ear injections sites and the three distal sites were relatively low in the rabbits of the terminal sacrifice. The inflammatory lesions mainly consisted of subacute inflammation and edema, ranging in trace to mild severity.

III. Subcutaneous Injection Tolerance Study In the Albino Rabbit (Study Nº 184/8601).

The local irritation effects of nalmefene following subcutaneous administration was assessed in rabbits at during the period from January 24 to January 39, 1986.

Male and female New Zealand White rabbits were subjects for this study. Three male and three female rabbits were randomly assigned to the two treatment groups. The rabbits were injected with 2 ml of a 1 mg/ml or 2 mg/ml solution of nalmefene subcutaneously beneath the dorsal skin of the trunk. The rabbits were observed daily for seven consecutive days following treatment; any signs of reaction to treatment and changes in body weight were recorded. On day 8 of the study, all rabbits were sacrificed and the injection site and surrounding tissue were examined for any reaction to nalmefene treatment.

Results. There were no observed drug-related clinical signs in both males and female rabbits treated with 2 mg or 4 mg of nalmefene. Macroscopic analysis of the injection site and surrounding tissue revealed ecchymosis formation on the surface of the muscles under the skin of four rabbits (3 males and 1 female) treated with the 2 mg/ml concentration of nalmefene. No macroscopic changes were observed in the rabbits receiving the 1 mg/ml concentration of nalmefene.

IV. Intra-Arterial Injection Tolerance Study In Albino Rabbit (Study Nº 185/8601).

The local irritation effects and tolerance to nalmefene following intra-arterial administration were assessed in New Zealand White rabbits at Toxicol Laboratories Limited (Ledbury, Herefordshire, England) during the period from January 24 to January 39, 1986.

Groups of three female New Zealand white rabbits received an acute injection of nalmefene (1 mg/ml) or 0.9% sodium chloride into the central artery of the ear. The injection volume was 1 ml. The rabbits were observed daily for seven consecutive days following treatment; any signs of reaction to treatment and changes in body weight were recorded. On day 8 of the study, all rabbits were sacrificed and microscopic analysis of the injection site and two levels distal to the injection site was performed.

Results. Tremors were observed in 1 of the nalmefene-treated rabbits immediately after the injection. Recovery was immediate, occurring within two minutes. In comparison to the control group, no significant differences were noted in body weight gain. Hematoma was observed frequently at the injection site in all rabbits in both group. The hematoma was observed with varying severity and was seen from days 2 to 8 of the study.

Microscopic analysis of the ear and sites surrounding the injection sites revealed some histological changes in the wall of the auricular artery at the injection site only in all rabbits in the nalmefene-treated group. Theses changes included: small areas of endothelial detachment, intimal thickening, splitting or fragmentation of the internal elastic membrane, minimal medial edema, and fibrin deposition with associated minimal heterophil and lymphocyte infiltration in segments of the adventitia. No histological changes were observed in the distal sites analyzed. Some minor changes were also observed in two rabbits in the control group. One rabbit in the control group had endothelial detachment in a small area near the injection site. Minimal heterophil and plasma cell infiltration were seen in the arterial adventitia distal to the injection site in another control rabbit.

IV. Intramuscular Irritation Study on Nalmefene (Study Nº KP12/JF1).

The potential of nalmefene to cause muscle irritation was studied in both male and female New Zealand White rabbits. This study was performed at
during the period of October 12 to October 16, 1982.

Three males and three females rabbits were injected with 1 ml of a 1 mg/ml solution of nalmefene into one erector spinae muscle. The contralateral erector spinae muscle of each rabbit served as the positive control; it was injected with 1 ml of a 4% formic acid solution. The animals were examined two times daily for four day for any signs of overt toxicity. 96 hours post-injection, all rabbits were weighted and sacrificed. The spinae erector muscle was moved and prepared for histological evaluation. Irritated muscle mass was dissected away from normal tissue and measured by displacement in a graduated cylinder containing 10 ml of buffered formalin; the mean volume of displacement for treated and positive control muscle was calculated.

Results. In comparison to the positive control, nalmefene did not induce any significant irritant effect in the spinae erector muscle in either female or male rabbits. Formic acid induced substantial swelling in all rabbits except for one which received a bad injection. The mean volume of displacement for the positive control and nalmefene treated rabbits was 4.2 and 0.03 ml, respectively.

V. Delayed Dermal Sensitization Study In The Guinea Pig (Study № 271/8510).

The potential of nalmefene to elicit a sensitization response in the guinea pig was studied [redacted]. The delayed contact hypersensitivity test was conducted [redacted]

Forty female guinea pigs were equally and randomly assigned to the two treatment conditions: test group and control group. The dorsal area between the shoulders of each subjects was shaved free of fur. Three pairs of intradermal injections were made within this shaved area, approximately 6 cm, 4 cm and bilateral to the midline. Guinea pigs in the test group were treated with two injections each of 0.1 ml Feund's Complete Adjuvant, two injections each of 0.1 ml of a 1% aqueous concentration of nalmefene and two injections each of 0.1 ml Feund's Complete Adjuvant mixed with an equal volume of a 2% concentration of nalmefene.

Seven days later this induction process was boosted by topically applying a 20% aqueous concentration of nalmefene over the injection site for 48 hours. Fourteen days after the boost, the challenge phase of the study was conducted. The rabbits were challenged by topically applying a 20% and 10% aqueous solution of nalmefene to the left and right flank of the rabbits, respectively, for a period of 24 hours. These concentrations of nalmefene were selected based on the results in a dose range finding study. Twenty-four and forty-eight hours after removing the nalmefene-treated dressing, the treated site was evaluated for degree of reaction (no reaction, scattered mild redness, moderate diffuse redness or intense redness and swelling) to the treatment.

Results. According to the Magnussson and Kligmar scale, nalmefene at a concentration of 20% was a weak (Grade I) sensitizer in the guinea pig; a sensitization rate of 0% was observed in all rabbits in the nalmefene and control groups.

VI. In Vitro Hemolysis Study on Nalmefene (Study № KP13/JF1).

The potential of nalmefene to induce *in vitro* hemolysis was examined in dog and rat blood.This study was conducted at [redacted] October 22, 1982.

Blood, approximately 1 ml, was collected from three male CD rats and three Beagle dogs. For each blood sample, 200 µl was pipetted into 3 separate tubes; a positive control tube, a negative control tube and a nalmefene tube. 200 µl of water was added to the positive control tube, 200 µl of normal saline was added to the negative control tube, and 200 µl of nalmefene (1.0 mg/ml) was added to nalmefene tube. The tubes were mixed, covered and incubated at room temperature. After a 1 hour incubation period, 5 ml of water was added to each positive control tube; 5 ml of saline was added to each negative control and nalmefene tubes. All tubes were mixed well and centrifuged for 5 minutes. Absorbance was measured with a spectrophotometer and the percent hemolysis was calculated for each nalmefene treated tube.

Results. There was no measurable hemolysis in the supernatant of dogs or rats blood samples treated with nalmefene.

REPRODUCTIVE AND TERATOLOGY STUDIES

REPRODUCTIVE TOXICITY STUDIES

ABSTRACT

The reproductive toxicity potential of nalmefene was evaluated in the standard three reproduction studies in rats and/or rabbits. Its effects on fertility and reproductive performance were assessed in rats following oral administration. The teratogenic potential of nalmefene was studied in both rats and rabbits. Nalmefene effects on peri- postnatal and neonatal development were evaluated in rats.

Nalmefene's effects on fertility and general reproductive performance were evaluated in male and female Sprague-Dawley rats (COBS:CD) after daily dosing throughout the period of gametogenesis, mating, gestation, parturition, and lactation. Nalmefene was tested at doses of 0, 2.0, 20.0, and 200 mg/kg/day during mating. After mating, the 200 mg/kg dose was reduced to 100 mg/kg as a result of the occurrence of convulsions in five out of fifteen female rat. Nalmefene treatment in this fertility study caused no effects on the numbers of males and female mating, fertility index, overall pregnancy rate, and time course of mating. The mean numbers of corpora lutea, pre-implantation and post-implantation loss were similar for all groups during the uterine examination on day 13 of gestation. Duration of gestation, duration of parturition and litter size were also not affected by nalmefene treatment. The viability index for the litters born to the rats treated with 100 mg/kg of nalmefene was significant reduced. This was attributed to maternal neglect and decreased nursing. The body weight of the pups was comparable to the control in all treatment groups except for the 100 mg/kg litters. No treatment-related malformations or adverse effects on ophthalmic, auditory or behavioral development and learning ability were evidence in the F_1 rats. Mating between F_1 animals revealed that nalmefene did not affect fertility or mating performance; no dose-related differences in the numbers of males and females mating, fertility index, overall pregnancy rate, and time course of mating. Also, the duration of gestation, litter size or pups survival were unaffected. Consistent with the results observed in the F_1 animals; no F_2 treatment-related abnormalities in necropsies of mortalities or terminal sacrifice and no effects on F_2 developmental parameters were seen.

In the tetralogy studies, Sprague-Dawley rats and Dutch Belted rabbits were dosed orally with 0, 1, 10, and 200 mg/kg of nalmefene daily during gestational days 6-15, and 6-18, respectively. No clinical signs of toxicity were observed in the rats receiving 1 or 10 mg/kg of nalmefene. Convulsions were observed in the rats administered 200 mg/kg of nalmefene. No treatment-related clinical signs of toxicity were observed in the rabbits at any of the dose levels. In the rat teratology study, maternal toxicity was evident as a statistically significant decrease in body weight gain and food consumption during days 6 thru 11 of gestation. In contrast, no effects were noted for body weight gain and food consumption in rabbits at any dose levels. Reproductive indices, i.e., number of corpora lutea, number of implantation sites, number of live fetuses, and pre- or post-implantation loss, were not affected by treatment with nalmefene in either species. Relative to the control fetuses, rat fetal body weights of the 200 mg/kg/day litter showed no differences; whereas, a significant increase in fetal body weight was observed in the two lower doses (1 and 10 mg/kg). In rabbits, fetal body weight of the 200 mg/kg/day litters was decreased relative to controls. This finding may be a result of the slightly larger litter size observed in this treatment group. Nalmefene was not a teratogen when administered during the fetal organogenesis period to pregnant rats or rabbits at doses up to 200 mg/kg/day. The incidence of malformations, visceral and skeletal anomalities were comparable in the nalmefene-treated and control groups. Rabbit fetuses in the 200 mg/kg group exhibited decreased ossification of the distal epiphysis of the femur and decreased body weights. These findings suggest that nalmefene may slightly retard fetal development/growth.

To further assess the teratogenic potential of nalmefene, gravid New Zealand White rabbits were intravenously injected with 0, 0.25, 1.25 or 8.0 mg/kg nalmefene daily on days 6-18 of gestation. The maternal No-Observable-Effect-Level (NOEL) was 0.25 mg/kg. The 1.25 mg/kg dose of nalmefene caused a reduction in food consumption during the post-dosing period. The highest dose of nalmefene studied produced overt signs of toxicity and caused a reduction in body weight and food consumption. The overt signs of toxicity were observed in the 8 mg/kg group included tremors, pupil constriction, biting movements, ataxia and hyperpnea. Nalmefene at dosages up to and including 8.0 mg/kg, had no affect on the mean number of corpora lutea, implantation sites and live and dead fetuses were observed. Fetal sex ratio, fetal body weights, and percentage of dead or resorbed conceptuses were also comparable to the control groups. No nalmefene-related fetal malformations or variations in gross external, soft tissue, or skeletal morphology were revealed. The developmental NOEL for nalmefene was also 0.25 mg/kg/day. The mid and high doses of nalmefene did cause some increase in total resorption of litters. The high dose (8.0 mg/kg) of nalmefene also did reduce the numbers of litters with live fetuses.

The effects of nalmefene on peri- and post-natal development were studied in Sprague-Dawley rats. The rats received oral doses of 2, 15, and 100 mg/kg of nalmefene from day 15 of gestation to day 20 post partum. Control animals received distilled water. One mortality occurred in the 100 mg/kg group. Preceding death, the following clinical signs were observed: loss of body weight, lethargy, hunched posture, hypothermia, and staining around the nose and mouth. No other clinical signs of toxicity were evident in the other treatment groups during the remaining period of the study. Maternal body weight gain and food consumption were significantly reduced in the high dose group from days 15 to 20. Nalmefene produced no adverse effects on perinatal development; duration of gestation or parturition, litter size, sex ratio, mean live birth, and lactation index were similar to the control. The viability index for the 100 mg/kg group was reduced relative to control; this was not statistically significant. Also, no treatment-related effects on postal-natal development were observed. Also no major malformations were observed.

In the neonatal toxicity study, treatment of neonatal Sprague-Dawley rats with nalmefene subcutaneously during day 1 to day 20 post partum had no adverse effects on bodyweight, viability index, lactation index, cumulative survival index, and developmental milestones (eye and ear openings, tooth eruption, hair growth, righting reflex and startle response). No nalmefene-induced mortalities or clinical signs indicative of toxicity were observed. Macroscopic or histological assessment of select organs did not reveal any treatment-related abnormalities.

The results of these studies indicate that nalmefene is not a reproductive toxicant in Sprague-Dawley rats or rabbits (New Zealand White or Dutch Belted). Segment I study in rats demonstrated that nalmefene had no direct effect on reproductive performance or fertility (pregnancy rate, implantation, gestation, and parturition). Segment II studies performed in pregnant rats and rabbits demonstrated that nalmefene does not induce any embryocidal and teratogenic effects following oral or intravenous administration. However, potential nalmefene-related early resorptions were observed in a few rabbits treated intravenously with 1.25 and 8.0 mg/kg. Segment III studies showed that nalmefene does not adversely affect perinatal, postnatal or neonatal development.

REPRODUCTIVE TOXICITY STUDIES.

The following studies were submitted in Volumes 1.17 and 1.18:

SUMMARY OF RE(REPRODUCTIVE TOXICITY STUDIES WITH NALMEFENE				
STUDY N°	TEST TYPE	STUDY TITLE	ROUTE	SPECIE
KP14/JF1	Dose Range Finding Study	Intravenous Dose Range Finding Study in the Pregnant Rat	PO	Rat
KPC/9/84	Segment I	Nalmefene - Rat Fertility and General Reproductive Performance Study	PO	Rat
KPC19/JF-1	Segment II	Rat Teratology Study	PO	Rat
KPC/10/84	Segment II	Nalmefene - Rat Teratology Study	PO	Rat
KP20/JF-1	Segment II	Rabbit Oral Teratology Study	PO	Rabbit
KPC/11/84	Segment II	Nalmefene - Rabbit Teratology Study	PO	Rabbit
KP16/JF-1	Dose Range Finding Study	Intravenous Dose Range Finding Study in the Pregnant Rabbit	IV	Rabbit
1101-001	Segment II	Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of Nalmefene Administered Intravenously to New Zealand White Rabbits	IV	Rabbit
KPC/12/84	Segment III	Nalmefene - Rat Peri and Postal Natal Study	PO	Rat
KPC/16/85	Segment III	Nalmefene - Rat Neonatal Toxicity Study	SC	Rat

Segment I.

Study Nº KPC/9/84.

The effects of nalmefene on fertility and general reproductive performance were evaluated in Sprague-Dawley rats (COBS:CD, Charles river, U.K.).

Dec. 1983 to July 1984. Prior to conducting this study a dose range finding study (KP14/JF1) was performed to determine the appropriate doses to use in the definitive study.

Male and female rats were given daily oral doses of nalmefene (2, 20, and 200 mg/kg/day) or vehicle (10 ml/kg/day) for 60 days and 14 days, respectively, prior to breeding. Dosing was continued through the mating period, gestation and lactation (Day 21). During gestation and lactation, the 200 mg/kg/day dose was reduced to 10 mg/kg/day. On day 13 of gestation, 15 females from each treatment group were sacrificed. They were examined for the numbers of corpora lutea, number and distribution of implantation sites (resorption, dead, and lived fetuses). The remaining females were allowed to litter normally. Their pups were examined immediately after birth for litter size, stillborn, and live born, and gross anomalities. All dead pups were evaluated for skeletal anomalies. Post weaning observations included: visual and auditory function test, Preyer's reflex test, open field behavior, and E-maze learning test. 15 F_1 males and 15 F_1 females were sacrificed after behavioral testing; another 15 F_1 males and F_1 females were evaluated for reproductive performance.

Results. 5 females in the high dose group developed convulsions between 74 and 82 days of dosing. One female (#224) died on day 82 following a convulsive episode on day 81. Food consumption and body weight were affected in the male rats. Food consumption was reduced intermittently in males treated with 20 mg/kg nalmefene and chronically reduced in the high dose (200 mg/kg) group. During the pre-mating period, the males in the high dose group rate of growth was reduced in comparison to the control animals. Females in the high dose group consumed less food initially. Nalmefene-treated female body weight were unaffected.

Relative to the control group, nalmefene treated rats did not show differences in their mating performance, fertility index, overall pregnancy rate, time course of mating, and vaginal smear patterns. Uterine examination on gestation day 13 revealed that the mean numbers of corpora lutea, pre-implantation loss, and post-implantation loss in the nalmefene treated females were similar to the control. Nalmefene was also without any direct effect on duration of gestation, duration of parturition, and litter size.

Nalmefene at doses up to 200 mg/kg was without any significant toxic effects. Nalmefene did not elicit any significant fetotoxic effects. Pups born to nalmefene treated rats did not show any significant differences in their developmental pattern in comparison to pups born to the control females. However, pups born to high dose dams had a slight growth retardation, but was not statistically significant throughout the lactation period. Pups in the high dose litters had a significant reduced viability index. This was attributed to maternal neglect and decreased nursing. The F_1 pups showed no treatment-related malformations or detrimental effects in the ophthalmic, auditory, or behavioral development and learning ability measures.

When the F_1 animals were mated, no treatment-related differences in the numbers of males and females mating, fertility index, overall pregnancy rate, time course of mating, and vaginal smear patterns were observed.

Segment II.

The potential developmental toxicity of nalmefene was evaluated in both rats and rabbits. Two separate teratology studies were conducted in rats. One Segment II rat study (Nº KP19/JF-1) was conducted by during the period Jan. 3 thru Jan. 13, 1983 in accordance to GLP. The second Segment II study (Nº KPC/10/84) was conducted riod Jan. 9 thru Jan. 30, 1984, according to GLP. Study Nº KP19/JF-1 Segment II reproduction study evaluated the effects of 1.0 and 10.0 mg/kg nalmefene during the period of organogenesis. The second Segment II reproduction study (Nº KP19/10-84) compared the effects of 200 mg/kg nalmefene to vehicle control.

Study Nº KP19/JF-1.

Study Nº KP19/JF-1 Segment II reproduction study was conducted in 72 time-mated Sprague-Dawley rats (COBS:CD); 36 of the rats were at day 1 of pregnancy and the other 36 rats were at day 2 of pregnancy upon arrival. The rats were equally and randomly divided into three treatment groups (N = 20/group). The treatment groups were: 1) vehicle control (sterile water for injection, 5 ml/kg/day); 2) 1 mg nalmefene/kg/day; 3) 10 mg nalmefene/kg/day. These doses were determined by the sponsor . The pregnant rats were treated orally with vehicle or nalmefene on days 6-15 of gestation. On day 20 of pregnancy, the rats were sacrificed and evaluated at necropsy. Measurements included bodyweight of live fetuses, number and distribution of implantation sites, number of resorptions, number of dead and live fetus, number of corpora lutea, type of abnormalities in major material organs, skeletal variants and external malformations in fetuses.

Results. There were no treatment-related mortalities. Maternal body weight was unaffected at these doses. Rats in the 10 mg/kg group had food consumption significantly greater than the control group. Nalmefene at doses of 1.0 and 10.0 mg/kg was without teratogenic or embryocidal effects; no significant effects on the mean number of corpora lutea, implantation sites, live fetuses, pre-implantation sites, visceral or skeletal anomalies/malformations. The mean fetal weight was significantly higher than controls in both treatment groups.

Study KPC/10/84.

This reproduction study was also conducted in Sprague Dawley rats (CD strain). Rats were bred by caging three females with one male rat. After mating, 40 pregnant females were equally and randomly assigned to the two treatment conditions: vehicle control (distilled water, 10 ml/kg), and 200 mg/kg of nalmefene. This dose of nalmefene was selected on the bases of a previous dose range finding study (Toxicol Report Nº KPC/13/84) in which 300 mg/kg was found to be toxic. Nalmefene or vehicle was administered orally on days 6-15 of pregnancy. The rats were sacrificed on Day 20 of pregnancy and the standard measures for development toxicity were conducted.

Results. Nalmefene-induced overt signs of toxicity were observed in one rat. Convulsions were observed within 5 to 10 minutes after each daily nalmefene treatment; they had a duration of 15 to 20 minutes. Maternal body weight was significantly reduced from days 6 to 11 of pregnancy and significantly increased on days 15 to 20 in the nalmefene-treated rats. Food consumption was significantly increased on days 15 to 20 of pregnancy. Nalmefene had no effect on the numbers of corpora lutea, the numbers of implantation sites, live fetuses, pre- and post-implantation losses, sex ratio and fetal bodyweight. External, visceral and ossification variations observed were within control ranges.

Study Nº KP20/JF-1.

Groups of 15 timed-mated Dutch belted rabbits were orally dosed once daily with 0, 1.0 and 10.0 mg/kg of nalmefene on days 6 to 18 of gestation. This range of dosages was derived from a previous dose-range finding study (Study Nº KP16/JF-1) conducted in pregnant rabbits. Nalmefene was dissolved in sterile water for injection. Animals in the vehicle control group received sterile water for injection.

On day 26 of gestation, the females were sacrificed and necropsy performed. The number of corpora lutea, implantation sites, resorption and live fetuses were assessed. Also, the fetuses were examined for signs of visceral and skeletal abnormalities.

Results. There was one maternal mortality during the study; one vehicle control rabbit died on day 26 of gestation. Necropsy findings revealed signs of pulmonary hemorrhaging and the presence of pulmonary exudate in the right lungs. No clinical abnormalities or overt sign of toxicity were observed in the nalmefene-treated rabbits. Body weight was unaffected. The mean number of corpora lutea, mean number of live fetuses, mean total number of implantation sites, implantation loss, and mean fetal weight did not differ statistically between the control and nalmefene-treated group. External examinations of the fetuses revealed two malformations. One fetus in the vehicle control group had gastroschisis, exencephaly, agenesis of sex organs, brachydactyly of fore and hind limbs and open eyes. One fetus in the 10 mg/kg group exhibited exencephaly with subcutaneous edema and limb fixture; neither malformation was considered to be nalmefene-related. It is concluded that nalmefene was not teratogenic in rabbits at doses up to 10.0 mg/kg following oral administration.

Study Nº KPC/11/84.

Another group of 15 time-mated Dutch belted rabbits were treated orally with vehicle (distilled water, 2 ml/kg) or 200 mg/kg/day of nalmefene on days 6 to 18 of pregnancy. Rabbits were sacrificed on day 28 and studied according to standard protocol.

Results. There were no nalmefene-induced mortality or clinical signs of toxicity observed. One control rabbit aborted on day 25 of pregnancy. Food consumption during the fetal development was significantly reduced during days 6 to 8 of gestation. However, this was not reflected in the body weight measures. Comparison of bodyweight of control and nalmefene-treated fetuses revealed a statistically significant decrease in fetal body weight. Paralleling this observed effect was an increase in the litter size (mean number of live fetuses) for the nalmefene-treated rabbits; this was not statistically significant. Nalmefene-treatment had no adverse effects on pregnancy rate, mean number of corpora lutea, pre- and post-implantation losses, mean number of implantation sites, or sex ratio of the fetuses. Two malformations were observed in fetus exposed to nalmefene. Two fetuses had ectopic testes and another fetus had agenesis of the thymus. Also, statistically significant ossification delays were observed in the distal epiphysis of the femur in 59% of the fetus in the treatment group. These findings suggest that nalmefene at a dose of 200 mg/kg may slightly affect fetal growth without producing any teratogenic effects.

Study Nº 1101-001.

New Zealand White rabbits [Hra:(NZW)SPF], artificially inseminated to induce pregnancy, were subjects for this reproductive study that evaluated the teratogenic and embroycidal potential of nalmefene following intravenous administration. The rabbits were assigned to four treatment groups (n=20/group): 1) vehicle control, physiological saline (1.0 ml/kg); 2) 0.25 mg nalmefene/kg/day; 3) 1.25 mg nalmefene/kg/base; and 4) 8.0 mg nalmefene/kg/day. The rabbits were dosed once daily during day 6 to day 18 of presumed gestation. On day 29 of gestation, the rabbits were sacrificed to examine fetuses for implantation, sex ratio, viability, resorption, visceral and skeletal malformations.

Results. No treatment-related maternal deaths occurred. Due to self-mutilation behavior, one doe in the high dose group was sacrificed on day 26 of pregnancy. Nalmefene-induced toxicity was also observed in this doe; they included pupil constriction, tremors, biting movements, rapid blinking, and ataxia. Self-mutilation of the vulva and fluid accumulation in the abdominal cavity were evident at necropsy. This doe had a litter size of nine fetuses that appeared normal for their developmental age.

No nalmefene-induced clinical signs were observed in the 0.25 and 1.25 mg/kg groups. Does in the high dose group developed tremors, pupil constriction, biting movements, ataxia, and hyperpnea immediately during injections; 30 to 60 minutes after treatment the animals appeared normal. Maternal body weight gain was significantly reduced in the 8.0 mg/kg group on days 6-8 of gestation. This group also had a reduction in food consumption during days 6-7 and days 19-29. Reduced food consumption was observed in both the 1.25 and 8.0 mg/kg groups. Relative to the control group, the mid dose group (1.25 mg/kg), and high dose group (8.0 mg/kg), food consumption was reduced on days 6 to 7, and days 19 to 29, respectively.

Although not statistically significant, early resorption was observed in all nalmefene-treatment groups. One doe in the low dose and mid dose groups had a litter of one and three early resorption, respectively. Two does in the 8.0 mg/kg group had one and six early resorptions, respectively. Relative to the control, does in the high dose groups had a reduced number of litters with live fetuses. No significant differences in the number of corpora lutea, number of implantation sites, number of live and dead fetuses, fetal sex ratio, and fetal body weight were observed at doses as high as 8.0 mg/kg/day. Among treated rabbits, no

statistically significant difference in soft tissue or skeletal development were observed. Ossification variations observed in all four treatment groups were within control range.

Segment III.

Study # KPC/12/84.

The effects of nalmefene on late fetal developmental, parturition, lactation, neonatal viability, and growth rate following oral administration were evaluated in rats. A peri- and postnatal reproductive toxicity study (Study 12/84) was performed for at period of April 2 to May 15, 1984. The study was conducted according to GLP.

Time-mated female Sprague Dawley rats (COBS:CD) were subjects for this study. The pregnant rats were allocated to four groups, 20 in each group, and given 0.0, 2.0, 15.0 or 100.0 mg/kg of nalmefene orally. The control group received the vehicle (distilled water) at a volume of 10 ml/kg. The dams were dosed from day 15 of pregnancy and up to day 20 post partum. Litters were examined as soon as possible after delivery for litter size, still born, sex ratio, viable pups, litter body weights, and gross abnormalities. Other parameters examined included: 1) mortality (viability index) was recorded up to day 4 post partum; 2) lactation index (survivors day 21/survivors day); 3) cumulative survival index; 4) pup developmental milestones: ears open, eyes open, static righting reflex (Day 5), startle reflex (Day 15). On day 21 post partum all dams and litters were sacrificed. Dams and pups were subjected to macroscopic examination limited to the thoracic and abdominal cavities.

Results. No overt maternal toxicity or death was observed in rats treated with 2 and 15 mg/kg of nalmefene. One dam in the high dose group died on day 21 of the study. Preceding her death, weight loss (from day 17) and lethargy, hunched posture, hypothermia, and blood staining around the nose and mouth (from Day 19) were observed. Necropsy findings revealed blood around the vulva, dark red spot (2 mm diameter) on stomach mucosa, and hemorrhage in uterus with 10 autolyzed immature fetuses. Administration of 100 mg/kg nalmefene resulted in a significant suppression in maternal weight gain from days 15 to 20 of pregnancy. Food consumption by the nalmefene treated rats was similar to that recorded for the control rats. One dam in the control group aborted on day 21 of the study. Nalmefene did not adversely affect the duration of gestation or parturition; no nalmefene-treated rats aborted.

Nalmefene did not adversely affect the duration of gestation or parturition. There were no significant differences between the treated and control groups in duration of gestation, duration of parturition, mean number of pups born, live birth index, mean lactation index and mean cumulative survival index. There were no statistically significant differences in any of the pup developmental milestones.

Study 16/85.

This study evaluated the effects of nalmefene on neonatal development. This study was conducted at June 25 thru August 7, 1985. It was performed in accordance to GLP regulations.

Neonates from Sprague Dawley rats (COBS:CD) were randomly divided into four treatment groups; ten pups were assigned to each group. The pups were treated daily with subcutaneous nalmefene from day 1 through day 25 of postpartum at doses of 0.0 (sterile water), 2.0, 10.0, and 50.0 mg/kg/day. Through

the period of treatment, potential clinical signs, body weight, and mortality were recorded daily for the pups. Physical developmental landmarks: ears open, eyes open, tooth eruption, hair growth were recorded at time of occurrence. Static righting reflex and startle response were examined on day 5 and day 15, respectively. On day 21 post partum all dams and pups were sacrificed. Macroscopic examination was performed on major organs from the abdominal, thoracic, and cranial cavities.

Results. No treatment-related clinical signs of toxicity were evident throughout the study. Several mortalities did occur during the study. Two pups from the control group, one pup from the low dose group and two pups from the mid dose group died. No significant differences were observed between the control and nalmefene-treated pups during lactation and for the following neonatal developmental landmarks: eye and ear openings, tooth eruption, hair growth, righting reflex, and startle response. Macroscopic evaluation of the examined organs (brain, heart, liver, kidneys, testes, and uterus) showed no substantial differences between the treated and control groups of pups.

MUTAGENICITY STUDIES

MUTAGENICITY STUDIES

ABSTRACT

The ability of nalmefene to cause gene mutation or chromosomal damage was evaluated in five genotoxicity assays. The assays performed were the: Bacterial Reverse Gene Mutation Assay (Ames Test), Mouse Lymphoma Cell Gene Mutation Test, Human Lymphocyte Metaphase Analysis, Mouse Micronucleus Test, and Rat Bone Marrow Cytogenic Analysis.

Nalmefene was assayed for mutagenicity in *Salmonella typhimurium* strains TA1535, TA1537, TA1536, TA98 and TA100 with and without the addition of S9 mix (Aroclor-induced rat liver S9). In experiment 1, nalmefene was tested in the concentration range of 0.4 to 1250 μg/plate. At the highest concentration tested, nalmefene caused a slight increase in the number of revertants in the absence of the metabolic activator; also, no cytotoxicity was observed at this concentration. In a second experiment, nalmefene was tested in the concentration range from 2 to 6250 μg/plate. There were no nalmefene-related increase in revertant colonies in any of the tester strains with or without metabolic activation. At the highest concentration tested, 6250 μg/plate, tested nalmefene was toxic to all five bacterial strains.

Nalmefene was analyzed for cytotoxicity and mutagenicity in cultured L5178Y mouse lymphoma cells. Nalmefene was cytotoxic at a concentration of 100 μg/ml and higher. Nalmefene did not induce either ouabain or 6-thioguanine resistant mutants in L5178Y mouse lymphoma cells. No statistically significant increase in mutant frequency in cells treated with Nalmefene (6.25 - 100.0 μg/ml) were evidence at the ouabain or 6-thioguanine locus.

The effects of nalmefene on chromosome aberration were evaluated in cultured human lymphocytes. The results demonstrated that nalmefene is a clastogen in human lymphocytes in the absence of the metabolic activator S9. At a concentration of 3.0 mg/ml, a statistically significant increase in chromosome aberrations (chromatid gaps) were found. However, in the presence of rat S9 mix activation, nalmefene had chromosomal aberration frequencies that were no different than negative control cells. These results suggest that nalmefene is metabolized to a non-clastogenic metabolite.

Nalmefene clastogenic potential was evaluated in the mouse micronucleus test at three dose level . No statistical significant increase over control values of the frequency of polychromatic erythrocyte-cor ining micronuclei was observed at any dose level or sampling time in either male or female mice.

The *in vivo* clastogenic potential of nalmefene was studied in the Cytogenic Analysis of Rat Bone Marrow Assay. Nalmefene was orally administered at doses of 62, 124, and 248 mg/kg; analysis were conducted at 6, 24, and 48 hours after treatment. Nalmefene at a single oral dose up to 248 mg/kg did not lead to an increase in structural chromosomal aberrations.

These data support the conclusion that nalmefene is not a mutagen in bacteria or mouse lymphoma cells in culture. Nalmefene was not a clastogen to rat or mouse bone marrow. However, Nalmefene, at a dose of 3.0 mg/ml, did have weak clastogenic activity in cultured human lymphocytes in the absence of an exogenous metabolic activator. It is reasonably safe to conclude that nalmefene lacks significant genotoxicity since nalmefene clastogenic effects were seen *in vitro* and were lacking in both the mouse and rat *in vivo* assays.

MUTAGENICITY STUDIES.

Bacterial Reverse Gene Mutation Assay.

The mutagenicity study was conducted at
during October 1981. The study was conducted in compliance with the Good Laboratory Practice regulations.

Salmonella typhimurium tester strains TA1535, TA1537, TA1538, TA98, and TA100 were used in this assay. The mutagenicity test was performed according to the plate-incorporation procedure of Ames and colleagues (1975). Nalmefene was tested over a broad range of concentrations in two separate experiments. In the first experiment it was tested in the concentration range of 0.4μg to 1250 μg and in the concentration range of 2 μg to 625 μg in the second experiment. Experiments were conducted both with and without the addition of the metabolic activator S9 (Aroclor-induced rat liver). Each concentration was ran in triplicate. The plates were incubated at 37°C for 48 hours and the number of revertant colonies were scored.

Appropriate negative and positive control experiments for each strain were ran simultaneously. The diagnostic mutagens were: N-methyl-N'-nitro-N-nitrosguanide (MNNG) at 1 μg/plate and 10 μg/plate for TA1535, TA1537, and TA100 without the S9 metabolic activator; 4-nitro-o-phenylenediamine (4NOPD) at 5 μg/plate and 50 μg/plate for TA1538 and TA98 without the S9 activator; and 2-aminoanthracene (2AA) at 5 μg/plate and 50 μg/plate for all five strains with 10% S9 activator.

Results. The results from experiment 1 are presented in Table 13. Both the negative and positive controls induced mutation frequencies as expected. Nalmefene (JF-1 HCL) was not active in the, TA1535, TA1537, TA98 or TA100 *Salmonella typhimurium* tester strains at any of the concentration evaluated. However, at a concentration of 1250 μg/plate, Nalmefene did show some activity in the TA1538 without S9; there was a slight increase in the number of revertants.

In the second experiment, JF-1 did not induce mutation frequencies in any of the *Salmonella typhimurium* strains tested with or without S9. Unlike the first experiment, the toxicity limit was reached. At a concentration of 625 μg/plate, the background lawn of bacterial growth was loss in all five strains.

Tests for Gene Mutation in L5178Y Mouse Lymphoma Cells treated with Nalmefene.

This mutagenicity study was conducted at
during October 1984 to May 1985. The study was conducted in compliance with the Good Laboratory Practice regulations.

In the cytotoxicity study, cultured L517BY mouse lymphoma cells were treated with nalmefene (0.1, 1.0, 10.0, 100.0 and 1000 μg/ml) in the presence and absence of the S9 metabolic activator, or the negative control DMSO for 2 hours. Following treatment, cells for cytotoxicity analyses were washed free of the test compounds, resuspended in fresh Fischer medium and allowed to recover during a 1 hour incubation period at 37°C. The cells were counted and resuspended in medium and allowed to incubate on molten

agar at 37°C for 14 days. Colonies resulting from the growth of surviving cells were counted and the percentage survival calculated against the negative control. The remaining cells were diluted to 1 x 10^5 cell/ml and incubated overnight. The cultures were counted daily and then diluted to 1 x 10^5 cells/ml until all the cultures were growing at the same rate as the negative control.

For the mutagenicity experiment, duplicate 10 ml cultures of L5178Y mouse lymphoma cells (5 x 10^5 cells/ml) in the logarithmic growth phase were treated with nalmefene (6.25,.25, and 100 μg/ml) in the presence and absence of the metabolic activator S9, ethylmethanesulphonate (EMS) in the absence of the S9 metabolic activator, benz(a)pyrene (BP) in the presence of the metabolic activator or DMSO for two hours. Following treatment, cells were centrifuged, washed twice with phosphate buffered saline, resuspended in fresh medium and incubated for one hour at 37°C to recover. The cells were then counted, diluted to 1 x 10^5 cells/ml in Fischer medium and incubated overnight. The cells were counted daily and diluted 1 x 10^5 cells/ml.

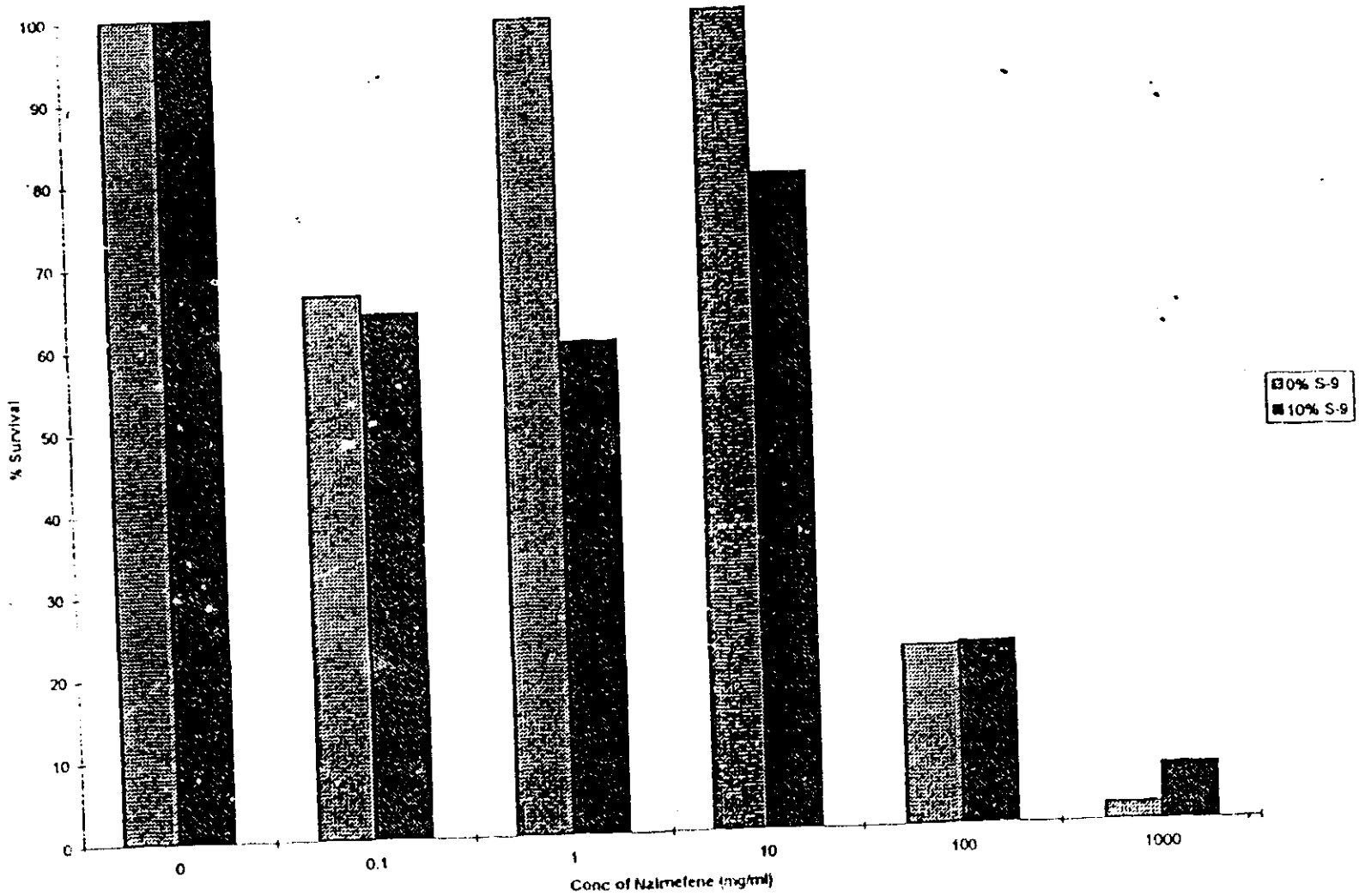
On days 4 and 5 (Experiment 1) and days 6 and 7 (Experiment 2) after treatment, cells were plated for ouabain resistant mutants and plated for survival on non-selective medium so that the numbers of mutants per 10^5 survivors could be calculated. The cells were incubated for 14 days to assess survival and for 3 weeks for mutation assessment. To assess resistance to ouabain, cultured cells (between 2 x 10^6 and 4 x 10^6) were treated with 4 ml of 10^{-2}M ouabain octahydrate.

On days 7 and 8 (Experiment 1) and days 12 and 13 (Experiment 2) after treatment, cells were plated for 6-thioguanine resistant mutants and plated for survival on non-selective medium so that the numbers of mutants per 10^5 survivors could be calculated. The cells were incubated for 14 days to assess survival and for 3 weeks for mutation assessment. To assess resistance to 6-thioguanine, cultured cells (between 1 x 10^6 and 2 x 10^6) were treated with 30 μg/ml of 6-thioguanine.

Results. The results from the cytotoxicity study are presented in figure 1. Cytotoxic doses of nalmefene were achieved in the study. Nalmefene was cytotoxic at 100 and 1000 μg/ml; these concentration killed 78 to 98 percent of the cells during the 2 hour incubation period. 100 μg/ml of nalmefene killed 78% of the cells in the presence and absence of the S-9 metabolic activator. 1000 μg/ml of nalmefene killed 98 and 93% of the cells in the absence and presence of S-9 metabolic activator, respectively.

Results from the mutagenicity assay are presented in Table 14. As anticipated, EMS and BP both induced statistically significant ($p<0.001$) ouabain and 6-thioguanine resistant mutants to the selective agents used. There were no indications that nalmefene induced a clear dose-related increase in the ouabain mutant frequency either with or without the S-9 metabolic activator. Nalmefene with S-9 metabolic activator appears to induce a dose related-increase in
6-thioguanine mutant frequency on colony day 8. This nalmefene-induced increase in 6-thioguanine was not statistically significant.

Figure 1. Cytotoxic Effects of Nalmefene Mouse Lymphoma Cells.



ug/ml

Table 13. Mutagenicity Testing Of Nalmefene In The Ames Assay.

TEST COMPOUND	DOSE (µg/plate)	SALMONELLA TYPHIMURIUM TEST STRAIN — MEAN No. OF REVERTANTS									
		TA1535		TA1537		TA1538		TA98		TA100	
		0% S9	10% S9	0% S9	10% S9	0% S9	10% S9	0% S9	10% S9	0% S9	10% S9
2AA	0.0		6.7		5.3		18.0		36.0		108.3
	5.0	NT	73.0	NT	57.0	NT	1069.5	NT	581.0	NT	267.3
	50.0		81.0		68.3		1129.0		1328.7		484.0
MNNG	0.0	3.3		4.0						88.0	
	1.0	203.0	NT	471.7	NT	NT	NT	NT	NT	699.3	NT
	10.0	1183.0		2195.0						2657	
4NOPD	0.0					13.7		30.7			
	5.0	NT	NT	NT	NT	590.3	NT	347.3	NT	NT	NT
	50.0					2174.3		1810			
JF-1 (HCL) (EXP. Nº 1)	0.0	10.7	5.7	11.7	7.0	10.7	18.3	31.3	39.7	150.3	125.7
	0.4	7.0	7.3	11.3	5.5	14.0	19.3	37.7	34.7	139.3	141.0
	2.0	7.0	5.0	8.7	8.0	7.7	17.0	37.7	29.7	150.0	143.3
	10.0	9.0	5.0	13.3	4.3	12.0	18.0	37.3	25.0	130.7	124.3
	50.0	10.0	7.3	11.3	7.0	10.0	18.7	45.3	32.3	152.7	132.7
	250.0	9.7	8.3	14.7	6.3	13.3	18.0	44.0	38.7	143.7	136.3
	1250.0	6.7	7.0	7.0	6.3	20.0	19.0	37.7	37.0	110.7	122.7
JF-1 (HCL) (EXP. Nº 2)	0.0	7.0	5.0	4.7	5.7	8.7	10.0	31.7	32.0	114.3	125.0
	2.0	3.7	5.0	5.3	5.3	7.7	14.3	50.7	32.3	115.3	140.0
	10.0	5.0	6.7	6.3	5.0	7.7	17.3	42.7	31.3	134.5	149.3
	50.0	5.3	4.7	4.0	4.0	10.7	12.7	51.0	28.0	137.0	122.7
	250.0	5.0	4.7	5.0	4.3	11.7	13.3	32.3	33.7	140.3	127.0
	1250	3.3	4.0	3.3	4.3	14.3	14.7	38.3	23.7	127.0	106.3
	6250	-	-	-	-	-	11.0*	22.7*	15.0*	-	-

*: Background lawn sparse or absence on some or all of the plates.
NT: Not tested

Table 14. Mutagenicity Testing Of almefene In The Mouse Lymphoma Cell Assay.

TEST COMPOUND	CONCENTRATION (µg/ml)	INDUCED MUTANT FREQUENCY/10⁶ SURVIVORS — OUABAIN								6-THIOGUANINE							
		0% S9				10% S9				0% S9				10% S9			
		DAY 4	DAY 5	DAY 6	DAY 7	DAY 4	DAY 5	DAY 6	DAY 7	DAY 7	DAY 8	DAY 12	DAY 13	DAY 7	DAY 8	DAY 12	DAY 13
NALMEFENE	0.00	0.0	0.0	0.00	0.79	0.0	0.46	0.97	0.00	5.9	9.0	5.38	8.14	11.8	10.4	5.84	11.79
	6.25	0.0	0.0	0.86	0.00	0.0	0.00	0.86	0.77	3.3	15.2	14.16	12.31	12.2	7.4	7.84	10.01
	25.0	0.0	0.0	1.04	0.58	0.0	0.96	0.00	0.98	10.6	13.1	7.02	7.88	10.8	11.8	13.3	6.38
	100.0	0.0	0.0	1.78	0.00	0.0	0.00	2.76	0.81	7.9	16.5	8.63	4.81	4.1	16.8	7.84	9.72
EMS	8mM	48.1	40.1	41.8	53.4	NT	NT	NT	NT	113.8	116.1	49.54	35.98	NT	NT	NT	NT
BP	2.0	NT	NT	NT	NT	47.2	33.0	47.7	37.6	NT	NT	NT	NT	137.1	91.3	79.28	52.97

NT: Not Tested

Metaphase Analysis of Human Lymphocytes Treated with Nalmefene.

This mutagenicity study was conducted a
during June 1985. The study was performed in compliance with the Good Laboratory Practice regulations.

Human lymphocytes collected from healthy donors were used for both the mutagenicity (n = 2) and cytotoxicity (n=1) analyses. Whole blood (0.8 ml) from the healthy donors was added to 10 ml of RPMI 1640 medium enriched with 15% fetal bovine serum, 0.1 ml of phytohaemagglutinin, and 100 units/ml penicillin and streptomycin to stimulate G_0 lymphocytes. After 44 hours of incubation at 37°C, the cultures were resuspended in treatment medium and incubated at 37°C for 3 hours. Cultures were treated in duplicate in all studies. The cultures were then re-incubated for 25 hours at 37°C. At the twenty-first hour of this incubation period, the cells were arrested in metaphase by adding 0.15 µg/ml of demecoline. Four hours later, the cells were harvested. After harvesting, chromosomal and cytological preparations were done.

The preliminary cytotoxicity assay was used to determine the test range for the definitive mutagenicity analyses. In this range-finding study, the treatment medium contained nalmefene at concentrations of 0.5, 1.0, 2.0, 5.0, and 10.0 mg/ml. Nalmefene was studied in the presence and absence of 10% S-9 metabolic activator. Toxicity was determined by counting a minimum of 500 cells and assessing dividing cells as a percentage of total lymphocytes (the mitotic index).

In the chromosomal aberration study, the treatment medium contained 0, 0.375, 0.75, 1.5, and 3.0 mg/ml of nalmefene in the presence of 10% S9 and 0, 0.75, 1.5, 3.0, and 6.0 mg/ml in the absence of 10% S9. The positive controls for the assay were: 25 µg/ml cyclophosphamide (CPA) in the presence of 10% S9 and methylmethane sulphonate (MMS) without 10% S9. In most cases, 200 cells were scored for chromosome damage; only well-spreaded metaphase with a chromosome count of 44 or more was scored. To determine whether the high dose of nalmefene inhibited mitosis, the mitotic index was calculated on 500 cells per culture.

Results. The results from the cytotoxicity study are presented in tables 15 and 16. In the presence of the metabolic activator, nalmefene caused a 15.9% to 77.8% suppression in the mitotic indices of human lymphocytes. Nalmefene, in the absence of the metabolic activator, caused a 43.8 to 28.2% inhibition of mitosis in the concentration range of 500 µg/ml to 5.0 mg/ml. At a concentration of 10.0 mg/ml, nalmefene inhibited mitosis at 100% in the absence of the metabolic inhibitor. The doses selected for the definitive study were based on these findings.

The results from the testing of nalmefene in the human lymphocyte for chromosomal aberrations are shown in Table 16. Drug concentrations of 0.75, 1.5, 3.0, and 6.0 mg/ml in the definitive study caused a 9.68-97.42% suppression in mitotic indices in the absence of the metabolic inhibitor S9. At the lowest concentration of nalmefene tested (0.75 mg/kg), the frequency of chromosome aberrations (5.5% with gaps; 2% without gaps) were the same as in the control level. The frequency of aberrations increased over the control at the concentrations of 1.5 and 3.0 mg/ml. The highest concentration (6.0 mg/ml) tested was very toxic; resulting in too few metaphase spreads for analysis. Most of the chromosome aberrations were chromatid gaps, chromatid deletions, and isolocus deletion; chromosome gaps, deletions, exchange and chromatid exchange were rare.

In the presence of an S9 activation mix, human lymphocytes exposed to 0.75 mg/ml was associated with 43.69% suppression of mitosis, but no significant increase in the percent abnormal cells.

Table 15. **Cytotoxic Effects of Nalmefene on Human Lymphocytes *In Vitro*.**

CONC. OF NALMEFENE	MITOTIC INDEX (M.I.) (%)		M.I. SUPPRESSION[a] (%)	
	0% S9	10% S9	0% S9	10% S9
0.0	3.86	9.0	0	0
500 µg/ml	2.17	7.57	43.8	15.9
1.0 mg/ml	2.91	5.9	24.6	34.4
2.0 mg/ml	2.98	5.0	22.8	44.4
5.0 mg/ml	2.77	3.8	28.2	57.8
10.0 mg/ml	0	2.0	100	77.8

a: M.I. suppression (%) expressed as the percent reduction of the treated mitotic index compared to the mitotic index of the solvent control.

Table 16. **Results of Chromosomal Aberration Analysis In Human Lymphocytes *In Vitro*.**

TREATMENT	% CELLS WITH ABERRATIONS		ABERRATIONS PER 100 CELLS						TOTAL ABERRATIONS		M.I. (%)	M.I. SUPPRESSION (%)
	+ GAPS	- GAPS	GAPS	CHROMOSOME DEL.	CHROMOSOME EXCH.	CHROMATID DEL.	CHROMATID EXCH.	ISOLOCUS DEL.	+ GAPS	- GAPS		
MMS	62	54	18	12	2	22	30	2	84[b]	66	-	-
CPA	32	18	16	0	0	14	4	0	34[a]	10	-	-
Nalmefene + 0% S9												
0.0 mg/ml	6	2	4	0	0.5	1	0	0.5	6	2	7.19	-
0.75 mg/ml	5.5	2	4.5	0	0	1	0	1	6.5	2	10.14	-
1.5 mg/ml	9	4.5	9	1	0	2	1	0.5	9.5	4.5	8.485	9.66
3.0 mg/ml	21.5	12.9	12.9[b]	1.8	0	6	1.2	1.9	25.9[b]	12.3	3.595	44.78
6.0 mg/ml	0	0	0	0	0	0	0	0	0	0	0.165	97.42
Nalmefene + 10% S9												
0.0 mg/ml	6	3	5	0.5	0	1.5	0.5	0.5	8	3	7.21	-
0.375 mg/ml	4	2.5	1.5	0	0.5	0.5	0.5	2.5	6.5	5	7.88	-
0.75 mg/ml	8	3	5	0	0	2	0.5	0.5	8	3	4.06	43.69
1.5 mg/ml	8.5	4	2.5	1	0	2	0.5	0.5	8.5	4	7.775	-
3.0 mg/ml	6	2.5	4	0	0	2	0	0.5	6.5	2.5	3.67	50.49

a: Significantly different from control ($p < 0.01$)
b: Significantly different from control ($p < 0.001$)

Mouse Micronucleus Test on Nalmefene.

The Mouse Micronucleus Assay mutagenicity study was conducted at in ıg March to May 1986. The assay was carried out in accordance to the guidelines and governmental standards of the Good Laboratory Practice (GLP).

The micronucleus test was conducted with young adult male and female CFLP mice . Thirty mice were randomly assigned to each treatment group. Nalmefene was orally administered at single doses of 55, 110, and 220 mg/kg for 2 consecutive days. Cyclophosphamide (CP), used as a positive reference compound, was administered orally into a group of 10 mice (5 males and 5 females) at a single dose of 40 mg/kg. Another group of 20 mice, receiving deionized water (20 ml/kg), served as negative control. The vehicle control was orally administered twice, with 24 hours between dosing.

Nalmefene-treated mice, 5 males and 5 females per time point, were sacrificed by CO_2 asphyxiation at 18, 42, and 66 hours after the second dosing. CP-treated mice were analyzed 18 hours after dosing. Bone marrow cells were aspirated from the femurs with a 1 ml syringe containing fetal calf serum. The bone marrow cells were centrifuged at 800 rpm for 5 minutes; the supernatant removed and the cells resuspended in a minimal volume of fetal calf serum. One drop of the cell suspension was placed on three slides and spread across the slide. All slides were allowed to air dry and age for 24 hours before staining. Slides were stained according to Gollapudi and Kamra (1979).

After staining, 1000 polychromatic erythrocytes (PCE) from each animal were scored and the number of micronucleated PCE, normo-chromatic erythrocytes (normocytes NCE) and mironucleuated NCE were recorded.

Results. The ratio of polychromatic erythrocytes to norchromatic erythrocytes is presented in Table 17. There was no indication of a reduction in PCE to NCE in the nalmefene-treated mice; the ratios for treated groups fell within the observed range for the vehicle control group. Thus, indicating no nalmefene-induced bone marrow toxicity in males or females.

Results of the micronucleus assay are presented in Table 18. As expected, cyclophosphamide clearly increased the numbers of micronucleated polychromatic erythrocytes. The number of micronucleated polychromatic erythrocytes (MN-PCE) per 1000 PCE was not statistically increased in female mice regardless of dose level or bone marrow collection time. Similarly, the bone marrow smears of the male mice in the 55.0 and 220 mg/kg group at all three evaluation point and the mice dosed with 100 mg/kg nalmefene and examined at the 18th and 48th hour collection time had similar frequencies of micronucleuated erythrocytes as the control mice. A small statistically significant decrease in MN-PCE was observed in the male mice 66 hours after treatment with 110 mg/kg nalmefene.

Table 17. Measure of Nalmefene-induced Bone Marrow Toxicity.

Treatment: Dose (mg/kg)	Sex	PCE/NCE Ratio		
		18 hr	42 hr	66 hr
Nalmefene: 0	M	0.68	0.86	0.26
	F	0.34	0.67	0.12
	M,F	0.51	0.76	0.19
Nalmefene: 55.0	M	0.59	0.91	0.69
	F	0.50	0.82	0.93
	M,F	0.54	0.86	0.81
Nalmefene: 110.0	M	0.48	0.79	0.65
	F	0.33	0.77	0.91
	M,F	0.40	0.78	0.78
Nalmefene: 220.0	M	0.59	0.77	0.81
	F	0.41	0.73	1.01
	M,F	0.50	0.75	0.92
CP: 40.0	M	0.42	NT	NT
	F	0.29	NT	NT
	M,F	0.35	NT	NT

Table 18. Effect of Nalmefene on the Frequency of Micronucleuated Polychromatic (MN-PCEs) in The Mouse Bone Marrow Assay.

Treatment: Dose (mg/kg)	Sex	INCIDENCE OF MICRONUCLEI/1000 POLYCHROMATIC ERYTHROCYTES											
		16 HOURS				42 HOURS				66 HOURS			
		Mean PCE	Mean MN-PCE	Mean MN-NCE	Mean Frequency of MN-PCE Formation (%)	MEAN PCE	MEAN MN-PCE	Mean MN-NCE	Mean Frequency of Formation of MN-PCE (%)	Mean PCE	Mean MN-PCE	Mean MN-NCE	Mean Frequency of Formation of MN-PCE (%)
Nalmefene: 2 X 0	M	1008.6	3.4	4.8	0.34	1012.2	3.6	1.8	0.36	1007.0	2.6	1.0	0.26
	F	1011.8	0.4	1.4	0.04	1006.0	0.8	1.0	0.08	1007.2	1.2	0.4	0.12
	M,F	1010.0	1.9	3.1	0.19	1009.1	2.1	1.4	0.21	1007.1	1.9	0.7	0.19
Nalmefene: 2X 55.0	M	1005.4	3.2	3.6	0.32	1018.6	4.4	1.8	0.43	1006.0	2.0	1.2	0.20
	F	1014.8	2.4	2.4	0.24	1023.6	0.8	0.8	0.08	1007.2	1.8	1.8	0.18
	M,F	1006.4	2.8	3.0	0.28	1020.2	2.6	1.3	0.26	1007.6	1.8	1.4	0.18
Nalmefene: 2 X 110.0	M	1008.6	4.6	7.0	0.46	1022.6	2.2	1.8	0.21	1006.6	0.6[a]	1.0	0.06
	F	1002.2	0.6	0.4	0.06	1008.4	0.8	0.6	0.08	1003.6	2.2	2.0	0.22
	M,F	1005.8	2.6	3.7	0.26	1016.1	1.5	1.3	0.15	1005.2	1.5	1.5	0.15
Nalmefene: 2 X 220.0	M	1013.6	3.6	4.4	0.36	1017.2	2.6	2.8	0.26	1025.6	3.25	1.25	0.32
	F	1008.0	1.2	0.4	0.12	1012.0	0.0	0.4	0.0	1011.8	2.4	0.6	0.24
	M,F	1010.6	2.4	2.4	0.24	1014.9	1.3	1.8	0.13	1017.8	2.8	0.89	0.27
CPA: 40	M	1008.8	9.2[b]	9.6	0.91	NT	NT	NT	NT	NT	NT	NT	NT
	F	1005.8	8.4[c]	0.4	0.84								
	M,F	1007.8	8.8	3.1	0.87								

a: One male dosed after the first dosing due to accidentally dosing into the lungs.
b: statistically significantly different from control (p = 0.028, Mann-Whitney U test)
c: statistically significantly different from control (p = 0.04, Mann-Whitney U test)
d: statistically significantly different from control (p = 0.048, Mann-Whitney U test)

Cytogenic Analysis of Rat Bone Marrow Treated With Nalmefene.

The mutagenicity study was conducted a
during September 1985 to January 1986. The assay was carried out in accordance to the guidelines and governmental standards of the Good Laboratory Practice (GLP).

This *in vivo* cytogenic experiment was conducted with groups of 30 (15 male and 15 female) Sprague-Dawley CD(SD)BR rats (Charles River U.K. Limited, Margate, Kent U.K.) per treatment group. The rats were treated with a single oral dose of 62, 124 or 248 mg/kg nalmefene dissolved in deionized water, and sacrifice either 6, 24 or 48 hours post-treatment. For positive controls, 5 male and 5 female rats were treated with 100 mg/kg methyl methane sulphonate (MMS) by a single intraperitoneal injection and were sacrificed 24 hours after treatment. For negative controls, 30 rats (15 male and 15 female) were administered vehicle (10 ml/kg) and sacrificed 2 hours following treatment. One hour prior to sacrifice, are animals were given an i.p. an injection of demecolcine (4.0 mg/kg).

Bone marrow cells were aspirated from the femurs by a syringe containing phosphate buffered saline. The cells were centrifuged (2000 rpm for 5 minutes) and resuspended in 2 ml of 0.075M KCL at 37°C for a 15-min hypotonic pre-treatment. This was followed by several fixations in ice-cold methanol:glacial acetic acid (3:1, v/v), the fixative procedure was terminated when a clear supernatant was obtained. Fixed cells were stored, suspended in fixative, at 4°C overnight. Cell suspensions were applied to slides, heated-dried, cooled to room temperature, and stained in 4% Gurr's Giemsa R66 for 5 minutes.

At least 50 metaphase figures from each set of 2 slides from each animal were examined; thus up to 500 cells per treatment condition at each time point could be scored. Only well spread metaphases with 40 or more chromosomes were scored. Aberrations were classified as chromatid or chromosomes gaps, breaks or exchange.

Results. Frequencies of aberrant mitoses including and excluding gaps, cells with deletions and exchanges are given in Table 19. There was no increase in either the number of mitoses containing structural alterations after the administration of nalmefene at any dose level or time point. MMS, the positive control substance for clastogenic activity, clearly increased the frequencies of structural aberrations.

Table 19. Results of Chromosomal Aberration Analysis In Rat Bone Marrow.

Treatment: (Dose (mg/kg))	Sex	CHROMOSOMAL ABERRATIONS PER 300 CELLS																									
		6 HOURS								24 HOURS								48 HOURS									
		Chromosome		Chromatid		Total Aberrations		% cells with aberrations		Chromosome		Chromatid		Total Aberrations		% Cells with Aberrations		Chromosome		Chromatid		Total aberrations		% cells with aberrations			
		-mid	-sce	-mid	-sce	+g	-g	+g	-g	-mid	-sce	-mid	-sce	+g	-g	+g	-g	-mid	-sce	-mid	-sce	+g	-g	+g	-g		
Vehicle	M	0.0	0.0	0.0	0.0	2.4	1.2	2.4	1.2	0.0	0.0	0.0	0.0	1.0	0.5	1.0	0.5	0.0	0.0	0.4	0.0	1.0	0.0	1.0	0.0		
	F	0.0	0.0	0.0	0.0	2.4	0.0	2.4	0.0	0.0	0.0	0.0	0.0	1.7	0.0	1.7	0.0	0.0	0.0	0.4	0.0	2.6	0.8	2.6	0.8		
	Mean	0.0	0.0	0.4	0.0	2.4	0.8	2.4	0.8	0.0	0.0	0.0	0.0	1.3	0.2	1.3	0.2	0.0	0.0	0.4	0.0	2.3	0.8	2.3	0.8		
Nalmefene: 62	M	0.0	0.0	0.5	0.0	2.8	0.5	2.8	0.5	0.0	0.0	0.0	0.0	1.7	0.8	1.7	0.8	0.0	0.0	0.4	0.0	1.2	0.4	1.2	0.4		
	F	0.0	0.0	0.0	0.0	3.6	1.2	2.8	1.2	0.4	0.0	0.6	0.0	3.2	1.2	2.4	1.2	0.0	0.0	0.0	0.0	2.4	0.4	2.4	0.4		
	Mean	0.0	0.0	0.2	0.0	3.2	0.8	2.8	0.8	0.2	0.0	0.4	0.0	2.4	1.0	2.0	1.0	0.0	0.0	0.2	0.0	1.8	0.4	1.8	0.4		
Nalmefene: 124	M	0.0	0.0	0.0	0.0	2.1	0.0	2.1	0.0	0.0	0.0	0.8	0.0	1.8	1.2	1.8	1.2	0.0	0.0	0.0	0.0	2.3	0.9	2.3	0.9		
	F	0.0	0.0	0.0	0.0	4.1	0.0	4.1	0.0	0.0	0.0	0.4	0.0	2.8	0.4	2.8	0.4	0.0	0.0	0.4	0.0	3.0	1.3	2.0	1.2		
	Mean	0.0	0.0	0.0	0.0	3.1	0.0	3.1	0.0	0.0	0.0	0.6	0.0	2.2	0.8	2.2	0.8	0.0	0.0	0.2	0.0	2.7	1.2	2.7	1.1		
Nalmefene: 248	M	0.0	0.0	0.4	0.0	3.4	0.4	3.4	0.4	0.0	0.0	0.0	0.0	2.5	1.2	2.5	1.2	0.0	0.0	0.0	0.0	0.9	0.0	0.9	0.0		
	F	0.0	0.0	0.4	0.0	2.4	0.4	2.4	0.4	0.0	0.0	0.0	0.0	1.0	0.0	1.0	0.0	0.0	0.0	0.8	0.0	3.2	0.8	3.2	0.8		
	Mean	0.0	0.0	0.4	0.0	2.9	0.4	2.9	0.4	0.0	0.0	0.0	0.0	1.8	0.7	1.8	0.7	0.0	0.0	0.4	0.0	2.1	0.4	2.1	0.4		
AMES: 100	M									0.0	0.0	2.8	0.0	6.5	4.8	6.9	4.8										
	F	NT	NT	NT	NT	NT	NT	NT	NT	0.0	0.0	1.8	0.8	13.6	5.6	9.6	4.4	NT	NT	NT	NT	NT	NT	NT	NT		
	Mean									0.0	0.0	2.8	0.4	11.0	5.2	8.3	4.6										

a: One male dosed after the first dosing du to accidentally dosing into the lungs.
b: statistically significantly different from control (p = 0.028, Mann-Whitney U test).
c: statistically significantly different from control (p = 0.04, Mann-Whitney U test).
NT: Not tested.

PHARMACOKINETICS

PHARMACOKINETICS

ABSTRACTS

The pharmacokinetic profile of nalmefene has been evaluated in dogs and rats following oral and intravenous administration. Nalmefene is well absorbed in both species following intravenous and oral administration; at least 88 and 85% of the radioactive dose was absorbed in rats and dogs, respectively. Because of extensive first-pass metabolism, the bioavailability of nalmefene was not quantifiable.

The distribution pattern of nalmefene has been evaluated in male and female rats following oral and intravenous administration. The results from the distribution studies indicate that there was no tissue-specific sequestration of nalmefene (or metabolites) following oral or intravenous administration. Twenty-fours hours after an intravenous injection of 4.3 n.g/kg of ^{14}C-nalmefene to male rats, 90% of the total radioactivity was detected in the excreta (44% urine, 36% feces), carcass (5%) and GI tract (5%).

Tissue distribution studies have been conducted in pregnant and lactating rats. The results indicated that intravenously administered ^{14}C-nalmefene (2 mg/kg) is rapidly distributed throughout the body. It was also relatively rapidly eliminated; approximately 55%, and 45% of the dose was excreted in the urine and feces, respectively, during 4 days. Radiolabelled nalmefene (or metabolites) was also secreted into milk. At 0.5 hours after dosing, the concentration of radioactivity was three times that in plasma. Nalmefene and/or metabolites also cross the placenta. The concentration of radioactivity in the fetuses was similar to or lower than, those in the corresponding plasma at all times. These findings are consistent with those observed with naloxone. Naloxone is rapidly transferred to the fetus of mothers after receiving an iv bolus of 400 μg naloxone; therapeutic plasma concentrations were obtained within 1 to 2 minutes. Fetal plasma concentrations of naloxone were approximately 2-fold lower than the corresponding maternal plasma concentrations at all times.

Using the ultrafiltration technique, the extent of ^{14}C-nalmefene serum protein binding was studied in rats and dogs over concentration range of 10-2000 ng/ml. The results revealed that the extent of serum protein binding was low; the mean percent bound was 33 ± 5, and 42 ±10 for rats and dogs, respectively. Statistical analyses (2-way ANOVA) revealed that serum protein binding was concentration and species dependent. Low plasma protein binding of nalmefene has also been observed in humans using the ultrafiltration technique. The extent of nalmefene serum binding, over a concentration range of 123 - 1845 ng/ml, was 45 ± 2. In contrast to the rat and dog data, the extent of protein binding is independent of the concentration.

The metabolic profile of nalmefene was examined in both the rat and dog following oral and intravenous administration. Nalmefene undergoes extensive and rapid metabolism in both the rat and dog; following intravenous administration the elimination half-lives was 1.0 and 2.5 hours, respectively. Following oral administration, nalmefene undergoes extensive first-pass metabolism in both species.

Analysis of nalmefene metabolites profiles following intravenous administration has revealed that the routes of nalmefene metabolism are species-dependent. In the rat, N-dealkylation of nalmefene to nornalmefene and subsequent conjugation with glucuronic acid are the major routes of metabolism. Nornalmefene glucuronide is the major metabolite of nalmefene in the rat. Nalmefene glucuronide and nornalmefene are other nalmefene metabolites detected in rats. In contrast to the rat, direct glucuronidation of nalmefene to nalmefene glucuronide and nalmefene diglucuronide was the predominant metabolic pathway of nalmefene in the dog. Nalmefene diglucuronide is the major nalmefene metabolite produced in dogs.

Nalmefene and nalmefene metabolites are eliminated in urine and feces. In both the rat and dog, the percent of total radioactivity recovered in the urine and feces within 24 hours were similar. The urinary metabolites consisted mainly of the conjugated metabolites (nalmefene glucuronide, nalmefene diglucuronide, and nornalmefene glucuronide), while the unconjugated metabolite nornalmefene was excreted in the feces. In the rat 10% and 15% of recovered radioactivity in the urine and feces was nalmefene, respectively; whereas in the dog 99% of the recovered radioactivity in the feces represented nalmefene.

In summary, the pharamcokinetic studies with nalmefene in dogs and rats have revealed the following points:

1. Nalmefene is rapidly absorbed following oral and intravenous administration in both species.

2. No specific tissue sequesters nalmefene and/or metabolites.

3. Like naloxone, nalmefene is rapidly transferred to the fetus of mothers.

4. Nalmefene has low binding affinity for serum protein.

5. Nalmefene is rapidly and extensively metabolized. The metabolism of nalmefene is species-dependent.

6. Nalmefene's elimination half-life is 1.0 and 2.5 hours in the rat and dog, respectively.

7. Interspecies comparisons of nalmefene pharmacokinetic parameters indicates that weight-normalized central volume of distribution are similar in rats, dogs and humans (3.5, 2.7, and 3.9 L/kg, respectively); and that there are marked differences seen for weight-normalize clearance (11.8, 3.7, and 0.8 L/hr/kg, respectively). The metabolic profile in the dog most closely resemble that reported in humans; nalmefene undergoes glucuronidation to yield the main metabolite nalmefene glucuronide. Nalmefene's plasma protein binding profile in rats, and dogs are similar to its profile in humans; nalmefene binds to serum protein with low affinity.

PHARMACOKINETICS STUDIES

Absorption, distribution, metabolism, and excretion:

Twelve preclinical pharmacokinetic studies (4 from the former sponsor and 8 from the current sponsor) were submitted in volume 1.21. The pharmacokinetic profile of nalmefene was studied in rats and dogs using unlabelled or ^{14}C-nalmefene.

Absorption.

The absorption profile of nalmefene was evaluated in three studies. Nalmefene is well and rapidly absorbed following oral and intravenous administration in both rats and dogs. Following intravenous and oral administration of ^{14}C-nalmefene, at least 88 and 85% of the radioactive dose was absorbed in rats and dogs, respectively. The bioavaliability of nalmefene after oral administration could not be calculated because of extensive absorption that occurred in both species. The peak plasma concentrations of nalmefene were non-measurable in both species because of extensive first-pass metabolism.

Distribution.

The distribution profile of nalmefene was evaluated in rats following oral and intravenous administration. A tissue distribution study in male rats treated with 2 mg/kg of ^{14}C-nalmefene HCL orally showed the highest radioactivity in the gastrointestinal tract, particularly the cecum (19.8% of the radioactive dose) and ileum (14.7% of the radioactive dose), two hours after dosing. By 72 hours only trace amounts of radioactivity were detected. Similar findings were observed in male rats dosed intravenously with 4.3 mg/kg of ^{14}C-nalmefene. Twenty four hours after intravenous administration, the carcass (5%) and the gastrointestinal tract (large intestine: 3.78%; caecum: 1.72%) had the highest concentration of radioactivity.

The extent of placenta transfer and tissue distribution of radiolabelled nalmefene was evaluated in pregnant rats. Twenty-five Sprague-Dawley rats (CD strain) were administered 2 mg/kg of ^{14}C-nalmefene intravenously on day 15 of gestation. Whole-body autoradiography was performed on five rats to qualitative the tissue distribution of nalmefene at 1, 3, 6, 24, and 72 hours after dosing (1 rat/time point). The concentration and amounts of total radioactivity were determined in the tissue of the remaining fifteen rats at 1, 3, 24, and 72 hours post-treatment (3 rats/time point).

The mean concentrations of radioactivity in all tissues measured were greatest at 1 hour after dosing. The kidneys (1.89 μg equivalents/g), liver (1.58 μg equivalents/g), lungs (1.5 μg equivalents/g) and mammary glands (1.11 μg equivalents/g) had the highest concentration of radioactivity at the 1 hr time point. Other tissues that had concentrations higher than the plasma were: ovaries (0.924 μg equivalents/g), uterus (0.745 μg equivalents/g), placenta (0.661 μg equivalents/g), brain (0.466 μg equivalents/g), and heart (0.427 μg equivalents/g). The mean concentrations of radioactivity in the fetuses were similar to the plasma mean concentration, 0.238 μg equivalents/g and 0.253 μg equivalents/g, respectively. 6 hours after treatment, the liver, kidneys, uterus and lungs still had higher concentrations of nalmefene than the plasma. Radioactivity was still detected in most tissues at 72 hours after dosing. At this time, the lungs kidneys, liver, and whole blood had the highest mean concentration of radioactivity.

Autoradiographic analysis revealed that radiolabelled nalmefene was rapidly and widely distributed throughout the tissues following intravenous administration. One hour after intravenous administration, the greatest concentrations were observed in the stomach contents, small intestine contents, intra-orbital

lacrimal gland, Harderean gland, submaxillary salivary gland, preputial gland, pituitary gland, and bladder. Lower concentrations were found in the spleen, liver (concentrated in the bile duct), lungs, kidneys, ovaries, bone marrow, adrenal glands, thyroid gland, and thymus. Blood and the fetuses had the lowest concentration of radiollabelled nalmefene. Three and six hours post-dosing, relatively high concentrations of radioactivity were in the gastrointestinal contents, bladder, oesophahageal contents, preputial gland, and submaxillary salivary gland. Only the lower gastrointestinal tract showed high concentrations of radioactivity 24 hours after administration. 72 hours post-treatment, only trace levels of radioactivity were defected in the tissues and the fetuses.

The distribution of 2 mg/kg (i.v.) of ^{14}C-nalmefene was studied in 15 lactating Sprague-Dawley rats (approximately 1 week after parturition). Nalmefene is secreted into milk; peak levels of radioactivity were measured in milk at 0.5 hours after dosing. The radioactivity was three times higher that found in plasma; mean concentrations of radioactivity in whole-blood, plasma, and milk were 0.389, 0.497, and 1.43 μg equivalents nalmefene base/ml, respectively. Thereafter, the concentrations of radioactivity declined rapidly. At 3 hours post-dosing, the concentrations of radioactivity were 0.077, 0.072, and 0.188 μg equivalents/ml, for whole-blood, plasma, and milk, respectively. The concentration of radiolabelled nalmefene in milk decreased to approximately half of the corresponding plasma levels by 24 hours after treatment; the mean concentrations of radioactivity in whole-blood, plasma and milk were 0.031, 0.017, and 0.008 μg equivalents/ml, respectively.

Plasma protein binding (PPB) was studied using ^{14}C-nalmefene in commercially obtained rat and dog serum (Pel Freez Biologicals, Rogers, AZ). Ultrafiltration methodology was used to determine extent of PPB. The extent of ^{14}C-nalmefene serum protein binding over a concentration range of 10 - 2000 ng/ml in rats and dogs was low, mean values of 33 and 42%, respectively. Statistical analysis (ANOVA) of rat and dog plasma binding data revealed significant differences between both between and within species ($p<0.01$). As depicted in table 20, an unusual bindings profile was observed; the percent binding increased throughout the 10-200 ng/ml concentration range and then decreased at the highest concentration tested in both species.

Table 20. Serum Protein Binding of ^{14}C-Nalmefene.

^{14}C-Nalmefene Concentration (ng/ml)	Mean % Bound ± SD	
	Rat	Dog
10.0	28 ± 4	31 ± 4
20.0	33 ± 4	44 ± 2
200.0	40 ± 5	54 ± 2
2000.0	29 ± 6	40 ± 3
Overall Mean	**33 ± 5**	**42 ± 10**

The distribution of ^{14}C-nalmefene in blood and plasma was evaluated in rat and dog whole blood. ^{14}C-nalmefene was equally distributed between blood and plasma; the mean ratios of C_b/C_p was 1.26 ± 0.02 and 1.33 ± 0.15 in rats and dogs, respectively. The difference in ratios between species was not statistically significant.

The results from these studies suggests the following:

1. No specific organ/tissue accumulates nalmefene (or metabolite) following oral or intravenous administration in male rats.

2. After intravenous administration of ^{14}C-nalmefene, radioactivity was rapidly distributed throughout body tissues of pregnant rats.

3. Naimefene and/or metabolite crosses the placenta; reaching concentrations in the fetuses that were similar to, or lower than, those in the corresponding plasma at all times.

4. Nalmefene and/or metabolite is/are secreted into milk, reaching concentrations 3 times that detected in plasma within 0.5 hours.

5. The extent of PPB for nalmefene was low in rats and dogs. These findings are consistent with results reported b iman plasma protein binding using similar methodology. The mean value for PPB was 45 ± 2% and it was found to be independent of concentration over a concentration range of 123 - 1845 ng/ml.

Metabolism.

In vitro and *in vitro* preparations have been used to study the metabolic profile of nalmefene in rats and/or dogs. Nalmefene undergoes extensive first-pass metabolism following oral administration in both the rat and dog. These studies have demonstrated that the routes of nalmefene metabolism are species-dependent. The metabolic fate of nalmefene in the rat and dog is depicted in figure 2.

In vitro studies have demonstrated that radiolabelled nalmefene undergoes extensive metabolism in rats and dogs following intravenous administration. In the rat, 35% and 27% of the total radioactivity was excreted in the urine and feces, respectively, within 24 hours. Thirty percent and 28% of the total radioactive dose was excreted in the urine and feces of dogs within 24 hours after dosing, respectively. In the rat, nornalmefene glucuronide was the major constituent of the urinary radioactivity, representing 65% of the total urinary radioactivity. Other metabolites identified in rat urine were: nalmefene glucuronide (5%), and nornalmefene (10%). In contrast to urine, nornalmefene (72%) was the major metabolite excreted in the rat feces. Nornalmefene was excreted in small quantity; it represented less than 2% of the total radioactivity.

In contrast to the rat, glucuronidation is the major metabolic pathway for nalmefene in the dog; N-dealkylation represents a very minor pathway of metabolism. Nalmefene glucuronide and nalmefene diglucuronide are the predominate metabolites of nalmefene found in the dogs; 78% and 22% of the urinary radioactivity were nalmefene glucuronide and nalmefene diglucuronide, respectively.

Using two *in vitro* systems, the rat liver microsome (phase I biotransformation) and precision-cut rat liver slice (phase I and phase II biotransformation) systems, metabolites of nalmefene were produced and characterized by chromatographic comparison with authentic standards and HPLC-MS. The results from these rat liver microsomal systems demonstrated that nalmefene is primarily metabolized to a Phase I, N-dealkylated metabolite (nornalmefene). In the precision-cut rat slice (PCRLS) system, nalmefene was

metabolized by Phase I and Phase II metabolic pathways to form nornalmefene and nalmefene glucuronide, respectively. Nornalmefene glucuronide, a major *in vivo* metabolite formed after intravenous administration of nalmefene to rats was not produced in the PCRLS study. In the rat, N-dealkylation of nalmefene to nornalmefene and subsequent conjugation with glucuronic acid is the major routes of metabolism. Nornalmefene glucuronide is the major metabolite found in the rat.

The cytochrome P-450 induction potential of nalmefene was investigated in rats by administering nalmefene (5 mg/kg, i.v.) for three consecutive days and studying its effects on antipyrine (20 mg/kg, i.v.) pharmacokinetics relative to phenobarbital- and saline-pretreated rats. The results from this study is presented in table 21 (as copied from the sponsor). In comparison to the classical P-450-inducing agent phenobarbital, 5 mg/kg nalmefene did not enhance the metabolism of antipyrine. Phenobarbital pretreatment caused a significant increase in the plasma clearance of antipyrine relative to saline; a 4-fold increase in clearance was observed. Nalmefene did not cause a significant alteration in the clearance of antipyrine relative to saline. Phenobarbital also altered other pharmacokinetic parameters of antipyrine; relative to the saline-pretreated group, a 3.7-fold decrease in antipyrine half-life and a slight increase in its volume of distribution were observed.

Table 21. Comparison of Antipyrine Pharmacokinetic Parameters in Saline-, Phenobarbital-, and Nalmefene Pretreated Rats (4/Group).

Parameter	Saline	Phenobarbital	Nalmefene
$t_{1/2}$ (min)	113 ± 30	30 ± 3*	87 ± 5
V_d (ml/kg)	755 ± 63	897 ± 46*	871 ± 176
Cl (ml/min/kg)	5 ± 1	21 ± 2*	7 ± 1
$AUC_{0-\infty}$ (ug.min/ml)	3948 ± 1069	980 ± 68*	2986 ± 515

Numbers represent Mean ± SD
***: Significantly difference compared to saline ($p \leq 0.05$).**

The ability of nalmefene to induce or inhibit rat microsomal liver enzymes was investigated in male Sprague-Dawley rats. Separate groups (n = 2/group) of rats were treated with nalmefene (5 mg/kg, i.v.), phenobarbital (80 mg/kg, i.p.) or saline (1 ml/kg, i.v.) for three consecutive days. After treatment, the first phase of nalmefene metabolism was evaluated by collecting and incubating liver microsomes with 5 - 40 μM nalmefene, 0.2 mg/ml microsomal protein and NADPH-regenerating system. Nornalmefene, the metabolite formed by N-dealkylation, concentration was assayed by HPLC. Nalmefene pretreatment for 3 consecutive days did not affect the K_m for N-dealkylation of nalmefene in rat liver microsomes; nalmefene did not show self induction potential. Nornalmefene was formed at a rate with K_m of 15.3 μM and a V_{max} of 1.6 μg/0.2 mg/2 minute. Rats pretreated with saline formed nornalmefene with a K_m of 12.3 μM and a V_{max} value of 1.6. In contrast, relative to saline, phenobarbital pretreatment produced a 3-fold decrease in the K_m (4.6 μM) and a slight decrease in V_{max} (1.1 μg/0/2 mg/2 minutes).

Excretion.

The pharmacokinetic profile of nalmefene was evaluated in male Sprague Dawley rats and Beagle dogs following intravenous administration. Intravenous bolus doses of nalmefene were administered to rats (4/group; 1 and 5 mg/kg) and dogs (n = 4; 1 and 4 mg/kg) and plasma was collected to analyze nalmefene levels. Nalmefene plasma concentrations were quantified using specific and sensitive radioimmunoassay procedures for 5 hours post-dosing. In both species at all doses, the concentration-time profiles appeared to decline in a biexponential fashion. The pharmacokinetic parameters obtained from analysis of the concentration-time data are presented in the following table:

Table 22. Pharmacokinetic Parameters of Nalmefene in Rats and Dogs following Intravenous Bolus Administration.

SPECIE	DOSE (mg/kg)	PHARMACOKINETIC PARAMETER (MEAN ± SD)			
		$T_{1/2}$ (min)	V_d (ml/kg)	Cl_{sys} (ml·min/kg)	$AUC_{0\text{-}inf}$ (ng·min/ml)
RAT	1.0	60.3 ± 6	2698 ± 854	239 ± 43	4006 ± 852
	5.0	66.0 ± 6	4317 ± 531	155 ± 24	30296 ± 4615
DOG	1.0	65.1 ± 10	2337 ± 534	73 ± 7	12849 ± 1367
	4.0	83.5 ± 22	3116 ± 397	50 ± 5	75457 ± 8444

Excretion via urine and feces was the major route of elimination of ^{14}C-nalmefene in the rat and dog after oral and intravenous administration. The excretion was considered rapid in rats because more than 90% of the dose was recovered in 72 hours. In rats dosed with 4.3 mg/kg (i.v.), a total of 86% of ^{14}C-nalmefene was excreted in 24 hours; 44% in the urine and 36% in the feces. Similar findings were observed in rats dosed with 2.0 mg/kg (i.v.) nalmefene, approximately 35% and 27% of the administered dose was recovered in the urine and feces within 24 hours, respectively. Analysis of the excreted radioactivity indicated that majority of the urinary radioactivity accounted for was conjugated and N-dealkyl nalmefene; 65%, 10%, 10%, and 5% of the radioactivity were identified as nornalmefene glucuronide, nornalmefene, nalmefene, and nalmefene glucuronide, respectively. The majority of the fecal radioactivity was nornalmefene (72%); the remaining fecal radioactivity was nalmefene (15%). Consistent with the findings following intravenous administration, 31% of the total dose of nalmefene following oral administration was recovered in the urine 24 hours after treatment. 18% and 30% were recovered in the feces 24 and 48 hours after dosing, respectively.

In dogs 42 - 58% of the total radioactive dose was excreted in the urine following intravenous and oral administration. Less than 1% of the urinary radioactivity was attributed to nalmefene. 29 - 45% and 45 - 56% of the radioactive dose were excreted in the feces over 96 hours following intravenous and oral administration, respectively. Following oral and intravenous administration, more than 50% of the fecal radioactivity was nalmefene.

LABELING.

The proposed draft labeling has been reviewed and the following changes are recommended:

WARNINGS

line 194 - 195:

The following sentence should be rephrased as following:

Original: Based on preclinical studies, reversal of buprenorphine-induced respiratory depression may be incomplete.

Replacement: Preclinical studies have shown that Revex® at doses up to 10 mg/kg (6 times the human dose) was ineffective in reversing buprenorphine-induced analgesic. Hence, Revex® may be ineffective in reversing buprenorphine-induced respiratory depression.

DRUG INTERACTIONS

LINE 228 - 229:

The following sentence should be rewritten as following:

Original: A preclinical interaction study with flumazenil did not show any deleterious interaction.

Replacement: Patients receiving flumazenil may exhibit an additive CNS excitation. Preclinical studies have shown that both drugs alone induced clonic seizures in rats; and the coadministration of flumazenil and Revex® (23:1 ratio) had a significant subadditive effect.

Clinical Pharmacology

The following sentence should be included in the label:

Nornalmefene, a metabolite of nalmefene possesses some pharmacological activity. Preclinical studies have shown that it produces antinociception following intrathecal administration and inhibits gastrointestinal contractions.

Carcinogenesis, Mutagenesis, Impairment of Fertility

The sentence on carcinogenicity should be deleted. The carcinogenicity studies that were submitted are not required for therapeutic drugs used acutely and were not reviewed. The following changes are recommended in this section:

Nalmefene did not have mutagenic activity in the Ames test with four bacterial strains, or in the mouse lymphoma assay. Clastogenic activity was not observed in the mouse micronucleus test or the cytogenic bone marrow assay in rats. However, nalmefene did exhibit a weak but significant clastogenic activity in the human lymphocyte metaphase assay in the absence but not in the presence of exogenous metabolic activation. Oral administration of nalmefene up to 200 mg/Kg/day did not affect fertility, reproductive performance, and offspring survival in rats.

Use in Pregnancy

Pregnancy Category B

The following changes are recommended in this section:

Reproduction studies have been performed in rats and rabbits by oral administration of nalmefene up to 200 mg/Kg/day (8000 times the human dose) and in rabbits by intravenous administration up to 8 mg/Kg/day (350 times the human dose). The results did not reveal evidence of impaired fertility or harm to the fetus. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers:

No changes are recommended.

EVALUATION AND COMMENTS.

Revex® (nalmefene hydrochloride injection) is an opioid antagonist indicated for the complete or partial reversal of opioid depression, including respiratory depression, and for the diagnosis and treatment of suspected opioid overdose. Revex® will be available as a sterile solution for intravenous, subcutaneous, and intramuscular administration in two concentrations (100 μg/mL and 1 mg/mL). In general, it is recommended that Revex® be titrated to reverse the undesired effects of opioids. In the case of reversal of postoperative opioid depression, the recommended dosage in individuals with no tolerance to, or dependence on, opioid is 0.1 to 0.5 μg/kg increments given at 2-5 minutes interval. For the management of opioid overdose, the recommended starting dose is 0.1 mg (IV); not to exceed a total dose of 1.6 mg.

Preclinical assessment of the pharmacological profile of nalmefene has demonstrated that nalmefene is a naloxone "me-to" compound. However, nalmefene appears to be more potent and to have a longer duration of action. Nalmefene is effective in reversing opioid agonist effects in animals in a variety of pharmacological preparations. Preclinical studies have demonstrated that nalmefene, like naloxone, is effective in reversing opioid-induced analgesia and suppression of gastrointestinal motility, attenuating opioid-type euphoria, and precipitating withdrawal symptoms in morphine-dependent primates.

Nalmefene's antagonistic properties are mediated via competitive inhibition of the δ, κ, μ, opioid receptors. It exhibits higher affinity for these opioid receptors than does naloxone or naltrexone. Nalmefene antagonistic effects at the μ opioid receptor is of clinical relevant. Activation of the μ receptors results in analgesia, respiratory depression, miosis, reduced gastrointestinal motility and euphoria.

Preclinical assessment of the pharmacological profile of nalmefene metabolites has revealed:

1. That nornalmefene also binds with relatively high affinity to the μ receptor, and with lower affinities to both the κ, and δ opioid receptors.

2. That nalmefene glucuronide has low affinities for the μ and κ opioid receptors, and has no affinity for the δ opioid receptors.

3. That nornalmefene possesses analgesic activity in the rat tail flick assay following intrathecal administration. It was equipotent as fentanyl and more potent than morphine.

The acute toxicity of nalmefene was assessed in CD-1 mice, CD rats, and Dutch Belted rabbits. Mortalities were observed in all three species. The mortality rate was dose-dependent. The time of death was route-dependent: intravenous > subcutaneous = oral. The toxic findings observed the most in all three species were of the CNS variety: tremors, convulsions, unsteady gait, and loss of balance/righting reflex. Other clinical signs observed frequently were: labored breathing, prostration, and hypoactivity. The clinical signs observed in the subacute, subchronic, and chronic toxicity studies were consistent with those seen in the acute studies, unsteady gait, tremors, convulsions, ataxia, and hypoactivity. Excessive salivation was observed consistently in these repeated dosing studies

The results from the special toxicity studies performed indicate:

1. That nalmefene will not produce any significant irritant effect following intravenous, intramuscular, or subcutaneous administration. In these studies nalmefene did not appear to have local irritant potential *in vivo* when injected intravascularly, perivasculary, intraarterially, intramuscularly or subcutaneously at level of 1 mg/ml. However, some irritant effects (eg. ecchymosis) were observed in rabbits receiving 2 mg/ml of nalmefene subcutaneously.

2. That nalmefene probably will not have hemolytic potential based on the *in vitro* hemolysis study.

3. That nalmefene could be regarded as being non-allergenic. Nalmefene did not act as a sensitizer in the delayed dermal sensitization study.

4. That the degradation product of nalmefene, bisnalmefene succinate, was three times less toxic than nalmefene; and that the major clinical signs associated with acute administration were lethargy, tremors, and convulsions..

Nalmefene probably will not have any reproductive toxicity potentials. In the Segment I reproduction study nalmefene did not affect fertility and reproductive performance in rats at doses up to 200 mg/kg (po). No teratogenic or embyrocidal effects were observed in rats or rabbits at doses up to 200 mg/kg (po) and in rabbits following intravenous administration of 8.0 mg/kg. However, nalmefene may slightly retard fetal growth,since rabbit fetuses in the 200 mg/kg group exhibited decreased ossification of the distal epiphysis of the femur and decreased body weight. Nalmefene also did not affect perinatal, postnatal or neonatal development in rats.

An individual probably will not be at significant genotoxic risk following the acute administration of nalmefene. Nalmefene did not cause gene mutation in bacteria or mouse cells in culture. Nalmefene lacked clastogenic properties in the rat bone marrow assay and mouse micronucleus assay. However, a significant positive clastogenic effect was observed *in vitro* at a concentration of 3.0 mg/ml of nalmefene in cultured human lymphocytes in the absence of the S-9 metabolic activator.

The pharmacokinetic data are similar in rats and dogs. Nalmefene is rapidly absorbed and extensively metabolized following oral and intravenous administration in both species. The terminal half-life was approximately 1 hour in the rat and 2.5 hours in the dog. This is in contrast to the reported half-life of 9 hours in man. Nalmefene and/or its metabolite do not distribute to a specific tissue in male or female rats following oral and/or intravenous administration. The excretion is rapid in both species. The nalmefene metabolic profile is species-dependent. In the rat, dealkylation of nalmefene to produce nornalmefene and its subsequent glucuronidation was the predominate pathway. The dog metabolic profile is similar to that in man; nalmefene undergoes direct glucuronidation. Consistent with the human protein binding profile, low protein binding was observed in the both rat and dog.

RECOMMENDATIONS.

We have no objections to the approval of this NDA, based on the preclinical pharmacology and toxicology. Minor labelling revisions will be necessary prior to approval.

BeLinda A. Hayes 2/28/95
BeLinda A. Hayes, Ph.D., Pharmacologist

Concurred by peer reviewer:

Almon W Coulter 2/28/95
Almon W. Coulter, Ph.D., Pharmacologist

CC: Orig NDA# 20-459
HFD-007/Div File
HFD-007/BHayes
HFD-007/BMcNeil
HFD-007/CWright
HFD-340
F/T by: BHayes/2-22-95

WRIGHT

JAN 13 1995

PILOT DRUG EVALUATION STAFF HFD-007
Review of Chemistry, Manufacturing, and Controls

NDA #: 20-459

REVIEW # 2 **DATE REVIEWED:** 1.9.95

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
AMENDMENT RESPONSE TO LETTER DATED 9.26.94	12.1.94	12.5.94	

NAME & ADDRESS OF APPLICANT:
Ohmeda Pharmaceutical Products Division Inc., 110 Allen Road, Liberty Corner, NJ 07938-0804, tel 908-604-7704, Robert Outwater, RA.

DRUG PRODUCT NAME
Proprietary: REVEX injection
Established: Nalmefene (or Nalmetrene) hydrochloride injection
Code Name/#: JF-1; SG 113H; ORF-11676
Chem.Type/Ther.Class:

PHARMACOL. CATEGORY: For reversal of opioid overdose at a maximum dose of 10 mg/patient; for reversal of post operative respiratory depression caused by the use of opiates for anesthesia at a maximum dose of 0.008 mg/kg with 0.1mg/ml solution; for treatment of drug addicts at a dose of 2 mg/patient and up to 5 doses with 1 mg/ml solution; for post operative patients at a dose of 0.001 mg/kg and up to 8 doses; if reversal is not observed after recommended dose, additional nalmefene is unlikely to be effective.

DOSAGE FORM: Injection.
STRENGTHS: 0.1 mg/ml/1ml amp (NDC 10019-315-21) and 1mg/ml/1ml amp (NDC 10019-311-21).
ROUTE OF ADMINISTRATION: IV; and IM, SC as alternate routes.
DISPENSED: __X__ Rx _____ OTC

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA AND WEIGHT:
Nalmefene's chemical name is 17-(cyclopropylmethyl)-4,5alpha epoxy-6-methylene morphinan-3,14-diol hydrochloride; empirical formula C21 H25 N 03. HCl with Mw = 375.9; CAS# 55096-26-9 base-USAN listing and CAS# 58895-64-0 HCl salt.

REMARKS:
The applicant has satisfactorily responded to the letter dated 9.26.94

4 pages
PURGED

CONCLUSIONS & RECOMMENDATIONS:

The applicant has PROVIDED COMMITMENTS to develop appropriate methods. Acceptable EER is attached.

On this basis, I suggest APPROVING

at RT.

cc:
Orig. NDA
HFD-007/Division File
HFD-007/PMaturu, CYaciw, BHayes, CWright, BMcNeal

P. Maturu
Pramod Maturu, Primary Review Chemist

1/13/95
Charlotte Yaciw, Secondary Review Chemist

filename: N20159.195

SATISFACTORY

SENSITIVE

REVIEW

OF

ENVIRONMENTAL ASSESSMENT

FOR

NDA 20-459

Revex Injection

(nalmefene hydrochloride)

HFD-007VIEW DIVISION

CENTER FOR DRUG EVALUATION AND RESEARCH

HFD-102

DATE COMPLETED 11/16/94

NDA 20-459
Revex (nalmefene HCl) Injection

Review of amended EA submitted September 27, 1994 (original EA review conducted by A. Mukherjee with deficiency letter to review division, HFD-007). I conducted second review only of material listed as deficient by A.Mukherjee in deficiency letter dated June 21, 1994.

The deficiency letter requested documentation of the applicant's request for an abbreviated EA. It appears that they are requesting an "infrequent use" abbreviated EA under 21 CFR 25.31a(b)(3). The information submitted in the amended EA (p.0025) and the low production volume seems to support the request for the abbreviated format. This is not a natural product therefore it cannot be granted a (b)(5) abbreviated EA--they also did not submit the correct information for that abbrev. EA format.

<u>Item 3</u>
Addresses provided for drug substance, drug product, and testing location.
Applicant's Response is Adequate.

<u>Item 4</u>
Discussion of environment surrounding the manufacturing places is adequate for the US sites.
Discussion of disposal is present. **Need permit number for Taylor waste disposal.**
Discussion of incineration is acceptable given it

The other incineration appears to occur at hospitals for which we do not need information on incineration.
Need documentation about incineration of returned goods. Need permit number, permitting authority, and discussion of packaging materials to be incinerated along with the drug product.

Need permit number and issuing authority for
Need permit number and issuing authority for Taylor waste water emissions.

Chemical identification of the drug substance, its impurities, additives, etc. is adequate. There is low solubility; however, there is little bioaccumulation likely.

DEFICIENT

Item 6
Certification from _ is the original permit to manufacture drugs.
Deficient--see above discussion in item 4.

This section adequately addresses the requests in the deficiency letter and the regulations, except as discussed below. MEEC is adequate. The production volume is stated and

6.b. Controls exercised on residual and emissions
This section should be revised to reflect all controls on emissions. Such information should include filters, recapturing, and other types of controls which limit emissions from the drug product manufacturing facility.

6.c. Effect of the proposed action on compliance with current emission requirements.
There should be a discussion of all permits, the permit numbers, and the issuing authorities. There should be permits for at least air and water. Under item 4 the company should discuss their waste disposal permits.

DEFICIENT

Toxicity data from TOMES attached.

SENSITIVE

REVIEW

OF

ENVIRONMENTAL ASSESSMENT

FOR

NDA 20-459

(Revex)

HFD-007 REVIEW DIVISION

CENTER FOR DRUG EVALUATION AND RESEARCH

HFD-102

DATE COMPLETED 6 /20 /94

25.31a Environmental assessment for proposed approvals of FDA-regulated products -- Format 1.

(a) For proposed actions to approve food or color additives, drugs, biological products, animal drugs, and class III medical devices, and to affirm food substances as generally recognized as safe (GRAS), the applicant or petitioner shall prepare an environmental assessment in the following format:

Environmental Assessment

1. Date: April 8, 1993

2. Name of applicant/petitioner:

Anaquest, Ohmeda Inc.

3. Address:

110 Allen Road, Liberty Corner, NJ 07938

Address for Drug Product Manufacturing:

Taylor Pharmacal Company
150 S. Wyckles Road
Decatur, IL 62522

Address for drug substance manufacturing:

Comment: Provide address where the drug substance would be manufactured if the street address is different from that has been submitted.

4. Description of the proposed action: Briefly describe the requested approval; need for the action; the locations where the products will be produced; to the extent possible, the locations where the products will be used and disposed of; and the types of environments present at and adjacent to those locations.

Nalmefene is an opiate antagonist and would be used for the treatment of opiate overdose. It will also be used for post

surgical recovery to overcome opiate induced respiratory depression. The drug product will be used throughout the United States. Returned and rejected samples would be collected at
All returned batches would be incinerated.

Malmefene hydrochloride injection will be prepared by Taylor Pharmacol Company. The drug substance will be provided to Taylor Pharmacol Company by Anaquest/Ohmeda.

Comment: Discuss the environment surrounding manufacturing places where the drug substance and the drug product will be manufactured. Discuss how much of unused and expired nalmefene will be disposed as per year basis on the fifth year of production. Provide incinerator permit where used and expired materials would be incinerated. List the packaging materials that would be incinerated along with the drug substance. Also discuss how unrecoverable nalmefene from the manufacturing process and from the formulation process would be disposed per year basis on the fifth year of production. All solid and liquid waste disposal, and emission from the incinerator should meet state, local and federal standards. Certificates for solid and liquid waste, and for emission to the air confirming the government

Nalmefene hydrochloride, sodium chloride, hydrochloric acid and water for injection will be used for the drug product.

Malmefene hydrochloride: CAS # 55096-26-9 for the base, chemical name: 17-(cyclopropylmethyl)-4,5 α-epoxy-6- methylenemorphinan-3,14-diol hydrochloride, molecular weight 375.9, impurities ranged 0.03 - 0.2% that include naltrexone, delta-7-nalmefene and 2,2'-bisnalmefene.

Comment: Provide Cas #, molecular weight, molecular formula, chemical name, solubility, physical form etc for the additives, known impurities, precursors and additives to the synthesis of the drug substance. Also provide solubility, physical form and physical properties of nalmefene.

6. Introduction of substances into the environment:

For the site(s) of production: list the substances expected to be emitted; state the controls exercised; include a citation of, and statement of compliance with, applicable emissions requirements (including occupational) at the Federal, State, and local level; and discuss the effect the approval of the proposed action will have upon compliance with current emissions requirements at the production site(s). Through use of calculations and/or direct measures, estimate to the extent possible the quantities and concentrations of substances expected to enter the environment as a result of use and/or disposal of products affected by the action.

Where are calculations

The sponsor stated that the amount of th it would be expected to reach the environme it of loss for the drug substance in the manuf product would be due to clean up of equi n of the finished product only. However expec e mentioned as per year basis. Most of the the Illinois plant would be discharged into [illegible] water system. The sponsor stated that there is no limit of nalmefene set by the state, local and federal authorities. Emission into air is considered to be negligible from manufacturing of the drug substance. Solid waste containing containers, papers and other combustible materials would be land filled. EPA landfill permit # is 11580402. Total amount of the drug substance consumed per year for he manufacturing of the drug

Comment: Provide an estimate of all the substances that would be emitted to the air, water and terrestrial environment as per year basis on the fifth year of production from the
State what type of control would be imposed to minimize the emission. Also calculate maximum estimated emitted concentration (MEEC) for the drug substance. Provide evidence that substances emitted either from the drug product or from the drug substance manufacturing are within the limit of state, local and federal regulations.

7. Fate of emitted substances in the environment: Predict environmental concentrations of and exposures to substances entering the environment as a consequence (direct or indirect) of the use and/or disposal of the products affected by the action for the following environmental compartments, including consideration of the major environmental transport and transformation processes involved:

(a) Air -- taking into account, to the extent possible, factors such as volatilization, photochemical and chemical degradation, rainout, and dispersion;

(b) Freshwater, estuarine, and marine ecosystems -- taking into account, to the extent possible, factors such as chemical and biological degradation, exchange between the water

For the site(s) of production: list the substances expected to be emitted; state the controls exercised; include a citation of, and statement of compliance with, applicable emissions requirements (including occupational) at the Federal, State, and local level; and discuss the effect the approval of the proposed action will have upon compliance with current emissions requirements at the production site(s). Through use of calculations and/or direct measures, estimate to the extent possible the quantities and concentrations of substances expected to enter the environment as a result of use and/or disposal of products affected by the action.

The sponsor stated that the amount of the drug substance that would be expected to reach the environment is minimal. Amount of loss for the drug substance in the manufacturing of the drug product would be due to clean up of equipments and filtration of the finished product only. However expected amount need to be mentioned as per year basis. Most of the drug substance from the Illinois plant would be discharged into the waste water system. The sponsor stated that there is no limit of nalmefene set by the state, local and federal authorities. Emission into air is considered to be negligible from manufacturing of the drug substance. Solid waste containing containers, papers and other combustible materials would be land filled. EPA landfill permit # is 11580402. Total amount of the drug substance consumed per year for he manufacturing of the

Comment: Provide an estimate of all the substances that would be emitted to the air, water and terrestrial environment as per year basis on the fifth year of production from the . State what type of control would be imposed to minimize the emission. Also calculate maximum estimated emitted concentration (MEEC) for the drug substance. Provide evidence that substances emitted either from the drug product or from the drug substance manufacturing are within the limit of state, local and federal regulations.

7. Fate of emitted substances in the environment: Predict environmental concentrations of and exposures to substances entering the environment as a consequence (direct or indirect) of the use and/or disposal of the products affected by the action for the following environmental compartments, including consideration of the major environmental transport and transformation processes involved:

(a) Air -- taking into account, to the extent possible, factors such as volatilization, photochemical and chemical degradation, rainout, and dispersion;

(b) Freshwater, estuarine, and marine ecosystems -- taking into account, to the extent possible, factors such as chemical and biological degradation, exchange between the water

column and sediments via sorption/desorption and biological processes, accumulation in animals, plants, and other organisms, introductions due to rainfall and losses due to volatilization;

(c) Terrestrial ecosystems -- taking into account, to the extent possible, factors such as chemical and biological degradation, sorption/desorption and leaching in soils, accumulation in animals and plants, introductions due to rainfall, losses due to volatilization, and entry into groundwater.

The sponsor has not addressed this issue considering an abbreviated format would be accepted for the EA.

8. Environmental effects of released substances: Given the information developed on the introduction (item 6) and fate (item 7) of substances which would be released as a consequence of the use and/or disposal of the products affected by the action, use any relevant toxicological data or other appropriate measures to predict, to the extent applicable, effects on animals, plants, humans, other organisms, and effects at the ecosystem-level in each of the environmental compartments listed in item 7.

The sponsor has not provided effect studies considering an abbreviate application would be accepted.

9. Use of resources and energy: Specify the natural resources, including land use, minerals, and energy, required to produce, transport, use, and/or dispose of a given amount of any product which is the subject of the action, including the resources and energy required to dispose of wastes generated from production, use, and/or disposal. Effects, if any, upon endangered or threatened species and upon property listed in or eligible for listing in the National Register of Historic Places must be discussed.

Sponsor has not provided any information for this section considering an abbreviated application would be accepted.

10. Mitigation measures: Describe measures taken to avoid or mitigate potential adverse environmental impacts associated with the proposed action.

The sponsor has not provided any information for this section considering an abbreviated format of the EA would be accepted.

11. Alternatives to the proposed action: If potential adverse environmental impacts have been identified for the

proposed action, describe in detail the environmental impact of all reasonable alternatives to the proposed action (including no action, and including measures that FDA or another government agency could undertake as well as those the applicant/petitioner would undertake). Describe particularly those alternatives that will enhance the quality of the environment and avoid some or all of the adverse environmental impacts of the proposed action. Discuss the environmental benefits and risks of the proposed action. Discuss the environmental benefits and risks of each alternative.

The sponsor has not provided any information for this section considering an abbreviated format EA would be accepted.

12. List of preparers: Those persons preparing the assessment together with their qualifications (expertise, experience, professional disciplines) shall be listed. Persons and agencies consulted shall also be listed.

1. Burkett, Ronald J, M.E chemical Engg.
   Director of Environmental Affairs
   Ohmeda

2. Taylor, Richard S. R.Ph.
   Consultant

13. Certification: The undersigned official certifies that the information presented is true, accurate, and complete to the best of the knowledge of the firm or agency responsible for preparation of the environmental assessment.

(Date)
April 8, 1993

Signed by Ronald J. Burkett
(Signature of responsible official)

Director of Environmental Affairs
Ohmeda

(Title)

14. References: List complete citations for all referenced material. Copies of referenced articles not generally available should be attached.

No reference has been provided.

15. Appendices: (a) Data summary charts (e.g., structural formula, vapor pressure, water solubility, n-octanol/water partition coefficient, biodegradation half-life, LC50 for each species tested, etc.).

(b) Test reports (for each experiment: research objective, experimental design and procedure, all data relevant to interpretation of the test result given in item 15(a), sample calculations and statistical analyses).

No data chart and study reports have been provided considering an abbreviated format would be acceptable.

(3) For approval of NDA's for human drugs and approval of licenses for biological products, when the drugs or biological products are intended for the prevention, treatment, or diagnosis of a rare disease or for a similarly infrequent use; for ophthalmic or topical application; or for local or general anesthesia; the following information is required for the items specified: (i) Format item 6. For the site(s) of production: list the substances expected to be emitted; state the controls exercised; include a citation of, and statement of compliance with, applicable emissions requirements (including occupational) at the Federal, State, and local level; and discuss the effect the approval will have upon compliance with current emissions requirements at the production site(s). Estimate the maximum yearly market volume of the drug product to aid in determining whether approval of the application could result in potentially significant environmental introductions from use of the product.

(ii) Format items 7 through 11 and 15. Documentation for these items is ordinarily not required.

(5) When the agency approves or issues, for a substance that occurs naturally in the environment, a food or color additive petition, GRAS affirmation petition, NDA, supplemental NDA, biological product license, NADA, supplemental NADA, or class III medical device, the following information is required for the format items specified:

(i) Format item 6. For the site(s) of production: list the substances expected to be emitted; state the controls exercised; and include a citation of, and statement of compliance with, applicable emissions requirements at the Federal, State, and local level; and discuss the effect the approval will have upon compliance with current emissions requirements at the production site(s).

(ii) Format item 7. Discuss whether the use of the

product can reasonably be expected on the basis of all available evidence to alter significantly the concentration and distribution of the product, its metabolites, degradation products, or its constituent parts in the environment.

(iii) Format item 8. Report existing data relating to the environmental effects of substances expected to be emitted into the environment as a consequence of use of the product. Report information obtained from the scientific literature on the toxicity of the product to laboratory animals, e.g., that information which is submitted to satisfy human safety requirements, and to organisms in the environment, e.g., fish, invertebrates, plants, fungi, and bacteria, that may be exposed to the product.

(6) For approval or issuance by the agency of a food or color additive petition, NDA, supplemental NDA, biological product license, NADA, or supplemental NADA for a product that has been approved by the Environmental Protection Agency (EPA) under section 4 or 5 of the Toxic Substances Control Act (TSCA) or under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the following information is required: (i) Format items 7 and 8. To address these items, rely on environmental information in studies submitted to EPA, in the application/petition submitted for FDA approval, and in the scientific literature. Describe any potential adverse environmental impacts determined by EPA.

(ii) Format item 15(b). For studies submitted to EPA or for relevant studies submitted in the application/petition to meet the requirements of the Federal Food, Drug, and Cosmetic Act, test reports may include only a brief description and summary of results of each study in lieu of attaching complete test reports.

Reviewed by: Asoke Mukherjee Ph.D., HFD-007/102

Concurrence: Phillip G. Vincent Ph.D., HFD-102

File: 20459E00.RAM

No advertising material has been submitted for NDA 20-459. This material is being requested in the approval letter.

There are no Federal Register Notices or OTC or DESI Documents relating to NDA 20-459.

NDA#: NDA020459
Trade and generic name & form: Nalmefene Injection (REVEX)
Sponsor: Ohmeda
Letter Date: 4/27/94
Date Completed: 7/31/94

Medical Officer Review
NDA 20495 Nalmefene Injection

"Revex"

Type of Submission: NME NDA
Date Received: 4/29/94
Review Completed : 7/20/94
Reviewer: Curtis Wright MD, MPH
Peer Reviewer: Robert Bedford
Date Cleared Peer: 7/29/94
Security Level: TradeSecret/Confidential (FDA and Special Government Employees)

ACCOUNTING DATA:

Abstract

Nalmefene is an opioid antagonist of the naloxone type, and the sponsor has shown that the drug is effective in reversing opioid agonist effects in animals, human laboratory studies, and in anesthesia and overdose settings in clinical trials. The duration of action of the drug appears to depend on dose, due to probable entero-hepatic recirculation, but was sufficiently long that re-sedation was not a problem in either the anesthetic setting or the management of overdose. As with all drugs of this class, careful attention to dosing is paramount, since too little will be ineffective and too much will risk reversal of analgesia and precipitated withdrawal. No idiosyncratic pulmonary edema of the naloxone type was recognized in the clinical trials, but as this is a rare event, it cannot be ruled out. Approval is recommended, but with careful attention to the dosing recommendations and precautions.

(Note: This review was completed prior to the final inspection of the sites by DSI and the conclusions are subject to modification if the submitted data are not confirmed by site inspection.)

INDEX

Background

The major clinical effects of opioid analgesics; pain relief, alteration of mentation and respiratory depression, are known to be mediated by specific opioid receptors located in central nervous system. Naloxone, and later naltrexone, were developed as specific opioid antagonists that bind to these receptors, but do not activate them. The result is that opioid agonists are displaced in a competitive fashion, and opioid effects are reversed. Specific opioid antagonists are well known to most practicing physicians, having been available for the last quarter century.

As with most agents, the introduction of naloxone into clinical practice led to its enthusiastic adoption. It appeared to offer in the treatment of overdose and in the reversal of opioid-induced anesthesia. As with most medicines, this enthusiasm was short lived, and two problems with the use of these agents emerged.

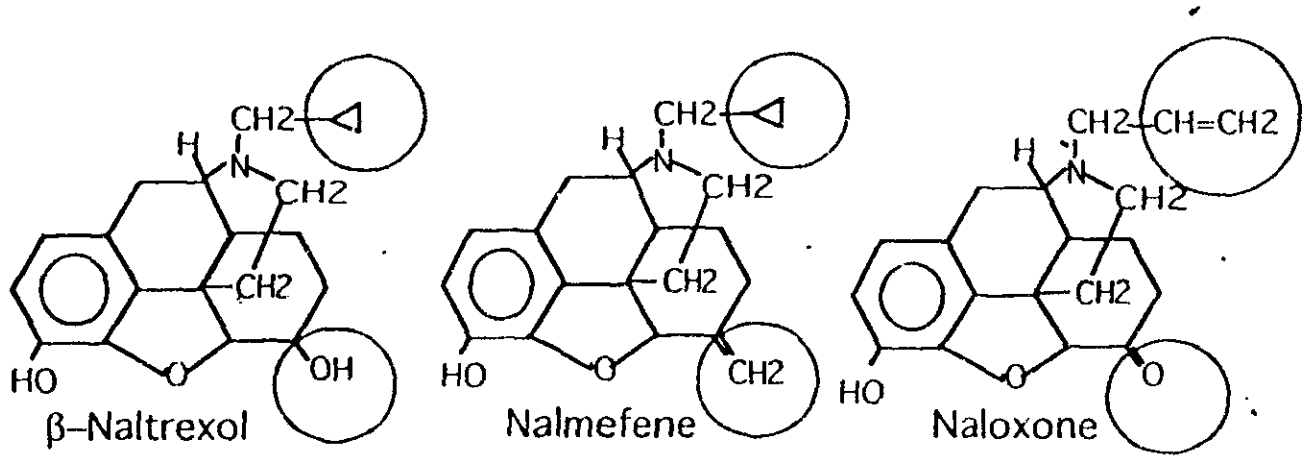
The first was purely pharmacological. Naloxone, given parenterally, has a short half life due to both redistribution and rapid elimination. In consequence, when given for an overdose of a long-acting opioid such as methadone, the antagonist wore off and the patient relapsed into coma.

Naltrexone, a similar agent, had a much longer half-life, but established a second problem with the use of these agents. Studies in normal volunteers, animals and patients established that the development of tolerance to opioids was a much faster process than previously thought. Biological adaptation to a dose of morphine began within fifteen minutes after administration, and resulted in a physiological withdrawal reaction when an opioid antagonist was given. This precipitated a withdrawal reaction, sometimes called naloxone sensitivity, consisted of reversal of analgesia, hyperalgesia, abdominal and joint pain, myalgia, nausea and vomiting. In the management of overdose it resulted in patients having severe withdrawal reactions in the emergency room, and in anesthesia it resulted in tachycardia, catecholamine release, dysrthymia and occasional sudden death in patients with occult compromised coronary circulation. Naltrexone-induced withdrawal was prolonged and difficult to manage, and the sponsor never made the drug available in a parenteral form.

The third problem, as yet unexplained, is a rare idiosyncratic acute pulmonary edema, occasionally seen with these agents in otherwise healthy patients. The syndrome has been described as anecdotally responding to phentolamine, but its cause and management remain obscure.

Ohmeda, saw the gap between the useful but short acting naloxone and the long-acting but oral naltrexone as a window of opportunity for a new product. They developed a naltrexone analog, called nalmefene, as an intermediate-duration opioid antagonist. The task in their clinical development program, therefore, was to show that the drug could be given in doses that were precise enough to reverse anesthesia without precipitating withdrawal, and robust enough to treat overdose without wearing off before the narcotic. It should also, of course, not induce the rare idiosyncratic pulmonary edema to any discernible extent.

The Drug Substance



As should be clear from the structures, nalmefene shares features with the structures of naloxone and naltrexol (the active moiety of naltrexone), sharing the double bond of the lower position with naloxone and the cyclopropane group of the upper position with naltrexol.

Preclinical pharmacology studies have shown that nalmefene has a high affinity for the mu opioid receptor, (IC_{50} 0.73 Nm), the kappa receptor, (IC_{50} 4.7 Nm), and for the delta receptor, (IC_{50} 9.3 Nm), values that show higher affinity than naltrexone (0.9/10/10 Nm) and much higher than naloxone (3/5/60/26 Nm).

The reader is directed to the pharmacologist's review for the details of the animal work, but nalmefene (which is metabolized in a similar fashion by animals and man) was examined in three animal models and found to be a more potent antagonist than naloxone, with a longer duration of action than naloxone in several species.

Pre-Clinical Pharmacokinetics

Pharmacokinetic studies in animals show that the elimination half-life in the rat is 1 hour and in the dog 2.5 hours. Conjugated metabolites appear rapidly in the plasma in both species. In the rat the majority of radioactivity following i.v. administration of radio labeled nalmefene is conjugated nalmefene and N-desalkyl nalmefene in the plasma and conjugated nalmefene and conjugated N-desalkyl nalmefene in the urine. For dogs conjugated nalmefene account for the majority of radioactivity in both plasma and urine.

Human Pharmacokinetics

The reader is directed to the kineticist's review for the details of the kinetic work, but nalmefene is both redistributed and directly metabolized to the glucuronide in man, with a central volume of distribution of about 4 liters/kg, a redistribution half life of about 20 minutes, and a half-life for metabolic clearance (as the glucuronide) of about an hour. Age, renal and hepatic disease caused mild reductions in clearance which are unlikely to be meaningful in single dose use.

As will be described in more detail below, it is more likely than not that there is some degree of entero-hepatic recirculation of the drug (the glucuronide is split by colonic bacteria and the nalmefene re absorbed) that becomes clinically meaningful at doses above 1/2 to 1 mg.

Human Pharmacodynamics
PET and SPECT ligands specific for the opioid receptor are available, and the sponsor was encouraged to study the uptake of these specific opioid ligands in imaging studies in man, both in the presence of placebo, naloxone, and nalmefene.

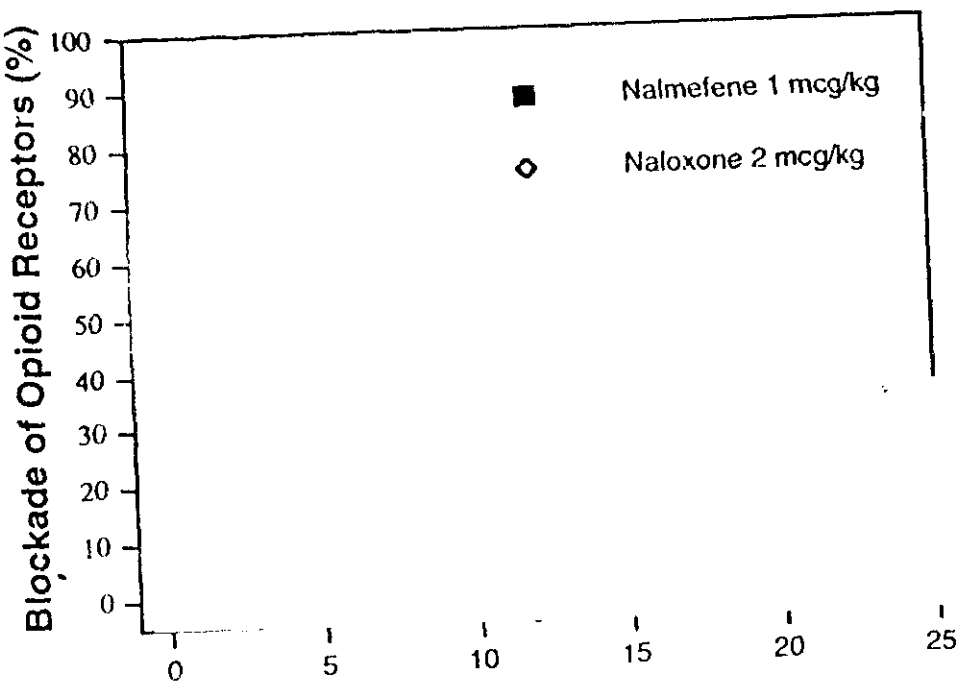
Study 24

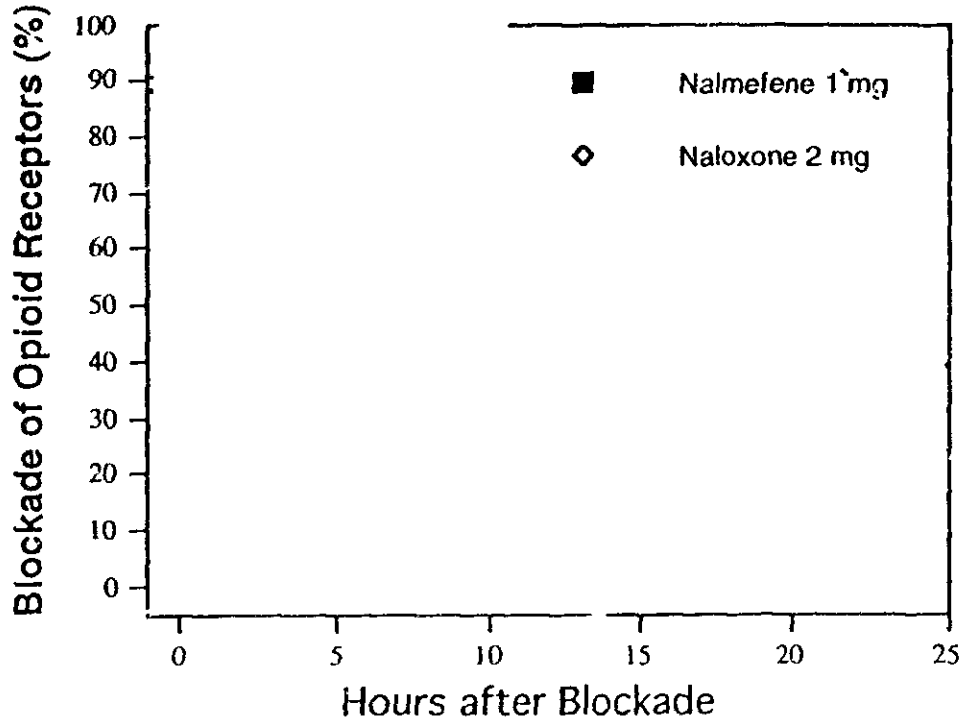
In Study 24 a dual detector system was used to measure the binding of a positron-emitting ligand, ^{11}C carfentanil, to opioid receptors in the brain. By observing the brain concentration vs time of ^{11}C carfentanil the percent of blockade of brain opioid receptors was measured in 4 healthy volunteers in a crossover study following nalmefene 1.0 **mg** i.v. and naloxone 2.0 **mg** i.v. In a second phase of the study, blockade of opioid uptake was studied in an additional 4 volunteers following nalmefene 1.0 **mcg/kg** i.v. and naloxone 2.0 **mcg/kg** i.v.

At the high dose, both nalmefene and naloxone blocked about 80% of the opioid receptors at 5 minutes. At 8 hours, nalmefene specific opioid blockade remained 75% while naloxone blockade was 20%.

At the low dose, nalmefene showed a 60% specific opioid blockade at 5 minutes. Nalmefene continued to block 40% of the receptors at 8 hours, while naloxone showed 35% blockade at 5 minutes and no specific blockade by 2 hours. The results are displayed in the following figures.

% Blockade of Opioid Receptors by Treatment Group following a single dose of opioid antagonist



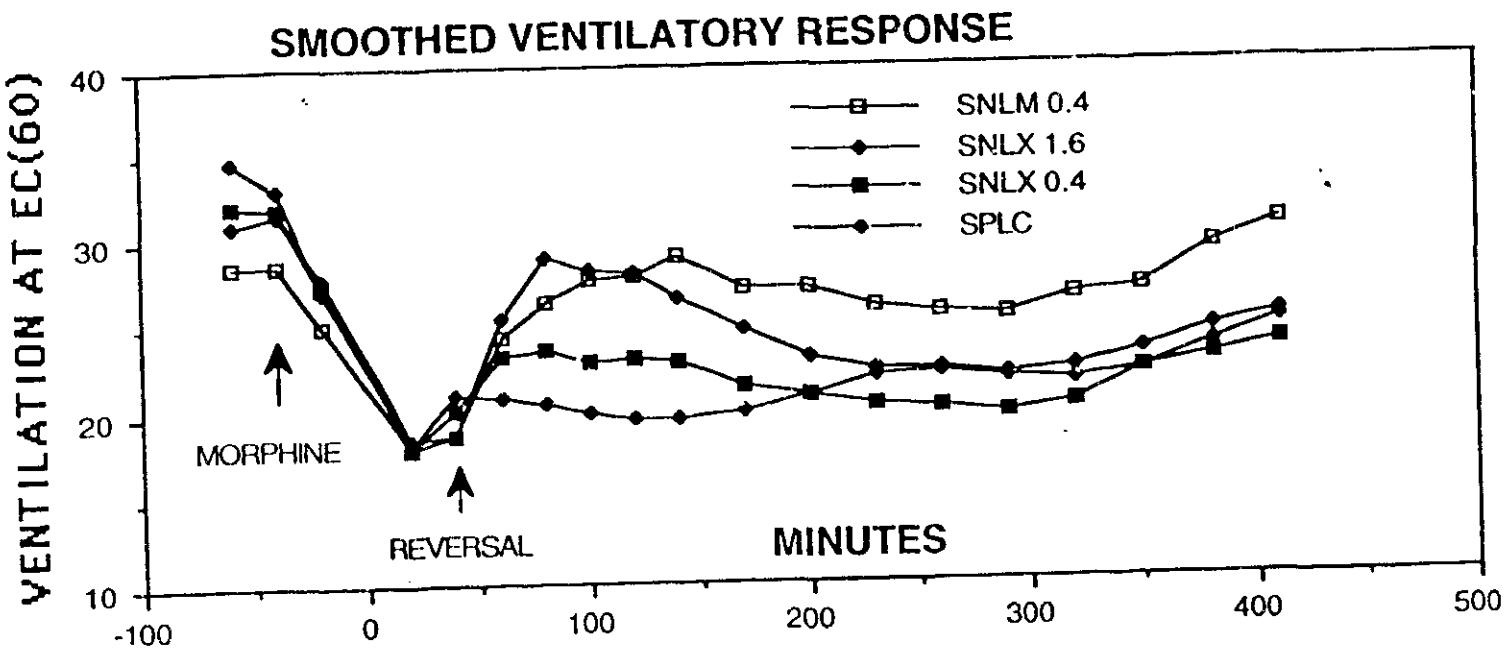


The study provides substantial evidence that nalmefene binds specifically to opioid binding sites in human brain and blocks the uptake of a receptor-specific PET ligand. It also suggests that nalmefene is more potent than naloxone (produces more blockade for a given dose) and has a longer duration of effect. Nalmefene seems to have a much longer duration of effect in high dose than naloxone, suggesting either a deep compartment or entero-hepatic recirculation. These data were consistent with the high binding affinity of nalmefene for opioid receptors.

The primary mechanism of respiratory depression by opioids involves a reduction in the brainstem respiratory centers sensitivity to CO_2. Therefore measurement of ventilatory response to CO_2 (minute ventilation at a specific partial pressure) is a sensitive and specific test of opioid effect on respiration and its reversal.

Study JF-145

Study JF-145 evaluated the effect of nalmefene 0.4 mg/70 kg, naloxone 0.4 mg/70 kg, naloxone 1.6 mg/70 kg or placebo on respiratory sensitivity to CO_2 following administration of 10 mg/70 kg of morphine. The study was conducted in 6 healthy, male volunteers as a double blind randomized placebo controlled, 4-way crossover study. The following figure shows the ventilatory responses in liters/minute after smoothing the data (using moving averages to reduce the noise) .



As can be seen from the graph, morphine caused a robust drop in minute ventilation under conditions of hypercarbia. Placebo did not materially affect this response, but there was a dose and drug dependent increase in hypercarbia-induced minute ventilation in the order:

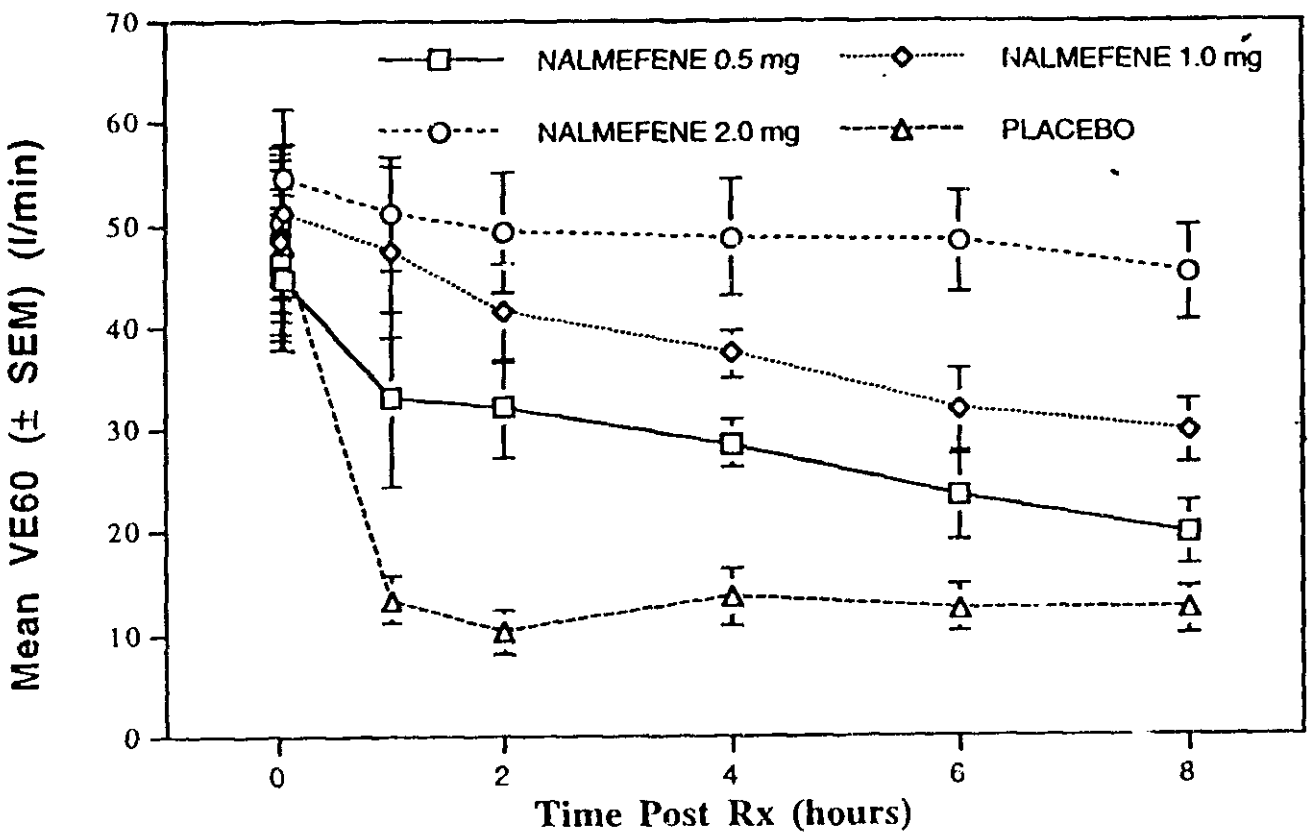
Placebo < naloxone 0.4 mg < naloxone 1.6 mg < nalmefene 0.4 mg.

The data suggest, but do not prove, that nalmefene is more potent (effective at lower dose) and has a longer duration of action than naloxone.

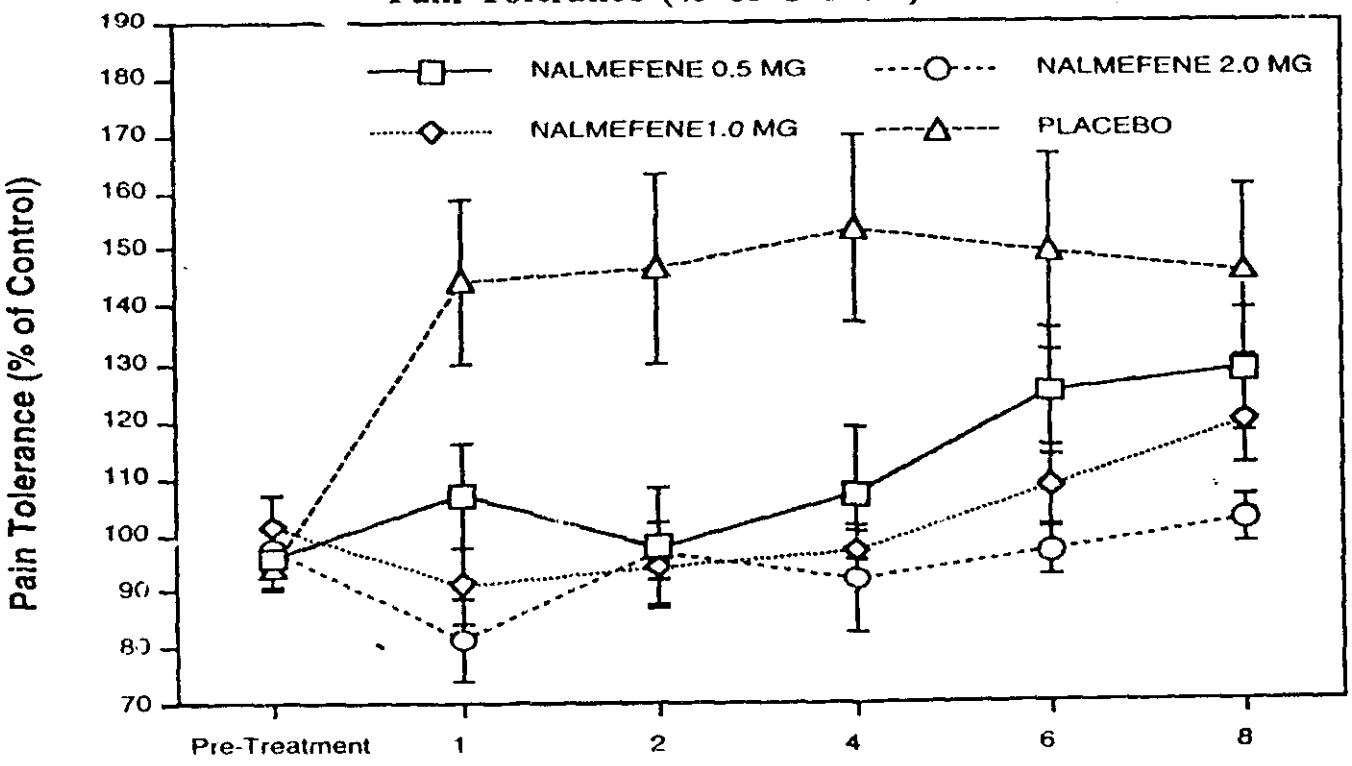
Study JF110

Another means of examining opioid effects is to look at subjective experiences and the response to (non-destructive) experimental pain. Study JF110 evaluated 0.5 mg, 1.0 mg and 2.0 mg IV of nalmefene plus placebo in 6 healthy male volunteers. After the test drug volunteers were challenged with fentanyl 2 mcg/kg at 1, 2, 4, 6 and 8 hours. Opioid induced respiratory depression was evaluated by determining the ventilatory response to hypercarbia and response to pain was determined using the tourniquet ischemia test. Nalmefene antagonized both the respiratory depressant and analgesic effects of fentanyl with the duration of antagonism being dose dependent. The following figure shows these results over the eight hours of the study. (The reader is advised that VE60= respiratory rate at 60 torr CO_2, high VE60 = little opioid effect, low VE60 means strong opioid effect).

Figure 2. Mean VE60 By Treatment Group



Pain Tolerance (% of Control) Over Time



This study provided strong evidence that nalmefene blocked opioid effects on respiration and experimental pain, but it also suggested that very large (2 mg) doses of nalmefene might cause hyperalgesia, as had been reported for naloxone.

There were a number of similar studies, weaker in design or execution, in the development plan, with similar results. They are provided in the package for examination if desired.

Clinical Studies

The following table shows the results of the sponsor's clinical development plan.

Study #	Type	Indication	N REVEX	N CTL	Dose µg/kg	Control	Outcome
JF-1-110	Clin Pharm	Reversal of Fentanyl	6	6	15-30	Dose	Supportive
JF-1-145	Clin Pharm	Reversal of Morphine	6	6	5	NLX/Dose	Supportive
24	Clin Pharm	Brain Binding in Man	8	8	1-15	NLX/Dose	Supportive
O5	Efficacy Study	Overdose	54	24/98	15-30	NLX/NEG	Substantial
JF-1-201	Efficacy Study	Overdose	42	26/66	15	NLX/NEG	Substantial
O2	Efficacy Study	Intrathecal Morphine	51	24	0.5-1.0	NLX/Dose	Substantial
15 US/CAN	Efficacy Study	Fentanyl Anesthesia	83	41	0.25-0.5	NLX/Dose	Substantial
13	Efficacy Study	Morphine Anesthesia	151	37	0.1-0.5	NLX/Dose	Substantial
O4A 1.1	Pilot Studies	Overdose	30	31	7-15	Dose	Supportive
O3	Efficacy Study	Fentanyl Anesthesia	34	14	0.25-0.5	Naloxone	Supportive
15UK	Efficacy Study	Fentanyl Anesthesia	27	18	0.25-0.5	Naloxone	Supportive
15 FR	Efficacy Study	Fentanyl Anesthesia	32	14	0.25-0.5	Naloxone	Supportive
JF-1-216	Pilot Studies	Narcotic Anesthesia	55	0	0.5-1.0	Dose	Supportive
12	Pilot Studies	Respiratory Depression	32	0	0.25-1.0	Dose	Supportive
O1	Pilot Studies	Intrathecal	24	0	0.5-1.0	Dose	Supportive
14	Clin Pharm	Pharmacodynamics	21	0	14-114	NLX/Dose	Supportive
16	Failed Design	Reversal of Sedation	36	12	1-15	PLC	Inconclusive
11	Failed Design	Conscious Sedation	154	53	15-50	PLC	Inconclusive
17	Failed Design	Conscious Sedation	22	8	15-30	PLC	Inconclusive
18	Failed Design	Conscious Sedation	151	52	15-50	NLX	Inconclusive
O4A 1.2	Failed Enrollment	Overdose	3	0	15	None	Inconclusive
O8	Failed Enrollment	Overdose	11	13/43	30	NLX	Inconclusive
O6A	Pilot Studies	Respiratory Depression	5	0	0.25	None	Inconclusive
O7	Pilot Studies	Epidural Pruritis	7	0	7	None	Inconclusive
JF-1-203	Failed Design	Conscious Sedation	48	?	15	NLX	No CRFs
Level III	Literature	Varied	495	?	7-15	?	No CRFs

It is unusual in that there were no "negative" studies, that is, studies of adequate power and design that failed to show efficacy. There were inconclusive studies, but these either failed enrollment or were of clearly inadequate design. Reviews of all studies are included in the package, but discussion will be limited to the trials which show substantial evidence of effectiveness or are strongly supportive.

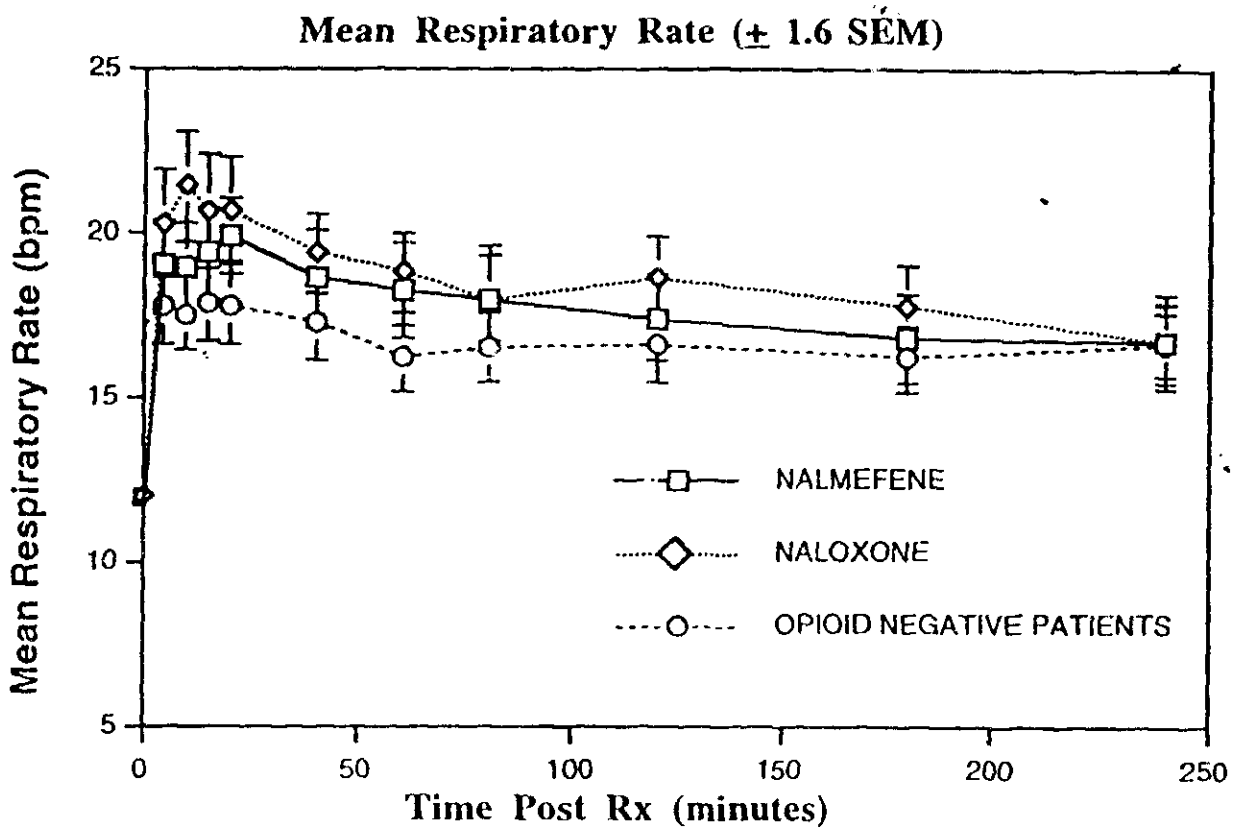
Demographics
Age, Gender, and Race were typical for the overdose and surgical populations, balanced for males and females, but light on the older patient and Orientals:

Age Group	N	Gender	N	Race	N
< 37	534	Male	705	Black	439
38-55	616	Female	1028	Caucasian	944
>56	32			Hispanic	304
				Oriental	9
				Other/Missing	16/21

Overdose
The major indication for opioid antagonists is the management of overdose in the Emergency Care setting. Most overdose cases are suffering from poly-drug ingestion, usually a mixture of alcohol, benzodiazepines, tricyclics, opioids, cocaine and "other". In the evaluation and analysis of such trials there has been an emerging standard. Patients are enrolled and treated "blind", but are analyzed in three groups after the toxicological test results come back. the three groups are the patients for whom the intended antidote might work given the test drug, the patients for whom the intended antidote might work given placebo or conventional treatment, and the patients for whom the treatment is expected not to work as a group.

In this case nalmefene is an opioid antagonist, and is not expected to have any effect in patients who do not have an opioid as part of their overdose. Thus the patients were divided into opioid (+) and opioid (-) on the basis of urine toxicology, and the opioid (-) patients were compared with the opioid (+) patients who were treated with either nalmefene or naloxone. This strategy is one way to evaluate the "non-specific" effects of the ER intervention (airway, fluids, glucose, oxygenation, stimulation) which have about as much effect regardless of the pharmacological cause of the intoxication. Better yet would be placebo, but in the presence of effective treatment (naloxone), use of placebo in this setting was rejected by most investigators and IRBs.

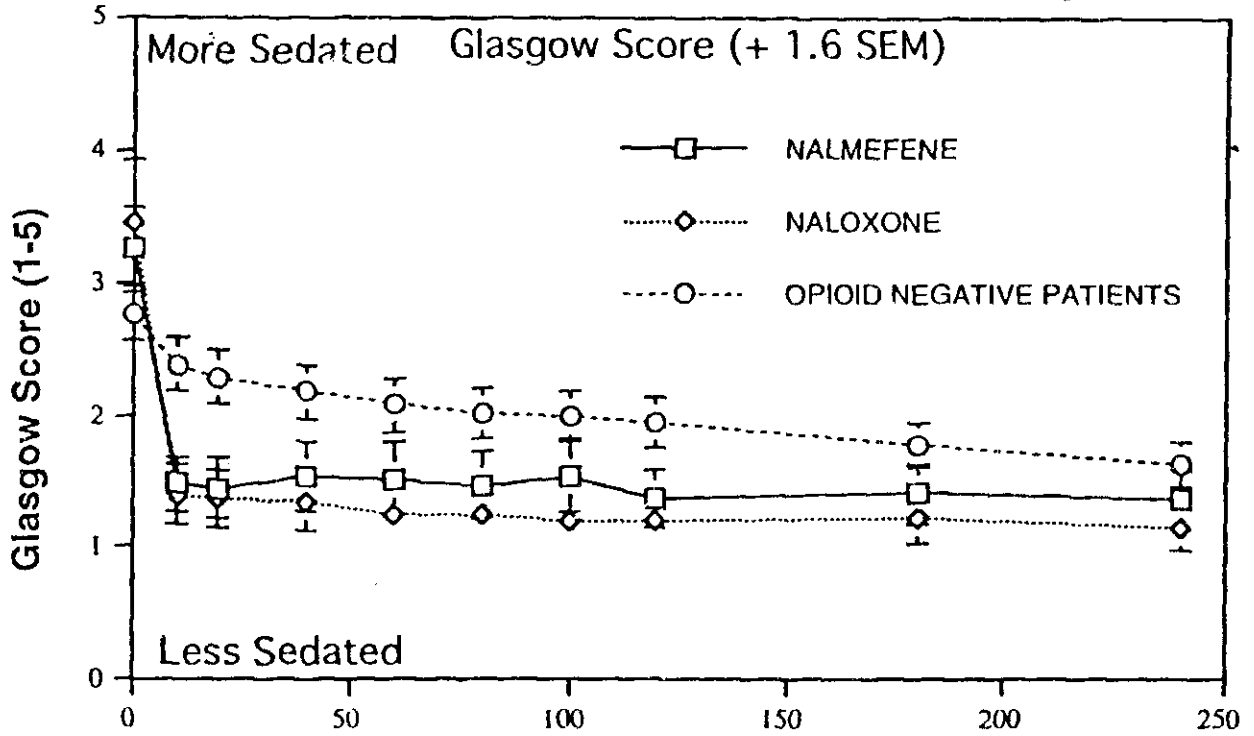
Study 05
Study 05 was a controlled trial of 176 patients, 77 opioid positive and 99 opioid negative, 162 males and 7 females, who were treated for overdose in the emergency room with increments of 1.0 mg or 2.0 mg of nalmefene or 2.0 mg of naloxone dosed to effect. All three treatments were effective in reversing the respiratory depression and other symptoms of opioid overdose. There was no difference between the two doses of nalmefene. The figure below displays the response of respiratory rate in opioid positive patients to both doses of nalmefene combined, naloxone, and in opioid negative patients to both nalmefene and naloxone.



Mean Respiratory Rate(± 1.6 SEM)											
	BASELINE	5	10	15	20	40	60	80	120	180	240
NALMEFENE											
N	54	54	54	54	54	51	49	50	48	44	44
MEAN	9.83	18.98	18.93	19.37	19.85	18.63	18.29	18.00	17.38	16.80	16.73
STD	6.54	6.06	6.27	6.41	5.35	6.69	6.28	5.74	5.63	5.50	5.95
SEM	0.89	0.83	0.85	0.87	0.73	0.94	0.90	0.81	0.81	0.83	0.90
1.6*SEM	1.42	1.32	1.37	1.39	1.16	1.50	1.44	1.30	1.30	1.33	1.43
NALOXONE											
N	24	24	24	23	23	22	22	23	23	23	21
MEAN	9.96	20.29	21.38	20.74	20.70	19.41	18.77	18.00	18.61	17.83	16.71
STD	7.20	4.96	5.12	5.19	4.65	3.50	3.53	4.72	3.92	3.61	3.45
SEM	1.47	1.01	1.05	1.08	0.97	0.75	0.75	0.98	0.82	0.75	0.75
1.6*SEM	2.35	1.62	1.67	1.73	1.55	1.19	1.20	1.57	1.31	1.21	1.21
OPIOID NEGATIVE											
N	97	98	98	96	97	93	91	92	92	93	91
MEAN	17.08	17.83	17.51	17.89	17.75	17.29	16.23	16.54	16.60	16.25	16.58
STD	7.18	7.28	6.94	7.51	7.25	6.90	6.06	6.41	6.55	6.13	5.64
SEM	0.73	0.74	0.70	0.77	0.74	0.72	0.64	0.67	0.68	0.64	0.59
1.6*SEM	1.17	1.18	1.12	1.23	1.18	1.15	1.02	1.07	1.09	1.02	0.95

The respiratory rate results show that opioid + patients had slowed respirations at baseline (9-10 breaths/min) and the opioid - patients had more normal respiratory rates (17 breaths/min). Both nalmefene and naloxone reversed the opioid-induced respiratory depression, but did not alter the respiratory rate of the opioid (-) group. Neither the naloxone or the nalmefene group suffered significant resedation.

Similar results were obtained using the Glasgow Coma Scale. Both nalmefene and naloxone promptly reversed the sedation associated with opioid (+) overdoses and not opioid (-) overdoses.



Glasgow Score (± 1.6 SEM)											
	TIME	0	10	20	40	60	80	100	120	180	240
NALMEFENE	N	54	54	54	51	51	50	50	49	46	44
	MEAN	3.26	1.49	1.45	1.53	1.52	1.47	1.55	1.37	1.41	1.36
	STD	1.48	0.94	1.02	1.19	1.19	1.10	1.20	0.96	0.99	0.92
	SEM	0.20	0.13	0.14	0.17	0.17	0.16	0.17	0.14	0.15	0.14
	1.6*SEM	0.32	0.20	0.22	0.27	0.27	0.25	0.27	0.22	0.23	0.22
NALOXONE	N	24	24	23	22	22	23	23	23	23	22
	MEAN	3.46	1.40	1.38	1.35	1.24	1.25	1.20	1.20	1.22	1.15
	STD	1.48	0.72	0.65	0.66	0.36	0.39	0.33	0.28	0.55	0.51
	SEM	0.30	0.15	0.14	0.14	0.08	0.08	0.07	0.06	0.11	0.11
	1.6*SEM	0.48	0.23	0.22	0.23	0.12	0.13	0.11	0.09	0.18	0.17
OPIOID	N	98	97	96	93	93	93	95	91	94	92
NEGATIVE	MEAN	2.77	2.38	2.28	2.18	2.08	2.02	1.99	1.94	1.78	1.64
	STD	1.29	1.25	1.25	1.18	1.20	1.20	1.16	1.10	1.04	1.02
	SEM	0.13	0.13	0.13	0.12	0.12	0.12	0.12	0.12	0.11	0.11
	1.6*SEM	0.21	0.20	0.20	0.20	0.20	0.20	0.19	0.18	0.17	0.17

In this measure, we see that the opioid + group is more sedated at baseline, and becomes more alert than the opioid - group after treatment. Again, both treatments were of similar efficacy.

Study JF-201

Study JF-201 compared nalmefene 1.0 mg to naloxone 2.0 mg dosed to effect in a pre-hospital setting for the treatment of overdose. The number of patients included was 134, 39 females and 95 males, 68 opioid positive and 66 opioid negative. Of the 134 patients, toxicology results were available for 97 patients. Of these 97, 69 tested positive for opioids in blood or urine and 28 did not. Among those who did test positive for opioids, the pupillary signs and respiratory status was as follows:

Pupillary Signs & Respiratory Status- Opioid (+) Patients				
	Dilated	Midpoint	Small	Pinpoint
Depressed Respirations	0	1	3	32
Not Depressed	1	1	8	23

Pupillary Signs & Respiratory Status- Opioid (-) Patients				
	Dilated	Midpoint	Small	Pinpoint
Depressed Respirations	0	1	0	4
Not Depressed	5	10	5	3

The interpretation is that in this patient cohort small and pinpoint pupils were predictive of opioid effects, as expected, and that the combination of pinpoint pupils and depressed respirations defined the population in which an opioid antagonist was most likely to be of use.

Statistically significant increases from baseline respiratory rate occurred at most or all three time points in all population groups. Improvements were particularly strong in the opioid positive patients with depressed respirations. Naloxone did statistically better than nalmefene at the 10 minute time point in both all opioid positive and the opioid positive patients with respiratory depression. The clinical importance of this difference is negligible, as the baselines were lower in the naloxone group and both nalmefene and naloxone restored respiratory rates to normal .

Respiratory Rate				
		Change from Baseline		
All Patients	Baseline	2 Minutes	10 Minutes	Last Observation
Nalmefene 1.0 mg	10.9	7.4*	6.7*	7.1*
Naloxone 2.0 mg	10.5	8.8*	9.7*	6.8*
Opioid Positive				
Nalmefene 1.0 mg	9.2	9.9*	8.3*	8.2*
Naloxone 2.0 mg	7.5	14.1*	13.0*†	9.2*
Opioid Negative				
Nalmefene 1.0 mg	13.4	4.1*	4.3*	5.7*
Naloxone 2.0 mg	12.6	4.9*	7.4*	5.1*

All Patients	Baseline	2 Minutes	10 Minutes	Last Observation
Opioid Positive & Respiratory Depression				
Nalmefene 1.0 mg	7.0	10.7*	9.5*	11.3*
Naloxone 2.0 mg	6.3	15.2*	13.6*†	12.0*
* Statistically significant at 0.05 significance level				
†Statistically significant between treatment difference				

Looking at the 50 patients with pinpoint pupils and depressed respirations:

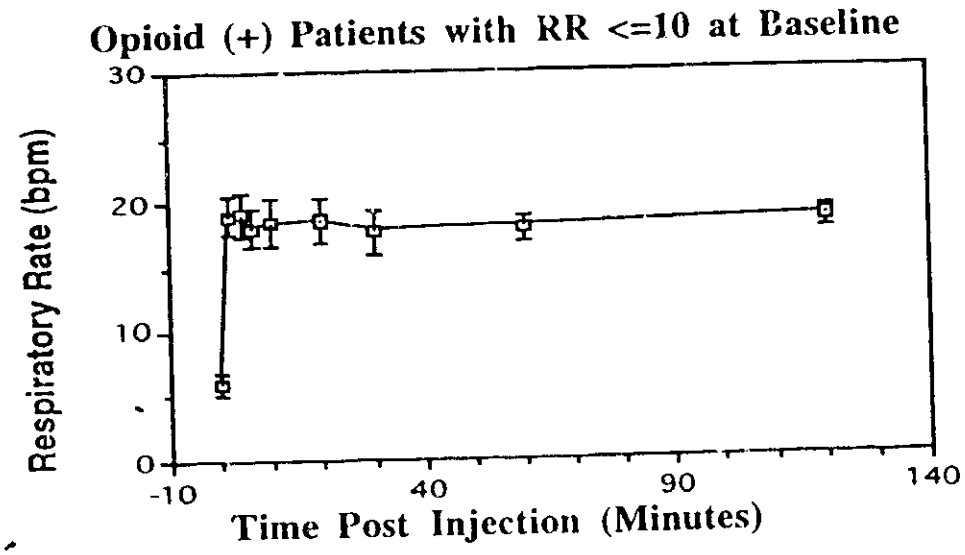
Mean Respiratory Rates-Opioid Subgroup							
	Base	2min	10min	30min	45min	60min	90min
N	50	50	50	50	47	48	48
Nalmefene 1 mg	4.84	15.2	16.1	18.4	18.7	20.1	18.2
Naloxone 2 mg	4.52	18.7	19.1	17.9	16.8	17.6	16.5

Respirations were returned to normal in the opioid affected subgroup by 2 minutes by both treatments, and there was a trend for nalmefene to hold higher rates longer than naloxone.

This study was less strong, since it had to be re-analyzed by the age[illegible] staff to select a clinically meaningful patient population. Nevertheless, in the clinically mean[illegible]l group (overdosed with pinpoint pupils and depressed respirations) that should get an opioi[illegible]agonist, nalmefene clearly restored respirations to normal as well or better then naloxone.

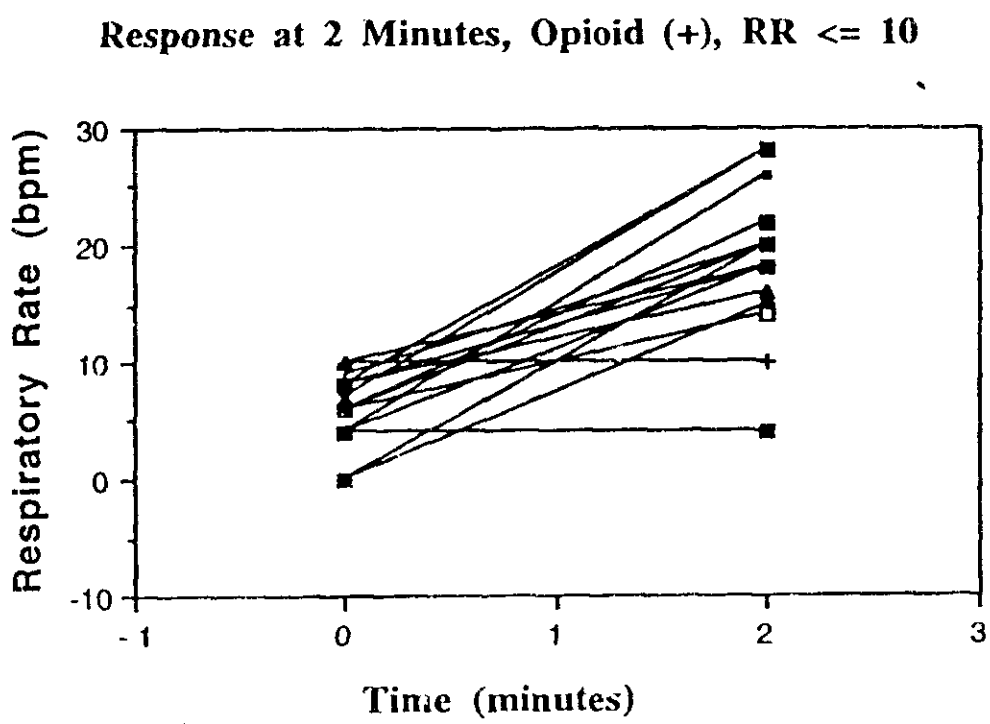
Study 04A1.1

Study 04A1.1 was an open-label study of incremental doses of nalmefene 0.5 mg and 1.0 mg dosed to effect in 61 male patients with overdose, 30 opioid positive, treated in the emergency room. The response of respiratory rate to nalmefene in the subgroup of 17 opioid positive overdose patients with respiratory rates less than 10 at baseline is shown in the figure below:



This pattern shows the immediate normalization of the respiratory rate within 1-2 minutes of injection, providing strong evidence of the efficacy of nalmefene in doses of 0.5-1.0 mg.

Individual responses are shown in the following wireplot:



Of the 17 patients in the subset, 15 of 17 responded with a restoration to normal respirations within two minutes with doses of 0.5-1.0 mg (the other two responded at 10 and 30 minutes respectively). The labeling should reflect that the efficacy of the drug is related to the probability that the patient has an opioid overdose. The corollary is that it appears without effect in patients that show no signs of opioid intoxication.

Pooled Analysis of Overdose Studies

The primary efficacy variable in all the overdose studies was respiratory rate. At baseline the investigators were inconsistent in their recording of the respiratory rate when patients required ventilatory assistance. Some recorded the patient's spontaneous rate, others recorded the rate at which the ventilator was set. Therefore, the respiratory rate for all patients who required ventilatory assistance was arbitrarily set at 4 for purposed of analysis throughout the studies.

The times of observation were fairly consistent to 20 minutes. Studies 04A1.1 and JF-201 had 2 minute time points. In studies 05 and 08 the first observation was at 5 minutes. After 20 minutes there was a high drop-out rate. In all the studies patients were classified as having an opioid positive overdose if any of the specimens obtained at baseline tested positive for any opioid.

The pooled efficacy analysis included all opioid positive patients from the four studies. Respiratory rate was the only variable analyzed. The other variables, pupil size, Glasgow Coma Scale, neurobehavioral assessment score, and sedation score have been analyzed in the individual study reports.

The table shows the respiratory rate response at 20 minutes for total doses administered in the first 19 minutes in all opioid positive patients.

Change of Respiratory Rate at 20 Minutes Opioid Positive Patients								
AGENT	TOTAL DOSE @ 20 MINUTES		N	MEAN	STD	BPM STD ERR	MIN	MAX
NAL	0.5 - 1.6MG	BASELINE	66	8.86	5.56	0.68	0	24
		20 MINUTES	65	18.20	5.23	0.65	4	30
		CHANGE	65	9.48	6.59	0.82	-12	24
NAL	> 1.6 MG	BASELINE	39	10.95	6.92	1.11	4	28
		20 MINUTES	40	19.08	5.45	0.86	4	36
		CHANGE	39	8.10	8.69	1.39	-10	30
NLX	> 1.6 MG	BASELINE	63	9.24	6.74	0.85	0	30
		20 MINUTES	62	20.21	5.20	0.66	8	40
		CHANGE	62	10.89	7.89	1.00	-10	28

The mean changes from baseline were not statistically significantly different among the groups (p=0.21).

Because not all opioid positive patients had respiratory depression at baseline, a second analysis was performed using only those patients whose baseline respiratory rates were 10 or less. The results at 20 minutes are presented in the next table.

Change of Respiratory Rate at 20 Minutes Opioid Positive Patients with Respiratory Depression								
AGENT	TOTAL DOSE @20 MINUTES		N	MEAN	STD	STD ERR	MIN	MAX
NAL	0.5 - 1.6MG	BASELINE	45	5.62	2.52	0.38	0	10
		20 MINUTES	45	17.53	4.79	0.71	4	28
		CHANGE	45	11.91	4.94	0.74	0	24
NAL	> 1.6 MG	BASELINE	19	4.84	1.92	0.44	4	10
		20 MINUTES	20	19.20	6.82	1.52	4	36
		CHANGE	19	14.32	6.87	1.58	0	30
NLX	> 1.6 MG	BASELINE	40	4.98	2.26	0.36	0	10
		20 MINUTES	39	19.62	3.93	0.63	12	28
		CHANGE	39	14.62	4.92	0.79	2	26

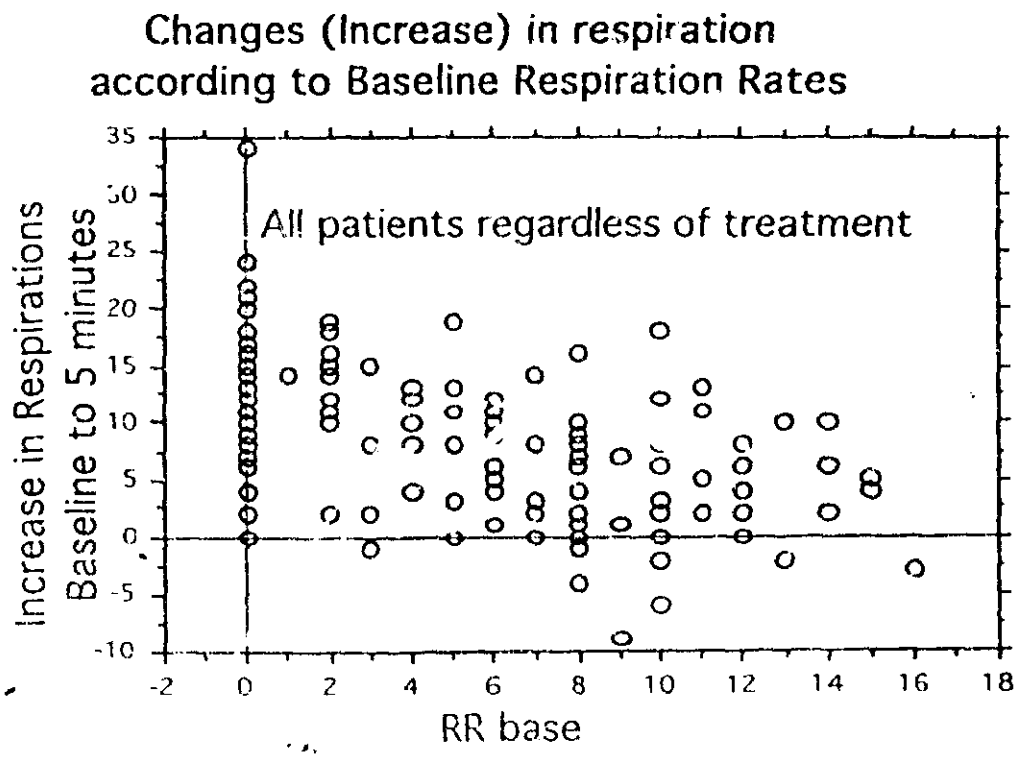
The mean changes from baseline were not statistically significantly different among the groups (p=0.76).

Conclusions on overdose.
Doses of 1-1.6 mg of nalmefene (15-22 mcg/kg) have been shown to be as effective in the emergency setting as the usual 2 mg dose of naloxone. In this setting, nalmefene trended toward holding its efficacy longer, but the trials were not designed to measure differences in resedation. The dose range was precisely established in this series of trials, but analysis of the grouped data suggests that doses above 1.6 mg provide no increase in efficacy, while examination of the individual trial reports suggests that doses below 0.5 mg show a trend toward reduced effectiveness. The sponsor recommends a titration approach in the range of 0.1 to 1 mg (titration steps 0.1, 0.5, 1.0 mg for a total dose of 1.6 mg). This seems very reasonable and is supported by the data.

Anesthesia
In the management of overdose, the drug had to have a robust effect, and it had to work in a setting where the upper limit on dosing was fairly robust. The research question in overdose management was, " Will a good big dose of this opioid antagonist wake this patient up?". In anesthesia, the need for control is paramount, and the question is more likely to be, " Will the drug reliably reverse opioid effects without the autonomic instability associated with hyperalgesia or precipitated withdrawal?

<u>Study 13</u>
Study 13 was a multicenter study of nalmefene at doses of 0.1, 0.25, 0.5, 1.0 mcg/kg and naloxone 1.0 mcg/kg in 188 patients, 41 males and 147 females, who received morphine as part of their anesthesia for abdominal or orthopedic surgical procedures.

Baseline was a significant covariate. The following figure plots the respiratory response to naloxone or nalmefene at 5 minutes versus the baseline respiratory rate.



As expected, those who had respiratory depression at baseline had the greatest response to the drug. The plot also shows that most of the response to either agent takes place in the first few minutes following the injection. Adjusting for baseline, the results at 5 minutes are given in the next two tables.

Improvement in Respiratory Rate at 5 Minutes After Adjusting for Differences in Baseline Respirations

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Treatment	4	449.694	112.412	3.207	0.0143
RR Baseline	1	1786.264	1786.264	50.961	0.0001
Residual	178	6239.199	35.052		

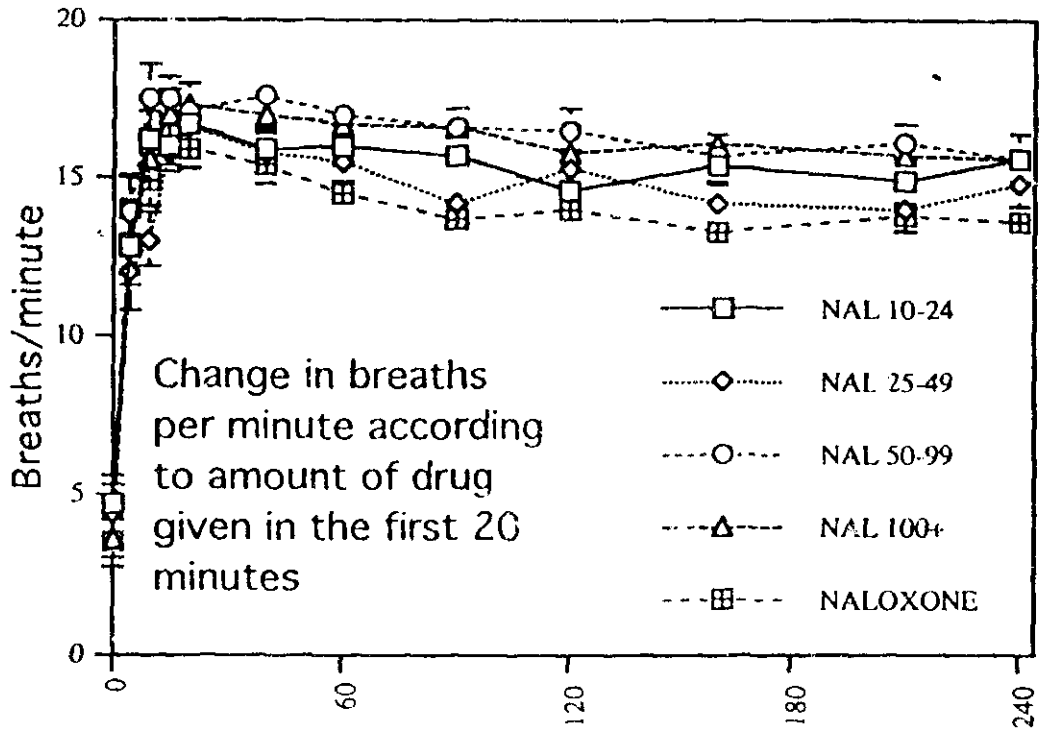
Increase in Respiratory Rate at 5 Minutes Adjusted to a Common Baseline Rate

	N	LS Mean	Std. Dev.	Std. Error
Nalmefene 0.10 mcg/kg	38	7.3	6.24	1.03
Nalmefene 0.25 mcg/kg	38	7.3	6.89	1.12
Nalmefene 0.50 mcg/kg	38	9.9	6.46	1.05
Nalmefene 1.0 mcg/kg	37	11.2	7.07	1.18
Naloxone 1.0 mcg/kg	37	10.2	6.79	1.15

The data show the observed improvement after the first dose of either nalmefene or naloxone across a ten-fold range of dosage (0.1-1.0 mg of nalmefene). The trial was sensitive (had a dose-response) and suggests that 1.00 mcg/kg naloxone is equivalent to .50-1.00 mcg/kg nalmefene in peak effect. Since one degree of improvement is expected just from the wearing off of anesthesia, the "no-effect" level cannot be determined precisely, but the minimum effective dose of nalmefene in this setting is likely to be in the 0.1-0.25 mcg/kg range.

Because this was an uncontrolled dose titration study, the original dose assignments were applicable only to the first evaluation (the 5 minute point described above). In order to evaluate duration of effect by dose, the total dose (mcg/kg) given in the first 19 minutes was calculated and sorted into the dose ranges 0.10-0.24, 0.25-0.49, 0.50-0.99, 1.00+ mcg/kg nalmefene or naloxone. The mean respiratory rates for the first four hours are shown in the graph below.

Respiratory Response to Treatment



Time after Baseline (minutes)

Respiratory Rate Over Time													
	Dose 20	RR	RR	RR	RR	RR	RR	RR	RR	RR	RR	RR	RR
		BASE	5	10	15	20	40	60	90	120	160	210	240
MEAN	NAL .10-.24	4.7	12.8	16.2	16.0	16.7	15.9	16.0	15.7	14.6	15.4	14.9	15.6
MEAN	NAL .25-.49	4.4	12.0	13.0	15.8	16.6	15.8	15.5	14.2	15.3	14.2	14.0	14.8
MEAN	NAL .50-.99	4.5	13.9	17.5	17.5	17.1	17.6	17.0	16.6	16.5	15.7	16.1	15.6
MEAN	NAL 1.00+	3.6	12.8	15.6	17.0	17.3	17.0	16.7	16.6	15.8	16.1	15.7	15.6
MEAN	NALOXONE	3.5	14.0	14.9	16.3	15.9	15.4	14.5	13.7	14.0	13.3	13.8	13.6
STDEV	NAL .10-.24	5.3	5.2	3.8	3.0	2.8	2.9	3.5	4.0	3.6	2.6	3.2	3.2
STDEV	NAL .25-.49	5.1	6.5	4..	3.2	2.6	3.4	3.2	2.9	3.7	3.3	3.7	3.8
STDEV	NAL .50-.99	4.8	6.5	5.7	3.5	3.2	3.3	2.9	3.5	3.9	3.6	3.5	2.8
STDEV	NAL 1.00+	4.5	6.4	4.7	5.2	4.9	4.5	3.8	3.6	3.5	3.4	2.9	3.3
STDEV	NALOXONE	4.4	6.3	4.3	3.6	3.7	4.0	3.6	3.3	3.3	3.4	2.8	3.4

COUNT	NAL .10-.24	30	29	27	27	30	28	29	29	27	27	27	28
COUNT	NAL 2: .49	29	29	28	29	27	29	28	29	29	27	28	28
COUNT	NAL .50-.99	35	35	30	33	35	35	35	35	35	33	34	34
COUNT	NAL 1.00+	57	56	54	51	55	56	57	57	57	54	53	54
COUNT	NALOXONE	37	35	32	35	36	37	37	37	36	37	36	36
SEM	NAL .10-.24	0.98	0.98	0.75	0.59	0.52	0.55	0.67	0.76	0.70	0.51	0.62	0.61
SEM	NAL .25-.49	0.96	1.22	0.84	0.60	0.51	0.64	0.62	0.54	0.60	0.64	0.72	0.73
SEM	NAL .50-.99	0.83	1.11	1.06	0.62	0.54	0.57	0.50	0.60	0.6[illegible]	0.63	0.61	0.50
SEM	NAL 1.00+	0.60	0.86	0.65	0.73	0.66	0.60	0.51	0.48	0.46	0.47	0.41	0.45
SEM	NALOXONE	0.73	1.08	0.78	0.62	0.62	0.67	0.59	0.56	0.56	0.56	0.47	0.58
Dose 20: dose (mcg/kg) given up to 19 minutes													

The naloxone group and all the nalmefene groups maintained normal respiratory rates to the end of the study. This suggests either that all the doses provided adequate reversal, or that the trial was insensitive to resedation. One argument that the trial was sensitive, however, was that patients in the lower dose groups of nalmefene were more likely to receive additional doses of opioid antagonist.

Study 15

Study 15 US/Can was a multicenter comparison of nalmefene 0.25 mcg/kg, nalmefene 0.5 mcg/kg and naloxone 0.5 mcg/kg in 124 patients, 33 male and 91 female, who received fentanyl as part of their anesthesia for abdominal surgery. In this study many patients had respiratory rates at baseline that were recorded as 0. However, based on their end-expiratory CO_2, these patients may or may not have had opioid-induced hypoventilation (they may have been hypocarbic from ventilation during surgery). The following table describes the patient population in terms of respiratory rate and end-expiratory CO_2.

Observed Frequencies for Baseline Ventilation x EE CO_2					
	Over-Ventilated EE CO_2<35mmHg	Physiologic EE $CO_2$35-55mmHg	Under-Ventilated EE CO_2>55mmHg	Missing	Totals
Apneic	17	22	11	18	68
< 8 BPM	2	11	3	25	41
> 8 BPM	1	7	1	7	16
Totals	20	40	15	50	125

Patients with low end-expiratory CO_2 may or may not have been experiencing an opioid effect and could have a substantial increase in respiratory rate after 5 minutes as their hypocarbia resolved even without opioid reversal. The following table shows the increase in respiratory rate (all patients.

Increase in Respiratory Rate (BPM) @ 5 Minutes (Adjusted to a Common Baseline Rate)				
	N	LS mean	Std. Dev.	Std. Error
Nalmefene 0.25 mcg/kg	39	7.37	6.77	1.08
Nalmefene 0.50 mcg/kg	41	9.12	7.04	1.10
Naloxone 0.50 mcg/kg	39	6.97	6.98	1.12

This table shows a 6-9 breaths per minute improvement after the first dose of either nalmefene or naloxone for the group as a whole. This analysis shows efficacy, but is degraded in resolution by the inclusion of individuals who are hypocarbic.

In order to evaluate the real effect of opioid reversal, individuals with respiratory rates at baseline less than 8 breaths/minute (a common cut-off in such studies) and an end-expiratory CO_2 more than 35 mmHg were selected. This subset clearly showed a full reversal effect, with the possibility of a lower rate of onset for nalmefene 0.25 than nalmefene 0.5 and naloxone 0.5.

Non-Hypocarbic Patients with Baseline Respiratory Rates < 8 BPM			
	N	5 Minutes (n, LS mean±SE)	10 Minutes (n, LS mean±SE)
Nalmefene 0.25 mcg/kg	12	12, 6.4±1.80	11, 13.3±2.15
Nalmefene 0.50 mcg/kg	15	14, 12.4±1.88	15, 14.8±0.76
Naloxone 0.50 mcg/kg	13	13, 12.8±1.80	13, 14.1±1.90
p-value		0.04	0.81

The subset of patients with respiratory rates at baseline less than 8 breaths/minute who had hypocarbia showed similar results in all three dose groups and probably did not have opioid-induced respiratory depression to begin with..

Hypocarbic Patients with Baseline Respiratory Rates < 8 BPM			
	N	5 Minutes (n, LS mean±SE)	10 Minutes (n, LS mean±SE)
Nalmefene 0.25 mcg/kg	6	5, 11.8±4.63	6, 13.1±3.07
Nalmefene 0.50 mcg/kg	6	6, 14.2±1.83	6, 16.1±1.62
Naloxone 0.50 mcg/kg	6	4, 12.0±3.26	6, 14.9±1.92
p-value		0.84	0.67

All patients maintained normal respirations for the duration of observation.

In this study (15 US/CAN) there was a significant difference in speed of onset for 0.25 mcg/kg as opposed to 0.50 mcg/kg, suggesting that 0.25 mcg/kg might be close to the minimum effective dose.

Study 02

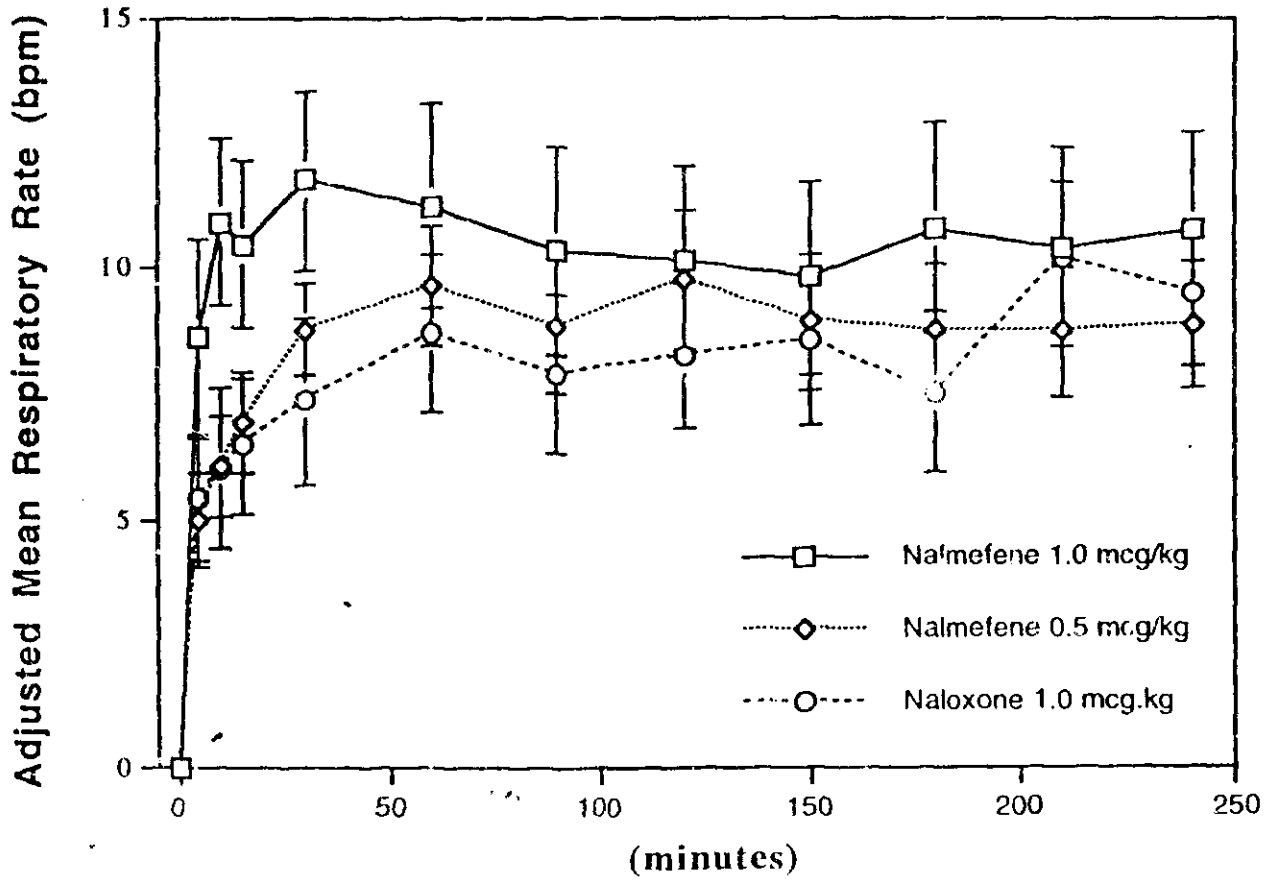
Study 02 studied the reversal of the systemic effects of 0.75 mg of morphine given intrathecally to 75 patients (32 females and 43 males) prior to major abdominal surgery.

The following tables and graphs show the results.

Summary of the Efficacy Parameters				
	Nalmefene 0.5 mcg/kg	Nalmefene 1.0 mcg/kg	Naloxone 1.0 mcg/kg	p value
Respiratory Rate				
Baseline	7.0	7.4	7.4	
Mean Change at 5 min	5.0*	8.6*	5.6*	
Mean Change at 15 min	6.9*	10.5*	6.7*	
Mean Change at 30 min	8.8*	11.8*	7.9*	0.027±
Mean Change at 240 min	8.9*	10.8*	9.5*	
PCO_2				
Baseline	52.5	56.7	53.1	
Mean Change at 5 min	-2.6	-5.8*	-3.7*	
Mean Change at 15 min	-4.5*	-10.7*	-3.3*	0.049±
Mean Change at 30 min	-6.0*	-11.3*	-1.2	0.003±
Mean Change at 240 min	-7.8*	-12.8*	-7.5*	
# of doses of study drug:				
median	2	2	2	
mean	2.77	2.32	3.71	0.027§

* $p<0.05$ based on Student t-test for change from baseline
± : Among treatments p-value by ANCOVA
§ : Among treatments p-value by proportional hazard regression

Adjusted Change in Respirations after Rx
(mean values +/- SEM)



Adjusted Change in Respirations after Rx									
	5 min	10 min	15 min	30 min	60 min	90 min	120 min	150 min	180 min
NALOXONE 1.00	5.6	6.5	6.7	7.9	8.7	7.8	8.3	8.6	7.5
NALMEFENE 0.50	5.0	6.0	6.9	8.8	9.6	8.8	9.8	8.9	8.8
NALMEFENE 1.00	8.6	10.9	10.5	11.8	11.2	10.3	10.2	9.8	10.8
"P" BETWEEN	0.06	0.001	0.009	0.01	0.01	0.02	0.008	0.03	0.001
NALOXONE 1.00*	a	a	a	a	a	a	a	a	a
NALMEFENE 0.50*	a	a	a	ab	a	a	a	a	ab
NALMEFENE 1.00*	a	b	b	b	a	a	a	a	b
*Turkey Test - Values associated with the same letter are comparable									

All three treatments improved respiratory rate for three hours. Both doses of nalmefene were more effective than naloxone 1.0 mcg/kg at 10 minutes and 15 minutes. Nalmefene 1.0 mcg/kg was more effective than naloxone 1.0 mcg/kg at 30 minutes and three hours as well.

Members of the nalmefene 1.0 mcg/kg group were also found to experience the most frequent and statistically significant reduction in the arterial PCO_2 from 5 through 240 minutes post-study drug administration compared to baseline.

Excluding patients who were terminated early (N=2), 8 of 24 (or 33%) and 11 of 23 (or 48%) patients in the nalmefene 0.5 and 1.0 mcg/kg groups, respectively reversed after 1 dose of study drug compared to only 5 of 18 (or 28%) in the naloxone 1.0 mcg/kg group. In the nalmefene 0.5 mcg/kg group 13 of 24 (or 54%) of patients reversed after 1 to 2 doses. In the nalmefene 1.0 mcg/kg group 17 of 23 (or 74%) of patients reversed under the same conditions. In the naloxone group, only 9 of 18 (or 50%) of patients reversed after 1 to 2 doses. Although these differences were not so robust as to establish a longer duration of action for nalmefene, they did suggest a trend in this direction.

Pooled Anatysis of Anesthesia Reversal

Studies 03, 12, 13, 15 US/Can, and 15 UK all used respiratory rate as the primary variable and had observations at comparable time points. A pooled analysis of the five studies was performed subdividing patients according to the total dose received within the first 19 minutes and examining the mean change in respiratory rate from baseline at 20 minutes. There were no significant differences among the dose groups of nalmefene or naloxone. All mean changes were significantly different from baseline.

Anesthesia Reversal									
Agent	Dose Received <19min	Observation	N	Mean	STD	SEM	Min	Max	"p"
NAL	0 - 0.25 mcg/kg	BASELINE	99	5.1	4.9	0.5	0	16	
		20 MINUTES	98	16.9	3.5	0.4	8	28	
		CHANGE	98	11.9	5.9	0.6	-2	28	<0.001
NAL	> 0.25 - 0.5 mcg/kg	BASELINE	119	4.2	5.2	0.5	0	20	
		20 MINUTES	119	17.0	4.0	0.4	6	28	
		CHANGE	119	12.8	6.1	0.6	-3	26	<0.001
NAL	> 0.5 - 1.0 mcg/kg	BASELINE	92	3.7	4.8	0.5	0	16	
		20 MINUTES	92	17.4	4.7	0.5	7	32	
		CHANGE	92	13.7	6.9	0.7	-4	32	<0.001
NAL	> 1.0 mcg/kg - 0.5 mg	BASELINE	16	3.3	4.3	1.1	0	13	
		20 MINUTES	15	17.0	5.1	1.3	6	26	
		CHANGE	15	13.5	6.5	1.7	0	24	<0.001
NLX	> 0.25 - 0.5 mcg/kg	BASELINE	32	3.8	4.7	0.8	0	14	
		20 MINUTES	32	15.8	4.4	0.8	8	24	
		CHANGE	32	12.5	6.2	1.1	2	24	<0.001
NLX	> 0.5 - 1.0 mcg/kg	BASELINE	48	3.8	4.8	0.7	0	14	
		20 MINUTES	48	16.8	4.8	0.7	10	35	
		CHANGE	48	13.0	6.4	0.9	0	35	<0.001
NLX	> 1.0 mcg/kg	BASELINE	30	2.7	3.5	0.6	0	10	
		20 MINUTES	29	16.0	3.8	0.7	10	28	
		CHANGE	29	13.3	4.9	0.9	4	28	<0.001

In conclusion, the pooled analysis of reversal of post operative opioid depression showed that by 20 minutes after administration all doses of nalmefene and naloxone were equally effective. This suggests that the design was not optimal to find the minimum dose (titration designs rarely are), but that the recommended doses, given as directed, are likely to be effective for all patients.

Effect on analgesia

No significant effect on analgesia was seen in the pivotal studies, although there were occasional patients that reported as worsening of analgesia from nalmefene. In one supportive study (15 UK), the investigator's reported a degradation of analgesia in the higher dose (0.50 mcg/kg) group, and stopped that dose level at that site. It is unclear how to interpret this, since other sites did not have this experience (see pain rating analyses in the pivotal trials).

The most conservative inference is that any long-lasting opioid antagonist would be expected to interfere with analgesia if given in excess, and nalmefene should be titrated to individual effect. The sponsor's recommendations for dosing are to individually titrate in three steps, 0.1, 0.25 & 0.50 mcg/kg, with a ceiling of 1.0 mcg/kg. This along with their suggested language in DOSAGE AND ADMINISTRATION ("larger doses may result in significant reversal of analgesia and increase in blood pressure," should convey the message.

Conclusion in Anesthesia

In terms of efficacy, (safety analysis to follow), doses of 0.25-0.5 mcg/kg appear to be the minimum effective dose of nalmefene, as contrasted with doses of 1-2 mcg/kg of naloxone. The data support that nalmefene is longer acting than naloxone, but the labeling must retain the precaution that re-sedation can occur, especially with long acting opioids.

The proposed titration to effect dosing, the dose steps, and the sponsor's warning about reversal of analgesia all appear appropriate.

Safety

Safety analysis for an NME breaks into two major parts; "things we expect from drugs of this class", and "things we had no idea might happen".

For drugs of this class, we expect, in order of frequency:
a. reversal of analgesia,
b. autonomic stimulation
c. precipitated withdrawal syndromes ("naloxone sensitivity")
d. re-sedation (inadequate duration of effect)
d. possible chemical hepatitis (naltrexone)
e. possible rare cases of pulmonary edema
f. (high doses) symptoms of endorphin reversal

For any new drug we expect:
a. Unexplained mortality
b. Unexplained morbidity
c. Unexplained discontinuations from study
d. Unusual events
e. Subjective symptoms from use

In the case of this drug in this indication, we have a single administration, relatively no chance for complications of chronic use, and precious little chance of dropouts (patients who are awake enough to drop out have achieved the full therapeutic effect). Because it is used in situations where serious risk of illness is prevalent (anesthesia and overdose), deaths are expected as are serious morbidity and significant adverse events.

Deaths

The table below provides a list of the 15 patient deaths in the whole population. Of the 15 deaths, ten occurred during or after the nalmefene administration period (incidence 0.9%), and five occurred during or after the naloxone administration period (incidence 1.4%). None of the 15 deaths was attributed to study drug administration. A discussion of the individual subject deaths by protocol is provided below:

Patient Deaths During Nalmefene Exposure		
Protocol	Patient	Cause
02	402	Cardiac Arrest, Septicemia
04A1.2	301	Acute GI bleed superimposed on chronic liver disease
04A1.2	303	Disseminated intravascular coagulation, multiple organ failure, cerebral contusion
05	101	Anoxic encephalopathy secondary to heroin and cocaine
05	169	Heroin Overdose
05	255	Metastatic Lung Cancer
05	401	Methadone Overdose
11	606	Rupture of thoracic aortic aneurysm
JF-1-201	102	Hypoxia, hypercarbia, hypoperfusion
JF-1-201	2104	Sepsis secondary to severe hepatorenal syndrome
During Naloxone Exposure		
02	406	Bilateral Adrenal Hemorrhage
05	156	Complications from multiple infections, hyperglycemia, seizures, and acidosis
05	361	Cardiac Arrest
05	954	Sepsis, hypovolemia, renal failure, and respiratory failure
JF-1-201	2089	Aspiration Pneumonia

Patient histories are presented by protocol.

PROTOCOL -02

Patient 402 was a 68-year-old white male with a history of COPD (the patient had been a heavy smoker for many years) who had a history of carcinoma of the lung with RUL resection and peptic ulcer disease in addition to hypertension, atrial fibrillation, and tachycardia.

The patient received one dose of nalmefene 1.0 mcg/kg at 1715 hours. During the course of surgery (abdominal aortic aneurysmectomy), the patient had significant desaturation managed with oxygen and tachycardia treated with labetalol.

The patient completed the study on March 1, 1990 and the physician judged the patient's response to the study drug to be good (as defined in the protocol). The patient died 17 days after study completion from clinical cardiac arrest, presumably secondary to known atherosclerosis, and clinical septicemia. The reviewer considers the relationship to study drug administration to be remote in this patient with COPD, status post lung resection and atherosclerotic disease to be remote.

Patient 406 was a 60-year-old white male with a history of gastritis and peptic ulcer disease, cholelithiasis, biliary colic, rectal bleeding, chronic pancreatitis (large pancreatic mass with invasion of the duodenum), hypertension, and alcoholism with accompanying delirium tremens. The patient had contracted malaria at the age of 22, and had prior surgeries for tonsillectomy, appendectomy, lipoma removal, iliac endarterectomy, lipectomy and thrombectomy. The patient had also been a smoker for many years and had been experiencing a non-productive cough for 5 months prior to study participation.

This patient underwent a successful cholecystectomy on June 14, 1990 and received 1 dose of naloxone 1.0 mcg/kg at 1300 hours. Fifteen minutes after receiving the study drug, the patient's respiration rate had increased from 8 to 13 breaths per minute, heart rate remained unchanged at 82 beats per minute, pH rose from 7.24 to 7.31, PCO_2 decreased from 59 to 44 mmHg, and PO_2 rose from 64 to 88 mmHg. Blood pressure increased from 108/51 (just prior to study drug administration) to 118/47. The investigator rated the patient's response to the study drug as excellent (per the protocol). The patient completed the study without problems, however, six days after study completion the patient died due to bilateral adrenal hemorrhage. The reviewer considers the relationship between the study drug (naloxone) and the adverse event to be remote.

PROTOCOL 04A1.2

Patient 301, a 52 year-old male, tested positive for methadone, diazepam, nordiazepam, and possibly ethchlorvynol. The patient presented with a history of hepatitis, hypertension, and IV drug abuse. At admission to the study the patient did not respond to verbal stimuli but did respond to pain. Nalmefene (0.5 mg) was administered and additional doses were given after 2, 4, 6 and 8 minutes. Since the patient did not respond clinically, 2.0 mg naloxone was administered 20 minutes after the first dose of nalmefene. The patient did not respond to this therapy. Two hours later the patient received intravenous morphine prior to intubation. Approximately 3 hours after the start of nalmefene therapy the patient experienced gastrointestinal bleeding and was treated with 50.0 mg cimetidine and one unit of packed red blood cells. The patient was hospitalized and died one day later.

Death was due to an acute gastrointestinal bleed superimposed on chronic liver disease. In the opinion of the investigator, death was not related to the use of nalmefene. The patient experienced changes in glucose, creatinine, SGOT, alkaline phosphatase, and WBC's which exceeded the maximum shift criteria. These changes, in the opinion of the investigator, were related to the patient's chronic liver disease, gastrointestinal bleeding and polydrug overdose, and were not related to the use of nalmefene. The reviewer agrees.

Patient 303, a 34 year-old obtunded male, with a history of alcohol and IV drug addiction, tested positive for alcohol, opiates and cocaine (lab results for this patient were obtained by phone, as source documentation was not contained in case report form). Although the patient exhibited one of the exclusion criteria (i.e. trauma to the head), in the opinion of the investigator, the patient's depressed level of consciousness was due to his overdose.

The patient was treated with 1.0 mg nalmefene and 5 minutes later was rated as having a complete response. Over the following 8 hours there was no deterioration of his response to therapy and he was discharged. The patient's pre-study laboratory data indicated chronic liver disease with either alcoholic or viral hepatitis (SGOT: 31608 IU/L; SGPT: 6750 IU/L; alkaline phosphatase: 534 IU/L), kidney dysfunction (creatinine: 3.2 mg/dl) and sepsis (WBC: 27.2 $10^3/mm^3$).

Five days after completing the study the patient was hospitalized and died. The cause of death was given as disseminated intravascular coagulation, multiple organ failure and cerebral contusion. In the opinion of the investigator, the patient's disseminated intravascular coagulation and organ failure were related to his alcoholism and IV drug abuse, and were not related to the use of nalmefene. The reviewer agrees

PROTOCOL 05

No patients died during the study enrollment period, although there were seven deaths after discharge from the study, three in the nalmefene 1.0 mg group, one in the nalmefene 2.0 mg group and three in the naloxone 2.0 mg group. In no case did the investigator feel that any of these deaths were related to study drug administration. Summaries of these patients are included below.

Patient Deaths in Protocol 05

Patient	Random Code	Study Drug	Enrollment	Opioid	Death	Cause of Death

P - positive | N - negative

Patient 101 was a 37 year old white male with a history of several suicide attempts while using intravenous drugs and depression, who was brought to the emergency room (August 1, 1990) after being found in his car. In the emergency room he was found to have a slow idioventricular rhythm with no pulse or pressure (i.e. electromechanical dissociation). He received 4 doses of study medication (nalmefene 1.0 mg) with no response. He then received a rescue dose of naloxone 2.0 mg. He was resuscitated, but his mental status never improved. His urine toxicology screen was positive for opiates and cocaine. The principle admitting diagnosis was anoxic encephalopathy with other diagnoses being heroine and cocaine overdose and aspiration pneumonia. On the fourth day of admission, his pupils became fixed and dilated. EEG's performed showed no cortical activity, only minimal brainstem responses. The family had the patient removed from ventilatory support on August 8, 1990 and the patient expired on August 9, 1990. Relationship to study drug remote.

Patient 156 was a 35 year old black male with a history of hepatitis, diabetes, rhabdomyolysis, seizures, endocarditis, hypertension and alcohol, heroin and cocaine addiction. He received on a chronic basis insulin -NPH, insulin regular and phenytoin. He was presented to the emergency room on 1/6/91 unresponsive. The patient received 4 doses of study medication (naloxone 2.0 mg) with no response. He subsequently received two 2.0 mg doses of naloxone as rescue medication. The patient had previously been admitted to the protocol as patient #153. He died on January 17, 1991 of complications from multiple infections, hyperglycemia, seizures and acidosis. In the investigators opinion no morbidity or mortality appears to be related to study drug. The reviewer agrees.

Patient 169 was a 53 year old black male with a history of seizures, hypertension and heroin, cocaine and alcohol abuse. He was admitted on March 14, 1991 unresponsive with pinpoint pupils, hypertension and tachycardia. No urine sample was taken for toxicologic screen, but heroin overdose was suspected. After one dose of study medication (nalmefene 2.0 mg) the patient was responsive. Within one hour of study enrollment, the patient signed out against medical advice. On March 20, the patient again overdosed, the paramedics found him apneic and he subsequently died. In the investigator's opinion, the death was not related to study drug. The reviewer agrees.

Patient 225 was a 72 year old white male with a history of metastatic lung carcinoma with diffuse liver metastasis. The patient was being treated with long term morphine sulfate for the control of severe pain and had been seen by his primary care oncologist who considered him terminal on the day prior to admission. At that time, because of severe pain, the dose of MS Contin had been increased from 15 mg p.o. q 12 h to 30 mg p.o q 12 h. The patient was presented to the Emergency Department of Jersey Shore Medical Center on January 22, 1991, after being found in his home obtunded with shallow agonal respirations by his son.

The patient received 3 doses of study medication (nalmefene 1.0 mg). The patient underwent withdrawal after receiving the first dose of study medication. He was given morphine sulfate to control the withdrawal and was subsequently given two additional doses of study medication. The patient became more alert and his respiratory rate increased, however, he began to complain of severe pain. Morphine sulfate was administered to control the pain. The consent form to participate in the nalmefene study was signed by the patient's daughter. The patient's daughter also signed a DNR order. The patient was diagnosed as being in a terminal state due to his cancer and was admitted to the hospital for comfort measures only. He was placed on a morphine drip to control his pain and expired at 19:00 hours on January 21, 1991. There was no evidence that treatment with nalmefene made his subsequent management more difficult once he was placed on morphine to control his pain and his withdrawal symptoms. The cause of death was listed as metastatic lung carcinoma. An autopsy was not performed. The reviewer considers the death to due to the primary condition.

Patient 361 was a 81 year old Caucasian male with a history of anemia, myocardial infarction and alcohol abuse. He was brought to the emergency room (3/8/91) in a lethargic state. His blood toxicology test was positive for ethanol 153 mg/dL. He received one dose of study medication (naloxone 2.0 mg). His blood pressure from 117/57 at baseline to 142/66 at 5 minutes and a maximum of 171/89 at 30 minutes. His heart rate remained between 60 and 82 bpm. His ECG showed a RBBB, first degree AV Block and questionable ST-T wave elevations in the lateral leads. He was admitted for altered mental status. On March 12, 1991 the patient expired after cardiac arrest. An autopsy revealed aspiration pneumonia and myocardial ischemia. The death was considered unrelated to study medication. The reviewer agrees.

Patient 401 was a 42 year old hispanic male with a history of hypertension, ethanol and intravenous drug abuse who was enrolled into the study and received one dose of study drug (nalmefene 1.0 mg). His drugs of abuse screen was positive for benzodiazepine, nicotine; caffeine, methadone, chlordiazepoxide and ibuprofen. He was admitted to the hospital for a methadone overdose on September 1, 1991. On September 4, he left the hospital against medical advice. On September 5 the patient was dead on arrival at the hospital. Cause of death was a methadone overdose.

Patient 954 was a 58 year old black male with a history of fatty liver, schizophrenia and seizure disorder presented obtunded on April 24, 1991 and entered into the protocol. His laboratory values indicated the presence of dehydration, renal failure and liver failure with evidence of rhabdomyolysis. He received two doses of study medication (naloxone 2.0 mg). His drugs of abuse screen found phenobarbital and phenytoin, both ongoing medications. On April 25, he developed hypotension, acidosis, disseminated intravascular coagulation, and worsened hepato-renal failure. He died 31 hours after study entry. In the investigator's opinion the cause of death was from complications of his myoglobinuria and hepatic failure and was not related to study drug.

PROTOCOL 11

Patient 606 was a 73-year-old white male. At the time of colonoscopy the patient was found to have a cecal mass and was transferred to the surgical service. A right colectomy was performed on March 13, 1991. The patient did fairly well postoperatively with a routine course until the day prior to discharge. An episode of arterial fibrillation with rapid ventricular response had occurred. This was managed effectively with digitalization. The cardiologist thought the patient could be discharged from the hospital to go home.

On the day of discharge, March 20, 1991, a hospital nurse found the patient lying in blood in the bathroom with no breathing and no pulse. CPR was initiated. The patient did not survive. An autopsy was performed and showed that the cause of death was due to rupture of a thoracic aortic aneurysm into his esophagus. The investigator believed that the patient's death had nothing to do with his participation in this study. The reviewer agrees.

PROTOCOL 1-201

Three patients died during the study. In all three cases, death occurred after the study observation period. Two of the patients were in the nalmefene group, the remaining patient was in the naloxone group. In no case did the investigator feel that the deaths were related to the study drug. Below are summaries of the patient data for each of the three deaths.

Patient 102 (opioid +): This patient was a 73 year old male Caucasian who was entered into the study on 10/5/86 for a suspected propoxyphene overdose. He had been severely depressed and had expressed a wish to die for several months, refusing to eat or drink. The patient was comatose upon entry into the emergency room. A blood screen showed a sub therapeutic amount of propoxyphene. The patient was given nalmefene as a study drug; no naloxone rescue was given. His level of consciousness and vital signs remained unchanged after administration of study drug (nalmefene). The BUN, alkaline phosphatase, and SGOT levels were abnormally high at post-study; the RBC, hemoglobin, and hematocrit were unusually low at post-study. The patient was transferred to Good Samaritan Hospital and died the next day. The final diagnosis was hypoxia, hypercarbia, and hypoperfusion. The reviewer considers the information to be inadequate to determine the cause of death and the relationship to the study drug is unknown.

Patient 2089 (opioid -): This patient was a 66 year old Caucasian female with a non-resectable esophageal carcinoma who was entered into the study on 8/31/86. At the time of entry, she was comatose because of a seconal overdose. She was in the naloxone treatment group. Thirty minutes after study drug was administered, the patient was given a 4.0 mg dose of naloxone as rescue, which did not result in an increase in respiratory rate (which was 20 bpm at time of rescue). The patient's medical history during the 48 hours prior to study entry included secobarbital, Percocet, prochlorperazine, Darvocet, ranitidine, hydroxyzine hydrochloride, triazolam, metoclopramide hydrochloride, and medroxyprogesterone. The patient died one month later of aspiration pneumonia. In the opinion of the investigator, the death was not related to study drug administration. The reviewer agrees.

Patient 2104 (opioid -): This patient was a 60 year old Caucasian man with a history of chronic ethanol intake and was admitted on 10/13/86 in coma that was secondary to ethanol and Valium intoxication. The patient was given nalmefene. Originally, the patient could not be intubated and showed no response to nalmefene. No naloxone rescue was given. The glucose, WBCs, and BUN were abnormally high at post-study; the bicarbonate, RBCs, hemoglobin, and hematocrit were abnormally low at post-study. The patient died on 11/2/86 of sepsis that was secondary to complications of severe hepatorenal syndrome. The investigator did not consider the death to be related to study drug administration. The reviewer considers a relationship remote, but possible.

Conclusion regarding deaths

The rate of death for nalmefene was equivalent to the rate of death for the comparator (naloxone) and appropriate for the severity of the patient's conditions. The deaths all appear to be related to the patient's underlying disorders. Of particular note are the patients who are successfully treated for overdose who presented within days with a second, fatal, overdose. This appears unrelated to nalmefene, but will be referred to the NIDA for further evaluation.

Discontinuations

As previously stated, discontinuations were not expected to be of great value in this NDA, although if the overdose patients had severe precipitated withdrawal, or the post-operative patients had severe pain, withdrawal would be expected.

There were a total of 8 patients who discontinued during the nalmefene trials due to adverse events. Of the 8 total subjects, 7 subjects (incidence 0.6 %) discontinued during the nalmefene administration period, 1 subject (incidence 0.25 %) discontinued during the naloxone administration period, and none of the subjects discontinued during placebo administration.

Patient Discontinuations Due to Adverse Events During nalmefene Exposure		
Protocol	Patient #	Adverse Event

Except for the hemorrhage and the circulatory collapse, the five adverse events resulting in discontinuation in the nalmefene group and the one in the naloxone group were considered possibly related to study drug.

PROTOCOL 01

Patient 10 was returned to surgery 4 hours after nalmefene administration, due to hypotension. The patient was found to have a severe hemorrhage secondary to the aortic reconstructive surgery.

PROTOCOL 02

Patient 116 was a 72-year-old white male with a history of hepatitis, chronically increased liver function tests, cholelithiasis, left nephrectomy for tumor, hypertension, coronary arteriosclerotic heart disease, coronary artery bypass graft, myocardial infarction, and transurethral resection of the prostate. An electrocardiogram performed 48 hours prior to study participation showed sinus bradycardia and possible left ventricular hypertrophy. This patient was taking metoprolol (for an unknown duration) for the treatment of hypertension, and also received 0.5 mg midazolam (as an anxiolytic) and 25 mcg of fentanyl (for pain) prior to surgery.

The patient received nalmefene 0.5 mcg/kg at 1400 hours. Approximately 15 minutes later, the patient began shaking and developed a fever. The shaking was treated with diphenhydramine, and the fever with acetaminophen. Both events resolved within 2 hours. The patient was terminated from the study at 1430 hours, 30 minutes after study drug administration. Both the shaking and fever were judged to be mild by the investigator. The relationship of both adverse events to the administration of study drug was considered possible. The patient's response to the study drug was considered marginal by the investigator. The reviewer considers it possible.

Patient 114 was a 60-year-old white male with a history of hepatitis (type unknown), alcoholism and transitional cell carcinoma of the bladder. The patient was scheduled for removal of the prostate, and had prior surgeries for orbital fracture, sinus surgery, cartilage repair to the left knee, vasectomy, and appendectomy. This patient also experienced arthritis in his right hand joints, and a rash on his right elbow (type unknown, which was present pre and post operatively). The patient's medication regimen included Septa, neomycin, erythromycin, potassium chloride, magnesium citrate, thiamine and ascorbic acid.

One dose of nalmefene 1.0 mcg/kg was administered to the patient at 1445 hours. Five minutes after study drug administration blood pressure changed from 181/76 to 160/90, heart rate increased from 94 to 136 beats per minute, respiration rate increased from 8 to 26 breaths per minute, while PCO_2 and PO_2 both decreased from 75 to 60 and 142 to 104 mmHg, respectively. pH was inadvertently not measured at baseline, but was recorded at 7.15 at the five minutes post study drug time point. According to the investigator, several minutes after study drug administration the patient began shaking vigorously all over. Then his temperature rose. Blood transfusion was stopped. Blood, urine and packed RBC's were cultured with no organism growth. The patient was actively cooled. The fever and shaking resolved within 1 1/2 - 2 hours without apparent problems. The patient received "rescue" narcotic antagonist therapy (0.2 mg naloxone) by infusion, but the time of rescue is not known. The nalmefene was analyzed and no pyrogens were found.

Postoperative concomitant medications included mezlocillin, potassium chloride, esmolol, sodium bicarbonate, dopamine, labetalol, glycopyrrolate, and neostigmine.

Both the shaking and fever were judged by the investigator to be moderate in severity and possibly related to study drug administration. Both adverse events resolved with treatment which included discontinuing the blood transfusion. The patient's response to study drug was considered poor, and he was discontinued from the study at 1515 hours, 30 minutes post study drug administration. The reviewer considers the clinical picture more typical of a blood transfusion reaction, but rates it possible.

Patient 120 was a 64-year-old white male with a history of respiratory problems including increased anterior-posterior diameter and decreased breath sounds, bilateral leg claudication, a fractured tibia, an enlarged prostate, peripheral vascular disease, coronary artery disease, carotid endarterectomy and alcohol abuse.

Surgery was completed at 1220. The patient received one dose of naloxone 1.0 mcg/kg at 1505 hours. At that time blood pressure was 150/62, heart rate was 117, respiration rate was 16, pH was 7.27 and PCO_2 and PO_2 were 49 and 122 mmHg respectively. Fifteen minutes after receiving naloxone the patient's blood pressure was 150/80, heart rate remained unchanged at 117, respiration rate actually decreased from 16 to 14, pH remained unchanged at 7.27, PCO_2 was 46 mmHg and PO_2 was 126 mmHg. The investigator rated the patient's response to study drug as poor. Subsequently, this patient developed shaking and fever. These adverse events were rated as moderate in severity and thought to be possibly related to the administration of study drug per the investigator. The patient was terminated from the study at 1530 hours, 25 minutes after study drug administration. At 1530, the patient received 25 mg of meperidine and, between 1400 and 0810 also received 3 mg of morphine. The adverse events resolved within 2 hours of onset. Reviewer considers relationship possible, but unproven, as post-operative rigors more likely.

PROTOCOL 06A

Patient 105, during the first hour after the first dose of nalmefene, had an increase in systolic and diastolic blood pressure. Two additional doses were administered at 65 and 90 minutes after the first dose. Thirty minutes after this last dose the patient's blood pressure was 202/80 mmHg and 20 minutes later 568 mcg intravenous sodium nitroprusside was administered. The patient immediately experienced circulatory collapse with sinus arrest. The patient was resuscitated easily and did well. He was discontinued from the study. In the opinion of the investigator the circulatory collapse was not related to treatment with nalmefene. In the opinion of the reviewer the hypertension might have been related, but the collapse was almost certainly a reaction to the anti-hypertensive.

PROTOCOL 11

Patient 424 (nalmefene 1.0 mg) was a 55-year-old Caucasian male who was withdrawn because he developed nausea which was considered possibly study drug related. It was noted that the patient had a pre-study history of nausea. This was observed when the patient was being prepared for colonoscopy. Reviewer considers relationship possible.

PROTOCOL 13

Patient 331 (0.50 mcg/kg nalmefene) was a 50 year old hispanic female with a past history of hypothyroidism, cholecystectomy removal of a right axillary mass, a diagnosis of endometrial carcinoma, on levothyroxine, premarin and medroxyprogesterone (antineoplastic). Prestudy her ECG showed bradycardia, her vital signs were HR 78 bpm, BP 110/60 mmHg, her hemoglobin was 11.3 g/dl and her Hct 33%. She underwent a radical hysterectomy with pelvic and periaortic node dissection. She received 30 mg of morphine intraoperatively, nitrous oxide 50% and isoflurane 0.3% and required treatment with labetalol and hydralazine for hypertension. Post-operatively she was reversed with two doses of nalmefene 0.50 mcg/kg and her respiratory rate increased from 0 to 13 bpm.

Within 10-15 minutes of the second dose she was given hydromorphone 0.5 mg for pain. Fifty minutes later she was treated with 1.0 mg of hydromorphone. The nalmefene plasma levels at 20, 60, and 120 minutes were 0.213, 0.099 and 0.092 ng/mL, respectively. Three hours later she complained of mild to moderate pain. Her respiratory rate was 9 bpm. Ten minutes later she was given another dose of nalmefene 0.50 mcg/kg. Her abdominal pain increased so no further study drug was given. She continued O_2 by mask for decreased O_2 saturation. She was observed for 24 hours and was treated subsequently with hydromorphone. She did not receive naloxone, but was considered by the investigator to have discontinued due to an adverse reaction which was possibly study drug related. Because the patient was observed for 24 hours and had no interventions that would invalidate the observations, her data were included in both the safety and efficacy analysis. Her response was considered marginal. The reviewer considers this a case of reversal of analgesia.

PROTOCOL 18

Patient 302 (nalmefene 1.0 mg) was a 35 year old white female with a history of cervical myospastic syndrome, diverticulosis, hematochezia and endometriosis status post TAH with BSO and appendectomy. At the time of study entry, this patient noted depression and migraines. She had a problem with pain medications in the past for which she underwent rehabilitation approximately three years ago. In addition, she was allergic to penicillin, Compazine and Darvocet.

Prior to study entry, this patient was taking amitriptyline (100 mg, P.O) for sleep/depression, Fiorinal #3 (3 Tabs per week, P.O.) for headaches, Dicyclomine (60 mg, P.O.) for colonic spasms and Carisoprodol (1400 mg, P.O.) for muscle relaxation. Her pre-study physical exam was within normal limits, 37.1 C, HR 83 bpm, respiration rate 20 bpm and BP 117/76 mmHg; however, her ECG showed evidence of sinus arrhythmia.

She underwent colonoscopy for a diagnosis of rectal bleeding with a family history of colon polyps and a non-diagnostic barium enema. She received 5.0 mg midazolam and 100 mg meperidine administered as per protocol to induce a state of conscious sedation. She was treated with 1.0 mg nalmefene post colonoscope removal. Upon administration of approximately 1.5 mL of study drug, the patient complained of feeling moderately flushed, hot and agitated, with a sudden cold sensation which later abated. The investigator immediately interrupted study drug therapy at the onset of these adverse events, which lasted approximately two and a half hours. Due to the acute nature of these adverse events, they were considered to be probably drug related. This patient was withdrawn from the study due to her inability to cooperate with the psychomotor evaluation and the nature of her reaction.

After further questioning, however, patient 302 admitted to frequent use of narcotic-containing compounds, including Fiorinal with codeine and other opioids, thus indicating that her adverse reaction to the study medication was due to precipitated narcotic withdrawal induced by intravenous nalmefene. The reviewer agrees.

Conclusions about Discontinuations
As is usual, the patients who discontinued had an increased frequency of attributable adverse events, most of which were properly classified by the investigator. The hyperalgesia, the precipitated withdrawal, and the chills/fever may need to be mentioned in precautions.

Serious Adverse Reactions
Reversal of opioids can result in reversal of analgesia, autonomic instability, precipitated withdrawal reactions, and rarely with pulmonary edema Patients with these potentially treatment related adverse events are discussed below, whether or not the investigator considered the event an adverse event or an intercurrent illness.

Because of the structural similarity of nalmefene to naltrexone, an opioid antagonist that has been associated with liver damage, patients were also observed for liver injury, coded as hepatitis or hepatic failure. Brief case histories follow the tables.

Withdrawal Cluster 4 - Seizure or Delirium

Nalmefene N=1127			Naloxone N=369		
Dose	Protocol	Patient #	Dose	Protocol	Patient #

Epileptiform seizures are uncommon in pure mu agonist withdrawal, or following opioid antagonist administration, but were clustered with delirium as the rigors of opioid withdrawal are frequently mis-classified this way. Seizures and delirium were limited to the overdose studies, occurred with equal frequency in both groups, (The frequency of seizure or delirium in the nalmefene group was 0.62% and in the naloxone group 1.08%), and were probably not related to reversal.

Hepatitis or Hepatic Injury					
Nalmefene N=1127			Naloxone N=369		
Dose	Protocol	Patient #	Dose	Protocol	Patient #

* Patient died. A summary is included in the section on DEATHS.

The frequency of hepatic injury or hepatitis was 0.27% in the nalmefene group and 0.27% in the naloxone group. The hepatic injuries were not considered treatment related in either group.

Pulmonary Edema					
Nalmefene N=1127			Naloxone N=369		
Dose	Protocol	Patient #	Dose	Protocol	Patient #

The frequency of pulmonary edema was 0.27% in the nalmefene group and 0.81% in the naloxone group. The episodes of pulmonary edema were more likely to be attributed to fluid overload than to opioid reversal in both groups.

Cardiac Arrhythmias or Ischemia							
Nalmefene N = 1127				Naloxone N = 369			
Dose	Protocol	Patient Number	Diagnosis	Dose	Protocol	Patient Number	Diagnosis

Dose	Protocol	Patient Number	Diagnosis	Dose	Protocol	Patient Number	Diagnosis
			CG				

The incidence of cardiac arrhythmias or ischemia was 0.16% in the nalmefene group and 0.14% in the naloxone group.

PROTOCOL 02

Patient 307 (nalmefene 0.5 mcg/kg), with a history of hypertension and pre-surgery premature ventricular contractions, experienced premature ventricular contractions which needed treatment for one hour after study drug administration. At time of discharge, she had experienced isolated unifocal premature ventricular contractions. Relationship?

PROTOCOL 04A1.1

Patient 410, a 57-year-old black male (opioid negative) with a previous history of cervical myelopathy, hypertension, and alcohol abuse, was admitted to the emergency department on April 20, 1990. The patient was in an obtunded state with questionable soft focal neurologic findings such as facial droop. Hypotension (systolic Blood Pressure of 60-80 mm Hg), hypothermia (temperature, 95°F), and dehydration were diagnosed at the initial assessment. Because of suspected opiate overdose, the patient was administered 10 doses of nalmefene, 1.0 mg per dose. Dopamine was administered to treat the hypotension, which subsequently resolved after 1 hour. The patient was noted to develop left-sided tremulousness during the nalmefene and dopamine administrations. It was thought that the patient might have been having a seizure, and intravenous phenytoin and Valium were administered. The patient subsequently became apneic for about 2 hours and required intubation.

The patient recovered fully and was extubated. A CAT scan was negative. It was thought that the underlying stenosis of the vertebral artery was exacerbated by the hypotension, thereby causing the tremor on the left side. Another possibility for the tremor might be related to acute ingestion of a large quantity of ethanol. The investigator considered the likelihood of a causal relationship between the tremulousness and study drug to be possible, and a relationship between the apnea and hypotension and the study drug to be remote. The reviewer agrees.

Patient 415 (nalmefene 1.0 mg), experienced delirium after an episode of unresponsiveness. The patient has a history of seizures and drug/alcohol abuse. The investigator felt the seizure was likely due to toxic encephalopathy/psychosis related to cocaine and alcohol. The patient's condition completely resolved. Relationship?

PROTOCOL 05

Patient 166 (nalmefene 2.0 mg), presented with ethanol (228 mg/dl) and cocaine overdose. During the study he experienced tachycardia (100-170 bpm) of unknown etiology for approximately one day. The tachycardia resolved spontaneously. Duration argues against nalmefene relationship.

Patient 214 (nalmefene 2.0 mg), experienced vomiting and delirium tremens secondary to acute opioid withdrawal after administration of study drug. He had a history of intravenous drug abuse, heroin and cocaine abuse and ethanol abuse. He presented to the emergency room positive for cocaine, codeine and morphine. Probable precipitated withdrawal.

Patient 258 (nalmefene 2.0 mg) developed pulmonary edema as a result of three liters of saline given to raise his blood pressure from 0/0 mmHg. Probable shock lung from hypotension and/or crystalloid overload.

Patient 1053 (naloxone 2.0 mg) with arrhythmia and hypertension had amitriptyline overdoses which are known to cause arrhythmias including tachycardia. Relationship?

PROTOCOL 08

Patient 1402 (naloxone 2.0 mg), presented to the emergency room with a head injury (epidural intracranial hematoma), his baseline heart rate was 40 bpm and continued to remain below 60 bpm for the first 30 minutes after study drug administration. He has a history of previous head injury and alcohol abuse. The head injury was diagnosed after study drug administration. The investigator felt the bradycardia was secondary to the head injury. The patient did not complete the study as he was take to surgery. Unrelated.

PROTOCOL 11

Patient 104 (nalmefene 2.0 mg) was a 62-year-old Caucasian female was noted to have an abnormal EKG reading during the post-study physical examination on January 13, 1992. The patient was described to have a possible first degree AV Block. The patient did not have a pre-study history of the noted effect. The investigator considered the event to be mild in severity and possibly related to study drug. Probably unrelated.

Patient 1204 (nalmefene 2.0 mg), pre-study electrocardiogram showed QT prolongation and right ventricular hypertrophy. On her post-study electrocardiogram, possible first degree AV block was seen. A cardiologist was contacted to follow up on this patient. No further information was collected in regard to the AV block. The patient completed the study. Probably unrelated.

PROTOCOL 12

Patient 7 (nalmefene 0.5 mcg/kg) had a history of mitral valve prolapse with premature ventricular contractions documented on her ECG prior to surgery. She held premature ventricular contractions during the study and received quinidine sulfate pre- and post-operatively. The investigator did not consider the arrhythmia nalmefene related. Reviewer agrees.

Patient 10 (nalmefene 0.5 mcg/kg) whose only significant preoperative history was menometrorrhagia with anemia, HCT 32%, developed premature ventricular contractions in the recovery room after nalmefene had been given. She was treated with lidocaine and the arrhythmia resolved. Both her pre- and post-study ECGs were normal. The investigator commented that premature ventricular contractions are occasionally seen in the post-operative period and are not usually significance. He considered them remotely drug related. Relationship?

Patient 21 (nalmefene 1.0 mcg/kg), received metoprolol prior to surgery which kept her heart rate borderline bradycardic; post-operatively, she experienced a short bradycardic period. The patient completed the study. Probably unrelated.

PROTOCOL 13

Patient 622 (0.10 mcg/kg nalmefene) was a 44 year old black female with a history of uterine fibroids and anemia. She had no cardiovascular history but her pre treatment ECG showed non-specific anterolateral T wave changes. Her heart rate was 74 bpm and her blood pressure 140/90. Her laboratory examination showed slightly elevated CPK (95 u/l) and uric acid (6.3 mg/dl). Her hemoglobin was 11.2 g/dl and her hematocrit 33.4%. She underwent a transabdominal hysterectomy, with morphine 37 mg, nitrous oxide 50%, isoflurane 0.25% anesthesia. She was reversed with nalmefene 0.1 mcg/kg. She was retreated after 2 hours 53 minutes and 4 hours 12 minutes. At 1 hour 12 minutes post her initial reversal she was treated with nitropaste for chest pain. Nausea and vomiting occurred 12 and 20 hours after initial reversal. The anterolateral T wave changes seen pre-operatively were not seen post-operatively. Her CPK was further elevated, but was felt to be consistent with her surgical procedure. The chest pain was considered remotely drug related and resolved with treatment. Rated "possibly related" by reviewer.

Patient 624 (naloxone 1.0 mcg/kg) was admitted to the telemetry unit for 48 hours of electrocardiogram monitoring for arrhythmia. The cardiologist's evaluation felt that it was a vagal event due to nausea. A 24 hour followup holter monitor demonstrated no further cardiac abnormalities. In the investigator's opinion, the event was not related to study drug.

Patient 705 (nalmefene 0.25 mcg/kg), experienced severe tachycardia immediately following study drug administration. At baseline, his heart rate was 120 bpm following study drug administration his heart rate went as high as 138 bpm at 10 minutes, by 40 minutes his heart rate was 97 bpm. The patient completed the study. Probable reaction to study drug.

PROTOCOL 15

Patient 126 (naloxone 1.0 mcg/kg), complained of post-operative chest pains. She was treated with nitroglycerine and all isoenzymes were normal. The pain was relieved by discharge from the PACU. This patient has a history of myocardial infarction, hypertension, hepatitis and recreational drug use. Relationship?

Patient 205 (nalmefene 0.5 mcg/kg) a 42 year old with a history of atrial arrhythmias, experienced a mild atrial arrhythmia post operatively. The patient received only one dose of study drug and was treated with atropine for the arrhythmia approximately 2 hours after study drug administration. The patient recovered and the investigator felt this was remotely related. The reviewer agrees.

There were five post-operative episodes of bradycardia, one with naloxone, four with nalmefene, that were probably unrelated to the drugs.

Patient 207 (naloxone 1.0 mcg/kg), present with bradycardia intra operatively and post study drug administration. The patient experienced a post operative heart rate of 40 bpm during two separate episodes. Probably unrelated.

Patient 230 (nalmefene 0.25 mcg/kg), 40 years of age, experienced mild post-operative bradycardia. The patient was treated with 0.2 mg of atropine and recovered. This patient was given one dose of study drug 87 minutes prior to the atropine intervention. The investigator felt this episode was remotely related to the study drug. Probably unrelated.

Patient 516 (nalmefene 0.25 mcg/kg), experienced bradycardia (45 bpm) at 5 minutes post study drug administration. No concomitant medication was given and the patient completed the study.

Patient 527 (nalmefene 0.25 mcg/kg), experienced bradycardia (44 bpm) at 5 minutes post study drug administration. No concomitant medication was given and the patient completed the study.

Patient 529 (nalmefene 0.25 mcg/kg), experienced bradycardia (46 bpm) at 5 minutes post study drug administration. No concomitant medication was given and the patient completed the study.

PROTOCOL -18
Patient 211 (nalmefene 1.0 mg), a 48 year old black male, reported mild chest pain during the post-study drug observation period. The pain lasted approximately two minutes and was mild in severity. As per the patient, the chest pain was likely to be due to gas. Pre- and post-study ECG, however, were abnormal, showing signs of a possible right ventricular hypertrophy (RVH) with borderline primary AV block.

Nonetheless, a medical history check was unremarkable for significant cardiovascular or respiratory history. Relation to study drug administration was classified as remote and the patient recovered promptly. Significance?

Patient 223 (nalmefene 2.0 mg), a 43 year old black female, with an unremarkable cardiovascular and respiratory medical history, presented with pre-study sinus bradycardia as indicated by baseline electrocardiogram (ECG). Post-study drug administration, the patient remained bradycardic with a lowering of T-waves on post-study ECG. The asymptomatic event was classified as clinically insignificant and remotely related to study drug administration.

PROTOCOL JF-201
Patient 119 (nalmefene 1.0 mg), experienced intermittent seizures and continuous adult respiratory distress syndrome during the study. Discharge diagnosis is acute pulmonary edema secondary to acute heroin overdose and seizures secondary to hypoxia. Secondary diagnosis was hypoxic encephalopathy (residual from previous heroin overdose). Reviewer concurs.

Patient 132 (naloxone 2.0 mg) with a history of alcoholic liver disease experienced hematemesis during the study. The discharge diagnosis was heroin overdose and aspiration pneumonia.

PROTOCOL 02
Patient 301 (naloxone 1 mcg/kg), experienced pulmonary edema due to volume overload, because intravenous fluids were needed to compensate for rapid blood loss.

Patient 311 (naloxone 1 mcg/kg), experienced pulmonary edema due to fluid overload given for metabolic acidosis.

There were a number of individuals whose conditions did not seem related to the study drugs:

PROTOCOL 03

Patient 134, a 48 year old female who received nalmefene 0.25 mcg/kg, had inverted T- waves in leads V2-V5 on her post-treatment ECG. She had no complaints of chest pain. Her SGOT remained normal (Pre 28, Post 31) but her hemoglobin and hematocrit fell from 11.3 g, 34% pre-surgery to 8.3 g, 25% post-surgery suggesting that anemia may have caused some ischemic changes on the ECG. The investigator considered the finding remotely drug related.

PROTOCOL 04A1.1

Patient 138 (nalmefene 1.0 mg), presented with constricted pupils, vomitus in oropharyngeal, aspiration pneumonia with diffuse inspiratory and expiratory rhonchi, shock liver versus hepatitis and mild upper gastrointestinal bleed, renal failure versus shock kidney all secondary to rhabdomyolysis and possible hypoxic brain injury. The patient has a history of rhabdomyolysis secondary to poly drug abuse over 15 years and participation in a methadone program for heroin abuse. Complications considered by reviewer to be due to the primary addictive disorder.

PROTOCOL 05

Patient 103 (naloxone 2.0 mg), has a long history of suspected pseudo-seizures and Munchhausen's syndrome. A workup has been negative for organic seizure causes. His discharge diagnosis was questionable seizure activity and Munchhausen's syndrome.

Patient 112 (nalmefene 1.0 mg), history of episodes of pseudo-seizures or seizures, hypertension and drug abuse. The patient presented positive for cocaine and cannabinoids. During the study, the patient did not have any seizure activity and the etiology for the seizure which was the cause of the hospital visit was unknown. His post-ictal state was felt to be the cause of lethargy seen during the study as well as brief episode of disorientation.

Patient 116 (naloxone 2.0 mg), presented to the emergency room in a post-ictal state with acute intoxication and intermittent profound depression of consciousness. The staff believe the patient's seizure threshold was lowered from cocaine and ethanol. This patient has a history of seizures, ethanol, cocaine and marijuana abuse.

Patient 157 (naloxone 2.0 mg), has a history of seizures. The investigator suspects a pseudoseizure, but not proven. The patient was intubated and anticonvulsant medication was initiated.

Patient 161 (nalmefene 2.0 mg), presented with a positive cocaine level and post-ictal. The discharge diagnosis was alcoholic seizure disorder.

Patient 253 (nalmefene 2.0 mg), was found unresponsive at home due to pulmonary edema. He had a history of hypertension, cardiomegaly and hypertensive nephrosclerosis (renal failure).

Patient 356 (naloxone 1.0 mg), was brought to the emergency room for evaluation of pulmonary edema due to alcohol abuse. He had a history of alcohol and heroin abuse. At the time of admittance, he had cyanosis small pupils (2mm) and impaired consciousness. He completed the study and the discharge diagnosis was pulmonary edema.

Patient 652 (nalmefene 2.0 mg), has a long standing seizure disorder secondary to old head trauma.

Patient 951 (nalmefene 1.0 mg), admitted with hypertension, tachycardia and possible seizure. It was in the investigator's opinion that the hypertension and tachycardia were related to alcohol withdrawal. The patient developed rhabdomyolysis and delirium tremens after admission.

PROTOCOL 13
Patient 218 (nalmefene 0.5 mcg/kg), experienced shallow rapid respirations with no complaint of pain. First believed to be respiratory depression due to narcotics. Following a chest xray, pulmonary edema was revealed. Furosemide 10 mg was given and the edema was resolved. Edema was felt to be from fluid overload.

PROTOCOL 18
Patient 818 (naloxone 1.0 mg), experienced tachycardia. In the investigator's opinion, the tachycardia was probably due to difficult procedure, pain and a hypertensive episode.

Possible Myocardial Ischemic Episode Protocol 21,266-21
Subject 103 was a normal control in the pharmacokinetic evaluation of nalmefene in patients with liver disease. He was a 34 year old Asian male with a pre-study (6/8/93) ECG showing sinus bradycardia, an inverted T wave in lead III, and flat T waves in leads II and AVF. His pre-study CPK was 129 IU/L and his SGOT was 26 IU/L. On the day of the study (6/22/93), two hours after receiving 2 mg of nalmefene intravenously, at 10:30 he complained of pressure, initially reported as numbness, in his chest. His heart rate and blood pressure were stable:

A post-study ECG on 6/22/93 showed inverted T waves in leads II, III, and AVF, read as compatible with inferior ischemia, new as compared to 6/8/93. The chest pressure was considered mild and possibly nalmefene related. It resolved without treatment. On 6/24/93 his CPK was 48 IU/L and his SGOT was 19 IU/L. On 7/21/93 a follow up ECG was normal. Coronary ischemia has not been previously associated with nalmefene.

This is new information, and it may be related to the rarer pulmonary edema associated with naloxone. High dose (> 1 mg nalmefene or > 2 mg naloxone) opioid antagonists may have some direct effect on the cardiac or pulmonary vasculature, but this is an isolated event. It should be mentioned, but causality is far from certain.

Clinical Laboratory Evaluations
Blood , serum and urine samples were obtained for hematology, serum chemistries, and urinalysis pre and post treatment in all studies. Laboratory normal values were examined for each participating institution for consistency. When there were large differences among the normal ranges for a test, all values for that test were normalized so that laboratory values could be pooled. Monitor's or Anaquest ranges were selected that allowed for expected changes in laboratory values during the perioperative period. Hematology values, electrolytes, blood sugar, BUN, serum proteins and urinalyses all showed changes consistent with the patients' perioperative condition or with hydration and infections in the overdose population.

Because of the naltrexone example, the measures of liver function, SGOT (AST), SGPT (ALT), bilirubin, and alkaline phosphatase were examined in the patient population as a whole. Clinically meaningful changes were defined as changes greater than the maximum allowed shift (change form baseline) or increases to above the monitor's range (upper limits of normal). The table presents a summary of the number of patients who had changes in liver function tests from pre to post study. Overall the frequencies of changes were comparable in the nalmefene, naloxone, and placebo groups.

Abnormal Laboratory Values

Lab Value	Nalmefene N=1127		Naloxone N=369		Placebo N=77	
	n	%	n	%	n	%
Alk.Phos.	7	0.62	4	1.08	1	1.30
SGOT	87	7.72	27	7.32	5	6.49
SGPT	56	4.97	15	4.07	2	2.6
Bilirubin	34	3.02	15	4.07	1	1.3
p>0.3 for all among treatment comparisons using Fisher's exact test						

Single-dose studies are not expected to be especialy sensitive to abnormal hepatic chemistries, but this is the setting in which the parenteral form of the drug will be used.

Special Populations

PATIENTS WITH ARTERIOSCLEROTIC CARDIOVASCULAR DISEASE (ASCVD)
Cardiac arrhythmias have been associated with reversal of opioid depression with naloxone. Because some patients with cardiac disease may be treated with reversal agents, a subset of all patients with arteriosclerotic cardiovascular disease (ASCVD) was created by selecting all patients with a pretreatment history of angina, previous myocardial infarction, previous coronary bypass surgery or ASCVD. The following table summarizes cardiovascular adverse events reported in this population.

Adverse Events in Patients with ASCVD

	Nalmefene N=81		Naloxone N=11		Placebo N=11	
ADE	n	%	n	%	n	%
Hypertension	1	1	2	18	0	0
Hypotension	1	1	0	0	1	9
Tachycardia	1	1	0	0	0	0
Vasodilation	4	5	0	0	0	0
Lung edema	1	1	0	0	0	0
Chest pain	0	0	1	9	0	0

Previous history of ASCVD did not increase the frequency of cardiovascular adverse events for the nalmefene group. However, the number of naloxone control patients may be too small to estimate accurately whether the frequency of hypertension and chest pain were increased.

PATIENTS WITH LIVER DISEASE

A pharmacokinetic study of nalmefene was performed in 12 patients with liver disease. There was a statistically significant decrease of 28% in nalmefene plasma clearance in patients with liver disease. This reduction in clearance is not so large as to require adjusting the single-doses of nalmefene recommended for acute reversal of opioid effects.

PATIENTS WITH RENAL INSUFFICIENCY

A pharmacokinetic study of nalmefene was performed in 8 patient volunteers with end-stage renal disease, both between and during hemodialysis. The investigator felt that it was necessary to administer the dose of nalmefene over one minute to reduce the incidence of side effects such as dizziness and hypertension. Plasma clearance of free nalmefene was significantly reduced compared to normal controls by about 27% and the elimination half life was prolonged. Dialysis clearance was estimated to be 405 l/hr. Adjustment of the dose of nalmefene for acute administration is probably not necessary.

INFORMATION FROM STUDIES BY OTHER SPONSORS

The nalmefene bibliography contains references to studies carried out by other companies on oral nalmefene used for indications not included in this new drug application. No serious adverse reactions were reported in these publications. The adverse events reported were similar to those observed and reported in the clinical trials summarized in this application.

Overall Adverse Event Frequencies

The database for nalmefene consists of 26 studies that were conducted by Ohmeda PPD (formerly Anaquest), and four (4) studies that were conducted by Key Pharmaceutical, the former sponsor, which were analyzed by Ohmeda PPD. These studies contain a total of 1733 volunteers or patients who received nalmefene or control treatments, whose data are included in the electronic database. In addition, another 30 studies including 865 volunteers or patients, 495 of whom were exposed to nalmefene, conducted by the previous sponsor for which case report forms are not available are summarized in the Key Safety Summary.

Adverse reactions were collected in all case report forms, were coded using COSTART standard terms and were entered into the database.

The 1279 people who received nalmefene in all studies reported in the electronic database, included 152 volunteers in pharmacokinetic or pharmacodynamic studies, and 1,127 patients given nalmefene to reverse opioid depression postoperatively or following an overdose. Fifty three (53) volunteers exposed to nalmefene and naloxone in crossover studies (Studies 10, 14, 16, and 24) were not counted either in the numerator or the denominator for calculation of adverse reaction frequencies in volunteers.

Adverse events occurring more than once and at a frequency above 1% in normal subjects, whether or not they received pre treatment with opioids, included dizziness, headache, nausea, somnolence, asthenia, increased blood pressure (coded as hypertension), back pain, chills, hypotension, vomiting, pain, and injection site inflammation.

In patients who had received opioids to provide analgesia or had known or suspected opioid overdose, the adverse events with a frequency above 1% included nausea, vomiting, tachycardia, increased blood pressure (coded as hypertension), fever, dizziness, headache, chills, hypotension, and vasodilatation.

In order to obtain a clear picture of the adverse event profile, similar types of adverse events were clustered together using clusters of symptoms developed in studies of opioid withdrawal. The analysis by cluster consisted of listing patients who had any symptom in the cluster. The frequencies of nausea, vomiting, tachycardia, and increased blood pressure were high as was the frequency of the Withdrawal 1 cluster. The frequencies of other withdrawal symptoms were less than 1% individually, but the overall frequency of the cluster, Withdrawal 2, was 2.3% for nalmefene and 1.6% for naloxone.

withdrawal (cluster) 1 - nausea, vomiting, hypertension, tachycardia;
withdrawal (cluster) 2 - sweating, diarrhea, chills, withdrawal syndrome, insomnia, leg cramps;
withdrawal (cluster) 3 - agitation, nervousness, increased reflexes, anxiety;
withdrawal (cluster) 4 - delirium, convulsion.

Because the frequency of increased pain was obscured by different codes for pain in different parts of the body, a cluster of "inadequate analgesia" was created including back pain, abdominal pain, pain, and chest pain.

Potentially serious cardiovascular effects have been reported in the setting of opioid reversal with naloxone. Therefore a cluster "severe tachycardia" including severe tachycardia and arrhythmia was created. A separate table of patients who had new onset of tachycardia (heart rate over 120 after drug treatment) was created by analyzing the vital signs and identifying all patients whose heart rates increased by more than 10 beats per minute (bpm) and whose heart rate was greater than 120.

Two other potentially serious and possibly drug related cluster categories included: the hepatitis cluster of hepatitis and hepatic failure and pulmonary edema (coded lung edema).

Adverse Events - Volunteers		
Adverse event	nalmefene N=99 n (%)	placebo N=6 n (%)
Dizziness	19 (19.2)	2 (33.3)
Headache	8 (8.1)	
Nausea	7 (7.1)	
Somnolence	6 (6.1)	
Asthenia	3 (3.0)	
Hypertension	3 (3.0)	
Back pain	2 (2.0)	
Chills	2 (2.0)	
Hypotension	2 (2.0)	
Vomiting	2 (2.0)	
Pain	2 (2.0)	
Injection site inflammation	2 (2.0)	

Adverse Events - Patients			
Adverse Event	Nalmefene N=1127 N (%)	Naloxone N=369 N (%)	Placebo N=77 N (%)
Nausea	201 (17.8)	68 (18.4)	5 (6.5)
Vomiting	107 (9.5)	26 (7.0)	3 (3.9)
Tachycardia	54 (4.8)	28 (7.6)	
Hypertension	52 (4.6)	25 (6.8)	
Fever	34 (3.0)	13 (3.5)	
Dizziness	29 (2.6)	8 (2.2)	1 (1.3)
Headache	17 (1.5)	3 (0.8)	3 (3.9)
Chills	16 (1.4)	5 (1.4)	
Hypotension	13 (1.2)	3 (0.8)	
Vasodilatation	13 (1.2)	2 (0.5)	

Intercurrent Illnesses/Symptoms						
	Nalmefene		Naloxone		Placebo	
Overdose	N=288 n	%	N=153 n	%		
Tachycardia	11	3.8	6	3.9		
Vomiting	7	2.4	4	2.6		
Hypertension	6	2.1	2	1.3		
Convulsion	4	1.4	5	3.2		
Postoperative	N=839	%	N=216	%	N=77	%
Hypertension	45	5.4	14	6.5	6	7.8
Tachycardia	20	2.4	8	3.7	1	1.3
Hypotension	8	1.0	3	1.4	1	1.3

Gender, age and race were not predictive of any adverse event when type of surgery (abdominal/gynecological) was taken into acount.

Adverse Events by Dose - Postoperative												
Adverse Event	Nalmefene 0.1 mcg/kg N=38		Nalmefene 0.25 mcg/kg N=138		Nalmefene 0.5 mcg/kg N=184		Nalmefene 1.0 mcg/kg N=49		Naloxone 0.5 mcg/kg N=73		Naloxone 1.0 mcg/kg N=51	
	n	%	n	%	n	%	n	%	n	%	n	%
Hypertension	3	7.9	18	13.0	18	9.8	4	8.2	12	16.4	9	17.6
Tachycardia	5	13.2	11	8.0	15	8.2	5	10.2	12	16.4	8	15.7
Nausea	15	39.5	49	35.5	63	34.2	21	42.9	31	42.5	22	43.1
Vomiting	4	10.5	20	14.5	23	12.5	9	18.4	7	9.6	7	13.7
Pain	2	5.3	4	2.9	9	4.9	2	4.1	3	4.1	2	3.9
p>0.05 for the among group comparisons for all adverse event rates using Fisher's exact test												

There was no dose effect for adverse events in the postoperative group.

In the overdose population, there was an apparent dose effect. The following table shows the frequency of any adverse effect and the frequency of vomiting.

Adverse Events by Dose - Overdose								
	Nalmefene 0.5 mg N=32		Nalmefene 1.0 mg N=166		Nalmefene 2.0 mg N=90		Naloxone 2.0 mg N=153	
	n	%	n	%	n	%	n	%
Any AE	4	**12.5**	32	**19.5**	27	**30.3**	25	16.7
Hypertension	0	**0**	1	**0.6**	2	**2.2**	2	1.3
Tachycardia	0	**0**	8	**4.8**	6	**6.7**	8	5.2
Nausea	0	**0**	2	**1.2**	4	**4.4**	4	2.6
Vomiting	1	**3.1***	12	**7.2***	20	**22.2***	11	7.2*
* p<0.05 for the among treatment differences using the Fisher's exact test								

The 0.5 mg dose of nalmefene had the lowest incidence of adverse events. Nalmefene 1.0 mg and naloxone 2.0 mg had comparable incidence of adverse events and nalmefene 2.0 mg had the highest incidence of adverse events. It is very likely that this rank order is due to drug effect, and nalmefene 2.0 mg is too great a dose in this setting.

Withdrawal Clusters								
	Nalmefene				Naloxone			
	Post Anesthesia N=409		Overdose N=288		Post Anesthesia N=124		Overdose N=153	
	n	%	n	%	n	%	n	%
Withdrawal 1	201	**49**	45	16	74	**60**	18	12
Withdrawal 2	11	3	6	2	3	2	2	1
Withdrawal 3	6	1	1	0	4	3	0	0

The overall incidence of withdrawal symptoms in the two patient populations, post anesthesia reversal of opioid depression and overdose was comparable in the nalmefene and naloxone groups. The high incidence of Withdrawal cluster 1, (nausea, vomiting, mild hypertension, mild tachycardia) is probably not very predictive, being a symptom cluster that is common after anesthesia.

Because investigators did not always consider tachycardia an adverse event, the vital sign report was screened for new onset tachycardia, defined as an increase of more than 20 bpm to a final heart rate greater than 120 bpm.

Adverse Events by Dose - Postoperative												
Adverse Event	Nalmefene 0.1 mcg/kg N=38		Nalmefene 0.25 mcg/kg N=143		Nalmefene 0.5 mcg/kg N=225		Nalmefene 1.0 mcg/kg N=90		Naloxone 0.5 mcg/kg N=73		Naloxone 1.0 mcg/kg N=75	
	n	%	n	%	n	%	n	%	n	%	n	%
New Onset Tachycardia	1	3	4	3	12	5	10	11	3	4	5	7

At doses of 0.1 mcg/kg to 0.5 mcg/kg, doses used in the post-operative reversal of opioid depression, nalmefene had an incidence of new tachycardia between naloxone 0.5 mcg/kg and naloxone 1.0 mcg/kg. At doses of 1.0 mcg/kg the incidence for nalmefene went up to 11% indicating that the 1.0 mcg/kg dose may be too high for post-operative reversal.

DRUG ABUSE AND OVERDOSAGE INFORMATION

Opioid agonists have a high potential to produce physical dependence, which is a major drawback to their therapeutic use. Opioid antagonists, however, do not have any dependence liability associated with them.

Nalmefene was studied to determine its overt effects and potential to produce physical dependence in drug-free rhesus monkeys. The rhesus monkeys received nalmefene 0.1 mg/kg and were observed closely for the first 6 hours, then examined again for 15 minutes at 12, 18 and 24 hours. Nalmefene produced occasional mild and transient signs and symptoms such as staring central nervous system depression coupled with mild tremors and occasional myoclonic jerks. The investigators indicated that most of the symptoms, except staring, occurred normally or after placebo medication.

Nalmefene was also tested in the single dose suppression test (of morphine withdrawal), precipitated withdrawal test (in morphine-dependent monkeys) and primary physical dependence test. Nalmefene did not substitute for morphine in the single dose suppression test and, in fact, exacerbated withdrawal in morphine-independent monkeys. In the primary physical dependence test, 2 of the 3 monkeys died following withdrawal from nalmefene. Death was due to water retention considered to be due to up-regulation of opioid receptors in the posterior pituitary.

In another study, nalmefene was made available to rhesus monkeys trained to self-administer codeine. Nalmefene did not substitute for codeine.

In a study of six healthy volunteers with a history of opioid abuse, nalmefene was administered in a double blind randomized latin square design. This study was to determine if nalmefene caused typical morphine like effects. A comparison of physiologic effects, as reported by subject or investigator, was made between morphine, 15 and 30 mg given intramuscularly, nalmefene 25, 50 and 100 mg given orally and placebo. Drowsiness or sleepiness was the most common drug effect reported after the administration of each treatment. Only morphine produced pupillary constriction and increased subject-reported euphoria and "drug-liking." Neither drug increased Addiction Research Inventory Scale scores measuring dysphoria or sedation or produced changes on the Profile of Mood States questionnaire. The data indicated that nalmefene did not produce typical morphine-like effects and has no apparent abuse potential.

In a single ascending dose study in volunteers, nalmefene 50, 100, 200 and 300 mg was given orally. Peak plasma concentrations increased proportionally with dose from 24.3 ± 11.0 ng/ml at 50 mg to 177 ± 18 ng/ml at 300 mg. Nalmefene was well tolerated with mild and transient lightheadedness being reported after 100-300 mg. In a similar study, volunteers received 0.5, 1.0 and 2.0 mg of nalmefene. Peak plasma levels for these doses were 1.77 ± 0.75, 3.16 ± 0.34 and 5.75 ± 0.67 ng/ml for 0.5, 1.0 and 2.0 mg, respectively.

These study designs are typically used for assessment of physical and psychological dependence liability and indicate that nalmefene has no abuse potential.

Dialysis

Nalmefene 1.0 mg was also studied in renally impaired patients on dialysis. The dialyziabilty of nalmefene is 5% over a three hour period.

Information available from outside the NDA

Nalmefene has been studied by private parties, other sponsors, and in intra-mural and extra-mural research. Review of the extant bibliography on nalmefene supports its being a pure opioid antagonist of the naltrexone/naloxone type. One paper of note was a pre-publication report by Cheskin, Chami, Johnson & Jaffe, in which oral nalmefene glucuronide was given to opioid-tolerant, methadone maintained opioid addicts. Doses of 0.225-0.450 mg of the glucuronide, given orally, produced opioid withdrawal symptoms significantly different from placebo in five subjects with an onset of 9 hours and a peak effect at 14 hours. This finding replicated similar findings in rats, where oral nalmefene hydrochloride produced opioid withdrawal in fifteen minutes, while the oral administration nalmefene glucuronide produced symptoms after 1-3 hours. Direct colonic administration of nalmefene glucuronide produced opioid withdrawal in dependent animals in 5-8 minutes.

These findings are most consistent with nalmefene glucuronide being split by colonic bacteria with resultant absorption of the glucuronide in animals and man. They suggest that there is a deep compartment caused by entero-hepatic recirculation, that is clinically relevant in the doses used in the management of overdose. This data fits well with the observed PET scanning data which showed a similar effect.

Integrated Efficacy & Safety Conclusion

Nalmefene appears to have the same kind of efficacy and safety profile as the two currently approved opioid antagonists. It has been given in doses from the minimally effective dose (about 0.1 mcg/kg) up to the largest dose that can reasonably be given (2 mg IM/IV & up to 300 mg orally).

The sponsor has provided substantial evidence that nalmefene is effective in reversing opioid agonist effects from binding, animal, human imaging, clinical laboratory, anesthesia and overdose studies. Nalmefene appears to induce the same series of effects as naloxone:

Reversal of respiratory depression ->
reversal of analgesia->
autonomic reactions (tachycardia, arousal)->
precipitated withdrawal symptoms->
severe withdrawal reactions

The tachycardia, hypertension, reversal of analgesia, stress and arrhythmia responses limit the use of these class of compounds in anesthesia, and make careful use by individual titration appropriate.

The sponsor wishes a claim of longer duration of action than naloxone. This is reasonable, but it must be tempered by the evidence that the long terminal half-life of the drug is due to entero-hepatic recirculation. Low doses of nalmefene probably have about twice the duration of action as naloxone, while doses large enough for the entero-hepatic recirculation to be meaningful (10 mcg/kg or above) show a duration of action perhaps four to five times longer.

Age, gender and race did not appear to affect the efficacy and safety of nalmefene, beyond the usual caveat that drugs of this class carry higher risk in patients with occult cardiovascular disease, which is a relative contraindication to its non-emergent use.

A claim of longer duration of action is reasonable, but limits must be set in the labeling and advertising (see advertising review).

Recommendation

Recommend approval in the recommended doses, but suggest:

1. Phase IV commitment for study of the pharmacokinetics of the glucuronide in man.
(to examine the possible role of enterohepatic recirculation.)

2. Phase IV commitment for observational studies in pediatric population looking at the drug's pharmacokinetics by sparse sampling techniques and/or appropriate trials establishing the safety of its use in anesthesia, conscious sedation, or overdose.

CC: Original NDA
HFD-007 Division File
HFD-007 C Wright
HFD-007 CSO Mcneal
HFD-340
R/D & F/T : CWIV
Location: CWIV Archive

7/31/94
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7/31/94
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NDA#: NDA020459
Trade and generic name & form: Nalmefene Injection (REVEX)
Sponsor: Ohmeda
Letter Date: 4/27/94
Date Completed:

Medical Officer Review (Appendum)
NDA 20495 Nalmefene Injection
"Revex"

Type of Submission: NDA General Correspondence
Date Received: Aug. 1994
Review Completed : 9/2/94
Reviewer: Curtis Wright MD, MPH
Peer Reviewer: Robert Bedford
Date Cleared Peer:
Security Level: TradeSecret/Confidential (FDA and Special Government Employees)

ACCOUNTING DATA:

Review Work as NDA-	6 hours
Report Preparation-	3 hour

Abstract

ADE analysis looking for dose-response for adverse events.

Background

The NDA for REVEX did not include a machine readable ADE file which used the population of ADE's as the denominator. This precluded a usual type of analysis. The sponsor was asked to provide this data, and promptly did so. This is the report of the subsequent analysis.

Methods

The analysis started with the following list of naive terms description of all the AEs & intercurrent illnesses reported in the studies, regardless of treatment or attribution. There were 1441 of them, which are listed in table 1 of the appendix to this report.

Preliminary Triage

Examination of the table revealed that the entries could be triaged using the usual kinds of criteria:

217 of the AE's occurred either before the study drug was given, or were transient in nature (transient hypotension, episode of arm pain) and occurred more than two hours after test drug. (Two hours was picked by the reviewer as it is highly unlikely that a transient response occurring two hours after a single IV injection is causally related in this class of drug.)

12 of the AE's were reported as NOT RELATED by the investigator and the reviewer agreed.

50 of the AE's were single occurrences of either a non-serious AE.
160 of the AE's were listed as REMOTE by the investigator and the reviewer agreed.
This left 1002 Adverse events with a possible relationship to the test drugs.

Occurrence Across Study Types

There were five major kinds of study in the nalmefene NDA; Clinical Pharmacology (CLINP), Conscious Sedation (CSED), Overdose, Postoperative (POSTOP), and reversal of systemic effects (EPIDURAL). For non-serious adverse events that were relatively frequent, it would be expected that such events would occur in more than one kind of study. If they did not, it is likely they are unrelated.

	Adverse Events by Study Type					
	CLINP	CSED	Overdose	Postop	Epidural	Total
ABDOMINAL PAIN	1	4	0	8	0	13
AGITATION	0	4	0	6	1	11
ASTHENIA	4	1	0	0	0	5
ATRIAL	0	1	3	2	0	6
BRADYCARDIA	0	4	1	9	0	14
CHEST PAIN	1	1	0	3	0	5
CHILLS	2	3	1	13	3	22
CONFUSION	0	1	1	1	1	4
CONVULSION	0	0	10	0	0	10
DIARRHEA	1	0	3	0	0	4
DIZZINESS	24	28	2	4	0	58
DRY MOUTH	0	4	2	1	0	7
DYSPHORIA	2	6	0	0	0	8
FEVER	**0**	**0**	**6**	**11**	**19**	**36**
HEADACHE	14	17	1	4	0	36
HYPERGLYCEMIA	**0**	**0**	**3**	**1**	**1**	5
HYPERTENSION	3	41	12	89	2	147
HYPOGLYCEMIA	**0**	**0**	**4**	**0**	**0**	**4**
HYPOTENSION	1	11	3	12	1	28
LUNG EDEMA	0	0	1	1	1	3
MYALGIA	2	1	2	7	1	13
MYOCLONUS	3	0	4	0	0	7
NAUSEA	17	34	9	140	31	231
NERVOUSNESS	0	3	0	4	2	9
PRURITUS	**0**	**0**	**0**	**3**	**6**	**9**
SCREAMING	0	1	0	1	0	2
SOMNOLENCE	6	2	0	2	0	10
SWEATING	1	2	0	1	0	4
SYNCOPE	1	1	0	1	0	3
TACHYCARDIA	0	6	37	72	2	117
TREMOR	1	0	2	3	5	11
URINARY RETENTION	**0**	**0**	**0**	**5**	**1**	**6**
VASODILATATION	4	11	1	0	1	17
VOMITING	5	16	47	50	9	127
VENTRICULAR	1	0	1	3	1	6
WITHDRAWAL	0	0	4	0	0	4
Total	94	203	160	457	88	1002

In the reviewer's opinion, FEVER, HYPERGLYCEMIA, HYPOGLYCEMIA, PRURITIS and URINARY RETENTION were all related to pre-existing conditions or other factors, not to nalmefene.

Relative Risk

There were 1127 patients in the NDA'd studies who received nalmefene, 369 who received naloxone, and 77 who received placebo. For events with a reasonable numerator which were non-serious, it is reasonable to examine the relative rates for each treatment:

	NALMEFENE	NALOXONE	PLACEBO	NALMEFENE	NALOXONE	PLACEBO
	1127	369	77	%	%	%
ABDOMINAL PAIN	12	1	0	1.06%	0.27%	0.00%
AGITATION	10	1	0	0.89%	0.27%	0.00%
ASTHENIA	**4**	**0**	**1**	**0.35%**	**0.00%**	**1.30%**
ATRIAL DYSRTHYMIA	4	2	0	0.35%	0.54%	0.00%
BRADYCARDIA	12	2	0	1.06%	0.54%	0.00%
CHEST PAIN	4	1	0	0.35%	0.27%	0.00%
CHILLS	18	4	0	1.60%	1.08%	0.00%
CONFUSION	3	1	0	0.27%	0.27%	0.00%
CONVULSION	5	5	0	0.44%	1.36%	0.00%
DIARRHEA	4	0	0	0.35%	0.00%	0.00%
DIZZINESS	**46**	**7**	**5**	**4.08%**	**1.90%**	**6.49%**
DRY MOUTH	**5**	**0**	**2**	**0.44%**	**0.00%**	**2.60%**
DYSPHORIA	**7**	**0**	**1**	**0.62%**	**0.00%**	**1.30%**
HEADACHE	**30**	**3**	**3**	**2.66%**	**0.81%**	**3.90%**
HYPERTENSION	**100**	**41**	**6**	**8.87%**	**11.11%**	**7.79%**
HYPOTENSION	**21**	**5**	**2**	**1.86%**	**1.36%**	**2.60%**
LUNG EDEMA	2	1	0	0.18%	0.27%	0.00%
MYALGIA	10	3	0	0.89%	0.81%	0.00%
MYOCLONUS	6	0	1	0.53%	0.00%	1.30%
NAUSEA	171	52	8	15.17%	14.09%	10.39%
NERVOUSNESS	6	3	0	0.53%	0.81%	0.00%
SCREAMING	2	0	0	0.18%	0.00%	0.00%
SOMNOLENCE	10	0	0	0.89%	0.00%	0.00%
SWEATING	3	1	0	0.27%	0.27%	0.00%
SYNCOPE	**1**	**1**	**1**	**0.09%**	**0.27%**	**1.30%**
TACHYCARDIA	78	38	1	6.92%	10.30%	1.30%
TREMOR	7	4	0	0.62%	1.08%	0.00%
VASODILATATION	16	1	0	1.42%	0.27%	0.00%
VOMITING	99	25	3	8.78%	6.78%	3.90%
VENTRICULAR DYS	6	0	0	0.53%	0.00%	0.00%
WITHDRAWAL SYN.	3	1	0	0.27%	0.27%	0.00%
Total	705	203	34	62.56%	55.01%	44.16%

In the reviewer's opinion, ASTHENIA, DIZZINESS, DRY MOUTH, DYSPHORIA, HEADACHE, HYPOTENSION, and SYNCOPE were likely not to be related to either active test drug.

Dose Relatedness

Nalmefene was given in microgram amounts to reverse anesthesia, and in milligram amounts in overdose and in Clinical Pharmacology Studies.

	Micrograms	Milligrams	Micrograms	Milligrams
N @ Risk	839	440	100.00%	100.00%
ABDOMINAL PAIN*	9	3	1.07%	0.68%
AGITATION	8	2	0.95%	0.45%
Atrial Dysrthymia	**1**	**3**	**0.12%**	**0.68%**
BRADYCARDIA	8	4	0.95%	0.91%
CHEST PAIN	3	1	0.36%	0.23%
CHILLS*	16	2	1.91%	0.45%
CONFUSION	2	1	0.24%	0.23%
CONVULSION	**2**	**3**	**0.24%**	**0.68%**
DIARRHEA	2	2	0.24%	0.45%
LUNG EDEMA	1	1	0.12%	0.23%
MYALGIA	7	3	0.83%	0.68%
MYOCLONUS	**2**	**4**	**0.24%**	**0.91%**
NAUSEA*	148	23	17.64%	5.23%
NERVOUSNESS	5	1	0.60%	0.23%
SCREAMING	1	1	0.12%	0.23%
SOMNOLENCE	6	4	0.72%	0.91%
SWEATING	2	1	0.24%	0.23%
TACHYCARDIA*	58	20	6.91%	4.55%
TREMOR	6	1	0.72%	0.23%
VASODILATATION*	**7**	**9**	**0.83%**	**2.05%**
VOMITING*	71	28	8.46%	6.36%
Ventricular	5	1	0.60%	0.23%
WITHDRAWAL SYN	1	2	0.12%	0.45%

Although the numbers are thin, there is a hint that atrial arrhythmias, vasodilatation, convulsion and myoclonus are more likely with larger doses. The relationship should be viewed with suspicion, however, since they are also much more common in cases of overdose which was confounded with the higher doses in these studies (see analysis in the primary safety report).

Discussion

Negative analysis. All of the over 1% adverse events appear to be more related to the setting and diagnosis of the patients (postop nausea, postop chills, postop vomiting) than to the use of nalmefene. There is a hint of some possible cardiovascular effect related to vasodilatation or vagal reflexes, but the numbers are too small and the confounding factors too great to have more than a ghost of a suspicion.