CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-964

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology OND IO
NDA: 21-964
Submission date: March 30, 2007
Drug: methylnaltrexone
Sponsor: Progenics Pharmaceuticals, Inc.
Indication: Treatment of opioid-induced constipation in patients receiving palliative care
Reviewing Division: Division of Gastroenterology Products

Comments:
I concur with the Division pharmacology/toxicology recommendation that the non-clinical studies submitted to this NDA provide sufficient information to support the use of methylnaltrexone for the treatment of opioid-induced constipation in patients receiving palliative care.

The proposed pregnancy labeling category is B. This appears appropriate since the teratogenicity studies conducted in rats and rabbits did not show any teratogenic potential. The high doses used in these studies were about 14 or 17 times the human dose of 0.3 mg/kg based on a body surface area comparison. It appears that a comparison based on AUC values may yield even higher animal to human dose ratios.

Section —of the labeling describes nonclinical studies on the cardiovascular safety of methylnaltrexone. The outcome of these studies was mixed in that some appeared to provide an adequate margin for safety while others showed some potential for cardiovascular effects. The reviewer noted that a thorough QT study in humans would help address this issue. Subsequent to the pharm/tox review, an acceptable clinical thorough QT study was submitted with intravenous administration of methylnaltrexone. This study did not show an effect on QT interval.

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/s/

Paul Brown
4/24/2008 01:23:22 PM
PHARMACOLOGIST

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**21-964**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**March 30, 2007**
PRODUCT:	**Methylnaltrexone (MNTX) Injection**
INTENDED CLINICAL POPULATION:	**Opioid-Induced Constipation in Patients Receiving Palliative Care**
SPONSOR:	**Progenics Pharmaceuticals, Inc.**
DOCUMENTS REVIEWED:	**eCTD submission (March 30, 2007)**
REVIEW DIVISION:	**Division of Gastroenterology Products (HFD-180)**
PHARM/TOX REVIEWER:	**Tamal K. Chakraborti, Ph.D.**
PHARM/TOX TEAM LEADER (ACTING):	**Sushanta K. Chakder, Ph.D.**
DIVISION DIRECTOR (ACTING):	**Daniel A. Shames, M.D.**
PROJECT MANAGER:	**Matthew C. Scherer**

Date of review submission to Division File System (DFS): November 29, 2007

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on Approvability: From a nonclinical perspective, this NDA may be approved.

B. Recommendation for Nonclinical studies: None

C. Recommendations on Labeling: The sponsor should be asked to modify the labeling as proposed in the text of this review.

II. Summary of nonclinical findings

A. Brief Overview of Nonclinical Findings: The systemic toxicity of MNTX was adequately evaluated in complete range of acute, subacute/subchronic and chronic toxicity studies in mice, rats and dogs. The potential genotoxicity of MNTX was examined in an adequate battery of genotoxicity tests. In addition, MNTX has been evaluated for fertility and reproductive performance (Segment I) in rats, teratology (Segment II) in rats and rabbits and peri- and post-natal development (Segment III) in rats. Adequate safety pharmacology studies were also conducted with MNTX.

Generally, bridging studies comparing the pharmacokinetics (PK) and toxicity of MNTX following single intravenous (IV) and subcutaneous (SC) dose in dogs and single IV, SC and oral (PO) dose in rats demonstrated comparable PK and toxicity profile between SC and IV route. Based on this, pivotal IV toxicity studies in rats and dogs were considered adequate to support recommended clinical dosing using SC route.

In safety pharmacology studies, MNTX at IV doses ranging from 1 to 20 mg/kg had no apparent toxicologically significant effects on the neuropharmacological profile in mice, gastrointestinal function in rats, pulmonary function in guinea pigs, or renal function in rats. Cardiovascular safety pharmacology studies were conducted using adequate battery of *in vitro* and *in vivo* tests. Methylnaltrexone showed significant cardiovascular effects in these studies. In hERG assay, MNTX caused concentration-dependent IKr inhibition (1%, 12%, 13% and 40% inhibition of hERG current at 30, 100, 300 and 1000 μM concentrations, respectively, compared to vehicle control; IC-50 > 1000 μM). In isolated canine (dog) Purkinje fibers, MNTX caused prolongations in action potential duration at 60% repolarization (APD_{60}: 13%, 21% and 15% at 1, 3 and 10 μM, respectively) and 90% repolarization (APD_{90}: 10%, 16% and 12% at 1, 3 and 10 μM, respectively) at basic cycle length (BCL) of 2 sec compared to baseline values. The highest tested concentration in the canine Purkinje fiber study (10 μM) was about 18 and 37 times the Cmax at human SC doses of 0.3 (Cmax = 234 ng/mL) and 0.15

mg/kg (Cmax = 117 ng/mL), respectively. In isolated rabbit Purkinje fibers, MNTX caused concentration-dependent increase in APD_{60} (2%, 4% and 10% at 1, 10 and 100 μM at BCL of 2 sec) and APD_{90} (1%, 3% and 6% at 1, 10 and 100 μM at BCL of 2 sec) when compared to baseline values at all three stimulus intervals (BCL of 2, 1 and 0.5 sec). The highest MNTX concentration (100 μM) tested in the rabbit Purkinje fiber study was about 186 and 373 times the Cmax at human SC doses of 0.3 and 0.15 mg/kg, respectively. In a cardiovascular safety study (0247DP45.001) in anesthetized dogs at 1, 5, and 25 mg/kg, IV, MNTX caused decreases in blood pressure (up to 13%), heart rate (8-17%), cardiac output (4-18%), left ventricular pressure (<5 to 10%), left ventricular end diastolic pressure (up to 6%), and +dP/dt (12-19%) at ≥ 1 mg/kg. The magnitude and duration of the effects were generally dose-related. In a second cardiovascular IV safety pharmacology study (940-001) in conscious Beagle dogs at 1, 5 and 20 mg/kg, MNTX caused a dose-related increase in QTc interval when compared to vehicle control (males: 2%, 5% and 10% increase at 1, 5 and 20 mg/kg, respectively, at 45 minutes after treatment; females: 3%, 5% and 12% increase at 1, 5 and 20 mg/kg, respectively, at 1 hour after treatment). Predicted exposures (C_{5min} and $AUC_{0-\infty}$ values of 56,483 ng/mL and 25,222 ng.h/mL, respectively) after a single IV dosage of 20 mg/kg to beagle dogs were approximately 482 and 144 times, respectively, the exposure (Cmax =117 ng/mL and AUC = 175 ng·h/mL) at a human SC dose of 0.15 mg/kg and 241 times and 66 times, respectively, the exposure (Cmax = 234 ng/mL and AUC = 382 ng·h/mL) at a human SC dose of 0.3 mg/kg. In conscious guinea pigs (tested at 1, 5 and 20 mg/kg), mild prolongation of QTc (4% over baseline) was observed at 20 mg/kg IV. Based on the prolongation of QT and increase in the action potential durations in cardiovascular safety pharmacology studies, MNTX appears to have significant potential for QT prolongations in humans. A thorough QT study in humans should address this issue.

In toxicity studies in rats and dogs, target organs appear to be the cardiovascular system (CVS) and the central nervous system (CNS). Methylnaltrexone caused prolongations of QTc interval (116-122% of the pretest values and 115% of the control) on Day 8 of the treatment at 750 mg/kg/day in one male and two females in the one-month oral toxicity study in dogs. The average AUC_{0-t} at 750 mg/kg/day on Day 8 in females (16,7397 ng.h/mL) was approximately 876 and 438 times the human AUC at 0.15 and 0.3 mg/kg, respectively. The average Cmax (29100 ng/mL) in females at 750 mg/kg/day on Day 8 was about 249 and 124 times the human Cmax at 0.15 (Cmax = 117 ng/mL) and 0.3 mg/kg (Cmax = 234 ng/mL), respectively. In a three-month intravenous toxicity study in dogs at 1, 5 and 20 mg/kg/day, there was a dose-related prolongation of QTc in both sexes (male at Day 6: 9 and 15% increase at 5 and 20 mg/kg/day, respectively; females at Day 6: 3%, 5% and 12% increase at 1, 5 and 20 mg/kg/day, respectively) at Day 6 and Day 83 (similar increases as at Day 6 in both sexes) post-treatment compared to vehicle control. In this study, Cmax values (average males and females) at

Day 90 at 1 (Cmax = 8034 ng/mL), 5 (Cmax = 35,413 ng/mL) and 20 (Cmax = 74,125 ng/mL) mg/kg/day were 69, 303 and 633 times the human Cmax (117 ng/mL) at 0.15 mg/kg, respectively. The Cmax values (average males and females) at Day 90 at 1 (Cmax = 8034 ng/mL), 5 (Cmax = 35,413 ng/mL) and 20 (Cmax = 74,125 ng/mL) mg/kg/day were 34, 151 and 316 times the human Cmax (234 ng/mL) at 0.30 mg/kg, respectively. The AUC_{0-t} values (average males and females) at Day 90 at 1 (AUC_{0-t} = 924 ng.h/mL), 5 (AUC_{0-t} = 6690 ng.h/mL) and 20 (AUC_{0-t} = 34,463 ng.h/mL) mg/kg/day were 5, 38 and 197 times the human AUC (175 ng.h/mL) at 0.15 mg/kg, respectively. The AUC_{0-t} values (average males and females) at Day 90 at 1 (AUC_{0-t} = 924 ng.h/mL), 5 (AUC_{0-t} = 6690 ng.h/mL) and 20 (AUC_{0-t} = 34,463 ng.h/mL) mg/kg/day were 2, 17 and 90 times the human AUC (382 ng.h/mL) at 0.30 mg/kg, respectively.

Test-article-related CNS effects in rats and dogs generally included tremors, convulsions, and decreased activity. Adverse CNS-related clinical signs were seen at IV dosages $\geq$ 15 mg/kg/day in rats and at IV dosages $\geq$ 20 mg/kg/day in dogs. These CNS adverse effects of MNTX suggested that possibly a limited amount of MNTX crosses the blood-brain barrier. The NOAELs in 90-day IV toxicity studies in rats and dogs were 1 mg/kg. Exposure at 1 mg/kg/day in rats (286 ng·h/mL) and 1 mg/kg in dogs (922 ng·h/mL) were approximately 1.6 and five times, respectively, the human AUC at 0.15 mg/kg, and approximately 0.75 and two times, respectively, the human AUC at 0.30 mg/kg.

Microscopic observations, clinical signs, and direct measurements of hemodynamic parameters indicated that MNTX may cause a decrease in blood pressure. Microscopically congestion, edema around major vessels, and increased interstitial inflammation were noted in rats following oral administration. In premature decedent rats, congestion was noted in the liver, adrenal and kidney at $\geq$ 500 mg/kg/day in the six-month oral study. In dogs, congestion was seen in various organs (primarily in the gastrointestinal tract) at $\geq$ 300 mg/kg/day in the 28-day oral study. In addition, hemorrhage occurred in the kidney, uterus and thymus of the high-dose and the mid-dose dogs that died prematurely. These findings suggested that MNTX may have adverse effect on the circulatory function.

Methylnaltrexone was not shown to be mutagenic in a battery of genotoxicity studies using Ames test, chromosome aberration assays in Chinese hamster ovary (CHO) cells and human peripheral blood lymphocytes (HPBL), and a mouse lymphoma forward mutation assay, and an *in vivo* mouse micronucleus test by IP or SC route.

In a Segment I, fertility and early embryonic development to implantation study in male and female rats, MNTX was tested at 5, 25 and 150 mg/kg/day by SC route. There were no significant treatment-related effects on fertility

and reproductive performances in both sexes. In intravenous Segment II, teratogenicity studies in rats and dogs, MNTX was not teratogenic in either species. In a subcutaneous Segment III pre and postnatal development study in rats, MNTX did not show any significant adverse effect on growth and development of the offspring.

B. Pharmacologic Activity: Methylnaltrexone is a quaternary derivative of opioid antagonist, naltrexone (NTX), developed for the treatment of opioid-induced side effects. Other opioid antagonists such as naltrexone, naloxone and nalmefene are fairly lipid soluble and readily cross the blood-brain barrier. The addition of the methyl group in the amine ring of naltrexone forms a compound with greater polarity and lower lipid solubility that provides limited access to the brain. These properties provide methylnaltrexone with the potential to block undesirable peripheral side effects (constipation, nausea and vomiting, biliary colic, urinary retention and pruritis) of opioid pain medications through peripheral opioid receptors, while sparing centrally mediated analgesic effects.

Methylnaltrexone was found to be more selective for the μ-type opioid receptors (IC50 = 300 nM) in the GI tract compared to the κ- (19-fold less potent) and the δ- (ineffective) type receptors in isolated gastric-brainstem preparation in neonatal rats. The Ki value for MNTX for human μ and κ receptors were 28 and 230 nM, respectively, whereas the Ki value for the δ receptor was > 10 μM. In several *in vivo* studies, MNTX was shown to inhibit morphine-induced effects (spike potential of duodenal smooth muscle, gastric transit time, emesis, cough, etc.) without sparing central analgesic effect. However, in rodents, MNTX was demethylated, possibly forming naltrexone. In the cardiovascular safety pharmacology study in dogs, MNTX showed cardiovascular effects. Methylnaltrexone delayed the gastrointestinal (GI) transit in rats. Overall, the results of pharmacology studies appeared to support the intended use.

C. Nonclinical Safety Issues Relevant to Clinical Use: Cardiovascular (QT prolongations) and CNS (tremor, convulsions and decreased activity) side effects were the most prominent findings in dogs and rats and dogs, respectively. Methylnaltrexone caused significant prolongations of action potential durations (APD_{60} and APD_{90}) in isolated canine and rabbit Purkinje fibers. In addition, MNTX caused prolongations of QTc intervals in cardiovascular safety pharmacology studies and 3-month intravenous toxicity studies in dogs. The QT prolongations were dose-related and were observed in both sexes consistently (similar degree of prolongation) at different time points and appear to be a true treatment (MNTX)-related effect. These QT prolongations were seen at approximately 300 and 150 times human Cmax at 0.15 and 0.3 mg/kg, respectively and 38 and 18 times human AUC at 0.15 and 0.3 mg/kg, respectively. Maximum QT prolongations of 12-15% were seen at

20 mg/kg, which is about 630 and 315 times human Cmax at 0.15 and 0.3 mg/kg, respectively and 197 and 90 times human AUC at 0.15 and 0.3 mg/kg/day, respectively. Exposure at the NOAEL of 1 mg/kg/day in rats (AUC = 286 ng·h/mL) and 1 mg/kg in dogs (AUC = 922 ng·h/mL) were approximately 2 and 5 times, respectively, the human exposure at recommended dose of 0.15 mg/kg, and approximately 0.75 and 2 times, respectively, the human exposure at maximum recommended dose of 0.30 mg/kg. Based on QT prolongations in dogs, MNTX appears to have significant potential for QT prolongations in humans. A thorough QT study in humans will help to address this issue.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

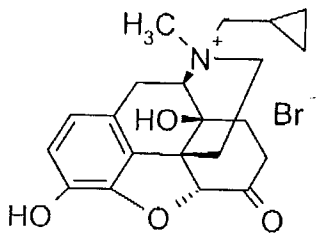
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-964
Review number: 000
Sequence number/date/type of submission: 000/March 30, 2007/Original
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Progenics Pharmaceuticals, Inc., Tarrytown, NY.
Manufacturer for drug substance: Mallinckrodt Inc., St. Louis, Missouri

Reviewer name: Tamal. K. Chakraborti
Division name: Division of Gastroenterology Products
HFD #: 180
Review completion date: November 29, 2007

Drug:

Trade name: Not provided
Generic name: N-Methylnaltrexone bromide/Naltrexone methobromide (MNTX)
Code name: MOA-728
Chemical name: N-(cyclopropylmethyl)-noroxymorphone methobromide
CAS registry number: 73232-52-7
Molecular formula/molecular weight: $C_{21}H_{26}NO_4.Br/436$
Structure:



Relevant INDs/NDAs/DMFs:

1. IND 64,583 (Methylnaltrexone Injection, Progenics, HFD-180)
2. IND

Drug class: Methylnaltrexone (MNTX) is a selective, peripheral μ-type opioid receptor antagonist.

Intended clinical population: Methylnaltrexone Injection is indicated for the treatment of opioid-induced constipation in patients receiving palliative care. The recommended human dose is mg/kg, SC and the maximum recommended human dose is approximately mg/kg, SC.

Clinical formulation: Methylnaltrexone Bromide Injection, 20 mg/mL is provided in a 3-mL clear, glass vial, gray rubber stopper, and aluminum overseal with flip-off-cap. The fill volume of the drug product is in order to provide a withdrawable volume of 0.6 mL. The complete list of components and composition of Methylnaltrexone Bromide Injection, 20 mg/mL is provided below (from page 1 of Sec. 2.3.P.1).

Table 2.3.P.1.1: Composition of Methylnaltrexone Bromide Injection, 20 mg/mL

Component	Grade	Quantity/ 0.6 mL	Concentration (mg/mL)	Function
Methylnaltrexone Bromide	In-house Standard	12 mg [a]		
Sodium Chloride	USP/Ph.Eur.	3.9 mg		
Edetate Calcium Disodium	USP	0.24 mg		
Glycine Hydrochloride	In-house	0.18 mg		
Hydrochloric Acid	NF/Ph.Eur.			
Sodium Hydroxide	NF/Ph.Eur.			
Water for Injection	USP/Ph.Eur.	q.s. to 0.6 mL		

a. Input based on 100% potency. This amount will be adjusted based upon actual assay of the drug substance.

The following table (from Section 2.3.S.3 of sponsor's submission) shows the impurity profile of MNTX.

1 Page(s) Withheld

X Trade Secret / Confidential

_____ Draft Labeling

_____ Deliberative Process

Withheld Track Number: Pharm/Tox-1

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission: The following table lists the studies reviewed in this submission.

STUDY	REPORT NO.	TESTING LAB.	LOT/ BATCH NO.	REV. PAGE #
PHARMACOLOGY*				
ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION				
ABSORPTION				
Pharmacokinetics of MNTX in rats following a single SC dose	0835RP45001, AA04298-1	2	D04957	33
Pharmacokinetics of MNTX in rats following a single SC dose	AA04298	2, 3	D04957	33
Phamracokinetics of MNTX in dogs following a single SC and IV dose	AA04300, 0832DP45001, AA04300-1	2, 3	D04957	34
Pharmacokinetic study in fasted male rats comparing single IV, SC and PO dose	65072	5	H10206	35
Pharmacokinetics in rats following oral administration of MNTX	020598	3	D04957	36
DISTRIBUTION				
Quantitative tissue distribution study in rats following an IV administration of ^{14}C-MNTX	013153	6	XPY MDSPSRS PG-45	37
In vitro protein binding in rats, dogs and human plasma	107N-0305PBR	4	E06275	50
Uptake of radioactivity and metabolite profiles in the brain of male rats following a single IV administration of ^{3}H-MNTX	65672	7	SEL/1674	52
Lacteal excretion and placental transfer of ^{3}H-MNTX following a single SC administration in rats	7434-120	8	SEL/1674, B06274	54
METABOLISM				
Glucoronidation of MNTX in human, rat and Beagle dog microsomes	107N-0407	4	NA	55
In vitro metabolism of MNTX in the mouse, rat, dog and human hepatic preparations	7434-112	8	SEL/1308 A	56
Metabolite profile of MNTX in the plasma, urine and feces from CD-1 mice following a single oral dose of ^{3}H-MNTX	107N-0521	4	SEL/1674	58
Metabolite profile in male rats following a single IV or oral dose of ^{3}H-MNTX	65621	7	SEL/1674	59
Mass balance, pharmacokinetic and metabolite profile in rats following IV administration of ^{14}C-MNTX	013151A	6	NA	61
Metabolite profile of MNTX in the bile and feces from bile duct cannulated rats following a single PO or IV dose of ^{3}H-MNTX	107N-0602	4	SEL/1674	62

Metabolite profiles in male Beagle dogs following a single IV and PO dose of ^{3}H-MNTX	65622	7	SEL/1674	64
Mass balance, pharmacokinetic and metabolite profile in Beagle dogs following IV and PO administration of ^{14}C-MNTX	013152A	6	NA	65
EXCRETION				
Excretion, mass balance and pharmacokinetics in CD-1 mice following a single oral dose of ^{3}H-MNTX	107-0520B	4	SEL/1674	66
Mass balance and pharmacokinetics of in rats following an IV dose of ^{14}C-MNTX	013151	6	XPY MDSPSRS PG-37	67
Biliary excretion of total radioactivity in bile duct cannulated male rats following a single PO or IV dose of ^{3}H-MNTX	107-0601	6	SEL/1674	68
Mass balance and pharmacokinetics in Beagle dogs following an IV and PO dose of ^{14}C-MNTX	013152	6	XPY MDSPSRS PG-37	69
TOXICOLOGY				
Acute				77
Rat				77
Single, SC	0406RP45001	2	D04957	77
Single, SC	64345	9	H10206	77
Single, SC	65328	9	H10207	77
Subacute/Subchronic/Chronic				
Mouse				
7-Day, PO (Gavage)	7434-102	9	D10795	80
90-Day, PO (Gavage)	7434-101	10	D10795	82
Rat				
14-Day, IV	PH437-UR-001-89	11	0151D1	89
14-Day, IV	64868	9	H010206, H10207	93
90-Day, IV	154-003	12	C13753, C13754	99
7-Day, PO	154-001	12	D05340	105
28-Day, PO	154-012	12	D04957	107
6-Month, PO	7434-103	10	D04957, D12461, D10785, D10125, E06275	112
Dog				
14-Day, IV	PH432-UR-001-89	11	0151D1	121
90-Day, IV	154-004	12	C13754	124
7-Day, PO	154-009	12	C13753	133
7-Day, PO	154-010	12	C13753, D05340	135
28-Day, PO	154-013	12	D10125	137
39-Week, PO	7434-104	8	D10785, E06274, E06276	146

GENOTOXICITY				
Chromosome aberration test in Chinese hamster ovary (CHO) cells	AA53RH331B TL	13	C13754	147
Ames test	AA53RH503B TL	13	C13754	152
L5178Y $TK^{+/-}$ Mouse lymphoma forward mutation assay	7434-105	10	D04957	155
Chromosome aberration test in human peripheral blood lymphocytes (HPBL)	7434-106	10	D04957	163
In vivo micronucleus test in mice, IP	AA53RH123B TL	13	C13754	171
In vivo micronucleus test in mice, SC	7434-100	10	D04957	175
REPRODUCTIVE TOXICITY				
Rat				
Segment I, SC	2516-004	14	D05340	179
Segment II, IV	2516-001	14	C13754	183
Segment III, SC	2516-003	14	D05340	198
Rabbits				
Segment II, IV	2516-002	14	C13574	190
Local Tolerance				
Rabbit, SC	0406LP45002	2	D04957	205

1. [redacted]
2. [redacted]
3. [redacted]
4. [redacted]
5. Wyeth Research, Pearl River, NY
6. [redacted]
7. Wyeth Research, Collegeville, PA
8. [redacted]
9. Wyeth Research, Chazy, NY
10. [redacted]
11. [redacted]
12. [redacted]
13. [redacted]
14. [redacted]

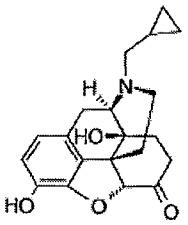
*: Studies reviewed either under IND 64,583 [redacted] The pharmacology reviews of these studies are incorporated in the appropriate sections of this review.

Studies not reviewed within this submission: [redacted] were not reviewed.

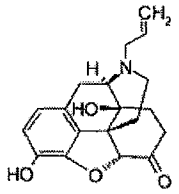
2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

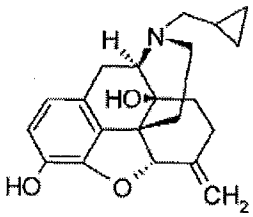
Methylnaltrexone is a quaternary derivative of naltrexone (NTX), an opioid antagonist. Other opioid antagonists such as naltrexone, naloxone and nalmefene (structures are shown below) are fairly lipid soluble and readily cross the blood-brain barrier. The addition of the methyl group in the amine ring of NTX forms a compound with greater polarity and lower lipid solubility that allows limited access to the blood-brain barrier in humans. These properties provide MNTX with the potential to block undesirable peripheral side effects (constipation, nausea and vomiting, biliary colic, urinary retention and pruritis) of opioid pain medications through peripheral opioid receptors, while sparing centrally mediated analgesic effects.



Naltrexone:



Naloxone:



Nalmefene

Pharmacology studies have been conducted with MNTX using several *in vitro* and *in vivo* experiments. In the NDA, the sponsor only submitted *in vitro* receptor binding study report in support of primary pharmacology. The sponsor submitted several pharmacology study reports under IND 64,583, which were reviewed previously under IND 64,583 initial submission. This brief summary includes the main findings of those studies including the study submitted under this NDA. Methylnaltrexone was found have lower affinity (IC50 = 305 nM) for the rat brain opiate receptors compared to naltrexone (IC50 = 7.2 nM). The drug was found to be more selective for μ-type opioid receptors (IC50 = 300 nM) in the GI tract compared to κ- (19-fold less potent) and δ- (ineffective) type receptors in isolated gastric-brainstem preparation in neonatal rats. The Ki value for MNTX for human μ and κ receptor were 28 and 230 nM, respectively, whereas the Ki value for the δ receptor was > 10 μM. In several *in vivo* studies, MNTX was shown to inhibit morphine-induced effects (spike potential of duodenal smooth muscle, gastric transit time, emesis, cough, etc.) without sparing central analgesic effect. Methylnaltrexone did not produce behavioral signs of opioid withdrawal (tremor, yawning, and restlessness) in acutely opioid-dependent dogs. However, in rodents,

MNTX was demethylated, possibly forming naltrexone. Methylnaltrexone delayed the GI transit in rats. Overall, pharmacology studies appear to support the intended marketing indication.

In safety pharmacology studies, MNTX at IV doses ranging from 1 to 20 mg/kg had no apparent toxicologically significant effects on the neuropharmacological profile in mice, gastrointestinal function in rats, pulmonary function in guinea pigs, or renal function in rats. Cardiovascular safety pharmacology studies were conducted using adequate battery of *in vitro* and *in vivo* tests. Methylnaltrexone showed significant cardiovascular effects in these studies. In hERG assay, MNTX caused concentration-dependent IKr inhibition (1%, 12%, 13% and 40% inhibition of hERG current at 30, 100, 300 and 1000 μM concentrations, respectively, compared to vehicle control; IC-50 > 1000 μM). In isolated canine (dog) Purkinje fibers, MNTX caused prolongations in action potential duration at 60% repolarization (APD_{60}: 13%, 21% and 15% at 1, 3 and 10 μM, respectively) and 90% repolarization (APD_{90}: 10%, 16% and 12% at 1, 3 and 10 μM, respectively) at basic cycle length (BCL) of 2 sec compared to baseline values. The highest tested concentration in the canine Purkinje fiber study (10 μM) was about 18 and 37 times the Cmax at human SC doses of 0.3 (Cmax = 234 ng/mL) and 0.15 mg/kg (Cmax = 117 ng/mL), respectively. In isolated rabbit Purkinje fibers, MNTX caused concentration-dependent increase in APD_{60} (2%, 4% and 10% at 1, 10 and 100 μM at BCL of 2 sec) and APD_{90} (1%, 3% and 6% at 1, 10 and 100 μM at BCL of 2 sec) when compared to baseline values at all three stimulus intervals (BCL of 2, 1 and 0.5 sec). The highest MNTX concentration (100 μM) tested in the rabbit Purkinje fiber study was about 186 and 373 times the Cmax at human SC doses of 0.3 and 0.15 mg/kg, respectively. In a cardiovascular safety study (0247DP45.001) in anesthetized dogs at 1, 5, and 25 mg/kg, IV, MNTX caused decreases in blood pressure (up to 13%), heart rate (8-17%), cardiac output (4-18%), left ventricular pressure (<5 to 10%), left ventricular end diastolic pressure (up to 6%), and +dP/dt (12-19%) at ≥ 1 mg/kg. The magnitude and duration of the effects were generally dose-related. In a second cardiovascular IV safety pharmacology study (940-001) in conscious Beagle dogs at 1, 5 and 20 mg/kg, MNTX caused a dose-related increase in QTc interval when compared to vehicle control (males: 2%, 5% and 10% increase at 1, 5 and 20 mg/kg, respectively, at 45 minutes after treatment; females: 3%, 5% and 12% increase at 1, 5 and 20 mg/kg, respectively, at 1 hour after treatment). Predicted exposures (C_{5min} and $AUC_{0-\infty}$ values of 56,483 ng/mL and 25,222 ng.h/mL, respectively) after a single IV dosage of 20 mg/kg to beagle dogs were approximately 482 and 144 times, respectively, the exposure (Cmax =117 ng/mL and AUC = 175 ng·h/mL) at a human SC dose of 0.15 mg/kg and 241 times and 66 times, respectively, the exposure (Cmax = 234 ng/mL and AUC = 382 ng·h/mL) at a human SC dose of 0.3 mg/kg. In conscious guinea pigs (tested at 1, 5 and 20 mg/kg), mild prolongation of QTc (4% over baseline) was observed at 20 mg/kg IV. Based on the prolongation of QT and increase in the action potential durations cardiovascular safety pharmacology studies, MNTX appears to have significant potential for QT prolongations in humans. A thorough QT study in humans will help to address this issue.

2.6.2.2 Primary pharmacodynamics

Mechanism of Action:

IN VITRO ACTIVITY AND SELECTIVITY OF NALTREXONE METHOBROMIDE
[redacted] Study No. 1026730, 2002)

Receptor Binding Screening Assay

N-Methylnaltrexone was tested at 10 μM in radioligand binding assays for a large number (81) of receptors, transporters, and ion channels. Significant binding activity was detected only at the μ and κ opioid receptors (human). As a follow-up, complete competition binding assays were performed for these receptors. The K_i values for the μ and κ receptors were 28 and 230 nM, respectively. Weak binding activity was detected at the δ opioid receptor (23% inhibition), α_{2B} adrenergic receptor (38% inhibition), CCK_B receptor (28% inhibition), and histamine H_1 receptor (33% inhibition).

Drug Activity Related to Proposed Indication:

In the NDA, the sponsor only submitted *in vitro* receptor binding study report in support of primary pharmacology. The sponsor submitted several pharmacology study reports under IND 64,583, which were reviewed previously under IND 64,583 Initial submission. This brief summary includes the main findings of those studies including the study submitted under this NDA.

In dogs, SC or IV (0.25 to 5.0 mg/kg) administration of MNTX was shown to inhibit morphine-induced spike potentials in a dose-related manner from duodenal smooth muscle. Methylnaltrexone was shown to inhibit dose-related morphine-induced slowing of gastrointestinal transit time in rats at 1 to 60 mg/kg, SC while sparing the opioid analgesic effects. Methylnaltrexone also dose dependently antagonized morphine induced-emesis in Mongrel dogs when administered at 0.06 to 1.0 mg/kg by either intramuscular (IM) or IV route. In Guinea pigs, MNTX administered intraperitoneally (IP) at 2.0 mg/kg blocked the incidence of cough induced by morphine. In several studies in mice and rats, MNTX failed to alter the central opioid activity. Methylnaltrexone (1 to 50 mg/kg) did not produce behavioral signs of opioid withdrawal (tremor, yawning, and restlessness) in acutely opioid-dependent dogs. However, in some species (rodents), MNTX was demethylated, possibly forming naltrexone, which can enter CNS and may precipitate CNS effects. Overall, pharmacology studies appear to support the proposed marketing indication.

2.6.2.3 Secondary pharmacodynamics

The sponsor submitted secondary pharmacology study reports under IND 64,583 initial submission, which were reviewed previously and are incorporated below from the pharmacology review dated November 8, 2002 of IND 64,583.

Secondary Pharmacodynamics:

Dose-Related Antagonism of the Emetic Effect of Morphine by Methylnaltrexone in Dogs

Opioids administered to produce analgesia cause unwanted emesis in patients (incidence 20%-30%, depending on situation). Tests in animals show that quaternary narcotic antagonists like methylnaltrexone (MNTX) do not affect the analgesic potency of morphine, but such compounds have not been examined for their potential to antagonize morphine-induced emesis. To determine the effects of MNTX on emetic response, mongrel dogs (n = 6 – 9) were challenged with morphine alone or morphine and various doses of MNTX i.m. (intramuscular) or i.v. Antagonism of the emetic response was dose related: MNTX, 0.25 mg/kg i.m. or 0.2 mg/kg i.v., completely blocked the emetic effect of morphine in dogs for approximately 60 minutes. These data indicate that opioid-induced emesis might be prevented by MNTX without affecting analgesia.

Prevention of Apomorphine- or Cisplatin-Induced Emesis in the Dog by a Combination of Methylnaltrexone and Morphine

Morphine can have either an emetic or an antiemetic effect. The emetic effect of morphine can be blocked by methylnaltrexone (MNTX), a quaternary opioid antagonist with peripheral action. This study was conducted to test the hypothesis that administering MNTX to block the peripheral emetic effect of morphine would unmask the central antiemetic effect of the morphine. The net result would be a reduction in apomorphine- or cisplatin-induced emesis. MNTX 0.25 mg/kg and morphine 1 mg/kg were administered to conscious dogs, which were then challenged with the potent emetic agents apomorphine or cisplatin. Emesis was assessed by the presence of characteristic retching motions accompanied by the regurgitation of gastric contents. Apomorphine challenges of 0.1 mg/kg and of 0.03 mg/kg produced 100% emesis in control animals. After pretreatment with MNTX and morphine, the frequency of emesis with the larger dose of apomorphine was reduced to 50% and with the smaller dose to 22%. MNTX alone did not block apomorphine-induced emesis. In animals challenged with cisplatin 3 mg/kg, the emetic response was 100%. Emesis did not occur in animals pretreated with MNTX 0.25 mg/kg and morphine 1 mg/kg before cisplatin. The results demonstrated that MNTX combined with morphine reduces apomorphine-induced emesis and blocks cisplatin-induced emesis. These results appear to support the hypothesis that the emetic effect of morphine is peripheral and its antiemetic action is central. Therefore, MNTX in combination with morphine may have a clinical role in the treatment of chemotherapy-induced emesis.

Antagonism of Inhibition of Gastric Acid Secretion by Morphine in the Rat by MNTX

In this study, the effects of MNTX on morphine-induced inhibition of gastric acid secretion were studied in the pylorus-ligated rat. Morphine decreased gastric acid secretion more potently after i.c.v. than after i.v. administration. The inhibitory effect of i.v. administered morphine on gastric acid secretion was not blocked by the quaternary opioid antagonist naltrexone methylbromide (MNTX, 10 mg/kg) when given s.c. However, when naltrexone methylbromide was administered i.c.v., it blocked completely the effects of i.c.v. morphine and partially antagonized the effects of

i.v. morphine, indicating a central site of action for morphine.

Effects of Methylnaltrexone on Morphine-Induced Cough Suppression in Guinea Pigs

In this study, MNTX was administered to guinea pigs previously injected with morphine sulfate to determine whether the compound could block opioid-induced cough suppression without blocking antinociception. The effects of methylnaltrexone (0.8, 1.6 and 2.0 mg/kg) and of naltrexone (0.01, 0.02, 0.16 and 0.32 mg/kg) were compared in animals who had been injected with morphine sulfate (8.1 mg/kg). At 2.0 mg/kg of MNTX, number of coughs returned to baseline value and nociception remained unaffected. At the two higher doses of naltrexone (0.32 and 0.16 mg/kg), morphine-induced antitussive effect was blocked, but antinociception was reversed. The results suggested that methylnaltrexone possesses opioid antagonist activity in receptors peripheral to the blood-brain barrier.

Lack of Interference with Central Opioid Activity

Role of Central Versus Peripheral Opioid Receptors in Analgesia Induced by Repeated Administration of Opioid Antagonists in Rats

Although analgesia induced by blockade of opioid receptors has been well established, it is still unknown whether its development is mediated by the blockade of centrally located opioid receptors. In this study, rats were treated with either systemically or i.c.v. applied naloxone or MNTX. Following MNTX administration, each animal was tested for paw lick latency on a 51^{0}C hot plate. Hot plate testing and drug injections were carried out for 4 consecutive days. Rats treated with i.c.v. microinjections of MNTX or naloxone displayed paw lick latencies that were significantly longer than those observed in control animals. In contrast, rats treated with s.c. injections of MNTX did not show any increase in paw lick latency, whereas rats treated with s.c. injections of naloxone displayed paw lick latencies that were significantly longer than those of control rats. These results support that MNTX does not easily penetrate the blood-brain barrier and the blockade of central opioid receptors underlies the development of an analgesic response.

Reversal of Morphine-Induced Catalepsy in the Rat by Methylnaltrexone

In this study, the effects of methylnaltrexone was examined in reversing the catalepsy induced by morphine in rats. Morphine, 20 mg/kg, induced rigid catalepsy that attained a peak effect (as manifested by duration of catalepsy) at 60-120 min and progressively declined thereafter. Subcutaneous administration of methylnaltrexone only partially reversed the catalepsy at doses up to 56 mg/kg 60-90 min post-morphine. The extent of the reversal of catalepsy produced by methylnaltrexone tended to increase with time. Methylnaltrexone, administered into the cerebral ventricle 70 min after the injection of morphine, completely suppressed the catalepsy with an ED_{50} of 1 μg/kg when tested at 90 min after morphine. These results indicate that opiate-induced catalepsy is predominantly mediated at sites within the central nervous system. Methylnaltrexone is about 10,000 times more potent in reversing catalepsy when administered centrally than when administered peripherally, which suggested its peripheral action.

Lack of Precipitation of Opioid Withdrawal

Withdrawal Precipitating Properties of MNTX in Monkeys

MNTX was studied for antagonism of acute opiate effects on precipitation of withdrawal in morphine-dependent rhesus monkeys. MNTX was ineffective in precipitating withdrawal in morphine-dependent rhesus monkeys up to 32 mg/kg, s.c. In contrast, naltrexone (0.004 mg/kg) and naloxone (0.01 mg/kg) and nalorphine (0.09 mg/kg), all of which enter the CNS, were found to be active in this model.

Central Regulation of Intestinal Function: Morphine Withdrawal Diarrhea

In this study in morphine-dependent rats, MNTX (30 mg/kg, s.c.) did not produce statistically significant occurrence of withdrawal diarrhea as measured by the incidence of diarrhea or fecal weight; however, MNTX precipitated withdrawal diarrhea when administered intracerebroventricularly (i.c.v.) at a dose of 20 µg. In contrast, peripheral administration of naloxone (0.03 to 0.12 mg/kg i.v. or s.c.) precipitated withdrawal diarrhea in this model. The results of this suggested peripheral action of MNTX.

2.6.2.4 Safety pharmacology

Neurological Effects:

Neuropharmacological Profile of Methylnaltrexone in Mice (Study No. 0200MP45.001)

Three groups of ten CD-1 mice were intravenously (i.v.) administered MNTX at 1, 4 and 16 mg/kg. The control animals were treated with saline. The mice were observed for signs (functional observational battery such as seizure/convulsion, motor activity, grip strength, ataxia, stereotypy etc.) of toxicity at 3, 15 and 30 min, 1, 2, 3, 4, and 24 hours following treatment. There were no MNTX-related findings at any of the tested doses.

Cardiovascular Effects:

In Vitro Studies

Effects of Methylnaltrexone on Cloned hERG Channels Expressed in Mammalian Cells (Study 030714.QSF)

In this study, the *in vitro* effects of MNTX on ionic currents were determined in voltage-clamped human embryonic kidney cells (HK293) stably expressing the human ether-à-go-go-related gene (hERG). Four concentrations of MNTX (30, 100, 300 and 1000 μM) were tested. MNTX inhibited hERG current by 1.1% (n = 3), 12.1% (n = 3), 12.8% (n = 5) and 39.8% (n = 4) at 30, 100, 300 and 1000 μM, respectively. In addition, four concentrations of cisapride (0.003, 0.01, 0.03 and 0.1 μM) were tested as positive control. Cisapride inhibited hERG current by 6.6 (n = 3), 14.0% (n = 3), 34.0% (n = 3) and 71.1% (n = 3) at 0.003, 0.01, 0.03 and 0.1 μM, respectively, with an IC-50 value of 0.051 μM. MNTX did not produce greater than 50% block at the highest concentration. The IC-50 for MNTX appeared to be >1000 μM. The following table (from page 20 of the study report) shows the summary results.

Table 2: Summary statistics for MNTX inhibition of hERG current
Mean percent inhibition at each MNTX concentration (Mean), standard deviation (SD), standard error of the mean (SEM), and number of cells (N). *Significantly different ($P<0.05$) from vehicle control.

Concentration (μM)	Mean	SD	SEM	N
0	0.4%	0.3%	0.2%	4
30	1.1%	1.2%	0.7%	3
100	12.1%	4.6%	2.7%	3
300	12.8%	8.1%	3.6%	5
1,000	39.8%*	14.6%	7.3%	4

Effects of Methylnaltrexone on Action Potentials in Isolated Canine Cardiac Purkinje Fibers (Study 030109.QSF)

The *in vitro* effects of MNTX on cardiac action potentials in isolated, canine (Beagle dogs) Purkinje fibers were evaluated at 1, 3, and 10 μM concentrations (n = 4 fibers). Three concentrations of MNTX (1, 3 and 10 μM) were applied sequentially in ascending order without washout between applications, to each Purkinje fiber. The vehicle was distilled water. At the end of the vehicle exposure period the positive control article (1 μM cisapride) was applied. Intracellular membrane potentials were recorded using conventional intracellular micro electrodes. MNTX had no marked effects on resting potential or on action potential amplitude or rate of rise. Statistically significant prolongation of APD60 and APD90 was observed at all concentrations. MNTX at 1, 3, and 10 μM, respectively, prolonged the APD60 by 13%, 21%, and 15% at a pacing interval of 2s (corresponding to a bradycardic heart rate of 30 beats/minute). At the same BCL, the APD90 was prolonged by 10, 16 and 12% at 1, 3 and 10 μM, respectively.

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In contrast, 1 μM cisapride prolonged the APD60 and APD90 by 42.1 % and 48.2% under identical experimental conditions. Overall, MNTX prolonged cardiac action potential duration. The following table (from page 17 of the study report) shows the summary results.

11 Tables

Table 1. Summary of the Effects (Mean ± S.E.M.) of MNTX on Action Potential Parameters at 2 s, 1 s and 0.5 s BCL's

BCL = 2 s					
	APD_{60}	APD_{90}	RMP	APA	Vmax
Concentration	Δ% ± S.E.M.	Δ% ± S.E.M.	ΔmV ± S.E.M.	ΔmV ± S.E.M.	Δ%
1 μM	13.0 ± 2.6 *	9.8 ± 2.7 *	-2.4 ± 0.5	-1.1 ± 1.0	0.2 ± 8.3
3 μM	21.1 ± 4.8 *	16.4 ± 4.5 *	-1.5 ± 1.4	-2.0 ± 3.2	1.0 ± 8.2
10 μM	15.4 ± 0.9 *	12.3 ± 0.9 *	-1.7 ± 0.8 *	-1.9 ± 1.7	0.6 ± 7.4

BCL = 1s					
	APD_{60}	APD_{90}	RMP	APA	Vmax
Concentration	Δ% ± S.E.M.	Δ% ± S.E.M.	ΔmV ± S.E.M.	ΔmV ± S.E.M.	Δ%
1 μM	11.5 ± 3.0 *	8.8 ± 2.8 *	-2.5 ± 0.7	1.3 ± 1.0	-3.9 ± 7.8
3 μM	12.4 ± 4.3 *	9.5 ± 4.1	-1.9 ± 1.2	-0.1 ± 3.3	0.1 ± 9.4
10 μM	10.9 ± 2.6 *	8.7 ± 2.2 *	-1.6 ± 0.6	0.7 ± 1.9	2.6 ± 7.8

BCL = 0.5 s					
	APD_{60}	APD_{90}	RMP	APA	Vmax
Concentration	Δ% ± S.E.M.	Δ% ± S.E.M.	ΔmV ± S.E.M.	ΔmV ± S.E.M.	Δ%
1 μM	9.1 ± 1.8 *	7.3 ± 1.7 *	-2.7 ± 0.6	0.9 ± 1.3	-4.9 ± 8.0
3 μM	7.0 ± 1.8 *	5.3 ± 1.1 *	-2.3 ± 1.0	-1.5 ± 3.2	-9.4 ± 10.4
10 μM	8.4 ± 0.9 *	7.0 ± 1.1 *	-2.2 ± 0.8	0.0 ± 1.7	-2.6 ± 4.8

BCL, Basic Cycle Length; APD_{60} and APD_{90}, action potential duration measured at 60% and 90% repolarization; Δ%, Percent change from baseline values; ΔmV, absolute change from baseline in millivolts; NA, not applicable; RMP, resting membrane potential; APA, action potential amplitude; Vmax, maximum action potential rate of rise. * Statistically different ($p<0.05$) from time matched vehicle controls (1 μM vs vehicle sequence 1; 3 μM vs vehicle sequence 2; 10 μM vs vehicle sequence 3 in Tables 9-14).

Effect of Methylnaltrexone on Action Potentials in Isolated Rabbit Cardiac Purkinje Fibers - Study 03 1222.QSF)

In this study, the *in vitro* effects of MNTX on cardiac action potentials in isolated rabbit Purkinje fibers were determined at 1, 10 and 100 μM concentrations. Drug solutions were added sequentially to four fiber preparations at three stimulus intervals [basic cycle lengths (BCL) of 2 (bradycardic, 30 beats/min), 1 (normocardic, 60 beats/min) and 0.5 (tachycardic, 120 beats/min) s] and the effects of MNTX on action potential parameters were compared to the vehicle control sequences. MNTX increased APD60 (action potential duration at 60% repolarization) and APD90 (action potential duration at 90% repolarization) at all three stimulus intervals (BCL of 2, 1 and 0.5) in a concentration-dependent manner. MNTX did not induce statistically significant changes in resting membrane potential (RP) or the maximum rate of depolarization (Vmax) at any of the tested concentrations. MNTX induced statistically significant increases (≤1.2 mV) in action potential amplitude (APA) at 100 μM concentrations when compared to vehicle controls. Cisapride at 100 and 500 nM (0.1 and 0.5 μM) concentrations (BCL = 2, 1 and 0.5 s) induced statistically significant prolongation of APD60 (29.4% - 89.5%) when compared to vehicle controls. Cisapride at 100 nM (0.1 μM; BCL = 2, 1 and 0.5) induced statistically significant prolongation of APD90 (36.1% - 44.2%). Cisapride at 500 nM (BCL = 1 and 0.5) induced statistically significant prolongation of APD90 (63.2% - 136.3%). The following table (from page 19 of the study report) shows the summary data for MNTX.

Table 1. Summary of the Effects of MNTX

2 s BCL					
MNTX	APD60	APD90	RMP	APA	Vmax
μM	(Δ%)	(Δ%)	(ΔmV)	(ΔmV)	(Δ%)
1	1.6 ± 2.9	1.0 ± 2.2	0.2 ± 0.3	-2.4 ± 1.2	-17.7 ± 8.3
10	4.4 ± 3.2	2.9 ± 2.5	-0.3 ± 0.6	-0.8 ± 1.2	-19.9 ± 10.9
100	10.5 ± 5.7	5.8 ± 4.7	-1.2 ± 0.7	0.3 ± 1.0*	-21.4 ± 12.1

1 s BCL					
MNTX	APD60	APD90	RMP	APA	Vmax
μM	(Δ%)	(Δ%)	(ΔmV)	(ΔmV)	(Δ%)
1	1.5 ± 3.0	0.8 ± 2.2	0.2 ± 0.5	-2.1 ± 1.2	-13.6 ± 5.9
10	3.1 ± 3.7	1.9 ± 2.9	-0.3 ± 0.5	-0.2 ± 0.7	-19.7 ± 9.1
100	8.8 ± 5.0	5.5 ± 4.3	-0.9 ± 0.7	1.2 ± 0.6*	-20.2 ± 9.8

0.5 s BCL					
MNTX	APD60	APD90	RMP	APA	Vmax
μM	(Δ%)	(Δ%)	(ΔmV)	(ΔmV)	(Δ%)
1	0.9 ± 2.1	1.3 ± 1.6	0.4 ± 0.9	-3.7 ± 1.7	-15.6 ± 5.3
10	1.8 ± 2.6	1.6 ± 2.1	0.7 ± 0.7	-1.4 ± 0.8	-23.8 ± 11.8
100	6.3 ± 3.4	4.9 ± 3.1	-0.2 ± 0.9	0.0 ± 1.0*	-23.1 ± 11.7

BCL, Basic Cycle Length; APD_{60} and APD_{90}, action potential duration measured at 60% and 90% repolarization; Δ%, Percent change from baseline values (Mean ± SEM, n = 4 fibers); ΔmV, absolute change from baseline in millivolts; RMP, resting membrane potential; APA, action potential amplitude; Vmax, maximum rate of depolarization.
*Denotes statistical significance ($P < 0.05$) when compared to vehicle control sequence.

In Vivo Studies

Cardiovascular (Hemodynamics) Evaluation of Methylnaltrexone in Dogs (Study No. 0247DP45.001)

The acute effects of intravenous (i.v.) doses of methylnaltrexone (MNTX) on cardiac and circulatory functions were examined in beagle dogs. The dogs were anesthetized and surgically prepared (open chest) and evaluated for systolic, diastolic and mean arterial pressures. Heart rate, left ventricular pressure, left ventricular end diastolic pressure, +dP/dt, cardiac output and lead II ECG were also evaluated. Each dog received single individual doses of MNTX at 1, 5 and 25 mg/kg in an escalating manner. MNTX showed varied effects among four animals. MNTX caused decrease (20 to 27%) in blood pressure, heart rate, cardiac output, left ventricular pressure, +dP/dt and left ventricular end diastolic pressure. Some of the changes (heart rate, cardiac output and +dP/dt) appeared to be dose-related. Transient changes in p-wave amplitude were also observed in 3 of 4 dogs. One dog exhibited decreased p-wave amplitude from 2 – 5 min after 25 mg/kg. Another two dogs showed increased p-wave amplitude at 5 and 25 mg/kg. The sponsor stated that these changes were indicative of autonomic nervous system effects of test article and not cardiac specific. It is to be mentioned here that abnormalities of the P wave seen during sinus rhythm are sometime associated with atrial fibrillation. The QT interval was also not measured in this study. The sponsor did not submit any data regarding the P wave duration also. The sponsor needs to conduct additional cardiac-hemodynamic studies (effect on P wave amplitude, duration and QT interval in sinus as well as in pacing heart) in dogs to address these issues further.

Cardiovascular Study (940-001) in Beagle Dogs

Methods: In a previous cardiovascular hemodynamic study at 1, 5 and 25 mg/kg, i.v. (0247DP.45.001) in Beagle dogs, MNTX caused decrease in p-wave amplitude at 5 and 25 mg/kg doses in one dog and increase in p-wave amplitude in another dog at 1 and 5 mg/kg but not at 25 mg/kg. In the absence of a dose response, p-wave amplitude changes were considered to be due to autonomic nervous system effect. To further evaluate the potential cardiovascular effects, the sponsor conducted this current study in conscious dogs using telemetry.
In this study, dogs (Group 2-4, n = 2/sex/dose, 9 months old) received intravenous bolus injection of MNTX at 1, 5, and 20 mg/kg (0.5 ml/kg). The control animals (Group 1) received the vehicle (0.9% sodium chloride for injection, USP). The dogs received each treatment once for four consecutive days by slow bolus intravenous injection over approximately one minute. The test article was administered in ascending dose order and an approximately 24 hour period separated each administration. The following parameters were examined from Day -1 (approximately 24 hours predose) to Day 5 (approximately 24 hours postdose): blood pressure, heart rate, body temperature, and the electrocardiograms (ECG: RR, PR, QRS, and QT intervals). The heart rate-corrected QT interval (QTc) was calculated using the method described by Spence et al [*Toxicological Sciences* 1988, 45 (2): 247-258] using analysis of covariance and linear regression model. Data were collected and analyzed in 15-minute bins during the course of the study. Physiological parameters were monitored for at least 24 hours predose and for at least 24 hours postdose. In addition, during the study, the animals were observed twice daily for mortality, morbidity, injury, and the availability of food and water. Detailed clinical examinations were conducted predose and approximately 2, 5, 10 and 30 minutes postdose and 1 and 2 hours postdose on Days 1-4. Body weights were collected prior to each dose administration. At the termination of the study, the animals were euthanized and discarded without further evaluation.

Results: The sponsor presented raw data in this submission. There was no concurrent positive control in this study. No significant changes were observed in body weight, body temperature, systolic, diastolic and mean arterial pressure and heart rate. ECG parameters (RR, PR, QRS intervals and morphology of tracings) were unaffected by treatment. In the vehicle control, QTc values did not appear to change over time. The maximum effects on QTc were observed at 45-60 minute after treatment especially at high dose in both sexes. In males, there were 2%, 5% and 10% increase in QTc over vehicle control (mean = 220 msec, n = 2/sex/dose) at 1, 5 and 20 mg/kg, respectively, at 45 minutes after treatment. In females, there were 2.8%, 9.4% and 11.7% increase in QTc over vehicle control (mean = 213 msec, n = 2/sex/dose) at 1, 5 and 20 mg/kg, respectively, at 1 hour after treatment. Overall, the results of this study indicated a dose-related increase in QTc interval when compared to vehicle control. The maximum increase of 10-11% over control was observed at 20 mg/kg (human equivalent dose of 11 mg/kg, which is about 6.2-fold higher than the proposed human dose of 1.8 mg/kg, i.v. for the proposed Phase 3 study).

Addendum: The incidence of QT_c prolongation that could be considered as clinically significant was limited to one female, which exhibited QT_c increases of up to 29 msec after administration of 20 mg/kg (relative to the maximum baseline value of 249 msec). QT_c values that were measured after administration of vehicle did not exceed the maximum value observed in the 24-hr baseline recording period.

Addendum: The following table shows the QTc data from the above study. This is addendum also corrects the percent increases of QTc in females mentioned in line 8 of the results section. In the results section above, the stating " In females, there was 2.8%, 9.4% and 11.7 % increase....." should be replaced by In females, there was 2.8%, 5% and 11.7% increase .....". This addendum also adds the following table for this study for better understanding of the study results.

MALE		QTc (msec)	
Group	**Animal Number**	**45 min**	**% Control (Average)**
0 mg/kg	902	225	100%
	903	216	
1 mg/kg	902	234	102%
	903	216	
5 mg/kg	902	241	105%
	903	222	
20 mg/kg	902	252	110%
	903	234	

FEMALE		QTc (msec)	
Group	**Animal Number**	**60 min**	**% Control (Average)**
0 mg/kg	904	217	100%
	905	209	
1 mg/kg	904	225	103%
	905	213	
5 mg/kg	904	229	105%
	905	217	
20 mg/kg	904	255	112%
	905	222	

Electrophysiological Assessment of the Effects of Methylnaltrexone on Electrocardiographic Function and Arterial Blood Pressure in Intact Awake Guinea Pigs (Study 400120-1)

The objective of this study was to determine the potential effects of MNTX at 1, 5, and 20 mg/kg administered intravenously on electrocardiographic function and arterial blood pressure in six intact awake guinea pigs. Recorded ECG parameters included PQ, RR, QRS, QT and corrected QT (QTc, Fridericia's correction) intervals. After baseline ECG recordings (at least 30 min), guinea pigs were administered the test article (six animals), the positive control cisapride (two animals), or vehicle (two animals). Solutions of MNTX or the vehicle were infused intravenously in volumes of 0.5 mL for 1 minute. MNTX solutions were administered in an escalating dose manner in each treated animal, with individual doses of 1, 5, or 20 mg/kg and at a rate of 0.5 mL/min. Fifteen second interval data were selected for analysis at 2, 5, 10, and 15 minutes after each injection. Cisapride (3 mg/kg) was infused over a 15-minute period (at a rate of 0.037 mL/min for one animal and 0.032 mL/min for the other animal) beginning at the conclusion of baseline, and animals were monitored for 45 minutes thereafter.

There were no statistically significant differences between the MNTX and vehicle-treated groups at any time for any ECG parameters. However, a few differences between MNTX 20 mg/kg and lower doses of MNTX were found to be statistically significant. The RR interval 2 minutes after injection of 20 mg/kg MNTX (mean, 165.8 msec) was significantly greater (a difference of 5%) than the RR interval at 2 minutes after 5 mg/kg MNTX (mean, 157.4 msec). The baseline RR interval was 165.0 msec. There were no statistically significant differences in the QT and QTc intervals between the vehicle and any dose of MNTX (1, 5, or 20 mg/kg) at any time point. However, the QT and QTc intervals 2 and 5 minutes after 20 mg/kg were 6% greater than the intervals at 2 and 5 minutes after 1 mg/kg. The intervals at 5 minutes after 20 mg/kg were 5% greater than those at 5 minutes after 5 mg/kg. These differences were statistically significant. Overall, MNTX caused an apparent dose-related increase in QT and QTc intervals. Mean arterial blood pressure ranged from 59.7 to 69.0 mmHg through all times prior to injection of 20 mg/kg MNTX but dropped to 35.4 and 53.6 mmHg at 2 and 5 minutes after that injection. The decrease in systemic blood pressure at the 2 min time point after injection of 20 mg/kg MNTX was statistically significant in comparison to values at the same time in the vehicle group. There were no significant differences in diastolic blood pressure (DBP) and mean arterial pressure (MAP) when comparisons were analyzed between the vehicle group and any dose of MNTX (1, 5, or 20 mg/kg) at any time point. The positive control cisapride produced increases in the RR, QT and QTc intervals when compared to baseline values.

Overall, IV infusion of MNTX produced significant reductions in systolic blood pressure and increase in QT, QTc and RR intervals. The following tables (from pages 25, 28 and 29 of sponsor's submission) show the effects of MNTX on RR, QT and QTc intervals.

IND 64,583 Serial No. 104 and 134

21. TABLES

Table 21.1. RR Interval (msec)

Dose Level	Time Following Dosing	Vehicle Group Mean (Standard Error) (N=2)	Test Article Group Mean (Standard Error) (N=6)	Sig. Diff. Between Test Article and Vehicle Dose Groups?[a]
Baseline	0	154.0 (5.3)	165.0 (7.3)	No
MNTX (1 mg/kg)	2 min	154.2 (7.0)	159.0 (5.5)	No
	5 min	153.4 (5.2)	159.0 (4.4)	No
	10 min	156.3 (6.8)	161.7 (6.3)	No
	15 min	152.8 (7.5)	162.2 (4.3)	No
MNTX (5 mg/kg)	2 min	155.0 (8.4)	157.4 (5.2)	No
	5 min	151.6 (6.8)	159.4 (5.1)	No
	10 min	157.8 (9.6)	159.5 (7.3)	No
	15 min	145.3 (.)	165.4 (12.9)	No
MNTX (20 mg/kg)	2 min	148.5 (.)	165.8 (8.6)	No
	5 min	155.7 (8.9)	162.5 (6.7)	No
	10 min	156.4 (5.8)	158.8 (5.7)	No
	15 min	156.1 (7.4)	159.2 (6.7)	No
Significant Diff. Between Test Article Concentrations at Corresponding Times Following Dosing?[b]		Yes MNTX (5 mg/kg) vs. MNTX (20 mg/kg) at 2 min		
Significant Diff. Between Test Article Concentrations and Baseline?[c]		Yes MNTX (1 mg/kg) at 2 min and 5 min MNTX (5 mg/kg) at 2 min		

a. As determined by conducting F-tests within a repeated measures ANOVA applied to the vehicle and test article group averages, and adjusting the p-values of the 13 tests using the Benjamini and Hochberg approach to controlling the false discovery rate to no higher than 0.05 across all 13 time points simultaneously. When the p-value in this column is less than 0.05, significant test article dose level effects were concluded versus vehicle. No significant differences among dose levels were observed at any of these time points.
b. A t-test for significant differences among test article dose levels at corresponding times following dosing was performed at the 0.05/3=0.0167 level within the repeated measures ANOVA.
c. A t-test for significant differences between test article dose levels versus baseline was performed at the 0.05 level within the repeated measures ANOVA.

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Table 21.4. QT Interval (msec)

Dose Level	Time Following Dosing	Vehicle Group Mean (Standard Error) (N=2)	Test Article Group Mean (Standard Error) (N=6)	Sig. Diff. Between Test Article and Vehicle Dose Groups?[a]
Baseline	0	89.4 (5.8)	88.7 (3.7)	No
MNTX (1 mg/kg)	2 min	88.1 (4.7)	87.4 (3.1)	No
	5 min	91.8 (9.1)	88.9 (3.0)	No
	10 min	90.4 (7.7)	88.8 (3.6)	No
	15 min	88.5 (6.1)	90.2 (4.4)	No
MNTX (5 mg/kg)	2 min	89.4 (7.3)	89.4 (3.8)	No
	5 min	88.3 (6.7)	90.2 (3.3)	No
	10 min	89.9 (7.7)	89.0 (4.2)	No
	15 min	81.3 (.)	87.5 (6.6)	No
MNTX (20 mg/kg)	2 min	82.5 (.)	93.9 (6.1)	No
	5 min	89.9 (7.6)	95.6 (5.7)	No
	10 min	90.1 (7.3)	90.0 (3.9)	No
	15 min	90.7 (7.8)	88.7 (4.4)	No
Significant Diff. Between Test Article Concentrations at Corresponding Times Following Dosing?[b]		Yes MNTX (1 mg/kg) vs. MNTX (20 mg/kg) at 2 min MNTX (1 mg/kg) vs. MNTX (20 mg/kg) at 5 min MNTX (5 mg/kg) vs. MNTX (20 mg/kg) at 5 min		
Significant Diff. Between Test Article Concentrations and Baseline?[c]		Yes MNTX (20 mg/kg) at 2 min and 5 min		

a. As determined by conducting F-tests within a repeated measures ANOVA applied to the vehicle and test article group averages, and adjusting the p-values of the 13 tests using the Benjamini and Hochberg approach to controlling the false discovery rate to no higher than 0.05 across all 13 time points simultaneously. When the p-value in this column is less than 0.05, significant test article dose level effects were concluded versus vehicle. No significant differences among dose levels were observed at any of these time points.

b. A t-test for significant differences among test article dose levels at corresponding times following dosing was performed at the 0.05/3=0.0167 level within the repeated measures ANOVA.

c. A t-test for significant differences between test article dose levels versus baseline was performed at the 0.05 level within the repeated measures ANOVA.

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Table 21.5. QTc(F) Interval (msec)

Dose Level	Time Following Dosing	Vehicle Group Mean (Standard Error) (N=2)	Test Article Group Mean (Standard Error) (N=6)	Sig. Diff. Between Test Article and Vehicle Dose Groups?[a]
Baseline	0	166.6 (6.8)	161.7 (4.7)	No
MNTX (1 mg/kg)	2 min	164.3 (6.2)	161.3 (4.4)	No
	5 min	171.4 (15.0)	164.0 (4.8)	No
	10 min	167.6 (11.9)	162.8 (5.1)	No
	15 min	165.4 (8.7)	165.2 (7.0)	No
MNTX (5 mg/kg)	2 min	168.3 (10.5)	165.5 (5.8)	No
	5 min	165.4 (10.0)	166.2 (4.8)	No
	10 min	166.2 (10.8)	163.9 (5.6)	No
	15 min	154.6 (.)	159.2 (7.9)	No
MNTX (20 mg/kg)	2 min	155.8 (.)	170.7 (9.1)	No
	5 min	167.0 (11.0)	174.9 (8.4)	No
	10 min	167.1 (11.5)	166.1 (5.9)	No
	15 min	168.2 (11.8)	163.6 (6.4)	No
Significant Diff. Between Test Article Concentrations at Corresponding Times Following Dosing?[b]		Yes MNTX (1 mg/kg) vs. MNTX (20 mg/kg) at 2 min MNTX (1 mg/kg) vs. MNTX (20 mg/kg) at 5 min MNTX (5 mg/kg) vs. MNTX (20 mg/kg) at 5 min		
Significant Diff. Between Test Article Concentrations and Baseline?[c]		Yes MNTX (20 mg/kg) at 2 min and 5 min		

a. As determined by conducting F-tests within a repeated measures ANOVA applied to the vehicle and test article group averages, and adjusting the p-values of the 13 tests using the Benjamini and Hochberg approach to controlling the false discovery rate to no higher than 0.05 across all 13 time points simultaneously. When the p-value in this column is less than 0.05, significant test article dose level effects were concluded versus vehicle. No significant differences among dose levels were observed at any of these time points.

b. A t-test for significant differences among test article dose levels at corresponding times following dosing was performed at the 0.05/3=0.0167 level within the repeated measures ANOVA.

c. A t-test for significant differences between test article dose levels versus baseline was performed at the 0.05 level within the repeated measures ANOVA.

Pulmonary Effects:

Pulmonary Assessment of Methylnaltrexone in the Guinea Pig (Study No. 1082GP45.001)

This study was conducted to determine the potential effects of MNTX on pulmonary function in the guinea pig. In this study, Hartley guinea pigs (4/dose), were intravenously administered single doses of MNTX at 0, 1, 4 and 16 mg/kg (dose volume = 1 ml/kg). The animals were anesthetized and surgically prepared to evaluate airway resistance, dynamic lung compliance, respiratory rate, tidal volume and minute volume. MNTX did not cause any statistically significant effect at any dose level compared to vehicle treated control (saline, 0.9%).

Renal Effects:

EVALUATION OF THE EFFECTS OF INTRAVENOUSLY ADMINISTERED METHYLNALTREXONE ON RENAL FUNCTION IN RATS LABORATORY STUDY IDENTIFICATION NUMBER 940-002, 2002)

Renal Effects:

Renal Function Study in Rats

Methods: Male CD®(SD)IGS BR rats (approximately 9 weeks old; 245-279 g) were treated intravenously with 0 (vehicle), 1, 5, or 20 mg/kg methylnaltrexone, using a dose volume of 1 ml/kg (10 rats/group). The vehicle was 0.9% NaCl. Immediately after dosing, the rats were treated orally by gavage with 5 ml/kg water. Urine was collected for about three hours following post-dose fluid loading. Blood was collected for measurement of clinical chemistry parameters after urine collection.

Results: One death occurred in the 20 mg/kg group as a result of a gavage error during administration of water. Breathing difficulty and shallow breathing was observed in all treatment groups. Rapid or slow breathing was observed at 20 mg/kg. Tremors and abdominal distension occurred once in the 20 mg/kg group. Clinical chemistry parameters (including urea nitrogen and creatinine) were unaffected.

Gastrointestinal Effects:

Gastrointestinal Propulsion Assay in Rats Following Intravenous Administration of Methlynaltrexone (Study No. 0239RP45.001)

In this study, MNTX was administered by a single intravenous injection to 4 groups of 10 male Sprague-Dawley rats per group, at treatment levels of 0, 1, 4 and 16 mg/kg. A 10% suspension of activated charcoal in 0.25% methylcellulose was orally administered approximately 5 minutes after the i.v. MNTX injection. The rats were sacrificed 30 min after the charcoal treatment. GI transit in each animal was determined by the total distance (centimetre), of charcoal movement from the pyloric sphincter to caecum. Although not statistically significant, MNTX caused a dose-related mean decrease of 4%, 6%, and 11% at 1, 4, and 16 mg/kg, respectively, when compared to control. MNTX might have the potential to decrease intestinal motility.

Abuse Liability:

Effects of Opiate Antagonists and Their Quaternary Derivatives on Heroin Self-Administration in the Rat

This study was conducted to examine the potential of MNTX for abuse or dependency in the rat. Naltrexone methobromide (MNTX), was compared with the parent compound (naltrexone) for their ability to antagonize the reinforcing properties of heroin as measured in an operant, i.v., self-administration paradigm. Lower doses (up to 0.2 mg/kg) of naltrexone produced dose-dependent increases in heroin self-administration, but at higher doses (10-30 mg/kg) these drugs produced transient decreases (20-100 min) in self-administration followed by recovery. Naltrexone was approximately 1.5 times more potent than naloxone in increasing heroin self-administration at the lower doses (up to 0.2 mg/kg) and had a slightly longer duration of action. MNTX was ineffective as antagonists of heroin self-administration in doses 200 times greater than the effective antagonist dose of naloxone or naltrexone. As MNTX has limited access to CNS, these results support the notion that acute reinforcing property of i.v. opiates involve opiate receptors located within the central nervous system and do not involve peripheral opiate receptors.

Other: None

2.6.2.5 Pharmacodynamic drug interactions

None

2.6.3 PHARMACOLOGY TABULATED SUMMARY

None included

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Generally, MNTX exposure (AUC and Cmax) was found to be greater than dose proportional after SC, IV, and PO dosing in rats and dogs. No significant gender differences were observed in pharmacokinetic parameters in rats and dogs. Methylnaltrexone was distributed mostly to the small intestine, liver and kidney, in the rat, with the brain and skeletal muscle having the lowest concentrations at one hour after dosing. Penetration to the brain was limited with lower concentrations in the brain than in all other tissues. Minimal amounts of MNTX were found in brain of rat and rabbit after administration of high IV or epidural doses. Plasma protein binding was found to be minimal. Metabolic pathways included hydroxylation, reduction, methylation, sulfation and glucuronidation. Plasma metabolite profiles suggested minimal metabolism of

MNTX in human subjects following IV administration. The most abundant metabolites in the human plasma were MNTX sulfate, and methyl-6α-naltrexol and methyl-6ß-naltrexol isomers. Methylnaltrexone sulfate and methyl-6ß-naltrexol were also produced in the rat plasma. The major circulating metabolite in dogs was MNTX glucuronide. In human liver microsomes, MNTX inhibited the activity of CYP2D6 with a Ki value of 8 µM. Drug-drug interactions involving MNTX and CYP2D6 substrates could be possible following IV administration. Methylnaltrexone did not induce any of the cytochrome P450 isoforms when tested *in vitro*. Methylnaltrexone was secreted into the bile in rats and dogs. In bile duct cannulated rats, about 16% of the administered dose was excreted in the bile following IV administration. In rats and dogs, the major route of excretion was via the urine after IV dosing and via the feces after oral dosing in mice and dogs. In pregnant rats, MNTX-derived radioactivity rapidly crossed the placenta. The fetal exposure was approximately 10% of the maternal exposure. In lactating rats, MNTX-derived radioactivity was excreted into the breast milk. Methylnaltrexone did not appear to be a p-glycoprotein substrate in Caco-2 cell monolayers. However, MNTX appeared to be a substrate of the human organic cation transporter (OCT1).

2.6.4.2 Methods of Analysis

[Discussed see under individual study reviews]

2.6.4.3 Absorption

PLASMA PHARMACOKINETICS OF METHYLNALTREXONE IN RATS ADMINISTERED A SINGLE SUBCUTANEOUS DOSE [redacted] STUDY NO. 0835RP45.001, [redacted] PROJECT NO. AA04298_1 AND [redacted] REPORT NO. AA04298, 2003)

Methods: This study was conducted to examine the plasma pharmacokinetics of MNTX in rats administered a single SC dose. In this study, three groups of male and female rats (n = 39/sex/group) received a single SC dose of MNTX at 1, 5 and 20 mg/kg. Blood samples were collected terminally from 3 animals/sex/time point/group. Plasma samples were analyzed for MNTX using a validated liquid chromatography-tandem mass spectroscopy (LC-MS/MS) method.

Results: Following a single SC dose of MNTX at 1, 5 and 20 mg/kg, the mean Cmax values were 564, 4613 and 14000 ng/mL in male rats and 461, 3013 and 18433 ng/mL in female rats, respectively. The corresponding mean AUC_{0-8} values were 279, 1613 and 8934 ng.hr/mL in male rats and 240, 1533 and 9873 ng.hr/mL in female rats, respectively. The mean Tmax values ranged from approximately 5 to 10 minutes post-dose in male and female rats. The mean elimination half-live values at doses of 1, 5, and

20 mg/kg were 2.70, 8.98 and 6.56 hours in male rats and 2.94, 12 and 11 hours in female rats, respectively. The PK parameters are shown in the table below (page 15 of the report AA04298).

25/Jul/2003

Table 1
REPORT NO. AA04298
PROTOCOL NO. 0835R
Summary of Results - Toxicokinetics Parameters for Methylnaltrexone in Plasma Following a Single Subcutaneous Administration of Methylnaltrexone to Male and Female Sprague-Dawley Rats

Group Number	Dose (mg/kg)	Gender	AUC_{0-t} (ng.h/mL)	AUC_{inf} (ng.h/mL)	AUC_{0-t}/AUC_{inf} (%)	Cmax (ng/mL)	tmax (h)	kel (1/h)	t½ (h)	CL/F (L/h/kg)	$V_{d\ area}/F$ (L/kg)	AUC_{inf}/Dose	Cmax/Dose
1	1	Male	279	279	100.0	553.7	0.0810	0.2570	2.70	3.58	13.9	279.0	553.7
2	5	Male	1606	1613	99.6	4613.3	0.0760	0.0772	8.98	3.10	40.2	322.6	922.7
3	20	Male	8918	8934	99.8	14000.0	0.1660	0.1057	6.56	2.24	21.2	446.7	700.0
1	1	Female	240	240	99.9	461.0	0.0760	0.2359	2.94	4.16	17.7	240.0	461.0
2	5	Female	1526	1533	99.5	3013.3	0.0840	0.0600	11.56	3.26	54.4	306.6	602.7
3	20	Female	9842	9873	99.7	18433.3	0.0830	0.0626	11.07	2.03	32.3	493.7	921.7

Note: Total plasma clearance (CL/F), calculated as $Dose/AUC_{inf}$
Volume of distribution ($V_{d\ area}/F$) was calculated as $Dose/(AUC_{inf}*kel)$

PLASMA PHARMACOKINETICS OF METHYLNALTREXONE IN DOGS ADMINISTERED A SINGLE SUBCUTANEOUS AND INTRAVENOUS DOSE STUDY NUMBER 0832DP45.001, PROJECT NO. AA04300_1 AND REPORT NO. AA04300, 2003)

Methods: This study was conducted to examine the plasma pharmacokinetics of MNTX in dogs administered a single SC or IV dose of MNTX. In this study, a group of six beagle dogs (n = 3/sex) received a single SC dose of MNTX at 1, 5 and 20 mg/kg and then a single IV dose of MNTX at 1, 5 and 20 mg/kg, with a one-week washout period between each dose in a cross-over fashion. Plasma samples were analyzed for MNTX using a validated LC-MS/MS method.

Results: Based on AUC values, systemic exposure to MNTX following SC and IV administration in male and female Beagle dogs appeared to be more than dose-proportional over the dose range studied. Systemic exposure to MNTX was similar in both sexes. At the 1 and 20 mg/kg dose levels, half-life, clearance and volume of distribution were independent of gender. Following a single SC dose of MNTX at 1, 5 and 20 mg/kg, the mean Cmax values were 437, 2543 and 14467 ng/mL in male dogs and 361, 3110 and 11037 ng/mL in female dogs, respectively. The corresponding mean AUC_{0-8} values were 667, 4457 and 24334 ng.hr/mL in male dogs and 725, 5240 and 25251 ng.hr/mL in female dogs, respectively. The mean Tmax values ranged from 0.23 to 0.67 hours. The mean elimination half-live values ranged from 9.5 to 14.1 hours regardless of sex. The mean absolute bioavailability ranged from 83.7 to 112% in male dogs and from 78.9 to 102% in female dogs. Overall, the exposure (AUC) values were

comparable following SC and IV dose in both sexes. The PK parameters are shown in the following table (from page 15 of the study report).

Table 1
REPORT NO. AA04300
PROTOCOL NO. 0832D
Summary of Results - Toxicokinetics Parameters for Methylnaltrexone in Plasma Following a Single Subcutaneous and Intravenous Administration of Methylnaltrexone to Male and Female Beagle Dogs

Gender	Dose (mg/kg)	Route of Administration	AUC_{0-t} (ng.h/mL)	AUC_{inf} (ng.h/mL)	AUC_{0-t}/AUC_{inf} (%)	Cmax (ng/mL)	Tmax (h)	kel (1/h)	t½ (h)	CL* (L/h/kg)	$V_{d\,area}$* (L/kg)	AUC_{inf}/Dose	Cmax/Dose	F (%)
Male	1	SC	836.5	866.5	95.83	437.3	0.233	0.06807	11.182	1.504	24.13	866.7	437.3	83.65
Male	5	SC	4306.4	4456.6	96.58	2543.3	0.419	0.07911	9.524	1.126	15.62	891.3	508.7	96.32
Male	20	SC	23742.9	24333.7	97.50	14466.7	0.592	0.06164	11.985	0.8272	14.52	1216.7	723.3	112.06
Male	1	IV	713.8	800.4	89.16	3646.7	0.000	0.04369	16.13	1.254	29.25	800.0	3646.7	N/A
Male	5	IV	4394.6	4670.8	94.13	15566.7	0.000	0.03965	18.08	1.076	27.81	934.2	3113.3	N/A
Male	20	IV	21315.8	21872.1	97.45	76000.0	0.000	0.05997	12.003	0.9171	15.81	1093.6	3800.0	N/A
Female	1	SC	686.8	725.0	94.71	361.0	0.508	0.05409	10.84	1.401	21.93	725.0	361.0	78.92
Female	5	SC	4953.8	5240.3	94.39	3110.0	0.494	0.05087	14.10	0.9609	19.85	1048.1	622.0	101.85
Female	20	SC	24316.7	25250.8	96.17	11035.7	0.668	0.05199	13.97	0.8007	16.45	1262.5	551.8	88.97
Female	1	IV	798.2	920.3	86.44	3696.7	0.000	0.04238	16.82	1.093	26.75	920.3	3696.7	N/A
Female	5	IV	4993.6	5215.7	95.51	20900.0	0.000	0.05779	12.340	0.9853	17.69	1043.1	4180.0	N/A
Female	20	IV	27733.0	28570.7	96.88	126166.7	0.000	0.05984	13.012	0.7132	13.76	1428.5	6308.3	N/A

*For subcutaneous administration, parameters should read CL/F and $V_{d\,area}$/F and were calculated as Dose/AUC_{inf} and Dose/(AUC_{inf}*kel), respectively.
N/A - Not applicable
SC - Subcutaneous
IV - Intravenous

SINGLE INTRAVENOUS, SUBCUTANEOUS AND ORAL (GAVAGE) DOSE PHARMACOKINETIC STUDY IN FASTED MALE RATS (REPORT NO. 65072)

Methods: In this study, MNTX was administered to male rats to estimate PK parameters of MNTX and its SC and oral bioavailability in the fasted state. Methylnaltrexone was administered as a single 2 mg/kg IV (bolus) or SC dose or as a single 100 mg/kg oral (gavage) dose to fasted male rats. Blood samples were collected at predetermined time points after dosing. The plasma MNTX concentrations were determined using a validated LC/MS/MS method.

Results: Following a single 2 mg/kg IV dose of MNTX to fasted male rats, the mean Cmax and AUC_{0-8} values were 1059 ng/mL and 397 ng.hr/mL, respectively. The mean $t_{1/2}$ value was 5.9 hr. Following a single 2 mg/kg SC dose of MNTX to fasted male rats, the mean Cmax and AUC_{0-8} values were 1252 ng/mL and 497 ng.hr/mL, respectively. The Tmax was observed at 5 min post-dose. The mean $t_{1/2}$ value was 7.5 hr. The mean apparent subcutaneous bioavailability was calculated to be 125% in fasted male rats. Following a single 100 mg/kg oral (gavage) dose of MNTX to fasted male rats, the mean Cmax and AUC_{0-8} values were 42.3 ng/mL and 147 ng.hr/mL, respectively. The Tmax was observed between 0.5 and 2 hr post-dose. The mean $t_{1/2}$ value was 4.2 hr. The mean apparent oral bioavailability was calculated to be 0.74% in fasted male rats. Overall, the PK parameters appeared to be comparable following SC and IV administration of MNTX at 2 mg/kg. The following table (from page 20 of the report) shows the PK parameters.

Table 6.0-4: Individual and Mean (± SD) Pharmacokinetic Parameters of MOA-728 in Fasted Male Rats Following a Single Dose Intravenous (IV, Bolus), Subcutaneous (SC) or Oral (Gavage) Administration of MOA-728 (Protocol 06_1501)

Dose mg/kg	Route	SAN	Body Weight (kg)	$C_{5min/max}$[a] (ng/mL)	t_{max} (hr)	AUC_{0-24} (ng•hr/mL)	$AUC_{0-\infty}$ (ng•hr/mL)	CL_T (L/hr/kg)	Vd_{ss} (L/kg)	$t_{1/2}$ (hr)	F[b] (%)
2	IV	1	0.22	1035	NA	372	372	5.37	2.12	7.1	NA
		2	0.22	1150	NA	441	ND	ND	ND	ND	NA
		3	0.23	959	NA	388	389	5.15	3.09	5.3	NA
		4	0.24	1093	NA	429	429	4.66	2.59	5.1	NA
		Mean ± SD	0.23 ± 0.01	1059 ± 82	NA	407 ± 33	397 ± 29	5.06 ± 0.36	2.60 ± 0.48	5.9 ± 1.1	NA
2	SC	5	0.23	1329	0.083	424	425	NA	NA	6.1	NA
		6	0.22	918	0.083	433	433	NA	NA	7.0	NA
		7	0.22	1466	0.083	580	581	NA	NA	8.3	NA
		8	0.26	1296	0.083	546	547	NA	NA	8.4	NA
		Mean ± SD	0.23 ± 0.02	1252 ± 235	0.083 ± 0.000	496 ± 79	497 ± 79	NA	NA	7.5 ± 1.1	125
100	Oral	9	0.22	52.5	0.50	122	124	NA	NA	5.0	NA
		10	0.22	17.5	2.00	114	121	NA	NA	5.4	NA
		11	0.23	68.5	0.50	223	224	NA	NA	3.8	NA
		12	0.23	30.8	2.00	119	120	NA	NA	2.7	NA
		Mean ± SD	0.23 ± 0.01	42.3 ± 22.6	1.25 ± 0.87	145 ± 53	147 ± 51	NA	NA	4.2 ± 1.2	0.74

SAN. Study Animal Number
a. C_{5min} for IV route; C_{max} for SC and oral route
b. $[(AUC_{0-\infty\ (SC\ or\ oral)} \bullet Dose_{(IV)})/(AUC_{0-\infty\ (IV)} \bullet Dose_{(SC\ or\ oral)})] \bullet 100$
NA. Not applicable
ND. Not determined due to insufficient data in the terminal phase

PHARMACOKINETIC STUDY IN MALE RATS FOLLOWING ORAL ADMINISTRATION OF N-METHYLNALTREXONE REPORT NO. 020598)

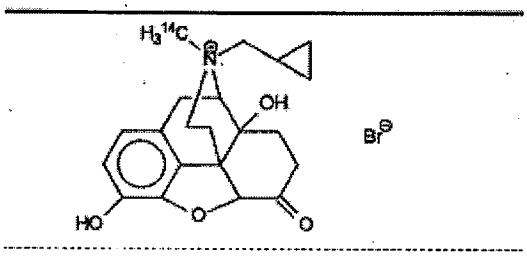
Methods: This study was conducted to determine the PK profiles and urine recovery of MNTX at various doses after oral administration to male Sprague Dawley (SD) rats. N-methylnaltrexone was administered as a single oral (gavage) dose to male rats (n = 39/dose group) at 80, 400, 1723 and 3169 mg/kg. Plasma and urine samples were quantified for MNTX concentrations by validated LC-MS/MS methods.

Results: Following a single oral dose of MNTX at 80, 400 and 1723 mg/kg, the mean Cmax values were 83, 1426 and 101100 ng/mL, respectively. The corresponding AUC_{0-24} values were 411, 2951 and 905243 ng.hr/mL, respectively. The mean Tmax values ranged from 0.25 to 0.75 hours post-dose. The MNTX elimination half-life values were estimated to be 12.5 and 9.8 hours for the 80 and 400 mg/kg groups, respectively; and the corresponding AUC_{0-8} values were 578 and 3126 ng.hr/mL, respectively. The PK parameters were not determined for the 3169 mg/kg dose group due to high mortality. The mean urine recoveries of MNTX up to 48 hours post-dose were 0.40, 2.38, 4.82 and 3.01% at 80, 400, 1723 and 3169 mg/kg, respectively, with the majority of the MNTX recovered within 6 hours post-dose.

2.6.4.4 Distribution

QUANTITATIVE TISSUE DISTRIBUTION STUDY FOLLOWING AN INTRAVENOUS ADMINISTRATION OF [^{14}C]MNTX TO SPRAGUE DAWLEY RATS (REPORT NO. 013153, REPORT NO. 013153)

Methods: This study was conducted to assess the tissue distribution of MNTX following a single IV administration of [^{14}C]-MNTX to male and female rats. In this study, 34 male and 34 female SD rats were divided into two groups. All rats in Group 1 received ^{14}C-MNTX (specific activity = 19.81 μCi/mg) at 10 mg/kg. The following figure (from page 39 of the study report) shows the position of radiolabeling.



Three male and 3 female rats were sacrificed at each of the following time-points: 1, 2, 3, 4, 6, 8, 12, 24, 48, 72 and 120 hr post-dose. Blood and selected tissues were collected from all rats. All samples were analyzed by liquid scintillation spectroscopy (LSS) for radioactivity content.

Results: In male rats, at 1 hr after a single (10 mg/kg) IV administration of [^{14}C]MNTX, the radioactivity concentrations in tissues were highest in the small intestine followed by the liver, kidney, brown fat, salivary glands, pancreas, large intestine, prostate, mesenteric lymph nodes, thymus and stomach. Tissue-to-plasma [^{14}C]MNTX-derived radioactivity concentration (T/P) ratios for male rats were calculated only up to 24 hr post-dose, due to undetectable plasma radioactivity concentrations after 24 hr. At 1 hr post-dose, the highest T/P ratio was found in the small intestine (133) followed by the liver (128), kidneys (75.9), salivary glands (12.8) and brown fat (12.6). The remaining tissues had T/P ratios less than 10 and 4 of these tissues (blood, testes, skeletal muscle and eyes) had ratios less than 1. From 2 to 12 hr post-dose, T/P ratios increased in most tissues with the majority of the values greater than 1. In general, T/P ratios decreased by 24 hr, with the exception of brown fat (365), thyroid (50.0), heart (34.9), peritoneal fat (20.6), eyes (13.2) and testes (8.05). These results suggested that [^{14}C]MNTX (parent or metabolite) may accumulate in these tissues.

In females, at 1 hr post-dose, the [^{14}C]MNTX-derived radioactivity concentrations in tissues were highest in the small intestine followed by the kidney, liver, brown fat, salivary glands, bone marrow, mesenteric lymph nodes, pancreas, large intestine, thymus, stomach and thyroid. The T/P ratios for female rats were calculated only up to 12 hr post-dose, due to undetectable plasma radioactivity concentrations after 12 hr. At 1 hr

post-dose, the highest T/P ratio was found in the small intestine followed by the kidneys, liver, brown fat, salivary glands and bone marrow. The remaining tissues had T/P ratios less than 10 and 5 tissues (bone, blood, peritoneal fat, skeletal muscle and brain) had ratios less than 1. From 2 to 12 hr post-dose, T/P ratios increased in most tissues, suggesting [^{14}C]MNTX (parent and/or metabolite) accumulation.

Overall, the highest amounts of [^{14}C]MNTX-derived radioactivity (>10 µg-equiv/g) in the males and females was found in the small intestine, liver and kidney, at 1 hr post-dose. The T/P [^{14}C]MNTX-derived radioactivity ratios were generally greater than 1, which indicated that [^{14}C]MNTX and its metabolites were widely distributed throughout the tissues. At 120 hr post-dose, less than 10% of the radioactivity equivalent to the respective Cmax values remained in tissues except for the testes (26.7%), eyes (25.0%), heart (10.7%) and thyroid (10.1%) in male rats, and the eyes (20.0%) and heart (10.8%) in female rats. Methylnaltrexone was distributed in the brain in both sexes. Maximum tissue/plasma (T/P) ratio for the brain was attained in the male (2.07) at 12 hr and in the female (2.06) at 6 hr postdose. At 24 hour postdose, the radioactivity disappeared from the brain in both sexes as evidenced by T/P ratio of zero. The following tables (form sponsor's submission) show the mean concentration of radioactivity in different tissues in male and female rats.

7.3. Mean Concentration of Drug-Derived Radioactivity (µg-equiv/g) in Tissues of Male Sprague Dawley Rats Following a Single Intravenous Dose of ^{14}C-MNTX

Tissue ID	Concentration (µg-equiv/g)			
	1 hr	2hr	3 hr	4 hr
Kidney	17.74	7.91	5.49	4.36
Liver	30.01	14.71	8.57	6.22
Plasma	0.24	0.08	0.07	0.04
Adrenals	0.45	0.42	0.61	0.65
Lymph N. (mesent.)	1.92	1.61	1.74	1.91
Lung	0.70	0.55	0.54	0.48
Blood	0.17	0.08	0.07	0.05
Spleen	0.59	0.49	0.49	0.40
Bone Marrow	0.47	0.42	0.41	0.38
Thyroid	0.79	1.26	0.82	0.87
Brown Fat	3.07	5.96	6.08	6.62
Skin	0.60	0.54	0.48	0.42
Thymus	1.71	1.28	1.70	1.73
Harderian Gland	0.35	0.25	0.28	0.29
Salivary Gland	3.00	2.65	2.89	2.98
Small Intestine	31.66	26.40	13.94	11.04
Heart	0.56	0.49	0.58	0.40
Prostate	2.00	0.75	0.39	0.29
Carcass	0.57	0.62	0.33	0.41
Seminal Vesicle	0.93	0.74	0.74	0.51
Testes	0.15	0.16	0.10	0.09
Pancreas	2.29	2.07	2.35	2.26
Epididymis	0.35	0.35	0.24	0.19
Large Intestine	2.23	2.39	4.49	7.34
Stomach	1.09	1.10	1.27	0.74
Bone	0.28	0.20	0.17	0.20
Skeletal Muscle	0.10	0.08	0.06	0.05
Eyes	0.38	0.19	0.18	0.14
Brain	0.10	0.07	0.05	0.04
Peritoneal Fat	0.26	0.27	0.38	0.29

7. 3. Mean Concentration of Drug-Derived Radioactivity (µg-equiv/g) in Tissues of Male Sprague Dawley Rats Following a Single Intravenous Dose of ^{14}C-MNTX (Cont'd.)

Tissue ID	Concentration (µg-equiv/g)			
	6 hr	8 hr	12 hr	24 hr
Kidney	1.87	1.65	1.34	0.69
Liver	2.10	1.13	0.46	0.23
Plasma	0.02	0.02	0.01	0.01
Adrenals	0.52	0.30	0.37	0.11
Lymph N. (mesent.)	1.24	1.65	0.81	0.38
Lung	0.41	0.30	0.24	0.12
Blood	0.03	0.02	0.00	0.00
Spleen	0.32	0.32	0.21	0.09
Bone Marrow	0.28	0.26	0.22	0.10
Thyroid	0.68	0.81	0.42	0.41
Brown Fat	3.74	3.77	2.96	2.77
Skin	0.34	0.30	0.24	0.15
Thymus	1.26[a]	0.72	0.58	0.23
Harderian Gland	0.25	0.26	0.23	0.14
Salivary Gland	2.34	1.78	1.25	0.55
Small Intestine	1.76	1.15	0.47	0.13
Heart	0.39	0.50	0.35	0.27
Prostate	0.23	0.25	0.17	0.07
Carcass	0.26	0.43	0.26	0.10
Seminal Vesicle	0.50	0.42	0.37	0.20
Testes	0.08	0.08	0.08	0.07
Pancreas	1.49	1.70	1.35	0.47
Epididymis	0.21	0.19	0.20	0.11
Large Intestine	7.90	5.96	1.99	0.28
Stomach	0.67	0.50	0.28	0.11
Bone	0.12	0.19	0.11	0.02
Skeletal Muscle	0.05	0.06	0.04	0.02
Eyes	0.12	0.12	0.10	0.09
Brain	0.03	0.02	0.02	0.00
Peritoneal Fat	0.20	0.34	0.26	0.15

a: Less Reliable Value for animal No. 1015

7.3. Mean Concentration of Drug-Derived Radioactivity (µg-equiv/g) in Tissues of Male Sprague Dawley Rats Following a Single Intravenous Dose of ^{14}C-MNTX (Cont'd.)

Tissue ID	Concentration (µg-equiv/g)		
	48 hr	72 hr	120 hr
Kidney	0.33	0.20	0.07
Liver	0.11	0.10	0.04
Plasma	0.00	0.00	0.00
Adrenals	0.04	0.00	0.00
Lymph N. (mesent.)	0.18	0.07	0.00
Lung	0.03	0.02	0.00
Blood	0.00	0.00	0.00
Spleen	0.03	0.02	0.00
Bone Marrow	0.04	0.01	0.00
Thyroid	0.27	0.18	0.08
Brown Fat	1.23	0.59	0.19
Skin	0.10	0.03	0.00
Thymus	0.03[a]	0.05	0.00
Harderian Gland	0.09	0.07	0.02
Salivary Gland	0.20	0.16	0.06
Small Intestine	0.04	0.03	0.00
Heart	0.20	0.15	0.06
Prostate	0.02	0.00	0.00
Carcass	0.08	0.08	0.02
Seminal Vesicle	0.07	0.04	0.02
Testes	0.05	0.04	0.04
Pancreas	0.06	0.02	0.00
Epididymis	0.07	0.05	0.03
Large Intestine	0.09	0.05	0.00
Stomach	0.01	0.00	0.00
Bone	0.00	0.00	0.00
Skeletal Muscle	0.00	0.00	0.00
Eyes	0.06	0.06	0.09
Brain	0.00	0.00	0.00
Peritoneal Fat	0.07	0.02	0.00

a: Less Reliable Value for animal No. 1026

7.4. Mean Concentration of Drug-Derived Radioactivity (µg-equiv/g) in Tissues of Female Sprague Dawley Rats Following a Single Intravenous Dose of ^{14}C-MNTX

Tissue ID	Concentration (µg-equiv/g)			
	1 hr	2 hr	3 hr	4 hr
Kidney	17.60	8.53	4.12	3.44
Liver	17.55	8.74	2.60	1.35
Plasma	0.33	0.10	0.04	0.03
Adrenals	0.54	0.49	0.35	0.28
Lymph N. (mesent.)	2.87	1.74	1.67	1.64
Lung	0.74	0.56	0.47	0.51
Blood	0.22	0.09	0.05	0.05
Spleen	0.78	0.51	0.45	0.41
Bone Marrow	3.23	0.47	0.36	0.36
Thyroid	1.28	0.98	1.02	1.03
Brown Fat	5.25	6.04	7.16	7.11
Skin	0.51	0.45	0.28	0.42
Thymus	1.60	2.03	1.52	1.28
Harderian Gland	0.34	0.27	0.31	0.33
Salivary Gland	4.89	4.35	3.54	3.00
Small Intestine	38.18	25.83	37.54	12.98
Heart	0.65	0.55	0.50	0.59
Ovaries	0.52	0.65	0.44	0.46
Carcass	0.48	0.38	0.48	0.52
Uterus	0.48	0.43	0.61	0.32
Pancreas	2.69	2.97	2.66	2.52
Large Intestine	2.35	2.13	11.00	9.92
Stomach	1.51	0.98	0.82	0.64
Bone	0.23	0.13	0.16	0.43
Skeletal Muscle	0.08	0.06	0.05	0.05
Eyes	0.35	0.19	0.13	0.13
Brain	0.10	0.06	0.04	0.04
Peritoneal Fat	0.22	0.17	0.16	0.30

7.4. Mean Concentration of Drug-Derived Radioactivity (µg-equiv/g) in Tissues of Female Sprague Dawley Rats Following a Single Intravenous Dose of ^{14}C-MNTX (Cont'd.)

Tissue ID	Concentration (µg-equiv/g)			
	6 hr	8 hr	12 hr	24 hr
Kidney	2.55	1.63	1.60	0.79
Liver	1.02	0.51	0.26	0.08
Plasma	0.02	0.01	0.01	0.00
Adrenals	0.33	0.13	0.15	0.07
Lymph N. (mesent.)	1.48	2.28	1.35	0.39
Lung	0.41	0.30	0.29	0.10
Blood	0.03	0.01	0.00	0.00
Spleen	0.33	0.28	0.21	0.07
Bone Marrow	0.34	0.27	0.22	0.09
Thyroid	1.11	1.09	0.71	1.00
Brown Fat	5.64	3.79	4.12	2.33
Skin	0.30	0.26	0.24	0.10
Thymus	1.50	0.76	0.67	0.20
Harderian Gland	0.23	0.21	0.21	0.14
Salivary Gland	3.43	2.39	1.61	0.63
Small Intestine	1.44	1.19	0.32	0.14
Heart	0.55	0.44	0.46	0.31
Ovaries	0.50	0.37	0.30	0.14
Carcass	0.58	0.39	0.20	0.10
Uterus	0.33	0.20	0.18	0.08
Pancreas	2.40	1.52	1.41	0.40
Large Intestine	7.36	16.77	2.00	0.21
Stomach	0.43	0.38	0.27	0.13
Bone	0.14	0.10	0.07	0.00
Skeletal Muscle	0.04[a]	0.03	0.03	0.00
Eyes	0.11	0.11	0.11	0.09
Brain	0.03	0.01	0.01	0.00
Peritoneal Fat	0.18	0.19	0.16	0.10

a: Less Reliable Value for animal No. 1514

7.4. Mean Concentration of Drug-Derived Radioactivity (µg-equiv/g) in Tissues of Female Sprague Dawley Rats Following a Single Intravenous Dose of ^{14}C-MNTX (Cont'd.)

Tissue ID	Concentration (ug-equiv/g)		
	48 hr	72 hr	120hr
Kidney	0.34	0.20	0.09
Liver	0.05	0.06	0.02
Plasma	0.00	0.00	0.00
Adrenals	0.01	0.00	0.00
Lymph N. (mesent.)	0.16	0.05	0.00
Lung	0.03	0.02	0.00
Blood	0.00	0.00	0.00
Spleen	0.02	0.00	0.00
Bone Marrow	0.03	0.03	0.00
Thyroid	0.26	0.20	0.00
Brown Fat	1.35	0.86	0.42
Skin	0.05	0.00	0.00
Thymus	0.05	0.00	0.00
Harderian Gland	0.09	0.07	0.03
Salivary Gland	0.21	0.13	0.10
Small Intestine	0.03	0.02	0.00
Heart	0.20	0.14	0.07
Ovaries	0.04	0.02	0.00
Carcass	0.06	0.07	0.02
Uterus	0.05	0.01	0.00
Pancreas	0.05[a]	0.01	0.00
Large Intestine	0.05	0.05	0.00
Stomach	0.02	0.00	0.00
Bone	0.00	0.00	0.00
Skefetal Muscle	0.00	0.00	0.00
Eyes	0.06	0.05	0.07
Brain	0.00	0.00	0.00
Peritoneal Fat	0.03	0.01	0.00

a: No sample was present for animal No. 1527

The following tables show (from sponsor's submission) mean tissue/plasma ratios of radioactivity in male and female rats.

7.5. Mean Tissue to Plasma Ratio of Drug-Derived Radioactivity in Tissues of Male Sprague Dawley Rats Following a Single Intravenous Administration of ^{14}C-MNTX

Tissue ID	Ratio			
	1 hr	2 hr	3 hr	4 hr
Kidney	75.85	95.80	79.92	109.09
Liver	128.02	181.11	123.33	152.39
Plasma	1.00	1.00	1.00	1.00
Adrenals	1.92	5.11	8.75	16.52
Lymph N. (mesent.)	8.18	19.25	25.57	47.19
Lung	3.01	6.66	7.85	11.65
Blood	0.71	0.94	0.99	1.21
Spleen	2.54	6.02	7.11	10.01
Bone Marrow	2.00	5.13	6.00	9.54
Thyroid	3.39	14.60	12.36	22.27
Brown Fat	12.55	72.48	88.71	165.10
Skin	2.57	6.38	6.89	10.53
Thymus	7.35	15.52	24.52	43.62
Harderian Gland	1.49	2.98	4.09	7.34
Salivary Gland	12.79	34.28	41.46	74.70
Small Intestine	133.01	319.39	204.86	266.73
Heart	2.36	5.90	8.38	10.25
Prostate	7.47	9.52	5.87	7.15
Carcass	2.44	7.22	4.82	10.36
Seminal Vesicle	3.99	8.90	10.51	12.82
Testes	0.65	1.98	1.49	2.31
Pancreas	9.83	24.76	34.67	57.19
Epididymis	1.47	4.51	3.39	4.82
Large Intestine	9.54	29.23	62.14	183.86
Stomach	4.56	13.13	18.22	18.69
Bone	1.21	2.40	2.46	4.90
Skeletal Muscle	0.44	1.02	0.90	1.37
Eyes	1.51	2.28	2.58	3.63
Brain	0.44	0.87	0.79	1.09
Perifoneal Fat	1.16	3.18	5.75	7.21

7.5. Mean Tissue to Plasma Ratio of Drug-Derived Radioactivity in Tissues of Male Sprague Dawley Rats Following a Single Intravenous Administration of ^{14}C-MNTX (Cont'd.)

Tissue ID	Ratio			
	6 hr	8 hr	12 hr	24hr
Kidney	78.07	100.94	126.18	90.86
Liver	84.58	68.90	43.29	31.36
Plasma	1.00	1.00	1.00	1.00
Adrenals	21.09	18.87	36.16	13.74
Lymph N. (mesent.)	50.21	99.64	75.93	52.20
Lung	16.55	18.34	23.14	15.09
Blood	1.38	0.96	0.00	0.00
Spleen	12.77	19.45	20.34	10.52
Bone Marrow	11.42	15.57	21.07	12.46
Thyroid	27.60	49.49	40.21	50.02
Brown Fat	151.77	232.41	282.15	365.46
Skin	14.00	18.28	22.87	18.77
Thymus	51.13	44.59	54.94	26.96
Harderian Gland	10.18	15.76	21.73	18.91
Salivary Gland	97.05	108.67	118.22	65.23
Small Intestine	73.14	70.48	44.72	15.86
Heart	16.31	30.39	33.11	34.85
Prostate	9.32	15.17	16.39	9.04
Carcass	10.87	25.98	24.36	13.19
Seminal Vesicle	20.22	25.69	35.19	28.56
Testes	3.34	4.64	7.49	8.05
Pancreas	61.31	102.32	128.71	61.25
Epididymis	8.61	12.00	18.74	12.34
Large Intestine	314.41	365.30	190.42	30.28
Stomach	27.40	29.43	26.38	14.71
Bone	4.99	11.58	10.91	0.00
Skeletal Muscle	1.98	3.41	3.65	3.37
Eyes	4.90	6.98	9.15	13.16
Brain	1.32	1.49	2.06	0.00
Peritoneal Fat	8.09	20.53	23.98	20.55

7.5. Mean Tissue to Plasma Ratio of Drug-Derived Radioactivity in Tissues of Male Sprague Dawley Rats Following a Single Intravenous Administration of ^{14}C-MNTX (Cont'd.)

Tissue ID	Ratio		
	48 hr	72 hr	120 hr
Kidney	NC	NC	NC
Liver	NC	NC	NC
Plasma	NC	NC	NC
Adrenals	NC	NC	NC
Lymph N. (mesent.)	NC	NC	NC
Lung	NC	NC	NC
Blood	NC	NC	NC
Spleen	NC	NC	NC
Bone Marrow	NC	NC	NC
Thyroid	NC	NC	NC
Brown Fat	NC	NC	NC
Skin	NC	NC	NC
Thymus	NC	NC	NC
Harderian Gland	NC	NC	NC
Salivary Gland	NC	NC	NC
Small Intestine	NC	NC	NC
Heart	NC	NC	NC
Prostate	NC	NC	NC
Carcass	NC	NC	NC
Seminal Vesicle	NC	NC	NC
Testes	NC	NC	NC
Pancreas	NC	NC	NC
Epididymis	NC	NC	NC
Large Intestine	NC	NC	NC
Stomach	NC	NC	NC
Bone	NC	NC	NC
Skeletal Muscle	NC	NC	NC
Eyes	NC	NC	NC
Brain	NC	NC	NC
Peritoneal Fat	NC	NC	NC

NC: Not calculated

7.6. Mean Tissue to Plasma Ratio of Drug-Derived Radioactivity in Tissues of Female Sprague Dawley Rats Following a Single Intravenous Administration of ^{14}C-MNTX

Tissue ID	Ratio			
	1 hr	2 hr	3 hr	4 hr
Kidney	57.23	84.00	112.37	114.91
Liver	54.06	85.35	75.98	44.69
Plasma	1.00	1.00	1.00	1.00
Adrenals	1.73	4.75	9.54	9.28
Lymph N. (mesent.)	9.62	17.17	51.22	60.90
Lung	2.42	5.52	12.86	16.96
Blood	0.69	0.86	1.39	1.57
Spleen	2.51	5.02	12.38	13.62
Bone Marrow	11.84	4.60	10.01	12.02
Thyroid	4.33	9.64	27.17	34.28
Brown Fat	17.13	59.69	197.38	236.67
Skin	1.66	4.36	7.62	14.09
Thymus	5.04	19.90	41.69	42.65
Harderian Gland	1.12	2.65	8.52	10.99
Salivary Gland	15.70	42.67	96.73	99.99
Small Intestine	130.82	258.71	1055.06	430.76
Heart	2.13	5.35	13.65	19.68
Ovaries	1.71	6.37	12.14	15.15
Carcass	1.57	3.69	13.02	17.07
Uterus	1.55	4.19	16.36	10.69
Pancreas	8.42	29.35	73.86	84.14
Large Intestine	7.50	21.22	297.98	330.47
Stomach	5.36	9.64	22.97	21.31
Bone	0.77	1.31	4.33	14.64
Skeletal Muscle	0.26	0.55	1.28	2.02
Eyes	1.17	1.86	3.64	4.17
Brain	0.32	0.63	1.15	1.20
Peritoneal Fat	0.71	1.68	4.49	10.07

7.6. Mean Tissue and Plasma Ratio of Drug-Derived Radioactivity in Tissues of Female Sprague Dawley Rats Following a Single Intravenous Administration of ^{14}C-MNTX (Cont'd.)

	Ratio			
Tissue ID	6 hr	8 hr	12 hr	24 hr
Kidney	149.28	135.01	206.69	NC
Liver	59.79	41.97	32.94	NC
Plasma	1.00	1.00	1.00	NC
Adrenals	19.40	11.32	18.79	NC
Lymph N. (mesent.)	85.78	182.90	176.03	NC
Lung	23.97	24.80	37.42	NC
Blood	1.97	0.70	0.00	NC
Spleen	18.93	23.88	27.15	NC
Bone Marrow	19.70	23.12	28.48	NC
Thyroid	63.66	94.02	92.62	NC
Brown Fat	331.04	318.79	537.98	NC
Skin	17.56	22.09	30.82	NC
Thymus	86.66	65.85	86.42	NC
Harderian Gland	13.66	17.76	26.76	NC
Salivary Gland	199.78	208.88	209.10	NC
Small Intestine	84.00	97.81	42.13	NC
Heart	32.33	37.52	59.66	NC
Ovaries	28.76	30.60	38.93	NC
Carcass	36.07	31.77	26.86	NC
Uterus	19.08	15.81	22.70	NC
Pancreas	140.33	127.23	182.97	NC
Large Intestine	440.47	1518.52	264.48	NC
Stomach	24.56	30.54	34.65	NC
Bone	8.07	7.92	9.41	NC
Skeletal Muscle	2.25	1.98	3.70	NC
Eyes	6.74	8.73	13.86	NC
Brain	2.07	1.05	1.92	NC
Peritoneal Fat	10.93	15.64	21.15	NC

NC: Not calculated

7.6. Mean Tissue and Plasma Ratio of Drug-Derived Radioactivity in Tissues of Female Sprague Dawley Rats Following a Single Intravenous Administration of ^{14}C-MNTX (Cont'd.)

Tissue ID	Ratio 48hr	72 hr	120 hr
Kidney	NC	NC	NC
Liver	NC	NC	NC
Plasma	NC	NC	NC
Adrenals	NC	NC	NC
Lymph N. (mesent.)	NC	NC	NC
Lung	NC	NC	NC
Blood	NC	NC	NC
Spleen	NC	NC	NC
Bone Marrow	NC	NC	NC
Thyroid	NC	NC	NC
Brown Fat	NC	NC	NC
Skin	NC	NC	NC
Thymus	NC	NC	NC
Harderian Gland	NC	NC	NC
Salivary Gland	NC	NC	NC
Small Intestine	NC	NC	NC
Heart	NC	NC	NC
Ovaries	NC	NC	NC
Carcass	NC	NC	NC
Uterus	NC	NC	NC
Pancreas	NC	NC	NC
Large Intestine	NC	NC	NC
Stomach	NC	NC	NC
Bone	NC	NC	NC
Skeletal Muscle	NC	NC	NC
Eyes	NC	NC	NC
Brain	NC	NC	NC
Peritoneal Fat	NC	NC	NC

NC: Not calculated

IN VITRO PROTEIN BINDING DETERMINATION OF METHYLNALTREXONE (MNTX) IN SPRAGUE-DAWLEY RAT, BEAGLE DOG, AND HUMAN PLASMA BY EQUILIBRIUM DIALYSIS AND LC/MS/MS [redacted] PROJECT NUMBER 107N-0305PBR)

This study has been reviewed under IND [redacted] The review of this study is incorporated below from the pharmacology review of IND [redacted] dated May 20, 2005.

***In Vitro* Protein Binding Study in Rats, Dogs, and Humans**

Methods: Plasma samples from Sprague Dawley rats, Beagle dogs, and humans were used. Plasma protein binding of methylnaltrexone was measured using an equilibrium dialysis method. The tested drug concentrations were 0.2, 2, 7.5, and 75 µg/ml in rat and dog plasma, and 0.2 and 2 µg/ml in human plasma. The cut-off molecular weight for the dialysis membranes was 5000. Plasma samples were dialyzed with potassium phosphate isotonic buffer pH 7.4 for 3 hr at 37°C.

Results: A preliminary study demonstrated that methylnaltrexone was stable for at least 3 hr at 37°C in plasma from rats, dogs, and monkeys, and in potassium phosphate isotonic buffer pH

7.4. The time needed to achieve equilibrium (i.e. a constant proportion of free drug) during dialysis was determined to be 2 hr in a preliminary study in human plasma using 1 µg/ml methylnaltrexone. The results of the main protein binding study are shown in the following table.

	%Unbound		
Methylnaltrexone (µg/ml)	**Rat**	**Dog**	**Human**
0.2	86.7	93.6	89.0
2	83.6	87.1	84.7
7.5	90.6	94.6	nt
75	87.5	99.6	nt

Values are the mean of 3 samples.
nt: not tested

The results indicate that only a small proportion of drug is protein-bound in rat, dog, and human plasma (9-16%, 0.4-13%, and 11-15%, respectively).

7.4. The time needed to achieve equilibrium (i.e. a constant proportion of free drug) during dialysis was determined to be 2 hr in a preliminary study in human plasma using 1 µg/ml methylnaltrexone. The results of the main protein binding study are shown in the following table.

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Methylnaltrexone (µg/ml)	**Rat**	**Dog**	**Human**
0.2	86.7	93.6	89.0
2	83.6	87.1	84.7
7.5	90.6	94.6	nt
75	87.5	99.6	nt

Values are the mean of 3 samples.
nt: not tested

The results indicate that only a small proportion of drug is protein-bound in rat, dog, and human plasma (9-16%, 0.4-13%, and 11-15%, respectively).

UPTAKE OF RADIOACTIVITY AND METABOLITE PROFILES IN BRAIN OF MALE SPRAGUE-DAWLEY RATS FOLLOWING A SINGLE 5 MG/KG INTRAVENOUS ADMINISTRATION OF [^{3}H]MNTX (REPORT NO. 65672)

Methods: In this study, brain uptake of [^{3}H]MNTX was examined in male SD rats after a single 5 mg/kg IV administration. Plasma and brain samples were collected at 0.25, 1, 4, 8 and 24 hr following the IV dose, and were analyzed for radioactivity content. Brain and plasma samples were also analyzed for metabolite profiles by high pressure liquid chromatography (HPLC) with radioactivity detection and for metabolite characterization by LC/MS.

Results: Mean plasma radioactivity concentrations declined rapidly from 2.45 µg equivalents/mL at 0.25 hr post-dose to 0.01 µg equivalents/mL at 24 hr post-dose. Mean brain radioactivity concentrations declined from 71.7 ng equivalents/g at 0.25 hr post-dose to 2.3 ng equivalents/g at 24 hr post-dose. The plasma and brain AUC_{0-24} values of radioactivity concentrations were 3407 and 279 ng equivalents.hr/mL(g), respectively. Mean brain-to-plasma ratios of radioactivity ranged from 0.03 to 0.29 between 0.25 and 24 hr post-dose. The brain-to-plasma ratio of radioactivity based on AUC_{0-24} values was 0.08. MNTX was the predominant radioactive component in the brain, representing greater than 89.7% of total radioactivity in brain extracts from 0.25 to 8 hr post-dose. Naltrexone, an N-demethylation product, was observed in brain samples, representing 4.0% of radioactivity at 0.25 hr and 5.2% at 1 hr post-dose. Trace amounts of methyl-6ß-naltrexol (M5) and hydroxy O-methyl MNTX (M6) were also observed in brain extracts at 0.25 and 1 hr post-dose.

Overall, following a single 5 mg/kg IV dose of [^{3}H]MNTX to rats, the brain-to-plasma ratio of radioactivity based on AUC_{0-24} values was 0.08, indicating limited uptake of [^{3}H]MNTX-related radioactivity into the brain. [^{3}H]Methylnaltrexone was the predominant radioactive component in the rat brain. The following tables (from page 21 and 22 of the study report) show the plasma and brain radioactivity.

MOA-728 RPT-65672

Table 6.0-1: Plasma and Brain Radioactivity Concentrations and Brain-to-Plasma Ratios of Radioactivity in Male Rats Following a Single 5 mg/kg Intravenous Dose of [^{3}H]MOA-728

Time (Hr)	Rat 1	Rat 2	Rat 3	Rat 4	Mean ± SD
Plasma (µg equivale					
0.25					2.45 ± 0.63
1					0.57 ± 0.20
4					0.06 ± 0.01
8					0.03 ± 0.00
24					0.01 ± 0.01
Brain (ng equiv					
0.25					71.7 ± 8.4
1					41.2 ± 4.3
4					14.7 ± 0.8
8					8.5 ± 0.6
24					2.3 ± 0.8
Brain-to-plasma ratios					
0.25	0.04	0.02	0.03	0.03	0.03 ± 0.01
1	0.07	0.08	0.12	0.05	0.08 ± 0.03
4	0.27	0.22	0.24	NA	0.24 ± 0.03
8	0.30	0.27	0.31	NA	0.29 ± 0.02
24	0.17	0.22	0.09	NA	0.16 ± 0.07

a. Three animals were dosed in 4, 8 and 24 hr groups.

MOA-728 RPT-65672

Table 6.0-2: Summary of MOA-728 Metabolites Observed in Rat Brain

Peak	Ret. Time (min)[a]	$[M]^+$	Site of Metabolism	Metabolite
Naltrexone		342	Methyl group	Naltrexone
M5		358	Carbonyl group	Methyl-6β-naltrexol
M6		386	Aromatic ring	Hydroxy O-methyl MOA-728
MOA-728		356	------	MOA-728

a. LC retention time taken from radiochromatograms and may differ from LC/MS retention times.

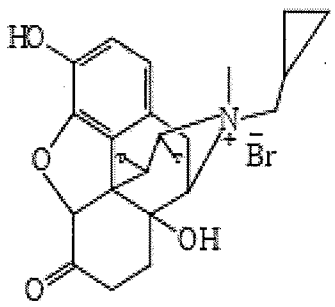
LACTEAL EXCRETION AND PLACENTAL TRANSFER OF ^{3}H-METHYLNALTREXONE FOLLOWING ADMINISTRATION OF A SINGLE SUBCUTANEOUS DOSE TO LACTATING AND PREGNANT RATS REPORT 7434-120, 2006)

Methods: This study was conducted to examine the extent of placental transfer and lacteal excretion of ^{3}H-MNTX-derived radioactivity following a single (10 mg/kg) SC administration of ^{3}H-MNTX to gravid (Group 1) and lactating (Group 2) Sprague-Dawley rats. In Group 1, a single (10 mg/kg, 300 µCi/kg, 129.8 µCi/mg) SC dose of ^{3}H-MNTX in saline solution was administered to 15 dams on gestational day 18 (GD18). The following figure (from page 8 of the study report) shows the position of radiolabeling.

Radiolabeled Test Article

Test article:	^{3}H-Methylnaltrexone
Chemical name:	[15,16-^{3}H]-N—Methylnaltrexone bromide
Molecular formula:	$C_{21}H_{24}BrNO_4$
Molecular weight:	438.16
Lot No.:	SEL/1674
Specific activity:	128.9 µCi/mg salt
Radiopurity:	
Storage conditions:	Approximately -70°C

Structure:



T- signifies the position of the radiolabel

Three dams were sacrificed at 0.5, 1, 2, 4 and 8 hr after dosing. Blood was obtained from six fetuses/dam and pooled by litter. ^{3}H-Methylnaltrexone-derived radioactivity concentrations in the maternal and fetal plasma were determined by liquid scintillation counting (LSC). In Group 2, a single (10 mg/kg, 300 µCi/kg) SC dose of ^{3}H-MNTX in saline solution was administered to 15 dams on postpartum day 10. Milk was collected from 3 dams at 0.5, 1, 2, 4 and 8 hr after dosing. Pups were removed from the mothers approximately 4 hr before collection of milk and the dams were given a SC injection of oxytocin before milking to stimulate lactation.

Results: In Group 1, the mean Cmax values for MNTX in the maternal and fetal plasma were 3.06 and 0.107 µg equiv/g and were observed at 0.5 and 1 hr, respectively. The mean radioactivity concentrations in the maternal and fetal plasma then declined to 0.0493 and 0.0382 µg equiv/g at 8 hr, respectively. The fetal to maternal plasma radioactivity concentration ratio was 0.1 at the Tmax (1 hr) in fetuses, which increased to

0.8 at the last sampling time point (8 hr), indicating that ^{3}H-MNTX and possible metabolites crossed the placenta.

In Group 2, the mean Cmax value in the maternal plasma was 2.08 µg equiv/g and was observed at 0.5 hr after dosing. The mean radioactivity concentrations in maternal plasma then declined to 0.0528 µg equiv/g at 8 hr. The concentrations of radioactivity in milk increased from 0.202 µg equiv/g at 0.5 hr to 1.25 µg equiv/g at 8 hr. The milk to maternal plasma concentration ratios at 0.5 and 8 hr after dosing were 0.1 and 24.0, respectively. These data indicated that ^{3}H-MNTX-derived radioactivity was excreted into the breast milk.

Overall, the results suggested that ^{3}H-MNTX was readily distributed to the fetus after SC administration to gravid rats and was excreted readily into the breast milk. Plasma concentrations of ^{3}H-MNTX and possible metabolites in the fetus appeared to be lower than that of the maternal plasma.

2.6.4.5 Metabolism

EVALUATION OF THE POTENTIAL FOR DIRECT GLUCURONIDATION OF N-METHYLNALTREXONE (MNTX) IN HUMAN, SPRAGUE-DAWLEY RAT, AND BEAGLE DOG MICROSOMES (NO. 107N-0407)

This study has been reviewed under IND The review is incorporated below from the pharmacology review dated May 20, 2005.

Glucuronidation of Methylnaltrexone in Liver Microsomes from Rats, Dogs, and Humans

Methods: Liver microsomes from Sprague Dawley rats, Beagle dogs, and humans were obtained from a commercial vendor (pooled microsomes from males and females). The microsomes (1 mg protein/ml) were mixed with 0.1 M potassium phosphate buffer, pH 7.1, and 50 µg/ml alamethicin, and placed on ice for 15 min. This was followed by the addition of 1 mM $MgCl_2$, 5 mM saccharolactone, and methylnaltrexone (0.5 or 5 µg/ml). After 3 min of pre-incubation at 37°C, the reaction was initiated by the addition of 5 mM UDPGA. The reaction was terminated at 0, 15, 30, or 60 min by the addition of one volume (0.5 ml) of acetonitrile containing 10 ng/ml [$^{13}CD_3$]methylnaltrexone (internal standard). The negative control samples contained 0.5 µg/ml methylnaltrexone and no UDPGA. The positive control samples contained 6 µg/ml oxymorphone. The reaction in the positive control samples was terminated by the addition of one volume of acetonitrile containing 10 ng/ml deuterated oxycodone. All samples were analyzed using a LC/MS/MS method.

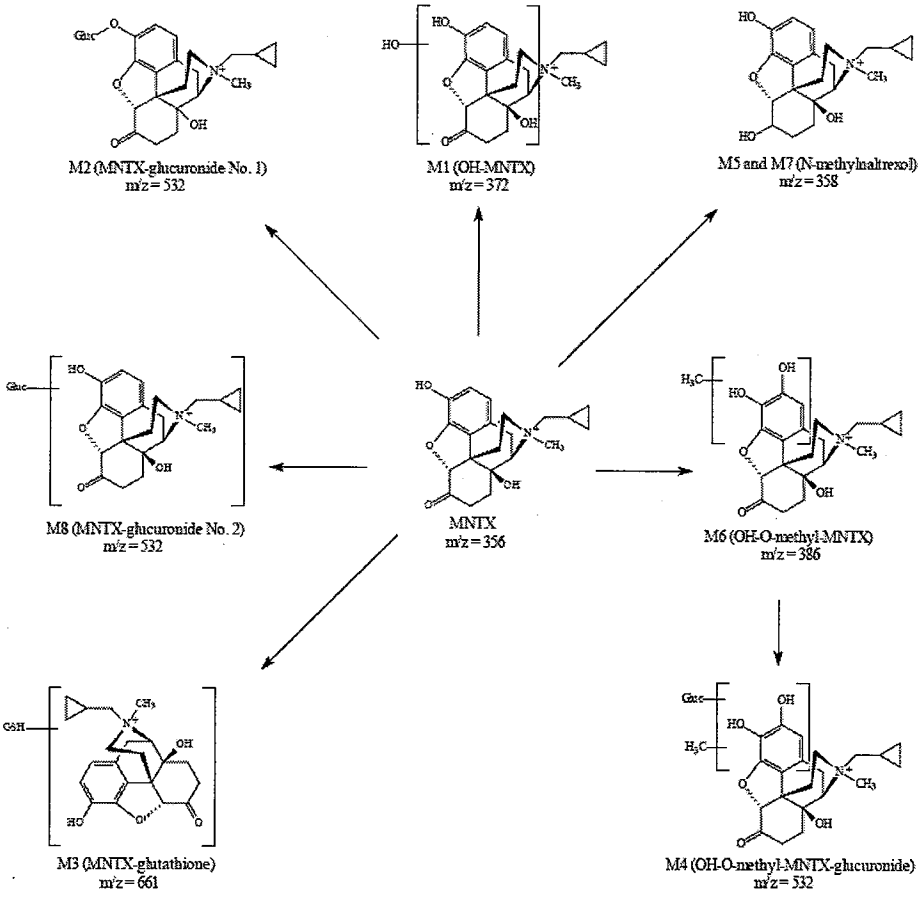
Results: The formation of methylnaltrexone-glucuronide was extremely limited in all of the tested species. As a result, no decrease in the amount of unchanged drug was detected following incubation for up to 60 min. The rank order for the rate of methylnaltrexone glucuronidation was rat>dog>human. Incubation of rat microsomes with oxymorphone resulted in extensive conversion to the glucuronide conjugate. Much lower amounts of oxymorphone-glucuronide were produced by dog and human microsomes.

IN VITRO METABOLISM OF N-METHYLNALTREXONE IN MOUSE, RAT, DOG, AND HUMAN HEPATIC PREPARATIONS
NO. 7434-112, 2005)

Methods: In the present study, the *in vitro* metabolism of MNTX was examined by incubating [^{14}C]MNTX (2.8 and 28 µM, 20.54 µCi/mg) with hepatocytes and hepatic S9 and cytosolic fractions of male CD-1 mice, Sprague Dawley rats, beagle dogs and humans. The samples were analyzed by HPLC with radioactive detection and by LC/MS for characterization of metabolites.

Results: The extent of metabolism of MNTX in hepatocytes was variable across the species examined. Mouse and rat hepatocytes had moderate metabolism of MNTX while there was little biotransformation in dog hepatocytes and trace metabolism in human hepatocytes. Mouse and rat had similar metabolite profiles. Methylnaltrexone glucuronide (M2), O-methylated hydroxy MNTX glucuronide (M4) and N-methylnaltrexol (M7) were the major metabolites in the mouse and rat hepatocytes, while hydroxy MNTX (M1) and MNTX glutathione conjugate (M3) were the major metabolites in the dog hepatocytes. Hydroxy MNTX (M1), N-methylnaltrexol (M5) and O-methylated hydroxy MNTX (M6) were also detected in the mouse and rat hepatocytes as minor metabolites, and MNTX glucuronide (M2), O-methylated hydroxy MNTX (M6) and MNTX glucuronide (M8) were also identified in dog hepatocytes. Metabolism of MNTX in human hepatocytes was too low to allow any metabolite characterization. Metabolism of MNTX in hepatic S9 and cytosolic fractions was not observed in any of the species examined. The following figure shows the proposed metabolic pathways for MNTX.

Figure 34
Proposed Biotransformation Pathway for MNTX



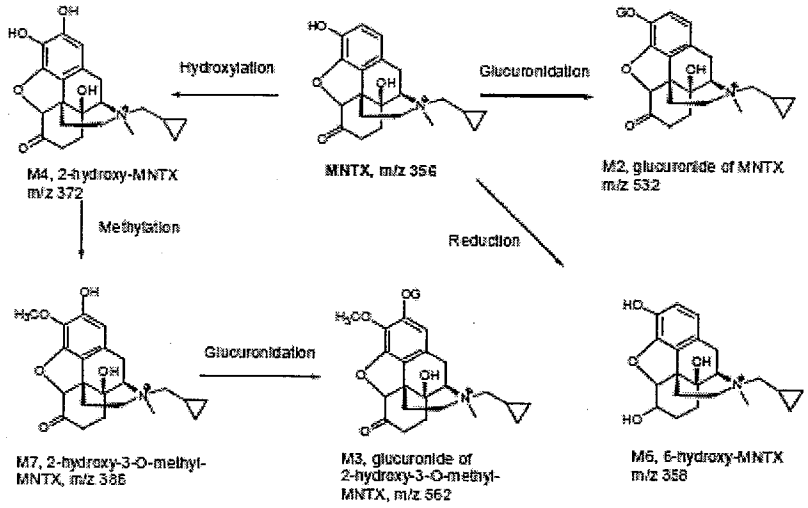
In conclusion, MNTX was moderately metabolized in the mouse and rat hepatocytes, and mildly metabolized in dog hepatocytes. The metabolic pathways of MNTX in animal species examined included hydroxylation, glucuronidation, methylation and glutathione conjugation. Methylnaltrexone underwent trace metabolism in human hepatocytes. Metabolism in human hepatocytes was too low to allow any metabolite characterization. There was no metabolism of MNTX in hepatic S9 or cytosolic fractions of all species examined.

IDENTIFICATION AND PROFILING OF METABOLITES OF MNTX IN PLASMA, URINE AND FECES FROM CD-1 MICE FOLLOWING ADMINISTRATION OF A SINGLE ORAL DOSE OF [3H]MNTX / [redacted] PROJECT NO. 107N-0521)

Methods: In the present study, plasma collected from male mice (n = 4/time point) at 0.5, 1, 2 and 4 hr, and urine and feces collected up to 96 hr post-dose from a separate group of mice (n = 8), following a single 50 mg/kg oral dose of [^{3}H]MNTX (56.49 mCi/mmol) were pooled and analyzed for metabolite profiles and metabolite identification by LC/MS coupled with radioactivity detection.

Results: About 8.3% of the administered dose was excreted in the urine 0-24 hr post-dose. [^{3}H]Methylnaltrexone was the major radioactive component in pooled urine samples, representing about 6% of the administered dose in 24 hrs. The major urinary metabolites included MNTX glucuronide (0.79% of the administered dose), methyl-6-naltrexol (0.54% of the administered dose) and hydroxy O-methyl MNTX (0.59% of the administered dose). Hydroxy MNTX and hydroxy O-methyl MNTX glucuronide were also observed in the 0-8 hr urine. About 89% of the administered dose was excreted through feces in 0-24 hr. [^{3}H]Methylnaltrexone was the predominant radioactive component in the pooled 0-24 hr fecal homogenate, representing 83.3% of the administered dose. Methyl-6-naltrexol was observed as the only fecal metabolite, representing 5.86% of the administered dose. [^{3}H]Methylnaltrexone (0.74% of the administered dose) was the only radioactive peak observed in the pooled 24-48 hr fecal homogenate. Naltrexone, the potential N-demethylation metabolite, was not observed in urine or fecal homogenate.

Overall, MNTX was moderately metabolized in mice, via hydroxylation, reduction, methylation and glucuronidation. Naltrexone, the N-demethylation product of MNTX, was not observed in the urine or feces. Methylnaltrexone was the major radioactive component in the urine and accounted for about 6% of the administered dose. The five urinary metabolites were: M4, a hydroxylation product of MNTX; M6, a reduction product of MNTX; M7, a methylation product of M4; M3, a glucuronide of M7; and M2, a glucuronide of MNTX. Each urinary metabolite accounted for less than 0.8 % of the administered dose. The major radioactive component in the feces was also MNTX (84% of the administered dose). Metabolite M6 that accounted for about 5.9 % of the administered dose was identified as a reduction product of MNTX. The following figure (from page 9 of the report) shows the proposed metabolic pathways.



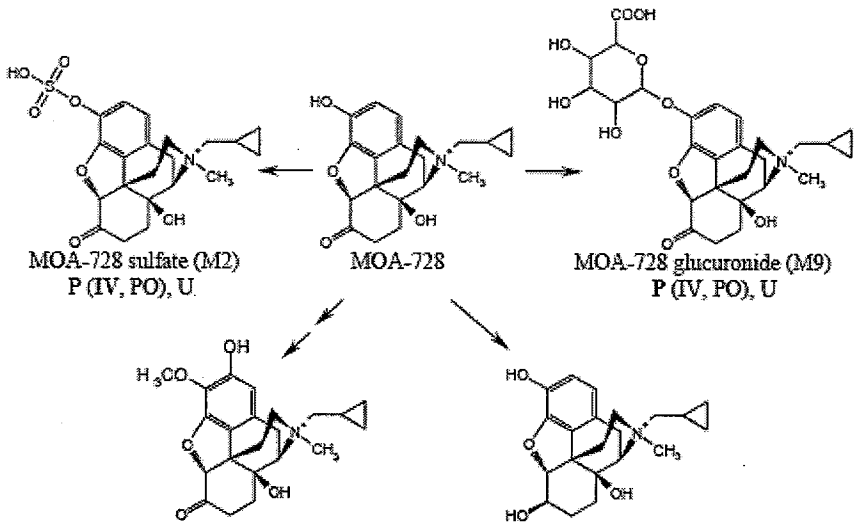
METABOLITE PROFILES IN MALE SPRAGUE-DAWLEY RATS FOLLOWING A SINGLE 5 MG/KG INTRAVENOUS OR 2000 MG/KG ORAL ADMINISTRATION OF [^{3}H]MNTX (REPORT NO. 65621)

Methods: In this study, the *in vivo* metabolism of [^{3}H]MNTX was examined in male SD rats after a single 5 mg/kg IV or 2000 mg/kg oral administration. Plasma samples were obtained at 0.25, 1, 4 and 24 hr following the IV dose, at 0.5, 2, 6 and 24 hr following the oral dose, and urine was collected 0-24 hr post-dose. Plasma and urine samples were analyzed for metabolite profiles by HPLC with radioactivity detection and for metabolite characterization by LC/MS.

Results: Tmax was observed at 0.25 hr and declined at 24 hr following the single 5 mg/kg IV dose. A Cmax of 13.5 µg equivalents/mL was observed at 0.5 hr and decreased to 2.60 µg equivalents/mL at 24 hr following the single 2000 mg/kg oral dose. Methylnaltrexone was the major radioactive component in the plasma samples, representing 93.2, 69.3 and 49.8% of total radioactivity at 0.25, 1 and 4 hr post-dose, respectively, following the single 5 mg/kg IV dose, and 79.3 to 83.0% of total radioactivity in the 0.5-6 hr post-dose period following the single 2000 mg/kg oral dose. Methylnaltrexone sulfate (M2) and MNTX glucuronide (M9) were the major metabolites in the plasma following the IV dose. The percentage of radioactivity attributed to these metabolites increased over time between 0.25 and 4 hr following the 5 mg/kg IV dose, representing 1.1-18.3% and 2.5-16.9% of total radioactivity, respectively. Following the 2000 mg/kg oral administration, MNTX glucuronide (M9) was the major metabolite in the plasma, representing 10.6 to 14.1% of total radioactivity between 0.5 and 6 hr post-dose. Small amounts (about 6% of plasma radioactivity) of methyl-6ß-naltrexol (M5)

and hydroxy O-methyl MNTX (M6) were also observed in the plasma following the IV or oral administration. Naltrexone, the demethylation product, was not observed in any of the plasma samples. About 56.8% of the administered IV dose and 5.6% of the administered oral dose was excreted in 0-24 hr urine. The parent drug represented 92.4% of total radioactivity in the pooled 0-24 hr urine sample following the IV dose and urinary metabolite profiles were qualitatively similar to the plasma profiles. Following the IV dose, urinary metabolites included MNTX sulfate (M2), MNTX glucuronide (M9), methyl-6ß-naltrexol (M5) and hydroxy O-methyl MNTX (M6) (each = 3% of urine radioactivity).

Overall, MNTX was metabolized in rats via sulfation, glucuronidation, reduction, hydroxylation and methylation. Naltrexone was not detected in the plasma. The following figure (from page 34 of the study report) shows the proposed metabolic pathways for MNTX in the rat.



P, plasma; U, urine.
Urine was assessed after the IV dose only.
Major metabolites are bolded.

METABOLITE PROFILES IN MALE SPRAGUE-DAWLEY RATS FOLLOWING A SINGLE 5 MG/KG INTRAVENOUS OR 2000 MG/KG ORAL ADMINISTRATION OF [^{3}H]MNTX (REPORT NO. 013151A)

This study has been reviewed under [redacted]. The review is incorporated below from the pharmacology review dated May 20, 2005.

Metabolism Study in Rats Using Intravenous Administration of [^{14}C]Methylnaltrexone

Note: The samples used in this study were collected from animals in the excretion study.

Methods: Male Sprague Dawley rats (approximately 53-59 days old) were treated intravenously with 10 mg/kg [^{14}C]methylnaltrexone using a dose volume of 1 ml/kg (2 rats). The radiolabel was located in the N-methyl group. Urine and feces were collected at 0-48 hr post-dose. The radioactivity in urine and feces was extracted, followed by analysis using HPLRC, LC/MS, and MS.

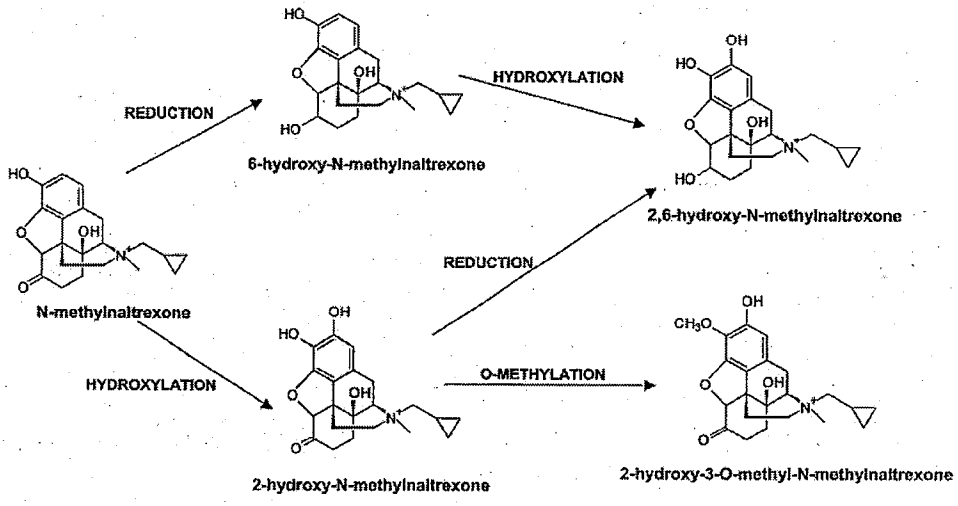
Results: Urine collected from the two animals contained 53% and 58% of the radioactive dose, and feces contained 35% and 34%. The proportion of extracted radioactivity was approximately 97-99% for urine and feces. All radioactivity injected in the HPLRC column was recovered during elution. The mean proportion of radioactive dose that was excreted and analyzed was 87.3%, adjusted for loss in the extraction procedure. The metabolic profile obtained by HPLRC analysis is shown in the table below.

Peak ID	%Radioactive Dose		
	Urine	Feces	Total
RM1	1.2	0.5	1.7
Methylnaltrexone	51.1	28.2	79.3
RM2	1.8	4.4	6.2

The peak designated as RM1 was identified as 2-hydroxy-N-methylnaltrexone. Peak RM2 was composed of two metabolites, 2-hydroxy-3-O-methyl-N-methylnaltrexone and 6-hydroxy-N-methylnaltrexone. The peak associated with methylnaltrexone contained low levels of the metabolite 2,6-hydroxy-N-methylnaltrexone. This metabolite contributed only 5.4% of the methylnaltrexone-associated peak. Therefore, approximately 75% of the dose was recovered as

unchanged drug. The proposed metabolic pathway is shown in the figure below (taken from the study report).

Figure 6. Proposed metabolite pathway of N-methylnaltrexone in rat



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Page 12

Conclusions: Metabolism of methylnaltrexone in rats was minimal following intravenous administration. Low levels of 2-hydroxy-N-methylnaltrexone, 2-hydroxy-3-O-methyl-N-methylnaltrexone, 6-hydroxy-N-methylnaltrexone, and 2,6-hydroxy-N-methylnaltrexone were detected in urine and feces.

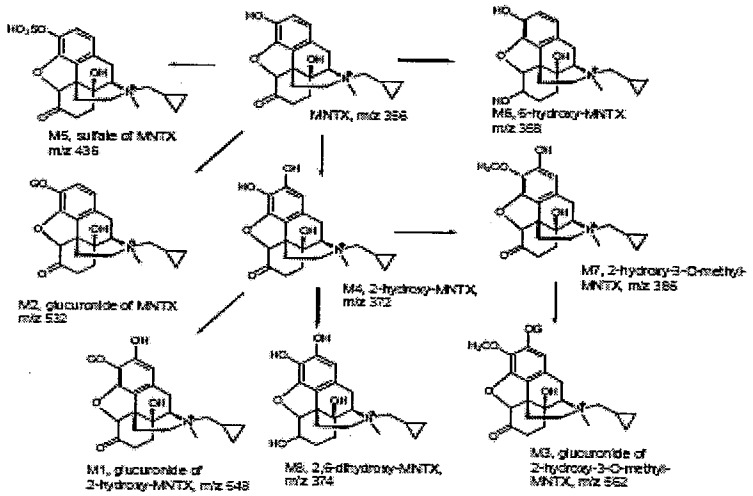
IDENTIFICATION AND PROFILING OF METABOLITES OF MNTX IN BILE AND FECES FROM BILE DUCT CANNULATED SPRAGUE-DAWLEY RATS FOLLOWING ADMINISTRATION OF A SINGLE ORAL OR INTRAVENOUS BOLUS DOSE OF [^{3}H]MNTX ——— PROJECT NO. 107N-0602)

Methods: In the present study, metabolite profiles of MNTX in the bile and feces of bile duct cannulated male SD rats (n = 3) were determined following a single oral or IV bolus dose of [^{3}H]MNTX (specific activity = 56.49 mCi/mmol) at 10 mg/kg. Bile was collected at 0-4, 4-8, 8-24, 24-48 and 48-72 hr postdose and feces were collected at 0-24, 24-48 and 48-72 hr post-dose.

Results: [^{3}H]Methylnaltrexone was the major radioactive component in the bile following the oral or IV administration. About 2.0 and 15.9% of administered dose was recovered in the bile following the oral and IV doses, respectively. [^{3}H]Methylnaltrexone in the bile accounted for 1.26% of the oral dose and 9.32% of the IV dose. Biliary metabolites included methyl-6-naltrexol, hydroxy MNTX, dihydroxy MNTX, hydroxy O-methyl MNTX, MNTX sulfate, MNTX glucuronide, hydroxy MNTX glucuronide and glucuronide of hydroxy O-methyl MNTX following the oral and IV administration. MNTX glucuronide and a glucuronide of hydroxy O-methyl MNTX were the major biliary metabolites. Biliary MNTX glucuronide represented approximately 0.18% and 2.62% of the oral and the IV doses, respectively, while the glucuronide of hydroxy O-methyl MNTX in the bile represented approximately 0.44 and 2.84% of the oral and the IV dose, respectively. [^{3}H]Methylnaltrexone was the predominant radioactive component in the feces following the oral or IV administration. About 100.4 and 9.1% of administered dose recovered in the feces following the oral and IV doses, respectively. [^{3}H]Methylnaltrexone in the feces accounted for approximately 98.0% of the oral dose and 8.23% of the IV dose. Methyl-6-naltrexol was the only metabolite observed in the feces following the oral dose, while methyl-6-naltrexol and hydroxy O-methyl MNTX was observed in the feces following the IV dose.

In conclusion, [^{3}H]MNTX was the major radioactive component in the bile and feces. Generally, MNTX was metabolized in rats via sulfation, glucuronidation, reduction, hydroxylation and methylation. The following figure (from page 99 of the study report) shows the proposed metabolic pathways in the rat.

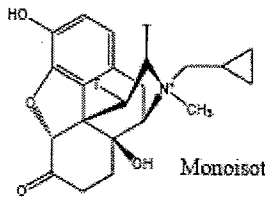
Figure 68. Proposed Metabolic Pathways of MNTX in Rats*



* The positions of hydroxylation, methylation and glucuronidation are tentative.

METABOLITE PROFILES IN MALE BEAGLE DOGS FOLLOWING A SINGLE 5 MG/KG INTRAVENOUS OR 200 MG/KG ORAL ADMINISTRATION OF [^{3}H]MOA-728 (REPORT NO. 65622)

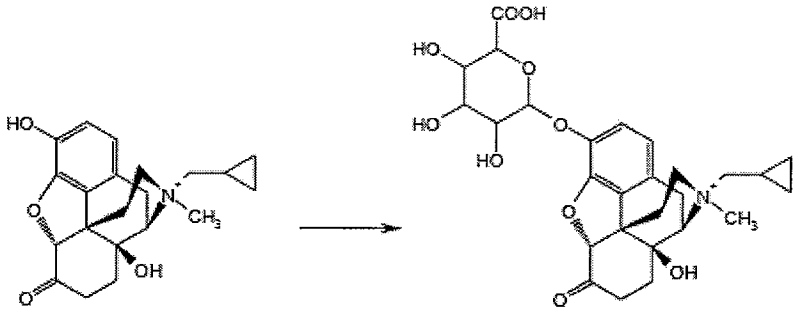
Methods: In this study, the *in vivo* metabolism of [^{3}H]MNTX (158.4 μCi/mg) was examined in male beagle dogs after a single 5 mg/kg IV or 200 mg/kg oral administration. Plasma samples were obtained at 0.25, 1, 4 and 24 hr following the IV dose and at 0.5, 2, 6 and 24 hr following the oral dose; urine was collected at 0-24 and 24-48 hr post-dose. Plasma and urine samples were analyzed for metabolite profiles by HPLC with radioactivity detection and for metabolite characterization by LC/MS. The following figure (from page 10 of the study report) shows the labeling of the tritium.



MOA-728
$C_{21}H_{25}NO_4$
Monoisotopic MW of unlabeled compound =
T = tritium label

Results: Mean plasma radioactivity concentrations were 6.28 μg equivalents/mL at 0.25 hr and declined to 0.06 μg equivalents/mL at 24 hr following the single 5 mg/kg IV dose. Mean plasma radioactivity concentrations reached a maximum of 7.82 μg equivalents/mL at 0.5 hr and decreased to 0.55 μg equivalents/mL at 24 hr following the single 200 mg/kg oral dose. Methylnaltrexone was the major radioactive component in the pooled dog plasma samples, representing 98.6, 77.2 and 66.4% of total radioactivity at 0.25, 1 and 4 hr post-dose, respectively, following the single 5 mg/kg IV dose, and 98.2, 90.6 and 82.4% of total radioactivity at 0.5, 2 and 6 hr post-dose, respectively, following the single 200 mg/kg oral dose. Methylnaltrexone glucuronide (M9) was the major metabolite in the plasma, representing 0-18.5% of total radioactivity in the 0.25 to 4 hr period following the IV dose and 0-12.6% of total radioactivity in the 0.5 to 6 hr post-dose period following the oral dose. About 66.0 and 4.4% of the administered IV dose was excreted in 0-24 and 24-48 hr urine, respectively, while 5.0 and 1.1% of the administered oral dose was excreted in 0-24 and 24-48 hr urine, respectively. Following the IV dose, the metabolite profile of the pooled 0-24 hr urine was similar to the plasma profiles, and MNTX glucuronide (M9) was the only metabolite in the urine, representing 3.2% of total radioactivity, while the parent drug represented 96.2% of total radioactivity. The following figure (from page 28 of the study report) shows the proposed metabolic pathways in the dog.

Figure 6.0-6: Proposed Metabolic Pathway for MOA-728 in Dogs



MOA-728 → MOA-728 Glucuronide (M9)
Plasma, urine

Urine was assessed after the IV dose only.
Bold face indicates major metabolite in the matrix.

In conclusion, MNTX was metabolized in dogs primarily via glucuronidation. This is in contrast to rats where sulfation, reduction, hydroxylation and methylation were observed in addition to glucuronidation.

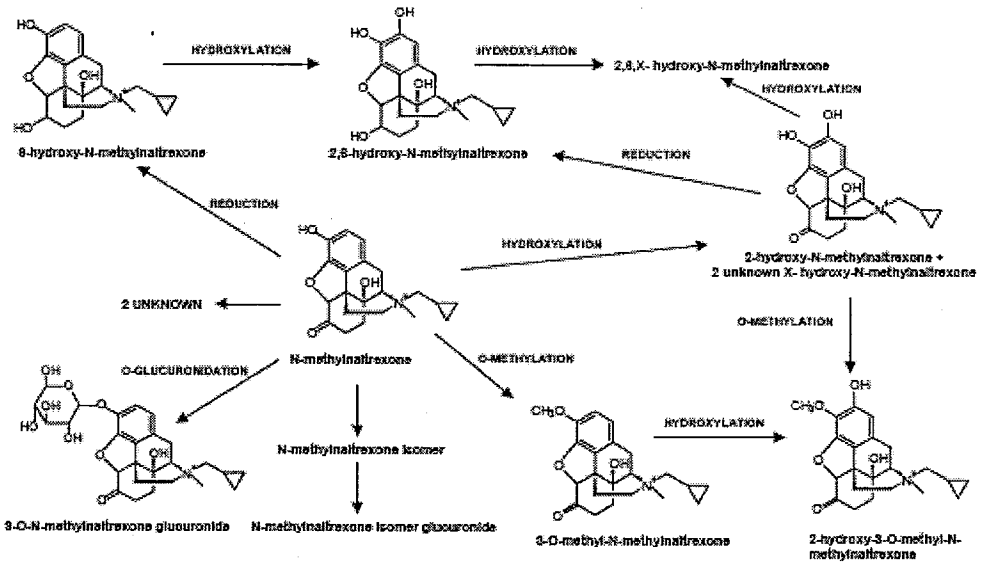
MASS BALANCE, PHARMACOKINETICS, AND METABOLITE PROFILE OF THE RADIOACTIVITY FOLLOWING INTRAVENOUS ADMINISTRATION AND ORAL GAVAGE OF ^{14}C-N-METHYLNALTREXONE TO BEAGLE DOGS [redacted] PROJECT NO. 013152A)

Methods: This study was conducted to examine mass balance and metabolic profiling of MNTX after IV or PO administration of MNTX to three male dogs. The dogs were initially dosed intravenously at 100 mg of [^{14}C]-MNTX (0.30 μCi/mg), followed by PO administration 650 mg of [^{14}C]-MNTX (specific activity of 0.62 μCi/mg), with a 2-week washout period in between dosing. Pooled urine and fecal excreta samples were analyzed by [redacted] and [redacted]

Results: Seven unknown metabolites (DM1, DM2, DM3, DM4, DM5, DM6, and DM7) were identified in the urine and feces. DM2 consisted of one metabolite, an unknown hydroxylation (X-hydroxy-N-methylnaltrexone). DM3 consisted of three metabolites, 2-hydroxy-N-methylnaltrexone, 3-O-N-methylnaltrexone glucuronide, and an unknown metabolite. DM4 consisted of three metabolites, another unknown hydroxylation, another unknown metabolite, and 2,6,X-hydroxy-N-methylnaltrexone. DM5 consisted of two metabolites, 3-O-methyl-N-methylnaltrexone, and a N-methylnaltrexone isomer glucuronide. DM6 consisted of one metabolite, an N-methylnaltrexone isomer. N-methylnaltrexone accounted for most of the percent of dose recovered.

In summary, MNTX was not extensively metabolized in the dog after PO gavage and IV administration. A total of 76.49% of the PO dose was recovered in the urine and feces samples. [^{14}C]-Methylnaltrexone accounted for about 72% of the PO dose, while all putative metabolites identified accounted for a total of about 5% of the PO dose and were categorized as insignificant metabolites. A total of about 68% of the IV dose was recovered in the urine and feces samples. [^{14}C]-Methylnaltrexone accounted for about 58.44 % of the IV dose while all putative metabolites identified accounted for a total of 9.08% of the IV dose and were categorized as insignificant metabolites. Based on the putative metabolite identifications, the proposed biotransformation pathways of [^{14}C]-MNTX included keto-reduction, hydroxylation, and O-methylation. The following figure (from page 45 of the study report) shows the proposed metabolic pathways in the dog.

Figure 14. Proposed metabolite pathway of N-methylnaltrexone in dog



2.6.4.6 Excretion

EXCRETION MASS BALANCE AND PHARMACOKINETICS OF TOTAL RADIOACTIVITY IN CD-1 MICE FOLLOWING ADMINISTRATION OF A SINGLE ORAL DOSE OF [^{3}H]MNTX ——————————— PROJECT NO. 107-0520B)

Methods: In this study, a single oral dose of [^{3}H]MNTX was administered as a saline solution at a target dose of 50 mg/kg to two groups of male CD-1 mice after an overnight

fast. Group 1 was comprised of 8 animals and was used to determine the percentage and time course of excretion of total [^{3}H]MNTX-derived radioactivity in the urine and feces. Urine and feces were collected up to 96 h post-dose from Group 1 animals. Carcasses of Group 1 mice were saved for possible radioanalysis. A daily cage rinse and a cage wash and wipe at termination were also performed for Group 1. Group 2 consisted of 40 male CD-1 mice in which the pharmacokinetics of plasma and blood [^{3}H]MNTX-derived radioactivity were evaluated. Blood samples were collected from Group 2 male mice up to 4 h post-dose (4 animals/time point). All urine, feces, plasma, blood, and cage residue (cage rinse, cage wash and cage wipe) specimens were analyzed for radioactivity.

Results: For Group 1, the mean recovery in the feces was 90.0% of the administered radioactive dose. Urinary excretion accounted for 8.7% of the administered dose. Excretion of radioactivity in feces and urine primarily occurred within the first 24 h post-dose. The mean total recovery of radioactivity in urine, feces, cage rinses, cage washes, and cage wipes was 99.2%. In Group 2 mice, mean peak plasma and blood concentrations (Cmax) of total radioactivity were 1.336 µg equivalents/mL and 0.588 µg equivalents/mL, respectively, which were both achieved at 2.0 h post-dose. The mean blood-to-plasma radioactivity concentration ratio was low (average 0.512). Overall, after administration of a single oral 50 mg/kg dose of [^{3}H]MNTX to CD-1 mice, 90.0% of the radioactivity was recovered in the feces, and 8.7% was recovered in the urine.

MASS BALANCE AND PHARMACOKINETICS OF RADIOACTIVITY FOLLOWING INTRAVENOUS ADMINISTRATION OF ^{14}C-N METHYLNALTREXONE IN RATS [redacted] STUDY NO. 013151)

This study has been reviewed under IND [redacted] The review is incorporated below from the pharmacology review dated May 20, 2005.

Excretion Study in Rats Using Intravenous Administration

Methods: Male Sprague Dawley rats (approximately 53-59 days old) were treated intravenously with 10 mg/kg [^{14}C]methylnaltrexone using a dose volume of 1 ml/kg (3 rats). The radiolabel was located in the N-methyl group. Urine was collected prior to dosing, at 0-6 and 6-24 hr post-dose, and at 24-hr intervals thereafter until 168 hr (seven days) post-dose. Feces was collected prior to dosing and at 24-hr intervals through 168 hr post-dose. Expired CO_2 was collected prior to dosing and at 0-4, 4-8, and 8-24 hr post-dose. Radioactivity content in urine, feces, CO_2, cage wash, and carcasses was measured.

Results: The cumulative excretion data are shown in the table below.

%Radioactive Dose					
Urine	Feces	CO_2	Cagewash (water)	Cagewash (methanol)	Total
57.5 ± 3.8	32.4 ± 3.9	0.04 ± 0.00	0.25 ± 0.22	0.03 ± 0.04	90.2 ± 2.0

Values are the mean ± S.D. of 3 rats.

Approximately 49% of the total radioactivity was excreted in urine at 6 hr post-dose, and about 31% was excreted in feces during the first 24 hr after dosing. Only minimal amounts of radioactivity were excreted after 24 hr post-dose. However, trace amounts of radioactivity were still present in urine and fecal samples collected at 144-168 hr post-dose. The carcasses contained 0.25 ± 0.28% of the total radioactive dose. Therefore, approximately 10% of the radioactivity was unaccounted for in this study.

BILIARY EXCRETION OF TOTAL RADIOACTIVITY IN BILE DUCT CANNULATED MALE SPRAGUE-DAWLEY RATS FOLLOWING ADMINISTRATION OF A SINGLE ORAL OR INTRAVENOUS BOLUS DOSE OF [^{3}H]MNTX PROJECT NO. 107-0601)

Methods: In the present study, biliary and fecal excretion of radioactivity in bile duct cannulated male SD rats (n = 3) was determined following a single oral or intravenous (IV) bolus dose of [^{3}H]MNTX (specific activity = 56.49 mCi/mmol) at 10 mg/kg. Bile was collected at 0-4, 4-8, 8-24, 24-48 and 48-72 hr post-dose and feces was collected at 0-24, 24-48 and 48-72 hr post-dose. Bile and feces were analyzed for total radioactivity.

Results: Following the oral dose, the mean recoveries in the bile and feces were 2.0% and 100.4% of the administered dose, respectively. The mean total recovery of radioactivity was 102.4% in three days. Excretion was rapid, with 2.0% and 96.8% of the administered dose excreted in the bile and feces in the first 24 hr post-dose, respectively. Less than 0.1% of the administered dose was recovered in bile and feces from 48 to 72 hr post-dose. Following the IV administration, the mean recoveries in the bile and feces were 15.9 and 9.1% of the administered dose, respectively. The mean total recovery of radioactivity was 24.9% in three days. Excretion of radioactivity in the bile and feces primarily occurred within the first 24 hr post-dose, with 15.7 and 7.3% of the administered dose excreted in bile and feces in the first 24 hr post-dose, respectively. Less than 0.5% of the administered dose was recovered in bile and feces from 48 to 72 hr post-dose.

In conclusion, excretion of radioactivity in the bile appeared to be low following an oral administration of [^{3}H]MNTX, with the majority of radioactivity recovered in the feces. Following an IV dose, about 16 and 7% of the administered dose was recovered in the bile and feces, respectively. These results indicated that the urinary route was the major route of excretion following IV administration of MNTX to rats.

MASS BALANCE, PHARMACOKINETICS AND METABOLITE PROFILE OF THE RADIOACTIVITY FOLLOWING INTRAVENOUS ADMINISTRATION AND ORAL GAVAGE OF ^{14}C-N-METHYLNALTREXONE TO BEAGLE DOGS REPORT NO. 013152)

Methods: In this study, three male beagle dogs received a single IV and an oral dose of ^{14}C-MNTX (specific activity = 13.16 μCi/mg) in a cross-over design at 10 mg/kg and 50 mg/kg, respectively. Administrations of the oral and intravenous dose were separated by a 2-week washout period. Urine, feces, and cage debris were collected at selected intervals to 168 hr following each dose administration. Cage washes were also collected at the end of each collection period (168 hr post-dose). In addition, blood samples were collected from each animal at pre-dose, 2 (intravenous dose only), 5 (intravenous dose only), 15 (intravenous dose only), 30, 45 min and again at 1, 1.5, 2, 3 (oral dose only), 4, 6, 8, 10 (oral dose only), 12, 16 (oral dose only), 24, 48 and 72 hr (intravenous dose only) following each dose administration. All samples collected (urine, feces, cage debris, cages washes, blood and plasma) were analyzed for total radioactivity content by liquid scintillation spectroscopy. In addition, selected samples of urine and feces were analyzed for parent drug and its metabolites by HPL and/or LC/MS.

Results: Following a single IV administration of ^{14}C-MNTX, drug-derived radioactivity was eliminated mostly in the urine (58.3% of the administered dose). The remainder of drug-derived radioactivity was eliminated in the feces (30.3% of the dose). A total of 91.7% of the dose administered was recovered in the excreta by 168 hr post-dose most of which (83.4%) was recovered within the first 48 hr following dose administration. Following a single oral administration of ^{14}C-MNTX, drug-derived radioactivity was eliminated primarily in the feces (70.9% of the dose). The remainder of recovered drug-derived radioactivity was eliminated in the urine (14.8% of the dose). A total of 87.9% of the dose administered was recovered in the excreta by 168 hr post-dose. Most of the drug-derived radioactivity (84.4%) was recovered in excreta within the first 48 hr following dose administration. Methylnaltrexone appeared to be eliminated relatively rapidly following IV administration in dogs, as suggested by short half-life values in both blood and plasma. Elimination of drug-derived radioactivity appears to proceed at a much slower rate following oral administration. The following tables (from page 30 and 31 of the study report) show PK parameters after IV and oral administration of MNTX in male dogs.

8.8. Individual and Mean Pharmacokinetic Parameters in Blood and Plasma Following a Single Intravenous Administration of ^{14}C-*N*-Methylnaltrexone Bromide to Male Beagle Dogs

Matrix	Animal No.	Pharmacokinetic Parameters							
		C_{max} (µg-equiv/g)	$t_{1/2}$ (hr)	T_{max} (hr)	AUC_{0-t} (µg-equiv•hr/mL)	$AUC_{0-\infty}$ (µg-equiv•hr/mL)	CL (mL/hr•kg)	Vdss (mL/kg)	Kel (1/hr)
Plasma	1001	50.1	0.349	0.03	13.0	13.3	753	318	1.99
	1002	57.7	2.49	0.03	15.8	17.6	568	720	0.278
	1003	56.6	0.509	0.03	11.9	12.3	812	364	1.36
	Mean	54.8	1.12	0.03	13.6	14.4	711	468	1.21
	%CV	6	107	NC	15	20	18	47	72
Blood	1001	28.7	0.3	0.03	7.15	8.13	1230	531	2.02
	1002	33.8	0.3	0.03	7.98	8.57	1170	399	2.64
	1003	34.9	0.3	0.03	6.70	7.29	1370	487	2.21
	Mean	32.5	0.31	0.03	7.28	8.00	1260	473	2.29
	%CV	10	13	0	9	8	8	14	14

NC: Not calculated

8.9 Individual and Mean Pharmacokinetic Parameters in Blood and Plasma Following a Single Oral Administration of ^{14}C-*N*-Methylnaltrexone Bromide to Male Beagle Dogs

Matrix	Animal No.	Pharmacokinetic Parameters							
		C_{max} (µg-equiv/g)	$t_{1/2}$ (hr)	T_{max} (hr)	AUC_{0-t} (µg-equiv•hr/mL)	$AUC_{0-\infty}$ (µg-equiv•hr/mL)	CL (mL/hr•kg)	F (%)	Kel (1/hr)
Plasma	1001	5.21	10.1	3.00	21.2	22.5	2220	33.9	0.0687
	1002	5.40	23.9	2.00	30.4	33.5	1490	38.1	0.0290
	1003	5.45	10.6	2.00	18.7	20.3	2460	33.0	0.0657
	Mean	5.35	14.8	2.33	23.5	25.5	2060	35.0	0.0545
	%CV	2	53	25	26	28	25	8	41
Blood	1001	3.23	0.9	2.00	9.84	11.6	4310	28.6	0.807
	1002	3.30	1.6	2.00	13.6	15.8	3180	36.8	0.441
	1003	3.30	1.4	2.00	9.03	9.95	5030	27.3	0.500
	Mean	3.28	1.27	2.00	10.8	12.4	4170	30.9	0.583
	%CV	1	29	0	23	24	22	17	34

NC: Not Calculated

2.6.4.7 Pharmacokinetic drug interactions

TRANSPORTER-MEDIATED DRUG UPTAKE MEASUREMENT IN OOCYTES WITH METHYLNALTREXONE BROMIDE (STUDY REPORT NO. 300777860)

Methods: This study was performed using *Xenopus levis* (frog) oocytes expressing human OAT1 (Organic Anion Transporter 1) and OCT1 (Organic Cation Transporter 1) uptake transporters, to determine whether MNTX is a substrate of these transporters. Water-injected oocytes served as controls, and radiolabeled probe substrates were used to confirm transporter expression. Positive controls were paraminohippuric acid (PAH) and tetratehylammonium acetate (TEA) and were used at 3 and 10 µM concentrations for OAT1 and OCT1, respectively.

Results: Incubation of 1 pM [^{3}H]-MNTX (specific activity 56.49 mCi/mmol) for 60 minutes and 90 minutes, respectively for OAT1 and OCT1- expressing oocytes demonstrated transporter-mediated uptake activity with OCT1, but not with OAT1. Uptake activity fold-difference ratios for 1 pM [^{3}H]-MNTX were 10.4 (OCT1) and 0.5 (OAT1), respectively. The results of this study indicated that MNTX is a substrate for human OCT1 and not for human OAT1.

ASSESSMENT OF POTENTIAL INTERACTIONS OF CO-ADMINISTERED DRUGS ON THE RENAL EXCRETION OF ^{3}H-MNTX (METHYLNALTREXONE) USING ISOLATED PERFUSED RAT KIDNEYS [redacted] STUDY NUMBER 022)

Methods: The objectives of this study were:

1. To investigate, using the cation and anion transporter substrates cimetidine and probenecid, respectively, the mechanisms of renal secretion of ^{3}H-MNTX (Stage 1, Specific activity = 56.49 mCi/mmol)
2. To determine whether therapeutic plasma concentrations of drugs that are likely to be clinically co-administered with MNTX, namely cimetidine, morphine and procainamide, could potentially compete/interfere with the renal excretion of ^{3}H-MNTX (Stage 2)

In this study, the left renal vein and artery, as well as the urethra, of anesthetized male Sprague-Dawley rats were surgically cannulated and attached to an apparatus. The kidneys were perfused *in situ* using a perfusate containing bovine red blood cells. The test compounds were added directly into a perfusate, and samples of the perfusate and urine were collected at the appropriate time points. The duration of each perfusion was 60 or 120 minutes in Stages 1 and 2, respectively. Renal clearance of radiolabeled inulin served as the measure of the glomerular filtration rate (GFR).

Results: ^{3}H-Methylnaltrexone was shown to be actively secreted into the tubular fluid with the mean CL_{MNTX}/CL_{inulin} of 7.20. Positive control cation and anion transporter substrates, ^{14}C- triethyl ammonium acetate (TEA) and ^{14}C- paraminohippuric acid (PAH), showed an average CLTEA/CLinulin of 5.88 and CL_{PAH}/CL_{inulin} of 2.36, respectively. Co-perfusion of 100 µM cimetidine inhibited the active tubular secretion of ^{3}H-MNTX and ^{14}C-TEA by approximately 70% and 75%, respectively. In contrast, 100 µM probenecid had minimal or no effect on the tubular secretion of ^{3}H-MNTX and ^{14}C-PAH. Concentration-dependent inhibition of active urinary secretion of 1 µM ^{3}H-MNTX was demonstrated with cimetidine at 2, 20 and 250 µM (34.9, 46.2 and 70.3%, respectively); procainamide at 20, 100 and 500 µM (30.3, 69.8 and 82.5%, respectively); and morphine at 0.5, 2.5 and 12.5 µM (21.5, 28.8 and 52.3%, respectively).

In humans, the Cmax following therapeutic regimens of cimetidine and procainamide are 2.41 µg/mL (9.6 µM) and 3.83 µg/mL (16.4 µM), respectively. The therapeutic plasma concentrations of morphine to achieve adequate analgesia are approximately 10 to 100

ng/mL (0.035 to 0.35 μM). Therefore, therapeutic concentrations of cimetidine, procainamide and morphine inhibited active urinary secretion of MNTX by < 50%. More significant inhibition was observed at concentrations 5- to 20-fold higher than the therapeutic concentrations of the three drugs. These results indicated that the tubular secretion of MNTX was inhibited by co-administration of cimetidine, morphine and procainamide.

INHIBITION OF METHYLNALTREXONE BROMIDE OCT-1 MEDIATED UPTAKE IN HOCT1-EXPRESSING OOCYTES
STUDY REPORT NO. 300777817)

Methods: This study was performed using Xenopus levis (frog) oocytes expressing human OCT1 (hOCT1) uptake transporter, to determine whether selected commercially available drugs (cimetidine, morphine, procainamide and ranitidine) can inhibit hOCT1-mediated uptake of MNTX. Additionally, the ability of MNTX to inhibit hOCT1-mediated uptake of probe substrate tetraethyl ammonium (10 μM [^{14}C]-TEA) was also investigated. Three concentrations of each test inhibitor were used. Water-injected oocytes served as controls and radiolabeled probe substrate TEA co-incubated with cimetidine was used to confirm transporter inhibition.

Results: Concentration-dependent inhibition of hOCT1-mediated uptake of 1 μM [^{3}H]-MNTX was demonstrated with cimetidine at 4, 20 and 100 μM (30.2%, 25.4% and 73.8%, respectively), ranitidine at 2, 10 and 50 μM (12%, 25% and 81% respectively), and procainamide at 20, 100 and 500 μM (25%, 82% and 97%, respectively). It is concluded that these compounds are inhibitors of OCT1-mediated uptake of MNTX. Morphine also caused inhibitions of uptake at 0.5, 2.5 and 12.5 μM (27.6%, 13.0% and 27.5%, respectively) concentrations. Inhibition of OCT1-mediated uptake of 10 μM TEA was not inhibited by MNTX at concentrations of 1 and 5 μM, however 25 μM MNTX inhibited TEA uptake by 42%. The results of this study indicated that MNTX is a relatively weak inhibitor of human OCT1 at concentrations 5 to 25 folds higher than anticipated Cmax following IV and SC therapeutic regimens.

2.6.4.8 Other Pharmacokinetic Studies

None

2.6.4.9 Discussion and Conclusions

Generally, MNTX exposure was found to be greater than dose proportional after SC, IV, and PO dosing in rats and dogs. No significant gender differences in rats and dogs were observed in pharmacokinetic parameters. Methylnaltrexone was distributed mostly to the small intestine, liver and kidney, in the rat, with the brain and skeletal muscle having the lowest concentrations at one hour after dosing. Penetration to the brain was limited with lower concentrations in the brain than in all other tissues. Minimal amounts of MNTX

were found in brain of rat and rabbit after administration of high IV or epidural doses. Plasma protein binding was found to be minimal. *In vivo*, MNTX was not extensively metabolized in mice, rats and dogs. The metabolic pathways included hydroxylation, reduction, methylation, sulfation and glucuronidation. Plasma metabolite profiles suggested minimal metabolism of MNTX in human subjects following IV administration. The most abundant metabolites in the human plasma were MNTX sulfate, and methyl-6α-naltrexol and methyl-6ß-naltrexol isomers. Methylnaltrexone sulfate and methyl-6ß-naltrexol were also observed in the rat plasma. The major circulating metabolite in dogs was MNTX glucuronide. In human liver microsomes, MNTX inhibited the activity of CYP2D6. Drug-drug interactions involving MNTX and CYP2D6 substrates could be possible following IV administration. Methylnaltrexone did not induce any of the cytochrome P450 isoforms when tested *in vitro*. Methylnaltrexone was secreted into the bile of rats and dogs. In rats and dogs, the major route of excretion was via the urine after IV dosing and via the feces after oral dosing. In pregnant rats, MNTX-derived radioactivity rapidly crossed the placenta. The fetal exposure was approximately 10% of the maternal exposure. In lactating rats, MNTX-derived radioactivity was excreted into breast milk. Methylnaltrexone did not appear to be a p-glycoprotein substrate in Caco-2 cell monolayers. However, MNTX appeared to be a substrate of the human organic cation transporter (OCT1).

2.6.4.10 Tables and figures to include comparative TK summary

None included

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

None included

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General Toxicology: The toxicity of MNTX administered by the SC route was evaluated in single dose acute toxicity studies in rats and rabbits, by the IV route for up to 90 days in rats and dogs and by the oral route up to 90 days in mice, and up to six months in rats, and up to nine months in dogs. A battery of genotoxicity studies were conducted with MNTX which included the Ames test, chromosome aberration assays in Chinese hamster ovary (CHO) cells and human peripheral blood lymphocytes (HPBL), a mouse lymphoma $TK^{+/-}$ forward mutation assay, and a mouse micronucleus test by intraperitoneal (IP) or SC route. In addition, MNTX has been evaluated for fertility and reproductive performance (Segment I) in rats, teratology (Segment II) in rats and rabbits and peri- and post-natal development (Segment III) in rats. Adequate safety pharmacology studies were also conducted with MNTX.

In different acute SC toxicity studies in rats, the maximum nonlethal dose ranged from 120 to 500 mg/kg, and clinical signs included irritation and discoloration at the injection site. In rats, the maximum nonlethal oral dose was 5000 mg/kg and there were no significant MNTX-related clinical signs. In dogs, the maximum nonlethal oral dose ranged from 75-2000 mg/kg. Clinical signs at 1500-2000 mg/kg included ataxia and decreased activity, convulsions, prostration, and tremors. Other treatment-related clinical signs included nictitating membrane protrusion, ptosis, bloodshot eyes, pupil dilation and excessive lacrimation at all dose levels, and abnormal respiratory signs in the male and female at 1500 mg/kg.

Pharmacokinetic bridging studies demonstrated comparable PK and toxicity profile following SC and IV administration and supported the use of IV toxicology studies for recommended SC route of administration in humans.

In a seven-day oral dose ranging study (7434-102) in mice, animals were administered MNTX at dosages of 80, 400, 2000 and 5000 mg/kg/day. Methylnaltrexone-related mortality occurred on day 4 at 5000 mg/kg/day. There were no MNTX-related clinical signs, effects on body weight, food consumption or hematology parameters. Based on mortality at 5000 mg/kg/day, 2000 mg/kg/day was considered as the tolerated dose.

In a 90-day oral toxicity study (7434-101) in mice, animals were administered MNTX at 80, 400 and 2000/1500 mg/kg/day. Mortality was observed at 400 and 2000 mg/kg/day and high dose was decreased to 1500 mg/kg/day on day 3. The NOAEL was considered as 80 mg/kg/day in males but was not established in females. The uterus (cystic endometrial hyperplasia) and liver (hepatocellular necrosis) was considered as the target organ of toxicity. The maximum tolerated dose (MTD) was considered as < 400 mg/kg/day.

In a 14-day intravenous toxicity study (437-UR-001-89) in rats, animals were administered MNTX at 5, 10 and 30 mg/kg/day (2.5, 5 and 15 mg/kg bid). Mortality and adverse clinical signs (including convulsions, decreased motor activity, tremors, ataxia, low carriage, dyspnea) was seen at 30 mg/kg/day. There were no significant histopathology findings. The NOAEL could not be determined as treatment-related effects were seen at all dose levels. The target organ appeared to be the CNS based on the clinical signs.

In a 14-day intravenous study in rats, animals were treated at 5 and 15 mg/kg/day administered in either vehicle (saline or CaEDTA). Mortality was observed at 15 mg/kg/day. The no observed adverse effect level (NOAEL) was considered as 5 mg/kg/day. The toxicity and toxicokinetic parameters of MNTX administered by intravenous injection were similar for a saline with CaEDTA vehicle and a saline-only vehicle. The target organ of toxicity appeared to be the central nervous system based on the clinical signs observed in animals died early.

In a three-month intravenous toxicity study (154-003) in rats, rats were administered MNTX at dosages of 1, 5, and 20 mg/kg/day. In this study, MNTX-related mortality and

adverse clinical signs (including whole body tremors, prostration, myoclonus, mixed convulsions, and labored breathing) occurred at 5 and 20 mg/kg/day. Most of the dead animals had congestion in the liver and lungs and hemorrhage in the thymus. The NOAEL was considered as 1 mg/kg/day. The CNS was considered as the target organ of toxicity based on clinical signs of tremors and convulsions.

In a seven-day oral dose ranging study (154-001) in rats, rats were administered MNTX at dosages of 80, 400 and 2000 mg/kg/day. There was no MNTX-related mortality, effects on body weight, food consumption, or macroscopic observations. The high dose formulation was determined to be 66.4% of the target concentration of 200 mg/mL. Therefore, the high dose was approximately 1300 mg/kg based on the dosing analysis. Many standard toxicity parameters were not examined in this study.

In a 28-day oral toxicity study (154-012) in rats, animals were administered MNTX as dosages of 80, 400 and 2000 mg/kg/day. Treatment-related clinical signs at the high dose included fecal alterations (soft/watery stools) and salivation. Liver and lungs were target organs of toxicity at 2000 mg/kg/day group. Two females in each of the 400 and 2000 mg/kg/day groups exhibited increases in the plasma ALT (> 2-fold). Hepatic lesions were not observed in the 2000 mg/kg/day females with elevated ALT levels. The pulmonary lesions included interstitial inflammation and alveolar histiocytosis. A NOAEL was not established because the lungs and liver were not examined in the low- and intermediate-dose group animals. However, 400 mg/kg/day was considered as the tolerated dose, based on the mild severity of the liver and lung lesions observed at 2000 mg/kg/day group.

In a six-month oral toxicity study (7434-103) in rats, animals were administered MNTX at 0, 100, 1000/500, and 3000/2000/1000 mg/kg/day. Mortality was seen at 3000, 2000, 1000 and 500 mg/kg/day. Decedent animals had moderate to severe congestions in lungs, liver, adrenals and kidneys suggestive of a decreased blood pressure/ or terminal cardiac insufficiency. The cardiovascular system (CVS) was considered as the target organ of toxicity. Meningeal edema was also observed in decedent animals. Based on these results, the NOAEL was considered as 100 mg/kg/day.

In a 14-day intravenous toxicity study (PH432-UR-001-89) in dogs, animals were administered MNTX at dosages of 0, 5, 20 and 40 mg/kg/day (administered as 2.5, 10 and 20 mg/kg/dose, BID). In this study, there were no adverse MNTX-related mortalities, effects on body weight, food consumption, changes in clinical pathology parameters, organ weights, macroscopic or microscopic observations. Thus, the NOAEL was 40 mg/kg/day. The target organ appears to be the CNS based on clinical signs (abnormal stance/gait and ataxia).

In a three-month intravenous toxicity study (154-004) in dogs, animals were administered MNTX at dosages of 0, 1, 5, and 25/20 mg/kg/day. Based on dose-limiting prostration observed on day 1 at 25 mg/kg/day, the high dosage was reduced to 20 mg/kg/day. No MNTX-related mortality occurred in this study. At 25/20 mg/kg/day, clinical signs included prostration, clonic tremors, and decreased activity. Prolongations (up to 37

msec, 15% increase compared to vehicle control) of QTc intervals were seen at 5 and 20 mg/kg/day. The CNS was a target organ of toxicity at 20 mg/kg/day, as indicated by the incidence of tremors. Heart was considered to be a target organ of toxicity, based on the significant prolongation of QT_c at 5 and 20 mg/kg/day. Spleen was a target organ of toxicity at 5 and 20 mg/kg/day, based on the incidence of fibrosis. The NOAEL was 1 mg/kg/day.

In a seven-day oral study (154-009) in dogs, animals were treated at 75 mg/kg/day by oral gavage. Oral administration of 75 mg/kg/day was associated with protrusion of nictitating membrane, dilatation of pupils, excessive lacrimation, bloodshot eyes, reduced activity, ptosis, and slight weight loss.

In another seven-day oral dose escalation study (154-010) in dogs, dogs (one male and one female) were treated orally via gavage with escalating doses of methylnaltrexone over the course of 44 days from 100 to 2000 mg/kg. Clinical signs included dilated pupils and protruding nictitating membrane at each dose level. Ptosis was observed at 100, 150, 700, 1000, 1500, and 2000 mg/kg. Excessive lacrimation occurred at 250, 350, 700, and 1000 mg/kg. Abnormal respiratory sounds occurred at 1500 and 2000 mg/kg. Ataxia was observed at 1500 and 2000 mg/kg. Tremors, convulsions and prostration were observed in the female at 1500 mg/kg. The MTD was considered as 1000 mg/kg.

In a 28-day oral toxicity study (154-013) in dogs, MNTX was administered at dosages of 60, 300, and 1500 mg/kg. Mortality was seen at 1500 and 300 mg/kg/day. Discoloration of the GI tract and histologic evidence of congestion of multiple organs, particularly various segments of the gastrointestinal tract, was observed in some of these unscheduled deaths. Furthermore, one dog had mild renal tubular necrosis and intratubular hemorrhage. Adverse clinical signs included prostration, ataxia, retching, and/or convulsions. At 750 mg/kg/day, adverse increases in the QTc interval occurred on day 4 in the male (50 msec, 22%) and on day 8 in two females (36 msec; 16%, and 53 msec; 23%) compared with pre-test values. The tolerated dose was considered as 60 mg/kg/day.

In a 39-week oral (gavage) toxicity study (7434-104) in dogs, animals were administered MNTX at 0, 20, 60, and 180 mg/kg/day. The high dose of 180 mg/kg/day was increased to 225 mg/kg/day on study day 43, and was further increased to 250 mg/kg/day on study day 71. In this study, 2 animals given the high dose of 180/225/250 mg/kg/day died due to gavage error. There were no adverse clinical signs. Compound-related tremors and ataxia were observed at 180/225/250 mg/kg/day but were not adverse because they were sporadic (only observed on days 43 and 71), infrequent, and did not affect the overall health of the animals. There were no compound-related effects on body weight or food consumption, ophthalmologic parameters, ECG (including QT and QTc interval), blood pressure, heart rate, clinical pathology parameters, urinalysis results, organ weights, macroscopic or microscopic observations. Based on these results, the NOAEL appeared to be 60 mg/kg/day. The target organ could not be identified in the absence of significant histopathological findings in any organ or tissue.

Genetic Toxicology: Methylnaltrexone was not shown to be mutagenic in a battery of genotoxicity studies using Ames test, chromosome aberration assays in Chinese hamster ovary (CHO) cells and human peripheral blood lymphocytes (HPBL), and a mouse lymphoma forward mutation assay, and a mouse micronucleus test by IP or SC injection.

Carcinogenicity: Not conducted

Reproductive Toxicology: Methylnaltrexone has been evaluated for fertility and reproductive performance (Segment I) in rats, teratology (Segment II) in rats and rabbits and peri- and post-natal development (Segment III) in rats. In a Segment I, fertility and early embryonic development to implantation study in male and female rats, MNTX was tested at 5, 25 and 150 mg/kg/day by subcutaneous route. There were no significant treatment-related effects on fertility and reproductive performances in both sexes. Methylnaltrexone was not teratogenic in rats (up to 25 mg/kg/day) and rabbits (up to 16 mg/kg/day) by IV route. In a subcutaneous Segment III pre- and postnatal development in rats, MNTX did not appear to cause any adverse effect on growth and development of the offspring.

Special Toxicology: None

2.6.6.2 Single-dose toxicity

Acute toxicity studies in rats

Report No.	Testing Laboratory	Species/ Route	Date Started	Date Completed	Batch No.
64345	Wyeth Research, Chazy, NY	SD Rats/SC	May 16, 2006	September 14, 2006	H10206
65328	Wyeth Research, Chazy, NY	SD Rats/SC	July 14, 2006	September 14, 2006	H10207

GLP Compliance: A statement of compliance was included.

Methods: The acute toxicity of MNTX was examined in rats using SC route. In study 64345, MNTX was administered as a single subcutaneous dosage to male and female SD rats (3 or 4/sex/group). Animals were administered 120 or 400 mg/kg/day in saline vehicle, 120 mg/kg MNTX in saline containing 1.2 mg/mL CaEDTA, or 400 mg/kg MNTX in saline containing [redacted] CaEDTA. Control animals were administered vehicle consisting of saline or saline + 4 mg/mL CaEDTA. The dose volume was 2 mL/kg. The following table (from page 10 of the report no. 64345) shows the study design.