

6.0 EXPERIMENTAL DESIGN

Table 6.0-1: Experimental Design

Dosage Group	Vehicle	Dosage ^a (mg/kg/day)	Dosage ^b (mg/m ²)	Concentration ^c (mg/mL)	Animal #	
					Males ^d	Females ^d
1 Old Vehicle	Saline	0	0	0	1-3	4-6
2 New Vehicle ^e	Saline + Ca EDTA	0	0	0	7-9	10-12
3 MOA-728	Saline	120	720	60	13-15	16-18
4 MOA-728 ^f	Saline + Ca EDTA	120	720	60	19-21	22-24
5 MOA-728	Saline	400	2400	200	25-27	28-30
6 MOA-728 ^e	Saline + Ca EDTA	400	2400	200	31-33	34-36

a. Based on the active moiety of the compound

b. The calculation for dose (mg/m²) = Km x (dose in mg/kg), where Km is a constant value of 6 for rats.

c. Concentrations of the dosing formulation were based on delivery of a constant volume of 2 mL/kg.

d. Animals will be euthanized on day 4.

e. Contains _____ mL CaEDTA

f. Contains _____ mL CaEDTA

The doses were selected based on the results of another dose ranging study (0406RP45.001) in SD rats at 0, 20, 30, 40, 50, 80, 120, 200, and 500 mg/kg/day (2 mL/kg). The vehicle used in the study was saline and the dose volume was 2 mL/kg. There were no unscheduled deaths, and there were no compound-related clinical signs observed at ≤ 200 mg/kg. Adverse injection site necrosis was observed at 500 mg/kg. Based on these results, the NOAEL was determined to be 200 mg/kg, and the highest non-lethal dose was 500 mg/kg. In the same study, male and female (1/sex) rats were also administered 400 and 500 mg/kg MNTX by SC injection. The vehicle used was saline, but the dose volume was 5 mL/kg. In these groups, there were no unscheduled deaths. Abnormal stance, body drop, body tremors, labored respiration and necrosis at the injection site occurred at = 400 mg/kg and vocalization upon touch and wet fur was observed at 500 mg/kg. Based on these results, the high dose selected for this study was 400 mg/kg (2 mL/kg dose volume).

In study 65328, MNTX was administered as a single subcutaneous dosage to male and female SD rats (5/sex/group) at 40 mg/kg or 120 mg/kg MNTX in saline or saline with 0.4 mg/mL CaEDTA. Control animals were administered vehicle consisting of saline or saline with 0.4 mg/mL CaEDTA. Animals were observed for 4 days for mortality, clinical observations, body weight, macroscopic observations in the major abdominal and thoracic organs and injection sites, and microscopic observations at the injection site. The following table (from page 8 of the study report) shows the study design.

3.1 Experimental Design

Table 3.1-1: Experimental Design

Dosage Group	Vehicle	Dosage ^a (mg/kg)	Dosage ^b (mg/m ³)	Concentration ^c (mg/mL)	Animal Numbers	
					Male ^d	Female ^d
1 Old Vehicle	Saline	0	0	0	1-5	6-10
2 New Vehicle ^e	Saline + CaEDTA	0	0	0	11-15	16-20
3 MOA-728	Saline	40	240	20	21-25	26-30
4 MOA-728 ^e	Saline + CaEDTA	40	240	20	31-35	36-40
5 MOA-728	Saline	120	720	60	41-45	46-50
6 MOA-728 ^e	Saline + CaEDTA	120	720	60	51-55	56-60

a. Dosages were administered based on the active moiety of the test article.

b. The calculation for dose (mg/m³) = Km x (dose in mg/kg), where Km is a constant value of 6 for rats.

c. Concentrations of the dosing formulation were based on delivery of a constant volume of 2 mL/kg.

d. Animals were euthanized on day 4.

e. Contained  CaEDTA

Results: The maximum nonlethal dose was 400 mg/kg, SC. Clinical signs at 400 mg/kg included adverse black discoloration at the injection site with alopecia occurred in males given 400 mg/kg MNTX in saline +  CaEDTA but not in males given 400 mg/kg in saline. Discoloration of the injection site correlated microscopically with degeneration/necrosis or subcutaneous hemorrhage at the injection site. These studies are considered deficient as the animals were observed for only 4 day and should have been observed for 14 days.

Acute Subcutaneous Toxicity Studies in SD Rats

Species	Route	Dose (mg/kg)	Maximum Nonlethal Dose (mg/kg)		Minimum Lethal Dose (mg/kg)		Time to death
			Male	Female	Male	Female	
SD Rats, 3-4/sex/dose	SC	120, 400	400	400	-	-	-
SD Rats, 5/sex/dose	SC	40, 120	120	120	-	-	-

Summary: In acute SC toxicity studies in rats, maximum nonlethal dose was 400 mg/kg. Clinical signs at 400 mg/kg included adverse black discoloration at the injection site with alopecia occurred in males given 400 mg/kg MNTX in saline +  CaEDTA but not in males given 400 mg/kg in saline. Discoloration of the injection site correlated microscopically with degeneration/necrosis or subcutaneous hemorrhage at the injection site. These studies are considered deficient as the animals were observed for only 4 day post-treatment and should have been observed for at least 14 days.

2.6.6.3 Repeat-dose toxicity

This study has previously been reviewed under IND ~~_____~~ The review is incorporated below from the pharmacology review of IND ~~_____~~ dated June 2, 2005.

Drug: lot # D10795: — pure

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Formulation/Vehicle: solution/deionized water

Animals: \ CD-1®(ICR)BR mice (approximately eight weeks old)
Males: 16.1-35.6 g
Females: 21.5-27.4 g

METHODS: Mice were treated orally with 0 (vehicle), 80, 400, 2000, or 5000 mg/kg/day methylnaltrexone for seven days (5 mice/sex/group). The test and control articles were administered by gavage, using a dose volume of 10 ml/kg. The basis of dose selection was not stated. The following parameters were recorded:

Mortality: twice daily

Clinical Signs: immediately after dosing and approximately 1 hr post-dose

Bodyweight: days 1 and 8

Food Consumption: measured on day 8 (day of sacrifice)

Hematology: blood was collected at termination (day 8)

Clinical Chemistry: blood was collected at termination (day 8)

Gross Pathology: day of sacrifice

RESULTS:

Mortality: One female in the 5000 mg/kg/day group died on day 4. This animal did not exhibit clinical signs.

Clinical Signs: None.

Bodyweight: Weight gain was significantly increased in the 5000 mg/kg/day females. No weight gain occurred in the control females. No dose-related changes in weight gain occurred in males.

Food Consumption: No effects.

Hematology: No effects were observed. Coagulation parameters were not measured.

Clinical Chemistry: The reported parameters were limited to alkaline phosphatase, AST, ALT, sodium, and chloride, which were measured only in the drug-treated groups. Since no control values are available, it is not possible to perform a conclusive evaluation of the data. However, AST values tended to increase with dose level.

Gross Pathology: No treatment-related changes were observed in the female that died prematurely, and no cause of death was apparent. Drug-related effects were absent in the animals sacrificed at termination.

Conclusions: One death occurred in mice treated orally with 5000 mg/kg/day methylnaltrexone. The drug was tolerated at doses of up to 2000 mg/kg/day.

90-DAY ORAL GAVAGE TOXICITY STUDY WITH METHYLNALTREXONE IN MICE STUDY NO. 7434-101)

This study has previously been reviewed under IND The review is incorporated below from the pharmacology review of IND dated June 2, 2005.

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On Original

90-Day Oral Gavage Toxicity Study with Methylnaltrexone in Mice

Key Study Findings: mortality at 400 and 2000/1500 mg/kg/day; uterus was a target organ of toxicity at 80 mg/kg/day and higher; NOAEL was 80 mg/kg/day in males, but was not established in females due to hyperplasia in uterus

Study # 7434-101

Amendment # 009, Vol. 3, Pg. 1

Conducting Laboratory and Location: _____

Date of Study Initiation: June 9, 2003 (report dated August 19, 2004)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ()

Drug: lot # D10795; _____ pure

METHODS: — CD-1®(ICR)BR mice (approximately eight weeks old; males: 21.0-37.7 g; females: 20.0-30.1 g) were treated orally with 0 (vehicle), 80, 400, or 2000/1500 mg/kg/day methylnaltrexone for 90 days (20 mice/sex/group). Additional groups were treated with 80, 400, or 1500 mg/kg/day po for measurement of toxicokinetic parameters (100 mice/sex/group). The high dose was reduced from 2000 to 1500 mg/kg/day on day 3 due to mortality. The animals which died on days 1 and 2 were replaced. The replacement animals were treated with 1500 mg/kg/day for the remainder of the study. The test and control articles were administered by gavage, using a dose volume of 10 ml/kg. Methylnaltrexone was administered as a solution in deionized water. Although not stated in the study report, dose selection was likely based on results of the 7-day oral toxicity study in mice, in which administration of up to 2000 mg/kg/day was tolerated. In the present study, the following parameters were recorded:

Observations and Times:

Mortality: twice daily

Clinical Signs: daily (30 min post-dose)

Bodyweight: weekly starting on day 1

Food Consumption: weekly

Ophthalmoscopy: prior to study initiation and on week 13

Hematology: blood was collected from half of the surviving mice in each group at termination

Clinical Chemistry: blood was collected from half of the surviving mice in each group at termination

Urinalysis: urine samples were collected at termination

Gross Pathology: at termination and immediately after premature death

Organ Weights: adrenals, brain, epididymides, heart, kidneys, liver with drained gallbladder, lungs, ovaries, pituitary, prostate, salivary gland, seminal vesicles, spleen, testes, thymus, thyroid/parathyroid, uterus; paired organs were weighed together

Histopathology: The following organs/tissues were examined in the control and high-dose groups, and in all animals that died prematurely, unless stated otherwise: adrenals, aorta, brain, cecum, colon, duodenum, epididymides, esophagus, eyes (with optic nerve), gallbladder, gross lesions (all groups), Harderian gland, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mesenteric), mammary gland (females), ovaries, pancreas, pituitary, prostate, rectum, salivary glands (mandibular), sciatic nerve, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, sternum (with marrow), stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus (all groups), vagina.

Adequate Battery: yes (x) no ()

Peer Review: yes () no (x)

Toxicokinetics: Blood samples were collected at 0.25, 0.5, 0.75, 1, 2, 3, 4, 8, 12, and 24 hr post-dose on days 1 and 90 (5 mice/sex/group/time-point). Plasma drug concentrations were measured using a LC/MS/MS method.

RESULTS:

Mortality: Nine mice in the 2000 mg/kg/day group were found dead on days 1 and 2. These mice were replaced. The incidence of deaths in the remainder of the study is summarized in the following table.

Dose (mg/kg/day)	Deaths	Days
400	2 males	11, 45
1500*	9 males 6 females	22-33, 87 7, 21-31

*Reduced to 1500 mg/kg/day on day 3.

Clinical signs of toxicity prior to death were limited to hypoactivity in one female treated with 1500 mg/kg/day. The incidence of deaths in the toxicokinetic groups was not reported.

Clinical Signs: No treatment-related signs were observed.

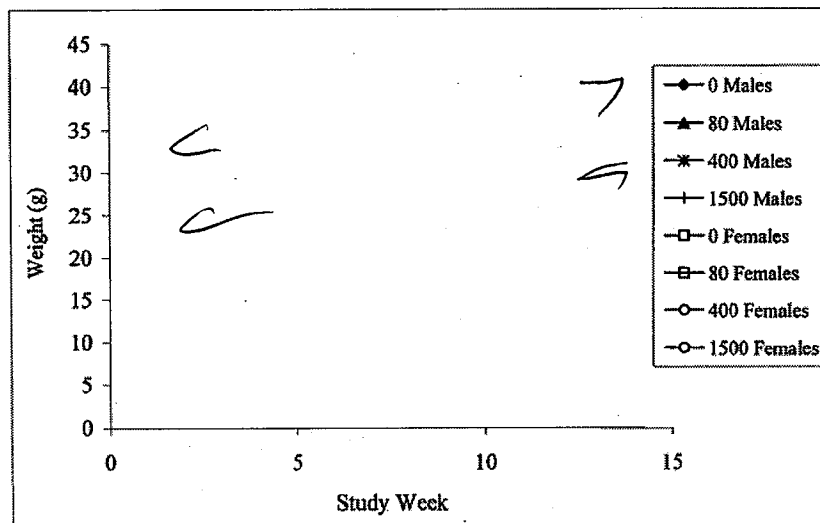
Bodyweight: Bodyweight data are summarized in the following table.

Dose (mg/kg/day)	Mean ($\pm$ S.D.) Bodyweight (g)		
	Initial	Week 4	Week 14
0	M: 30.1 $\pm$ 4.1 F: 24.8 $\pm$ 2.2	M: 34.2 $\pm$ 3.2 F: 28.2 $\pm$ 1.7	M: 37.5 $\pm$ 3.3 F: 30.4 $\pm$ 2.3
80	M: 30.6 $\pm$ 3.7 F: 25.4 $\pm$ 1.0	M: 34.9 $\pm$ 2.9 F: 27.2 $\pm$ 1.4	M: 38.4 $\pm$ 3.4 F: 29.4 $\pm$ 1.7
400	M: 31.8 $\pm$ 2.8 F: 24.7 $\pm$ 1.5	M: 35.3 $\pm$ 3.2 F: 26.9 $\pm$ 2.3	M: 39.3 $\pm$ 3.8 F: 30.2 $\pm$ 1.6
2000/1500	M: 31.5 $\pm$ 2.6 F: 25.9 $\pm$ 1.2	M: 33.9 $\pm$ 3.4 F: 28.3 $\pm$ 1.2	M: 39.0 $\pm$ 3.5 F: 31.0 $\pm$ 1.8

M: males
F: females

No significant changes in mean bodyweight values were observed. Weight gain in the 80, 400, and 2000/1500 mg/kg/day females was decreased by 28%, 2%, and 9%, respectively, on week 14. The strong inhibition of weight gain in the low-dose females is not considered to be biologically significant, given the clear absence of a dose-dependent response. Weight gain in males was not affected. The changes in bodyweight during the course of the study are shown in the figure below (taken from amendment # 009).

**APPENDIX: NINETY-DAY ORAL GAVAGE TOXICITY STUDY WITH MNTX:
BODY WEIGHTS: MALE AND FEMALE MICE**



Food Consumption: Weekly food consumption was frequently increased in the 400 and 2000/1500 mg/kg/day males (8-16% and 7-17%, respectively). In the high-dose females, increases of 12-19% occurred on six different weeks of the treatment period. Food consumption was increased by 15% in the 400 mg/kg/day females on week 13.

Ophthalmoscopy: No treatment-related effects were observed.

ECG: Not performed.

Hematology: No major effects occurred, as shown in the table below.

Parameter	Dose (mg/kg/day)	Change	Week
MCV	2000/1500 ^m	7% increase	14
MCH	2000/1500 ^m	10% increase	14
	2000/1500 ^f	6% increase	14
Monocytes	400 ^m , 2000/1500 ^m	2-fold increase	14
Eosinophils	2000/1500 ^m	2.5-fold increase	14

m: males

f: females

Coagulation tests were not performed.

Clinical Chemistry: For many of the reported parameters, the low number of samples (0-4 mice/sex/group) precluded any evaluation of the effect of methylnaltrexone. These parameters included glucose, urea, creatinine, cholesterol, total protein, albumin, globulin, A/G ratio, calcium, total bilirubin, triglycerides, and inorganic phosphorus. For the remaining parameters, no drug-related effects on the mean group values were observed. However, individual mice in the 400 and 2000/1500 mg/kg/day group exhibited increased ALT levels, relative to the mean control value. ALT was increased by 2.3- and 3.2-fold in two females treated with 400 mg/kg/day, and by 2.4- and 2.5-fold in two females treated with 2000/1500 mg/kg/day. Each of these animals exhibited similar increases in AST. No hepatic lesions were associated with the increase in ALT at 2000/1500 mg/kg/day. Liver was not examined in the 400 mg/kg/day group.

Urinalysis: The reported parameters include specific gravity, pH, urobilinogen, clarity, color, glucose, ketones, protein, bilirubin, occult blood, nitrite, RBC, WBC, epithelial cells, amorphous sediment, crystals, bacteria, casts, yeast, sperm, and mucus. No parameters were affected.

Organ Weights: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio for seminal vesicles were increased by 22%, 18%, and 23%, respectively, in the 400 mg/kg/day group. At 2000/1500 mg/kg/day, these parameters were increased by 9-16% (not significant).

Gross Pathology:

Premature Deaths

Brain: Soft texture was observed in the 400 and 2000/1500 mg/kg/day groups (1/2 and 1/15 mice, respectively).

Kidneys: Pale area was observed in the 2000/1500 mg/kg/day group (1/15 mice).

Lungs: Dark appearance occurred in the 2000/1500 mg/kg/day group (2/15 mice). Mottled appearance occurred in the 2000/1500 mg/kg/day group (1/15 mice).

Terminal Sacrifice

No treatment-related changes were observed.

Histopathology:

Premature Deaths

Adipose Tissue: Necrosis occurred in one mouse in the 400 mg/kg/day group (examined because of abnormal gross appearance).

Esophagus: Muscle degeneration was observed in the 400 and 2000/1500 mg/kg/day groups (1/2 and 1/15 mice, respectively).

Harderian Gland: Glandular atrophy and fatty infiltrate occurred in the 2000/1500 mg/kg/day group (1/15 mice).

Lungs: Hemorrhage occurred in the 2000/1500 mg/kg/day group (2/15 mice).

Pancreas: Hemorrhage was observed in the 2000/1500 mg/kg/day group (1/15 mice).

Terminal Sacrifice

Liver: Hepatocellular necrosis occurred in the 2000/1500 mg/kg/day group (2/25 mice). The nature of this lesion (i.e. single cell or focal) was not stated. The low- and intermediate-dose groups were not examined.

Uterus: Cystic endometrial hyperplasia occurred in the 80 and 2000/1500 mg/kg/day groups (1/20 and 5/14 females, respectively). Dilatation was observed in the 80, 400, and 2000/1500 mg/kg/day groups (1/20, 1/20, and 3/20 females, respectively). Examination of uterus was performed in only 4/20 females in each of the 80 and 400 mg/kg/day groups.

Toxicokinetics: The plasma kinetic parameters are shown in the following table.

Dose (mg/kg/day)	C _{max} (ng/ml)	t _{max} (hr)	t _{1/2} (hr)	AUC _{0-t} (ng•hr/ml)	AUC _{ss (0-t)} (ng•hr/ml)
Day 1					
80	M: 265 F: 460	M: 3.0 F: 0.25	M: nc F: nc	M: 1621 F: 1469	na
400	M: 1323 F: 1118	M: 0.5 F: 0.25	M: nc F: 5.4	M: 3903 F: 2921	na
1500	M: 2040 F: 26987	M: 0.25 F: 0.75	M: 5.0 F: nc	M: 8687 F: 30955	na
Day 90					
80	M: 153 F: 231	M: 1.0 F: 0.25	M: nc F: 11	na	M: 594 F: 490
400	M: 1923 F: 1227	M: 0.5 F: 0.25	M: nc F: 2.8	na	M: 1998 F: 2231
1500	M: 1883 F: 2877	M: 2.0 F: 0.5	M: 1.8 F: 2.1	na	M: 5523 F: 6765

Values were determined from 3-5 mice/sex/group/time-point.

M: males

F: females

nc: Not calculated due to the absence of a defined terminal phase.

na: not applicable

AUC and C_{max} values were increased with dose level, although the proportionality relative to dose increment was highly variable. No consistent sex-related differences were observed, although the AUC and C_{max} values were markedly higher in the 1500 mg/kg/day females on day 1. The AUC values on day 90 were lower than those observed on day 1.

Conclusions: Mortality occurred in a dose-dependent manner at 400 and 2000/1500 mg/kg/day. In the dead animals, lesions occurred sporadically in adipose tissue, Harderian gland, lungs, and pancreas. Therefore, the cause of death was undetermined, but should be considered as drug-related. Uterus is considered as a target organ of toxicity at 80 mg/kg/day and higher, based on the incidence of cystic endometrial hyperplasia (surviving animals only). Weight gain in the 80, 400, and 2000/1500 mg/kg/day females was decreased by 28%, 2%, and 9%, respectively, on week 14. The strong inhibition of weight gain in the low-dose females is not considered to be biologically significant, given the clear absence of a dose-dependent response. Weight gain in males was not affected. The NOAEL in males was 80 mg/kg/day. A NOAEL was not established in females due to hyperplasia in uterus. The MTD is estimated to be 300 mg/kg/day, based on the low incidence of mortality at 400 mg/kg/day (2/20 mice), histopathology data, and the absence of weight gain suppression.

14-DAY INTRAVENOUS STUDY IN RATS

STUDY NO. PH 437-UR-001-89)

This study has previously been reviewed under IND 64,583. The review is incorporated below from the pharmacology review of IND 64,583 dated November 7, 2002.

Study title: 14-Day Intravenous Toxicity Study in Rats

Key study findings: Treatment-related mortality was observed at 15 mg/kg/day b.i.d. No significant treatment-related histopathological changes were observed at any tested dose. The NOEL could not be determined, as drug-induced effects were seen at all dose levels. The target organ of toxicity appeared to be the central nervous system (CNS) based on the clinical signs (abnormal gait, tremor, and clonic convulsion).

Study no: PH 437-UR-001-89

Volume #, and page #: Vol. 1.4; Page: 1045

Conducting laboratory and location: _____

Date of study initiation: March 16, 1989

GLP compliance: A statement of compliance was included.

QA report: yes (X) no ()

Drug, lot #, and % purity: Methylnaltrexone bromide, Lot. No. 0151D1. Purity data not provided.

Formulation/vehicle: 0.9% normal saline

Methods:

Dosing:

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Species/strain: Rats/Sprague Dawley

#/sex/group or time point (main study): 10/sex/dose (control = 5/sex)

Satellite groups used for toxicokinetics or recovery: None.

Age: 43 days old

Weight: 133 – 188 g

Doses in administered units: 2.5, 10, 20 mg/kg twice daily or 5, 20 and 40 mg/kg/day. Due to mortality on Day 1 at 20 mg/kg/day, the animals were sacrificed and discarded. A dose level of 15 mg/kg twice daily or 30 mg/kg/day was added to the study. The animals were administered MNTX at 5, 20 or 30 mg/kg/day intravenously for 14 consecutive days. The basis of dose selection was not mentioned.

Route, form, volume, and infusion rate: Intravenous, solution, and 1 ml/kg.

Observations and times:

Clinical signs: Daily

Body weights: Weekly

Food consumption: Weekly

Ophthalmoscopy: Prior to treatment initiation and before study termination

Hematology: At termination

Clinical chemistry: At termination

Gross pathology: At necropsy

Organs weighed: The following organs were weighed from all animals: liver, adrenal glands, kidneys, ovaries, testes and brain.

Histopathology: The following organs/tissues from all animals in the control and high dose groups were examined for histopathological examinations: brain, pituitary, thyroid/parathyroid, thymus, aorta, lungs, trachea, heart, sternum with bone marrow, salivary glands, liver, spleen, kidneys/adrenals, pancreas, gonads, uterus, accessory genital organs (epididymides, prostate and seminal vesicles), esophagus, nasal turbinates, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, urinary bladder, lymph nodes (mesenteric), mammary gland, thigh musculature, peripheral nerve (sciatic), eyes, femur, spinal cord (cervical, midthoracic and lumbar) and lacrimal glands.

Reviewer: Tamal K. Chakraborti, Ph.D.

IND No. 64, 583

Toxicokinetics: None.

Results:

Mortality: One control and one male rat at 30 mg/kg/day died. The control animal died due to bacterial infection. The death of the treated rat was considered to be due to treatment, as this rat showed severe clonic convulsion prior to death.

Clinical signs: Treatment related clinical signs included chromodacryorrhea, flaccid body tone, dyspnea, eye vascularization and lacrimation, decreased activity, abnormal stance/gait/body drop, tremors and clonic convulsion.

Body weights: The mean initial and final body weights of the control males were 188 and 251 g, respectively. The mean initial and final body weights of the control females were 130 and 173 g, respectively. No significant treatment related changes were observed.

Food consumption: The mean initial and final food consumption in control males were 19.8 and 21.5 g/animal/day, respectively. The mean initial and final food consumption in control females were 17.1 and 18.2 g/animal/day, respectively. No treatment related changes were observed.

Ophthalmoscopy: No significant treatment related changes were observed.

Hematology: No significant treatment related changes were observed.

Clinical chemistry: The following changes were observed in the high dose group: Male: calcium: 81% of control, control = 11.4 mg/dl; total bilirubin: 390% of control, control = 0.10 mg/dl; Female: Calcium: 91% of control, control = 11.24 mg/dl; total bilirubin = 231% of control, control = 0.19 mg/dl.

Organ weights: No significant treatment related changes were observed.

Gross pathology: No significant treatment related changes were observed.

Histopathology: There were no significant treatment related changes. Incidental histopathological changes were observed in lungs (perivascular/bronchial mononuclear cell infiltration, multifocal focal areas of nonsuppurative pneumonitis or foci of foamy macrophages), kidney (early stages of chronic progressive neuropathy: multifocal nonsuppurative nephritis, tubular regeneration and/or tubular dilation, cortical crystals, dilated pelvis and tubular mineralization), liver (nonsuppurative hepatitis and/or foci of mononuclear cells), hearts (iatrogenic lesions: multifocal areas of myocardial fiber degeneration/or hemorrhage). However, these changes were observed in the high dose as well as in some control animals.

Summary of individual study findings: In a 14-Day i.v. toxicity study in Sprague-Dawley rats (10/sex/group), MNTX was administered via i.v. bolus injection at 5, 10 and 30 mg/kg/day (2.5, 5 and 15 mg/kg b.i.d.). Treatment-related mortality was observed at 15 mg/kg/day b.i.d. No significant treatment-related histopathological changes were observed at any of the tested doses.

The NOEL could not be determined, as drug-induced effects were seen at all dose levels. The target organ of toxicity appeared to be the central nervous system (CNS) based on the clinical signs (abnormal gait, tremor, and clonic convulsion). The basis of dose selection was not mentioned.

Study Title: 14-Day IV Toxicity Study in Rats

Key study findings: In a 14-day IV 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.

Study no.: 64868

Volume #, and page #: N/A

Conducting laboratory and location: Wyeth Research, Chazy, NY

Date of study initiation: June 8, 2006

GLP compliance: A statement of compliance was included

QA report: yes (X) no ()

Drug, lot #, and % purity: MNTX. Lot No. H10206 and 10207, —

Methods: The purpose of the study was to evaluate and compare the toxicity and toxicokinetics of MNTX when administered to male and female rats intravenously in saline only and in saline with CaEDTA.

Doses: 5 and 15 mg/kg/day

Species/strain: SD rats

Number/sex/group or time point (main study): 10/sex/group

Route, formulation and dose volume: Intravenous (bolus), saline/saline with CaEDTA, 0.25 mL/kg

Satellite groups used for toxicokinetics or recovery: None

Age: Approximately 9 weeks

Weight: Males: 289-356 g; Females: 174-241 g

Study design: METHYLNALTREXONE in saline only or in saline with CaEDTA (—) and — with glycine HCl was administered intravenously (bolus) to male and female — CD(SD) rats (10/sex/group) at dosages of 5 or 15 mg/kg/day once daily for at least 14 consecutive days. The vehicle-control group (10/sex) received saline only. Plasma pharmacokinetics of MOA-728 in each vehicle was evaluated following the 1st and 14th dose. The following table (from page 12 of the study report) shows the study design.

3.1 Experimental Design

Table 3.1-1: Experimental Design

Dosage Group	Vehicle	Dosage ^a (mg/kg/day)	Dosage ^b (mg/m ²)	Concentration ^c (mg/mL)	Animal Numbers	
					Male	Female
1 Vehicle-Control	Saline	0	0	0	1-10	11-20
2 MOA-728	Saline	5	30	20	21-30	31-40
3 MOA-728	Saline	15	90	60	41-50	51-60
4 MOA-728	Saline + CaEDTA ^{d,e}	5	30	20	61-70	71-80
5 MOA-728	Saline + CaEDTA ^{d,e}	15	90	60	81-90	91-100
6 Vehicle-Control TK	Saline	0	0	0	101-103	104-106
7 MOA-728 TK	Saline	5	30	20	107-115	116-124
8 MOA-728 TK	Saline	15	90	60	125-142	143-160
9 MOA-728 TK	Saline + CaEDTA ^{d,e}	5	30	20	161-169	170-178
10 MOA-728 TK	Saline + CaEDTA ^{d,e}	15	90	60	179-196	197-214

a. Dosages were administered based on the active moiety of the test article.

b. The calculation for dose (mg/m²) = Km x (dose in mg/kg), where Km is a constant value of 6.

c. Concentrations of the dosing formulations were based on delivery of a constant volume of 0.25 mL/kg.

d. Concentration of CaEDTA was _____ mL.

e. _____ solution of glycine HCl was used to adjust the _____

TK. Toxicokinetic study animals

Observations and times:

Mortality: Mortality was observed twice daily.

Clinical signs: Clinical signs were observed once daily.

Body weights: Body weight was recorded on a weekly basis.

Food consumption: Food consumption was recorded on a weekly basis.

Ophthalmoscopy: Ophthalmoscopic examinations were conducted at pretest and during week 2.

Hematology: Hematology was conducted at necropsy.

Clinical chemistry: Clinical chemistry was conducted at necropsy.

Gross pathology: Gross pathology was conducted at necropsy from animals from Groups 1-5.

Organ weights: The following (from page 19 of the study report) organs were weighed from all Group 1-5 animals.

Adrenal Glands	Pituitary Gland
Brain	Prostate Gland
Epididymides	Spleen
Heart	Testes
Kidneys	Thymus
Liver	Thyroid/Parathyroid Glands
Ovaries	Uterus

Histopathology: The following (from page 18 of the study report) organs/tissues from animals from Group 1, Group 3 and Group 5 were examined for histopathology.

Adrenal Glands (cortex and medulla)	Mammary Gland
Aorta (thoracic)	Optic Nerves
Bone and Joint (femoral-tibial joint)	Ovaries
Bone Marrow (sternum)	Pancreas
Brain	Peripheral Nerve (sciatic)
Cecum	Pituitary Gland
Colon	Prostate Gland
Duodenum	Salivary Gland (submandibular)
Epididymides	Seminal Vesicles
Esophagus	Skeletal Muscle (thigh)
Eyes	Skin
Gut Associated Lymphoid Tissue	Spinal Cord (cervical and lumbar)
Harderian Gland	Spleen
Heart	Stomach (squamous and glandular)
Ileum	Testes
Injection sites (tail)	Thymus
Jejunum	Thyroid/Parathyroid Glands
Kidneys	Tongue
Liver	Trachea
Lungs	Urinary Bladder
Lymph Nodes (mandibular and mesenteric)	Uterus
Macroscopic Lesions	Vagina

Toxicokinetics: Blood samples were collected from TK animals as per the following schedule (from page 19 of the study report).

3.11 Bioanalytical Evaluations

Table 3.11-1: Blood Collection Schedule for Measurement of Test Article Concentration

Day 1 Sampling Interval (hours after dosing)	Animal Numbers			
	Group 8		Group 10	
	Male	Female	Male	Female
0.083 ^a	125-127	143-145	179-181	197-199
1 ^a	128-130	146-148	182-184	200-202
3 ^a	131-133	149-151	185-187	203-205
7 ^b	125-127	143-145	179-181	197-199
10 ^b	128-130	146-148	182-184	200-202
24 ^b	131-133	149-151	185-187	203-205

a. Sample was collected from the retro-orbital sinus.

b. Sample was collected terminally from the vena cava or abdominal aorta.

Table 3.11-2: Blood Collection Schedule for Measurement of Test Article Concentration

Day 14 Sampling Interval (hours after dosing)	Animal Numbers									
	Group 6		Group 7		Group 8		Group 9		Group 10	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
0.083 ^a	101-103 ^b	104-106 ^b	107-109	116-118	134-136	152-154	161-163	170-172	188-190	206-208
1 ^a			110-112	119-121	137-139	155-156	164-166	173-175	191-193	209-211
3 ^a			113-115	122-124	140-142	158, 160	167, 169	176-178	194-196	212-214
7 ^b			107-109	116-118	134-136	152-154	161-163	170-172	188-190	206-208
10 ^b			110-112	119-121	137-139	155-156	164-166	173-175	191-193	209-211
24 ^b			113-115	122-124	140-142	158, 160	167, 169	176-178	194-196	212-214

a. Sample was collected from the retro-orbital sinus.

b. Sample was collected terminally from the vena cava or abdominal aorta.

Results:

Mortality: There were five treatment-related deaths in this study. The compound-related deaths included one male (#42) and two female rats (#157 and #159) administered MNTX at 15 mg/kg/day in saline-only, these animals were found dead soon after dosing on days 10, 13, or 14, respectively. Clinical signs observed in these animals after dosing on the day they died included general convulsions, decreased motor activity, and tremors (#42 only). The convulsions occurred soon after dosing and lasted approximately 30 to 60 seconds. No gross lesions were present at necropsy, and there were no MNTX-related microscopic observations. One male rat, #P84 (pretest animal number), administered MNTX at 15 mg/kg in saline with CaEDTA, was found dead approximately 3 hours after dosing on day 1 and was replaced in the study to maintain a sufficient number of animals for toxicokinetic analyses. There were no clinical observations for this animal. No macroscopic observations were found at necropsy. One male animal (#168) in the TK group administered 5 mg/kg/day MNTX in saline with CaEDTA was inadvertently given a 50 mg/kg dose on day 10 and died soon after dosing. Clinical signs prior to death in this animal were tremors and decreased motor activity. No gross lesions were found at necropsy, and clinical pathology was not performed.

Clinical signs: Treatment-related clinical signs included general convulsions, decreased motor activity, tremors, ataxia, low carriage, dyspnea, bradypnea, rales, tachypnea, and

discoloration (blue color in hind feet). Most episodes of convulsions and tremors initiated soon after dosing and lasted approximately 30 seconds to 2 minutes, after which the animals either recovered (majority of animals) or died (animals #42, #157, and #159).

Body weights: The mean initial and final body weights of control males were 326.6 and 383.0 g, respectively. The mean initial and final weight of control females were 209.1 and 234.0 g, respectively. There were no treatment-related changes.

Food consumption: There were no treatment-related effects on food consumption. The data were not presented.

Ophthalmoscopy: There were no MNTX-related ocular findings.

Hematology: No significant treatment-related changes were observed.

Clinical chemistry: There were no treatment-related clinical chemistry alterations.

Gross pathology: There were no treatment-related macroscopic observations.

Organ weights: There were no treatment-related changes.

Histopathology: There were no significant treatment-related microscopic observations.

Toxicokinetics: After once-daily IV administration for 14 days, exposure to MNTX was similar when administered in saline-only and in saline with CaEDTA at any dosage. When administered in either vehicle, exposure to MNTX in both male and female rats increased with increasing doses in a greater than dose-proportional manner over the dosage range of 5 to 15 mg/kg/day. Overall exposures were similar in male and female rats regardless of vehicle and dosage. No apparent accumulation was observed in male rats, while low to moderate accumulation was observed in female rats after multiple dosing at 15 mg/kg/day regardless of vehicle. Mean TK parameters are shown in the following table (from page 30 of the study report).

Table 4.9-1: Mean ($\pm$ SE) MOA-728 Pharmacokinetics in Rats

Day	Dosage (mg/kg/day)	Vehicle	Sex	C_{5min} (ng/mL)	AUC_{0-24} (ng•hr/mL)	$C_{5min}/Dose$	$AUC_{0-24}/Dose$	R
1	15	Saline	Male	19552 $\pm$ 976	11453 $\pm$ 555	1303 $\pm$ 65	764 $\pm$ 37	NA
1	15	Saline	Female	13696 $\pm$ 3646	8133 $\pm$ 1984	913 $\pm$ 243	542 $\pm$ 132	NA
1	15	Saline + CaEDTA	Male	22624 $\pm$ 2234	13342 $\pm$ 1269	1508 $\pm$ 149	889 $\pm$ 85	NA
1	15	Saline + CaEDTA	Female	14710 $\pm$ 5553	8936 $\pm$ 3012	981 $\pm$ 370	596 $\pm$ 201	NA
14	5	Saline	Male	1416 $\pm$ 465	1375 $\pm$ 332	283 $\pm$ 93	275 $\pm$ 66	NA
14	5	Saline	Female	2798 $\pm$ 929	1723 $\pm$ 503	560 $\pm$ 186	345 $\pm$ 101	NA
14	5	Saline + CaEDTA	Male	3942 $\pm$ 1079	2712 $\pm$ 598	788 $\pm$ 216	542 $\pm$ 120	NA
14	5	Saline + CaEDTA	Female	2972 $\pm$ 887	2016 $\pm$ 532	594 $\pm$ 177	403 $\pm$ 106	NA
14	15	Saline	Male	14759 $\pm$ 3502	11765 $\pm$ 2316	984 $\pm$ 233	784 $\pm$ 154 ^a	1.0
14	15	Saline	Female	16327 $\pm$ 2097	10497 $\pm$ 1353	1088 $\pm$ 140	700 $\pm$ 90 ^a	1.3
14	15	Saline + CaEDTA	Male	20287 $\pm$ 3704	13388 $\pm$ 2113	1352 $\pm$ 247	893 $\pm$ 141	1.0
14	15	Saline + CaEDTA	Female	24463 $\pm$ 4146	14718 $\pm$ 2335	1631 $\pm$ 276	981 $\pm$ 156 ^a	1.6

AUC_{0-24} : Area under the concentration-versus-time-curve from 0 to 24 hours.

C_{5min} : Peak plasma concentration at 5 minutes

SE: Standard error

R: Accumulation factor, calculated as the ratio of $AUC_{0-24} (day 14)/AUC_{0-24} (day 1)$

NA: Not applicable

a: Significantly different from that of 5 mg/kg/day, same day, same vehicle, same sex

The analytical and statistical methods and results are presented in separate reports.^{2,3}

Summary: In a 14-day IV toxicity 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 decedent animals.

Study Title: 14-Day Intravenous Study in Rats (Report No. 65648)

Methods: This is a toxicokinetic study. In this study, MNTX was administered to male and female rats by once-daily intravenous (IV) injection at dosages of 0, 5 and 15 mg/kg/day for up to 14 days when delivered in saline and in saline with CaEDTA. For toxicokinetic (TK) evaluation, blood samples were collected from satellite TK groups of animals at pre-determined time points on day 1 and day 14. The plasma MNTX concentrations were determined using a validated LC/MS/MS method, and pharmacokinetic parameters were determined.

Results After once-daily IV administration for 14 days, exposure to MNTX appeared to be similar when delivered in saline only and in saline with CaEDTA at any dosage and for each sex. When delivered in either vehicle, exposure to MNTX in both male and female rats generally increased with increasing doses in a greater than dose-proportional manner over the dosage range of 5 to 15 mg/kg/day. Exposure was similar in male and female rats at each dosage level. No accumulation was observed in male rats, while low to moderate accumulation was observed in female rats after multiple dosing at 15 mg/kg/day regardless of vehicle. The following table (from page 9 of the study report) shows the TK parameters.

Table 1: Mean ($\pm$ SE) MOA-728 C_{5min} and AUC_{0-24} Values in Rats After Once-Daily Intravenous Dosing of MOA-728 for up to 14 Days - Day 1 and Day 14 Data (Protocol 06_1185)

Day	Dosage (mg/kg/day)	Vehicle	Sex	C_{5min} (ng/mL)	AUC_{0-24} (ng•hr/mL)
1	15	Saline	Male	19552 $\pm$ 976	11453 $\pm$ 555
1	15	Saline	Female	13696 $\pm$ 3646	8133 $\pm$ 1984
1	15	Saline + CaEDTA	Male	22624 $\pm$ 2234	13342 $\pm$ 1269
1	15	Saline + CaEDTA	Female	14710 $\pm$ 5553	8936 $\pm$ 3012
14	5	Saline	Male	1416 $\pm$ 465	1375 $\pm$ 332
14	5	Saline	Female	2798 $\pm$ 929	1723 $\pm$ 503
14	5	Saline + CaEDTA	Male	3942 $\pm$ 1079	2712 $\pm$ 598
14	5	Saline + CaEDTA	Female	2972 $\pm$ 887	2016 $\pm$ 532
14	15	Saline	Male	14759 $\pm$ 3502	11765 $\pm$ 2316
14	15	Saline	Female	16327 $\pm$ 2097	10497 $\pm$ 1353
14	15	Saline + CaEDTA	Male	20287 $\pm$ 3704	13388 $\pm$ 2113
14	15	Saline + CaEDTA	Female	24463 $\pm$ 4146	14718 $\pm$ 2335

NINETY-DAY STUDY OF METHYLNALTREXONE ADMINISTERED VIA INTRAVENOUS INJECTION TO SPRAGUE-DAWLEY RATS
STUDY NO. 154-003, 2003)

This study has previously been reviewed under IND . The review is incorporated below from the pharmacology review of IND dated June 2, 2005.

Ninety-Day Study of Methylalntrexone Administered Via Intravenous Injection to Sprague-Dawley Rats

Key Study Findings: mortality occurred at 5 and 20 mg/kg/day; CNS was a target organ of toxicity in the 20 mg/kg/day group; NOAEL was 1 mg/kg/day

Study # 154-003

Vol. 2, Pg. 46

Testing Laboratory: _____

Study Dates: January 14, 2002-April 8, 2003

GLP Compliance: A statement of compliance was included.

QA Report: (x)Yes ()No

Drug: lot # C13754; _____
lot # C13753; _____

Formulation/Vehicle: solution/0.9% NaCl for injection, USP

Animals: _____CD®(SD)IGS BR rats (approximately 7 weeks old)
Males: 195-235 g
Females: 130-176 g

METHODS: The rats were treated intravenously with 0 (vehicle), 1, 5, or 20 mg/kg/day methylalntrexone for 90 days (15 rats/sex/group). Additional groups were treated with 1, 5, or 20 mg/kg/day iv for measurement of toxicokinetic parameters (45 rats/sex/group). The dose volume was 2 ml/kg. Dose selection was based on results from a 14-day intravenous toxicity study in rats, in which methylalntrexone produced mortality at 15 mg/kg b.i.d. Signs of CNS

toxicity were observed at 2.5, 5, and 15 mg/kg b.i.d. (Pharmacology/Toxicology review of IND 64,583 dated November 8, 2002). The present study included the following parameters:

Clinical Signs: once daily (twice daily for mortality)

Bodyweight: once weekly

Food Consumption: once weekly

Ophthalmoscopy: prior to study initiation and at termination

Hematology: blood was collected on days 30/31 and at termination

Clinical Chemistry: blood was collected on days 30/31 and at termination

Urinalysis: urine was collected on days 30/31 and at termination

Gross Pathology: day of sacrifice (day 91 for males and day 92 for females)

Organ Weights: adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testes, thymus, and thyroids; paired organs were weighed together

Histopathology: The following organs/tissues were examined in the control and high-dose groups, except where stated otherwise: adrenals, aorta, bone with marrow (sternum and femur), brain (brain stem, cerebellum, and cerebrum), cecum, cervix, colon, duodenum, epididymides, esophagus, eyes, gross lesions (all groups), heart, injections site (all groups), ileum, jejunum, kidneys, liver, lungs, lymph nodes (mandibular, mesenteric), mammary glands, optic nerve, ovaries, pancreas, pituitary, prostate, rectum, salivary glands (mandibular), sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, lumbar, thoracic), spleen, stomach (forestomach, glandular), testes, thymus, thyroids, tongue, trachea, urinary bladder, uterus, vagina. Examination was also performed for each animal that died prematurely.

Toxicokinetics: Blood was collected at 0.03, 0.08, 0.25, 1, 4, 8, and 24 hr post-dose on days 1, 30, and 90 (5 rats/sex/group/time-point). Plasma drug concentrations were measured using a validated LC/MS/MS method.

RESULTS:

Mortality: The incidence of deaths is summarized in the following tables.

Dose (mg/kg/day)	Main Study Groups	
	# Deaths	Day(s)
0	0	-
1	0	-
5	1	26
20	5	15, 43, 67, 86, 90

Dose (mg/kg/day)	Toxicokinetic Groups	
	# Deaths*	Day(s)
0	0	-
1	0	-
5	1	26
20	1	18

*Deaths that occurred shortly after blood sampling are not included.

The two deaths in the 5 mg/kg/day group and three deaths in the 20 mg/kg/day group were considered as treatment related. One female in the 20 mg/kg/day group exhibited convulsions after dosing and died two minutes later on day 43. Two deaths in the 20 mg/kg/day group were attributed to a dosing complication. One death occurred after the accidental repeat administration of 20 mg/kg at 10 min after the daily dose was given.

Clinical Signs: A high incidence of labored breathing, prostration, and whole body tremors occurred in the 20 mg/kg/day group. Convulsions occurred in 2/15 females in the 20 mg/kg/day group (one incidence in both females). Myoclonus occurred in the 20 mg/kg/day males (11/15). Each of these signs occurred within 30 min after dosing. Purple skin discoloration and swelling of the tail in all study groups was attributed to repeated injections.

Bodyweight: Weight gain was not affected. The control males and females weighed 210.0 ± 9.0 g and 158.6 ± 8.0 g, respectively, on day 1, and 499.7 ± 43.1 g and 274.7 ± 20.5 g, respectively, at termination.

Food Consumption: No effects.

Ophthalmoscopy: The authors stated that no drug-related effects were observed. However, the observations were not included in the study report.

Electrocardiography: Not performed.

Hematology: The results are shown in the following table.

Parameter	Dose (mg/kg/day)	Change	Day
RBC	20 ^m	7% decrease	30
Hematocrit	20 ^m	5% decrease	30
WBC	5 ^f	20% decrease	30
Lymphocytes	5 ^f	22% decrease	30

m: males
f: females

APTT and PT were unaffected.

Clinical Chemistry: Phosphorus was increased by 8% and 24% in the 1 and 20 mg/kg/day females, respectively, on day 30. One female in the 5 mg/kg/day group exhibited a 2.7-fold increase in ALT and a 2.8-fold increase in AST on day 91, relative to the mean control value. One female in the 20 mg/kg/day group exhibited a 7-fold increase in ALT and a 6.5-fold increase in AST on day 91. One female in the 5 mg/kg/day group exhibited a 2.7-fold increase in AST on day 91.

Urinalysis: The following parameters were reported: glucose, bilirubin, ketone, specific gravity, occult blood, pH, protein, urobilinogen, nitrite, leukocytes, RBC, WBC, epithelial cells, casts, crystals, color, and clarity. No treatment-related changes were observed.

Organ Weights: Absolute weight, organ/bodyweight ratio, and organ/brain weight, ratio were reported. There were no effects.

Gross Pathology: No treatment-related lesions were observed.

Histopathology:

Premature Deaths (1 male in the 5 mg/kg/day group, 3 males and 2 females in the 20 mg/kg/day group)

Adrenals: Congestion in cortex and medulla occurred in the 20 mg/kg/day group (1/5 rats).

Heart: Dilatation of atrium was observed in the 20 mg/kg/day group (1/5 rats).

Injection Site: Slight fibrosis (20 mg/kg/day) and hemorrhage (5 and 20 mg/kg/day) was observed.

Kidney: Congestion occurred in the 20 mg/kg/day group (2/5 rats).

Liver: Mild to moderate congestion was observed in the 5 and 20 mg/kg/day groups (all rats). Lymphocytic infiltration occurred in the 20 mg/kg/day group (1/5 rats).

Lungs: Congestion was observed in the 5 and 20 mg/kg/day group (3/5 rats at 20 mg/kg/day).

Ovaries: Congestion occurred in the 20 mg/kg/day group (one rat).

Pituitary: Congestion occurred in the 20 mg/kg/day group (one female).

Thymus: Hemorrhage was observed in the 5 and 20 mg/kg/day groups (4/5 rats at 20 mg/kg/day).

Terminal Sacrifice

No treatment-related lesions were observed.

Toxicokinetics: The plasma kinetic parameters are shown in the following tables. The toxicokinetic data from day 90 were not analyzed due to a potential error in the drug concentration measurement.

Study Day 1

	Group 2 1 mg/kg/day		Group 3 5 mg/kg/day		Group 4 20 mg/kg/day	
	Male	Female	Male	Female	Male	Female
AUC ₀₋₁ (ng·h/mL)	269	232	1133	1256	8841	9111
AUC _{inf} (ng·h/mL)	269	232	1135	1258	8850	9126
C _{max} (ng/mL)	1380.41	2417.93	7623.52	11783.86	53150.80	66370.84
t _{1/2} (h)	3.7	4.0	4.1	4.6	4.7	6.6
CL (L/h/kg)	3.7	4.3	4.4	4.0	2.3	2.2
V _{d area} (L/kg)	20	25	26	26	15	21

Study Day 30

	Group 2 1 mg/kg/day		Group 3 5 mg/kg/day		Group 4 20 mg/kg/day	
	Male	Female	Male	Female	Male	Female
AUC ₀₋₁ (ng·h/mL)	242	330	1393	1399	8399	7915
Accumulation Factor	0.90	1.4	1.2	1.1	0.95	0.87
C _{max} (ng/mL)	1382.99	1186.13	8823.88	7040.29	47193.93	45534.54
t _{1/2} (h)	2.9	7.2	4.4	11	6.5	7.8
CL (L/h/kg)	4.1	3.0	3.6	3.6	2.4	2.5
V _{d area} (L/kg)	17	32	23	56	22	28

Values were determined from 5 rats/sex/group/time-point.

The increases in AUC and C_{max} values in the 20 mg/kg/day group were disproportionately large relative to the dose increment. There were no consistent sex-related differences in the plasma kinetic parameters. Accumulation of drug in plasma was not evident.

Animals: —CD®(SD)IGSBR rats (approximately six weeks old)

Males: 208-243 g

Females: 152-184 g

Methods: In the rising dose tolerance (acute) study, rats were treated with a single oral administration of 2000, 3000, 4000, or 5000 mg/kg methylnaltrexone (2 rats/sex/group). The drug was administered by gavage using a dose volume of 10 ml/kg. In the repeat-dose toxicity study, rats were treated orally with 0 (vehicle), 80, 400, or 2000 mg/kg/day for seven days, using a dose volume of 10 ml/kg (5 rats/sex/group). The high-dose formulation was determined to contain 66.4% of its target concentration of 200 mg/ml. Therefore, the high dose was approximately 1300 mg/kg/day in the 7-day study. The high-dose formulation was a suspension, whereas the low- and intermediate-dose formulations were solutions.

Results:

Rising Dose Tolerance Study

Mortality: None.

Clinical Signs: Increased salivation was observed for up to 40 min post-dose in the 3000, 4000, and 5000 mg/kg groups.

Bodyweight: Each rat gained weight (8-17 g) at one day after dosing.

7-Day Repeat Dose Study

Mortality: None.

Clinical Signs: Soft stool was observed in the 2000 mg/kg/day group. Two high-dose females exhibited increased activity for up to 2 hr 40 min post-dose.

Bodyweight: Weight gain was unaffected.

Food Consumption: No effects.

Gross Pathology: No treatment-related changes were observed.

Conclusions: In the acute (rising dose tolerance) study, methylnaltrexone was not lethal at oral doses of up to 5000 mg/kg. Salivation occurred at 3000 mg/kg and higher. The only abnormalities observed in the 7-day repeat dose study were soft stool and increased activity, which occurred in the 1300 mg/kg/day group. Many of the standard toxicity parameters such as histopathologic examination were not included in the 7-day study.

**28-DAY TOXICITY AND TOXICOKINETIC STUDY OF METHYLNALTREXONE
ADMINISTERED VIA ORAL GAVAGE TO SPRAGUE-DAWLEY RATS
(STUDY NO. 154-012)**

This study has previously been reviewed under IND () The review is incorporated below from the pharmacology review of IND () dated February 8, 2006.

28-Day Toxicity and Toxicokinetic Study of Methylalntrexone Administered Via Oral Gavage to Sprague Dawley Rats

Key Study Findings: tolerated dose was 400 mg/kg/day; liver and lungs were target organs of toxicity at 2000 mg/kg/day

Study # 154-012

Vol. 9, Pg. 1

Testing Laboratory: ()

Study Dates: August 1, 2002-April 23, 2003

GLP Compliance: A statement of compliance was included.

QA Report: (x)Yes ()No

Drug: lot # D04957; ()

Formulation/Vehicle: solution/deionized water

Animals: () CD®(SD)IGSBR rats (approximately six weeks old)
Males: 169-203 g
Females: 108-165 g

METHODS: Rats were treated orally with 0 (vehicle), 80, 400, or 2000 mg/kg/day methylalntrexone for 28 days (15 rats/sex/group). Additional groups were treated orally with 80, 400, or 2000 mg/kg/day for measurement of toxicokinetic parameters (30 rats/sex/group). The test and control articles were administered by gavage, using a dose volume of 10 ml/kg. Dose levels were selected based on results from a 7-day oral toxicity study in rats (reviewed above), in which methylalntrexone was tolerated at doses of up to 1300 mg/kg/day. The present study included the following parameters:

Clinical Signs: once daily (twice daily for mortality)

Bodyweight: once weekly

Food Consumption: once weekly

Ophthalmoscopy: prior to study initiation and on day 26

Hematology: blood was collected at termination (day 29 for males, day 30 for females); bone marrow smears were prepared from the femur, but were not examined

Clinical Chemistry: blood was collected at termination (day 29 for males, day 30 for females)

Urinalysis: urine was collected overnight prior to necropsy (day 29 for males, day 30 for females)

Gross Pathology: day of sacrifice

Organ Weights: adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testes, thymus, and thyroids; paired organs were weighed together

Histopathology: The following organs/tissues were examined in the control and high-dose groups, except where stated otherwise: adrenals, aorta, bone with marrow (sternum and femur), brain (brain stem, cerebellum, and cerebrum), cecum, cervix, colon, duodenum, epididymides, esophagus, eyes, gross lesions (all groups), heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mandibular, mesenteric), mammary glands, optic nerve, ovaries, pancreas, parathyroids, pituitary, prostate, rectum, salivary glands (mandibular), sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, lumbar, thoracic), spleen, stomach (forestomach, glandular), testes, thymus, thyroids, tongue, trachea, urinary bladder, uterus, vagina.

Toxicokinetics: Blood was collected at 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hr post-dose on days 1 and 28 (3 rats/sex/group/time-point). Plasma drug concentrations were measured using a validated LC/MS/MS method.

RESULTS:

Mortality: No deaths occurred.

Clinical Signs: Soft stool was observed on multiple occasions in the 2000 mg/kg/day group (15/30 rats). Increased salivation occurred in the 400 and 2000 mg/kg/day groups within 30 min post-dose (1/30 and 4/30 rats, respectively).

Bodyweight: Weight gain was reduced by 23% in the 2000 mg/kg/day males during days 22-28 (weight gain in control males was 24.3 ± 6.2 g during this period). Over the entire treatment period, weight gain in the 2000 mg/kg/day males was decreased by 8%, although this change was not statistically significant. Weight gain was increased by 21-22% in the 400 and 2000 mg/kg/day females during days 1-8. The control males and females weighed 188 ± 6 g and 152 ± 9 g, respectively, on day 1, and 360 ± 26 g and 244 ± 18 g, respectively, at termination.

Food Consumption: Food intake was increased by 20% in the 2000 mg/kg/day females. No changes were observed in males.

Ophthalmoscopy: No effects.

Electrocardiography: Not performed.

Hematology: The results are shown in the following table.

Parameter	Dose (mg/kg/day)	Change	Day
RBC	2000 ⁱ	9% increase	30
Hemoglobin	2000 ^m	6% increase	29
	2000 ^f	4% increase	30
Hematocrit	2000 ^m	9% increase	29
	2000 ^f	7% increase	30
WBC	2000 ^m	23% increase	29
Platelets	80 ^m	11% increase	29
	2000 ^m	12% decrease	29
	2000 ^f	18% decrease	30
Lymphocytes (absolute)	2000 ^m	19% increase (ns)	29
Neutrophils (absolute)	2000 ^m	69% increase	29
	2000 ^f	78% increase	30

ns: not significant

m: males

f: females

The most notable effect was an increase in neutrophil count in the 2000 mg/kg/day group. RBC, hemoglobin, and hematocrit were slightly increased in the 2000 mg/kg/day group. APTT (activated partial thromboplastin time) and PT (prothrombin time) were unaffected.

Clinical Chemistry: The results are shown in the following table.

Parameter	Dose (mg/kg/day)	Change	Day
Glucose	2000 ^m	23% increase	29
BUN	400 ^m , 2000	13-20% decrease	29/30
AST	2000 ^m	19% increase	29
Alkaline Phosphatase	2000 ^f	25% decrease	30
Albumin	2000 ^m	5% increase	29
	2000 ^f	10% increase	30
Globulin	2000 ^f	10% increase	30
Total Protein	2000 ^f	10% increase	30
Potassium	2000 ^f	15% decrease	30
Chloride	80, 400, 2000	4%, 11%, 36% increase	29/30
Phosphorus	400 ^f , 2000 ^f	9%, 13% decrease	30

ns: not significant

m: males

f: females

The most notable effect was a dose-dependent increase in chloride concentration. ALT and AST was increased by 11.3- and 8-fold, respectively, in one female in the 400 mg/kg/day group, relative to the mean control value. One female in the 2000 mg/kg/day group exhibited a 2.4-fold increase in ALT and a 2.3-fold increase in AST. ALT was increased by 2.4- and 2.2-fold in one female in each of the 400 and 2000 mg/kg/day groups, respectively. One female in each of the 80 and 2000 mg/kg/day group exhibited a 2.5- and 2.4-fold increase in AST, respectively.

Urinalysis: The following parameters were reported: glucose, bilirubin, ketone, specific gravity, occult blood, pH, protein, urobilinogen, nitrite, leukocytes, RBC, WBC, epithelial cells, casts, crystals, color, and clarity. Leukocytes were present in urine from the 2000 mg/kg/day males.

Organ Weights: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were reported.

Adrenals: Absolute weight was reduced by 12% and 14% in the 400 and 2000 mg/kg/day females, respectively. Organ/bodyweight ratio was reduced by 15% in the 400 and 2000 mg/kg/day females. Organ/brain weight was reduced by 14% in the 400 mg/kg/day females.

Epididymides: Absolute weight was reduced by 13% in the 2000 mg/kg/day males.

Kidneys: Organ/brain weight was reduced by 9% in the 2000 mg/kg/day females.

Liver: Absolute weight and organ/bodyweight ratio were reduced by 12% and 6%, respectively, in the 2000 mg/kg/day males.

Testes: Absolute weight was reduced by 8% in the 2000 mg/kg/day males.

Thymus: Absolute weight and organ/bodyweight ratio were reduced by 15% in the 2000 mg/kg/day females.

Gross Pathology:

No treatment-related changes were observed.

Histopathology:

Liver: Hepatocellular vacuolation occurred in the 2000 mg/kg/day group (6/30 rats). This lesion was not associated with increased ALT in the affected animals. The authors considered this change to be related to metabolic stress rather than evidence of hepatotoxicity. Low- and intermediate-dose groups were not examined.

Lungs: Alveolar histiocytosis was observed in the control and 2000 mg/kg/day groups (1/30 and 5/30 rats, respectively). Interstitial inflammation occurred in the control and 2000 mg/kg/day groups (4/30 and 13/30 rats, respectively). Low- and intermediate-dose groups were not examined. The interstitial inflammation was characterized by focal or multifocal thickening of the alveolar walls, hypertrophy of pneumonocytes, "desquamation" of alveolar macrophages, and slight lymphocytic infiltration. The severity of this change was graded as trace level in all of the affected control rats, and in most of the affected high-dose rats. In two high-dose rats, the severity was graded as mild or moderate.

Uterus: Dilatation and hemorrhage was observed in the 2000 mg/kg/day group (3/15 and 1/15 females, respectively).

Toxicokinetics: The plasma kinetic parameters are shown in the following tables.

Study Day 1							
		Group 2 80 mg/kg/day		Group 3 400 mg/kg/day		Group 4 2000 mg/kg/day	
		Male	Female	Male	Female	Male	Female
AUC _{0-t}	(ng·h/mL)	181	203	1118	1133	6625	4541
AUC _{inf}	(ng·h/mL)	181	204	1121	1141	6703	4651
C _{max}	(ng/mL)	26.663	31.433	249.000	179.000	1490.000	1083.000
t _{1/2}	(h)	2.2	3.2	2.9	3.8	4.2	5.2
CL/F	(L/h/kg)	441	392	357	351	298	430
V _{d,area} /F	(L/kg)	1418	1828	1482	1927	1816	3213

Values were determined from 3 rats/sex/group/time-point.

Study Day 28						
	Group 2 80 mg/kg/day		Group 3 400 mg/kg/day		Group 4 2000 mg/kg/day	
	Male	Female	Male	Female	Male	Female
AUC _{0-t} (ngh/mL)	146	213	747	858	7671	7141
Accumulation Factor*	0.81	1.04	0.67	0.75	1.14	1.54
C _{max} (ng/mL)	14.667	117.700	117.100	183.667	908.667	3049.333
t _{1/2} (h)	3.8	7.3	4.7	N/C	3.3	3.2
CL/F (L/h/kg)	549	376	536	466	261	280
V _{d,area} /F (L/kg)	2978	3939	3611	N/C	1242	1305

N/C- Not Calculated. T_{1/2} and V_{d,area}/F could not be estimated because the concentration-vs.-time profile did not exhibit a log-linear terminal elimination phase.

*Accumulation factor was calculated as AUC_{0-t}(Study Day 28)/AUC_{0-t}(Study Day 1)

Values were determined from 3 rats/sex/group/time-point.

C_{max} values tended to be greater in females on day 28. In the 2000 mg/kg/day males, the increase in the AUC value was disproportionately large, relative to the dose increment. The increase in C_{max} values in males was also disproportionately large. No consistent sex-related differences in AUC values were observed. A low level of plasma drug accumulation appeared to occur in the 2000 mg/kg/day females, based on a comparison of the AUC values on days 1 and 28.

METHODS: CD(SD)IGS BR rats (approximately 6 weeks old; males: 167-209 g; females: 127-167 g) were treated orally with 0 (vehicle), 100, 1000/500, or 3000/2000/1000 mg/kg/day methylnaltrexone for at least 26 weeks (20 rats/sex/group). Additional groups were treated orally with the same doses of methylnaltrexone for measurement of toxicokinetic parameters (70 rats/sex/group). The high dose level was reduced from 3000 to 2000 mg/kg/day on day 21, and reduced further to 1000 mg/kg/day on day 45 due to mortality. The intermediate-dose level was decreased from 1000 to 500 mg/kg/day on day 45. The surviving animals in the high-dose toxicokinetic group were added to the high-dose main study group on day 92 due to mortality. Thereafter, these animals were subjected to the procedures designated for the main study groups. The test and control articles were administered by gavage using a dose volume of 10 ml/kg. The vehicle was deionized water. Dose selection was based on results of a 28-day oral toxicity study in rats, in which mild hepatic and pulmonary toxicity was observed at 2000 mg/kg/day.

Observations and Times:

Mortality: twice daily

Clinical Signs: daily

Bodyweight: weekly starting on day 1

Food Consumption: weekly

Ophthalmoscopy: prior to study initiation and on week 26

Hematology: blood samples were collected from the first 10 males and females in each group on day 90, and from all animals at termination

Clinical Chemistry: blood samples were collected from the first 10 males and females in each group on day 90, and from all animals at termination

Urinalysis: urine samples were collected on day 90 and at termination

Gross Pathology: at termination and immediately after premature death

Organ Weights: adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, salivary gland, seminal vesicles, spleen, testes, thymus, thyroid/parathyroid, uterus; paired organs were weighed together

Histopathology: The following organs/tissues were examined in the control and high-dose groups, and in all animals that died prematurely, unless stated otherwise: adrenals, brain, cecum, colon, duodenum, epididymides, esophagus, eyes, femur (with marrow), gross lesions (all groups), heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mesenteric), mammary gland (females), ovaries, pancreas, pituitary, prostate, rectum, salivary glands (mandibular), sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen.

sternum (with marrow), stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus, vagina.

Adequate Battery: yes (x) no ()

Peer Review: yes () no (x)

Toxicokinetics: Blood samples were collected at 0.25, 0.5, 0.75, 1, 2, 3, 4, 8, 12, and 24 hr post-dose on days 1 and 90 (5 and 3 rats/sex/group/time-point on days 1 and 90, respectively). Plasma drug concentrations were measured using a LC/MS/MS method.

RESULTS:

Mortality: Many deaths occurred in the intermediate- and high-dose groups. The incidence of deaths and the associated dose levels are shown in the table below.

Dose Group	Dose (mg/kg/day)	Deaths	Days
Intermediate	1000	1 male 5 females	35-41
	500	1 male 3 females	90 and 175
High	3000	3 males 3 females	2-20
	2000	1 male 3 females	21-35
	1000	16 males 13 females	52-170

Deaths occurred at dose levels of 500 mg/kg/day and higher. On day 90, the rats were dosed after blood was collected for clinical pathology. Within approximately 2 hr after dosing, death occurred in three rats in the intermediate-dose group and 16 rats in the high-dose group. The cause of deaths on day 90 could not be determined. However, the authors suggested that the high incidence of deaths on this day could be attributed to an increased concentration of drug in blood with a consequent increase in toxicity, as a result of lower blood volume following blood collection. At terminal sacrifice, the intermediate-dose group contained 18 males and 12 females, and the high-dose group had 14 males and 10 females (high-dose toxicokinetic animals were transferred to the high-dose main study group on day 92). The exact cause for any of the deaths was not stated in the report. However, moderate to severe congestion was observed in several organs, including lungs, liver, adrenals, and kidneys. These observations are suggestive of a decrease in blood pressure and/or terminal cardiac insufficiency, and are consistent with the hemodynamic effects observed in a cardiovascular function study in dogs (e.g. reductions in heart rate, blood pressure, cardiac output, and +dP/dt) (Pharmacology Review of IND dated November 8, 2002). Meningeal edema was also observed.

Clinical Signs: Red nasal discharge was observed in the 100, 1000/500, and 3000/2000/1000 mg/kg/day groups during days 1-91 (1/40, 7/40, and 6/40 rats, respectively). Squinting (one or both eyes) occurred in the 1000/500 and 3000/2000/1000 mg/kg/day groups during days 1-91 (2/40 and 10/40 rats, respectively). An increased incidence of rough hair coat was observed in the 3000/2000/1000 mg/kg/day group during days 1-91 (13/40 rats, compared with 1/40 rats in the control group). Reddening of ears occurred in the 1000/500 and 3000/2000/1000 mg/kg/day groups during days 1-91 (4/40 and 3/40 rats, respectively). During days 92-185, the only drug-related effect was reddening of ears in the 1000/500 and 3000/2000/1000 mg/kg/day groups (2/31 and 4/32 rats, respectively).

Bodyweight: Bodyweight data are summarized in the following tables.

TABLE 1

Dose (mg/kg/day)	Mean ($\pm$ S.D.) Bodyweight (g)			
	Initial	Week 4	Week 13	Week 27
0	M: 194 $\pm$ 9.4 F: 147 $\pm$ 10.2	M: 353 $\pm$ 23.8 F: 222 $\pm$ 14.1	M: 507 $\pm$ 67.1 F: 297 $\pm$ 18.0	M: 588 $\pm$ 83.2 F: 331 $\pm$ 19.9
100	M: 192 $\pm$ 8.8 F: 146 $\pm$ 10.8	M: 348 $\pm$ 22.7 F: 227 $\pm$ 16.5	M: 508 $\pm$ 45.1 F: 304 $\pm$ 33.4	M: 596 $\pm$ 61.9 F: 342 $\pm$ 46.7
1000/500	M: 192 $\pm$ 9.4 F: 147 $\pm$ 9.6	M: 342 $\pm$ 20.8 F: 228 $\pm$ 19.6	M: 483 $\pm$ 36.1 F: 302 $\pm$ 39.3	M: 539 $\pm$ 40.2 F: 335 $\pm$ 28.8
3000/2000/1000	M: 190 $\pm$ 10.5 F: 144 $\pm$ 9.8	M: 315 $\pm$ 31.4* F: 217 $\pm$ 17.6	M: 423 $\pm$ 52.1* F: 288 $\pm$ 31.6	M: 480 $\pm$ 59.0* F: 316 $\pm$ 37.6

M: males

F: females

* $p \leq 0.05$

TABLE 2

Dose (mg/kg/day)	Increase in Mean Bodyweight (g)	
	Week 4	Week 13
0	M: 159 F: 75	M: 313 F: 150
100	M: 156 F: 81	M: 316 F: 158
1000/500	M: 150 F: 81	M: 291 F: 155
3000/2000/1000	M: 125 F: 73	M: 233 F: 144

M: males

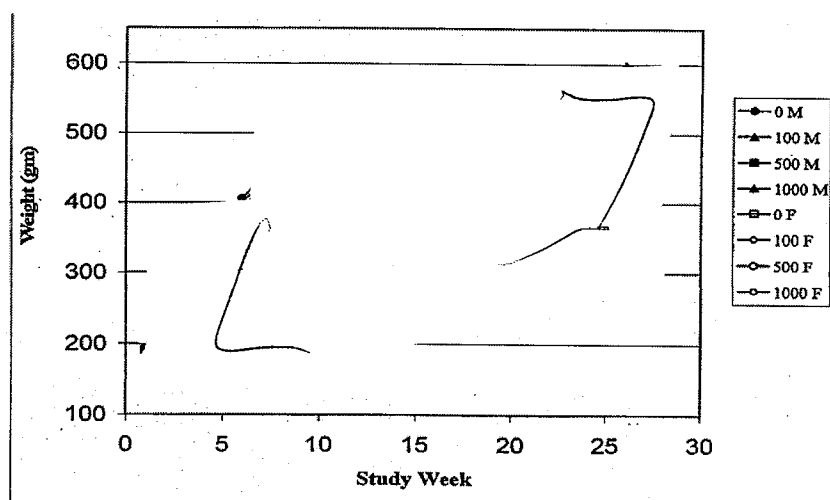
F: females

TABLE 3

Dose (mg/kg/day)	%Control Bodyweight at Week 27	Increase in Mean Bodyweight at Week 27 (g)
0	M: 100 F: 100	M: 394 F: 184
100	M: 101 F: 103	M: 404 F: 196
1000/500	M: 92 F: 101	M: 347 F: 188
3000/2000/1000	M: 82 F: 95	M: 290 F: 172

M: males
F: females

Mean bodyweight was significantly reduced in the high-dose males on weeks 4, 13, and 27 (table 1). Weight gain in the high-dose males was reduced by 21% at week 4, and by 26% at week 13 (table 2). Weight gain in the intermediate-dose males was suppressed by 6-7% at weeks 4 and 13, whereas no effect occurred in the low-dose males. The high-dose females exhibited a 4% decrease in weight gain on week 13, whereas increases of 5% and 3% occurred in the low- and intermediate-dose females, respectively. At week 27, weight gain was decreased by 12% and 26% in the intermediate- and high-dose males, respectively. The high-dose females exhibited a 7% decrease in weight gain on week 27, whereas increases of 7% and 2% occurred in the low- and intermediate-dose females, respectively. The changes in bodyweight during the course of the study are shown in the figure below (taken from the study report).



Food Consumption: Methylnaltrexone produced a dose-dependent increase in weekly food consumption in females (7-12% at 100 mg/kg/day, 9-16% at 1000/500 mg/kg/day, and 11-22% at 3000/2000/1000 mg/kg/day). Increased food intake in females occurred in at least one dose group on 17/26 weeks of treatment. Food intake was increased by 8-10% in the high-dose males on weeks 1 and 2. The food consumption was approximately 28-35 g/animal/day in control males, and 21-25 g/animal/day in the control females.

Ophthalmoscopy: No drug-related effects were observed.

ECG: Not performed.

Hematology: The results are shown in the table below.

Parameter	Dose (mg/kg/day)	Change	Week
Reticulocyte count	1000/500 ^m , 3000/2000/1000 ^m	28%, 33% decrease	13
	3000/2000/1000 ^f	18% decrease (ns)	13
	1000/500 ^m , 3000/2000/1000 ^m	21%, 29% decrease	27
Hemoglobin	1000/500 ^m , 3000/2000/1000 ^m	7% increase	13
	1000/500 ^m , 3000/2000/1000 ^m	5% increase	27
MCV	3000/2000/1000 ^m	4% increase	13, 27
MCH	3000/2000/1000 ^m	7% increase	13
	3000/2000/1000 ^m	5% increase	27
Platelets	1000/500 ^f , 3000/2000/1000 ^f	20%, 21% decrease	13
	1000/500 ^m , 3000/2000/1000 ^m	14%, 16% decrease	27
	3000/2000/1000 ^f	20% decrease	27
WBC	3000/2000/1000 ^f	30% decrease (ns)	13
Neutrophils	1000/500 ^f , 3000/2000/1000 ^f	55%, 67% increase	27
Lymphocytes	3000/2000/1000 ^f	36% decrease	13
Monocytes	1000/500 ^f , 3000/2000/1000 ^f	50% increase	27

ns: not significant

m: males

f: females

The most notable effects were decreased reticulocyte count in the intermediate- and high-dose groups, increased neutrophil count in the intermediate- and high-dose females, and decreased lymphocyte count in the high-dose females. PT (prothrombin time) and APTT (activated partial thromboplastin time) were not affected.

Clinical Chemistry: The results are shown in the table below.

Parameter	Dose (mg/kg/day)	Change	Week
Glucose	100 ^f , 1000/500 ^f , 3000/2000/1000 ^f	10-11% increase	13
Urea	3000/2000/1000 ^m	27% decrease	13
	100 ^f , 1000/500 ^f , 3000/2000/1000 ^f	13%, 20%, 20% decrease	13
	1000/500 ^m , 3000/2000/1000 ^m	14%, 21% decrease	27
	3000/2000/1000 ^f	20% decrease	27
ALT	1000/500 ^m , 3000/2000/1000 ^m	22-31% decrease	13, 27
Bilirubin	1000/500 ^m , 3000/2000/1000 ^m	50% decrease	13, 27
	100 ^f , 1000/500 ^f , 3000/2000/1000 ^f	50% decrease	13
Alkaline phosphatase	1000/500 ^m , 3000/2000/1000 ^m	17-20% decrease	13, 27
	3000/2000/1000 ^f	27% decrease	27
Cholesterol	1000/500 ^m , 3000/2000/1000 ^m	31%, 39% decrease	27
Triglycerides	1000/500 ^f , 3000/2000/1000 ^f	18%, 28% increase (ns)	27
Globulin	3000/2000/1000 ^m	13% decrease	27
A/G ratio	3000/2000/1000 ^m	20% increase	27
Potassium	3000/2000/1000 ^m	9% decrease	13
Chloride	1000/500 ^m , 3000/2000/1000 ^m	5%, 11% increase	13
	1000/500 ^f , 3000/2000/1000 ^f	4%, 8% increase	13
	1000/500 ^m , 3000/2000/1000 ^m	6%, 11% increase	27
	3000/2000/1000 ^f	7% increase	27

ns: not significant

m: males

f: females

None of the observed changes in the mean group values were clinically significant. However, large increases in ALT and AST were observed in individual females. These changes are shown in the table below.

Dose (mg/kg/day)	Rat #	Increase in AST	Increase in ALT	Week
1000/500	B70090	2.1-fold	2.9-fold	13
	B70091	1.6-fold	2.6-fold	13
	B70095	1.9-fold	3.0-fold	13
	B70104*	4.2-fold	5.6-fold	27
3000/2000/1000	B70180**	1.6-fold	2.3-fold	13
	B70191**	1.9-fold	2.3-fold	13
	B70192**	2.3-fold	3.4-fold	13
	B70266	4-fold	4.6-fold	27

Increases in AST and ALT are expressed relative to the mean control value in females.

* Blood was not collected on week 13.

** Animal died prior to study termination.

The increased ALT levels in the individual 3000/2000/1000 mg/kg/day females were not associated with drug-related liver lesions (1000/500 mg/kg/day females were not examined).

Urinalysis: The following parameters were reported: specific gravity, pH, glucose, bilirubin, ketones, nitrite, occult blood, protein, urobilinogen, appearance, color, casts, crystals, epithelial cells, leukocytes, erythrocytes, sperm, yeast, mucus, amorphous sediment, and bacteria. A slight reduction in pH occurred in the intermediate- and high-dose groups. An increase in leukocytes was observed in the intermediate- and high-dose females.

Organ Weights: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio for adrenals were increased by 5.9-fold, 7-fold, and 6-fold, respectively, in the high-dose males. These changes were not statistically significant, due to the high variability of the data. Organ/bodyweight ratio for adrenals was increased by 19% (not significant) in the high-dose females. Slight reductions in absolute weight occurred in epididymides, heart, kidneys, liver, lungs, salivary glands, seminal vesicles, and spleen. These changes are not considered to be biologically significant. The terminal bodyweight of high-dose males was reduced by 18%, compared with the control value. The reduction in bodyweight resulted in an increased organ/bodyweight ratio for brain, testes, and epididymides, in the absence of increased absolute organ weight.

Gross Pathology:

Premature Deaths

Liver: Dark appearance occurred in the intermediate- and high-dose groups (1/10 and 1/39 rats, respectively).

Lungs: Dark discoloration was observed in the intermediate- and high-dose groups (5/10 and 15/39 rats, respectively). Mottled appearance was observed in the intermediate- and high-dose groups (1/10 and 4/39 rats, respectively).

Terminal Sacrifice

Liver: Pale area occurred in the high-dose group (2/24 rats).

Uterus: Distension with fluid in lumen was observed in the low-, intermediate-, and high-dose groups (2/20, 1/12, and 1/10 females, respectively).

Histopathology:

Premature Deaths

Adrenals: The authors stated that moderate to marked congestion was observed, although this observation was not listed in the histopathology summary table.

Brain: Edema in meninges occurred in the intermediate- and high-dose groups (2/10 and 13/39 rats, respectively).

Kidneys: The authors stated that moderate to marked congestion was observed, although this observation was not listed in the histopathology summary table.

Liver: The authors stated that moderate to marked congestion was observed, although this observation was not listed in the histopathology summary table.

Lungs: Congestion was observed in the intermediate- and high-dose groups (9/10 and 32/39 rats, respectively). Hemorrhage occurred in the high-dose group (2/39 rats).

Terminal Sacrifice

No drug-related effects were observed.

Toxicokinetics: The plasma kinetic parameters are shown in the following table.

Dose (mg/kg/day)	C _{max} (ng/ml)	t _{max} (hr)	t _{1/2} (hr)	AUC _{0-24 hr} (ng•hr/ml)	AUC _{0-∞} (ng•hr/ml)
Day 1					
100	M: 20.0	M: 2	M: 3.9	M: 99.8	M: 101
	F: 21.2	F: 2	F: 3.7	F: 107	F: 108
1000	M: 429	M: 0.3	M: nc	M: 1423	M: nc
	F: 310	F: 0.5	F: 2.3	F: 1101	F: 1127
3000	M: 15983	M: 0.5	M: nc	M: 8392	M: nc
	F: 61909	F: 1	F: 1.6	F: 44967	F: 45061
Day 90					
100	M: 48.0	M: 2.0	M: 5.3	M: 191	M: 198
	F: 39.0	F: 0.5	F: 3.5	F: 147	F: 148
500	M: 10233	M: 0.3	M: 5.3	M: 3871	M: 3910
	F: 19871	F: 0.5	F: 5.5	F: 6306	F: 6327
1000	M: 516	M: 0.8	M: 4.7	M: 2280	M: 2311
	F: 44645	F: 0.5	F: 4.0	F: 17494	F: 17531

Values were determined from 5 and 3 rats/sex/group/time-point on days 1 and 90, respectively.

M: males

F: females

nc: Not calculated due to the absence of a defined terminal phase.

The increases in C_{max} and AUC values in the high-dose group on day 1 and the intermediate- and high-dose groups on day 90 tended to be disproportionately large relative to the dose increment, particularly in females. For example, the AUC_{0-24 hr} value in the 3000 mg/kg/day females on day 1 was 420-fold greater than the 100 mg/kg/day value, whereas the dose increment was only 30-fold. The AUC and C_{max} values in the low- and intermediate-dose groups on day 90 were higher than those observed on day 1, suggestive of plasma drug accumulation. The AUC and C_{max} values were higher in females in the high-dose group.

Conclusions: Mortality was observed at oral dose levels of 500 mg/kg/day and higher. The authors did not conclusively identify the cause of death. However, the dead animals exhibited moderate to severe congestion in lungs, liver, adrenals, and kidneys. These observations are suggestive of a decrease in blood pressure and/or terminal cardiac insufficiency, and are consistent with the hemodynamic effects observed in a cardiovascular function study in dogs (e.g. reductions in heart rate, blood pressure, cardiac output, and +dP/dt) (Pharmacology Review of IND, dated November 8, 2002). Therefore, the cardiovascular system is considered to be a target organ of toxicity at 500 mg/kg/day and higher. Meninges in brain is also considered as a target organ of toxicity, based on the incidence of edema in the 1000/500 and

3000/2000/1000 mg/kg/day groups (dead animals only). Weight gain in males was suppressed by 26% at the highest dose (3000/2000/1000 mg/kg/day) on week 13. The final bodyweight in the high-dose males was decreased by 18% relative to the control value. The NOAEL was 100 mg/kg/day. Therefore, the MTD is estimated to be 300 mg/kg/day, based on the incidence of mortality at 500 mg/kg/day and higher, histopathology data (i.e. congestion in multiple organs and meningeal edema at 500 mg/kg/day and higher), suppressed weight gain in males at 3000/2000/1000 mg/kg/day, and the absence of adverse effects at 100 mg/kg/day.

14-DAY INTRAVENOUS TOXICITY STUDY IN DOGS

STUDY NO. PH 432-UR-001-89)

This study has previously been reviewed under IND 64,583. The review of this study is incorporated below from the pharmacology review of IND 64,583 dated

Study title: 14-Day Intravenous Toxicity Study in Dogs

Key study findings: The NOEL was identified as 40 mg/kg/day. The target organ of toxicity appeared to be the central nervous system (CNS) based on the clinical signs (abnormal gait, ataxia).

Study no: PH 432-UR-001-89

Volume #, and page #: Vol. 1.3; Page: 664

Conducting laboratory and location: _____

Date of study initiation: March 6, 1989

GLP compliance: A statement of compliance was included.

QA report: yes (X) no ()

Drug, lot #, and % purity: Methylnaltrexone bromide, Lot. No. 0151D1. Purity data not provided.

Formulation/vehicle: 0.9% normal saline

Methods:

Dosing:

Species/strain: Dogs/Beagle

#/sex/group or time point (main study): 4/sex/dose

Satellite groups used for toxicokinetics or recovery: None.

Age: 188 – 246 days old

Weight: 7.9 – 10.8 kg

Reviewer: Tamal K. Chakraborti, Ph.D.

IND No. 64, 583

Doses in administered units: 2.5, 10, 20 mg/kg twice daily or 5, 20 and 40 mg/kg/day. The animals were administered MNTX at 5, 20 or 40 mg/kg/day intravenously for 14 consecutive days. The basis of dose selection was not mentioned.

Route, form, volume, and infusion rate: Intravenous, solution, and 0.5 ml/kg.

Observations and times:

Clinical signs: Daily

Body weights: Weekly

Food consumption: Daily

Ophthalmoscopy: Prior to treatment initiation and before study termination

Hematology: At termination

Clinical chemistry: At termination

Gross pathology: At necropsy

Organs weighed: The following organs were weighed from all animals: liver, adrenal glands, kidneys, gonads, thyroid and parathyroid.

Histopathology: The following organs/tissues from all animals in the control and high dose groups were examined for histopathological examinations: thyroid/parathyroid, thymus, aorta, lungs, trachea, heart, sternum with bone marrow, liver, spleen, kidneys/adrenals, pancreas, ovaries, testes, uterus, accessory genital organs (epididymides, prostate and seminal vesicles), esophagus, skin, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, urinary bladder, lymph nodes (mesenteric), mammary gland, thigh musculature, peripheral nerve, eyes, gall bladder, femur, spinal cord (cervical, midthoracic and lumbar) and lacrimal glands.

Toxicokinetics: None.

Results:

Mortality: None.

Clinical signs: Treatment related clinical signs included relaxation of the nictitating membrane, decreased activity, abnormal stance/gait, vascularizations and excessive licking and ataxia (especially in high dose group).

Body weights: The mean initial and final body weights of the control males were 10.7 and 10.8 kg, respectively. The mean initial and final body weights of the control females were 8.1 and 7.9 kg, respectively. No significant treatment related changes were observed.

Food consumption: The mean initial and final food consumption in control males were 0.271 and 0.373 kg/animal/day, respectively. The mean initial and final food consumption in control females were 0.155 and 0.217 kg/animal/day, respectively. At high dose, the food consumption in males was significantly decreased (66% of control, control = 0.313 kg) in males whereas food consumption was increased (149% of control, control = 0.203 kg) in females on Day 11 compared to control. The cause of this apparent sex-related difference in food consumption at high dose is not clear.

Ophthalmoscopy: No significant treatment related changes were observed.

Hematology: No significant treatment related changes were observed.

Clinical chemistry: There were no apparent significant treatment related changes.

Organ weights: No significant treatment related changes were observed.

Gross pathology: Several gross observations were reported at necropsy. These observations included: prescapular lymph nodes enlarged, dark, red or pitted green; cervical lymph nodes enlarged; mandibular lymph nodes enlarged, dark; mesenteric lymph nodes enlarged, dark or exhibiting scattered red foci; dilated brain ventricles; clear fluid-filled cyst on the pituitary gland; mottled lung lobes; raised subpleural cyst on costal surface of the lung, red nodule on the costal surface of the lung; firm tan nodule on the lung; raised dark and dark red area on the spleen, raised red linear area of the spleen and multiple red raised areas of the spleen; polycystic areas of the kidneys; clear fluid-filled ovarian cysts; mucosal reddening of the duodenum, the jejunum and a red slightly depressed area of the jejunum; enlarged payers patches of the ileum, raised red nodule of the ileocecal junction, red raised linear area of the ileum at the junction with the cecum, red areas on the mucosa of the ileum; red foci on the colon; red foci on the rectum, axillary lymph nodes red, dark red or enlarged. The relation of these changes with the treatment was not elucidated.

Histopathology: There were no significant treatment related changes.

Summary of individual study findings: In a 14-Day i.v. toxicity study in beagle dogs, animals were administered MNTX by intravenous injection (0.5 ml/kg) at 5, 20 and 40 mg/kg/day (2.5, 10 and 20 mg/kg b.i.d.). The NOEL was considered as 40 mg/kg/day (20 mg/kg b.i.d.). The target organ of toxicity appeared to be the central nervous system (CNS) based on the clinical signs (abnormal stance/gait, ataxia).

NINETY-DAY TOXICITY STUDY OF METHYLNALTREXONE ADMINISTERED
VIA INTRAVENOUS INJECTION TO BEAGLE DOGS
(STUDY NO. 154-004)

This study has previously been reviewed under IND _____. The review is incorporated below from the pharmacology review of IND _____ dated _____

Study Title: 90-Day Intravenous Toxicity Study in Dogs

Key Study Findings: In a 90-Day i.v. toxicity study in beagle dogs, animals were administered MNTX by intravenous bolus injection (0.5 ml/kg) at 1, 5 and 20 mg/kg/day. The NOAEL was considered as 1 mg/kg/day. The target organ of toxicity appeared to be the central nervous system (CNS) based on the clinical signs (abnormal stance/gait, ataxia). The heart could also be considered as a target organ based on the prolongation of QTc.

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Study No: 154-004

Volume #, and Page #: Vol. C15.2; Page: 1

Conducting Laboratory and Location: _____

Date of Study Initiation: January 14, 2002

GLP Compliance: A statement of compliance was included.

QA Report: yes (X) no ()

Drug, Lot #, and % Purity: Methylnaltrexone bromide, lot # C13753; _____ pure lot # C13754; _____ pure

Formulation/Vehicle: Solution/0.9% normal saline (NaCl)

Methods:

Dosing:

Species/strain: Dogs/Beagle

#/sex/group or time point (main study): 4/sex/dose

Satellite groups used for toxicokinetics or recovery: None.

Age: Approximately 7 months old.

Weight: Males: 10.9-14.8 kg; Females: 8.6-11.7 kg

Doses in administered units: Beagle dogs were treated intravenously with 0 (0.9% NaCl), 1, 5, or 25/20 mg/kg/day methylnaltrexone for 90 days (4 dogs/sex/group). The high dose in males was reduced to 20 mg/kg/day i.v. on Day 2, due to the incidence of prostration for 15-30 min and other clinical signs after the administration of 25 mg/kg on Day 1. Dosing in females was initiated one day after the males. The high-dose females were treated with 20 mg/kg/day i.v. throughout the entire study. The high dose will be referred to hereafter as 20 mg/kg/day. Dose selection was based on results from a 14-day intravenous toxicity study in dogs, in which dose levels of 2.5, 10, and 20 mg/kg b.i.d. were tested. No limiting toxicity was observed in this study, although treatment-related clinical signs indicative of CNS effects were noted (e.g. decreased activity, abnormal gait, and ataxia).

Route, form, volume: Intravenous, solution, and 0.5 ml/kg.

Observations and times:

Clinical signs: Clinical signs were observed on a daily basis.

Body weights: Body weights were recorded on a weekly basis.

Food consumption: Food consumption was recorded on a weekly basis.

Ophthalmoscopy: Ophthalmoscopic examinations were conducted prior to treatment initiation and on Week 6 and at study termination.

Electrocardiography (ECG): Electrocardiographic examinations were conducted at Weeks 1 and 12 and were performed within 0.5 hr of dosing.

Hematology: Blood was collected for hematology prior to study initiation, on Week 4, and at termination.

Clinical chemistry: Blood was collected for clinical chemistry prior to study initiation, on Week 4, and at termination.

Urinalysis: Urine was collected prior to study initiation and on weeks 4 and 12.

Gross pathology: Gross pathology was conducted at necropsy.

Organs weighed: The following organs were weighed from all animals: adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testes, thymus, and thyroids.

Histopathology: The following organs/tissues were examined in all groups: adrenals, aorta, bone (femur), bone with marrow (sternum), brain (brain stem, cerebellum, and cerebrum), cecum, cervix, colon, duodenum, epididymides, esophagus, eyes, gallbladder, gross lesions, heart, ileum, injection site, jejunum, kidneys, liver, lungs, lymph nodes (mandibular, mesenteric), mammary glands, optic nerve, ovaries, pancreas, pituitary, prostate, rectum, salivary glands (mandibular), sciatic nerve, skeletal muscle, skin, spinal cord (cervical, lumbar, thoracic), spleen, stomach (cardiac, fundic, pyloric), testes, thymus, thyroids, tongue, trachea, urinary bladder, uterus, vagina.

Toxicokinetics: Blood was collected at 0.03, 0.08, 0.25, 1, 4, 8, and 24 hr post-dose on days 1, 31, and 90. Plasma drug concentrations were measured using a validated LC/MS/MS method.

Results:

Mortality: None.

Clinical signs: Clinical signs included a high incidence of bloodshot eyes, dilated pupils, protruding nictitating membrane, and ptosis were seen in all treatment groups. Excessive lacrimation was also observed in each of the treatment groups. Retching and increased salivation were also observed at 5 and 20 mg/kg/day. A high incidence of ataxia was observed at 20 mg/kg/day. Reduced activity, prostration, and tremors occurred on a few occasions at 20 mg/kg/day.

Body weights: The mean initial and final body weights of the control males were 12.0 and 13.2 kg, respectively. The mean initial and final body weights of the control females were 9.7 and 10.6 kg, respectively. No significant treatment related changes were observed.

Food consumption: The mean initial and final food consumption in control males were 405.8 and 395.2 g/animal/day, respectively. The mean initial and final food consumption in control females were 369.6 and 378.3 g/animal/day, respectively. No significant treatment-related changes were observed.

Ophthalmoscopy: No significant treatment related changes were observed.

Electrocardiography: ECG was recorded within 0.5 hr of dosing. The reported parameters included morphology, heart rate, RR, PR, QRS, QT, QT_c(a) (Fridericia's formula), and QT_c(b) (Vandenhende's formula). On Day 6 in males, there were 8.6% and 15% increase in QT_c over vehicle control (mean = 242 msec) at 5 and 20 mg/kg, respectively, at 30 minutes after treatment. Similar increases were also seen in males at Day 83. In females at Day 6, there were 3.3%, 5.4% and 12% increase in QT_c over vehicle control (mean = 242 msec) at 1, 5 and 20 mg/kg, respectively, at 0.5 hour after treatment. Similar increases were also seen in females at Day 83. The data is shown in the following tables.

Dose ***	Males								
	Pre-Study			Day 6			Day 83		
	QT (msec)	QT _c (a) (msec)	QT _c (b) (msec)	QT (msec)	QT _c (a) (msec)	QT _c (b) (msec)	QT (msec)	QT _c (a) (msec)	QT _c (b) (msec)
0	188 ± 13	246 ± 6	221 ± 9	185 ± 6	242 ± 17	217 ± 10	193 ± 10	248 ± 13	224 ± 11
1	185 ± 19	242 ± 14	217 ± 16	180 ± 8	239 ± 10	213 ± 7	200 ± 0	256 ± 7	232 ± 4
5	190 ± 12	231 ± 11	214 ± 10	190 ± 12	263 ± 14	231 ± 12	214 ± 11*	263 ± 12	242 ± 9*
20	183 ± 13	229 ± 14	209 ± 13	213 ± 19*	279 ± 12**	250 ± 15**	205 ± 6	271 ± 11	243 ± 8*

Values are the mean ± S.D. of 4 dogs.

*p<0.05

**p<0.01

*** mg/kg/day

Dose ***	Females								
	Pre-Study			Day 6			Day 83		
	QT (msec)	QT _c (a) (msec)	QT _c (b) (msec)	QT (msec)	QT _c (a) (msec)	QT _c (b) (msec)	QT (msec)	QT _c (a) (msec)	QT _c (b) (msec)
0	188 ± 15	246 ± 16	221 ± 14	190 ± 8	242 ± 19	220 ± 11	195 ± 13	245 ± 10	223 ± 11
1	198 ± 10	254 ± 9	230 ± 9	195 ± 6	250 ± 12	226 ± 6	193 ± 10	256 ± 15	228 ± 10
5	185 ± 13	241 ± 19	217 ± 15	194 ± 16	255 ± 23	229 ± 20	205 ± 6	263 ± 11	238 ± 8
20	195 ± 6	258 ± 5	231 ± 3	205 ± 6	271 ± 7	242 ± 6	208 ± 5	271 ± 9*	244 ± 7*

Values are the mean ± S.D. of 4 dogs.

*p<0.05

**p<0.01

*** mg/kg/day

Hematology: No significant treatment related changes were observed.

Clinical chemistry: There were no apparent significant treatment related changes.

Urinalysis: No significant treatment-related effects were observed.

Organ weights: The following organ weight changes were observed: Spleen: Absolute weight, organ/bodyweight ratio, and brain/bodyweight ratio were reduced by 22-31%, 21-33%, and 19-32%, respectively, in all treatment group males (not significant). Thymus: Absolute weight, organ/bodyweight ratio, and brain/bodyweight ratio were reduced by 32-42%, 31-46%, and 33-40%, respectively, in all treatment group females (not significant).

Gross pathology: White focus or foci in capsule of spleen was observed at 20 mg/kg/day group (2 of 8 dogs). Multiple white scars in the capsule of the spleen were also observed at 5 mg/kg/day group (1 of 8 dogs).

Histopathology: Histopathological changes were observed in the spleen (focal fibrosis at 5 and 20 mg/kg/day in 1 of 8 and 2 of 8 dogs, respectively).

Toxicokinetics: There was no evidence of drug accumulation following repeated exposure for 90 days. The C_{max} and AUC appeared to increase in a greater than dose-proportional manner in both sexes at 5 and 20 mg/kg/day. The mean TK parameters are shown in the following table (from page 95 and 96 of Vol. C15.2 of sponsor's submission).

			Group 2 1 mg/kg/day		Group 3 5 mg/kg/day		Group 4 20/25* mg/kg/day	
			Male	Female	Male	Female	Male	Female
Study Day 1	n		4	4	4	4	4	4
	AUC ₀₋₂₄	(ng·h/mL)	652	889	5779	6098	40772	32028
	C _{max}	(ng/mL)	2958	3035	18350	19650	195150	72075
Study Day 31	n		4	4	4	4	4	4
	AUC ₀₋₂₄	(ng·h/mL)	708	776	6131	6142	33579	32087
	Accumulation Factor		1.1	0.87	1.1	1.0	N/A	1.0
Study Day 90	n		4	4	4	4	4	4
	AUC ₀₋₂₄	(ng·h/mL)	991	858	6609	6770	33811	35115
	Accumulation Factor		1.5	1.0	1.1	1.1	0.83	1.1
			C _{max}	(ng/mL)	12213	3855	48950	21875
							62425	95825

* Following a dose of 25 mg/kg on Study Day 1 for Group 4 males, the animals became prostrate. Per Protocol Amendment #2, the dose level for Group 4 was reduced from 25 mg/kg to 20 mg/kg on Study Day 2 for the male dogs and female dogs in Group 4 were started at 20 mg/kg.

N/A - Not Applicable

			Group 2 1 mg/kg/day		Group 3 5 mg/kg/day		Group 4 20/25* mg/kg/day	
			Male	Female	Male	Female	Male	Female
Study Day 1	n		4	4	4	4	4	4
	t _{1/2}	(h)	7.4	8.8	8.0	8.5	8.2	11
	CL**	(L/h/kg)	1.5	1.2	0.88	0.83	0.62	0.63
Study Day 31	n		4	4	4	4	4	4
	t _{1/2}	(h)	14	12	11	11	10	12
	CL	(L/h/kg)	1.4	1.3	0.82	0.84	0.60	0.64
Study Day 90	n		4	4	2**	2**	4	3**
	t _{1/2}	(h)	11	13	11	13	10	14
	V _d AUC	(L/kg)	18	23	10	14	9.0	11
Study Day 90	n		4	4	4	4	4	4
	CL	(L/h/kg)	1.1	1.2	0.81	0.78	0.62	0.59

* Following a dose of 25 mg/kg on Study Day 1 for Group 4 males, the animals became prostrate. Per Protocol Amendment #2, the dose level for Group 4 was reduced from 25 mg/kg to 20 mg/kg on Study Day 2 for the male dogs and female dogs in Group 4 were started at 20 mg/kg.

** Kel, t_{1/2}, and V_d AUC could not be calculated for some animals because the concentration versus time profile did not exhibit a log-linear terminal elimination phase.

Summary: In a 90-day i.v. toxicity study in beagle dogs, animals were administered MNTX by intravenous injection (0.5 mL/kg) at 1, 5 and 20 mg/kg/day. The NOAEL was considered as 1 mg/kg/day. The target organ of toxicity appeared to be the central nervous system (CNS) based on the clinical signs (abnormal stance/gait, ataxia). The heart could also be considered as a target organ based on the prolongation of QTc.

Addendum: Spleen appeared to be a target organ of toxicity, based on the incidence of fibrosis at 5 and 20 mg/kg/day.

**DOSE ESCALATING TOXICITY STUDY OF METHYLNALTREXONE
ADMINISTERED VIA ORAL GAVAGE TO BEAGLE DOGS**
(NO. 154-008, 2002)

This study has previously been reviewed under IND — The review is attached below from the pharmacology review of IND dated February 8, 2006.

Acute Oral Dose-Ranging Toxicity Study of Methylnaltrexone Administered Via Oral Gavage to Beagle Dogs

Key Study Findings: 25 mg/kg po produced no clinical signs, whereas signs were observed at 50 and 75 mg/kg

Study # 154-008

Vol. 8, Pg. 172

Testing Laboratory: _____

Study Dates: March 12, 2002-August 19, 2002

GLP Compliance: The study was not conducted under GLP guidelines.

QA Report: (x)Yes ()No

Drug: lot # C13753; _____

Formulation/Vehicle: solution/deionized water

Animals: Beagle dogs (approximately 9 months old)
1 male, age 16 months, 14.4 kg
1 female, age 19 months, 8.7 kg

Methods: Dogs (one male and one female) were treated orally with methylnaltrexone according to the following schedule: 75 mg/kg on day 1, 50 mg/kg on day 8, and 25 mg/kg on day 13. The drug was administered by gavage using a dose volume of 3 ml/kg. The animals were observed through day 15.

RESULTS:

Mortality: None.

Clinical Signs: Administration of 50 mg/kg in the male produced protrusion of nictitating membrane (both eyes), pupil dilatation, and excessive lacrimation. The same dose produced only pupil dilatation in the female. Administration of 75 mg/kg in the male produced protrusion of nictitating membrane (both eyes), pupil dilatation, excessive lacrimation, white vomitus, retching, bloodshot eyes, ptosis, and decreased activity. The female exhibited pupil dilatation and reduced activity after dosing with 75 mg/kg. All of these effects were reversible.

Bodyweight: Bodyweight in the male and female was reduced by 0.3 and 0.9 kg, respectively, during the study period.

Food Consumption: Food intake in the female was reduced by about 2000 g on days 8-15, as compared with days 1-8. Food intake could not be measured in the male on days 8-15 due to spillage of food.

Conclusions: Administration of 25 mg/kg po produced no clinical signs, whereas signs were observed at 50 and 75 mg/kg po.

Dose-Escalation Toxicity Study of Methylnaltrexone Administered Via Oral Gavage to Beagle Dogs

Key Study Findings: maximum tolerated dose was 1000 mg/kg

Study # 154-010

Vol. 4, Pg. 1

Testing Laboratory

Study Dates: April 20, 2002-October 8, 2002

GLP Compliance: The study was not conducted under GLP guidelines.

QA Report: (x)Yes ()No

Drug: lot # C13753;
lot # D05340

Formulation/Vehicle: solution/deionized water
suspension/0.5% carboxymethylcellulose

Animals: Beagle dogs (approximately 9 months old)
1 male, 14.4 kg; 1 female, 8.7 kg

Methods: Dogs (one male and one female) were treated orally via gavage with escalating doses of methylnaltrexone over the course of 44 days, as described in the following table.

Dose (mg/kg)	Day(s)	Concentration (mg/ml)	Dose Volume (ml/kg)	Vehicle
100	1, 2	33.3	3	water
150	3	50	3	water
250	4	83.3	3	water
350	5	116.7	3	water
700	6	233.3	3	water
1000	38	333.3	3	water
1500	42	500	3	0.5% CMC
1500*	43	300	5	0.5% CMC
2000*	44	200	10	0.5% CMC

* male only

The gavage became clogged during the dosing procedure on day 42. Therefore, this dose (1500 mg/kg) was repeated on day 43. The authors stated that the 2000 mg/kg dose was difficult to administer due to the drug settling out of suspension in the dosing syringe. The animals were observed through day 52. Dose selection was based on results from an oral dose escalation study and a 7-day pilot oral study in dogs (study # 154-009, reviewed in this submission).

RESULTS:

Mortality: None.

Clinical Signs: Dilated pupils and protruding nictitating membrane were observed 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.

Bodyweight: The male gained 0.8 kg during the study. The female lost 0.2 kg.

Food Consumption: No effects.

Conclusions: The maximum tolerated dose is considered to be 1000 mg/kg po. Clinical signs were observed at all dose levels.

SEVEN-DAY PILOT TOXICITY STUDY OF METHYLNALTREXONE ADMINISTERED VIA ORAL GAVAGE TO BEAGLE DOGS **STUDY NO. 154-009)**

This study has previously been reviewed under IND The review is attached below from the pharmacology review of IND dated February 8, 2006.

Seven-Day Pilot Toxicity Study of Methylnaltrexone Administered Via Oral Gavage to Beagle Dogs

Key Study Findings: oral administration of 75 mg/kg/day produced clinical signs and slight weight loss

Study # 154-009

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Vol. 8, Pg. 211

Testing Laboratory: F

Study Dates: April 4, 2002-June 20, 2002

GLP Compliance: The study was not conducted under GLP guidelines.

QA Report: (x)Yes ()No

Drug: lot # C13753;

Formulation/Vehicle: solution/deionized water

Animals: Beagle dogs

1 male, age 18 months, 10.1 kg

1 female, age 19 months, 16.9 kg

Methods: Dogs (one male and one female) were treated orally with 75 mg/kg/day for seven days. The drug was administered by gavage using a dose volume of 3 ml/kg. Dose selection was based on results from an oral dose-ranging study in dogs (study # 154-008, reviewed above).

RESULTS:

Clinical Signs: No signs were observed on day 1. During days 2-7, the following signs were observed: protrusion of nictitating membrane (male only), dilatation of pupils, excessive lacrimation, bloodshot eyes, reduced activity (male only), and ptosis. No signs were observed on day 8.

Bodyweight: Weight was reduced by 0.3 and 0.5 kg in the male and female, respectively.

Food Consumption: Daily food intake varied during the study. No treatment-related effect was apparent.

Hematology (pre-study and day 8): No substantial changes from baseline values were observed.

Clinical Chemistry (pre-study and day 8): No substantial changes from baseline values were observed.

Conclusions: 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.

SEVEN-DAY PILOT TOXICITY STUDY OF METHYLNALTREXONE
ADMINISTERED VIA ORAL GAVAGE TO BEAGLE DOGS (
STUDY NO. 154-010)

This study has previously been reviewed under IND The review is attached below from the pharmacology review of IND dated February 8, 2006.

Dose-Escalation Toxicity Study of Methylnaltrexone Administered Via Oral Gavage to Beagle Dogs

Key Study Findings: maximum tolerated dose was 1000 mg/kg

Study # 154-010

Vol. 4, Pg. 1

Testing Laboratory

Study Dates: April 20, 2002-October 8, 2002

GLP Compliance: The study was not conducted under GLP guidelines.

QA Report: (x)Yes ()No

Drug: lot # C13753;
lot # D05340;

Formulation/Vehicle: solution/deionized water
suspension/0.5% carboxymethylcellulose

Animals: Beagle dogs (approximately 9 months old)
1 male, 14.4 kg; 1 female, 8.7 kg

Methods: Dogs (one male and one female) were treated orally via gavage with escalating doses of methylnaltrexone over the course of 44 days, as described in the following table.

Dose (mg/kg)	Day(s)	Concentration (mg/ml)	Dose Volume (ml/kg)	Vehicle
100	1, 2	33.3	3	water
150	3	50	3	water
250	4	83.3	3	water
350	5	116.7	3	water
700	6	233.3	3	water
1000	38	333.3	3	water
1500	42	500	3	0.5% CMC
1500*	43	300	5	0.5% CMC
2000*	44	200	10	0.5% CMC

* male only

The gavage became clogged during the dosing procedure on day 42. Therefore, this dose (1500 mg/kg) was repeated on day 43. The authors stated that the 2000 mg/kg dose was difficult to administer due to the drug settling out of suspension in the dosing syringe. The animals were observed through day 52. Dose selection was based on results from an oral dose escalation study and a 7-day pilot oral study in dogs (study # 154-009, reviewed in this submission).

RESULTS:

Mortality: None.

Clinical Signs: Dilated pupils and protruding nictitating membrane were observed 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.

Bodyweight: The male gained 0.8 kg during the study. The female lost 0.2 kg.

Food Consumption: No effects.

Conclusions: The maximum tolerated dose is considered to be 1000 mg/kg po. Clinical signs were observed at all dose levels.

TWENTY-EIGHT DAY TOXICITY STUDY OF METHYLNALTREXONE ADMINISTERED VIA ORAL GAVAGE TO BEAGLE DOGS / STUDY NO. 154-013)

This study has previously been reviewed under IND — The review of this study is attached below from the pharmacology review of IND — dated February 8, 2006.

QA Report: (x)Yes ()No

Drug: lot # D10125, pure

Formulation/Vehicle: solution/deionized water

Animals: Beagle dogs, age 6-8 months

Males: 7.1-12.5 kg

Females: 6.2-8.0 kg

METHODS: Dogs were treated orally with 0 (vehicle), 60, 300, and 1500/1000/750 mg/kg/day (4 dogs/sex/group). The test and control articles were administered by gavage using a dose volume of 8 ml/kg. The 0, 60, and 300 mg/kg/day groups were treated for 28 days. Dosing of the high-dose males was interrupted due to the death of one male on day 1 after treatment with 1500 mg/kg. This animal was replaced. The remaining high-dose males were allowed a 5-day washout period before the resumption of dosing with 1000 mg/kg for one day only, which produced three more deaths. The only surviving high-dose male was then treated with 750 mg/kg/day for an additional four days. The start of treatment of the high-dose females was delayed by one day and was initiated at 1000 mg/kg/day, given for five days. The high dose in females was then reduced to 750 mg/kg/day due to mortality, and was given for an additional eight days. All animals were sacrificed the day after termination of treatment. Dose selection

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was based on results from a 7-day oral toxicity study (#154-009) and a dose-escalation study in dogs (#154-010), reviewed above.

Clinical Signs: once daily (twice daily for mortality)

Bodyweight: once weekly

Food Consumption: once weekly

Ophthalmoscopy: prior to study initiation and at termination

ECG: pre-study in all animals, day 27 in the control, 60, and 300 mg/kg/day groups, day 4 in one high-dose male (750 mg/kg/day), and day 8 in two high-dose females (750 mg/kg/day); performed within approximately 0.5 hr of dosing

Hematology: Blood was collected prior to study initiation in all groups, on days 15 and 29 (termination) in the control, 60, and 300 mg/kg/day groups, on day 4 in the high-dose males, and on day 8 in the high-dose females. Bone marrow smears were prepared from each animal, but were not examined.

Clinical Chemistry: Blood was collected prior to study initiation in all groups, on days 15 and 29 (termination) in the control, 60, and 300 mg/kg/day groups, on day 4 in the high-dose males, and on day 8 in the high-dose females.

Urinalysis: Urine was collected prior to study initiation in all groups and within five days of termination in the control, 60, and 300 mg/kg/day groups.

Gross Pathology: day of sacrifice or day of death

Organ Weights: adrenals, brain, epididymides, heart, kidneys, liver, ovaries, pituitary, spleen, testes, thymus, and thyroids; paired organs were weighed together

Histopathology: The following organs/tissues were examined in all groups: adrenals, aorta, bone (femur), bone with marrow (sternum), brain (brain stem, cerebellum, and cerebrum), cecum, cervix, colon, duodenum, epididymides, esophagus, eyes, gallbladder, gross lesions, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mandibular, mesenteric), mammary glands, optic nerve, ovaries, pancreas, pituitary, prostate, rectum, salivary glands (mandibular), sciatic nerve, skeletal muscle, skin, spinal cord (cervical, lumbar, thoracic), spleen, stomach (cardiac, fundic, pyloric), testes, thymus, thyroids, tongue, trachea, urinary bladder, uterus, vagina.

Toxicokinetics: Blood was collected at 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hr post-dose. The days of blood sampling for the individual dose groups are shown in the following table.

Day	Dose Group (mg/kg/day)
1	60, 300, and 1500/1000/750
4	1500/1000/750 (males)
8	1500/1000/750 (females)
15	0, 60, and 300
28	0, 60, and 300

Plasma drug concentrations were measured using a validated LC/MS/MS method.

RESULTS:

Mortality: Several deaths occurred in the intermediate- and high-dose groups, as shown in the following table.

MORTALITY TABLE

Study Day	Date	Dose (mg/kg)	Group	Sex	Animal #	Disposition
1	9-12-02	1500	4	Male	2587	FD
2	9-14-02	1000	4	Female	2584	FD
1	9-17-02	1000	4	Male	2581	FD
1	9-17-02	1000	4	Male	2585	FD
1	9-17-02	1000	4	Male	2617	FD
2	9-18-02	300	3	Male	2573	FD
3	9-19-02	300	3	Male	2579	FD
5	9-17-02	1000	4	Female	2586	FD
5	9-21-02	300	3	Male	2577	FD

FD = found dead

The remaining high-dose animals were sacrificed prematurely due to severe clinical signs, including clonic whole body convulsions, prostration, ataxia, and retching.

Clinical Signs: Bloodshot eyes, protrusion of nictitating membrane, and ptosis were frequently observed in all treatment groups. Clonic whole-body convulsions, ataxia, prostration, and retching occurred occasionally in the intermediate- and high-dose groups. Dilated pupils were observed in the 300 mg/kg/day group. A low incidence of excessive lacrimation was observed in all treatment groups (females only). Soft stool was observed sporadically in all treatment groups. One high-dose male exhibited otitis externa.

Bodyweight: Weight gain was unaffected. The mean bodyweight of control males and females was 10.4 ± 1.6 kg and 7.4 ± 0.4 kg, respectively, on day 1, and 10.8 ± 1.9 kg and 7.8 ± 0.6 kg, on day 28.

Food Consumption: Food intake for the entire treatment period was increased by 17% and 19% in the 60 and 300 mg/kg/day females, respectively. Food consumption in the surviving 300 mg/kg/day male was 20% higher than the control value.

Ophthalmoscopy: One high-dose female exhibited ptosis and prolapse of nictitating membrane on the final day of treatment.

Electrocardiography: The ECG recordings were performed within approximately 0.5 hr of dosing. The reported parameters included morphology (rhythm), heart rate, RR, PR, QRS, QT, and QT_c (Fridericia's formula). Heart rate, RR, and QRS were unaffected. No treatment-related abnormalities in morphology were observed. PR was reduced by 21% in the 60 mg/kg/day females. Increases in QT and QT_c were observed in the three surviving animals in the high-dose (750 mg/kg/day) group, relative to the pre-study values. Each of the QT_c values in these animals exceeded the maximum value (259 msec) in the vehicle control group. QT_c was not affected at 300 mg/kg/day or lower. The QT_c values are shown in the following tables.

Dose (mg/kg/day)	Sex	Pre-study QT _c (msec)	Day 27 QT _c (msec)
0 (vehicle)	M	242 ± 15	245 ± 13
	F	228 ± 12	237 ± 18
60	M	238 ± 7	252 ± 12
	F	244 ± 6	244 ± 11
300	M	246 ± 9	253*
	F	238 ± 10	247 ± 9
1500/1000/750	M	228 ± 6	NS
	F	242 ± 13	NS

Values are the mean ± S.D. of 4 dogs/sex/group, except where stated otherwise.

*Only one animal was available due to mortality

NS: No surviving animals remained by day 27.

Dose (mg/kg/day)	Dog #	Sex	Day	Pre-study QT _c (msec)	QT _c (msec)
750	2583	M	4	227	277
750	2582	F	8	235	288
750	2588	F	8	231	267

Due to mortality, ECG was not performed in three dogs in the 300 mg/kg/day group, or in five dogs in the 1500/1000/750 mg/kg/day group.

Hematology:

RBC: The surviving 300 mg/kg/day male exhibited a 10% and 19% decrease on days 15 and 29, respectively. The 60 and 300 mg/kg/day females exhibited a reduction of 10% and 8% (not significant), respectively, on day 29. A 12% decrease occurred in the high-dose females at premature sacrifice (day 14), relative to the mean pre-study value.

Hemoglobin: The surviving 300 mg/kg/day male exhibited a 13% decrease on day 29. An 11% decrease occurred in the high-dose females at premature sacrifice (day 14), relative to the mean pre-study value.

Hematocrit: A decrease of 15% occurred in the surviving 300 mg/kg/day male on day 29. A reduction of 10% occurred in the high-dose females at premature sacrifice (day 14), relative to the mean pre-study value.

Coagulation: PT and APTT were unaffected.

Clinical Chemistry:

ALT: Increased by 89% in the surviving 300 mg/kg/day male on day 29; increased by 1.9- and 2.7-fold in two high-dose females at premature sacrifice (day 14), relative to the baseline values. AST: Increased by 3- and 4.4-fold in two high-dose females at premature sacrifice, relative to the baseline values; increased by 32% in the 300 mg/kg/day females.

Cholesterol: Reduced by 26% in the surviving high-dose male at premature sacrifice, relative to the pre-study value.

Triglycerides: Increased by 54% in the high-dose females at premature sacrifice (day 14), relative to the baseline values.

Chloride: Increased by 21% in the surviving high-dose male at premature sacrifice, relative to pre-study value; increased by 19% in the high-dose females at premature sacrifice, relative to the baseline values; increased by 11-15% in the surviving 300 mg/kg/day male on days 15 and 29; increased by 12-13% in the 300 mg/kg/day females on days 15 and 29; increased by 5-6% in the 60 mg/kg/day males on days 15 and 29.

Urinalysis: The following parameters were reported: glucose, bilirubin, ketone, specific gravity, occult blood, pH, protein, urobilinogen, nitrite, leukocytes, RBC, WBC, epithelial cells, casts, crystals, color, and clarity. No changes occurred.

Organ Weights: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were reported.

Heart: Absolute weight and organ/brain weight ratio was increased by 30% and 20%, respectively, in the surviving 300 mg/kg/day male.

Kidneys: Absolute weight was increased by 18% in the surviving 300 mg/kg/day male.

Liver: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were increased by 55%, 19%, and 42%, respectively, in the surviving 300 mg/kg/day male.

Gross Pathology:

Premature Deaths (3 at 300 mg/kg/day, 9 at 1500/1000/750 mg/kg/day)

Gastrointestinal Tract: Red, black, and white foci, and red discoloration in mucosa were observed throughout all segments in each animal.

Terminal Sacrifice

Gastrointestinal Tract: Red foci, thick mucosa, and red discoloration were observed in the duodenum-rectum segment of the GI tract at 60 and 300 mg/kg/day (4/8 and 4/5 dogs, respectively).

Stomach: Multiple white nodules in pyloric mucosa occurred in the 60 mg/kg/day group (1/4 males).

Histopathology:

Premature Deaths (3 at 300 mg/kg/day, 9 at 1500/1000/750 mg/kg/day)

Cecum: Congestion occurred in the 300 and 1500/1000/750 mg/kg/day groups (3/3 and 8/9 dogs, respectively).

Colon: Congestion was observed in the 300 and 1500/1000/750 mg/kg/day groups (3/3 and 8/9 dogs, respectively).

Duodenum: Congestion occurred in the 300 and 1500/1000/750 mg/kg/day groups (3/3 and 8/9 dogs, respectively).

Ileum: Congestion was observed in the 300 and 1500/1000/750 mg/kg/day groups (1/3 and 7/9 dogs, respectively).

Jejunum: Congestion occurred in the 300 and 1500/1000/750 mg/kg/day groups (all dogs).

Kidneys: Congestion was observed in the 1500/1000/750 mg/kg/day group (3/9 dogs). Tubular necrosis and hemorrhage occurred in the 1500/1000/750 mg/kg/day group (1/9 dogs).

Liver: Congestion was observed in the 300 and 1500/1000/750 mg/kg/day groups (2/3 and 6/9 dogs, respectively).

Lungs: Congestion and edema occurred in the 1500/1000/750 mg/kg/day group (1/9 dogs).

Lymph Node: Hemorrhage of mandibular node occurred in the 300 and 1500/1000/750 mg/kg/day groups (1/3 and 1/9 dogs, respectively). Sequestered blood was observed in the mesenteric lymph node in the 300 and 1500/1000/750 mg/kg/day groups (1/3 and 3/9 dogs, respectively).

Pancreas: Congestion occurred in the 1500/1000/750 mg/kg/day group (3/9 dogs).

Pituitary: Congestion was observed in the 1500/1000/750 mg/kg/day group (2/9 dogs).

Rectum: Congestion occurred in the 300 and 1500/1000/750 mg/kg/day groups (2/3 and 6/9 dogs, respectively).

Stomach: Congestion was observed in the 300 and 1500/1000/750 mg/kg/day groups (2/3 and 7/9 dogs, respectively).

Thymus: Congestion occurred in the 1500/1000/750 mg/kg/day group (4/9 dogs). Hemorrhage was observed in the 300 and 1500/1000/750 mg/kg/day groups (2/3 and 5/9 dogs).

Urinary Bladder: Congestion and hemorrhage occurred in the 1500/1000/750 mg/kg/day group (1/9 dogs).

Uterus: Hemorrhage was observed in the 1500/1000/750 mg/kg/day group (2/4 females).

Terminal Sacrifice (0, 60, and 300 mg/kg/day)

Cecum: Congestion occurred in the 300 mg/kg/day group (1/5 dogs).

Duodenum: Congestion was observed in the 300 mg/kg/day group (1/5 dogs).

Jejunum: Congestion occurred in the control and 300 mg/kg/day groups (1/8 and 1/5 dogs, respectively).

Lymph Nodes: Sequestered blood in the mandibular node was observed in the 60 and 300 mg/kg/day groups (1/8 and 2/5 dogs, respectively). Sequestered blood in the mesenteric node was observed in the 60 and 300 mg/kg/day groups (1/8 and 5/5 dogs, respectively).

Thymus: Hemorrhage occurred in the 60 and 300 mg/kg/day groups (2/8 and 1/5 dogs, respectively).

Toxicokinetics: Plasma kinetic parameters are shown in the following tables.

Table C: Mean Toxicokinetics Parameters for Methylnaltrexone in Male and Female Beagle Dogs – Study Day 1

		Group 2 60 mg/kg/day		Group 3 300 mg/kg/day		Group 4 1000 (750)* mg/kg/day	
		Male	Female	Male	Female	Male	Female
n		4	4	4	4	1	4
AUC ₀₋₄	(ng·h/mL)	7194	8877	60775	96955	246550	273325
AUC ₀₋₈	(ng·h/mL)	7447	9213	61821	98041	249521	241888
C _{max}	(ng/mL)	2515	2474	21375	33393	110000	72450
t _{1/2}	(h)	8.2	9.7	8.1	7.4	5.8	5.0
CL/F	(L/h/kg)	14	20	6.7	3.8	4.0	6.3
V _{d, area} /F	(L/kg)	154	271	111	45	34	46

* Per Protocol Amendment No. 2, due to deaths of male dogs at the 1000 mg/kg dose level, the dose level for Group 4 was further reduced to 750 mg/kg, on Study Days 2 and 6 for male and female dogs, respectively.

Table D: Mean Toxicokinetics Parameters for Methylnaltrexone in Male and Female Beagle Dogs – Study Days 4, 8 and 15

Study Days 4 and 8

	Group 4 1000 (750)* mg/kg/day			
	Study Day 4		Study Day 8	
	Male	Female	Male	Female
n	1	-	-	2
AUC ₀₋₁₂ (ng·h/mL)	49806	N/D	N/D	167397
Accumulation Factor	N/D	N/D	N/D	N/C
C _{max} (ng/mL)	6920	N/D	N/D	29100
t _{1/2} (h)	7.7	N/D	N/D	4.7
CL/F (L/h/kg)	15	N/D	N/D	7.9
V _{d area} /F (L/kg)	167	N/D	N/D	60

* Per Protocol Amendment No. 2, due to deaths of additional dogs at the 1000 mg/kg dose level, the dose level for Group 4 was further reduced from 1000 mg/kg to 750 mg/kg, on Study Day 2 for males and on Study Day 5 for females. N/D – Not determined. Per Protocol Amendment No. 3, dosing in Group 4 was terminated and surviving animals sacrificed after four days of dosing in males and eight days of dosing in females. N/C – Not calculated.

Study Day 15

	Group 2 60 mg/kg/day		Group 3 300 mg/kg/day	
	Male	Female	Male	Female
N	4	4	1	4
AUC ₀₋₁₂ (ng·h/mL)	2289	1722	76812	10167
Accumulation Factor	0.31	0.19	1.24	0.10
C _{max} (ng/mL)	898	801	22000	2235
t _{1/2} (h)	13	14	8.4	10
CL/F (L/h/kg)	41	43	3.9	30
V _{d area} /F (L/kg)	784	569	48	426

Mean Toxicokinetic Parameters for Methylnaltrexone in Male and Female Beagle Dogs – Study Day 28

	Group 2 60 mg/kg/day		Group 3 300 mg/kg/day	
	Male	Female	Male	Female
n	4	4	1	4
AUC ₀₋₂₄ (ng·h/mL)	6710	4915	33183	40384
Accumulation Factor	0.90	0.53	0.54	0.41
C _{max} (ng/mL)	3251	1288	6290	9203
t _{1/2} (h)	12	16	13	9.7
CL/F (L/h/kg)	22	44	9.0	13
V _d (L/kg)	388	985	169	163

The increase in AUC and C_{max} values tended to be disproportionately large relative to the dose increment, with the exception of the high-dose group on day 1, the 300 mg/kg/day females on day 15, and the surviving 300 mg/kg/day males on day 28. There were no consistent sex-related differences in the plasma kinetic parameters. The AUC and C_{max} values were sharply reduced on days 15 and 28, as compared to day 1. The t_{max} values ranged from 0.5 to 4 hr (data not shown).

Conclusions: Dose levels of 300 mg/kg/day po and higher produced mortality. All animals in the high-dose group died early in the study or were sacrificed prematurely due to severe toxicity (i.e. clonic whole body convulsions, prostration, ataxia, and retching). Kidney is considered to be a target organ of toxicity, based on the incidence of tubular necrosis in one high-dose animal that died prematurely. Congestion was observed in many organs in the 300 and 1500/1000/750 mg/kg/day groups, mostly in dogs that died prematurely. This suggests that cardiovascular function was impaired. No lesions in heart were observed. CNS was a target organ of toxicity in the 300 and 1500/1000/750 mg/kg/day groups, based on the incidence of convulsions in these groups. Prolongation of QT_c was observed after administration of 750 mg/kg in the three surviving animals of the high-dose group. The clinical significance of the QT_c prolongation is uncertain, given the severe toxicity and mortality that occurred at this dose level. A NOAEL was not established, due to the incidence of bloodshot eyes, nictitating membrane protrusion, and ptosis in all treatment groups. The tolerated dose was 60 mg/kg/day po.

39-WEEK ORAL GAVAGE CHRONIC TOXICITY AND TOXICOKINETIC STUDY WITH METHYLNALTREXONE IN DOGS (STUDY NO. 7434-104)

The report of this study was submitted under IND — Amendment 032 dated August 2, 2006 and will be reviewed under IND — However, a brief summary of this study is given below.

Methods: In this study, MNTX was administered orally by gavage to male and female Beagle dogs (4/sex/group) at dosages of 0, 20, 60 or 180/225/250 mg/kg/day, once daily, for 39-weeks at a dose volume of 5 mL/kg. The control group received water. Based on the lack of clinical signs of toxicity, the highest dose that was 180 mg/kg/day for days 1 through 42 was escalated twice to 225 mg/kg/day for days 43 through 70 and 250

mg/kg/day for day 71 through sacrifice. The following parameters were examined: mortality, clinical observations, electrocardiography, blood pressure, ophthalmologic observations, body weights, food consumption, clinical observations, organ weights, hematology, coagulation, clinical chemistry, urinalysis, and macroscopic and microscopic observations. Plasma was collected for toxicokinetic evaluations on days 1, 42, and 268.

Results: Two females died before the scheduled necropsy. The first animal died on day 23 at 180 mg/kg/day and the cause of death was considered to be gavage-related, as red, foamy contents were found in lungs at necropsy, and death was not preceded by an MNTX-related clinical signs (ataxia, tremors). The second animal's death (180/225/250 mg/kg/day) occurred on day 104 due to gavage accident. Treatment-related clinical signs included tremors and ataxia in 1 male and 1 female at 180/225/250 mg/kg/day on days 43 and 71, respectively. There were increased incidences of elevated third eyelids or red sclera in both sexes at ≥ 20 mg/kg/day. There were no treatment-related effects on mean body weights, body weight gains, food consumption, ophthalmic findings, hematology values, clinical chemistry values, urinalysis results, organ weights, or macroscopic and microscopic observations in animals examined at the scheduled necropsy. There were no treatment-related effects on blood pressure, electrocardiograms, heart rate, QT interval, or QTc. Methylnaltrexone was absorbed rapidly after oral administration to dogs, with T_{max} at approximately 2 hours. Exposures generally increased in a slightly greater than dose-proportional manner over the dosage range of 20 to 250 mg/kg/day. There was no apparent gender difference in the toxicokinetics and the elimination half-life was independent of the dosage after daily treatment for 268 days. There was no apparent accumulation of MNTX over time. In conclusion, the NOAEL appeared to be 60 mg/kg/day. The target organ of toxicity could not be identified in the absence of significant histopathological findings in any organ or tissue.

2.6.6.4 Genetic toxicology

IN VITRO MAMMALIAN CHROMOSOME ABERRATION TEST
STUDY NO. AA53RH.331.BTL, 2002)

This study has previously been reviewed under IND 64,583 Serial No. 002. The review of this study is incorporated below from the pharmacology review of IND 64,583 dated November 7, 2002.

Study title: In vitro Mammalian Chromosome Aberration Test

Key findings: Negative.

Study no: AA53RH.331.BTL

Study type: *In vitro* cytogenetic assay with mammalian cells (Chinese hamster ovary or CHO).

Volume #, and page #: Amendment # 002, 131

Conducting laboratory and location: _____

Date of study initiation: January 7, 2002

GLP compliance: A statement of compliance was included.

QA reports: yes (X) no ()

Drug, lot #, and % purity: Methylnaltrexone bromide, C13754, and _____

Formulation/vehicle: Solution/water

Methods: The chromosome aberration assay was performed using standard procedures by exposing duplicate cultures of CHO cells to the test article as well as positive and solvent controls. CHO cells were seeded at approximately 5×10^5 cells/25 cm² flask and were incubated at 37°C in a humidified atmosphere of 5% CO₂ in air for 16 – 24 hours. Treatment was carried out by refeeding duplicate flasks with 4.5 ml complete medium (McCoy's 5A medium supplemented with 10% Fetal bovine serum (FBS), 100 units penicillin/ml and 100 µg streptomycin/ml, and 2 mM L-glutamine) for the non-activated study or 4.5 ml S9 reaction mixture for the S9 activated study, to which 500 µL of dosing solution of test or control article in solvent or solvent alone was added. In the non-activated system, the cells were exposed to the test article for 4 hours or continuously for 20 hours up to the cell harvest at 37°C in a humidified atmosphere of 5% CO₂ in air. In the 4 hour exposure group, after the exposure period, the treatment medium was removed, the cells washed with buffer, re-fed with complete medium and returned to the incubator. Two hours prior to the scheduled cell harvest, Colcemid® was added to duplicate flasks for each treatment condition at a final concentration of 0.1 µg/ml and the flasks returned to the incubator until cell collection.

A concurrent toxicity test was conducted in both the non-activated and the S9 activated test systems. After cell harvest, an aliquot of the cell suspension was removed from each culture and counted using a Coulter counter. The presence of test article precipitate was assessed visually. Cell viability was determined by using trypan blue dye exclusion method. The cell counts and percent viability were used to determine cell growth inhibition relative to the solvent control.

Two hours after the addition of Colcemid®, metaphase cells were harvested for both the non-activated and S9 activated studies by trypsinization. The cells were fixed after centrifugation with

2 ml of Camoy's fixative (methanol: glacial acetic acid, 3: 1, v/v). To prepare slides, the fixed cells were resuspended after centrifugation to opalescence in fresh fixative. A sufficient amount of suspension was dropped on to the center of the glass slide and allowed to dry and stained with 5% Giemsa, air dried and permanently mounted. To ensure that a sufficient number of metaphase cells were present on the slides, the percentage of cells in mitosis per 500 cells scored (mitotic index) was determined for each treatment group. A minimum of 200 metaphase spreads (100 per duplicate flask) were examined and scored for chromatid-type and chromosome-type aberrations.

Strains/cell line: Chinese hamster ovary (CHO) cells

Dose selection criteria:

Basis of dose selection: Cell growth inhibition

Range finding studies: Preliminary range finding assay was conducted at 0.436 to 4360 µg/ml concentrations in the presence and absence of metabolic activation (S9). Substantial toxicity was not observed at any of the tested doses in the non-activated 4 and 20 hour exposure groups or in the S9 activated 4 hour exposure group. Based on the results of this assay, the following doses were selected for the chromosome aberration assay: 1090, 2180 and 4360 µg/ml (± S9).

Test agent stability: Stable under test and storage conditions.

Metabolic activation system: The S9 liver microsomal fraction was prepared from livers obtained from Aroclor 1254 pretreated (500 mg/kg, i.p.) male SD rats.

Controls:

Solvent controls: Water

Positive controls: Without S9 mix: 0.1 and 0.2 µg/ml mitomycin C (MMC)
With S9 mix: 10 and 20 µg/ml cyclophosphamide (CP)

Comments: None.

Exposure conditions:

Incubation and sampling times: 4 h and 20 h in the non-activated system and 4 h in the activated system and cells were harvested at 20 hours after treatment.

Doses used in definitive study: 1090, 2180 and 4360 µg/ml

Study design: The study design is shown in the following table.

Treatment Condition	Treatment Time (hr)	Recovery Time (hr)	Dose (µg/ml)
-S9	4	16	1090, 2180, 4360
	20	0	1090, 2180, 4360
+S9	4	16	1090, 2180, 4360

Analysis:

No. of replicates: Two/dose

Counting method: Manual

Criteria for positive results: The test article was considered to induce a positive response when the percentage of cells with aberrations is increased in a dose-responsive manner with one or more concentrations being statistically significant ($p < 0.05$). However, values that are statistically significant but do not exceed the range of historic solvent controls was considered as not biologically significant.

Summary of individual study findings:

Study validity: The criteria for a valid response were as follows: 1) the frequency of cells with structural chromosome aberrations in the solvent control must be within the range of the historical solvent control and 2) the percentage of cells with chromosome aberrations in the positive control must be statistically increased ($p < 0.05$, Fisher's exact test) relative to the solvent control. The study was considered valid as it met the above criteria.

Study outcome: Negative. The results are shown in the following table (Amendment # 002, page 154 of sponsor's submission).

TABLE 10
SUMMARY

Treatment (µg/mL)	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored	Aberrations Per Cell (Mean +/- SD)	Cells With Aberrations Numerical (%)	Structural (%)
Water	-	4	13.8	200	0.000 ±0.000	1.5	0.0
Methylnaltrexone							
1090	-	4	12.9	200	0.000 ±0.000	1.5	0.0
2180	-	4	12.3	200	0.010 ±0.100	1.0	1.0
4360	-	4	11.9	200	0.000 ±0.000	0.0	0.0
MMC, 0.2	-	4	8.1	200	0.300 ±0.940	3.0	17.5**
Water	+	4	14.6	200	0.000 ±0.000	0.0	0.0
Methylnaltrexone							
1090	+	4	13.9	200	0.000 ±0.000	1.5	0.0
2180	+	4	13.8	200	0.000 ±0.000	1.0	0.0
4360	+	4	13.1	200	0.005 ±0.071	2.5	0.5
CP, 10	+	4	11.8	200	0.335 ±1.029	2.5	18.0**
Water	-	20	13.5	200	0.000 ±0.000	0.5	0.0
Methylnaltrexone							
1090	-	20	13.2	200	0.000 ±0.000	0.5	0.0
2180	-	20	11.7	200	0.005 ±0.071	0.0	0.5
4360	-	20	13.3	200	0.015 ±0.122	1.5	1.5
MMC, 0.1	-	20	7.8	200	0.370 ±1.217	2.0	19.0**

Treatment: Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.

Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.

Percent Aberrant Cells: ** $p \leq 0.01$; using Fisher's exact test.

BACTERIAL REVERSE MUTATION ASSAY STUDY NO.
AA53RH.503.BTL, 2002)

This study has previously been reviewed under IND 64,583 Serial No. 002. The review of this study is incorporated below from the pharmacology review of IND 64,583 dated November 7, 2002.

*Appears This Way
On Original*

Study no: AA53RH.503.BTL

Study type: Ames test

Volume #, and page #: Amendment # 002, 1

Conducting laboratory and location: _____

Date of study initiation: January 9, 2002

GLP compliance: A statement of compliance was included.

QA reports: yes (X) no ()

Drug, lot #, and % purity: Methylnaltrexone bromide, C13754, and _____

Formulation/vehicle: Solution/water

Methods: MNTX was tested using *Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* tester strain WP2 uvrA in the presence and absence of Aroclor-induced rat liver S9. The assay was performed in two phases, using the plate incorporation method. The first phase or preliminary dose ranging experiment was conducted to establish the dose range for the confirmatory assay. The second phase or the confirmatory assay was conducted to evaluate and confirm the mutagenic potential of the test article.

Strains/cell line: *Salmonella typhimurium* TA1535, TA1537, TA 98, TA 100 and *Escherichia coli* tester strain WP2 uvrA

Dose selection criteria:

Basis of dose selection: Cytotoxicity and solubility.

Range finding studies: Preliminary dose ranging assay was conducted at 2.5, 7.5, 25, 75, 200, 600, 1800 and 5000 µg per plate concentrations (2 plate/dose) in the presence or absence of S9. In this assay, no cytotoxicity or precipitate was observed. Based on the results of this assay, the maximum dose for the confirmatory assay was selected as 5000 µg/plate.

Test agent stability: Test article was found to be stable in water at room temperature.

Metabolic activation system: The S9 liver microsomal fraction was prepared from livers obtained from Aroclor 1254 pretreated (500 mg/kg, i.p.) male SD rats.

Controls:

Vehicle: Water

Positive controls: Without metabolic activation: sodium azide (1.0 µg/plate) for TA 1535, TA100; 9-aminoacridine (75 µg/plate) for TA1537 and 2-nitrofluorene (1.0 µg/plate) for TA 98; methyl methane sulfonate (MMS, 1 µl/plate) for WP2 uvrA. With metabolic activation: 2-Aminoanthracene (1 µg/plate for all strains except 10 µg/plate for WP2 uvrA).

Comments: None

Exposure conditions:

Incubation and sampling times: 48 to 72 hours

Doses used in definitive study: 75, 200, 600, 1800 and 5000 µg/plate

Study design: The assay was performed in two phases, using the plate incorporation method. The first phase or preliminary dose ranging experiment was conducted to establish the dose range for the confirmatory assay. The second phase or the confirmatory assay was conducted to evaluate and confirm the mutagenic potential of the test article.

Analysis:

No. of replicates: All dose levels of test article, vehicle control and positive control were plated in triplicate.

Counting method: The colonies were counted by using an automated colony counter.

Criteria for positive results: 1) a dose-related increase in the number of revertants and/or 2) a reproducible biologically relevant positive response (TA 98, TA 100 and WP2 uvrA: the number of revertants is at least twice as high as compared to the spontaneous reversion rate or control. Strain TA 1535, TA 1537: the number of revertants is at least three times higher than the control) for at least one of the test points in at least one strain with or without metabolic activation.

Summary of individual study findings:

Study validity: The study was considered as valid as all criteria for a valid study were met.

Study outcome: Negative. The summary of results is shown in the following table (from page 038 of Amendment 002 dated May 28, 2002 of sponsor's submission).

Bacterial Mutation Assay
Summary of Results

Table 22

Test Article Id : Methylnaltrexone
Study Number : AA53RH.503.BTL Experiment Nos : B2/B4

Average Revertants Per Plate $\pm$ Standard Deviation

Liver Microsomes: None

Dose (μ g/plate)	TA98	TA100	TA1535 ^a	TA1537	WP2 <i>uvrA</i> ^a
Vehicle	12 $\pm$ 2	173 $\pm$ 28	18 $\pm$ 3	7 $\pm$ 3	11 $\pm$ 3
75	11 $\pm$ 1	157 $\pm$ 28	16 $\pm$ 3	8 $\pm$ 3	10 $\pm$ 4
200	10 $\pm$ 0	158 $\pm$ 7	15 $\pm$ 3	8 $\pm$ 4	14 $\pm$ 5
600	11 $\pm$ 1	180 $\pm$ 27	18 $\pm$ 4	8 $\pm$ 3	12 $\pm$ 3
1800	12 $\pm$ 1	136 $\pm$ 20	15 $\pm$ 1	5 $\pm$ 1	13 $\pm$ 4
5000	9 $\pm$ 2	162 $\pm$ 14	14 $\pm$ 3	6 $\pm$ 2	10 $\pm$ 5
Positive	155 $\pm$ 5	689 $\pm$ 32	316 $\pm$ 46	858 $\pm$ 46	119 $\pm$ 7

Liver Microsomes: Rat liver S9

Dose (μ g/plate)	TA98	TA100	TA1535 ^a	TA1537	WP2 <i>uvrA</i> ^a
Vehicle	19 $\pm$ 6	142 $\pm$ 24	14 $\pm$ 6	7 $\pm$ 1	13 $\pm$ 4
75	19 $\pm$ 8	157 $\pm$ 40	13 $\pm$ 5	8 $\pm$ 3	11 $\pm$ 5
200	19 $\pm$ 8	152 $\pm$ 13	12 $\pm$ 2	8 $\pm$ 3	12 $\pm$ 2
600	16 $\pm$ 4	170 $\pm$ 19	15 $\pm$ 5	10 $\pm$ 4	13 $\pm$ 4
1800	17 $\pm$ 3	146 $\pm$ 12	13 $\pm$ 4	9 $\pm$ 3	15 $\pm$ 4
5000	18 $\pm$ 3	135 $\pm$ 8	12 $\pm$ 3	9 $\pm$ 1	12 $\pm$ 4
Positive	490 $\pm$ 111	797 $\pm$ 63	76 $\pm$ 13	99 $\pm$ 11	560 $\pm$ 42

Vehicle = Vehicle Control

Positive = Positive Control

Plating aliquot: 100 μ L

a = Data from Experiment B4

L5178Y TK+/- MOUSE LYMPHOMA FORWARD MUTATION ASSAY WITH A
CONFIRMATORY ASSAY WITH METHYLNALTREXONE
STUDY NO. 7434-105)

This study has previously been reviewed under IND Serial No. 008. The review of this study is incorporated below from the pharmacology review of IND dated February 8, 2006.

**Appears This Way
On Original**

Conclusions: Methylnaltrexone was not clastogenic under the study conditions.

L5178Y TK^{+/+} Mouse Lymphoma Forward Mutation Assay with a Confirmatory Assay with Methylnaltrexone

Key Findings: negative

Study # 7434-105 (24831-0-431ICH)

Amendment # 008, Vol. 7, Pg. 283

Conducting Laboratory and Location: /

Date of Study Initiation: September 2, 2003 (final report dated April 20, 2004)

GLP Compliance: A statement of compliance was included.

QA Report: yes(x) no()

Drug: lot # D04957; / pure

Vehicle: saline for injection, USP

Methods: The mutagenic potential of methylnaltrexone was tested using the mouse lymphoma L5178Y tk^{+/+} - 3.7.2C cell line. RPMI 1640 medium supplemented with 10% horse serum, F68, L-glutamine, sodium pyruvate, penicillin, and streptomycin was used as the growth medium. A preliminary toxicity assay was performed using drug concentrations of 0 (vehicle), 8.65, 17.3, 34.5, 69, 138, 275, 550, 1100, 2200, and 4400 µg/ml (single cultures). Cells were incubated with methylnaltrexone for 4 hr at 35-38°C in the presence or absence of S9 liver fraction, and for 24 hr at 35-38°C in the absence of S9. Following the treatment period, the cells were washed, resuspended in growth medium, counted, diluted as needed, and incubated overnight. At the end of this incubation, a cell count was determined to measure the effect on growth relative to the vehicle control cultures. These data were used as the basis for dose selection in the main assay.

In the initial mutation assay, lymphoma cells were incubated with 0 (vehicle), 31.3, 62.5, 125, 250, 500, 1000, 2000, 2500, 3000, or 4400 µg/ml methylnaltrexone for 4 hr at 35-38°C in the absence or presence of S9. All concentrations above 62.5 µg/ml were eventually used for evaluation of mutant frequency. In the confirmatory mutation assay, cells were incubated with 0 (vehicle), 250, 500, 1000, 1500, 2000, 2500, 3000, 3500, or 4400 µg/ml for 4 hr at 35-38°C in the presence of S9, and for 24 hr in the absence of S9. All concentrations above 250 µg/ml were eventually used for evaluation of mutant frequency. Single cultures, triplicate cultures, and duplicate cultures were used for the test article, vehicle control, and positive control, respectively. The drug-treatment incubation was performed in culture tubes, which contained 6×10^6 cells suspended in 10 ml of medium at the start of treatment. At the end of treatment, cells