

were washed and resuspended in 20 ml of medium at a density of 3×10^5 cells/ml. A period of 2 days was allowed for cell growth and mutation expression. At the end of the growth period, the cells were counted and appropriate cultures were selected for cloning and mutant selection. A total of 3×10^6 cells from each selected culture were suspended in selection medium, which was similar to the growth medium, but also contained 3 $\mu\text{g/ml}$ 5-trifluorothymidine and 0.24% Noble agar. Each culture sample was distributed into three 100-mm dishes. Cloning efficiency was assessed by seeding three dishes with approximately 600 cells in cloning medium containing 0.24% Noble agar. The cells were incubated in selection medium or cloning medium for 14 days and were counted using an automated colony counting system. Colony sizing analysis was performed for each assay.

The mutant frequency (MF), cloning efficiency (CE), and relative cloning efficiency were calculated using the following equations:

$$\text{MF} = (\text{total number of mutant colonies in selection medium} \div \text{total number of viable colonies in cloning medium}) \times 2 \times 10^{-4}$$

$$\text{CE} = (\text{total number of viable colonies in cloning medium} \div \text{total number of cells seeded}) \times 100$$

$$\text{Relative cloning efficiency for drug-treated cultures} = (\text{CE in presence of drug} \div \text{mean CE in vehicle control cultures}) \times 100$$

Toxicity was expressed as relative total growth (RTG), which was obtained from the following equation:

$$\text{RTG} = (\text{relative suspension growth} \times \text{relative cloning efficiency}) \div 100$$

Relative suspension growth was measured after the 2-day growth and expression period used for the 4-hr treatment, and after the 24-hr treatment + 2-day growth and expression period. The S9 liver fraction (obtained from a commercial vendor) was prepared from male Sprague Dawley rats treated with Aroclor 1254 (500 mg/kg). The positive control compounds are listed in the following table.

Chemical	S9	4-hr Treatment	24-hr Treatment
		($\mu\text{g/ml}$)	($\mu\text{g/ml}$)
Methyl methanesulfonate (MMS)	-	13	6.5
Methylcholanthrene (MCA)	+	2	-

The criteria for a valid study were the following: average absolute cloning efficiency is between 60% and 130%; average suspension growth for vehicle control cultures is 8-fold during the growth/expression period; at least one of the positive control cultures exhibits a mutant frequency of at least 200×10^{-6} ; if the test article has weak or no mutagenic activity, the tested concentrations should reduce the RTG to 10-20% of the average vehicle control, unless the

highest concentration was at least two-times the solubility limit in culture medium; relative cloning efficiency is 10% or greater and the total number of viable clones exceeds 60; at least three cultures are analyzed for mutant colonies. The criteria for a positive response were one of the following: a dose-dependent increase of at least 2-fold in mutant frequency; an increase of at least 4-fold at a single dose which is equal or nearly equal to the highest testable dose, without evidence of a dose response. The criteria for a negative response were one of the following: a drug concentration range that produced RTG values of 10-20% is not associated with an increase of at least 2-fold in mutant frequency; a drug concentration range that includes 5 mg/ml or 10 mM (whichever is lower) is not associated with an increase of at least 2-fold in mutant frequency; a drug concentration range that includes a value approximately 2-fold greater than the solubility limit in culture medium is not associated with an increase of at least 2-fold in mutant frequency; an increase in mutant frequency is not repeated in the confirmatory assay.

Results:

Study validity: The study methods used and the criteria for a positive result were adequate. The criteria for a valid study were fulfilled.

Study outcome: In the preliminary toxicity assay, treatment of lymphoma cells with methylnaltrexone for 4 hr produced mild, non-dose-dependent reductions in cell density at the higher dose levels (550 µg/ml and higher). In contrast, 24-hr exposure to methylnaltrexone in the absence of S9 produced a robust decrease in cell density. At the highest dose level (4400 µg/ml), the cell density was reduced to 4.7% of the vehicle control culture. In the mutation assay, a similar pattern of toxicity was observed. In the 4-hr treatment assays with or without S9, RTG was either slightly decreased in a non-dose-dependent manner, or was unaffected. In the 24-hr treatment assay, RTG was reduced to 10-18% of the mean vehicle control value at concentrations of 3000-4400 µg/ml. The cytotoxic effect of methylnaltrexone in the 24-hr assay was clearly dose-dependent. No increases of at least 2-fold in mutant frequency were associated with methylnaltrexone. The proportion of small and large colonies was not affected. The results are shown in the following tables (taken from the study report).

TABLE 2: INITIAL MUTATION ASSAY WITHOUT ACTIVATION

A. TEST ARTICLE: Methylalntrexone
 B. ASSAY NO.: 24831-0-4311CH
 C. VEHICLE: Saline
 D. SELECTIVE AGENT: TFT 3.0 µg/mL
 E. TREATMENT DATE: 10/01/2003
 F. CELLS ANALYZED: 3 x 10⁶
 G. TREATMENT PERIOD: -4 hours
 H. EXPRESSION PERIOD: 2 days

Test Condition	Daily Cell Density/mL (x 10 ⁶)		Cumulative RSG ^a	Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency ^b	Relative Growth (%) ^c	Mutant Frequency (x 10 ⁻⁶) ^d
	Day 1	Day 2						
Nonactivation Controls ^e			AVG VC		AVG VC			
Vehicle Control	14.1	10.0	15.7	201	843	140.6	112.6	47.6
Vehicle Control	12.9	8.8	12.6	195	757	126.2	81.4	51.6
Vehicle Control	13.1	11.6	16.9	200	738	122.9	129.9	54.1
MMS 13 µg/mL	8.3	7.4	6.8	695	560	93.3	32.6	248.3 ^f
MMS 13 µg/mL	10.8	13.0	15.6	201	716	119.3	95.2	56.1
Test Article (µg/mL)			Relative to Vehicle Control (%)			Relative to Vehicle Control (%)		
125	12.0	9.6	85.0	227	846	108.5	92.2	53.7
250	11.7	10.7	92.4	257	807	103.6	95.7	63.8
500	13.3	9.3	91.3	176	834	107.0	97.6	42.1
1000	8.5	8.1	50.8	237	814	104.4	53.1	58.2
2000	10.9	9.4	75.6	194	748	96.0	72.6	51.9
2500	9.2	9.0	61.1	201	911	116.9	71.4	44.1
3000	8.2	11.0	66.6	170	744	95.5	63.6	45.7
4400	11.7	8.4	72.5	221	876	112.4	81.5	50.6

^aRSG = (Day 1 Count/3) x (Day 2 Count)/3 (or Day 1 Count if not subcultured)

^bCloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100

^cRelative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100

^dMutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10⁻⁶)

Decimal is moved to express the frequency in units of 10⁻⁶

^eVehicle Control = 10% Saline

Positive Control: MMS = Methyl methanesulfonate

^fMutagenic. Exceeds Minimum Criterion of 102.2 x 10⁻⁶

TABLE 4: CONFIRMATORY MUTATION ASSAY WITHOUT ACTIVATION

A. TEST ARTICLE: Methylalntrexone
 B. ASSAY NO.: 24831-0-4311CH
 C. VEHICLE: Saline
 D. SELECTIVE AGENT: TFT 3.0 µg/mL
 E. TREATMENT DATE: 10/28/2003
 F. CELLS ANALYZED: 3 x 10⁶
 G. TREATMENT PERIOD: ~24 hours
 H. EXPRESSION PERIOD: 2 days

Test Condition	Daily Cell Density/mL (x 10 ⁵)			Cumulative RSG ^a	Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency ^b	Relative Growth (%) ^c	Mutant Frequency (x 10 ⁻⁶) ^d
	Day 1	Day 2	Day 3						
Nonactivation Controls ^e									
Vehicle Control	12.0	10.2	10.6	48.1	149	722	120.4	95.2	41.4
Vehicle Control	10.2	11.6	12.1	53.0	134	628	104.7	91.4	42.7
Vehicle Control	11.6	11.2	12.3	59.2	130	697	116.2	113.8	37.2
				AVG VC			AVG VC		
MMS 6.5 µg/mL	10.1	5.1	7.6	14.5	520	321	53.5	12.8	324.5 ^f
MMS 6.5 µg/mL	9.3	6.6	7.8	17.7	529	228	38.0	11.1	464.1 ^f
Test Article (µg/mL)				Relative to Vehicle Control (%)			Relative to Vehicle Control (%)		
500	13.5	9.2	10.2	87.8	194	726	106.3	93.4	53.5
1000	10.1	7.3	15.1	77.2	148	628	92.1	71.1	47.2
1500	9.7	10.0	8.4	56.5	206	790	115.7	65.4	52.2
2000	8.6	7.1	8.4	35.6	249	754	110.4	39.3	66.0
2500	8.3	8.0	7.3	33.6	241	631	92.4	31.0	76.5
3000	6.9	6.1	7.6	22.2	164	553	81.0	18.0	59.2
3500	8.4	5.2	6.2	18.8	176	555	81.4	15.3	63.3
4000	6.2	5.9	5.6	14.2	124	503	73.7	10.5	49.5

^aRSG = [Treatment termination (Day 1) cell density/3] x [Day 2 cell density/3 or Day 1 density if not split back] x [Day 3 cell density/3 or Day 2 density if not split back]

^bCloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100

^cRelative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100

^dMutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10⁻⁴)

Decimal is moved to express the frequency in units of 10⁻⁶

^eVehicle Control = 10% Saline

Positive Control: MMS = Methyl methanesulfonate

^fMutagenic. Exceeds Minimum Criterion of 80.9 x 10⁻⁶

TABLE 6: INITIAL MUTATION ASSAY WITH ACTIVATION

A. TEST ARTICLE: Methylalntrexone
 B. ASSAY NO.: 24831-0-431ICH
 C. VEHICLE: Saline
 D. SELECTIVE AGENT: TFT 3.0 µg/mL
 E. TREATMENT DATE: 10/01/2003
 F. CELLS ANALYZED: 3×10^6
 G. TREATMENT PERIOD: ~4 hours
 H. EXPRESSION PERIOD: 2 days

Test Condition	Daily Cell Density/mL ($\times 10^5$)		Cumulative RSG ^a	Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency ^b	Relative Growth (%) ^c	Mutant Frequency ($\times 10^{-6}$) ^d	
	Day 1	Day 2							
Activation Controls ^e			AVG VC		AVG VC				
Vehicle Control	12.0	11.5	15.3	203	717	119.5	111.3	56.6	
Vehicle Control	11.8	8.4	11.0	292	774	128.9	86.4	75.6	
Vehicle Control	12.3	8.8	12.0	12.8	231	822	136.9	128.4	100.2
MCA 2 µg/mL	5.9	11.6	7.6	682	552	92.0	42.6	247.0 ^f	
MCA 4 µg/mL	3.6 ^g	4.7	1.6	212	115	19.1	1.8	369.5 ^f	
Test Article (µg/mL)			Relative to Vehicle Control (%)			Relative to Vehicle Control (%)			
125	14.5	10.2	128.5	267	1005	130.4	167.5	53.2	
250	12.6	13.6	148.9	178	673	87.4	130.0	52.8	
500	12.3	12.5	133.6	236	691	89.6	119.7	68.2	
1000	9.9	11.3	97.2	193	726	94.1	91.5	53.2	
2000	10.8	10.8	101.3	266	900	116.8	118.3	59.2	
2500	10.3	15.1	135.1	156	704	91.3	123.4	44.3	
3000	10.1	16.0	140.4	179	651	84.5	118.6	54.9	
4400	8.9	15.7	121.4	224	572	74.2	90.0	78.2	

^aRSG = (Day 1 Count/3) x (Day 2 Count)/3 (or Day 1 Count if not subcultured)

^bCloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100

^cRelative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100

^dMutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2×10^{-6})

Decimal is moved to express the frequency in units of 10^{-6}

^eVehicle Control = 10% Saline

Positive Control: MCA = Methylcholanthrene

^fMutagenic. Exceeds Minimum Criterion of 125.7×10^{-6}

^gNot subcultured

TABLE 8: CONFIRMATORY MUTATION ASSAY WITH ACTIVATION

A. TEST ARTICLE: Methylalntrexone
 B. ASSAY NO.: 24831-0-4311CH
 C. VEHICLE: Saline
 D. SELECTIVE AGENT: TFT 3.0 µg/mL
 E. TREATMENT DATE: 10/28/2003
 F. CELLS ANALYZED: 3 x 10⁶
 G. TREATMENT PERIOD: ~4 hours
 H. EXPRESSION PERIOD: 2 days

Test Condition	Daily Cell Density/mL (x 10 ⁵)		Cumulative RSG ^a	Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency ^b	Relative Growth (%) ^c	Mutant Frequency (x 10 ⁻⁶) ^d
	Day 1	Day 2						
Activation Controls ^e			AVG VC				AVG VC	
Vehicle Control	13.1	10.6	15.4	212	573	95.5	104.3	73.9
Vehicle Control	11.8	10.9	14.3	183	538	89.6	90.7	68.2
Vehicle Control	15.5	10.3	17.7	159	496	82.7	89.3	64.2
MCA 2 µg/mL	8.2	11.0	10.0	719	370	61.6	43.7	388.8 ^f
MCA 4 µg/mL	6.8	11.3	8.5	746	329	54.9	33.2	453.0 ^f
Test Article (µg/mL)			Relative to Vehicle Control (%)			Relative to Vehicle Control (%)		
500	12.9	10.8	97.9	212	685	127.9	125.2	61.8
1000	12.8	11.1	99.8	201	583	108.8	108.5	68.9
1500	14.2	9.4	93.8	201	666	124.2	116.5	60.3
2000	12.4	10.2	88.8	220	649	121.2	107.7	67.9
2500	10.3	10.5	76.0	199	686	128.1	97.3	57.9
3000	15.9	10.6	118.4	168	627	117.1	138.6	53.6
3500	13.8	11.6	112.4	168	574	107.1	120.4	58.6
4400	15.5	12.0	130.6	136	538	100.4	131.2	50.7

^aRSG = (Day 1 Count/3) x (Day 2 Count)/3 (or Day 1 Count if not subcultured)

^bCloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100

^cRelative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100

^dMutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10⁻⁶)

Decimal is moved to express the frequency in units of 10⁻⁶

^eVehicle Control = 10% Saline

^fPositive Control: MCA = Methylcholanthrene

Conclusion: Methylalntrexone was not mutagenic under the assay conditions.

CHROMOSOMAL ABERRATIONS IN CULTURED HUMAN PERIPHERAL BLOOD LYMPHOCYTES _____ **STUDY NO. 7434-106)**

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.

Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes

Key Findings: negative

Study # 7434-106 (24831-0-449OECD).

Amendment # 008, Vol. 7, Pg. 214

Conducting Laboratory and Location:

Date of Study Initiation: September 2, 2003 (final report dated April 13, 2004)

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GLP Compliance: A statement of compliance was included.

QA Report: yes(x) no()

Drug: lot # D04957; % pure

Vehicle: water

Methods: Peripheral blood lymphocytes were obtained from healthy human donors. Lymphocyte cultures were initiated by adding 0.6 ml of blood to approximately 9.4 ml RPMI 1640 medium supplemented with 25 mM HEPES, 20% fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM L-glutamine, and 2% phytohemagglutinin M. The cultures were incubated at 37°C in 5% CO₂/air mixture for two days. No preliminary toxicity assay was performed. In the initial chromosomal aberration assay, lymphocytes were incubated with 30.5, 43.6, 62.3, 89, 127, 182, 259, 371, 529, 756, 1080, 1540, 2210, 3150, or 4500 µg/ml methylalntrexone for 3 hr in the absence or presence of S9 liver fraction (duplicate cultures). Cells that were treated with 1540, 2210, 3150, or 4500 µg/ml were selected for assessment of chromosomal aberrations. In the confirmatory chromosomal aberration assay, lymphocytes were incubated with 125, 250, 500, 1000, 1500, 2500, 3500, or 4500 µg/ml methylalntrexone for 22 hr in the absence of S9, and with 1000, 1500, 2500, 3500, or 4500 µg/ml for 3 hr in the presence of S9 (duplicate cultures). Cells that were treated with 1500, 2500, 3500, or 4500 µg/ml in the absence of S9, and cells treated with 1000, 1500, 3500, or 4500 µg/ml in the presence of S9 were selected for assessment of chromosomal aberrations. Negative control and vehicle control cultures were used in each assay (duplicate cultures). The selection of the highest dose was based on OECD Testing Guidelines. Toxicity was evaluated based on the mitotic index (% of cells in mitosis observed in the examination of 100 cells/culture). Mitomycin C was used as a positive control in the absence of S9 (1 µg/ml in the 3-hour treatment, 0.3 µg/ml in the 22-hour treatment, duplicate cultures). Cyclophosphamide (25 µg/ml) was used as a positive control in the presence of S9 in the 3-hour treatment assay (duplicate cultures). The incubation procedures for the 3-hour and 22-hour treatments are described in the following table.

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Without S9			
1. 3 hr incubation with test article or control articles	2. Cells were washed and resuspended in fresh medium; incubation was continued for 17 hr	3. 0.1 µg/ml Colcemid® was added to medium and incubation continued for 2 hr	4. Cells were harvested, fixed, mounted on slides, stained with 5% Giemsa solution, and analyzed for chromosomal aberrations
1. 20 hr incubation with test article or control articles	2. 0.1 µg/ml Colcemid® was added to medium and incubation continued for 2 hr	3. Cells were harvested, fixed, mounted on slides, stained with 5% Giemsa solution, and analyzed for chromosomal aberrations	
With S9			
1. 3 hr incubation with test article or control articles	2. Cells were washed and resuspended in fresh medium; incubation was continued for 17 hr	3. 0.1 µg/ml Colcemid® was added to medium and incubation continued for 2 hr	4. Cells were harvested, fixed, mounted on slides, stained with 5% Giemsa solution, and analyzed for chromosomal aberrations

Cells in metaphase (100/culture) were examined for chromosomal aberrations. The incidence of polyploid cells and endoreduplication per 100 metaphase cells was also determined. The S9 liver fraction (obtained from a commercial vendor) was prepared from male Sprague Dawley rats treated with 500 mg/kg ip Aroclor 1254. The assay was considered as valid based on the following criteria: the negative and vehicle control cultures contain less than approximately 5% cells with aberrations; the proportion of cells with aberrations in the positive control cultures is increased in a statistically significant manner ($p \leq 0.01$), relative to the vehicle control cultures; the assay includes the highest applicable dose (10 mM) or a dose exceeding the solubility limit if the assay yields a negative result and no significant decrease ($\geq 50\%$) in mitotic index occurs; at least three drug concentrations are analyzable. The criteria for a positive response were a statistically significant increase ($p \leq 0.01$) in the number of cells with chromosomal aberrations at one or more dose levels, and evidence of a dose-response. The results were considered as negative if no significant increase in the number of cells with chromosomal aberrations occurred at any dose level.

Results:

Study validity: The study methods used and the criteria for a positive result were adequate. The criteria for a valid study were fulfilled.

Study outcome: Methylnaltrexone produced minimal toxicity. Mitotic index was reduced by 21-29% at 3150 and 4500 µg/ml in the initial 3-hr treatment assay without S9. No toxicity was observed at lower dose levels. In the confirmatory assay using similar doses, no reduction in mitotic index occurred in the 3-hr assay without S9. In the confirmatory assay performed in the presence of S9, mitotic index was reduced by 16-57% in a non-dose-dependent manner. In contrast, mitotic index was not affected in the initial assay using S9. Methylnaltrexone produced no significant increase in chromosomal aberrations. The results are shown in the following tables (taken from the study report).

Table 8: Chromosomal Aberrations in Human Lymphocytes - With Metabolic Activation - ~3-Hour Treatment, ~22-Hour Harvest

Assay No.: 24831-0-4490ECD	Trial No.: C1	Date: 10/16/03	Lab No.: CY101803	Test Article: Methylnitroreoxone	# Cells Scored for Aberrations	% Mitotic Index Reduction ^a	# of pp Cells	# of er Cells	Judg- ment (+/-)	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations					Judge- ment (+/-)
										Totals ^b					
										eggs	simple breaks	clite	maib	pp	
Controls															
Negative: RPMI 1640															
	A	100	0	0	0	0	0	0	0	0	0	2	2	2	
	B	100	0	0	0	0	0	0	0	0	0	0	0	1	
	Total	200	0	0	0	0	0	0	0	0	0	2	2	3	
	Average %		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	1.5	
Vehicle: CCGW 100 µL/mL															
	A	100	0	0	0	0	0	0	0	0	0	2	2	2	
	B	100	0	0	0	0	0	0	0	0	0	1	1	1	
	Total	200	0	0	0	0	0	0	0	0	0	3	3	3	
	Average %	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	1.5	1.5	
Positive: CP 25.0 µg/mL															
	A	50	0	0	0	0	0	0	0	0	0	3	3	23	
	B	50	0	0	0	0	0	0	0	0	0	1	1	23	
	Total	100	0	0	0	0	0	0	0	0	0	4	4	46	
	Average %		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0	4.0	46.0	
Test Article 1000 µg/mL															
	A	100	0	0	0	0	0	0	0	0	0	1	1	1	
	B	100	0	0	0	0	0	0	0	0	0	2	2	2	
	Total	200	0	0	0	0	0	0	0	0	0	3	3	3	
	Average %		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	1.5	
1500 µg/mL															
	A	100	0	0	0	0	0	0	0	0	0	1	1	1	
	B	100	0	0	0	0	0	0	0	0	0	3	3	5	
	Total	200	0	0	0	0	0	0	0	0	0	4	4	6	
	Average %	27	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	1.5	2.5	
3500 µg/mL															
	A	100	0	0	0	0	0	0	0	0	0	1	1	1	
	B	100	0	0	0	0	0	0	0	0	0	3	3	3	
	Total	200	0	0	0	0	0	0	0	0	0	4	4	4	
	Average %	33	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	2.0	2.5	
4500 µg/mL															
	A	100	0	0	0	0	0	0	0	0	0	1	1	1	
	B	100	0	0	0	0	0	0	0	0	0	2	2	2	
	Total	200	0	0	0	0	0	0	0	0	0	3	3	3	
	Average %	16	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	1.5	2.0	
Total Average %															
	A	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	1.5	1.9	
	B	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	
	Total	200	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	2.0	2.5	

clite: chromatid exchange chire: chromosome exchange
 % Mitotic Index reduction as compared to the vehicle control.
^a Significantly greater in % polyploidy and % endoreduplication than the vehicle control, p ≤ 0.01.
^b g = # or % of cells with chromosome aberrations; *g = # or % of cells with chromosome aberrations + # or % of cells with gaps.
^c Significantly greater in -g than the vehicle control, p ≤ 0.01. RPMI 1640 = culture medium CCGW = cell culture grade water CP = Cyclophosphamide
 maib: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

MAMMALIAN ERYTHROCYTE MICRONUCLEUS TEST STUDY
NO. AA53RH.123.BTL)

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 November 7, 2002.

Reviewer: Tamal K. Chakraborti, Ph.D.

IND No. 64, 583

Study title: Mammalian Erythrocyte Micronucleus Test by Intraperitoneal Route**Key findings:** Negative. However, the route of administration (intraperitoneal) was not appropriate, as the intended route of drug administration is subcutaneous.**Study no:** AA53RH.123.BTL**Study type:** *In vivo* mouse bone marrow micronucleus assay using intraperitoneal route.**Volume #, and page #:** Amendment # 002, 81**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: Male and female mice (5/sex/group/time point) were dosed intraperitoneally with 25, 50 or 100 mg (20 ml/kg) test article/kg body weight or with the negative (vehicle) or positive control article. An additional 5 mice/sex were treated in the high dose group to insure enough animals would be evaluated for micronuclei at this dose. Bone marrow cells collected 24 and 48 hours after treatment from the femur and smears were taken on slides. The slides were fixed in methanol, stained with May-Gruenwald-Giemsa and permanently mounted. Bone marrow cells polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) were analyzed for the presence of micronuclei. Polychromatic erythrocytes are young, immature red blood cells that stain bluish while normochromatic erythrocytes or normocytes are mature red blood cells that stain pink. Micronuclei are round, darkly-staining nuclear fragments with a sharp contour and diameters usually from 1/20th to 1/5th of an erythrocyte. Micronuclei can occur in both PCEs (MPCEs) or NCEs (MNCEs). Two thousand (2000) polychromatic erythrocytes per animal were scored for the presence of micronuclei. The number of micronucleated normochromatic erythrocytes in the field of 2000 polychromatic erythrocytes was enumerated for each animal. The proportion of polychromatic erythrocytes to total erythrocytes was also recorded per 1000 erythrocytes.

Strains/species: ICR mice (5 – 15/sex/dose per time point)**Dose selection criteria:****Basis of dose selection:** The assay was performed in two phases. The first phase (dose range-finding phase), designed to assess toxicity of the test article and set dose

levels for the definitive study consisted of a pilot toxicity study followed by a toxicity study. The second phase, the definitive micronucleus study, was designed to evaluate the potential of the test article to increase the incidence of micronucleated polychromatic erythrocytes in bone marrow of male and female ICR mice. In both phases of the study, test and control articles were administered at a dose volume of 20 ml/kg body weight by a single intraperitoneal injection.

Range finding studies: In the pilot toxicity study, male mice (2/group) were dosed with 1, 10, 100, or 1000 mg test article/kg body weight and male and female mice (5/sex/group) were dosed with 2000 mg/kg. Mortality was observed in 2 of 2 male mice at 1000 mg/kg and in 5 of 5 male mice and 5 of 5 female mice at 2000 mg/kg. Clinical signs immediately following treatment included convulsions in males at 1000 mg/kg and in males and females at 2000 mg/kg. In addition, piloerection was observed in males at 1, 10 and 100 mg/kg.

In the toxicity assay, male and female mice (5/sex/group) were dosed with 100, 150 or 200 mg test article/kg body weight. Mortality was observed in 4 of 5 males and 4 of 5 females at 150 mg/kg and in 5 of 5 males and 5 of 5 females at 200 mg/kg. Clinical signs following dose administration included: convulsions in males and females at 150 and 200 mg/kg, lethargy in males at 100 mg/kg and in one male and one female mouse at 150 mg/kg. Piloerection was also seen in one surviving male and one female mouse at 150 mg/kg. The high dose for the micronucleus test was set at 100 mg/kg, which was estimated to be the maximum tolerated dose.

Test agent stability: Stable under test and storage conditions.

Controls:

Vehicle: Water

Positive controls: Cyclophosphamide (50 mg/kg, i.p., 20 ml/kg)

Comments: None.

Exposure conditions:

Incubation and sampling times: Bone marrow sampling at 24 and 48 h post-dose.

Doses used in definitive study: Male and female mice (5/sex/group/time point) were dosed intraperitoneally with 25, 50 or 100 mg (20 ml/kg) test article/kg body weight. It is to be mentioned here that this intraperitoneal route is inappropriate in this case, as the drug is intended to be administered by subcutaneous route.

Study design: The study design is shown in the following table (from Amendment # 002, page 90 of sponsor's submission).

	Number of Mice Per Sex Dosed	Number of Mice Per Sex Used for Bone Marrow Collection After Dose Administration	
		24 hr	48 hr
Vehicle Control Water	10	5	5
Test Article Methylnaltrexone			
Low test dose (25 mg/kg)	5	5	0
Mid test dose (50 mg/kg)	5	5	0
High test dose (100 mg/kg)	15*	5	5
Positive Control CP (50 mg/kg)	5	5	0

*including 5 replacement animals per sex to ensure the availability of five animals for micronucleus analysis

Analysis:

Counting method: Two thousand (2000) polychromatic erythrocytes per animal were scored for the presence of micronuclei. The number of micronucleated normochromatic erythrocytes in the field of 2000 polychromatic erythrocytes was enumerated for each animal. The proportion of polychromatic erythrocytes to total erythrocytes was also recorded per 1000 erythrocytes.

Criteria for a valid test: The mean incidence of micronucleated polychromatic erythrocytes must not exceed 5/1000 polychromatic erythrocytes (0.5%) in the vehicle control. The incidence of micronucleated polychromatic erythrocytes in the positive control group must be significantly increased relative to the vehicle control group.

Criteria for positive results: The test article was considered to induce a positive response if a dose-responsive increase in micronucleated polychromatic erythrocytes was observed and one or more doses were statistically elevated relative to the vehicle control ($p < 0.05$, Kastenbaurn-Bowman Tables) at any sampling time. If a single treatment group was significantly elevated at one sacrifice time with no evidence of a dose-response, the assay was considered a suspect or unconfirmed positive and a repeat assay was recommended. The test article was considered negative if no statistically significant increase in micronucleated polychromatic erythrocytes above the concurrent vehicle control was observed at any sampling time.

Summary of individual study findings:

Study validity: The study was deemed valid as it met all criteria for a valid study as mentioned above.

Study outcome: Negative. The following table summarizes the results of the *in vivo* mouse bone marrow micronucleus assay (from Amendment # 002, page 101 of sponsor's submission).

**Table 6: Summary of Bone Marrow Micronucleus Analysis
After a Single Dose of Methylnaltrexone in ICR Mice**

Treatment (20 mL/kg)	Sex	Time (hr)	Number of Mice	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Micronucleated Polychromatic Erythrocytes	
						Number per 1000 PCEs (Mean +/- SD)	Number per PCEs Scored ¹
Water							
	M	24	5	0.477 ± 0.05	---	0.4 ± 0.22	4 / 10000
	F	24	5	0.472 ± 0.05	---	0.5 ± 0.35	5 / 10000
Methylnaltrexone							
25 mg/kg	M	24	5	0.477 ± 0.04	0	0.4 ± 0.22	4 / 10000
	F	24	5	0.449 ± 0.02	-5	0.4 ± 0.22	4 / 10000
50 mg/kg	M	24	5	0.465 ± 0.09	-3	0.6 ± 0.42	6 / 10000
	F	24	5	0.477 ± 0.02	1	0.6 ± 0.42	6 / 10000
100 mg/kg	M	24	5	0.445 ± 0.02	-7	0.5 ± 0.35	5 / 10000
	F	24	5	0.437 ± 0.03	-7	0.4 ± 0.42	4 / 10000
CP							
50 mg/kg	M	24	5	0.350 ± 0.02	-27	20.1 ± 5.26	*201 / 10000
	F	24	5	0.316 ± 0.04	-33	21.3 ± 6.06	*213 / 10000
Water							
	M	48	5	0.430 ± 0.02	---	0.3 ± 0.27	3 / 10000
	F	48	5	0.448 ± 0.03	---	0.3 ± 0.27	3 / 10000
Methylnaltrexone							
100 mg/kg	M	48	5	0.461 ± 0.04	7	0.5 ± 0.35	5 / 10000
	F	48	5	0.449 ± 0.03	0	0.2 ± 0.27	2 / 10000

¹*, p<0.05 (Kastenbaum-Bowman Tables)

IN VIVO MOUSE MICRONUCLEUS ASSAY WITH METHYLNALTREXONE
STUDY NO. 7434-100)

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

In Vivo Mouse Micronucleus Assay with Methylnaltrexone (Subcutaneous Administration)**Key Findings:** negative**Study #** 7434-100 (24831-0-455OECD)**Amendment #** 008, Vol. 7, Pg. 187**Conducting Laboratory and Location:** (**Date of Study Initiation:** February 17, 2003 (final report dated January 20, 2004)**GLP Compliance:** A statement of compliance was included.**QA Report:** yes(x) no()**Drug:** D04957; $\frac{1}{4}$ pure**Vehicle:** 0.9% saline for injection, USP

Methods: $\frac{1}{4}$ CD-1 \otimes (ICR)BR mice (age 8 weeks; males: 27.1-32.6 g; females: 20.8-26.6 g) were used. A dose range-finding study was performed using subcutaneous administration of 100, 200, 300, or 400 mg/kg/dose methylnaltrexone, administered twice for a total dose of 200, 400, 600, or 800 mg/kg, respectively (3 mice/sex/group). The doses were separated by approximately 6 hr. The dose volume was 5 ml/kg. The animals were observed five times during the first hour after each dose, and at least once daily for the duration of the assay (2 days). The study design for the micronucleus assay is shown in the following table.

Dose (mg/kg/dose)	Route	Dose Volume (ml/kg)	24-hr Sacrifice	48-hr Sacrifice
			# Mice/Sex	# Mice/Sex
Methylnaltrexone				
50 ^a	sc	5	6	0
100 ^a	sc	5	6	0
200 ^a	sc	5	6	6
Vehicle	sc	5	6	6
Cyclophosphamide	po	10	6	0
80				

a: administered twice, approximately 6 hr apart for a total dose of 100, 200, or 400 mg/kg

Cyclophosphamide was used as a positive control compound. Mice were observed for signs of toxicity, as described for the dose range-finding study. Bone marrow was collected from tibias at sacrifice (5 mice/sex/group). Marrow cells were washed in fetal bovine serum, mounted on slides, fixed in methanol, and stained with May-Grünwald solution followed by Giemsa. The proportion of PCE (polychromatic erythrocytes) was determined from observation of 500 erythrocytes per animal. The frequency of micronucleated cells was determined from observation of 2000 PCE/animal. Additional groups were treated with 0 (saline), 50, 100, or 200 mg/kg/dose methylnaltrexone for measurement of toxicokinetic parameters. Blood samples were collected at 0.08, 0.17, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12, and 24 hr after the second administration of methylnaltrexone (3 mice/sex/group/time-point). In the toxicokinetic control group, blood was collected at 1 hr after the second administration of saline (3 mice/sex). Plasma drug concentrations were measured using an unstated method. The criteria for a valid assay were the following: the mean vehicle control value for micronucleated PCE incidence is less than 0.4% and is within the historical control range; the mean positive control value is significantly increased ($p \leq 0.01$) and is consistent with the historical positive control data; the high dose produced clinical signs of toxicity or mortality. The criteria for a positive response were the following: a statistically significant increase in the incidence of micronucleated PCE for at least one dose level and a statistically significant dose-related response. The results were considered as negative if no significant increases in micronuclei and/or no evidence of a dose-dependent response was observed. The biological relevance of the results was also considered in the evaluation of micronuclei induction.

Results:

Study validity: The study methods used and the criteria for a positive result were adequate. The criteria for a valid assay were fulfilled.

Study outcome: In the dose range-finding assay, all animals treated with 300 or 400 mg/kg/dose were found dead or were sacrificed in moribund condition. Most of these deaths occurred after the initial dose. Clinical signs observed in mice prior to death included hypoactivity, rapid or irregular respiration, ataxia, tremors, convulsions, and recumbent position. Hypoactivity and rapid respiration were observed immediately after dosing in the 200 mg/kg/dose group. No signs were observed in the 100 mg/kg/dose group.

In the micronucleus assay, 3/12 females in the 200 mg/kg/dose group were found dead (one designated for the 24-hr sacrifice, two designated for the 48-hr sacrifice). Administration of 200 mg/kg/dose produced a low incidence of clinical signs, including hypoactivity, tremors, flattened posture, slow respiration, and squinting. The proportion of PCE (i.e. immature erythrocytes) and the incidence of micronucleated PCE were not affected by methylnaltrexone, as shown in the following table (taken from the study report).

TABLE I: MICRONUCLEUS DATA SUMMARY TABLE

ASSAY NO.: 24831
 TEST ARTICLE: Methylnaltrexone
 DATE DOSED: 11-Mar-03

TREATMENT	DOSE	HARVEST TIME	% MICRONUCLEATED PCEs MEAN OF 2000 PER ANIMAL ± S.E.		RATIO PCE:NCE MEAN ± S.E.	
			MALES	FEMALES	MALES	FEMALES
CONTROLS						
VEHICLE	0.9% Saline	24 hr	0.01 ± 0.01	0.01 ± 0.01	0.49 ± 0.04	0.64 ± 0.05
		48 hr	0.02 ± 0.01	0.02 ± 0.01	0.44 ± 0.04	0.62 ± 0.04
POSITIVE	CP mg/kg 80	24 hr	2.27 ± 0.27*	1.79 ± 0.21*	0.61 ± 0.07	0.68 ± 0.04
TEST ARTICLE	50 mg/kg/dose*	24 hr	0.02 ± 0.01	0.05 ± 0.02	0.49 ± 0.02	0.61 ± 0.05
		48 hr	0.03 ± 0.01	0.04 ± 0.02	0.53 ± 0.07	0.52 ± 0.03
	200 mg/kg/dose*	24 hr	0.06 ± 0.02	0.02 ± 0.01	0.41 ± 0.03	0.57 ± 0.05
		48 hr	0.01 ± 0.01	0.01 ± 0.01	0.59 ± 0.06	0.68 ± 0.06

* Administered twice approximately 6 hours apart for a total dose of 100, 200, or 400 mg/kg.

* Significantly greater than the corresponding vehicle control, p ≤ 0.01.

CP = Cyclophosphamide

PCE = Polychromatic erythrocyte

NCE = Normochromatic erythrocyte

The drug was detected in plasma in all treatment groups. Plasma exposure was increased with dose level. Results from the toxicokinetic measurements are shown in the following table.

Dose (mg/kg/dose)	C _{max} (ng/ml)	t _{max} (hr)	t _{1/2} (hr)	AUC _{0-t} (ng·hr/ml)
50	M: 20333	M: 0.17	M: 10.0	M: 10108
	F: 21933	F: 0.17	F: 8.6	F: 8696
100	M: 24633	M: 0.25	M: 10.4	M: 14524
	F: 33100	F: 0.17	F: 9.2	F: 16763
200	M: 59867	M: 0.17	M: 7.4	M: 38881
	F: 75167	F: 0.17	F: 9.0	F: 62234

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

M: males

F: females

Conclusions: Methylnaltrexone tested negative for induction of micronuclei under the assay conditions.

2.6.6.5 Carcinogenicity

Not conducted

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: SUBCUTANEOUS FERTILITY AND GENERAL REPRODUCTION TOXICITY STUDY OF METHYLNALTREXONE IN RATS (STUDY NO. 2516-004, 2003)

Key study findings: In a Segment I, fertility and early embryonic development to implantation study in male and female rats, MNTX was tested at 5, 25 and 150 mg/kg/day by subcutaneous route. Body weight, food consumption was reduced at high dose in both sexes. There were no significant treatment-related effects on fertility and reproductive performances in both sexes. Based on these data, the no-observable-adverse-effect-level (NOAEL) for effects on fertility and reproductive performance was considered as 25 mg/kg/day.

Study no.: 2516-004

Volume #, and page #: N/A

Conducting laboratory and location: _____

Date of study initiation: March 4, 2003

GLP compliance: A statement of compliance was included

QA reports: yes (X) no ()

Drug, lot #, and % purity: MNTX, Lot No. D05340, _____

Methods

Doses: 0 (0.9% NaCl), 5, 25 and 150 mg/kg/day

Species/strain: Sprague Dawley rats

Number/sex/group: 25/sex/group

Route, formulation, and volume: Subcutaneous, solution in 0.9% NaCl, 2 mL/kg,

Satellite groups used for toxicokinetics: None

Study design: Methylnaltrexone was administered subcutaneously once daily to male rats (beginning 28 days before cohabitation with treated females and continuing through the day before euthanasia) and female rats (beginning 15 days before cohabitation with treated males and continuing through gestation day [GD] 7). Sprague-Dawley (S-D) rats (25/sex/group) were given dosages of 0 (vehicle), 5, 25, or 150 mg/kg/day at concentrations of 0, 2.5, 12.5 and 75 mg/mL, respectively. The control group received the vehicle consisting of 0.9% sodium

chloride injection, USP. The dose volume was 2 mL/kg. The study design is shown below (from page 16 of the study report).

2.7.1. Dosage Administration

Dosage Group	Dosage ^a (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Rats per Sex	Assigned Numbers	
					Male Rats	Female Rats
I	0 (Vehicle)	0	2	25	7801 - 7825	7901 - 7925
II	5	2.5	2	25	7826 - 7850	7926 - 7950
III	25	12.5	2	25	7851 - 7875	7951 - 7975
IV	150	75	2	25	7876 - 7900	7976 - 7993, 1784 ^b , 7995 - 8000

- a. The test article was considered 100% active for the purpose of dosage calculations.
 b. Female rat 7994 was found to have a red substance in the cage pan and a torn nail on the right front paw prior to administration of the test article, and was replaced with female rat 1784.

Parameters and endpoints evaluated: Evaluations consisted of mortality, clinical observations, body weight, food consumption, estrous cycles, fecundity parameters (mating and fertility indices, time to mating), hysterotomy findings on GD 13 (corpora lutea, implants, embryo mortality), placental appearance, weights of testes, epididymides, seminal vesicles (with and without fluid) and prostate, epididymal sperm count and motility, and postmortem observations.

Results

Mortality: All rats survived until scheduled necropsy.

Clinical signs: Methylnaltrexone-related clinical signs were observed at 150 mg/kg/day and consisted of tremors and decreased motor activity in males and discoloration and scab(s) at the injection site(s) in males and females.

Body weight: The mean initial and final body weights of control males were 353.2 and 507.6 g, respectively. The mean initial and final body weight of control females were 275.9 (GD0) and 346.5 g (GD13), respectively. Compared to controls, body weights and body weight gains were decreased in males and females at 150 mg/kg/day. Body weights in males at 150 mg/kg/day were decreased by 5% compared to controls, and this magnitude of difference first occurred on study day 39 and generally continued until study termination. In females, at 150 mg/kg/day, final group mean body weight was similar to controls, but body weight gains were decreased beginning the second week of dosing (decreased 43%) and during first week of gestation (decreased 16%) compared to controls.

Food consumption: The mean initial and final food consumption in control males were 26.3 and 26.6 g/animal/day, respectively. The mean initial and final food consumption in control females were 18.0 and 18.8 g/animal/day. In males, food consumption was

reduced at 25 and 150 mg/kg/day (5-6% reduction). However, food consumption was unaffected by treatment in females.

Toxicokinetics: None

Necropsy: The right epididymis of one rat at 25 mg/kg/day dosage group was separated from right testis. This was considered not test article-related because it was a single non-dosage-dependent event. No other gross lesions were observed at necropsy.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): In males, all mating and fertility parameters (number of days to inseminate, rats that mated, fertility index, sperm motility etc.) were unaffected. However, the amount of fluid in the seminal vesicles was decreased (15% to 18%) in males at 25 mg/kg/day compared to controls, and this decrease in fluid was considered to be compound-related. In females, estrous cycling was unaffected by MNTX treatment. The number of estrous stages per 14 days did not differ significantly among the groups. All mating and fertility parameters (the number of days in cohabitation, rats that mated, fertility index,) were unaffected by treatment. There were no effects of MOA-728 on estrous cyclicity, but there was a slight increase in the number of days in cohabitation at 150 mg/kg/day (2.6 days) compared with controls (2 days). There were no effects on mating, but the fertility rate was slightly decreased at 150 mg/kg/day (88%) compared with controls (96%). There were no remarkable placental observations. Pregnancy occurred in 24 (96.0%), 25 (100.0%), 24 (96.0%) and 22 (88.0%) rats in the four respective dosage groups. The litter averages for corpora lutea; implantations, litter sizes and viable and nonviable embryos were comparable among the four dosage groups. The following tables show the fertility parameters in males (page B-8 and B-12 of the study report) and females (page C-11 and C-13 of the study report).

PROTOCOL 2516-064: SUBCUTANEOUS FERTILITY AND GENERAL REPRODUCTION TOXICITY STUDY OF METHYLMALTREXONE IN RATS

TABLE B6 (PAGE 1): MATING AND FERTILITY - SUMMARY - MALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 5	III 25	IV 150
RATS IN COHABITATION	N	25	25	25	25
DAYS IN COHABITATION a	MEAN±S.D.	2.0 ± 1.1	2.0 ± 1.1	2.2 ± 1.3	2.6 ± 1.8
RATS THAT MATED b	N(%)	25(100.0)	25(100.0)	25(100.0)	25(100.0)
FERTILITY INDEX c	N/N (%)	24/25 (96.0)	25/25 (100.0)	24/25 (96.0)	22/25 (88.0)
RATS WITH CONFIRMED MATING DATES	N	25	25	25	25
MATED WITH FIRST FEMALE e					
DAYS 1-7	N(%)	25(100.0)	25(100.0)	25(100.0)	24(96.0)
DAYS 8-14	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
MATED WITH SECOND FEMALE e					
DAYS 15-21	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
RATS PREGNANT/RATS IN COHABITATION d	N/N (%)	24/25 (96.0)	25/25 (100.0)	24/25 (96.0)	22/25 (88.0)

- a. Restricted to rats with a confirmed mating date and rats that did not mate.
- b. Includes only one mating for each male rat.
- c. Number of pregnancies/number of rats that mated.
- d. Includes only one pregnancy for each rat that impregnated more than one female rat.
- e. Restricted to rats with a confirmed mating date.

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PROTOCOL 2516-004: SUBCUTANEOUS FERTILITY AND GENERAL REPRODUCTION TOXICITY STUDY OF METHYLNALTREXONE IN RATS

TABLE B10 (PAGE 1): SPERM MOTILITY, COUNT AND DENSITY - SUMMARY - MALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)	0 (VEHICLE)	5	25	150	150
RATS TESTED	N	25	25	25	25
VAS DEFERENS SPERM MOTILITY					
NUMBER MOTILE	MEAN±S.D.	321.0 ± 91.8	337.5 ± 85.5	313.5 ± 83.6	301.9 ± 88.0
MOTILE PERCENT	MEAN±S.D.	95.0 ± 2.2	95.2 ± 2.7	94.2 ± 3.3	94.8 ± 2.9
STATIC COUNT (NONMOTILE)	MEAN±S.D.	16.3 ± 7.1	16.8 ± 10.7	19.6 ± 11.8	16.1 ± 9.9
TOTAL COUNT a	MEAN±S.D.	337.3 ± 94.0	354.3 ± 87.7	333.1 ± 87.1	318.0 ± 90.8
CAUDA EPIDIDYMAL SPERM COUNT					
SPERM COUNT b	MEAN±S.D.	133.6 ± 22.6	131.5 ± 29.7	129.4 ± 23.9	125.9 ± 19.0
SPERM DENSITY c	MEAN±S.D.	1311.6 ± 291.2	1307.2 ± 246.6	1194.3 ± 266.6	1241.8 ± 184.0

- a. Sum of number motile and static count. Groups of five fields were evaluated until a sperm count of at least 200 was achieved or 20 fields were evaluated.
- b. Sperm count used in the calculation of sperm density. Ten fields were evaluated.
- c. The sperm density was calculated by dividing the sperm count by the volume in the image area (34.3×10^{-6} mL), multiplying by 2 (dilution factor) and multiplying by 10^6 to obtain the sperm concentration. The calculated sperm concentration value (rounded to 1 decimal place) was multiplied by 50 (volume) and divided by the weight of the left cauda epididymis (see Table B16 for the weight of the left cauda epididymis) to obtain the sperm density. The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.

PROTOCOL 2516-004: SUBCUTANEOUS FERTILITY AND GENERAL REPRODUCTION TOXICITY STUDY OF METHYLNALTREXONE IN RATS

TABLE C10 (PAGE 1): MATING AND FERTILITY, ESTROUS CYCLING AND DAYS IN COHABITATION - SUMMARY - FEMALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) a	0 (VEHICLE)	5	25	150	150
ESTROUS CYCLING OBSERVATIONS					
RATS EVALUATED	N	25	25	25	25
PRECOITAGE ESTROUS CYCLING					
ESTROUS STAGES/ 14 DAYS	MEAN±S.D.	3.3 ± 1.0	3.4 ± 0.8	3.6 ± 0.6	3.4 ± 0.9
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF DIESTRUS	N	0	0	0	0
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF ESTRUS	N	0	0	0	0
PRECOHABITATION ESTROUS CYCLING					
ESTROUS STAGES/ 14 DAYS	MEAN±S.D.	3.3 ± 0.5	3.0 ± 0.5	3.2 ± 0.6	3.2 ± 0.9
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF DIESTRUS	N	0	1	1	0
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF ESTRUS	N	0	0	0	0

- a. Dosage occurred on day 1 of study through day 7 of gestation.

PROTOCOL 2516-004: SUBCUTANEOUS FERTILITY AND GENERAL REPRODUCTION TOXICITY STUDY OF METHYLNALTREXONE IN RATS
 TABLE C11 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY - FEMALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) ^a		0 (VEHICLE)	5	25	150
RATS TESTED		N	25	25	25
PREGNANT	N(%)	24 (96.0)	25 (100.0)	24 (96.0)	22 (88.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 13 OF GESTATION	N	24	25	24	22
CORPORA LUTEA	MEAN±S.D.	17.1 ± 2.9	17.4 ± 2.3	17.3 ± 2.4	18.0 ± 1.7
IMPLANTATIONS	MEAN±S.D.	15.3 ± 2.2	15.3 ± 2.8	14.9 ± 1.7	15.1 ± 2.7
LITTER SIZES	MEAN±S.D.	14.4 ± 2.6	14.8 ± 2.7	14.3 ± 2.1	14.1 ± 2.9
VIABLE EMBRYOS	N	346	371	344	311
	MEAN±S.D.	14.4 ± 2.6	14.8 ± 2.7	14.3 ± 2.1	14.1 ± 2.9
NONVIABLE EMBRYOS	N	22	11	14	21
	MEAN±S.D.	0.9 ± 1.5	0.4 ± 0.6	0.6 ± 1.0	1.0 ± 1.1
DAMS WITH ANY NONVIABLE EMBRYOS	N(%)	12 (50.0)	9 (36.0)	9 (37.5)	14 (63.6)
DAMS WITH ALL NONVIABLE EMBRYOS	N(%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
DAMS WITH VIABLE EMBRYOS	N(%)	24 (100.0)	25 (100.0)	24 (100.0)	22 (100.0)
PLACENTAE APPEARED NORMAL	N(%)	24 (100.0)	25 (100.0)	24 (100.0)	22 (100.0)

a. Dosage occurred on day 1 of study through day 7 of gestation.

Summary: In a Segment I study in male and female rats, MNTX was tested at 5, 25 and 150 mg/kg/day by SC route. Body weight, food consumption was reduced at high dose in both sexes. There were no significant treatment-related effects on fertility and reproductive performances in both sexes. Based on these data, the no-observable-adverse-effect-level (NOAEL) for effects on fertility and reproductive performance was considered as 25 mg/kg/day.

Embryofetal development

INTRAVENOUS DEVELOPMENTAL TOXICITY STUDY OF METHYLNALTREXONE IN RATS (STUDY NO. 2516-001, 2002)

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

Intravenous Developmental Toxicity Study of Methylnaltrexone in Rats (Segment II Study)

Key Study Findings: no evidence of teratogenicity or embryofetotoxicity; mortality and signs of CNS toxicity occurred in pregnant females treated with 25 mg/kg/day

Study # 2516-001

Vol. 6, Pg. 1

Testing Laboratory: _____

Study Dates: January 10, 2002 - July 31, 2002

GLP Compliance: A statement of compliance was included.

QA Report: (x)Yes ()No

Drug: lot # C13754; ~~100~~ % pure

Formulation/Vehicle: solution/0.9% NaCl

Animals: ✓ CD®(SD)IGS BR VAF/Plus® rats

Females: 223-243 g (age at study initiation was not stated)

Males: 471-798 g (age at study initiation was not stated)

METHODS: Male and female rats were cohabitated (1:1) for up to five days. Mating was confirmed by observation of spermatozoa in vaginal smears and/or the presence of a copulatory plug. The day of mating confirmation was designated as day 0 of gestation. Mated female rats were treated intravenously with 0 (vehicle), 1, 5, or 25 mg/kg/day methylnaltrexone on gestation days 7 through 17 (25 rats/group). The dose volume was 2 ml/kg. The authors stated that dose selection was based on results from "previous intravenous studies conducted in rats and dogs". It is unclear as to which studies are referenced by this statement. In the present study, mated females were sacrificed on day 21 of presumed gestation, and C-sections were performed. All fetuses were examined for external abnormalities. Approximately one half of the fetuses in each litter were examined for visceral abnormalities. These fetuses were subsequently fixed in Bouin's solution, and the heads were examined by sectioning. The remaining fetuses were

eviscerated and stained with Alizarin red for examination of skeletal anomalies. The following parameters were reported:

Clinical Signs: three times daily on days 7-21

Maternal Bodyweight: day 0 and days 7-21

Food Consumption: days 0, 7, 10, 12, 15, 18, and 21

Gross Pathology: day 21

Examination of Reproductive Tract: day 21, day of death, or day of premature delivery

Fetal Observations: external, visceral, and skeletal examination; sex; bodyweight

RESULTS:

In-Life Observations:

Mortality: Five deaths occurred in the 25 mg/kg/day group on days 7-10. The deaths occurred shortly after dosing and were preceded by clinical signs such as whole body tremors and labored breathing.

Clinical Signs: All high-dose rats exhibited tremors, labored breathing, and decreased motor activity. The high-dose group also exhibited a high incidence of impaired righting reflex, mydriasis, ataxia, body jerks, and clonic convulsions. Gasping and pale extremities occurred sporadically in the high-dose group.

Bodyweight: Weight gain was unaffected.

Food Consumption: Methylalntrexone had no effect on food intake.

Terminal and Necroscopic Evaluations:

Gross Pathology: No changes were observed.

C-Section Observations: The contents of the reproductive tract were examined in each animal. One female in the 25 mg/kg/day group delivered prematurely on day 21. This female exhibited decreased motor activity, labored breathing, tremors, mydriasis, and body jerks during the treatment period. Weight gain and food consumption were unaffected in this animal. The litter consisted of 14 live fetuses, all of which appeared normal in the gross, visceral, and skeletal examination. One control female delivered prematurely on day 21. The only clinical sign in this animal was the appearance of a brown perivaginal substance on day 15. The litter consisted of 13 viable fetuses and one dead fetus. One live fetus had missing digits, missing metacarpals, and unossified distal phalanges. The results from the surviving animals are shown in the table below.

Parameter	Dose (mg/kg/day)			
	0	1	5	25
Mated Females	25	25	25	25
Pregnant (n)	24	24	25	24
Deaths (n)	0	0	0	5
Delivered Prematurely (n)	1	0	0	1
Pregnant with C-section on day 21 (n)	23	24	25	18
Corpora Lutea	17.7 ± 2.0	17.4 ± 2.1	17.6 ± 3.2	17.6 ± 2.8
Implantations	15.5 ± 1.6	15.5 ± 1.4	15.6 ± 1.8	15.3 ± 1.9
Live Fetuses	14.3 ± 2.2	14.8 ± 1.7	15.0 ± 1.9	14.7 ± 1.8
Dead Fetuses	0	0	0	0
Early Resorptions	1.2 ± 1.6	0.8 ± 0.9	0.5 ± 0.6	0.6 ± 0.8
Late Resorptions	0	0	0.1 ± 0.3	0
% Dams with Resorptions	52.2	54.2	52.0	39.8
% Live Male Fetuses/Litter	52.6 ± 13.6	49.8 ± 14.5	54.7 ± 13.3	51.0 ± 12.1
Normal Placenta (%)	95.6	100	100	100

Values are the mean ± S.D. per litter, unless stated otherwise.

Methylnaltrexone had no effects on the C-section parameters.

Fetal Observations: Fetal weights are listed in the following table.

Sex	Vehicle	Fetal Weight (g)		
		1 mg/kg/day	5 mg/kg/day	25 mg/kg/day
Males	5.42 ± 0.34	5.48 ± 0.24	5.43 ± 0.24	5.56 ± 0.29
Females	5.11 ± 0.31	5.18 ± 0.26	5.15 ± 0.23	5.24 ± 0.30

Fetal weight was unaffected. The results from external examination are shown in the table below.

GROSS OBSERVATIONS	DOSE (mg/kg/day)				
		0	1	5	25
Litters Evaluated	N	23	24	25	18
Fetuses Evaluated	N	329	355	375	265
Live Fetuses	N	329	355	375	265
ABNORMALITIES					
Head					
Papilla absent					
Fetal Incidence	N (%)	0	0	0	1 (0.4)
Litter Incidence	N (%)	0	0	0	1 (5.6)
Eye bulge depressed					
Fetal Incidence	N (%)	0	1 (0.3)	0	0
Litter Incidence	N (%)	0	1 (4.2)	0	0
Protruded tongue					
Fetal Incidence	N (%)	0	0	0	1 (0.4)
Litter Incidence	N (%)	0	0	0	1 (5.6)
Micrognathia of jaw					
Fetal Incidence	N (%)	0	0	0	1 (0.4)
Litter Incidence	N (%)	0	0	0	1 (5.6)
Limbs/Extremities					
Short tail					
Fetal Incidence	N (%)	0	0	0	1 (0.4)
Litter Incidence	N (%)	0	0	0	1 (5.6)
Constricted tail					
Fetal Incidence	N (%)	0	0	0	1 (0.4)
Litter Incidence	N (%)	0	0	0	1 (5.6)
Digits absent					
Fetal Incidence	N (%)	0	0	0	1 (0.4)
Litter Incidence	N (%)	0	0	0	1 (5.6)
Hindlimbs rotated medially					
Fetal Incidence	N (%)	0	0	0	1 (0.4)
Litter Incidence	N (%)	0	0	0	1 (5.6)
Torso					
Anal opening absent					
Fetal Incidence	N (%)	0	0	0	1 (0.4)
Litter Incidence	N (%)	0	0	0	1 (5.6)
Edema					
Fetal Incidence	N (%)	0	0	0	1 (0.4)
Litter Incidence	N (%)	0	0	0	1 (5.6)

One fetus in the 25 mg/kg/day group exhibited the following abnormalities: absence of papilla, protrusion of tongue, micrognathia in jaw, short tail, absence of digits, rotated hindlimbs, absence of anal opening, and edema. Visceral observations are shown in the following table.

Fetal Incidence	N (%)	0	0	0	1 (0.7) ¹
Litter Incidence	N (%)	0	0	0	1 (5.6)
Six right ribs missing					
Fetal Incidence	N (%)	0	0	0	1 (0.7) ¹
Litter Incidence	N (%)	0	0	0	1 (5.6)
Three left ribs missing					
Fetal Incidence	N (%)	0	0	0	1 (0.7) ¹
Litter Incidence	N (%)	0	0	0	1 (5.6)
LIMBS:					
Missing digits in forelimbs and hindlimbs					
Fetal Incidence	N (%)	0	0	0	1 (0.7) ¹
Litter Incidence	N (%)	0	0	0	1 (5.6)

1: This fetus exhibited multiple malformations.

SKELETAL VARIATIONS	DOSE (mg/kg/day)				
		0	1	5	25
Litters Evaluated	N	23	24	25	18
Fetuses Evaluated	N	329	355	375	265
Live Fetuses	N	329	355	375	265
VERTEBRAE:					
Extra rib present at 7 th cervical vertebra					
Fetal Incidence	N (%)	2 (1.2)	2 (1.1)	3 (1.6)	2 (1.4)
Litter Incidence	N (%)	2 (8.7)	2 (8.3)	3 (12)	2 (11.1)

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DELAYED OSSIFICATION	DOSE (mg/kg/day)				
		0	1	5	25
Litters Evaluated	N	23	24	25	18
Fetuses Evaluated	N	329	355	375	265
Live Fetuses	N	329	355	375	265
SKULL:					
Tympanic rings not ossified					
Fetal Incidence	N (%)	0	0	0	1 (0.7) ¹
Litter Incidence	N (%)	0	0	0	1 (5.6)
RIBS:					
Incompletely ossified					
Fetal Incidence	N (%)	1 (0.6)	0	0	0
Litter Incidence	N (%)	1 (4.3)	0	0	0
Not ossified					
Fetal Incidence	N (%)	0	0	0	1 (0.7) ¹
Litter Incidence	N (%)	0	0	0	1 (5.6)
LIMBS:					
Metacarpals not ossified in forelimbs					
Fetal Incidence	N (%)	0	0	0	1 (0.7) ¹
Litter Incidence	N (%)	0	0	0	1 (5.6)
Phalanges not ossified in forelimbs					
Fetal Incidence	N (%)	0	0	0	1 (0.7) ¹
Litter Incidence	N (%)	0	0	0	1 (5.6)
Phalanges not ossified in hindlimbs					
Fetal Incidence	N (%)	0	0	0	1 (0.7) ¹
Litter Incidence	N (%)	0	0	0	1 (5.6)
Metatarsals not ossified in hindlimbs					
Fetal Incidence	N (%)	0	0	0	1 (0.7) ¹
Litter Incidence	N (%)	0	0	0	1 (5.6)

1: This fetus exhibited multiple malformations.

No treatment-related malformations, variations, or delayed ossification was observed. One fetus in the 25 mg/kg/day group exhibited multiple malformations and delayed ossification. The number of ossification sites per fetus was unaffected by treatment.

Conclusions: Methylnaltrexone was not teratogenic and did not produce embryofetotoxicity at intravenous doses of up to 25 mg/kg/day. Intravenous administration of 25 mg/kg/day produced severe maternal toxicity. All rats in this dose group exhibited CNS-related clinical signs. Several deaths occurred in this group (5/25 rats). Given the adverse effects in the high-dose group, the dose selection is considered as adequate.

TOXICOKINETIC ASSESSMENT FOLLOWING INTRAVENOUS ADMINISTRATION TO PREGNANT RATS ON GESTATION DAY 15 (REPORT NO. 65526 and 65835)

Methods: MOA-728 was administered intravenously (IV; slow bolus) to mated female —CD(SD) rats at dosages of 1, 5 (9/group), or 25 (12/group) mg/kg once on gestation day (GD) 15. Blood samples were collected from 3 animals per group at 2 designated

time-points as follows: at 10 minutes and 7 hours, 1 and 12 hours, and 3 and 24 hours after dosing on GD 15.

Results: Exposure increased with increasing dosage in a greater than dose-proportional manner. The mean AUC_{0-24hr} values were 312, 2116, and 30838 ng.hr/mL at 1, 5 and 25 mg/kg/day, respectively. The mean TK parameters are shown in the following table (from page 14 of the study report).

Table 4.3-1: Mean (\pm SE) MOA-728 Pharmacokinetics in Pregnant Rats Following a Single Intravenous Dose on Gestation Day 15

Dose (mg/kg)	C _{10min} (ng/mL)	AUC ₀₋₂₄ (ng•hr/mL)	AUC ₀₋₂₄ /Dose
1	409 \pm 28	312 \pm 17	312 \pm 17
5	3288 \pm 515	2116 \pm 300	423 \pm 60
25	50444 \pm 1322	30838 \pm 785	1234 \pm 31

Individual and group mean plasma concentrations and PK parameters for MOA-728 are presented in a separate report.²

INTRAVENOUS DEVELOPMENTAL TOXICITY STUDY OF METHYLNALTREXONE IN RABBITS (————— STUDY NO. 2516-002, 2002)

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

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Intravenous Developmental Toxicity Study of Methylnaltrexone in Rabbits (Segment II Study)

Key Study Findings: not teratogenic or embryofetotoxic at doses of up to 16 mg/kg/day iv; mortality and CNS toxicity were observed in rabbits treated with 24/16 mg/kg/day iv (two days at 24 mg/kg/day)

Study # 2516-002

Vol. 6, Pg. 235

Testing Laboratory:

Study Dates: January 28, 2002 - July 31, 2002

GLP Compliance: A statement of compliance was included.

QA Report: (x)Yes ()No

Drug: lot # C13754; pure

Formulation/Vehicle: solution/0.9% NaCl

Animals: Hra:(NZW)SPF rabbits
Females: 2.9-4.4 kg (age 6-6.5 months)

METHODS: Mated rabbits were treated intravenously with 0 (vehicle), 1, 8, or 24/16 mg/kg/day methylnaltrexone on days 6-18 of gestation (20 females/group). The day of mating confirmation was considered as day 0 of gestation. The high dose was reduced to 16 mg/kg/day on days 7-8 due to mortality. The dose volume was 1 ml/kg. Dose selection was based on results from a dose-ranging teratogenicity study in rabbits. In this study, administration of 24 mg/kg/day iv produced mortality and signs of CNS toxicity in pregnant rabbits, as well as a reduction in fetal bodyweight. In the present study, mated females were sacrificed on day 29 of presumed gestation, and C-sections were performed. All fetuses were examined for external abnormalities and visceral abnormalities. The brain was also examined. All fetuses were eviscerated and stained with Alizarin red for examination of skeletal anomalies. The following parameters were reported:

Clinical Signs: twice daily

Maternal Bodyweight: day 0 and days 6-29

Food Consumption: daily

Gross Pathology: day 29, day of death, or day of premature delivery

Examination of Reproductive Tract: day 29, day of death, or day of premature delivery

Fetal Observations: external, visceral, and skeletal examination; sex; bodyweight

RESULTS:

In-Life Observations:

Mortality: Four deaths occurred in the high-dose group following administration of 24 mg/kg/day on days 6-7. The deaths occurred immediately after dosing and were preceded by clinical signs such as clonic convulsions, body jerks, and labored breathing in 3/4 animals. One death occurred in each of the 1 and 8 mg/kg/day groups (days 11 and 9, respectively), but these were not considered as treatment-related.

Clinical Signs: The high-dose group (24/16 mg/kg/day) exhibited ataxia, decreased motor activity, body jerks, labored breathing, and loss of righting reflex. Ataxia was the most frequently observed sign after the high-dose was reduced to 16 mg/kg/day. One high-dose female delivered on day 29.

Bodyweight: Weight gain was unaffected.

Food Consumption: Food intake was unaffected.

Terminal and Necroscopic Evaluations:

Gross Pathology: Spongy appearance of lungs was observed in a female that died on day 7 after administration of 24 mg/kg. A trichobezoar was present in the stomach of the 1 mg/kg/day rabbit that was sacrificed in moribund condition on day 11.

C-Section Observations: The contents of the reproductive tract were examined in each animal. All rabbits that died prematurely were found to be pregnant, except for one high-dose female. Embryofetotoxicity was not observed in these animals. One female in the high-dose group delivered four dead fetuses on day 29. Ten corpora lutea were found in this animal. The results from surviving rabbits are shown in the table below.

Parameter	Dose (mg/kg/day)			
	0	1	8	24/16
Mated Females	20	20	20	20
Pregnant (n)	20	18	18	19
Deaths (n)	0	1	1	4
Premature Delivery	0	0	0	1
Pregnant with C-section on day 29 (n)	20	17	17	15
Corpora Lutea	10.4 ± 2.1	9.9 ± 2.0	9.2 ± 2.2	9.7 ± 2.2
Implantations	9.8 ± 2.5	9.1 ± 2.3	8.6 ± 2.2	9.0 ± 2.4
Live Fetuses	9.3 ± 2.6	8.4 ± 2.4	8.0 ± 2.3	8.6 ± 2.7
Dead Fetuses (total)	1	1	0	0
Early Resorptions	0.1 ± 0.3	0.1 ± 0.3	0.2 ± 0.4	0.2 ± 0.4
Late Resorptions	0.4 ± 0.5	0.5 ± 1.3	0.4 ± 0.7	0.2 ± 0.4
Post-implantation Loss (%)	5.8 ± 7.0	7.0 ± 14.3	6.6 ± 8.9	6.0 ± 8.4
% Dams with Resorptions	45.0	29.4	41.2	40.0
% Live Male Fetuses/Litter	52.6 ± 19.0	50.7 ± 23.8	48.2 ± 12.3	54.5 ± 19.5
Normal Placenta (%)	100	100	100	100

Values are the mean ± S.D. per litter, unless stated otherwise.

C-section parameters were not affected by methylalntrexone.

Fetal Observations: Fetal weights are listed in the following table.

Sex	Fetal Weight (g)			
	Vehicle	1 mg/kg/day	8 mg/kg/day	24/16 mg/kg/day
Males	42.7 ± 5.6	46.9 ± 4.1	46.5 ± 6.9	45.7 ± 4.9
Females	43.1 ± 6.4	44.8 ± 4.4	45.0 ± 6.9	44.1 ± 4.7

Values are the mean ± S.D.

Fetal weight was unaffected. The results from external examination are shown in the table below.

GROSS OBSERVATIONS	DOSE (mg/kg/day)				
		0	1	8	24/16
Litters Evaluated	N	20	17	17	15
Fetuses Evaluated	N	187	144	137	129
Live Fetuses	N	186	143	137	129
ABNORMALITIES					
Head					
Domed					
Fetal Incidence	N (%)	0	0	1 (0.7)	0
Litter Incidence	N (%)	0	0	1 (5.9)	0
Short snout					
Fetal Incidence	N (%)	0	1 (0.7) ¹	0	0
Litter Incidence	N (%)	0	1 (5.9)	0	0
Nares absent					
Fetal Incidence	N (%)	0	1 (0.7) ¹	0	0
Litter Incidence	N (%)	0	1 (5.9)	0	0
Teeth absent					
Fetal Incidence	N (%)	0	1 (0.7) ¹	0	0
Litter Incidence	N (%)	0	1 (5.9)	0	0
Torso					
Edema					
Fetal Incidence	N (%)	0	1 (0.7) ²	0	0
Litter Incidence	N (%)	0	1 (5.9)	0	0
Purple areas on skin					
Fetal Incidence	N (%)	0	1 (0.7) ²	0	0
Litter Incidence	N (%)	0	1 (5.9)	0	0

1: This fetus had other gross abnormalities.

2: This fetus had other gross abnormalities.

No drug-related abnormalities were observed. The results of the visceral examination are shown in the following table.

VISCERAL OBSERVATIONS	DOSE (mg/kg/day)				
		0	1	8	24/16
Litters Evaluated	N	20	17	17	15
Fetuses Evaluated	N	187	144	137	129
Live Fetuses	N	186	143	137	129
MALFORMATIONS					
Dilated brain ventricles					
Fetal Incidence	N (%)	0	0	1 (0.7)	0
Litter Incidence	N (%)	0	0	1 (5.9)	0
Extra papillary muscles in heart					
Fetal Incidence	N (%)	0	0	1 (0.7)	0
Litter Incidence	N (%)	0	0	1 (5.9)	0
Gallbladder absent					
Fetal Incidence	N (%)	2 (1.1)	1 (0.7)	0	0
Litter Incidence	N (%)	1 (5.0)	1 (5.9)	0	0
Mass in thoracic cavity					
Fetal Incidence	N (%)	0	1 (0.7)	0	0
Litter Incidence	N (%)	0	1 (5.9)	0	0
VARIATIONS					
Absence of Intermediate lobe of lungs					
Fetal Incidence	N (%)	1 (0.5)	0	0	1 (0.8)
Litter Incidence	N (%)	1 (5.0)	0	0	1 (6.7)

No treatment-related anomalies were observed. The skeletal observations are shown in the following tables.

SKELETAL MALFORMATIONS	DOSE (mg/kg/day)				
		0	1	8	24/16
Litters Evaluated	N	20	17	17	15
Fetuses Evaluated	N	187	144	137	129
Live Fetuses	N	186	143	137	129
SKULL:					
Nasals contained an internasal					
Fetal Incidence	N (%)	3 (1.6)	0	0	0
Litter Incidence	N (%)	3 (15.0)	0	0	0
Nasals contained an intranasal					
Fetal Incidence	N (%)	0	0	1 (0.7)	0
Litter Incidence	N (%)	0	0	1 (5.9)	0
Irregular shape and fusion of nasals					
Fetal Incidence	N (%)	0	1 (0.7) ¹	0	0
Litter Incidence	N (%)	0	1 (5.9)	0	0
Irregular shape and fusion of frontals					
Fetal Incidence	N (%)	0	1 (0.7) ¹	0	0
Litter Incidence	N (%)	0	1 (5.9)	0	0
Parietal contained a hole					
Fetal Incidence	N (%)	0	0	1 (0.7) ²	0
Litter Incidence	N (%)	0	0	1 (5.9)	0
Extra ossification sites in anterior fontanelle					
Fetal Incidence	N (%)	1 (0.5)	0	0	0
Litter Incidence	N (%)	1 (5.0)	0	0	0
Large posterior fontanelle					
Fetal Incidence	N (%)	0	0	1 (0.7) ²	0
Litter Incidence	N (%)	0	0	1 (5.9)	0
Close set eye sockets					
Fetal Incidence	N (%)	0	1 (0.7) ¹	0	0
Litter Incidence	N (%)	0	1 (5.9)	0	0
VERTEBRAE:					
Thoracic hemivertebra					
Fetal Incidence	N (%)	0	0	1 (0.7)	0
Litter Incidence	N (%)	0	0	1 (5.9)	0
Lumbar hemivertebra					
Fetal Incidence	N (%)	0	1 (0.7)	0	0
Litter Incidence	N (%)	0	1 (5.9)	0	0
RIBS:					
Small					
Fetal Incidence	N (%)	0	1 (0.7)	0	0
Litter Incidence	N (%)	0	1 (5.9)	0	0
STERNEBRAE:					
Fused centra					
Fetal Incidence	N (%)	4 (2.2)	4 (2.8)	3 (2.2)	2 (1.6)
Litter Incidence	N (%)	2 (10.0)	3 (17.6)	3 (17.6)	2 (13.3)

1: This fetus exhibited other malformations.

2: This fetus exhibited other malformations.

SKELETAL VARIATIONS	DOSE (mg/kg/day)				
		0	1	8	24/16
Litters Evaluated	N	20	17	17	15
Fetuses Evaluated	N	187	144	137	129
Live Fetuses	N	186	143	137	129
SKULL:					
Displaced midline suture in nasals					
Fetal Incidence	N (%)	0	0	1 (0.7)	0
Litter Incidence	N (%)	0	0	1 (5.9)	0
Irregular suture in nasal-frontal					
Fetal Incidence	N (%)	1 (0.5)	0	0	0
Litter Incidence	N (%)	1 (5.0)	0	0	0
Angulated hyoid					
Fetal Incidence	N (%)	5 (2.7)	2 (1.4)	4 (2.9)	2 (1.6)
Litter Incidence	N (%)	5 (25.0)	1 (5.9)	2 (11.8)	2 (13.3)
LIMBS:					
Irregular shape of scapulae alae					
Fetal Incidence	N (%)	0	1 (0.7)	0	1 (0.8)
Litter Incidence	N (%)	0	1 (5.9)	0	1 (6.7)
RIBS:					
Extra					
Fetal Incidence	N (%)	0	1 (0.7)	0	0
Litter Incidence	N (%)	0	1 (5.9)	0	0
Thickened					
Fetal Incidence	N (%)	0	1 (0.7)	0	1 (0.8)
Litter Incidence	N (%)	0	1 (5.9)	0	1 (6.7)

DELAYED OSSIFICATION	DOSE (mg/kg/day)				
		0	1	8	24/16
Litters Evaluated	N	20	17	17	15
Fetuses Evaluated	N	187	144	137	129
Live Fetuses	N	186	143	137	129
SKULL:					
Premaxillae not ossified					
Fetal Incidence	N (%)	0	1 (0.7)	0	0
Litter Incidence	N (%)	0	1 (5.9)	0	0
STERNEBRAE:					
Sternal centra incompletely ossified					
Fetal Incidence	N (%)	1 (0.5)	1 (0.7)	0	1 (0.8)
Litter Incidence	N (%)	1 (5.0)	1 (5.9)	0	1 (6.7)
PELVIS:					
Pubis not ossified					
Fetal Incidence	N (%)	0	0	1 (0.7)	0
Litter Incidence	N (%)	0	0	1 (5.9)	0

Methylaltrexone had no effect on the incidence of skeletal malformations or variations. Ossification was also unaffected.

Conclusions: Methylnaltrexone was not teratogenic or embryofetotoxic at doses of up to 16 mg/kg/day iv. Deaths occurred at the initial high-dose level of 24 mg/kg/day, and appeared to be the result of CNS toxicity (e.g. convulsions). Other signs of CNS-related toxicity (e.g. ataxia, decreased motor activity) were observed at 24 mg/kg/day, and continued after reduction of the dose to 16 mg/kg/day. Therefore, the dose selection is considered as adequate.

TOXICOKINETIC ASSESSMENT FOLLOWING INTRAVENOUS ADMINISTRATION TO PREGNANT RABBITS ON GESTATION DAY 15 (REPORT NO. 65525)

Methods: Blood samples were collected from all animals at 10 minutes and 1, 3, 7, 12, and 24 hours after dosing on GD 15. Methylnaltrexone concentrations in rabbit plasma were determined using a validated LC/MS/MS method.

Results: Following a single dosage (IV; bolus) to pregnant rabbits, exposure (AUC_{0-24hr}) increased with increasing dosage in an apparently greater than dose proportional manner. The $t_{1/2}$ at 16 mg/kg/day was determined to be 6.9 hours. The mean AUC_{0-24hr} values were 365, 5345, and 15382 ng·hr/mL at 1, 8 and 16 mg/kg/day, respectively. The following table (from page 14 of the study report) shows the TK parameters in rabbits.

Table 4.3-1: Mean (\pm SD) MOA-728 Plasma Concentration - Time Profiles in Pregnant Rabbits Given Intravenous (Bolus) Doses of MOA-728-Gestation Day 15 Data

Dose (mg/kg)	C_{10min} (ng/mL)	AUC_{0-24} (ng·hr/mL)	$AUC_{0-\infty}$ (ng·hr/mL)	$AUC_{0-24}/Dose$	$t_{1/2}$ (hr)
1	555 \pm 34	365 \pm 29	ND	365 \pm 29	ND
8	8639 \pm 2466	5345 \pm 1560	ND	668 \pm 195	ND
16	24696 \pm 5697	15382 \pm 3532	19.7 ^a	961 \pm 221	6.9

ND. Not determined. The terminal phase was not well defined by at least 3 data points.

a. N = 1

Prenatal and postnatal development

Study title: SUBCUTANEOUS DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF METHYLNALTREXONE IN RATS, INCLUDING A POSTNATAL

BEHAVIORAL/FUNCTIONAL EVALUATION STUDY NO.
2516-003, 2004, and STUDY NO. AA04310 1)

Key study findings: In a Segment III pre- and postnatal development study in rats, animals were treated subcutaneously from gestation day 6 to postpartum day 20 at dosages of 0, 5, 25, or 150/100 mg/kg/day. Maternal toxicity in the form of mortality (150/100 mg/kg/day), clinical observations, and decreased body weight gain and food consumption occurred at dosages > 25 mg/kg/day. Therefore, the No Observed Adverse Effect Level (NOAEL) for maternal toxicity was considered 25 mg/kg/day. Based on decreased body weight in pups at 150/100 mg/kg/day that were reduced throughout the preweaning period, the NOAEL for perinatal/postnatal toxicity was considered 25 mg/kg/day. Growth and development (physical, sensory, behavioral and reproductive) of the F1 generation were unaffected.

Study no.: 2516-003

Volume #, and page #: N/A

Conducting laboratory and location: _____

Date of study initiation: March 2, 2003

GLP compliance: A statement of compliance was included.

QA reports: yes (X) no ()

Drug, lot #, and % purity: MNTX, Lot No. D05340, _____

Methods:

Doses: 5, 25, or 150/100 mg/kg/day

Species/strain: Sprague Dawley rats

Number/sex/group: 25/sex/group

Route, formulation, and volume: Subcutaneous, solution, 2 mL/kg

Satellite groups used for toxicokinetics: Yes

Study design: Methylnaltrexone was administered subcutaneously to mated female Sprague-Dawley (SD) rats (25/group) at dosages of 0 (vehicle), 5, 25, or 150/100 mg/kg/day once daily from gestation day (GD) 7 through postpartum day 20 (rats that delivered a litter) or GD 24 (rats that did not deliver a litter). Due to mortality and adverse clinical signs on GDs 13 and 14, the 150 mg/kg/day dosage was reduced to 100 mg/kg/day, and rats were in GDs 13 to 16 at the time the dosage was changed. Injection sites were rotated to minimize irritation. The control group received the vehicle consisting of 0.9% sodium chloride injection, USP. F1 generation rats were not directly treated with the test article, but may have been exposed to the drug either in utero exposure or via maternal milk during the lactation period. The following tables (from page 23 of the study report) show the study design.

2.6.1.1. Fo Generation Rats

Dosage Group	Dosage ^a (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Rats	Assigned Rat Numbers	
					Main Study	Toxicokinetic Study
I	0 (Vehicle)	0	2	25	8001 - 8025	Not applicable
II	5	2.5	2	25 + 24 ^b	8026 - 8050	8101 - 8124
III	25	12.5	2	25 + 24 ^b	8051 - 8075	8125 - 8132, 5475 ^c , 8134 - 8148
IV	150 ^d	75 ^d	2	25 + 24 ^b	8076 - 8100	8149 - 8172

- The test article was considered 100% active for the purpose of dosage calculations.
- Twenty-four rats assigned to toxicokinetic sample collection.
- Rat 8133 was removed from the study due to adverse clinical observations on DG 7, prior to administration of the test article, and was replaced with rat 5475.
- Effective 19 March 2003, the dosage was changed to 100 mg/kg/day and the concentration to 50 mg/mL.

2.6.1.2. F1 Generation Rats

Dosage Group	Maternal Dosage ^a (mg/kg/day)	Number of Rats per Sex	Assigned Rat Numbers	
			Male Rats	Female Rats
I	0 (Vehicle)	25	9601 - 9625	9701 - 9725
II	5	25	9626 - 9650	9726 - 9750
III	25	25	9651 - 9675	9751 - 9775
IV	150 ^b	25	9676 - 9700	9776 - 9800

- The test article was considered 100% active for the purpose of dosage calculations.
- Effective 19 March 2003, the dosage was changed to 100 mg/kg/day and the concentration to 50 mg/mL.

Parameters and endpoints evaluated: Evaluations consisted of the following: F0 mortality, clinical observations, body weight, food consumption, postmortem observations, and maternal behavior; and F1 litter size, mortality, clinical observations, sex distribution, weight, male organ weights (testes and epididymides), feed consumption and morphological development (including sexual maturation), learning and memory (passive avoidance and water maze), reproductive performance (mating and fertility indices, time to mating), hysterotomy findings, postmortem observations, and placental appearance; and F2 fetal sex, weight, and external anomalies. Plasma toxicokinetics of MOA-728 after one and 11 days of dosing were evaluated in satellite groups of gravid treated females (24/group)

Results:

F₀ in-life: Two pregnant rats at 150 mg/kg/day were found dead on GDs 13 or 14, and the cause of death was attributed to the treatment. Clinical signs prior to death consisted of discoloration of the injection site in both animals and lost righting reflex, clonic intermittent convulsions, whole body tremors, labored breathing and decreased motor activity in one animal. One pregnant rat at 100 mg/kg/day was found dead on GD 20, and the cause of death was attributed to MNTX. Prior to death, this animal displayed discoloration and scabs at the injection site and continuous whole body tremors. All other animals survived until scheduled sacrifice. Methylnaltrexone-related clinical signs

at 150 mg/kg/day included lost righting reflex, prostration, clonic convulsions, labored breathing and dyspnea, and these clinical signs appeared as early as GD 12. MOA-728-related clinical signs at ≥ 100 mg/kg/day included pale extremities, splayed limbs, tremors, twitches, decreased motor activity, low carriage, discoloration and scab(s) at the injection sites, grade 1 (slight) erythema and localized alopecia on the back. The mean initial (GD0) and the final (GD20) body weights of control females were 239 and 379 g, respectively. Transient periods of reduced weight gain and reduced food consumption were observed in animals at 150/100 mg/kg/day during gestation. During the lactation phase, body weights were lower at various time points compared to controls. The mean initial and final food consumption in control females were 23.4 and 23.7 g/animal/day, respectively. Slight transient reductions in body weight gain and food consumption were observed for 1 to 2 days at 25 mg/kg/day also. There were no effects of MNTX on litters or deliveries.

Systemic exposure (based on AUC) to MNTX increased with dosage across the dose range studied, although these increases were not strictly proportional to dose on GD 7 or 17. Half-lives on GDs 7 and 17 were comparable between dosages (ranging from 4.5 to 6.2 hr). There was no evidence of accumulation following multiple doses at 5 and 25 mg/kg/day. The TK parameters are shown in the following tables (from page 170 of the study report).

**Table B: Toxicokinetics Parameters for Methylnaltrexone
in Gravid Female Rats**

Gestation Day 7			
	Group 2 5 mg/kg/day	Group 3 25 mg/kg/day	Group 4 150 mg/kg/day
AUC ₀₋₂₄ (ng h/mL)	813	6994	98654
AUC ₀₋₁₇ (ng h/mL)	918	6976	98758
C _{max} (ng/mL)	1476.7	15400.0	45300.0
t _{1/2} (h)	5.7	5.5	4.7
CL/F (L/h/kg)	5.5	3.6	1.8
V _{d,area} /F (L/kg)	45	29	10

Gestation Day 17			
	Group 2 5 mg/kg/day	Group 3 25 mg/kg/day	Group 4 100 mg/kg/day
AUC ₀₋₂₄ (ng h/mL)	977	6651	58818
Accumulation Factor*	1.1	0.95	N/A
C _{max} (ng/mL)	1627.7	8926.7	36400.0
t _{1/2} (h)	4.5	5.4	6.2
CL/F (L/h/kg)	5.1	3.8	1.7
V _{d,area} /F (L/kg)	33	29	15

*Accumulation factor was calculated as AUC₀₋₂₄ (Study Day 17)/AUC₀₋₂₄ (Gestation Day 7)
N/A – Not Applicable

F₀ necropsy: One female rat at 5 mg/kg/day had marked dilatation in the right pelvis of the kidney. Another female had umbilical hernia at 5 mg/kg/day. There were no other significant treatment-related findings. The following table (from page B-14 of the study report) shows the delivery data for the F₀ females.

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TABLE B11 (PAGE 1): NATURAL DELIVERY OBSERVATIONS - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY): ^a		0 (VEHICLE)	5	25	100b
RATS ASSIGNED TO NATURAL DELIVERY		N	25	25	25
PREGNANT	N(%)	23(92.0)	24(96.0)	21(84.0)	22(88.0)
INCLUDED IN ANALYSES	N	23	24	21	19c
DELIVERED A LITTER	N(%)	23(100.0)	23(95.9)	21(100.0)	19(100.0)
DURATION OF GESTATION ^d	MEAN±S.D.	22.6 ± 0.5	22.8 ± 0.4	22.8 ± 0.7	22.7 ± 0.4
IMPLANTATION SITES PER DELIVERED LITTER	MEAN±S.D.	3.20 13.9 ± 3.7	3.31 14.4 ± 3.9	2.98 14.2 ± 3.0	2.76 14.5 ± 1.9
DAMS WITH STILLBORN PUPS	N(%)	3(13.0)	2(9.1)	4(19.0)	2(11.0)
DAMS WITH NO LIVEBORN PUPS	N	0(0.0)	0(0.0)	0(0.0)	0(0.0)
GESTATION INDEX ^e	\bar{x} N/N	100.0 23/ 23	95.9 23/ 24	100.0 21/ 21	100.0 19/ 19
DAMS WITH ALL PUPS DYING DAYS 1-4 POSTPARTUM	N(%)	0(0.0)	0(0.0)	1(4.9)	0(0.0)
DAMS WITH ALL PUPS DYING DAYS 5-21 POSTPARTUM	N	0(0.0)	0(0.0)	0(0.0)	0(0.0)

- a. Dosage occurred on day 7 of gestation through day 20 of lactation.
- b. Dosage changed from 150 mg/kg/day to 100 mg/kg/day on day 13, 14 or 16 of gestation.
- c. Excludes values for rats that were found dead during gestation.
- d. Calculated as the time (in days) elapsed between confirmed mating (arbitrarily defined as day 0) and the time (in days) the first pup was delivered.
- e. Number of rats with live offspring/number of pregnant rats.

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F₁ physical development: No test article-related clinical observations, postmortem observations, or mortality were observed. Body weights were significantly reduced on days 1, 8 and 15 at 150/100 mg/kg/day, but postweaning body weight gains were similar across groups. There were no effects of MNTX on food consumption in the F1 generation.

F₁ behavioral evaluation: There were no significant treatment-related effects on learning, short- or long-term retention or response inhibition in either males or females as evaluated in a passive avoidance paradigm.

F₁ reproduction: Mating, fertility, and hysterotomy parameters of the F1 were unaffected by MNTX treatment. The following table (from pages C-27 and C-28 of the study report) shows the mating and fertility data for the F1 male and female rats.

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TABLE C21 (PAGE 1): MATING AND FERTILITY - SUMMARY - F1 GENERATION MALE RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	5	25	100
RATS IN COHABITATION	N	25	25	24a	25
DAYS IN COHABITATION d	MEAN±S.D.	2.6 ± 1.1 [23]c	3.0 ± 1.4 [24]	2.6 ± 2.1	3.1 ± 1.7
RATS THAT MATED d	N(%)	25(100.0)	25(100.0)	24(100.0)	25(100.0)
FERTILITY INDEX e	N/N (%)	22/ 25 (88.0)	25/ 25 (100.0)	23/ 24 (95.8)	25/ 25 (100.0)
RATS WITH CONFIRMED MATING DATES	N	24	24	24	25
MATED WITH FIRST FEMALE b					
DAYS 1-7	N(%)	24(100.0)	24(100.0)	23(95.8)	24(96.0)
DAYS 8-14	N(%)	0(0.0)	0(0.0)	1(4.2)	1(4.0)
RATS PREGNANT/RATS IN COHABITATION	N/N (%)	22/ 25 (88.0)	25/ 25 (100.0)	23/ 24 (95.8)	25/ 25 (100.0)

- [] - NUMBER OF VALUES AVERAGED
- a. Excludes values for rat 9674, which was not assigned to cohabitation because there were no available female rats.
 - b. Restricted to rats with a confirmed mating date.
 - c. Excludes rat 9602, which had a mating date that was incorrectly identified.
 - d. Includes only one mating for each male rat.
 - e. Number of pregnancies/number of rats that mated.

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TABLE C22 (PAGE 1): MATING AND FERTILITY - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	5	25	100
RATS IN COHABITATION	N	25	25	24a	25
DAYS IN COHABITATION d	MEAN±S.D.	2.6 ± 1.1 [23]c	3.0 ± 1.4 [24]	2.6 ± 2.1	3.1 ± 1.7
RATS THAT MATED	N(%)	25(100.0)	25(100.0)	24(100.0)	25(100.0)
FERTILITY INDEX d	N/N (%)	22/ 25 (88.0)	25/ 25 (100.0)	23/ 24 (95.8)	25/ 25 (100.0)
RATS WITH CONFIRMED MATING DATES	N	24	24	24	25
MATED WITH FIRST MALE b					
DAYS 1-7	N(%)	24(100.0)	24(100.0)	23(95.8)	24(96.0)
DAYS 8-14	N(%)	0(0.0)	0(0.0)	1(4.2)	1(4.0)
RATS PREGNANT/RATS IN COHABITATION	N/N (%)	22/ 25 (88.0)	25/ 25 (100.0)	23/ 24 (95.8)	25/ 25 (100.0)

- [] - NUMBER OF VALUES AVERAGED
- a. Excludes values for rat 9759, which was moribund sacrificed on day 50 postweaning.
 - b. Restricted to rats with a confirmed mating date.
 - c. Excludes rat 9717, which had a mating date that was incorrectly identified.
 - d. Number of pregnancies/number of rats that mated.

The following table (from page C-29 of the study report) shows the cesarean section data of the F1 female rats.

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TABLE C23 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	5	25	100
RATS TESTED	N	25	25	24a	25
PREPREGNANT DELIVERED AND SACRIFICED	N(%)	22 (88.0)	25 (100.0)	23 (95.8)	25 (100.0)
	N(%)	1 (4.5)	0 (0.0)	0 (0.0)	0 (0.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION	N	21b	25b	23	25
CORPORA LUTEA	MEAN±S.D.	17.7 ± 2.8	19.1 ± 2.2	18.2 ± 2.5	17.5 ± 2.9
IMPLANTATIONS	MEAN±S.D.	15.4 ± 1.3	17.2 ± 1.9**	15.6 ± 2.5	15.9 ± 2.3
LITTER SIZES	MEAN±S.D.	14.9 ± 1.5	15.4 ± 2.1	15.1 ± 2.8	15.2 ± 2.6
LIVE FETUSES	N	313	411	347	379
	MEAN±S.D.	14.9 ± 1.5	16.4 ± 2.3	15.1 ± 2.8	15.2 ± 2.6
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.5 ± 0.8	0.8 ± 1.0	0.5 ± 0.8	0.7 ± 0.8
EARLY RESORPTIONS	N	11	20	10	18
	MEAN±S.D.	0.5 ± 0.9	0.8 ± 1.0	0.4 ± 0.8	0.7 ± 0.8
LATE RESORPTIONS	N	0	0	1	0
	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.0
DAMS WITH ANY RESORPTIONS	N(%)	7 (33.3)	13 (52.0)	7 (30.4)	13 (52.0)
DAMS WITH ALL CONCEPTUSES RESORBED	N(%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
DAMS WITH VIABLE FETUSES	N(%)	21 (100.0)	25 (100.0)	23 (100.0)	25 (100.0)
PLACENTAE APPEARED NORMAL	N(%)	21 (100.0)	25 (100.0)	23 (100.0)	25 (100.0)

a. Excludes rat 9759, which was moribund sacrificed on day 50 postweaning.
 b. Includes values for dams 9708 and 9749, which did not have a confirmed mating date.
 ** Significantly different from the vehicle control group value (p<0.01).

F₂ findings: There were no MOA-728-related effects on external morphology of the F₂. The following table (from page C-31 of the study report) shows the F₂ fetal gross external observations.

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TABLE C25 (PAGE 1): FETAL GROSS EXTERNAL ALTERATIONS - SUMMARY - P2 GENERATION LITTERS/PETUSES

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	5	25	100
LITTERS EVALUATED	N	20	24	23	25
FETUSES EVALUATED	N	298	396	347	379
LIVE	N	298	396	347	379
SKIN: BLACK AREA					
LITTER INCIDENCE	N(%)	1(5.0)	1(4.2)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.3)	1(0.2)	0(0.0)	0(0.0)
HINDLIMBS: ROTATED MEDIALY					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.3)
ANUS: NO OPENING PRESENT					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.2)a	0(0.0)	0(0.0)
TAIL: ABSENT					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.2)a	0(0.0)	0(0.0)

a. Fetus 9735-8 had other gross external alterations.

Summary: In a Segment III pre- and postnatal development study in rats, animals were treated subcutaneously from gestation day 6 to postpartum day 20 at dosages of 0, 5, 25, or 150/100 mg/kg/day. Maternal toxicity in the form of mortality (150/100 mg/kg/day), clinical observations, and decreased body weight gain and food consumption occurred at dosages > 25 mg/kg/day. Therefore, the No Observed Adverse Effect Level (NOAEL) for maternal toxicity was considered 25 mg/kg/day. Based on decreased body weight in pups at 150/100 mg/kg/day that were reduced throughout the preweaning period, the NOAEL for perinatal/postnatal toxicity was considered 25 mg/kg/day. Growth and development (physical, sensory, behavioral and reproductive) of the F1 generation were unaffected.

2.6.6.7 Local tolerance

EXPLORATORY SUBCUTANEOUS TOXICITY STUDY WITH VARIOUS FORMULATIONS OF METHYLNALTREXONE IN RABBITS

(Study No. 0406LP45.002, 2006)

Methods: The purpose of this study was to determine the potential dermal toxicity of six different formulations of MNTX when administered to 3 male rabbits per group as a single subcutaneous dose. Each animal served as its own control, receiving the vehicle and 10, 40, and 70 mg/mL of MNTX at a dose volume of 0.5 mL/kg administered subcutaneously on the clipped dorsal area of each rabbit. The six formulations (all expressed as mg/g of water) evaluated were:

A) _____

- B) _____
- C) _____
- D) _____
- E) _____
- F) MNTX (_____) NaCl _____

All formulations were _____ sterilized using a _____ and administered on day 1. Evaluations were based on mortality, clinical signs, and body weight effects. In addition, the Draize method was used to score for signs of dermal irritation. The injection sites were scored at 1, 4, 24, 48, and 72 hours and on day 5 of the study.

Results: There was no mortality in this study. No compound-related changes in body weight, clinical signs or signs of dermal irritation were observed in any animals receiving dose levels of 10, 40, or 70 mg/mL at a dose volume of 0.5 mL/kg. Only very slight dermal irritation (based on the Draize scores) was observed at various concentrations of formulations of A, B, C, and D. The irritation was minimal and of low incidence. The findings were not considered compound-related because the irritation observed was similar to the reactions seen in the respective control sites (0 mg/ml MNTX). Formulations E and F were non-irritating at any of the concentrations tested. Overall, there was no evidence of skin irritation in any of the formulations at any of the concentrations evaluated.

2.6.6.8 Special toxicology studies

None

2.6.6.9 Discussion and Conclusions

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

Generally, bridging studies comparing the pharmacokinetics (PK) and toxicity of MNTX following single intravenous (IV) and subcutaneous (SC) dose in dogs and single IV, SC and oral (PO) dose in rats demonstrated comparable PK and toxicity profile between SC

2.6.6.10 Tables and Figures

Incorporated in the appropriate sections of this review

2.6.7 TOXICOLOGY TABULATED SUMMARY

Pivotal toxicology studies were tabulated under section: “**Studies reviewed within this submission**”.

LABELING

The draft labeling of MNTX generally conforms to the format specified under 21CFR 201.57(c)(14) Requirements for PLR Prescription Drug Labeling. However, the following changes should be incorporated.

8.1 Pregnancy

Sponsor’s Version:



Evaluation: The text is not in accordance with 21CFR 201.57(c)(14). However, the labeling should be modified as proposed below.

Proposed Version:

8.1 Pregnancy

Pregnancy Category B.



8.3. Nursing Mothers

Sponsor's Version:

8.3 Nursing Mothers

Evaluation: The text is not in accordance with 21CFR 201.57(c)(14) and acceptable.

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Sponsor's Version:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Evaluation: The text is not in accordance with 21CFR 201.57(c)(14). However, the labeling should be modified as proposed below.

Proposed Version:

Carcinogenesis, Mutagenesis, Impairment of Fertility



13.2 Animal toxicology and/or pharmacology



OVERALL CONCLUSIONS AND RECOMMENDATIONS

Methylnaltrexone is a quaternary derivative of the pure opioid antagonist, naltrexone developed for the prevention and treatment of opioid-induced side effects. Other opioid antagonists such as naltrexone, naloxone and nalmefene are fairly lipid soluble and readily cross the blood-brain barrier. The addition of the methyl group in the amine ring of methylnaltrexone forms a compound with greater polarity and lower lipid solubility that does not cross the blood-brain barrier in humans. These properties provide methylnaltrexone with the potential to block undesirable peripheral side effects (constipation, nausea and vomiting, biliary colic, urinary retention and pruritis) of opioid pain medications through peripheral opioid receptors, while sparing centrally mediated analgesic effects.

In this NDA, the sponsor has provided the following study reports: pharmacology; absorption, distribution, metabolism, and excretion; safety pharmacology; acute toxicity studies in mice, rats and dogs; 90-day oral toxicity study in mice, 7-, 28- and 6-month oral toxicity studies in rats, 14-day and 90-day IV studies in rats, 7-, 28- and 9-month oral studies in dogs, 14- and 90-day IV studies in dogs; Ames test, chromosome aberration test in HPBL, mouse lymphoma assay and in vivo micronucleus test in mice; fertility and reproductive performance (Segment I) in rats, teratology (Segment II) in rats and rabbits and peri- and post-natal development (Segment III) in rats.

Pharmacology studies have been conducted with MNTX using *in vitro* and *in vivo* experiments. In the NDA, the sponsor only submitted *in vitro* receptor binding study reports in support of primary pharmacology. The sponsor submitted several pharmacology study reports under IND 64,583, which were reviewed previously under IND 64,583 Initial submission. This brief summary includes the main findings of those studies including the study submitted under this NDA. Methylnaltrexone was found have lower affinity (IC-50 = 305 nM) for rat brain opiate receptors compared to naltrexone (IC-50 = 7.2 nM). The drug was found to be more selective for μ -type opioid receptors (IC-50 = 300 nM) in the GI tract compared to κ - (19-fold less potent) and δ - (ineffective) type receptors in isolated gastric-brainstem preparation in neonatal rats. The K_i value for MNTX for human μ and κ receptor were 28 and 230 nM, respectively, whereas the K_i value for the δ receptor was $> 10 \mu\text{M}$. In several *in vivo* studies, MNTX was shown to inhibit morphine-induced effects (spike potential of duodenal smooth muscle, gastric transit time, emesis, cough, etc.) without sparing central analgesic effect. Methylnaltrexone did not produce behavioral signs of opioid withdrawal (tremor, yawning, and restlessness) in acutely opioid-dependent dogs. However, in rodents, MNTX was demethylated, possibly forming naltrexone, which may precipitate withdrawal symptoms due to its CNS access. However, MNTX did not precipitate withdrawal or reversal of opioid analgesia in various animal models as mentioned above. However, MNTX showed cardiovascular effects in dogs. Overall, the drug appeared to be more selective for peripheral μ -type opioid receptors and results of pharmacology studies appeared to support the intended marketing indication.

In safety pharmacology studies, MNTX at IV doses ranging from 1 to 20 mg/kg had no apparent toxicologically significant effects on the neuropharmacological profile in mice, gastrointestinal function in rats, pulmonary function in guinea pigs, or renal function in rats. Cardiovascular safety pharmacology studies were conducted using adequate battery of *in vitro* and *in vivo* tests. Methylnaltrexone showed significant cardiovascular effects in these studies. In hERG assay, MNTX caused concentration-dependent IKr inhibition (1%, 12%, 13% and 40% inhibition of hERG current at 30, 100, 300 and 1000 μM concentrations, respectively, compared to vehicle control; IC-50 $> 1000 \mu\text{M}$). In isolated canine (dog) Purkinje fibers, MNTX caused prolongations in action potential duration at 60% repolarization (APD₆₀: 13%, 21% and 15% at 1, 3 and 10 μM , respectively) and 90% repolarization (APD₉₀: 10%, 16% and 12% at 1, 3 and 10 μM , respectively) at basic cycle length (BCL) of 2 sec compared to baseline values. The highest tested concentration in the canine Purkinje fiber study (10 μM) was about 18 and 37 times the C_{max} at human SC doses of 0.3 (C_{max} = 234 ng/mL) and 0.15 mg/kg (C_{max} = 117 ng/mL), respectively. In isolated rabbit Purkinje fibers, MNTX caused concentration-dependent increase in APD₆₀ (2%, 4% and 10% at 1, 10 and 100 μM at BCL of 2 sec) and APD₉₀ (1%, 3% and 6% at 1, 10 and 100 μM at BCL of 2 sec) when compared to baseline values at all three stimulus intervals (BCL of 2, 1 and 0.5 sec). The highest MNTX concentration (100 μM) tested in the rabbit Purkinje fiber study was about 186 and 373 times the C_{max} at human SC doses of 0.3 and 0.15 mg/kg, respectively. In a cardiovascular safety study (0247DP45.001) in anesthetized dogs at 1, 5, and 25 mg/kg, IV, MNTX caused decreases in blood pressure (up to 13%), heart rate (8-17%), cardiac

output (4-18%), left ventricular pressure (<5 to 10%), left ventricular end diastolic pressure (up to 6%), and +dP/dt (12-19%) at ≥ 1 mg/kg. The magnitude and duration of the effects were generally dose-related. In a second cardiovascular IV safety pharmacology study (940-001) in conscious Beagle dogs at 1, 5 and 20 mg/kg, MNTX caused a dose-related increase in QTc interval when compared to vehicle control (males: 2%, 5% and 10% increase at 1, 5 and 20 mg/kg, respectively, at 45 minutes after treatment; females: 3%, 5% and 12% increase at 1, 5 and 20 mg/kg, respectively, at 1 hour after treatment). Predicted exposures (C_{5min} and $AUC_{0-\infty}$ values of 56,483 ng/mL and 25,222 ng.h/mL, respectively) after a single IV dosage of 20 mg/kg to beagle dogs were approximately 482 and 144 times, respectively, the exposure ($C_{max} = 117$ ng/mL and $AUC = 175$ ng.h/mL) at a human SC dose of 0.15 mg/kg and 241 times and 66 times, respectively, the exposure ($C_{max} = 234$ ng/mL and $AUC = 382$ ng.h/mL) at a human SC dose of 0.3 mg/kg. In conscious guinea pigs (tested at 1, 5 and 20 mg/kg), mild prolongation of QTc (4% over baseline) was observed at 20 mg/kg IV. Based on the prolongation of QT and increase in the action potential durations in cardiovascular safety pharmacology studies, MNTX appears to have significant potential for QT prolongations in humans. A thorough QT study in humans will help to address this issue.

Generally, MNTX exposure (AUC and C_{max}) was found to be greater than dose proportional after SC, IV, and PO dosing in rats and dogs. No significant gender differences in rats and dogs were observed in pharmacokinetic parameters. Methylnaltrexone was distributed mostly to the small intestine, liver and kidney, in the rat, with the brain and skeletal muscle having the lowest concentrations at one hour after dosing. Penetration to the brain was limited with lower concentrations in the brain than in all other tissues. Minimal amounts of MNTX were found in brain of rat and rabbit after administration of high IV or epidural doses. Plasma protein binding was found to be minimal. *In vivo*, MNTX was not extensively metabolized in mice, rats and dogs. The metabolic pathways included hydroxylation, reduction, methylation, sulfation and glucuronidation. Plasma metabolite profiles suggested minimal metabolism of MNTX in human subjects following IV administration. The most abundant metabolites in human plasma were MNTX sulfate, and methyl-6 α -naltrexol and methyl-6 β -naltrexol isomers. Methylnaltrexone sulfate and methyl-6 β -naltrexol were also observed in the rat plasma. The major circulating metabolite in dogs was MNTX glucuronide. In human liver microsomes, MNTX inhibited the activity of CYP2D6 with a K_i value of 8 μ M. Drug-drug interactions involving MNTX and CYP2D6 substrates could be possible following IV administration. MNTX did not induce any of the cytochrome P450 isoforms when tested *in vitro*. Methylnaltrexone was secreted into the bile in rats and dogs. In bile duct cannulated rats, about 16% of the administered dose was excreted in the bile following IV administration. In rats and dogs, the major route of excretion was via the urine after IV dosing and via the feces after oral dosing in mice and dogs. In pregnant rats, MNTX-derived radioactivity rapidly crossed the placenta. The fetal exposure was approximately 10% of the maternal exposure. In lactating rats, MNTX-derived radioactivity was excreted into breast milk. Methylnaltrexone did not appear to be a p-glycoprotein substrate in Caco-2 cell monolayers. However, MNTX appeared to be a substrate of the human organic cation transporter (OCT1).

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

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

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

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

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

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

In a three-month IV toxicity study (154-003) in rats, rats were administered MNTX at dosages of 1, 5, and 20 mg/kg/day. In this study, MNTX-related mortality and adverse clinical signs (including whole body tremors, prostration, myoclonus, mixed convulsions, and labored breathing) occurred at 5 and 20 mg/kg/day. Most of the dead animals had congestion in the liver and lungs and hemorrhage in the thymus. The NOAEL was considered as 1 mg/kg/day. The CNS was considered as the target organ of toxicity based on clinical signs of tremors and convulsions.

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

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

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

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

In a three-month intravenous toxicity study (154-004) in dogs, animals were administered MNTX at dosages of 0, 1, 5, and 25/20 mg/kg/day. Based on dose-limiting prostration observed on day 1 at 25 mg/kg/day, the high dosage was reduced to 20 mg/kg/day. No

MNTX-related mortality occurred in this study. At 25/20 mg/kg/day, clinical signs included prostration, clonic tremors, and decreased activity. In this study, there was a dose-related prolongation of QTc in both sexes (male at Day 6: 9 and 15% increase at 5 and 20 mg/kg/day, respectively; females at Day 6: 3%, 5% and 12% increase at 1, 5 and 20 mg/kg/day, respectively) at Day 6 and Day 83 (similar increases at Day 6 in both sexes) post-treatment compared to vehicle control. Prolongations (up to 37 msec, 15% increase compared to vehicle control) of QTc intervals were seen at 5 and 20 mg/kg/day. The CNS was a target organ of toxicity at 20 mg/kg/day, as indicated by the incidence of tremors. Heart was considered to be a target organ of toxicity, based on the significant prolongation of QT_c at 5 and 20 mg/kg/day. Spleen was a target organ of toxicity at 5 and 20 mg/kg/day, based on the incidence of fibrosis. The NOAEL was 1 mg/kg/day. In this study, C_{max} values (average males and females) at Day 90 at 1 (C_{max} = 8034 ng/mL), 5 (C_{max} = 35,413 ng/mL) and 20 (C_{max} = 74,125) mg/kg/day were 69, 303 and 633 times the human C_{max} (117 ng/mL) at 0.15 mg/kg, respectively. The C_{max} values (average males and females) at Day 90 at 1 (C_{max} = 8034 ng/mL), 5 (C_{max} = 35,413 ng/mL) and 20 (C_{max} = 74,125 ng/mL) mg/kg/day were 34, 151 and 316 times the human C_{max} (234 ng/mL) at 0.30 mg/kg, respectively. The AUC_{0-t} values (average males and females) at Day 90 at 1 (AUC_{0-t} = 924 ng.h/mL), 5 (AUC_{0-t} = 6690 ng.h/mL) and 20 (AUC_{0-t} = 34,463 ng.h/mL) mg/kg/day were 5, 38 and 197 times the human AUC (175 ng.h/mL) at 0.15 mg/kg, respectively. The AUC_{0-t} values (average males and females) at Day 90 at 1 (AUC_{0-t} = 924 ng.h/mL), 5 (AUC_{0-t} = 6690 ng.h/mL) and 20 (AUC_{0-t} = 34,463 ng.h/mL) mg/kg/day were 2, 17 and 90 times the human AUC (382 ng.h/mL) at 0.30 mg/kg, respectively.

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

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

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

23%) compared with pre-test values. The tolerated dose was considered as 60 mg/kg/day. The average AUC_{0-t} at 750 mg/kg/day on Day 8 in females (16,7397 ng.h/mL) was approximately 876 and 438 times the human AUC at 0.15 and 0.3 mg/kg, respectively. The average C_{max} (29100 ng/mL) in females at 750 mg/kg/day on Day 8 was about 249 and 124 times the human C_{max} at 0.15 (C_{max} = 117 ng/mL) and 0.3 mg/kg (C_{max} = 234 ng/mL), respectively.

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

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

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

All potential impurities of MNTX have been evaluated per ICH guidelines.

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which is in compliance with the Agency standard for an impurity containing a structural alert for genotoxicity (as per Type B pre-NDA CMC Meeting on November 1, 2004 and September 22, 2006).

Overall, systemic toxicity of MNTX was adequately evaluated in a complete range of general toxicity (acute, subacute and chronic), genotoxicity, and reproductive toxicity studies. Methylnaltrexone was not genotoxic. In fertility and reproductive performance study in rats, MNTX did not cause any adverse effect. It was also not teratogenic in rats or rabbits and did not cause any adverse effect on pre- and post-natal development in rats. However, target organs appear to be the cardiovascular system (QT prolongations in dogs) and the CNS (tremor, convulsions and decreased activity in rats and dogs). Methylnaltrexone caused significant prolongations of action potential durations (APD₆₀ and APD₉₀) in isolated canine and rabbit Purkinje fibers. In addition, MNTX caused prolongations of QTc intervals in *in vivo* cardiovascular safety pharmacology studies as well as in oral and intravenous toxicity studies in dogs. These QT prolongations were seen at approximately 300 and 150 times human C_{max} at 0.15 and 0.3 mg/kg, respectively, and 38 and 18 times human AUC at 0.15 and 0.3 mg/kg, respectively. Maximum QT prolongations of 12-15% were seen at 20 mg/kg, which is about 630 and 315 times human C_{max} at 0.15 and 0.3 mg/kg, respectively and 197 and 90 times human AUC at 0.15 and 0.3 mg/kg/day, respectively. Exposure at the NOAEL of 1 mg/kg/day in rats (AUC = 286 ng·h/mL) and 1 mg/kg in dogs (AUC = 922 ng·h/mL) were approximately 2 and 5 times, respectively, the human exposure at recommended dose of 0.15 mg/kg, and approximately 0.75 and 2 times, respectively, the human exposure at maximum recommended dose of 0.30 mg/kg. Based on QT prolongations in dogs, MNTX appears to have significant potential to cause QT prolongations in humans. A thorough QT study in humans will help to address this issue. Non-clinical studies conducted with MNTX appear to adequately support its use at the intended therapeutic dosage and in accordance with the proposed product labeling.

The labeling of MNTX conforms to the format specified under 21CFR, Subpart B. However, the suggested changes described in the text, should be incorporated.

Conclusions: From a nonclinical standpoint, this submission satisfies the criteria for marketing authorization of MNTX and is recommended for approval for the proposed use. However, based on QT prolongations in dogs, MNTX appears to have significant

potential to cause QT prolongations in humans. A thorough QT study in humans will help to address this issue.

Unresolved Toxicology Issues: None

Recommendations:

1. From a nonclinical standpoint, this NDA may be approved.
2. Based on QT prolongations in dogs, methylnaltrexone appears to have significant potential to cause QT prolongations in humans. A thorough QT study in humans will help to address this issue.

Suggested Labeling: The sponsor should be asked to modify the proposed label of methylnaltrexone as suggested in the text of this review.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

cc:

- Original NDA
- HFD-180
- HFD-180/RPM/MScherer
- HFD-180/Dr. Chakder
- HFD-180/Dr. Chakraborti

APPENDIX/ATTACHMENTS

None

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

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