

Study no.: ENDO # SP0002-220-03
Volume #, and page #: Electronic Submission (7/22/2003)
Conducting laboratory and location: _____
Date of study initiation: March 27, 2003
GLP compliance: Yes
QA reports: yes (X) no ()
Drug, lot #, and % purity: _____ Lot D14182, _____

Methods: The ability for _____ to induce chromosome aberrations in cultured Chinese Hamster ovary (CHO) cells with and without exogenous metabolic activation was determined via a standard assay. Metabolic activation mixture consisted of phenobarbital/ β -naphthoflavone-induced rat liver homogenate (S9) and the cofactor pool. This was purchased from _____. The S9 was tested for its potential to induce acceptable level of aberrations in CHO cells with cyclophosphamide (CP). Following treatment, a total of 1000 cells were scored from each concentration tested (500 from each duplicate flask) and the number of dividing cells was recorded. The mitotic index (MI = number of dividing cells from 1000 cells/10) and relative mitotic index (RMI = MI of test concentration/MI of solvent concentration x 100) was determined.

Strains/species/cell line: The clone _____ of the CHO cell line was used in the study. The cells originated at _____. Doubling time of the cell line is approximately 12 hours and its modal chromosome number is 21.

Doses used in definitive study: 0.1, 0.5, 1, 5 and 7.5 $\mu\text{g}/\text{mL}$ for non-activated system and 0.1, 0.5, 1, 5 and 10 $\mu\text{g}/\text{mL}$ for activated system. Based on the relative mitotic index results, the chromosome aberrations 1.0, 5.0 and 7.5 $\mu\text{g}/\text{mL}$ for the non-activated system and 1.0, 5.0 and 10 $\mu\text{g}/\text{mL}$ in the activated system were scored. One hundred metaphases were scored for chromosomal aberrations from each of two duplicate flasks (total of 200 metaphase cells per concentration). Only cells with 19-23 chromosomes were scored, and the microscopic coordinates of each cell with aberrations were recorded. The number of polyploid and endoreduplicated cells in a total of 100 dividing cells were scored and recorded for each cell culture.

A confirmatory assay was performed in both the non-activated and activated systems. The procedure was the same as the definitive study, except that the test article treatment period was extended to 20 hours for the non-activated system and a repeat of the 4-hours for the activated system. Concentrations tested were 0.05, 0.1, 0.5, 1, 5 and 7.5 $\mu\text{g}/\text{mL}$ and 0.05, 0.1, 0.5, 1, 5 and 10 $\mu\text{g}/\text{mL}$ for the non-activated and activated systems, respectively. A total of 100 metaphase cells were scored from duplicate slides except for the highest concentration of 10 $\mu\text{g}/\text{mL}$ in the activated system where only 64 metaphases were scorable for slide A.

Basis of dose selection: Dose selection was based upon solubility testing and a range finding study. For the range finding study, cells were treated with concentrations of 5,

Negative controls: The solvent control was acidic water as one or two drops of 1N HCl was added to the test article and diluted with water for solubility purposes. The untreated controls were treated with water alone.

Positive controls: Mitomycin-C (MMC) and Cyclophosphamide (CP) were dissolved in sterile water and diluted to 40 and 80 µg/mL (MMC) and 1.25 and 1.5 mg/mL (CP) stock solutions. The concentration of MMC used was 0.4 and 0.8 µg/mL in the non-activated system for the definitive assay and 0.2 and 0.4 µg/mL for the confirmatory assay. The concentration of CP used in the presence of metabolic activation was 7.5 and 12.5 µg/mL.

Incubation and sampling times: In the non-activated and activated systems, the cultures were treated for 4 hours, the medium removed and the cells were treated with PBS containing Ca²⁺ and Mg²⁺ for an additional 16 hours prior to harvest. In the confirmatory assay, the cells were treated with _____ for a total of 20 hours without metabolic activation. For the confirmatory assay with metabolic activation, the study was repeated as in the definitive assay.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The following criteria for a valid assay were employed: 1) the percentage of cells with aberrations in the solvent control should not have exceeded 4%, 2) the positive control should produce at least 25% of the cells with one or more chromosome aberrations, and 3) At least one of the test concentrations scored should show greater than 50% reduction in the relative cell growth and/or relative mitotic index (unless a maximum soluble concentration or highest allowable dose is reached without apparent toxicity). The study appears to fulfill the above criteria for a valid study. In addition, the study appears to be valid as the appropriate positive controls were used, an acceptable number of mitotic cells were evaluated and the protocols appear to be consistent with those described in the OECD Guidelines.

Study outcome: The mitotic indexes for the concentrations used in the definitive chromosome aberration assay are presented in the sponsor's table 4 below. The highest concentrated tested without metabolic activation (7.5 µg/mL) produced a relative mitotic index of 47%. The highest concentration tested with metabolic activation (10 µg/mL) produced a relative mitotic index of 32%.

Appears This Way
On Original

TABLE 3
CHROMOSOME ABERRATION ASSAY IN CHO CELLS
CHROMOSOME ABERRATIONS - DEFINITIVE ASSAY

TEST ARTICLE: ██████████
 SPONSOR: Mallinckrodt, Inc.
 SOLVENT: Aqueous water

TREATMENT TIME: 4 Hours
 HARVEST TIME: 20 Hours

STUDY NO.: 0795-3110
 TRIAL NO.: B1
 METABOLIC ACTIVATION: Yes () No (X)

TREATMENT AND CONC. (µg/mL)	CELLS Scored	NUMBER AND TYPE OF ABERRATIONS																		NO. OF ABS. PER CELL	% CELLS WITH ABS.	P-VALUE IN CHI-SQUARE**
		NOT COMPUTED				Chromatid Type						Chromosome Type						Others				
		tr	sr	%e	%pp	Simple			Complex			Simple			Complex			di	sd*			
						tb	bb	tr	or	cr	id	ci	sb	d	r	dm						
Untreated A	100			0	0															0.00	0.0	
Untreated B	100		1	0	1	1														0.01	1.0	
Untreated A+B	200		1	0.0	0.5	1														0.005	0.5	
Solvent A	100			0	1		1						1							0.02	2.0	
Solvent B	100			0	0								1							0.01	1.0	
Solvent A+B	200			0.0	0.5		1						2							0.015	1.5	
1.0 A	100	1		0	0								1							0.01	1.0	
1.0 B	100			0	1								1							0.01	1.0	
1.0 A+B	200	1		0.0	0.5								2							0.010	1.0	<solvent
5.0 A	100			0	0	3		1	1											0.05	5.0	
5.0 B	100	1		0	1	4		2												0.06	6.0	
5.0 A+B	200	1		0.0	0.5	7		3	1											0.055	5.5	p=0.0568
7.5 A	100			0	0	6	1	5	2	3	1	1	2							0.21	17.0	
7.5 B	100			0	0	9		1	1		2		4							0.17	13.0	
7.5 A+B	200			0.0	0.0	15	1	6	3	3	3	1	6							0.190	15.0	p<0.0001
MMC 0.4 A	100			0	0	16	6	4	6	11	1	3	5			1	1			0.63	33.0	
MMC 0.4 B	100	2		0	0	11	6	3	10	6	4	7	6				1			0.64	35.0	
MMC 0.4 A+B	200	2		0.0	0.0	27	12	7	16	17	5	10	11			1	1	1		0.685	34.0	p<0.0001

MMC = Mitomycin-C

Trend test: P<0.0001

* sd = 10 aberrations in calculations.

**Statistical analysis was performed on the % cells. The results are considered significant if p-value is ≤ 0.05. In the Chi-square test, all test article concentrations were compared to solvent. In the Chi-square test, MMC was compared to Untreated control data since the solvent for MMC was water.

In the presence of metabolic activation, the two highest concentrations (5 and 10 µg/mL) produced a significant increase in the % of cells with aberrations compared to controls. The relative mitotic indexes at these concentrations were 32 and 111%, respectively, suggesting that the aberrations can not be attributed to excessive cytotoxicity.

Best Possible Copy

**Appears This Way
 On Original**

TABLE 6
CHROMOSOME ABERRATION ASSAY IN CHO CELLS
CHROMOSOME ABERRATIONS - DEFINITIVE ASSAY

TEST ARTICLE: ██████████
 SPONSOR: Mallinckrodt, Inc.
 SOLVENT: Aqueous water

TREATMENT TIME: 4 Hours
 HARVEST TIME: 20 Hours

STUDY NO.: 0795-3110
 TRIAL NO.: B1
 METABOLIC ACTIVATION: Yes (X) No ()

TREATMENT AND CONC. (µg/mL)	CELLS Scored	NUMBER AND TYPE OF ABERRATIONS																NO. OF ABS. PER CELL	% CELLS WITH ABS.	P-VALUE IN CHI-SQUARE**
		NOT COMPUTED				Chromatid Type						Chromosome Type								
		tg	ey	% e	% pp	fb	lb	rr	qr	cr	ld	cl	sb	d	r	dn	pu			
Untreated A	100			4	0													0.00	0.0	
Untreated B	100			3	0				1									0.01	1.0	
Untreated A+B	200			3.5	0.0				1									0.005	0.5	
Solvent A	100			0	0	2			1			1	1					0.05	5.0	
Solvent B	100			1	0		1		1			1						0.03	3.0	
Solvent A+B	200			0.5	0.0	2	1		2			2	1					0.040	4.0	
1.0 A	100			2	0				2		1	1						0.04	4.0	
1.0 B	100			2	0	1			1	1		1						0.04	4.0	
1.0 A+B	200			2.0	0.0	1			3	1	1	2						0.040	4.0	p=solvent
5.0 A	100			0	0	6	1	2	1		1	2	1					0.14	12.0	
5.0 B	100			0	0	2		2	1		2	1	1					0.09	8.0	
5.0 A+B	200			0.0	0.0	8	1	4	2		3	3	2					0.115	10.0	p=0.0311
10 A	100			0	0	3	1		8	2			2					0.14	9.0	
10 B	100	1		0	0	3	2	1	7	1	1	2	3			1		0.21	17.0	
10 A+B	200	1		0.0	0.0	6	3	1	13	3	1	2	5		1			0.175	13.0	p=0.0023
CP 7.5 A	100			0	0	9	10	8	12	8	8	2	10			10	4	1.18	45.0	
CP 7.5 B	100			0	0	7	17	10	11	4	8	4	11			5	7	1.47	45.0	
CP 7.5 A+B	200			0.0	0.0	16	27	18	23	12	16	6	21			15	11	1.325	45.0	p<0.0001

CP = Cyclophosphamide

Trend test: P<0.0001

* ad = 10 aberrations in calculations.

**Statistical analysis was performed on the % cells with aberrations. The results are considered significant if p-value is ≤ 0.05.

In the Chi-square test, all test article concentrations were compared to solvent.

In the Chi-square test, CP was compared to Untreated control data since the solvent for CP was water.

Best Possible Copy

Appears This Way
 On Original

In the confirmatory assay where cells were treated for the full 20 hours, both concentrations of 5 and 7.5 µg/mL produced a relative mitotic index of 0% in the absence of metabolic activation. A concentration of 1 µg/mL produced a relative mitotic index of 60% in the absence of metabolic activation. In the presence of metabolic activation, concentrations of 5 and 10 µg/mL produced a relative mitotic index of 137 and 17%, respectively. The mitotic index values are presented in the sponsor's table below.

TABLE 8
CHROMOSOME ABERRATION ASSAY IN CHO CELLS
MITOTIC INDEX - CONFIRMATORY ASSAY

SPONSOR: Mallinckrodt, Inc.
TEST ARTICLE: ██████████

SOLVENT: Aqueous water

STUDY NO.: 0795-3110
TRIAL NO.: B2

Without Activation - Treatment: 20 Hours Harvest: 20 Hours					With Activation - Treatment: 4 Hours Harvest: 20 hours				
Test Article Concentration (µg/mL)	Tube No.	No. of Dividing Cells/500	Mean Mitotic Index (MI)	Relative Mitotic Index (RMI)	Test Article Concentration (µg/mL)	Tube No.	No. of Dividing Cells/500	Mean Mitotic Index (MI)	Relative Mitotic Index (RMI)
Untreated	A	51	7.3	78%	Untreated	A	25	4.8	117%
	B	22				B	23		
Solvent	A	43	9.3	100%	Solvent	A	21	4.1	100%
	B	50				B	20		
0.05	A	43	7.4	80%	0.05	A	26	4.7	115%
	B	31				B	21		
0.1	A	21	5.0	54%	0.1	A	35	5.9	144%
	B	29				B	24		
0.5	A	21	4.9	53%	0.5	A	33	4.9	120%
	B	28				B	16		
1.0	A	28	5.6	60%	1.0	A	19	3.8	93%
	B	28				B	19		
5.0	A	0	0	0	5.0	A	28	5.6	137%
	B	0				B	28		
7.5	A	0	0	0	10	A	2	0.7	17%
	B	0				B	5		
MMC 0.2	A	31	5.1	70%	CP 7.5	A	4	0.9	19%
	B	20				B	5		
MMC 0.4	A	14	2.6	36%	CP 12.5	A	1	0.1	2%
	B	12				B	0		

All test article concentrations and untreated were compared to solvent.

The positive controls were compared to Untreated since the solvent for MMC and CP was water.

MI = No. of dividing cells scored from 1000 cells

RMI = $\frac{\text{Test Dose MI}}{\text{Solvent Control MI}} \times 100$

10

Based upon the cytotoxicity results above, concentrations of 1, 0.5 and 0.05 µg/mL were scored in the absence of metabolic activation. The highest concentration scored (1 µg/mL) produced a statistically significant increase in the % of cells with chromosomal aberrations. As this concentration produced a 60% relative mitotic index, these results do not appear to be due to cytotoxicity. The number and type of chromosomal aberrations is reproduced in the sponsor's table 9 below:

TABLE 9
CHROMOSOME ABERRATION ASSAY IN CHO CELLS
CHROMOSOME ABERRATIONS - CONFIRMATORY ASSAY

TEST ARTICLE: _____
 SPONSOR: Mallinckrodt, Inc.
 SOLVENT: Acidic water

TREATMENT TIME: 20 Hours
 HARVEST TIME: 20 Hours

STUDY NO: 0795-3110
 TRIAL NO: B2
 METABOLIC ACTIVATION: Yes () No (X)

TREATMENT AND CONC. (µg/mL)	CELLS Scored	NUMBER AND TYPE OF ABERRATIONS														NO. OF ABER. PER CELL	% CELLS WITH ABER.	P-VALUE IN CHI-SQUARE**		
		NOT COMPUTED				Chromosid Type						Chromosome Type							Others	
		tg	sg	%e	%pp	tb	lcb	tr	qr	cr	id	cl	sb	d	r					dm
Untreated A	100			0	0													0.00	0.0	
Untreated B	100			0	0													0.00	0.0	
Untreated A+B	200			0.0	0.0													0.000	0.0	
Solvent A	100			0	1													0.00	0.0	
Solvent B	100			0	0													0.00	0.0	
Solvent A+B	200			0.0	0.5													0.000	0.0	
0.05 A	100			0	0													0.00	0.0	
0.05 B	100			0	0													0.00	0.0	
0.05 A+B	200			0.0	0.0													0.000	0.0	=solvent
0.5 A	100			0	0				1									0.01	1.0	
0.5 B	100			0	1	1		3		1								0.05	3.0	
0.5 A+B	200			0.0	0.5	1	1	3		1								0.030	2.0	p=0.1918
1.0 A	100			0	0	4			1		1		1					0.07	7.0	
1.0 B	100			0	0								3					0.03	3.0	
1.0 A+B	200			0.0	0.0	4			1		1		4					0.050	5.0	p=0.0061
MMC 0.2 A	100	1		0	0	10	7	7	19	4	2	6	3			1		0.59	41.0	
MMC 0.2 B	100			0	0	14	8	5	22	6	2	4	6			2	1	0.50	39.0	
MMC 0.2 A+B	200	1		0.0	0.0	24	16	12	41	10	4	10	9			3	1	0.695	40.0	p<0.0001

MMC = Mitomycin-C

Trend test: P<0.0001

*ad = 10 aberrations in calculations.

**Statistical analysis was performed on the % cells with aberrations. The results are considered significant if p-value is ≤ 0.05.

In the Chi-square test, all test article concentrations were compared to _____ historical Untreated(water) control data(0.22%) since the solvent for test article was IN HCl and water, and concurrent value is 0%.

In the Chi-square test, MMC was compared to _____ historical Untreated control data(0.22%) since the solvent for MMC was water and concurrent value is 0%.

As listed in sponsor's table 10, in the presence of metabolic activation, _____ at the two highest concentrations tested (5 and 10 µg/mL) significantly increased the % of cells with chromosomal aberrations. As indicated earlier, these concentrations produced 137% and 17% relative mitotic index, suggesting that the clastogenic effect can not be attributed entirely to cytotoxicity.

Appears This Way
 On Original

Best Possible Copy

TABLE 10
CHROMOSOME ABERRATION ASSAY IN CHO CELLS
CHROMOSOME ABERRATIONS - CONFIRMATORY ASSAY

TEST ARTICLE: _____
 SPONSOR: MAI Research, Inc.
 SOLVENT: AcdE: water

TREATMENT TIME: 4 Hours
 HARVEST TIME: 20 Hours

STUDY NO.: 079-3114
 TRIAL NO.: B2
 METABOLIC ACTIVATION: Yes (X) No ()

TREATMENT AND CONC. (µg/ml)	CELLS Scored	NUMBER AND TYPE OF ABERRATIONS																				NO. OF ABS. PER CELL	% CELLS WITH ABS.	P-VALUE IN CHI-SQUARE**						
		NOT CATEGORIZED				Chromosomal Type								Chromosome Type																
		%	sg	%e	%pp	Single				Complex				Single				Complex							Other					
						th	sb	tr	cp	cr	td	ed	ab	d	r	ds	po	ac*												
Untreated A	100	1		5	0	1																	0.01	1.0						
Untreated B	100			5	1																			0.00	0.0					
Untreated A+B	200	1		5.0	0.5	1																		0.005	0.5					
Solvent A	100			4	0	1			1															0.02	2.0					
Solvent B	100			3	0				1																0.01	1.0				
Solvent A+B	200			3.5	0.0	1		1	1																0.018	1.5				
1.0 A	100			4	0	1				3	2														0.06	5.0				
1.0 B	100			6	0																				0.00	0.0				
1.0 A+B	200			5.0	0.0	1				3	2														0.090	2.5	p=0.7210			
5.0 A	100			0	0	4	1	1	4	1			1												0.12	0.0				
5.0 B	100			0	0	1			8	1	1		1												0.12	11.0				
5.0 A+B	200			0.0	0.0	5	1	1	12	2	1		2												0.120	10.0	p=0.0006			
10 A	64			0	1	2		1	2		1														0.09	9.4				
10 B	100	1		0	0	3	4	3	2	2	2		2												0.18	18.0				
10 A+B	164	1		0.0	0.5	5	4	4	4	2	3		2												0.135	12.7	p<0.0001			
CP 7.5 A	100			0	0	9	5	9	9	7	14		2	6											15	4	1.18	47.0		
CP 7.5 B	100			0	0	5	10	5	12	8	13		6	2												13	7	0.78	42.6	
CP 7.5 A+B	200			0.0	0.0	14	15	14	21	15	27		8													28	4	0.980	44.5	p<0.0001

CP = Cyclophosphamide

Trend test: P<0.0001

*ad = 10 aberrations in calculations.

**Statistical analysis was performed on the % cells with aberrations. The results are considered significant if p-value is ≤ 0.05.

In the Chi-square test, all test article concentrations were compared to solvent.

In the Chi-square test, CP was compared to Untreated control data since the solvent for CP was water.

Study title: In Vitro Chromosome Aberrations Study with _____
 _____ in Chinese Hamster Ovary Cells

Key findings:

- The impurity, _____ tested positive for its potential to induce structural chromosome aberrations in cultured CHO cells both with and without metabolic activation. Under the conditions of the study _____ is therefore considered to be clastogenic.

Study no.:

_____ # AA85YE331.BTL

Volume #, and page #:

Volume 3, page 884 DMF _____

Conducting laboratory and location:

Date of study initiation:

January 15, 2004

GLP compliance:

Yes

QA reports:

yes (X) no ()

Drug, lot #, and % purity:

_____, B4968P171R, _____

Methods: The ability for _____ to induce chromosome aberrations in cultured Chinese Hamster ovary (CHO) cells with and without exogenous metabolic activation was determined via a standard assay. Metabolic activation mixture was prepared of Aroclor 1254-induced rat liver homogenate (S9) and the cofactor pool. The S9 was tested for its potential to induce acceptable level of aberrations in CHO cells with cyclophosphamide (CP). Following treatment, a total of 1000 cells were scored from each concentration tested (500 from each duplicate flask) and the number of dividing cells was recorded. The mitotic index (MI = number of dividing cells from 1000 cells/10) and relative mitotic index (RMI = MI of test concentration/MI of solvent concentration x 100) was determined.

Strains/species/cell line: The clone _____ of the CHO cell line was used in the study. The cells originated at _____. Doubling time of the cell line is approximately 12 hours and its modal chromosome number is 21.

Doses used in definitive study: Concentrations tested were 1.25, 2.5, 5, 10, 15, 20, and 25 µg/mL in the initial test for 4 hrs with activation system. In the repeat test concentration tested were 2.5, 5, 10 µg/mL with activation system. Concentrations tested with the non-activated systems were 0.079, 0.157, 0.313, 0.625, 1.25, and 2.5 µg/mL for 4 hr and 0.01, 0.02, 0.04, 0.79, 0.157, 0.313, 0.625, 1.25, and 2.5 µg/mL for 20 hr, respectively. A total of 100 metaphase cells were scored from duplicate slides.

Basis of dose selection: Dose selection was based upon osmolarity, solubility testing, and a range finding study. For the range finding study, cells were treated with concentrations of 0.29, 0.897, 2.99, 8.97, 29.9, 89.7, 299, 897, and 2990 µg/mL under both activated and non-activated conditions. Untreated water was employed as control. Cytotoxicity was evaluated as a reduction in the relative mitotic index or relative cell growth. Relative cell growth was determined by the number of cells in the test flask/number of cells in the solvent flask x 100. A concentration causing greater than 50% reduction in RCG and/or RMI was selected as the highest test concentration for the chromosome aberration assay. In addition, three or more lower concentrations were included in the assay. The sponsor's table 1, 2, and 3 (shown below) summarizes the results of the mitotic index range-finding test. As indicated in the above mentioned tables, substantial toxicity >50% were noted at 4 hrs in the presence and absence of the metabolic activation and in the absence of metabolic activation at 20 hrs with 29.9 µg/mL. The osmolarity of the solvent in the treatment medium was 280 mmol/kg and is considered acceptable (because osmolarity of the solvent was ≤ 20 %).

Negative controls: The solvent control was water; the test article was diluted with water for solubility purposes. The untreated controls were treated with water alone.

Positive controls: Mitomycin-C (MMC) and Cyclophosphamide (CP) were dissolved in sterile water and diluted to 0.1 and 0.2 µg/mL (MMC) and 10 and 20 µg/mL (CP) stock solutions.

Incubation and sampling times: In the non-activated and activated systems, the cultures were treated for 4 hours, the medium removed and the cells were treated with PBS containing Ca^{2+} and Mg^{2+} for an additional 20 hours prior to harvest. In the confirmatory assay, the cells were treated with _____ HCl for 4 and 20 hours without metabolic activation and 4 hrs with metabolic activation.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The following criteria for a valid assay were employed: 1) the percentage of cells with aberrations in the solvent control should not have exceeded 4%, 2) the positive control should produce at least 25% of the cells with one or more chromosome aberrations, and 3) At least one of the test concentrations scored should show greater than 50% reduction in the relative cell growth and/or relative mitotic index (unless a maximum soluble concentration or highest allowable dose is reached without apparent toxicity). The study appears to fulfill the above criteria for a valid study. In addition, the study appears to be valid as the appropriate positive controls were used, an acceptable number of mitotic cells were evaluated, and the protocols appear to be consistent with those described in the OECD Guidelines.

Study outcome: The mitotic indexes for the concentrations used in the definitive chromosome aberration assay with metabolic activation are presented in the sponsor's table 4 and 5 below. Cytotoxicity was 56 % at the highest concentrated tested without metabolic activation (10 $\mu\text{g}/\text{mL}$); a reduction in the mitotic index of 29% was seen at this dose compared to that of the solvent control. As shown in the table 6 below, a significant ($p < 0.01\%$) increase in structural chromosomal damage was observed at 5 (8.5%), and 10 (16%) $\mu\text{g}/\text{mL}$ compared control (1%). Positive control, 10 $\mu\text{g}/\text{mL}$ CP showed an increase in structural chromosomal damage of 26%.

The results of the chromosomal aberrations test in the absence of metabolic activation are presented in sponsor's table 7, and 8 reproduced on the next page. As noted, the high concentration tested (5 $\mu\text{g}/\text{mL}$) produced cytotoxicity (49%) with 4 hrs incubation in the absence of the metabolic activation. At this dose relative mitotic index reduced to 52%. Three concentrations 1.25, 2.5, and 5 $\mu\text{g}/\text{mL}$ were selected for microscopic examinations. There was a significant increase ($p < 0.01$) in the structural chromosomal damage (sponsor's table 10) in the cells treated _____ with all doses. The percentage increase in the structural chromosomal damage with 0, 1.25, 2.5, 5 $\mu\text{g}/\text{mL}$, and MMC (0.2 $\mu\text{g}/\text{mL}$) were 0, 3.0, 5.0, 23, and 18 respectively. Although the increase at low and mid dose was within the historical control range, the finding is considered biologically relevant because of two reasons. Firstly, the increase in the structural chromosomal aberration is dose responsive and secondly, the increase at high dose is even higher than the positive control.

Therefore, _____ was concluded to be a clastogen under the present experimental condition.

PRELIMINARY TOXICITY TEST USING ██████████
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

4 HOUR TREATMENT, 16 HOUR RECOVERY PERIOD

Treatment (µg/mL)	Cell Count (x10 ⁶)	Cell Viability (%)	Viable Cells/Flask (x10 ⁶)	Cell Growth Index (%)	Cell Growth Inhibition (%)
Water	1.48	100%	1.48	100%	
0.299	1.60	98%	1.57	106%	-6%
0.897	1.66	97%	1.61	109%	-9%
2.99	0.96	98%	0.94	64%	36%
8.97	0.97	96%	0.94	63%	37%
29.9	0.51	*	0.00	0%	100%
89.7	0.33	**	0.00	0%	100%
299	0.49	**	0.00	0%	100%
897	0.09	**	0.00	0%	100%
2990	0.18	**	0.00	0%	100%

* Due to excessive toxicity, no viable cells were observed.
 ** No cells were present.
 Treatment: CHO cells were treated in the absence of an exogenous source of metabolic activation for 4 hours at 37°C.
 Cell Viability: determined by trypan blue dye exclusion.
 Viable Cells/Flask = cell count / % viable cells
 Cell Growth Index = (cells per flask treated group / cells per flask control group), expressed as a percentage.
 Cell Growth Inhibition = 100% - % cell growth index; not calculated for negative controls.

Best Possible Copy

Appears This Way
On Original

PRELIMINARY TOXICITY TEST USING
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

4 HOUR TREATMENT, 16 HOUR RECOVERY PERIOD

Treatment (µg/mL)	Cell Count (x10 ⁶)	Cell Viability (%)	Viable Cells/Flask (x10 ⁵)	Cell Growth Index (%)	Cell Growth Inhibition (%)
Water	1.44	98%	1.41	100%	
0.299	1.79	100%	1.79	127%	-27%
0.897	1.32	99%	1.31	93%	7%
2.99	1.18	98%	1.16	82%	18%
8.97	1.30	98%	1.27	90%	10%
29.9	0.55	*	0.00	0%	100%
89.7	0.29	**	0.00	0%	100%
299	0.16	**	0.00	0%	100%
897	0.22	**	0.00	0%	100%
2990	0.45	**	0.00	0%	100%

* Due to excessive toxicity, no viable cells were observed.

** No cells were present.

Treatment: CHO cells were treated in the presence of an exogenous source of metabolic activation for 4 hours at 37±1°C.

Cell Viability: determined by trypan blue dye exclusion.

Viable Cells/Flask = cell count x % viable cells

Cell Growth Index = (cells per flask treated group/cells per flask control group), expressed as a percentage.

Cell Growth Inhibition = 100% - % cell growth index; not calculated for negative controls.

Appears This Way
On Original

TABLE 3
PRELIMINARY TOXICITY TEST USING
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

20-HOUR CONTINUOUS TREATMENT

Treatment (µg/ml)	Cell Count (x10 ⁶)	Cell Viability (%)	Viable Cells/Flask (x10 ⁶)	Cell Growth Index (%)	Cell Growth Inhibition (%)
Water	1.35	99%	1.34	100%	
0.299	1.41	98%	1.38	101%	-3%
0.897	1.35	98%	1.33	99%	1%
2.99	0.86	99%	0.85	63%	37%
8.97	0.80	97%	0.78	58%	42%
29.9	0.53	*	0.00	0%	100%
89.7	0.42	**	0.00	0%	100%
299	0.57	**	0.00	0%	100%
897	0.56	**	0.00	0%	100%
2990	0.20	**	0.00	0%	100%

* Due to excessive toxicity, no viable cells were observed.

** No cells were present.

Treatment: CHO cells were treated in the absence of an exogenous source of metabolic activation for 20 hours at 37±1°C.

Cell Viability: determined by trypan blue dye exclusion.

Viable Cells/Flask = cell count x % viable cells

Cell Growth Index = (cells per flask treated group/cells per flask control group), expressed as a percentage.

Cell Growth Inhibition = 100% - % cell growth index; not calculated for negative controls.

Appears This Way
 On Original

Best Possible Copy

TABLE 4
CONCURRENT TOXICITY TEST USING
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

4 HOUR TREATMENT, 16 HOUR RECOVERY PERIOD - INITIAL ASSAY

Treatment (µg/mL)	Flask	Cell Count Averages (x10 ⁵)	Cell Viability (%)	Mean Cells per Flask (x10 ⁵)	Cell Growth Index (%)	Cell Growth Inhibition (%)																																																																																						
Water	A	1.82	98%	1.61	100%																																																																																							
	B	1.47	98%				1.25	A	1.51	99%	1.45	90%	10%	B	1.39	100%	2.5	A	1.25	97%	1.42	88%	12%	B	1.66	98%	5	A	0.99	99%	1.17	73%	27%	B	1.37	99%	10	A	0.76	98%	0.71	44%	56%	B	0.67	100%	15	A	0.66	100%	0.70	43%	57%	B	0.75	97%	20	A	0.63	96%	0.56	35%	65%	B	0.53	98%	25	A	0.63	*	0.00	0%	100%	B	0.56	*	CP, 10	A	1.00	98%	1.10	68%	32%	B	1.25	97%	CP, 20	A	0.95	99%	0.93	58%
1.25	A	1.51	99%	1.45	90%	10%																																																																																						
	B	1.39	100%				2.5	A	1.25	97%	1.42	88%	12%	B	1.66	98%	5	A	0.99	99%	1.17	73%	27%	B	1.37	99%	10	A	0.76	98%	0.71	44%	56%	B	0.67	100%	15	A	0.66	100%	0.70	43%	57%	B	0.75	97%	20	A	0.63	96%	0.56	35%	65%	B	0.53	98%	25	A	0.63	*	0.00	0%	100%	B	0.56	*	CP, 10	A	1.00	98%	1.10	68%	32%	B	1.25	97%	CP, 20	A	0.95	99%	0.93	58%	42%	B	1.00	92%						
2.5	A	1.25	97%	1.42	88%	12%																																																																																						
	B	1.66	98%				5	A	0.99	99%	1.17	73%	27%	B	1.37	99%	10	A	0.76	98%	0.71	44%	56%	B	0.67	100%	15	A	0.66	100%	0.70	43%	57%	B	0.75	97%	20	A	0.63	96%	0.56	35%	65%	B	0.53	98%	25	A	0.63	*	0.00	0%	100%	B	0.56	*	CP, 10	A	1.00	98%	1.10	68%	32%	B	1.25	97%	CP, 20	A	0.95	99%	0.93	58%	42%	B	1.00	92%																
5	A	0.99	99%	1.17	73%	27%																																																																																						
	B	1.37	99%				10	A	0.76	98%	0.71	44%	56%	B	0.67	100%	15	A	0.66	100%	0.70	43%	57%	B	0.75	97%	20	A	0.63	96%	0.56	35%	65%	B	0.53	98%	25	A	0.63	*	0.00	0%	100%	B	0.56	*	CP, 10	A	1.00	98%	1.10	68%	32%	B	1.25	97%	CP, 20	A	0.95	99%	0.93	58%	42%	B	1.00	92%																										
10	A	0.76	98%	0.71	44%	56%																																																																																						
	B	0.67	100%				15	A	0.66	100%	0.70	43%	57%	B	0.75	97%	20	A	0.63	96%	0.56	35%	65%	B	0.53	98%	25	A	0.63	*	0.00	0%	100%	B	0.56	*	CP, 10	A	1.00	98%	1.10	68%	32%	B	1.25	97%	CP, 20	A	0.95	99%	0.93	58%	42%	B	1.00	92%																																				
15	A	0.66	100%	0.70	43%	57%																																																																																						
	B	0.75	97%				20	A	0.63	96%	0.56	35%	65%	B	0.53	98%	25	A	0.63	*	0.00	0%	100%	B	0.56	*	CP, 10	A	1.00	98%	1.10	68%	32%	B	1.25	97%	CP, 20	A	0.95	99%	0.93	58%	42%	B	1.00	92%																																														
20	A	0.63	96%	0.56	35%	65%																																																																																						
	B	0.53	98%				25	A	0.63	*	0.00	0%	100%	B	0.56	*	CP, 10	A	1.00	98%	1.10	68%	32%	B	1.25	97%	CP, 20	A	0.95	99%	0.93	58%	42%	B	1.00	92%																																																								
25	A	0.63	*	0.00	0%	100%																																																																																						
	B	0.56	*				CP, 10	A	1.00	98%	1.10	68%	32%	B	1.25	97%	CP, 20	A	0.95	99%	0.93	58%	42%	B	1.00	92%																																																																		
CP, 10	A	1.00	98%	1.10	68%	32%																																																																																						
	B	1.25	97%				CP, 20	A	0.95	99%	0.93	58%	42%	B	1.00	92%																																																																												
CP, 20	A	0.95	99%	0.93	58%	42%																																																																																						
	B	1.00	92%																																																																																									

* Due to excessive toxicity, no viable cells were observed.

Treatment: CHO cells were treated in the presence of an exogenous source of metabolic activation for 4 hours at 37±1°C.

Cell Viability: determined by trypan blue dye exclusion.

Viable Cells/Flask = cell count x % viable cells, reported as mean of Flasks A and B.

Cell Growth Index = (mean cells per flask treated group/mean cells per flask control group), expressed as a percentage.

Cell Growth Inhibition = 100% - % cell growth index; not calculated for negative controls.

Appears This Way
 On Original

Best Possible Copy

TABLE 5
CYTOGENETIC ANALYSIS OF CHO CELLS TREATED WITH
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

4 HOUR TREATMENT, 16 HOUR RECOVERY PERIOD - INITIAL ASSAY

Treatment (µg/mL)	Flask	Mitotic Index (%)	Cells Scored		% Aberrant Cells		Total Number of Structural Aberrations					Severely Damaged Cells	Average Aberrations Per Cell	
			Numerical	Structural	Numerical	Structural	Gaps	Chromatid		Chromosome				
								Br	Ex	Br	Dic			Ring
Water	A	7.4	100	100	4	1	0	1	0	0	0	0	0	0.010
	B	7.8	100	100	5	1	0	1	0	0	0	0	0	0.010
2.5	A	8.0	100	100	4	2	0	0	0	0	0	2	0	0.020
	B	9.0	100	100	6	3	0	1	0	0	2	0	0	0.030
5	A	7.0	100	100	6	9	0	3	0	0	5	1	0	0.090
	B	6.0	100	100	7	8	0	1	0	1	6	1	0	0.090
10	A	5.8	100	100	4	13	0	8	6	1	1	0	0	0.160
	B	5.0	100	100	4	19	0	8	11	0	2	0	0	0.210
CP, 10	A	5.4	100	50	4	28	0	1	15	0	0	0	2	0.720
	B	5.0	100	50	2	24	0	6	11	1	0	1	1	0.580

Treatment: CHO cells were treated for 4 hours at 37±1°C in the presence of an exogenous source of metabolic activation. An additional dose level of 1.25 µg/mL was tested as a safeguard against excessive toxicity at higher dose levels but was not required for microscopic examination. Dose levels 15, 20 and 25 µg/mL were not analyzed due to excessive toxicity.

Mitotic Index = number mitotic figures x 100/500 cells counted.

% Aberrant Cells: numerical cells include polyploid and endoreduplicated cells; structural cells exclude cells with only gaps.

Chromatid breaks (Br) include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Ex) include quadriradials, triradials and complex rearrangements.

Chromosome breaks (Br) include breaks and acentric fragments; Dic, dicentric chromosome.

Severely damaged cells includes cells with one or more pulverized chromosome and cells with 10 or more aberrations.

Average aberrations per cell: severely damaged cells and pulverizations were counted as 10 aberrations.

Appears This Way
 On Original

Best Possible Copy

TABLE 6
SUMMARY - INITIAL ASSAY

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean ± SD)		Cells With Aberrations	
				Numerical	Structural			Numerical (%)	Structural (%)
Water	+S9	4	7.6	200	200	0.010	±0.100	4.5	1.0
2.5	+S9	4	8.3	200	200	0.025	±0.157	5.0	2.5
5	+S9	4	6.3	200	200	0.090	±0.304	6.5	8.5**
10	+S9	4	5.4	200	200	0.185	±0.449	4.0	16.0**
CP, 10	+S9	4	5.2	200	100	0.650	±1.811	3.0	26.0**

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

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

Percent Aberrant Cells: *, p<0.05; **, p<0.01; using Fisher's exact test.

Appears This Way
On Original

Best Possible Copy

TABLE 7
CONCURRENT TOXICITY TEST USING
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

4 HOUR TREATMENT, 16 HOUR RECOVERY PERIOD - REPEAT ASSAY

Treatment (µg/ml)	Flask	Cell Count Averages (x10 ⁵)	Cell Viability (%)	Mean Cells per Flask (x10 ⁵)	Cell Growth Index (%)	Cell Growth Inhibition (%)																																																																																																
Water	A	1.77	99%	1.73	100%																																																																																																	
	B	1.74	98%				0.079	A	1.58	99%	1.52	88%	12%	B	1.49	99%	0.157	A	1.49	98%	1.48	85%	15%	B	1.53	97%	0.313	A	1.52	98%	1.53	88%	12%	B	1.59	98%	0.625	A	1.52	98%	1.47	85%	15%	B	1.46	99%	1.25	A	1.53	98%	1.44	83%	17%	B	1.42	97%	2.5	A	0.88	99%	0.88	51%	49%	B	0.91	98%	5	A	0.73	97%	0.76	44%	56%	B	0.85	97%	7.5	A	0.73	94%	0.74	43%	57%	B	0.80	98%	MMC, 0.1	A	1.12	97%	1.07	62%	38%	B	1.10	96%	MMC, 0.2	A	1.31	96%	1.32	76%
0.079	A	1.58	99%	1.52	88%	12%																																																																																																
	B	1.49	99%				0.157	A	1.49	98%	1.48	85%	15%	B	1.53	97%	0.313	A	1.52	98%	1.53	88%	12%	B	1.59	98%	0.625	A	1.52	98%	1.47	85%	15%	B	1.46	99%	1.25	A	1.53	98%	1.44	83%	17%	B	1.42	97%	2.5	A	0.88	99%	0.88	51%	49%	B	0.91	98%	5	A	0.73	97%	0.76	44%	56%	B	0.85	97%	7.5	A	0.73	94%	0.74	43%	57%	B	0.80	98%	MMC, 0.1	A	1.12	97%	1.07	62%	38%	B	1.10	96%	MMC, 0.2	A	1.31	96%	1.32	76%	24%	B	1.45	95%						
0.157	A	1.49	98%	1.48	85%	15%																																																																																																
	B	1.53	97%				0.313	A	1.52	98%	1.53	88%	12%	B	1.59	98%	0.625	A	1.52	98%	1.47	85%	15%	B	1.46	99%	1.25	A	1.53	98%	1.44	83%	17%	B	1.42	97%	2.5	A	0.88	99%	0.88	51%	49%	B	0.91	98%	5	A	0.73	97%	0.76	44%	56%	B	0.85	97%	7.5	A	0.73	94%	0.74	43%	57%	B	0.80	98%	MMC, 0.1	A	1.12	97%	1.07	62%	38%	B	1.10	96%	MMC, 0.2	A	1.31	96%	1.32	76%	24%	B	1.45	95%																
0.313	A	1.52	98%	1.53	88%	12%																																																																																																
	B	1.59	98%				0.625	A	1.52	98%	1.47	85%	15%	B	1.46	99%	1.25	A	1.53	98%	1.44	83%	17%	B	1.42	97%	2.5	A	0.88	99%	0.88	51%	49%	B	0.91	98%	5	A	0.73	97%	0.76	44%	56%	B	0.85	97%	7.5	A	0.73	94%	0.74	43%	57%	B	0.80	98%	MMC, 0.1	A	1.12	97%	1.07	62%	38%	B	1.10	96%	MMC, 0.2	A	1.31	96%	1.32	76%	24%	B	1.45	95%																										
0.625	A	1.52	98%	1.47	85%	15%																																																																																																
	B	1.46	99%				1.25	A	1.53	98%	1.44	83%	17%	B	1.42	97%	2.5	A	0.88	99%	0.88	51%	49%	B	0.91	98%	5	A	0.73	97%	0.76	44%	56%	B	0.85	97%	7.5	A	0.73	94%	0.74	43%	57%	B	0.80	98%	MMC, 0.1	A	1.12	97%	1.07	62%	38%	B	1.10	96%	MMC, 0.2	A	1.31	96%	1.32	76%	24%	B	1.45	95%																																				
1.25	A	1.53	98%	1.44	83%	17%																																																																																																
	B	1.42	97%				2.5	A	0.88	99%	0.88	51%	49%	B	0.91	98%	5	A	0.73	97%	0.76	44%	56%	B	0.85	97%	7.5	A	0.73	94%	0.74	43%	57%	B	0.80	98%	MMC, 0.1	A	1.12	97%	1.07	62%	38%	B	1.10	96%	MMC, 0.2	A	1.31	96%	1.32	76%	24%	B	1.45	95%																																														
2.5	A	0.88	99%	0.88	51%	49%																																																																																																
	B	0.91	98%				5	A	0.73	97%	0.76	44%	56%	B	0.85	97%	7.5	A	0.73	94%	0.74	43%	57%	B	0.80	98%	MMC, 0.1	A	1.12	97%	1.07	62%	38%	B	1.10	96%	MMC, 0.2	A	1.31	96%	1.32	76%	24%	B	1.45	95%																																																								
5	A	0.73	97%	0.76	44%	56%																																																																																																
	B	0.85	97%				7.5	A	0.73	94%	0.74	43%	57%	B	0.80	98%	MMC, 0.1	A	1.12	97%	1.07	62%	38%	B	1.10	96%	MMC, 0.2	A	1.31	96%	1.32	76%	24%	B	1.45	95%																																																																		
7.5	A	0.73	94%	0.74	43%	57%																																																																																																
	B	0.80	98%				MMC, 0.1	A	1.12	97%	1.07	62%	38%	B	1.10	96%	MMC, 0.2	A	1.31	96%	1.32	76%	24%	B	1.45	95%																																																																												
MMC, 0.1	A	1.12	97%	1.07	62%	38%																																																																																																
	B	1.10	96%				MMC, 0.2	A	1.31	96%	1.32	76%	24%	B	1.45	95%																																																																																						
MMC, 0.2	A	1.31	96%	1.32	76%	24%																																																																																																
	B	1.45	95%																																																																																																			

Treatment: CHO cells were treated in the absence of an exogenous source of metabolic activation for 4 hours at 37±1°C.

Cell Viability: determined by trypan blue dye exclusion.

Viable Cells/Flask = cell count x % viable cells, reported as mean of Flasks A and B.

Cell Growth Index = (mean cells per flask treated group/mean cells per flask control group), expressed as a percentage.

Cell Growth Inhibition = 100% - % cell growth index; not calculated for negative controls.

Appears This Way
 On Original

TABLE 8
CYTOGENETIC ANALYSIS OF CHO CELLS TREATED WITH [REDACTED]
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

4 HOUR TREATMENT, 16 HOUR RECOVERY PERIOD - REPEAT ASSAY

Treatment (µg/mL)	Flask	Mitotic Index (%)	Cells Scored		% Aberrant Cells		Total Number of Structural Aberrations						Severely Damaged Cells	Average Aberrations Per Cell
			Numerical	Structural	Numerical	Structural	Gaps	Chromatid Br.	Ex	Chromosomal Br.	Dic	Ring		
Water	A	8.8	100	100	3	0	0	0	0	0	0	0	0	0.000
	B	8.2	100	100	2	0	9	0	0	0	0	0	0	0.000
1.25	A	7.2	100	100	4	2	0	1	0	0	1	0	0	0.020
	B	8.0	100	100	2	4	0	1	0	1	1	1	0	0.040
2.5	A	8.2	100	100	3	3	0	2	0	0	1	0	0	0.030
	B	6.0	100	100	3	7	0	3	1	2	1	0	1	0.170
5	A	4.2	100	50	2	22	0	7	3	0	1	0	2	0.620
	B	4.0	100	50	1	24	0	8	4	0	2	0	2	0.680
MMC, 0.2	A	5.0	100	50	2	20	0	2	9	1	0	1	2	0.660
	B	4.6	100	50	4	16	0	3	5	2	0	1	0	0.270

Treatment: CHO cells were treated for 4 hours at 37±1°C in the absence of an exogenous source of metabolic activation. Additional dose levels of 0.079, 0.157, 0.313 and 0.625 µg/mL were tested as a safeguard against excessive toxicity at higher dose levels but were not required for microscopic examination. Dose level 7.5 µg/mL was not analyzed due to excessive toxicity.

Mitotic Index = number mitotic figures x 100/500 cells counted.

% Aberrant Cells: numerical cells include polyploid and endoreduplicated cells; structural cells exclude cells with only gaps.

Chromatid breaks (Br) include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Ex) include quadriradials, triradials and complex rearrangements.

Chromosome breaks (Br) include breaks and acentric fragments; Dic, dicentric chromosome.

Severely damaged cells includes cells with one or more pulverized chromosome and cells with 10 or more aberrations.

Average aberrations per cell: severely damaged cells and pulverizations were counted as 10 aberrations.

TABLE 10
SUMMARY - REPEAT ASSAY

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean ± SD)	Cells With Aberrations	
				Numerical	Structural		Numerical (%)	Structural (%)
Water	-S9	4	8.5	200	200	0.000 ±0.000	2.5	0.0
1.25	-S9	4	7.6	200	200	0.030 ±0.171	3.0	3.0*
2.5	-S9	4	6.1	200	200	0.100 ±0.743	3.0	5.0**
5	-S9	4	4.1	200	100	0.650 ±1.997	1.5	23.0**
MMC, 0.2	-S9	4	4.8	200	100	0.440 ±1.506	3.0	18.0**

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

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

Percent Aberrant Cells: *, p<0.05; **, p<0.01; using Fisher's exact test.

Best Possible Copy

Appears This Way
On Original

Study title: Bacterial Reverse Mutation Assay

Key findings: _____ was tested in the Ames Reverse Mutation Assay at concentrations of 15, 50, 150, 500, 1500, and 5000 µg/plate. There was no evidence for precipitate at any concentration. Cytotoxicity was observed ≥ 500 µg/plate. Under the conditions of the study, _____ was concluded to be negative in the bacterial reverse mutation assay.

Study no: AA85YE.503.BTL
Study type (if not reflected in title): Bacterial Reverse Mutation Assay (Ames Test)
Volume #, and page #: Volume 3, page 805 (DMF _____)
Conducting laboratory and location: _____
Date of study initiation: January 15, 2004
GLP compliance: Yes
QA reports: yes (X) no ()
Drug, lot #, radiolabel, and % purity: _____ : B4968P171-R,

Formulation/vehicle: Water was the solvent of choice

Methods: The assay was conducted in two phases using the plate incorporation method. The first phase was the toxicity-mutation assay used to establish the dose-range for the mutagenicity assay and get a preliminary mutagenicity evaluation. The second phase was the mutagenicity assay. Dosing was adjusted to compensate for test article activity. Tester strain TA98 and TA1537 are reverted from histidine dependence to histidine independence by frameshift mutagens. Tester strain TA1535 is reverted by mutagens that cause base pair substitution. Tester strain TA100 is reverted by mutagens that cause both frameshift and base pair substitution mutations. Specificity in *E.coli* is sensitive to base-pair substitution mutations, rather than frameshift.

Strains/species/cell line: *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2 *uvrA*.

Dose selection criteria: Toxicity and formation of a precipitate.

Basis of dose selection: Doses used in initial toxicity-mutation assay were 2.5, 7.5, 25, 75, 200, 600, 1800 and 500 µg/plate for _____. The test article was soluble and clear in water at the highest concentrations tested. NO precipitate was observed. Toxicity was seen at ≥600 µg/plate. No positive mutagenic responses were observed in any tester strains either with or without S9 activation. The definitive study therefore used the maximum recommended concentrations of test article as the basis for dose selection.

Range finding studies: Initial toxicity-mutation assay

Test agent stability: Result indicated that drug solutions of 0.025 mg/mL and 50 mg/mL in water were stable up to at least _____ days under ambient conditions.

Metabolic activation system: Aroclor 1254-induced rat liver S9 (male). Each bulk preparation of S9 was assayed for its ability to metabolize 2-aminoanthracene and 7,12-dimethylbenz(a)anthracene to forms mutagenic to *Salmonella typhimurium* TA100. S9 was tested at 10%.

Controls:

Vehicle: Water

Negative controls: Water

Positive controls:

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
All <i>Salmonella</i> strains	Rat	2-aminoanthracene	1.0
WP2 <i>uvrA</i>			10
TA98	None	2-nitrofluorene	1.0
TA100, TA1535		Sodium azide	1.0
TA1537		9-aminoacridine	75
WP2 <i>uvrA</i>		Methyl methanesulfonate	1,000

Comments: Controls are acceptable according to current standards.

Exposure conditions:

Incubation and sampling times: 48 to 72 hours at 37°C

Doses used in definitive study: Concentrations of _____
 _____ 15, 50, 150, 500, 1500, and 5000 µg/plate were tested in the definitive assay

Study design: Plate incorporation method as described above.

Analysis:

No. of replicates: 3

Counting method: Either entirely by automated colony counter or entirely by hand (microscope).

Criteria for positive results: Test article was considered positive if it caused a dose-dependent increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article. For strains TA1535 and TA1537, increase in mean revertants must be equal to or greater than three times the mean vehicle control value. For strains TA98, TA100 and WP2*uvrA*, increase in mean revertants at peak must be equal to or greater than two times the mean control value.

Summary of individual study findings:

Study validity: The study appears to be valid for the following reasons: 1) the appropriate controls were used, 2) the appropriate strains were tested, 3) the positive control substances produced reliable positive results, 4) the highest concentration of _____ tested, reached the maximum recommended concentration was the molar equivalent of 5,000 µg/plate 5) Evidence for increase in revertants following positive control treatment was noted.

Study outcome: No precipitate was observed, cytotoxicity was observed in all different strain with 500 µg/plate. _____ did not produce any increases in the number of revertants in any tester stain under the conditions tested. This is in concurrence with the Sponsor's conclusions.

Bacterial Mutation Assay
Summary of Results - Confirmatory Mutagenicity Assay

Table 22

Article Id : _____
Study Number : AA85YE.503.BTL Experiment No : B2

Average Revertants Per Plate ± Standard Deviation

Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	17 ± 2	124 ± 6	21 ± 7	5 ± 2	18 ± 3
15	20 ± 4	118 ± 11	14 ± 4	6 ± 3	19 ± 1
50	20 ± 2	113 ± 7	20 ± 2	6 ± 2	21 ± 5
150	16 ± 2	109 ± 9	16 ± 1	6 ± 1	16 ± 3
500	8 ± 5	103 ± 8	11 ± 5	3 ± 3	13 ± 4
1500	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 1
5000	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Positive	137 ± 10	617 ± 13	190 ± 42	418 ± 189	108 ± 13

Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	32 ± 2	129 ± 1	12 ± 2	9 ± 1	20 ± 4
15	29 ± 10	147 ± 9	15 ± 8	11 ± 2	19 ± 4
50	34 ± 2	139 ± 10	13 ± 1	8 ± 1	22 ± 4
150	23 ± 6	137 ± 4	13 ± 3	8 ± 4	17 ± 4
500	24 ± 5	130 ± 4	11 ± 3	5 ± 4	15 ± 3
1500	2 ± 2	25 ± 12	2 ± 1	1 ± 0	4 ± 2
5000	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Positive	958 ± 104	1539 ± 241	181 ± 13	223 ± 86	918 ± 15

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot: 100 µL

Best Possible Copy

Study title: Bacterial Reverse Mutation Assay _____

Key findings: Under the conditions of this study, _____ was not genotoxic either with or without metabolic activation in the Bacterial Reverse Mutation Assay.

Study no.: _____ # AA85YD.503.BTL

Volume #, and page #: _____ The study was submitted to the DMF.

Conducting laboratory and location: _____

Date of study initiation: January 15, 2004

¹ The Sponsor of this study was Mallinckrodt, Inc., St. Louis, MO

GLP compliance: Yes
 QA reports: yes (X) no ()
 Drug, lot #, and % purity: _____ Lot B5586P44-R,
 determined authentic via IR, MS, NMR and DSC. Purity ranged from _____ over
 the course of the study period.

Methods

The assay employed the plate incorporation method. Bacterial cultures were incubated for approximately 48 to 72 hours at 37±2°C. The condition of the bacterial lawn was evaluated for potential test-article related toxicity. Precipitation of drug was monitored for visually. Tester strain and test article were incorporated into the top agar layer and overlaid onto a minimal bottom agar. Plates were inverted and incubated for 48 to 72 hours at 37±2°C. Potential toxicity due to the test article was examined visually and scored compared to the vehicle as described in the sponsor's table below:

Code	Description	Characteristics
1	Normal	Distinguished by a healthy microcolony lawn.
2	Slightly reduced	Distinguished by a noticeable thinning of the microcolony lawn and possibly a slight increase in the size of the microcolonies compared to the vehicle control plate.
3	Moderately Reduced	Distinguished by a marked thinning of the microcolony lawn resulting in a pronounced increase in the size of the microcolonies compared to the vehicle control late.
4	Extremely Reduced	Distinguished by an extreme thinning of the microcolony lawn resulting in an increase in the size of the microcolonies compared to the vehicle control plate such that the microcolony lawn is visible to the unaided eye as isolated colonies.
5	Absent	Distinguished by a complete lack of any microcolony lawn over more than or equal to 90% of the plate.
6	Obscured by Particulate	The background bacterial lawn cannot be accurately evaluated due to microscopic test article particulate.
NP	Non-Interfering Precipitate	Distinguished by precipitate on the plate that is visible to the naked eye but any precipitate particles detected by the automated colony counter total less than or equal to 10% of the revertant colony count (e.g., less than or equal to 3 particles on a plate with 30 revertants).
IP	Interfering Precipitate	Distinguished by precipitate on the plate that is visible to the naked eye and any precipitate particles detected by the automated colony counter exceed 10% of the revertant colony count (e.g., more than 3 particles on a plate with 30 revertants). These plates are counted manually.

Revertant colonies were counted either entirely by automated colony counter or by hand.

The identity of the _____ structure in samples and the concentrations of the formulations of _____ were verified using high performance liquid chromatography (HPLC). In addition, drug solutions were determined to be stable at room temperature and 2 or 8°C.

Strains/species/cell line: *Salmonella typhimurium* histidine auxotrophs TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2uvrA.

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
All Salmonella Strains	Rat	2-aminoanthracene _____ Lot No. 09106PS Exp. Date 14-Sep-2005 CAS No. 613-13-8; Purity _____	1.0
WP2uvrA			10
TA98	None	2-nitrofluorene _____ Lot No. 08708HS Exp. Date 08-Mar-2006; CAS No. 607-57-8 Purity _____	1.0
TA100, TA1535		sodium azide _____ Lot No. 073KO119 Exp. Date 07-Jan-2009; CAS No. 26628-22-8 Purity _____	1.0
TA1537		9-aminoacridine _____ Lot No. 106F06681 Exp. Date 08-Nov-2004; CAS No. 90-45-9 Purity _____	75
WP2 uvrA		methyl methanesulfonate _____ Lot No. 04115KA Exp. Date 04-Sep-2005; CAS No. 66-27-3 Purity _____	1,000

Doses used in definitive study: The confirmatory assay examined 6 concentrations of test article, each plated in triplicate.

Basis of dose selection: Final doses were chosen based upon an initial toxicity-mutation assay with preliminary mutagenicity evaluation. Bacterial lawn was evaluated of test article toxicity via the dissecting microscope. There were no solubility problems at the limit dose of 50 mg/mL.

Negative controls: Water

Positive controls: See table above.

Incubation and sampling times: Plates were incubated for 48-72 hours.

Results: The results of the initial toxicity/mutagenicity study are presented in the sponsor's table 21 reproduced below:

**Bacterial Mutation Assay
Summary of Results - Initial Toxicity-Mutation Assay**

Table 21

Test Article Id : _____									
Study Number : AA85YD.503.B1L		Experiment No : B1							
Average Revertants Per Plate ± Standard Deviation									
Liver Microsomes: None									
Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2	uvrA			
Vehicle	22 ± 4	162 ± 18	25 ± 3	7 ± 0	19 ± 1				
2.5	15 ± 6	162 ± 23	21 ± 6	7 ± 6	19 ± 0				
7.5	15 ± 2	145 ± 10	27 ± 1	7 ± 2	20 ± 6				
25	20 ± 2	151 ± 2	18 ± 2	7 ± 1	24 ± 4				
75	20 ± 2	155 ± 4	20 ± 2	5 ± 1	22 ± 6				
200	12 ± 5	153 ± 15	16 ± 1	7 ± 2	20 ± 2				
600	12 ± 2	147 ± 24	20 ± 3	6 ± 0	13 ± 0				
1800	4 ± 1	39 ± 1	7 ± 1	1 ± 0	6 ± 1				
5000	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0				
Positive	121 ± 2	578 ± 79	380 ± 0	629 ± 11	108 ± 12				
Liver Microsomes: Rat liver S9									
Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2	uvrA			
Vehicle	29 ± 2	128 ± 3	18 ± 1	6 ± 0	24 ± 0				
2.5	27 ± 3	154 ± 20	15 ± 2	8 ± 1	20 ± 2				
7.5	33 ± 8	125 ± 35	15 ± 1	8 ± 1	18 ± 1				
25	24 ± 7	145 ± 5	18 ± 2	5 ± 3	24 ± 1				
75	25 ± 2	190 ± 30	18 ± 1	3 ± 1	15 ± 3				
200	27 ± 1	168 ± 11	15 ± 1	7 ± 1	27 ± 3				
600	24 ± 5	161 ± 1	8 ± 1	6 ± 4	16 ± 4				
1800	16 ± 1	105 ± 13	8 ± 2	2 ± 1	5 ± 3				
5000	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0				
Positive	224 ± 100	581 ± 1	148 ± 1	146 ± 25	842 ± 199				
Vehicle = Vehicle Control									
Positive = Positive Control (50 µL plating aliquot)									
Plating aliquot: 100 µL									

Best Possible Copy

The data from the table above indicate that concentrations of 600, 1800 and 5000 µg/plate produced toxicity either with or without metabolic activation with S9. There was no evidence of mutagenicity in any strain tested under these conditions. Although toxicity was noted at 5000 ng/plate, the limit dose was repeated for the confirmatory assay.

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The sponsor indicated that positive result should demonstrate dose-related increase in the mean revertants per plate of at least one tester strain over at least two increasing concentrations. For strains TA1535 and TA1537, a positive response required an increase in mean revertants at the peak of the dose response of at least 3 times the mean vehicle control value. For the other strains, a positive required an increase of at least 2-fold compared to controls.

According to the sponsor, study validity was established as follows:

- All Salmonella tester strain cultures must demonstrate the presence of the deep rough mutation (rfa) and the deletion in the uvrB gene.
- Cultures of tester strains TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor.
- All WP2 uvrA cultures must demonstrate the deletion in the uvrA gene. All cultures must demonstrate the characteristic mean number of spontaneous revertants in the vehicle controls as follows (inclusive): TA98, 10 - 50; TA100, 80 - 240; TA1535, 5 - 45; TAI537, 3 - 21 ; WP2 uvrA, 10-60.
- The mean of each positive control must exhibit at least a 3.0-fold increase in the number of revertants over the mean value of the respective vehicle control.
- A minimum of three non-toxic dose levels is required to evaluate assay data.
- A dose level is considered toxic if one or both of the following criteria are met:
(1) A >50 % reduction in the mean number of revertants per plate as compared to the mean vehicle control value. This reduction must be accompanied by an abrupt dose-dependent drop in the revertant count.
- At least a moderate reduction in the background lawn (background lawn code 3, 4 or 5).

Study outcome: The results of the confirmatory mutagenicity assay are reproduced in the sponsor's summary table 22 reproduced below. The results indicate that the test article _____ was not mutagenic either with or without metabolic activation under the conditions tested.

**Appears This Way
On Original**

**Bacterial Mutation Assay
Summary of Results - Confirmatory Mutagenicity Assay**

Table 22

Test Article Id : _____
 Study Number : AA85YD.503.BTL Experiment No : B2

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2	uvrA
Vehicle	11 ± 2	160 ± 9	19 ± 1	3 ± 1	20 ± 2	2
15	15 ± 3	167 ± 13	17 ± 2	5 ± 2	16 ± 2	2
50	15 ± 3	151 ± 3	17 ± 1	5 ± 1	16 ± 3	3
150	13 ± 1	165 ± 4	16 ± 1	4 ± 2	12 ± 5	5
500	11 ± 2	176 ± 9	15 ± 3	4 ± 3	12 ± 2	2
1500	7 ± 1	87 ± 10	8 ± 2	3 ± 0	6 ± 1	1
5000	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0
Positive	164 ± 33	603 ± 22	372 ± 10	486 ± 71	138 ± 6	

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2	uvrA
Vehicle	24 ± 4	155 ± 15	11 ± 2	6 ± 3	14 ± 3	3
15	27 ± 2	149 ± 5	8 ± 2	6 ± 1	19 ± 2	2
50	28 ± 3	158 ± 16	11 ± 1	8 ± 4	16 ± 5	5
150	26 ± 4	153 ± 17	14 ± 3	6 ± 2	13 ± 2	2
500	23 ± 5	141 ± 15	10 ± 2	5 ± 4	11 ± 2	2
1500	10 ± 1	116 ± 13	8 ± 2	2 ± 1	8 ± 2	2
5000	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0
Positive	1395 ± 250	1596 ± 274	151 ± 7	166 ± 9	896 ± 50	

Vehicle = Vehicle Control
 Positive = Positive Control (50 µL plating aliquot)
 Plating aliquot: 100 µL

Study title: In Vitro Mammalian Chromosome Aberration Test _____

Key findings: Under the conditions of the assay, _____ was negative for the induction of structural and numerical chromosome aberrations in the in vitro mammalian chromosome aberration assay using human peripheral blood lymphocytes.

Study no.: _____ Study² #AA85YD.341.BTL

Volume #, and page #: _____ Study submitted to DMF

Conducting laboratory and location: _____

Date of study initiation: January 15, 2004

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: _____, Lot number B5586P44-R, determined authentic via IR, MS, NMR and DSC. Purity ranged from _____ over the course of the study period.

² The Sponsor of this study was Mallinkrodt, Inc., St. Louis, MO.

Methods

Strains/species/cell line: Peripheral blood lymphocytes were obtained from a healthy non-smoking 45 year old female for the preliminary toxicity assay and from a healthy 27-year old female for the definitive study. Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from male Sprague-Dawley rats via a single ip injection of Aroclor 1254, 500 mg/kg, 5 days prior to sacrifice. Each bulk preparation was assayed for its ability to metabolize 2-aminoanthracene and 7,12-dimethyl-benz(a)anthracene to forms mutagenic to *Salmonella typhimurium* TA100. The _____ was soluble in water.

Doses used in definitive study: Doses for the definitive study are presented below:

Treatment Condition	Treatment Time	Recovery Time	Dose levels ($\mu\text{g/mL}$)
Non-activated	4 hr	16 hr	1.25, 2.5, 5, 35, 50, 65, 75
	20 hr	0 hr	1.25, 2.5, 5, 10, 15, 20, 25
S9 activated	4 hr	16 hr	5, 10, 20, 35, 50, 65, 75

Basis of dose selection: Dose selection for the definitive study was based upon a preliminary toxicity assay (reduction of mitotic index relative to solvent control).

The results of the preliminary toxicity study which examined concentrations of _____ up to 3200 $\mu\text{g/mL}$ (10 mM, limit dose), are reproduced in the sponsor's table below. Significant toxicity was demonstrated at concentrations of $\geq 32 \mu\text{g/mL}$ _____ by a reduction in the mitotic index of at least 50% relative to control in the non-activated 4 and 20 hour exposure conditions. In the presence of S9, substantial toxicity was noted at doses of $\geq 96 \mu\text{g/mL}$ _____.

Appears This Way
On Original

Preliminary Toxicity Test Using _____						
Metabolic Activation?	No		Yes		No	
Duration Treatment	4 hours		4 hours		20 hours	
	MITOTIC INDEX (%)	PERCENT CHANGE (%)	MITOTIC INDEX (%)	PERCENT CHANGE (%)	MITOTIC INDEX (%)	PERCENT CHANGE (%)
Water	8.4		8.6		7.8	
_____ (mg/mL)						
0.32	8.0	-5	9.2	7	8.4	8
0.96	9.0	7	8.8	2	8.4	8
3.2	9.2	10	8.0	-7	8.8	13
9.6	7.2	-14	7.0	-19	6.8	-13
32	1.0	-88	5.2	-40	0.4	-95
96	0.0	-100	0.2	-98	0.0	-100
320	0.0	-100	0.0	-100	0.0	-100
960	0.0	-100	0.0	-100		
3200	0.0	-100	0.0	-100		

Negative controls: Water

Positive controls: Mitomycin C (MMC) was used as a positive control in the non-activated study at final concentrations of 0.3 and 0.6 µg/mL. Cyclophosphamide (CP) was used as the positive control in the presence of S9 at a final concentration of 20 and 40 µg/mL. One dose from each positive control was chosen for analysis.

Incubation and sampling times: For the preliminary toxicity, the cells were exposed to solvent control and 9 concentrations of test article for 4 hours both with and without S9 and for 20 hours in the absence of S9. Cells were incubated at 37°C and 5% CO₂ in air. After 4 hours treatment cells were washed with calcium and magnesium free phosphate buffered saline, re-fed and returned to the incubator for an additional 16 hours. Following treatments, cells were collected by centrifugation, fixed, stained and the number of mitosis per 500 cells scored was determined. Isolated cells were fixed on slides for analysis.

Results: The results of the definitive study are provided in the sponsor's Tables 4, 5 and 6 below. In each case, the highest concentration examined was associated with a mitotic index of just below 5 (50%) indicating that the concentrations examined produced significant toxicity. Although the positive controls produced a clear clastogenic effect, _____, under the conditions of the assay was not clastogenic.

TABLE 4
CYTOGENETIC ANALYSIS OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES TREATED WITH [REDACTED] IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

DEFINITIVE ASSAY: 4 HOUR TREATMENT, 20 HOUR HARVEST

Treatment (µg/mL)	Flask	Mitotic Index (%)	Cells Scored		% Aberrant Cells		Total Number of Structural Aberrations						Severely Damaged Cells	Average Aberrations Per Cell
			Numerical	Structural	Numerical	Structural	Gaps	Chromatid		Chromosome				
								Br	Ex	Br	Dic	Ring		
Water	A	9.0	100	100	0	0	0	0	0	0	0	0	0	0.000
	B	9.2	100	100	0	0	1	0	0	0	0	0	0	0.000
1.25	A	8.6	100	100	0	1	0	1	0	0	0	0	0	0.010
	B	8.8	100	100	0	0	0	0	0	0	0	0	0	0.000
2.5	A	7.2	100	100	0	0	0	0	0	0	0	0	0	0.000
	B	6.8	100	100	0	0	0	0	0	0	0	0	0	0.000
5	A	4.4	100	100	0	0	1	0	0	0	0	0	0	0.000
	B	4.6	100	100	0	0	0	0	0	0	0	0	0	0.000
MMC, 0.6	A	5.6	100	50	0	20	1	3	8	0	0	0	0	0.220
	B	6.2	100	50	0	22	0	5	7	0	0	0	0	0.240

TABLE 5
CYTOGENETIC ANALYSIS OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES TREATED WITH [REDACTED] IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

DEFINITIVE ASSAY: 4 HOUR TREATMENT, 20 HOUR HARVEST

Treatment (µg/mL)	Flask	Mitotic Index (%)	Cells Scored		% Aberrant Cells		Total Number of Structural Aberrations						Severely Damaged Cells	Average Aberrations Per Cell
			Numerical	Structural	Numerical	Structural	Gaps	Chromatid		Chromosome				
								Br	Ex	Br	Dic	Ring		
Water	A	9.8	100	100	0	0	0	0	0	0	0	0	0	0.000
	B	9.2	100	100	0	0	0	0	0	0	0	0	0	0.000
5	A	8.8	100	100	0	0	0	0	0	0	0	0	0	0.000
	B	9.0	100	100	0	0	0	0	0	0	0	0	0	0.000
10	A	7.8	100	100	0	0	2	0	0	0	0	0	0	0.000
	B	7.6	100	100	1	0	0	0	0	0	0	0	0	0.000
20	A	4.4	100	100	0	1	1	1	0	0	0	0	0	0.010
	B	4.8	100	100	0	1	1	1	0	0	0	0	0	0.010
CF, 20	A	6.8	100	100	0	14	0	12	4	0	0	0	0	0.160
	B	6.4	100	100	0	14	0	12	2	1	0	0	0	0.150

Treatment: Human peripheral blood lymphocytes were treated for 4 hours at 37 ± 1°C in the presence of an exogenous source of metabolic activation. Dose levels 35, 50, 65 and 75 µg/mL were not analyzed due to excessive toxicity.

Mitotic Index = number mitotic figures x 100/500 cells counted.

% Aberrant Cells: numerical includes polyploid and endoreduplicated cells; structural excludes cells with only gaps.

Chromatid breaks include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Ex) include quadriradials, triradials and complex rearrangements.

Chromosome breaks include breaks and acentric fragments; Dic, dicentric chromosome.

Severely damaged cells includes cells with one or more pulverized chromosomes and cells with 10 or more aberrations.

Average Aberrations Per Cell: severely damaged cells and pulverizations were counted as 10 aberrations.

TABLE 6
CYTOGENETIC ANALYSIS OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES TREATED WITH _____ IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

DEFINITIVE ASSAY: 20 HOUR TREATMENT, 20 HOUR HARVEST

Treatment (µg/mL)	Flesh	Mitotic Index (%)	Cells Scored		% Aberrant Cells		Total Number of Structural Aberrations						Severely Damaged Cells	Average Aberrations Per Cell	
			Numerical	Structural	Numerical	Structural	Gaps	Chromatid		Chromosome					
								Br	Ex	Br	Die	Ring			
Water	A	10.2	100	100	0	0	1	0	0	0	0	0	0	0	0.000
	B	10.8	100	100	0	0	0	0	0	0	0	0	0	0	0.000
2.5	A	8.8	100	100	0	0	0	0	0	0	0	0	0	0	0.000
	B	9.0	100	100	0	0	0	0	0	0	0	0	0	0	0.000
5	A	8.6	100	100	0	1	0	1	0	0	0	0	0	0	0.010
	B	8.4	100	100	0	1	0	1	0	0	0	0	0	0	0.010
10	A	5.2	100	100	0	1	1	1	0	0	0	0	0	0	0.010
	B	4.8	100	100	0	1	0	1	0	0	0	0	0	0	0.010
MMC, 0.3	A	6.0	100	50	0	26	1	2	11	0	0	0	0	0	0.260
	B	6.4	100	50	0	26	1	6	10	0	0	0	0	0	0.320

Treatment: Human peripheral blood lymphocytes were treated for 20 hours at 37 ± 1°C in the absence of an exogenous source of metabolic activation. An additional dose level of 1.25 µg/mL was tested as a safeguard against excessive toxicity at higher dose levels but was not required for microscopic examination. Dose levels 15, 20 and 25 µg/mL were not analyzed due to excessive toxicity.

Mitotic Index = number mitotic figures x 100/500 cells counted.

% Aberrant Cells: numerical includes polyploid and endoreduplicated cells; structural excludes cells with only gaps.

Chromatid breaks include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Ex) include quadriradials, triradials and complex rearrangements.

Chromosome breaks include breaks and acentric fragments; Dio, dicentric chromosome.

Severely damaged cells includes cells with one or more pulverized chromosomes and cells with 10 or more aberrations.

Average Aberrations Per Cell: severely damaged cells and pulverizations were counted as 10 aberrations.

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The Sponsor's criteria for a valid test noted that the frequency of cells with structural chromosome aberrations in the solvent controls must be within historical controls. For a positive response, the % of cells with chromosome aberrations in the positive control must be statistically increased relative to solvent control.

COMMENT: Statistical significance alone is not an adequate criterion for a valid study; however, the study does appear to be valid.

Study outcome: The overall results of the study are summarized in the table below, reproduced from the sponsor's study report.

Appears This Way
On Original

TABLE 7
SUMMARY

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations	
				Numerical	Structural	Numerical (%)	Structural (%)		
Water	-S9	4	9.1	200	200	0.000	±0.000	0.0	0.0
1.25	-S9	4	8.7	200	200	0.005	±0.071	0.0	0.5
2.5	-S9	4	7.0	200	200	0.000	±0.000	0.0	0.0
5	-S9	4	4.5	200	200	0.000	±0.000	0.0	0.0
MMC, 0.6	-S9	4	5.9	200	100	0.230	±0.468	0.0	21.0**
Water	+S9	4	9.5	200	200	0.000	±0.000	0.0	0.0
5	+S9	4	8.9	200	200	0.000	±0.000	0.0	0.0
10	+S9	4	7.7	200	200	0.000	±0.000	0.5	0.0
20	+S9	4	4.6	200	200	0.010	±0.100	0.0	1.0
CP, 20	+S9	4	6.6	200	200	0.155	±0.402	0.0	14.0**
Water	-S9	20	10.5	200	200	0.000	±0.000	0.0	0.0
2.5	-S9	20	8.9	200	200	0.000	±0.000	0.0	0.0
5	-S9	20	8.5	200	200	0.010	±0.100	0.0	1.0
10	-S9	20	5.0	200	200	0.010	±0.100	0.0	1.0
MMC, 0.3	-S9	20	6.2	200	100	0.290	±0.518	0.0	26.0**

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

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

Percent Aberrant Cells: *, p<0.05; **, p<0.01; using the Fisher's exact test.

Appears This Way
On Original

Conclusions and Recommendations

The DMF holder submitted two in vitro genetic toxicology studies for _____, a drug substance impurity that contains an _____ structure, i.e., a structural alert for mutagenicity. The results of this minimal genetic toxicology screen were negative for the _____ impurity. Therefore, as previously communicated to the sponsor, the levels of this impurity have been adequately qualified.

2.6.6.5 Carcinogenicity

Study title: A 2-Year Oral Gavage Carcinogenicity Study of Oxymorphone- HCl In The Albino Mouse.

Key study findings:

- A 2-year mouse carcinogenicity study was conducted in CD-1 mice with oxymorphone HCL administration by oral gavage using 10, 25, 75, and 150 mg/kg/day doses for males and females.
- The clinical signs of self-mutilation of the skin, excessive grooming, and skin lesions were noted. There was an increase in mortality at intermediate high and high dose in male mice. In addition, a decrease in body weight was noted in all treatment groups >10 mg/kg/day indicating MTD has been reached in this study. Findings of the gross pathological observation included dilation of the pelvis, ureters, and urinary bladder wall in male mice receiving 75 or 150 mg/kg/day of oxymorphone HCl at an incidence that was greater than either controls or lower dose groups. Protrusion of the penis, commonly associated with obstructive uropathy, was also increased in males at these doses (75 and 150 mg/kg/day). Similar changes in the urinary bladder and pelvis were noted in females at the high dose.
- Animals from the 75 and 150 mg/kg/day dosage groups showed increased incidence and severity of urogenital tract lesions consistent with obstructive uropathy. This incidence was more prominent in males than in females and was associated with preterminal morbidity. In male animals, some marginal increase was noted in transitional cell hyperplasia of the urinary bladder. In female animals an increase was noted in subepithelial edema and/or connective tissue thickening. The findings are treatment related and might be correlated with the primary pharmacological function associated with urinary retention in animals.
- Increased incidences of prostate and seminal vesicular inflammation were also noted in high and high-intermediate dose group male animals.
- No evidence of carcinogenic potential was observed in mice. Although not statistically significant nor dose-dependent, there was an increase incidence of malignant lymphoma in several treatment groups compared to controls. The

incidence was higher in females than males that may be due to the higher exposure of the compound in females.

- The dose for human will be titrated based on individual need. The systemic drug exposure (AUC ng•h/mL) at the 10 mg/kg/day in mice was 0.35x (in males) and 0.42x (in females) times the human exposure at a dose of 260 mg (the dose for human needs to be titrated based on individual needs).

Adequacy of the carcinogenicity study and appropriateness of the test model: CD-1 mice were dosed once daily by oral gavage with oxymorphone HCl at doses of 10, 25, 75, and 150 mg/kg/day for males and females. The test model (CD-1 mice) is appropriate because the mouse is a universal model routinely used for evaluating the toxicity and carcinogenicity of various classes of chemicals and for which there is a large historical database. The study was adequate because the study duration met the regulatory required duration for carcinogenicity studies (104 weeks), the doses evaluated were judged to have reached the MTD based on mortality in high dose group males and decrease in body weight in males and females.

Evaluation of tumor findings:

There was a greater incidence of malignant lymphoma in the low-mid dose and high-mid dose treatment groups compared to controls. However, the incidence in the high-dose group was below that of the control group, and therefore not dose-dependent or statistically significant. The historical control data from the [redacted] laboratories for male and female mice showed incidence of spontaneous malignant lymphoma is high 62/805 (7%) in males and 175/805 (21%) in females. In contrast, the historical control data from [redacted] laboratories show incidence of malignant lymphoma in males and females of 3/2874 (0.1%) and 317/3192 (9%) respectively.

Opioids have a well documented effect on the immune cells. In the present study a distinct change in the monocyte/lymphocyte percent was noted at termination. In the rat carcinogenicity study malignant lymphoma was noted.

Dr Moh Jee Ng reviewed the statistical analysis of the tumor findings, the tables (showing neoplastic data) incorporated in the review is adapted from DR. Ng's analysis.

Study no.: [redacted]. Project No: 77070

Volume # and page #: EDR; Page 3000-6149

Conducting laboratory and location: [redacted]

Date of study initiation: August 15, 2002

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: Mallinckrodt D04069 (NCH Sample ID: 103941A), Mallinckrodt C03785 ([redacted] Lot No.: 010232; [redacted] Sample ID 84503A); Purity: [redacted]

CAC concurrence: NO

Methods

Doses: Males and Females: 10, 25, 75, and 150 mg/kg/day.

Basis of dose selection (MTD, MFD, AUC etc.): The dose selection was based on MTD, and eCAC concurrence was obtained prior to study initiation.

Species/strain: — CD-1® (ICR) BR —————

Number/sex/group (main study): 65/ sex/group

Route, formulation, volume: Oral gavage, the compound was formulated in clear water ————— and 5 mL/kg/day was administered daily via oral gavage.

Frequency of dosing: Daily

Satellite groups used for toxicokinetics or special groups: 23/sex/group

Age: Approximately 6-8 weeks old.

Animal housing: 1/sex/ in suspended stainless steel cage with bedding, temperature 18-26°C and relative humidity 30-70%, a minimum of 10 air changes/hour 10%. Lighting cycle 12 hours light/12 hours dark per 24 hours. Water and food (rodent diet: ————— pellets) were given ad libitum.

Restriction paradigm for dietary restriction studies: None.

Drug stability/homogeneity: Dose formulations were determined by the Sponsor to be stable for — days at 0.01, 1 and 50 mg/mL under ambient conditions.

Stability of the compound in the dosing solutions was determined at intervals for at least —days of refrigeration (2 to 8°C) storage and 8 hours at room temperature. Dose analyses results indicated that all dose formulations were within ± 5% of target appropriate for use on study.

Dual controls employed: No, but 100 animals were used in the control group.

Study Design:

The protocol employed a dose-escalation phase in order reach higher exposures of the opioid. All the animals from the treatment group were dosed with 10 mg/kg/day in the first week of the study; this dose is known as a tolerated dose in mice. The dose escalation procedures is illustrated in the table below, reproduced from the sponsor's submission.

Group No. Identification	Dose Level (mg/kg/day)				Animal Number			
	Week 1	Week 2	Week 3	Weeks 4 - 104	Main Study		Toxicokinetic* [†]	
					Males	Females	Males	Females
1. Vehicle Control	0	0	0	0	1001-1048, 1150-1101	1501-1518, 1520-1598, 1600-1602	-	-
2. Oxymorphone Hydrochloride	10	10	10	10	2001-2010, 2012-2065, 2089	2501-2565	2066-2088	2566-2583, 2585-2589
3. Oxymorphone Hydrochloride	10	25	25	25	3001-3065	3501-3511, 3513-3565, 3590	3066-3088	3566-3588
4. Oxymorphone Hydrochloride	10	25	75	75	4001-4065	4501-4565	4066-4088	4566-4588
5. Oxymorphone Hydrochloride	10	25	75	150	5001-5015, 5017-5036, 5038-5065, 5089, 5090	5501-5565	5066-5088	5566-5588
6. Health Screen	0	0	0	0	6001-6010	6501-6510	-	-

* euthanized following Week 26 toxicokinetic blood collection

[†] a reserve of 5 animals/sex/group was maintained until Week 26 to be sampled from in the event of unexpected deaths during the toxicokinetic sampling schedule

Interim sacrifices: None

Deviations from original study protocol: None

Observation times:

Mortality: The animals were observed twice daily for mortality and moribundity.

Clinical signs: The animals were observed twice daily for clinical signs.

Body weights: Body weights were recorded once pre treatment and weekly throughout the treatment period.

Food consumption: Food consumption was measured individually and recorded weekly throughout the study.

Histopathology: Peer review: Yes;

Toxicokinetics: During Week 26 of the treatment period, blood samples (0.5 mL) were collected into heparinized tubes from 5 toxicokinetic animals/sex/Groups 2-4/time point at nominally 0 (predose), 0.5, 1, 2, 4, 8, 16, and 24 hours post dose.

Results

Mortality:

As shown in the table below the mortality rate for male mice was higher than the controls in the 75 and 100 mg/kg/day treated groups from Week-78. However, a dose related increase in the survival rate was observed in the male mice treated 10 and 25 mg/kg/day from Week-79. At Week-103, the number of male mice survived from the high, medium high, medium low, low and control dose group was 37, 42, 65, 63, and 50 respectively. The increase in the survival rate was observed in the female mice from Week 53. This trend for the higher survival rate in the female rats (see table below) continued up to the end of the experimental period. At Week 103, the number of female mice survived from the high, medium high, medium low, and control dose group was 46, 55, 52, 57, and 38 respectively.

An increase in the incidence of obstructive uropathy and renal inflammatory changes was described as major cause of death in male mice. In female mice there was some evidence of an treatment effect at the highest dosage level, the major cause of death is histiocytic sarcoma which was observed at a higher incidence in control and the malignant lymphoma the incidence of which may be considered as dose related (5.8/65, 8/65, 11/65, 10/65, and 12/65 in the control, low, low mid, high mid, and high dose respectively) in females.

Other spontaneous causes of death were hepatocellular tumors and amyloidosis. A reduction in amyloidosis as a cause of death was present in male animals at the higher dose levels.

Cumulative Survival (%) in Mice

Sex	Male					Female				
	CD	LD	MIL	MHI	HID	CD	LD	MIL	MHI	HID
	0	10	25	75	150	0	10	25	75	150
oxycodone HCl (mg/kg/day)										
Weeks 0 - 52	96	99	94	92	92	97	97	94	91	92
53 - 78	73	86	88	80	69	76	85	86	86	79
79 - 91	62	75	79	57	54	54	71	80	77	71
92 - 103	50	63	65	42	37	38	57	52	55	46

Appears This Way
On Original

Major Factors Contributory to the Deaths of Preterminal Decedent Mice

	Males					Females				
	1	2	3	4	5	1	2	3	4	5
Mice per group	100	65	65	65	65	100	65	65	65	65
Number of preterminal deaths	50	26	24	39	42	62	30	33	30	36
Cause of death not determined	5	3	5	10	7	6	3	4	1	9
Neoplastic lesions										
Lymphoma/leukocytic sarcomata	8	4	5	5	1	19	11	9	10	12
Pulmonary tumours	7	3	—	—	—	3	3	—	3	—
Liver tumours	3	2	2	1	—	—	—	—	—	—
Mammary tumours	—	—	—	—	—	2	—	1	4	2
Hemangiosarcomata	3	—	—	—	—	1	—	1	—	1
Other neoplasms	1	—	—	—	—	7	4	3	4	2
Non-neoplastic lesions										
Amyloidosis	13	4	3	4	2	2	3	5	3	1
Obstructive uropathy	6	7	7	12	26	—	—	—	—	3
Glomerulonephropathy etc.	1	1	—	—	—	7	1	1	—	1
Pyelitis/pyelonephritis etc.	—	1	2	2	4	—	—	1	1	3
Other non-neoplastic lesions	3	1	—	5	2	15	5	8	4	2

Clinical signs:

Best Possible Copy

All treatment groups displayed clinical signs typical of a potent opioid. These include dilated pupils, circling, self-biting, excess grooming, licking and/or scratching. Other treatment-related clinical signs included oily fur (primarily cervical, cranial and interscapular) and yellow fur staining (many sites). An increased incidence of protruding penis and other urogenital findings (skin lesions, swelling, redness of penis, prepuce and/or scrotum) in treated males were likely related to pharmacologically-mediated urinary retention. Clinical signs (thin fur cover, severed tail, various skin lesions/scabs) were also observed, these changes might have resulted from the self mutilation.

Body weights:

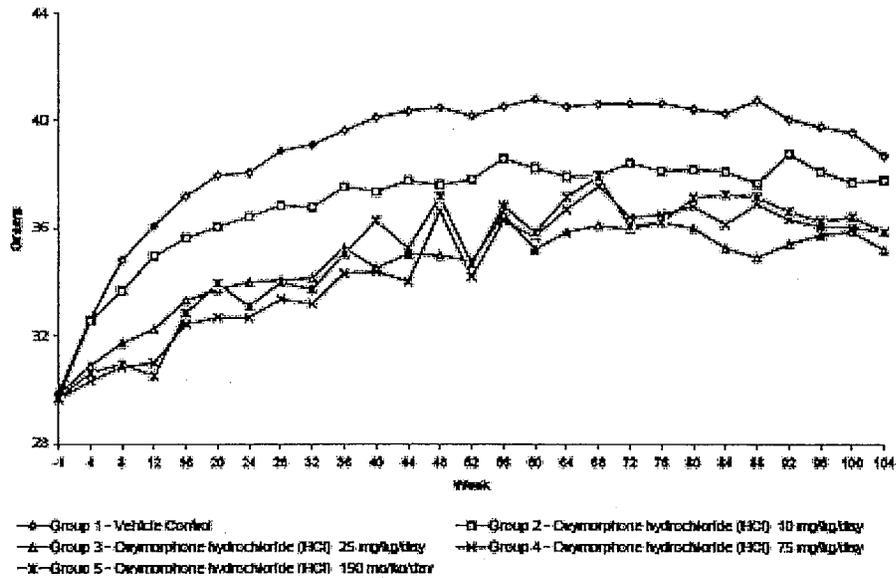
Oxymorphone treatment resulted in statistically significant reductions in mean body weight compared to controls generally throughout the treatment period at all doses. In males, mean body weight at Week 104 was 98%, 91%, 93% and 93% of controls at 10, 25, 75 and 150 mg/kg/day, respectively. Corresponding mean absolute body weight gain over the treatment period (Week -1 to 104) was 94%, 63%, 72% and 78% of the controls. In females, mean body weight at Week 104 was 91%, 87%, 87% and 86% of controls at 10, 25, 75 and 150 mg/kg/day, respectively. Corresponding mean absolute body weight gain over the treatment period (Week -1 to 104) was 74%, 65%, 66% and 64% of the controls.

Mean Body Weight (%) for MICE

Sex	Dose Groups	Mean Body Weight (grams)		Mean Body Weight Gain (MBWG)	% Differences in MBWG
		Beginning Study (week 1)	End of Study (week 104)		

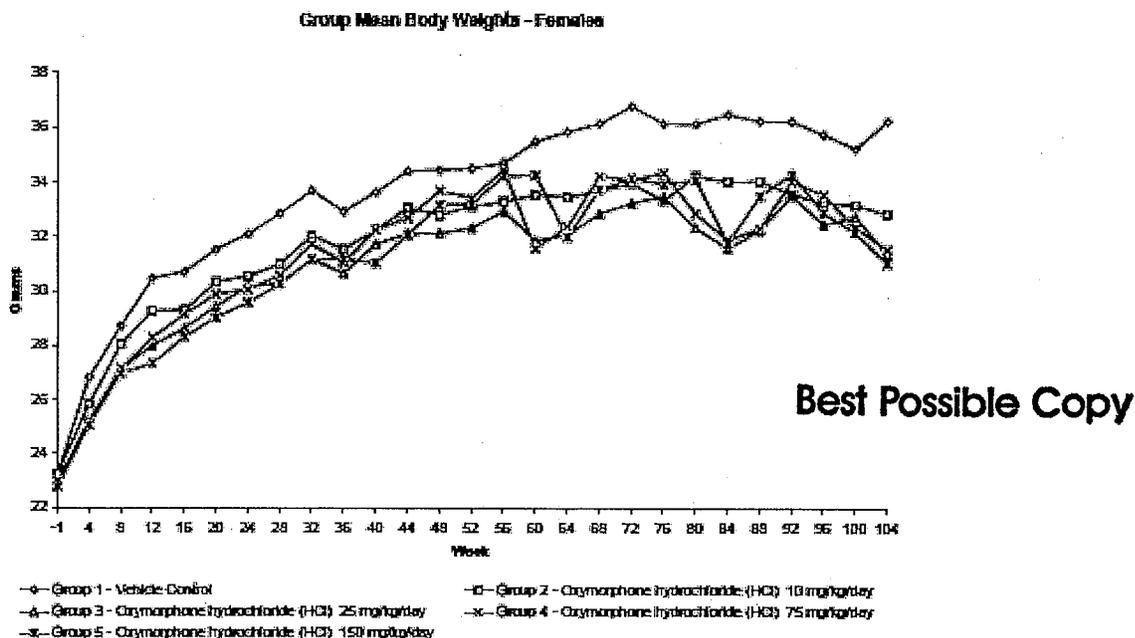
Male	0 mg/kg/day	30.76	38.71	7.95	
	10 mg/kg/day	29.80	37.81	8.01	1
	25 mg/kg/day	29.97	35.22	5.25	-34
	75 mg/kg/day	30.22	35.87	5.65	-29
	150 mg/kg/day	30.16	35.89	5.73	-28
Female	0 mg/kg/day	23.77	36.23	12.46	
	10 mg/kg/day	22.76	32.82	10.06	-19
	25 mg/kg/day	22.49	31.37	8.88	-29
	75 mg/kg/day	23.19	31.53	8.34	-33
	150 mg/kg/day	22.19	31.02	8.83	-29

Group Mean Body Weights - Males



Best Possible Copy

Appears This Way
On Original



Food consumption:

The changes in food consumption in males and females were variable throughout the experimental period. There was a statistically significant (P=0.05-0.001) decrease in food consumption in males and females between Week 5 and 14 in all dose groups. The food consumption was close to the control animals after Week 20 in both males and females from all dose groups. The food consumption decreased again significantly in all treatment groups around Week 50-62. No statistically significant differences in the food consumption between treatment groups from Week 70 until the end of the experimental period.

Hematology:

The hematological parameters were assessed at Week-52 and Week-103. As evident from the table below there were not much changes in the WBC --related parameters in Week-52. There was a significant decrease in monocyte counts at termination in both males (60%) and females (51%). A decrease in lymphocytes was also apparent at termination in males and females. The decrease in lymphocytes and monocytes were also seen at Week-52 but the changes were less prominent. An increase in the mean neutrophil percent was seen in males and females at Week 52 and Week 103; however, the changes were more prominent at termination. Circulating neutrophils are known to increase with stress which may be associated with long term administration of the compound. The decrease in monocytes and lymphocytes may be associated with the known effect of morphine and the related compounds on the immune system. The clinical relevance of the findings is not known.

Hematological Changes at Week 52

Parameters	Low		Medium Low		Medium High		High	
	Male	Female	Male	Female	Male	Female	Male	Female
Neutrophil (%)	NC	NC	N3↑	4NC↑	6↑	9↑	14↑	12↑

Lymphocyte (%)	NC	6↓	N4↓	N5↓	9↓	6↓	10↓	8↓
Monocyte (%)	NC	NC	NC	NC	NC	NC	32↓	NC

NC: No change

Hematological Changes at Week 103

Parameters	Low		Medium Low		Medium High		High	
	Male	Female	Male	Female	Male	Female	Male	Female
Neutrophil (%)	14↑	7↑	16↑	11↑	24↑	9↑	25↑	21↑
Lymphocyte (%)	12↓	NC	13↓	15↓	20↓	14↓	21↓	15↓
Monocyte (%)	29*↓	27*↓	29*↓	24*↓	52*↓	29*↓	60*↓	51*↓

NC: No change

*Statistically significant P=0.001

Ophthalmoscopy:

There were no ophthalmological findings in mouse.

Gross pathology:

Dilation of the pelvis, ureters, and urinary bladder wall was observed in male mice receiving 75 or 150 mg/kg/day of oxymorphone HCl than in controls or lower dose groups. Protrusion of the penis, commonly associated with obstructive uropathy, was also increased in males at this dose. Similar changes in the urinary bladder and pelvis were noted in females at high dose.

In male mice receiving 75 or 150 mg/kg/day of oxymorphone HCl a reduction in thymic size was recorded more frequently than in control or lower dose group animals.

**Appears This Way
On Original**

Principal Treatment-related or Possibly Treatment-related Gross Changes Observed at Necropsy

	Males					Females				
	1	2	3	4	5	1	2	3	4	5
Mice per group	100	65	65	65	65	100	65	65	65	65
Number of preterminal deaths	50	26	24	39	42	63	30	33	30	36
(a) Increases in incidence										
Kidney - pelvic dilatation	13	10	18	28	32	4	6	4	13	16
Ureter - dilatation	16	21	24	36	37	1	9	4	14	19
Urinary bladder - dilatation	19	18	19	37	50	4	3	3	12	25
Urinary bladder - thickening	8	11	11	20	13	4	8	17	23	18
Penis - protrusion	7	10	8	14	18					
Thyroid - small	31	25	25	42	44	23	19	17	25	22
(b) Decreases in incidence										
Cecum - dark foci	18	6	1	1	—	8	3	1	2	—
Liver - mass	33	10	10	5	6	6	1	3	1	1
Lung - mass	16	7	4	5	2	6	13	3	6	1
Lung - nodule	19	15	17	12	6	15	11	8	13	10
Spleen - enlargement	31	13	10	8	10	56	23	15	18	20
Lymph node - enlargement	19	16	12	14	12	35	23	16	17	16
LN (mandibular) - enlargement	12	3	3	3	2	8	5	6	5	5
LN (mesenteric) - enlargement	17	11	7	9	3	14	13	8	9	9
Prostate - pale areas	27	14	11	9	8					
Ovary - cyst						67	50	52	50	37
Ovary - mass						23	12	9	6	4
Uterus - mass						30	17	13	1	4
Uterus - thickening						18	2	4	2	1

Histopathology:

Best Possible Copy

Neoplastic

There was an apparent increased incidence of malignant lymphoma in treatment group in general compared to controls. The incidence was higher in females than males may be due to the higher exposure of the compound in females. The finding is not always dose related, nor statistically significant. The historical control data from the _____ laboratories for male and female mice showed incidence of spontaneous malignant lymphoma is high 62/805 (7%) in males and 175/805 (21%) in females. The historical control data from the _____ laboratories show incidence of malignant lymphoma in males and females are 3/2874 (0.1%) and 317/3192 (9%) respectively. Morphine and related substances have well documented effects on immune cells. In the present study a distinct change in the monocyte/lymphocyte percent was noted at termination. In the rat study malignant lymphoma was also noted. The present finding was not statistically significant and discussed by the sponsor as a common tumor finding as noted in the historical control data. As mentioned above the compound has known pharmacologic effect on the hematological cell population, a higher incidence of malignant lymphoma is noted in treatment groups compare to controls, and similar findings were noted in other species.

Tumor	CD	LD	ML	MH	HD
Oxymorphone	0	10	25	75	100

HCl (mg/kg/day)	mg/kg/day (incidence %)	mg/kg/day (incidence %)	mg/kg/day (incidence %)	mg/kg/day (incidence %)	mg/kg/day (incidence %)
Number of animals examined	100	65	65	65	65
Percent HEMOLYMPHORET- ICULAR TISSUE/malignant lymphoma: FEMALE	13 13%	8 12%	11 17%	10 15%	12 12%
Percent HEMOLYMPHORET- ICULAR TISSUE/malignant lymphoma: MALE	9 9%	3 5%	7 13%	5 11%	1 1.5%

Source data: dataset received on 3/22/2006, analysis data RIM1M and F56919

†: p-value presents for dose groups CD, LD, MD and HD trend.

Non Neoplastic:

Best Possible Copy

Animals from the 75 and 150 mg/kg/day dosage groups showed increased incidence and severity of urogenital tract lesions consistent with obstructive uropathy.

These animals were found to have renal pelvic dilatation, renal tubular dilatation, pyelitis/pyelonephritis, dilatation of the ureters with or without inflammation, and dilatation of the urinary bladder with or without inflammation. This incidence was more prominent in males than in females and was associated with preterminal morbidity. In male animals some marginal increase was noted in transitional cell hyperplasia of the urinary bladder, although this may have been a secondary response to dilatation, inflammation and urinary retention. In female animals an increase was noted in subepithelial edema and/or connective tissue thickening. The findings are treatment related and might be correlated with the primary pharmacological function associated with urinary retention in animals.

Increased incidences of prostate and seminal vesicular inflammation were also noted in high and high-intermediate dose group male animals.

**Appears This Way
On Original**

Best Possible Copy

Incidence of Major Urogenital Lesions

	Males					Females				
	1	2	3	4	5	1	2	3	4	5
Mice per group	100	65	65	65	65	100	65	65	65	65
Number of preterminal deaths	50	36	24	39	42	62	30	33	30	36
Kidney										
Pyelitis/pyelonephritis	3	4	5	13	16	3	—	1	2	5
Tubular dilatation	—	2	6	8	9	28	8	5	9	9
Pelvic dilatation	16	17	21	31	37	4	10	4	16	18
Ureter										
Dilatation	12	18	18	28	28	1	8	4	12	14
Inflammation	—	2	1	3	1	—	—	—	2	3
Urinary Bladder										
Dilatation	19	16	18	36	49	4	1	1	10	19
Hyperplasia: transitional epithelium	7	6	5	12	10	1	2	1	2	3
Edema/subepithelial thickening	1	4	3	5	1	1	3	2	17	13
Inflammation	2	3	1	7	6	3	1	1	1	3
Prostate										
Inflammation	9	9	5	16	26					
Seminal Vesicle										
Inflammation	5	4	2	12	11					

Toxicokinetics: The exposure especially the C_{max} was higher in all dose groups in females compare to that of males. The increase in C_{max} and AUC is generally dose proportional however, at lower doses the increase is more than dose proportional. An increase in dose from 10-25 mg/kg/day (2.5 fold) gave rise to about 4 fold increase in C_{max} and AUC in males. In females, an increase in dose from 10-25 mg/kg/day (2.5 fold) gave rise to about 8 and 5 fold increase in C_{max} and AUC respectively. A 6 fold increase in dose in males, (from 25-150 mg/kg/day) resulted in 6 and 10 fold increase in C_{max} and AUC respectively. A 6 fold increase in dose in females, (from 25-150 mg/kg/day) resulted in 5 and 12 fold increase in C_{max} and AUC respectively.

Summary of Toxicokinetics Findings:

Parameters	Low (10 mg/kg)		Medium Low (25 mg/kg)		Medium High (75 mg/kg)		High (150 mg/kg)	
	Male	Female	Male	Female	Male	Female	Male	Female
C _{max} (ng/mL)	37	31	142	248	325	432	919	1249
T _{max} (hr)	1	2	1	1	1	1	1	1
AUC (0-24 h) ng.h/mL	98	116	371	523	1040	1758	4002	4767

Study title: A 2-Year Oral Gavage Carcinogenicity Study Of Oxymorphone- HCl In The Albino Rats

Key study findings:

- A 104 week carcinogenicity study was performed in SR rats by oral gavage. The doses administered in the male and females rats were 2.5, 5, and 10 mg/kg/day

and 5, 10, and 25 mg/kg/day respectively. There was a decrease in the body weight ≥ 10 gm in all doses in males and females indicating that the MTD has been achieved in this experiment.

- Ophthalmological and histopathological observation noted increased eye lesions including corneal inflammation and retinal degeneration in all doses in females and mid and high dose in males, the incident is considered treatment related.
- Gross pathological findings included pale and raised area in lungs in both males and females, histopathological findings in lung include increased histiocyte infiltration and increase incidence of granuloma/inflammation in both males and females. The lung is a target organ for the compound and the findings observed are dose related, therefore, the effect is considered treatment related.
- Histopathological findings in skin showed dose related increase in the incidence of ulceration and scabs which may have resulted from self mutilation and excessive licking as observed in the clinical signs; the finding is considered treatment related. The clinical signs observed are known non clinical manifestations of morphine and related compounds; however, the clinical relevance of the findings is not known.
- Neoplastic lesion findings include malignant lymphoma in male rat (1/100 in control vs 4/65 in 10 mg/kg), kidney tubular cell adenoma in males rats (0/100 in control vs 2/65 at high dose), all body leiomyoma in female rats (0/100 in control vs 3/65 at high dose) and all body squamous cell carcinoma in males (2/100 in control, 4/65, 2/65, and 4/65 at low, mid, and high dose respectively) and females (0/100, 2/65, 2/65, and 3/65 in control, low, mid, and high dose respectively), and all body leiomyoma in females (0/100 in control vs 3/65 at high dose). The lymphoma and adenoma in the tubular cell in the kidney and the skin carcinoma is considered as neoplastic finding related to the target organ of toxicity and historical control data showed that the tumors observed in the above mentioned organs are in the borderline range and can not be considered as common tumor.
- The dose for human will be titrated based on individual need. For the opioid tolerant patient maximum dose tested was 260 mg AUC (ng.h/mL) = 273. At 10 mg/kg in rat different tumor incidences were noted. At 10 mg/kg the AUCs (ng.h/mL) were 93 and 415 in male and female rats respectively. The safety ratio in human exposure for tumor incidences compare to rat is 0.34x and 1.5x in males and females respectively.

Moh Jee Ng reviewed the statistical analysis of the tumor findings, the tables (showing neoplastic data) incorporated in the review is adapted from Ng's analysis.

Study no.: ~~██████████~~ Project No: 77069

Volume # and page #: EDR; Page 1-3000

Conducting laboratory and location: ~~██████████~~

Date of study initiation: July 25, 2002

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: ~~██████████~~ D04069 (~~██████████~~ Sample ID: 103941A),

_____ C03785 (_____ Lot No.: 010232; _____ Sample
ID 84503A); Purity: _____

CAC concurrence: NO

Methods

Doses: Males: 2.5, 5, 10 mg/kg/day. Females: 5, 10, 25 mg/kg/day

Basis of dose selection (MTD, MFD, AUC etc.): The dose selection was based on MTD.

Species/strain: Sprague Dawley Rats

Number/sex/group (main study): 65/sex/group

Route, formulation, volume: Oral gavage, the compound was formulated in clear water (_____) and 5 mL/kg/day was administered daily via oral gavage

Frequency of dosing: Daily

Satellite groups used for toxicokinetics or special groups: 12/sex/group

Age: Approximately 6-8 weeks old

Animal housing: 1/sex/ in suspended stainless steel cage with bedding, temperature 18-26°C and relative humidity 30-70%, a minimum of 10 air changes/hour 10%. Lighting cycle 12 hours light/12 hours dark per 24 hours. Water and food (rodent diet: _____ Teklad pellets) were given ad libitum.

Restriction paradigm for dietary restriction studies: None.

Drug stability/homogeneity: Dose formulations were determined by the Sponsor to be stable for _____ days at 0.01, 1 and 50 mg/mL under ambient conditions. Stability of the compound in the dosing solutions was determined at intervals for at least _____ days of refrigeration (2 to 8°C) storage and 8 hours at room temperature. Dose analyses results indicated that all dose formulations were within $\pm 5\%$ of target appropriate for use on study

Dual controls employed: No, but 100 animals were used in the control group.

Study Design:

Group No. Identification	Dose Level (mg/kg/day)		Number of Animals			
	Males	Females	Main Study		Toxicokinetic*	
			Males	Females	Males	Females
1. Vehicle Control	0	0	100	100	-	-
2. Oxymorphone-HCL - Low Dose	2.5	5	65	65	12	12
3. Oxymorphone-HCL - Mid Dose	5	10	65	65	12	12
4. Oxymorphone-HCL - High Dose	10	25	65	65	12	12
5. Health Screen	-	-	10	10	-	-

* euthanized following Week 26 toxicokinetic blood collection

Interim sacrifices: None

Best Possible Copy

Deviations from original study protocol: None

Observation times:

Mortality: The animals were observed twice daily for mortality and moribundity.

Clinical signs: The animals were observed twice daily for clinical signs.

Body weights: Body weights were recorded once pre treatment and weekly throughout the treatment period.

Food consumption: Food consumption was measured individually and recorded weekly throughout the study.

Histopathology: Peer review: Yes;

Toxicokinetics: During Week 26 of the treatment period, blood samples (0.5 mL) were collected into heparinized tubes from 3 toxicokinetic animals/sex/time point at nominally 0 (predose), 0.5, 1, 2, 4, 8, 12, and 24 hours post dose.

Results

Mortality:

As shown in the table below the survival rate for male rats was higher in the controls than that of the treated groups up to Week-78. However, a dose related increase in the survival rate was observed in the male rats from Week-79. At Week-103, the number of male rats survived from the high, medium, low and control dose group was 60, 48, 43, and 38 respectively. The increase in the survival rate was observed in the female rats from Week 53. This trend for the higher survival rate in the female rats (see table below) continued up to the end of the experimental period. At Week 103, the number of female rats survived from the high, medium, low and control dose group was 57, 40, 34, and 30 respectively. A decreasing trend in the number of chronic progress nephropathy was observed in the treated male rats when compared to that of the

controls. The treated male and female rats showed a decrease in the number of the pituitary tumors compared to those of the controls. The cause of death could not be determined in 44 males and 33 females. The causes of death for the rest of the animals were spontaneous in nature.

Mortality (%) in Rats

Oxymorphone-HCl (mg/kg/day)	Male				Female			
	(C)	1.0	2.5	5.0	(C)	1.0	2.5	5.0
Weeks 0-52	4	12	38	9	5	3	5	3
53-78	15	21	25	21	32	17	25	11
79-91	37	35	38	28	51	46	43	32
92-103	62	57	52	40	70	66	60	43

Cause of Death:

Best Possible Copy

	Males				Females			
	1	2	3	4	1	2	3	4
Rats per group	100	65	65	65	100	65	65	65
Number of pre-terminal deaths	64	38	35	27	71	43	40	28
Cause of death not determined	13	13	12	6	13	6	9	5
Neoplastic lesions								
Lymphoma/histiocytic sarcoma	2	1	2	5	3	—	1	—
Pituitary tumors	20	5	9	6	35	25	20	12
Mammary tumors	—	—	—	—	9	4	4	7
Subcutaneous tumors	5	3	4	3	1	1	—	1
Uterine tumors	—	—	—	—	1	3	2	—
Other neoplasms	7	8	2	4	5	1	2	—
Non-neoplastic								
Chronic Progressive Nephropathy	10	3	—	—	—	1	—	—
Cardiomyopathy	1	2	1	—	—	—	—	—
Pyelitis/pyelonephritis	—	—	1	1	—	—	—	—
Other non-neoplastic	4	1	4	2	2	1	2	1
Accidental deaths	1	2	—	—	2	1	—	2

Clinical signs:

There were no dose related changes in the clinical signs in rats. The behavioral changes observed in the treated animals were excessive licking/self biting causing swollen paw, wet fur, thin fur cover, and skin scabs. These clinical signs are related to the pharmacological effect of Oxymorphone HCl. The biological significance of the findings is yet to be determined.

Body weights:

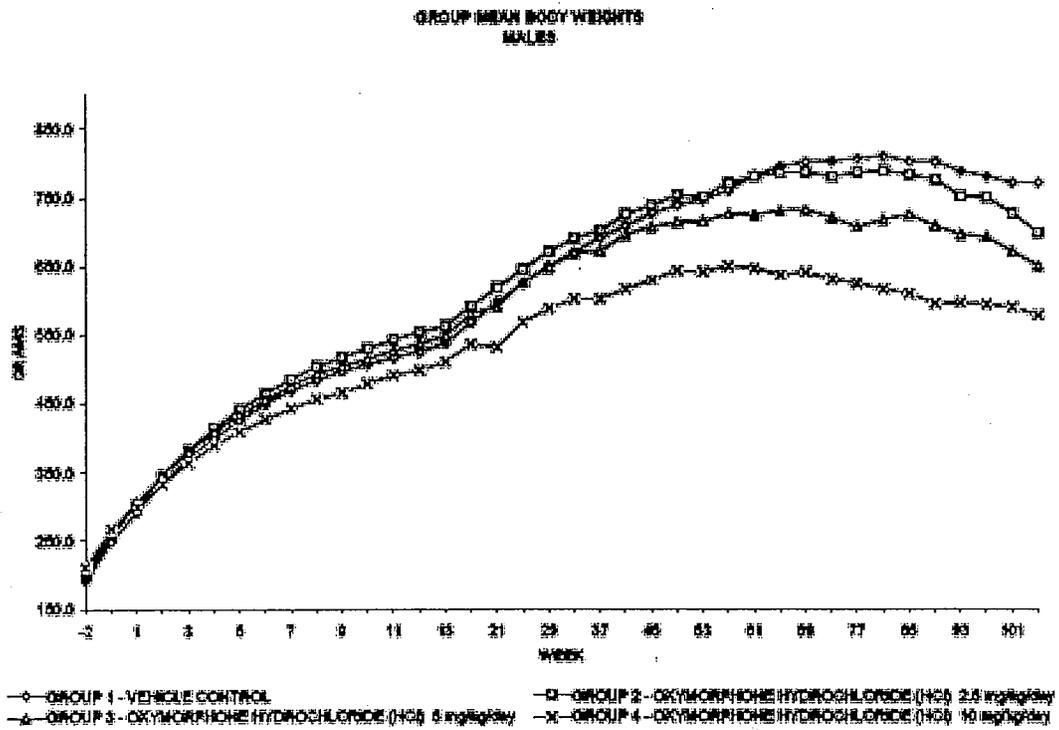
A dose dependent decrease in the mean body weights were observed in both males and females as shown in the table below. A 10% decrease in body weight was observed in males and females at 2.5 and 5 mg/kg/day dosing at the end of the experimental period (Week-103). The decrease in body weight gain noted in both sexes was believed by the Sponsor to have contributed to the higher survival rate of treated animals when compared with the control animals. The

present reviewer agreed with these observations based on the data from different studies reviewed before.

Mean Body Weight (%) for Rats

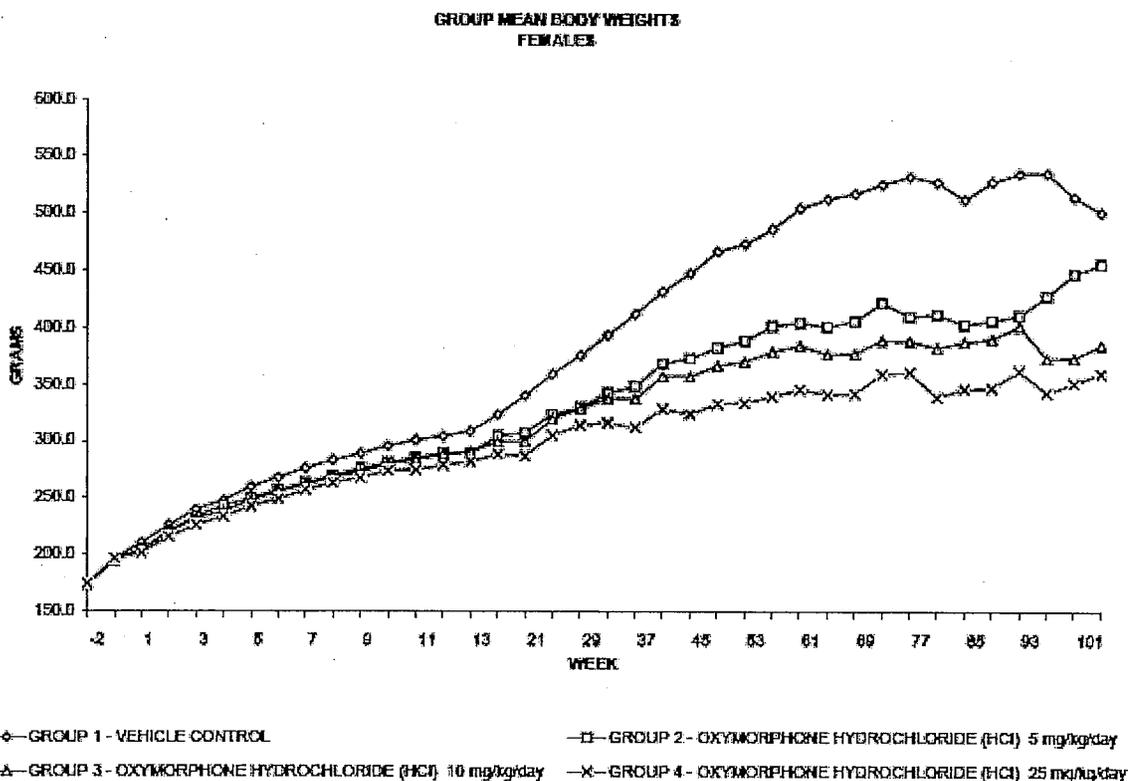
	Dose Groups	Mean Body Weight (grams)		% Differences in MBW
		Beginning Study (week 0)	End of Study (week 104)	
Male	0 mg/kg/day	291.2	770.0	
	2.5 mg/kg/day	302.1	697.9	-10
	5 mg/kg/day	305.6	649.9	-16
	10 mg/kg/day	299.3	578.1	-25
Female	0 mg/kg/day	210.3	501.0	
	5 mg/kg/day	204.2	455.8	-10
	10 mg/kg/day	204.5	385.2	-24
	25 mg/kg/day	201.5	360.1	-29

Appears This Way
On Original



Best Possible Copy

Appears This Way
On Original



Food Consumption:

There was a statistically significant decrease in food consumption <15% in females from all dose groups at Week-7. The decrease in food consumption after Week-7 was sporadic in female rats. In male rats at 10 mg/kg a statistically significant decrease in food consumption was noted up to Week-8 and at Week 56-85. The changes in food consumption in male rats at other times were sporadic. The decrease in food consumption in male and female rats might be related to the decrease in body weight.

Hematology:

The hematological parameters were assessed at Week-52 and Week-103. A statistically significant increase in the percentages of neutrophil was observed in males (5 and 10 mg/kg/day) and females (5, 10, and 25 mg/kg/day) at Week- 52. At termination this increase was still apparent in female rats at 25 mg/kg/day. There was a decrease in percentages of lymphocytes and monocytes in male and female rats at mid and high dose at Week-52. At termination female rats still showed decrease in the WBC percentages at high dose. The increase and decrease in the percentages of the WBCs are within the historical control range. Due to the statistically significant changes in these parameters, these findings are considered treatment related by the

reviewer. Morphine and its metabolites are known to have substantial effects on the immune system. However, potential clinical significance of these data is not clear.

Hematological Changes at Week 52

Parameters	Low Dose (mg/kg/day)		Medium Dose (mg/kg/day)		High Dose (mg/kg/day)	
	Male 2.5	Female 5	Male 5	Female 10	Male 10	Female 25
Neutrophil (%)	NC	37*↑	35*↑	37*↑	38*↑	58*↑
Lymphocyte (%)	NC	9↓	7↓	8↓	11↓	14↓
Monocyte (%)	NC	15*↓	15↓	30*↓	21*↓	31*↓
Eosinophil (%)	18↓	NC	18↓	NC	22↓	NC
Basophil (%)	19↓	NC	25↓	NC	27↓	NC

NC=No Change

Hematological Changes at Week 103

Parameters	Low Dose (mg/kg/day)		Medium Dose (mg/kg/day)		High Dose (mg/kg/day)	
	Male 2.5	Female 5	Male 5	Female 10	Male 10	Female 25
Neutrophil (%)	NS	9↑	NS	9↑	NS	30*↑
Lymphocyte (%)	NS	6↓	NS	7↓	NS	22*↓
Monocyte (%)	NS	28*↓	NS	35*↓	NS	37*↓

*Statistically significant, P=0.001

NC-No Change

Ophthalmoscopy:

An indirect ophthalmoscopy and biomicroscopic examination was performed on all animals at Week-53. There was an increased incidence of superficial punctate keratopathy (corneal opacity) in female rats at high dose (25 mg/kg/day). An increase in the incidence of diffuse retinal degeneration was also noted in the mid (10 mg/kg/day) and high dose in both female and male rats. Peer review of the ophthalmoscopic observations indicated that the findings might be related to the drug induced inhibition of the blink response. Also, these observations are related to the histological findings of increased acute to chronic corneal inflammation in high dose males and females and retinal atrophy in mid and high dose animals. The findings are treatment related; however, the toxicological significance of the findings is not known.

Summary of Ophthalmological Findings:

Parameters	Control		Low		Medium		High	
	Male	Female	Male	Female	Male	Female	Male	Female
Superior Punctate	18	-	26	33	87	75	57	50

Keratopathy								
Diffuse Retinal Degeneration	-	22	-	22	-	37	-	25

Gross pathology:

A dose related increased incidence of skin ulceration (males and females) and skin scab (females only) was recorded in animals given oxymorphone HCl at all dose levels compared with control group (see table below). These changes may be related to the clinical behaviors observed (excessive licking and self-biting/self mutilation). This finding is considered treatment related by the reviewer. An increased incidence of pale area and/or raised area was observed in the lung at necropsy in males and females given ≥ 10 mg/kg/day (high dose males and mid and high dose females) compared to the respective control group. There is no histological correlation of this finding. Lung, however, is a known target organ for morphine and respiratory distress following morphine administration is evident clinically and non clinically from the published literature. Therefore, this gross pathological finding in lung is considered treatment related and might have resulted from the pharmacodynamics effect of the compound.

Histopathology:**Neoplastic:**

A positive trend test for uterine endometrial polyp (females), malignant lymphoma (males) and renal tubular adenoma (males) with $p=0.0328$, $p=0.0264$ and $p=0.0414$, respectively was observed. The Sponsor mentioned that these tumors are considered as common tumors; therefore, this increasing trend was not considered statistically significant (nominal level of significance used when applying a trend test for common tumors is 0.005).

The historical control data from the ██████████ laboratory shows that in the control animals from thirty studies where 2146 kidneys were examined, a lesion related to the adenoma was found in nine kidneys in the male rats. The percentage ranges from 1.33-4.0. The historical control from the ██████████ laboratory where 647 control animals were examined, three kidneys showed adenoma of the tubular cells. The percentage ranges from 0.46-1.54. The reviewer believes that there is possibility that this neoplastic lesion might have been resulted from the secondary pharmacodynamics effect of the compound. The urinary retention is well documented in animals after morphine administration and has been noted in the gross pathological observation with this compound under the present experimental condition. The finding although might not be considered as rare tumor and therefore is not statistically significant should be presented in the label.

The reviewer concurs that the uterine polyp is a common tumor finding as evident by the historical control data from the ██████████ Laboratory (70/2143) and ██████████

laboratories (52/647). Since there is no morphine related pharmacological or toxicological findings noted under this experimental condition and with morphine in the public literature the occurrence uterine polyp may be considered as incidental.

The incidence of malignant lymphoma observed was 3/65 males from the high dose (10 mg/kg/day) in the pre terminal phase. No malignant lymphoma was noted in control group at this phase of the study. At terminal sacrifice 1/100 and 1/65 male rats were observed to have malignant lymphoma in the control and 10 mg/kg/day dose group respectively. In the historical control data from the _____ Laboratories 36 lymphomas were identified in 30 studies, the percentage of the occurrence of this lesion was 1.68% and the incidence was found to range between 0.91-6.0 percent. In the historical control data from the _____ laboratories 10/647 animals were found to have malignant lymphoma. The percentage of the occurrence of this lesion was 1.55 and it ranges between 0-2.67. Due to the nature of the lesion and well documented pharmacological effect of morphine on the lymphocytes, the present reviewer believes that this finding is related to the target organ effect of the compound. The current study also, demonstrated the effect of the compound on neutrophils, monocytes and lymphocytes. Therefore, this finding is recommended to be incorporated in the label. There were no changes in the incidence of malignant lymphoma formation in females compared to those of the controls.

There was an increase incidence of all body squamous cell carcinoma in both male and females; there was an increase incidence of the skin squamous cell carcinoma. The effect of the compound on skin was observed in the clinical signs as well as in the histopathological analysis. The historical control data from the _____ laboratories indicated 2/647 (0.31%, range: 0-1.79) females and 6/647 (0.93% range: 0-1.79) males showed squamous cell carcinoma of the skin. The historical control data from the _____ laboratories documented that 0.51% of males (range 0.91-4.0) and 0.21% of females (0.56-2.0) has squamous cell carcinoma of the skin. Due to an increase in squamous cell carcinoma in both male and female rats in all body the reviewer suggests that the findings may be mentioned in the label.

The incidence of leiomyoma increased in females in the all body analysis at high dose (one in each of the following organs: uterus, vagina, and cecum). In the historical control data from _____ laboratories 1/647 (0.15%) leiomyoma was described in cecum. No such lesions were mentioned in vagina and 5/647 leiomyoma (0.7 %) was mentioned in the uterus. In the historical control data from the _____ laboratories, the occurrence of leiomyoma was described as 2.9 % and 0.4% in the uterus and vagina, no such lesion was described in the cecum. Due to the increase in the incidence of the leiomyoma in the whole body it is recommended by the reviewer that this finding may be incorporated in the label.

Summary of Results of Trend Tests in Tumors for Rats (NOTE: The table below was reproduced from the Statistical Review of the carcinogenicity studies completed by Moh-Jee Ng)

Incidence Summary of the Malignant Lymphoma:

Tumor	CD 0	LD 2.5	MD 5	HD 10	P-values
Oxymorphone-HCl (mg/kg/day)	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day	
Number of animals examined; Preterminal	64	38	35	27	
Hemolymphoreticular Tissue/Malignant lymphoma	0	0	0	3	
Number of animals examined, Terminal	36	27	30	38	
Hemolymphoreticular Tissue/Malignant lymphoma	1	0	0	1	
Number of animals examined;Total	100	65	65	65	
Hemolymphoreticular Tissue/Malignant lymphoma	1	0	0	4 0.0639 [†]	0.0162*

Organ/Tumor	CD 0	LD 2.5	MD 5	HD 10	P-values
Oxymorphone-HCl (mg/kg/day)	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day	
Male					
Skin miscellaneous/papilloma & carcinoma: squamous cell	2	4	2	4	0.4262
All bodies/carcinoma: squamous cell	1	2	0	4	0.1366
Kidney / adenoma: tubular cell	0	0	0	2	0.0410
Female					
All bodies/carcinoma: squamous cell	0	2	2	3	0.0604
All bodies/Leiomyoma	0	0	0	3	0.1052
UTERUS / Polyp: endometrial stromal	5	1	5	9	0.0328

[†]: p-values present for dose groups CD, LD, MD and HD trends.

Source data: dataset received on 3/22/2006, analysis data R1M56919

Best Possible Copy

Non Neoplastic:

An increased incidence of acute to chronic corneal inflammation in males and females given 10 mg/kg/day and 25 mg/kg/day, respectively were observed at the study termination. In addition, an increase in the incidence and severity of retinal atrophy was observed in males and females given ≥5 mg/kg/day (mid and high dose males and all dose groups in females). This change, graded minimal to moderate in severity, was characterized by a partial to total loss of the outer nuclear layer of the retina as described in the peer review by the pathologist. The findings in the eye were dose related and therefore considered treatment related by the Sponsor. Reduce lubrication in the cornea and dryness of eye might be the likely cause for the inflammation noted.

An increase in the incidence of pulmonary histiocytosis was observed in males and females given ≥5 mg/kg/day (mid and high dose males and low, mid and high dose females) compared with the control group. This was accompanied with an increase in the incidence of pulmonary granulomas and/or subacute inflammation

which is often associated with the presence of foreign material (bedding) in the bronchi, bronchioles and/or alveoli. These changes were graded minimal to severe.

The increase in the airway inflammatory lesions, and the associated plant material, was most likely the result of decrease in swallowing efficiency and/or suppression of the cough reflex. This is a morphine related well known pharmacodynamics effect, the clinical relevance of the finding with the present compound should be adequately analyzed.

Summary of Gross Pathology and Histopathological Findings:

Parameters	Sex/Dosages (mg/kg/day)							
	Male /0	Female /0	Male/2.5	Female/5	Male/5	Female/10	Male/10	Female/25
Atrophy: Retina	10	14	11	49	20	48	37	66
Inflammation Cornea	4	6	5	9	2	8	14	23
Histiocytes:Lung	22	19	29	35	32	62	62	86
Inflammation Lung	7	11	8	8	17	15	54	57
Lung: pale area	5	5	15	9	15	29	34	48
Lung: raised area	5	6	5	3	2	11	12	28
Skin:Scab	36	15	48	34	45	46	48	51
Skin:Ulceration	21	7	42	34	54	31	60	25

Adequacy of the carcinogenicity study and appropriateness of the test model:

SD rats were dosed once daily by oral gavage with oxymorphone HCl at doses of 2.5, 5, and 10 mg/kg/day in males and 5, 10, and 25 mg/kg/day in females. The test model (SD rats) is appropriate because the rat is a universal model routinely used for evaluating the toxicity and carcinogenicity of various classes of chemicals and for which there is a large historical database. The study was adequate because the study duration met the regulatory required duration for carcinogenicity studies (104 weeks), the doses evaluated were judged to have reached the MTD based on decrease in body weight in males and females.

Evaluation of tumor findings:

Neoplastic lesion findings include malignant lymphoma in male rat (1/100 in control vs 4/65 at 10 mg/kg/day), kidney tubular cell adenoma in males rats (0/100 in control vs 2/65 at high dose), all body leiomyoma in female rats (0/100

in control vs 3/65 at high dose) and all body squamous cell carcinoma in males (2/100 in control, 4/65, 2/65, and 4/65 at low, mid, and high dose respectively) and females (0/100, 2/65, 2/65, and 3/65 in control, low, mid, and high dose respectively), and all body leiomyoma in females (0/100 in control vs 3/65 at high dose). The lymphoma and adenoma in the tubular cell in the kidney and the skin carcinoma is considered as neoplastic finding related to the target organ of toxicity and historical control data showed that the tumors observed in the above mentioned organs are in the borderline range and can not be considered as common tumor. Therefore, it is recommended that the findings may be incorporated in the label.

Toxicokinetics: The exposure was higher in all dose groups in females compare to that of males. The Cmax and AUC increased with increasing doses, however; the increase was found to be more than dose proportional. An increase in dose from 2.5-10 mg/kg/day (4 fold) gave rise to about 6 fold increase in Cmax and approximately 7 fold AUC in males. In females, an increase in dose from 5-25 mg/kg/day (5 fold) gave rise to about 17 and 15 fold increase in Cmax and AUC respectively.

Summary of Toxicokinetics Findings:

Parameters	Low Dose (mg/kg/day)		Medium Dose (mg/kg/day)		High Dose (mg/kg/day)	
	Male 2.5	Female 5	Male 5	Female 10	Male 10	Female 25
Cmax (mg/mL)	4	6	7	27	24	103
Tmax (hr)	1	1	0.5	1	1	1
AUC (0-24h) mg.h/mL	14	27	37	120	93	415

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: A dose range-finding study of the effect of oxymorphone hydrochloride on fertility and early embryonic development to implantation in rats

Key study findings: Oxymorphone (0, 0.1, 1, 10, 25 or 50 mg/kg/day) was administered to male and female rats to determine appropriate dosing for the definite Segment I study. The findings indicated the following:

1. Mortality was noted in the high dose males and females.

Study design: Toxicology males were dosed daily for 29 days prior to pairing and continued until one day prior to euthanasia. Females for toxicology were dosed daily for 14 days prior to pairing and through mating, continuing through gestation day 7.

Parameters and endpoints evaluated:

Clinical signs: Animals were examined twice daily for mortality and moribundity for the duration of the study. In addition to general clinical observations, each animal was examined for signs of toxicity both 15 minutes and 1 hour following dosing.

Body weights: Male body weights were recorded twice weekly throughout the dosing period until euthanasia (males day 59). Female body weights were measured twice weekly beginning at the initiation of treatment through day 15 of gestation.

Food consumption: Individual food consumption was recorded twice weekly corresponding to the body weight days described above.

Toxicokinetics: Blood was collected for toxicokinetics prior to dosing, 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20 and 24 hours following treatment.

Estrous Cycles: Vaginal smears for determining the stage of estrous were evaluated daily beginning with the first dose and continuing until evidence of copulation was observed.

Mating and fertility indices were calculated as follows:

$$\text{Male (Female) Mating Index (\%)} = \frac{\text{No. of Males (Females) with Evidence of Mating or Confirmed Pregnancy}}{\text{Total No. of Males (Females) Used for Mating}} \times 100$$

$$\text{Male Fertility Index (\%)} = \frac{\text{No. of Males Siring at Least 1 Litter}}{\text{Total No. of Males Used for Mating}} \times 100$$

$$\text{Female Fertility Index (\%)} = \frac{\text{No. of Females with Confirmed Pregnancy}}{\text{Total No. of Females Used for Mating}} \times 100$$

Gestation Day 15 Uterine Examinations: Animals were euthanized by CO₂ inhalation on gestation day 15. The abdominal and thoracic cavities were opened and the contents were examined. The uterus and ovaries were examined. The number of corpora lutea on each ovary was recorded. The number and location of all embryos, early resorptions, and the total number of implantation sites were recorded.

Necropsy: Macroscopic examination was completed for all toxicology animals at the scheduled euthanasia, as well as on animals that died or were euthanized *in extremis*. Necropsy included examination of the external surface, all orifices, the cranial cavity, the external surface of the brain and spinal cord and the thoracic, abdominal and pelvic cavities, including viscera. No tissue was preserved for histopathological examination in this dose range-finding study.

Organ weights: Weights of the following were obtained: adrenal gland, brain, epididymides, kidneys, liver, ovaries, pituitary gland and testes. Paired testes and epididymides were weighed separately. Absolute and organ-to-final-body-weight ratios were recorded.

Statistics: Statistical analysis was not completed for this study.

Results

Mortality: One male in the 10 mg/kg group and 3 males in the 50 mg/kg group were found dead between study days 2 and 20. One male in the 50 mg/kg group was euthanized *in extremis* on study day 6. One female in the 50 mg/kg group was found dead on study day 12.

Disposition	Summary of Animal Disposition											
	Males (mg/kg)						Females (mg/kg)					
	0	0.1	1	10	25	50	0	0.1	1	10	25	50
Found Dead	0	0	0	1	0	3	0	0	0	0	0	1
Euthanized in Extremis	0	0	0	0	0	1	0	0	0	0	0	4
Euthanized and Discarded	0	26	26	25	26	23	0	26	26	26	26	26
Terminal Necropsy	10	10	10	10	10	9	10	10	10	10	10	10

n/n = total occurrence/number of animals

Clinical signs: Prior to death CNS effects included excessive chewing of the tail and hypoactivity, exophthalmia, paleness and/or lacrimation. Other clinical signs included whole body tetany, convulsions, gasping, lethargy, repetitive movement of the mouth and jaws, Straub tail, rocking, lurching and swaying, exophthalmia, lacrimation, decreased defecation, and extreme aggravation on handling. The incidence of clinical signs is provided in the table below:

**Appears This Way
On Original**

Parameter	Incidence of Clinical Observations (n=10/group)											
	Males (mg/kg)						Females (mg/kg)					
	0	0.1	1	10	25	50	0	0.1	1	10	25	50
Behavior/CNS												
Hypoactive	0/0	0/0	0/0	0/0	0/0	3/3	0/0	0/0	0/0	0/0	0/0	1/1
Rocks, lurches and sways	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0	1/1	0/0
Hunched appearance	0/0	0/0	0/0	0/0	0/0	2/1	--	--	--	--	--	--
Extremely aggressive upon Handling	0/0	0/0	0/0	0/0	51/5	22/4	--	--	--	--	--	--
Convulsions	--	--	--	--	--	--	0/0	0/0	0/0	0/0	0/0	1/1
Eyes/Ears/Nose												
Wet red material around nose	0/0	0/0	0/0	0/0	1/1	4/4	0/0	0/0	0/0	0/0	0/0	0/0
Dried red material around nose	0/0	1/1	4/3	17/7	12/7	60/22	0/0	0/0	0/0	0/0	0/0	0/0
Dried red material around eye (rt)	0/0	2/1	0/0	26/2	5/3	11/6	0/0	0/0	0/0	0/0	9/3	45/18
Dried red material around eye (lt)	0/0	6/1	0/0	27/2	3/2	8/6	0/0	0/0	0/0	0/0	12/4	39/17
Dried red material around mouth	0/0	0/0	0/0	2/1	0/0	7/6	0/0	0/0	0/0	0/0	0/0	18/10
Excreta												
Urine red in color	0/0	0/0	0/0	0/0	0/0	4/3	--	--	--	--	--	--
Soft stool	1/1	2/2	9/5	7/6	7/5	9/7	0/0	0/0	1/1	5/3	8/6	7/4
Decreased defecation	0/0	0/0	0/0	0/0	0/0	6/3	0/0	0/0	0/0	0/0	0/0	17/13
Feces appear light in color	--	--	--	--	--	--	0/0	0/0	0/0	0/0	5/5	5/5
Feces absent	--	--	--	--	--	--	0/0	0/0	0/0	0/0	0/0	1/1
Cardio-Pulmonary												
Gaspings	--	--	--	--	--	--	0/0	0/0	0/0	0/0	0/0	2/2

n/n = total occurrence/number of animals; -- = no data recorded

Parameter	Incidence of Clinical CNS Observations 15 minutes post dose (n=10/group)											
	Males (mg/kg)						Females (mg/kg)					
	0	0.1	1	10	25	50	0	0.1	1	10	25	50
Behavior/CNS												
Whole body tetany	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0	0/0	8/7
Hypoactive	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0	0/0	1/1	6/5
Straub tail	0/0	0/0	0/0	4/3	3/3	5/4	0/0	0/0	0/0	4/4	10/8	9/8
Rocks, lurches and sways	0/0	0/0	0/0	0/0	2/2	1/1	0/0	0/0	0/0	0/0	0/0	1/1
Head sways from side to side	1/1	2/2	9/4	29/14	49/22	41/13	0/0	18/7	15/10	16/10	25/17	13/9
Popcorn seizure	0/0	0/0	4/3	8/6	9/4	6/5	0/0	0/0	1/1	4/2	5/5	7/5
Excessive chewing of cage bottom	0/0	0/0	3/1	18/8	28/15	50/15	0/0	2/2	10/4	26/9	16/12	28/16
Excessive chewing of tail	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/1
Hyperactive	0/0	0/0	0/0	12/8	14/5	12/7	0/0	0/0	0/0	0/0	0/0	0/0
Hunched appearance	0/0	0/0	0/0	0/0	1/1	1/1	0/0	0/0	0/0	1/1	0/0	0/0
Repetitive movement of mouth	0/0	0/0	0/0	1/1	5/3	3/3	0/0	0/0	0/0	1/1	0/0	4/4
Excessive chewing of rt forelimb	0/0	1/1	5/4	10/6	29/10	24/12	--	--	--	--	--	--
Excessive chewing of lt forelimb	0/0	1/1	0/0	21/10	29/7	33/9	--	--	--	--	--	--
Excessive paw licking	0/0	2/2	2/2	32/10	22/8	22/8	0/0	3/3	12/6	19/10	25/11	26/8
Excessive pawing of bottom cage	0/0	0/0	0/0	0/0	1/1	2/2	--	--	--	--	--	--
Excessive licking of cage bottom	0/0	0/0	0/0	1/1	11/3	25/8	0/0	0/0	0/0	1/1	0/0	0/0
Excessive licking of cage sides	0/0	0/0	0/0	0/0	2/2	7/4	--	--	--	--	--	--

n/n = total occurrence/number of animals; -- no observations recorded

Body weight: Body weights and body weight gains in male animals were reduced in the 10, 25 and 50 mg/kg/day groups beginning on day 7 and continuing until termination on day 59. On study day 59, mean body weight were reduced by approximately 12.6%, 19.9% and 18.5% following treatment with 10, 25 and 50 mg/kg/day, respectively. Body weights in females treated with 25 or 50 mg/kg were decreased by approximately 10% during the first two weeks of the pre-mating period. During the gestation period,

female animal body weights were decreased by 10% or greater following treatment with 10, 25 or 50 mg/kg/day on Day 7 and by 50 mg/kg/day on Day 15.

Parameter	Body Weights (% change of control; N=9-10)				
	Oxymorphone (mg/kg/day)				
	0.1	1	10	25	50
Males					
Day 0 to 59	+1	0	-12.6	-19.9	-18.5
Females					
Pre-Mating Period Day 7	-6	-3	-5	-8	-11
Pre-Mating Period Day 14	-6	-3	-6	-10	-11
Gestation Period Day 7	-2	-4	-10	-15	-13
Gestation Period Day 15	-1	-3	-3	-8	-13

n/n = total occurrence/number of animals

Food consumption: Food consumption in males was reduced by doses of 10, 25 and 50 mg/kg/day. Some evidence of tolerance to this effect was noted following day 21; however the three highest doses continued to reduce food consumption in males throughout the study.

Parameter	Male Food Consumption (gm/animal/day, % change of control; N=9-10)					Female Food Consumption (gm/animal/day, % change of control; N=9-10)				
	Oxymorphone (mg/kg/day)					Oxymorphone (mg/kg/day)				
	0.1	1	10	25	50	0.1	1	10	25	50
Day 0-3	0	-4	-17	-13	-18	-16	-16	-16	-21	-58
Day 3-7	0	-4	-8	-12	-23	0	-5	-16	-21	-26
Day 7-10	0	-4	-19	-23	-27	0	0	-11	-17	-11
Day 10-14	-4	-8	-15	-15	-15	0	-5	-11	-11	-11
Day 14-17	-4	-4	-12	-19	-23	*				
Day 17-21	0	+4	-8	-12	-4	*				
Day 21-24	0	0	-7	-11	-7					
Day 24-28	0	0	-7	-15	-11					
Day 56-59	+4	+4	-11	-19	-15					

n/n = total occurrence/number of animals.

* NOTE: Food consumption was not recorded during gestation (days 14-21).

Toxicokinetics: Plasma levels of oxymorphone in the range-finding study are reproduced in the sponsor's table below. The table contains data from both the range-finding study as well as the definitive study. The results indicate that the rats were exposed to a significant amount of oxymorphone. The levels increased with increased dose, but did not appear to be altered from day 1 compared to day 14 suggesting no alterations in the metabolism of oxymorphone under the conditions tested.

Table 1: Comparison of 1 hour Plasma Levels from the Definitive Study versus that for the Range-finding Study.

Study Day	Sex	Dose Group (mg/kg/day)	Time (hr)	Plasma Concentration (ng/mL)	
				Range-finding Study (411001)	Definitive Study (411004)
1 st Day	M	1	1	0.78 (1.22*)	
1 st Day	M	5	1		4.86
1 st Day	F	1	1	1.04 (1.76*)	
1 st Day	F	5	1		5.90
1 st Day	M	10	1	7.24	
1 st Day	F	10	1	8.85	
1 st Day	M	25	1	16.63	11.85
1 st Day	F	25	1	21.96	15.97
1 st Day	M	50	1	25.63	
1 st Day	F	50	1	139.97	
14 th Day	M	1	1	18.33 (1.23*)	
14 th Day	M	5	1		6.10
14 th Day	F	1	1	2.14*	
14 th Day	F	5	1		6.17
14 th Day	M	10		69.52 (28.17*)	
14 th Day	F	10		18.16	
14 th Day	M	25	1	69.52	43.17
14 th Day	F	25	1	49.80	36.71
14 th Day	M	50	1	172.49	
14 th Day	F	50	1	184.63	

* - Method 2 value.

Necropsy: Although several necropsy findings were noted in surviving animals, the only potentially treatment-related changes were noted in the bladder of one male animal and 2 female animals treated with the highest dose of 50 mg/kg/day.

Parameter	N	Incidence of Gross Necropsy Observations (Scheduled Necropsy)											
		Males (mg/kg/day)						Females (mg/kg/day)					
		0	0.1	1	10	25	50	0	0.1	1	10	25	50
Urinary Bladder Thickened		0	0	0	0	0	1	0	0	0	0	0	2
Calculi		0	0	0	0	0	1	0	0	0	0	0	0

Organ weights: There was an apparent decrease in absolute and relative liver weights in males treated with 10, 25 or 50 mg/kg/day oxymorphone. Relative adrenal weights in males appeared to increase in a dose-related manner. A similar trend was noted in absolute adrenal weights in males, although the effect was not statistically significant. There were no clear changes in brain, kidneys, testes, epididymis or pituitary in male rats. Likewise, there were no clear effects of oxymorphone on the organ weights in female rats.

Parameter	N	Summary of Organ Weight Changes (% C of control; Scheduled Necropsy)									
		Males (mg/kg/day)					Females (mg/kg/day)				
		0.1	1	10	25	50	0.1	1	10	25	50
Liver		10	10	10	10	9	9	10	8	10	9

Absolute	+1	-1	-26*	-42*	-40*	+2	-3	-3	-8	-15
Relative to body weight	+1	-1	-19	-27*	-26*	+2	0	-2	+1	-2
Adrenal										
Absolute	-1	+2	-1	+5	+15	+11	-1	-3	+12	-4
Relative to body weight	0	0	+17	+33*	+42*	+13	+4	0	+9	+9

* p < 0.05 compared to controls.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

There was no effect of oxymorphone on either male mating index or male fertility index noted. Male mating index (%) was calculated by dividing the number of males with evidence of mating (or confirmed pregnancy) by the total no of males used for mating (x100). Male fertility index was calculated by dividing the number of males siring a litter by the total number of males used for mating (x100).

Parameter	Summary of Male Reproductive Performance (N=9-10)					
	Males (mg/kg)					
	0	0.1	1	10	25	50
Male Mating Index (%)	100	90	90	100	100	100
Male Fertility Index (%)	90	80	90	90	100	88.9

In females, the female mating index and female fertility index were not changed by oxymorphone treatment. The mean pre-coital interval was increased in the animals above the ~~historical~~ historical control values (range 2.0-3.3 days). Estrus cycle length was increased in animals treated with 50 mg/kg (6.9 days) compared to the control group value (4.1 days), as depicted in the sponsors table below:

Parameter	Summary of Female Reproductive Performance (N=9-10)					
	Males (mg/kg)					
	0	0.1	1	10	25	50
Female Mating Index (%)	100	100	100	100	100	100
Female Fertility Index (%)	90	90	100	90	100	90
Mean pre-coital intervals (days)	3.3	3.7	3.8	3.4	2.6	4.1
Mean estrus cycle length	4.1	4.4	4.9	4.6	4.5	6.9

n/n = total occurrence/number of animals

Embryonic Data. Mean embryonic data is presented in the table below. Although statistical analysis was not completed on this data, doses of oxymorphone of 50 mg/kg/day appeared to decrease the mean number of viable embryos and decrease the number of implantation sites and corpora lutea. There were no apparent changes noted in the number of dead embryos, resorptions, post-implantation losses or pre-implantation losses. Doses of 25 mg/kg/day were associated with reduced mean numbers of corpora lutea, implantation sites and viable embryos, however, the number of implantation sites and viable embryo changes were within historical control values. All other parameters did not appear to be altered in a dose-dependent manner by oxymorphone treatment.

PROJECT NO. **411001P** -411001P
 SPONSOR: ENDO PHARM., INC.
 TABLE 28 (TOXICOLOGY PHASE)
 R-F STUDY OF OXYMORPHONE HYDROCHLORIDE IN RATS
 SUMMARY OF MEAN EMBRYONIC DATA AT THE SCHEDULED NECROPSY

PAGE 1

GROUP	VIABLE EMBRYOS	DEAD EMBRYOS	RESORPTIONS		POST IMPLANTATION LOSS	IMPLANTATION SITES	CORPORA LUTEA	PRE IMPLANTATION LOSS	NO. OF GRAVID FEMALES
			EARLY	LATE					
1 TOTAL	136	0	5	0	5	141	149	8	9
MEAN	15.1	0.0	0.6	0.0	0.6	15.7	16.6	0.9	
S.D.	1.45	0.00	0.73	0.00	0.73	1.22	1.88	1.17	
2 TOTAL	123	0	7	0	7	130	157	27	9
MEAN	13.7	0.0	0.8	0.0	0.8	14.4	17.4	3.0	
S.D.	4.44	0.00	1.09	0.00	1.09	3.97	3.32	3.35	
3 TOTAL	143	0	6	0	6	149	161	12	10
MEAN	14.3	0.0	0.6	0.0	0.6	14.9	16.1	1.2	
S.D.	1.49	0.00	0.97	0.00	0.97	1.10	1.66	1.14	
4 TOTAL	102	0	12	1	13	115	134	19	8
MEAN	12.8	0.0	1.5	0.1	1.6	14.4	16.8	2.4	
S.D.	2.38	0.00	1.85	0.35	2.13	2.67	1.16	2.92	
5 TOTAL	140	0	8	0	8	148	157	9	10
MEAN	14.0	0.0	0.8	0.0	0.8	14.8	15.7	0.9	
S.D.	1.49	0.00	0.79	0.00	0.79	1.48	1.42	1.20	
6 TOTAL	101	0	8	0	8	109	133	24	9
MEAN	11.2	0.0	0.9	0.0	0.9	12.1	14.8	2.7	
S.D.	2.68	0.00	1.05	0.00	1.05	2.62	2.33	2.83	

1- 0 MG/KG/DAY 2- 0.1 MG/KG/DAY 3- 1 MG/KG/DAY 4- 10 MG/KG/DAY 5- 25 MG/KG/DAY 6- 50 MG/KG/DAY

PLS04.02
12/21/2000

Study title: A study of the effect of oxymorphone hydrochloride on fertility and early embryonic development to implantation in rats

Key study findings: Oxymorphone was administered to male rats for 28 days prior to mating and continued throughout mating until one day prior to euthanasia. Female rats were treated for a total of 14 days prior to mating, throughout mating and through gestation day 7. The following findings regarding fertility and early embryonic development were obtained:

1. The study appears to be valid as significant toxicity was noted in both males and females at the higher doses.
2. Reproductive performance in males was not altered under the conditions tested.
3. There were no effects of oxymorphone on mean testicular and epididymal sperm numbers, sperm production rate, motility or morphology compared to control animals.
4. Reproductive performance in females was not altered by any dose of oxymorphone tested, however, mean estrus cycle length was slightly but significantly increased in the 25 mg/kg/day group.
5. Early embryonic development was significantly altered by oxymorphone treatment. Specifically, the mean number of viable embryos and the mean number of implantation sites were reduced by 14% in the 10 and 25 mg/kg/day group. The mean number of corpora lutea was significantly reduced only in the high dose group (25 mg/kg/day).
6. Overall, due to parental systemic toxicity, the NOAEL for parental toxicity was < 5 mg/kg/day. The NOAEL for reproductive performance in males was > 25

mg/kg/day. The NOAEL for reproductive performance in females was 5 mg/kg/day due to an increase in estrus cycle length.

Study no.: ~~411004~~
 Volume #, and page #: EDR
 Conducting laboratory and location: ~~_____~~
 Date of study initiation: February 16, 2001
 GLP compliance: Yes
 QA reports: yes (X) no ()
 Drug, lot #, and % purity: Oxymorphone hydrochloride, Lot 0881 A 42771, ~~_____~~ purity (note doses were calculated based on purity of the test article).

Methods

Doses: 0, 5, 10 and 25 mg/kg
 Species/strain: ~~_____~~ CD₀₁(SD)IGS BR rats
 Number/sex/group: 25/sex/group
 Route, formulation, volume, and infusion rate: Oral gavage, 10 ml/kg volume,
 Satellite groups used for toxicokinetics: 5/sex/group
 Study design: For toxicology, male animals were treated for 28-days prior to mating and continued throughout mating until one day prior to euthanasia. For females, animals were treated for a total of 14 days prior to mating, throughout mating and continuing through gestation day 7.

Group	Test Article	Dosage Level ^a (mg/kg/day)	Dosage Concentration ^a (mg/ml)	Dosage Volume (ml/kg)	Number of Animals	
					Males	Females
1	Vehicle	0	0	10	25	25
2	Oxymorphone HCl	5	0.5	10	25	25
3	Oxymorphone HCl	10	1	10	25	25
4	Oxymorphone HCl	25	2.5	10	25	25

^a - Expressed in terms of the oxymorphone salt

Parameters and endpoints evaluated:

Clinical signs: Animals were examined twice daily for mortality and moribundity for the duration of the study. In addition to general clinical observations, each animal was examined for signs of toxicity 1 hour following dosing. Due to excessive chewing and licking of the forelimbs, paws and/or digits, Nyla-Bones were provided on study day 8 to further reduce self-mutilation.

Body weights: Male body weights were recorded twice weekly throughout the dosing period until euthanasia (through day 62). Female body weights were measured twice weekly beginning at the initiation of treatment (day 14 through day 28). Once evidence of mating occurred females were weighed on gestation days 0, 3, 7, 10, 13 and 15.

Food consumption: Individual food consumption was recorded twice weekly corresponding to the body weight days described above.

Toxicokinetics: Blood was collected for toxicokinetics prior to dosing, 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20 and 24 hours following treatment.

Estrous Cycles: Vaginal smears for determining the stage of estrous were evaluated daily beginning with the first dose and continuing until evidence of copulation was observed. Positive evidence of mating was confirmed by the presence of sperm or a vaginal smear or a vaginal copulatory plug.

Mating and fertility indices were calculated as follows:

$$\text{Male (Female) Mating Index (\%)} = \frac{\text{No. of Males (Females) with Evidence of Mating or Confirmed Pregnancy}}{\text{Total No. of Males (Females) Used for Mating}} \times 100$$

$$\text{Male Fertility Index (\%)} = \frac{\text{No. of Males Siring at Least 1 Litter}}{\text{Total No. of Males Used for Mating}} \times 100$$

$$\text{Female Fertility Index (\%)} = \frac{\text{No. of Females with Confirmed Pregnancy}}{\text{Total No. of Females Used for Mating}} \times 100$$

Gestation Day 15 Uterine Examinations: Animals were euthanized by CO₂ inhalation on gestation day 15. The abdominal and thoracic cavities were opened and the contents were examined. The uterus and ovaries were examined. The number of corpora lutea on each ovary was recorded. The number and location of all embryos, early resorptions, and the total number of implantation sites were recorded.

Spermatogenic endpoints: Upon euthanasia, reproductive tract of males were exposed and the epididymis weighed. An incision was made in the distal region of the right cauda epididymis and the epididymis was placed in PBS with 10% BSA for 10 minutes to collect sperm. Sperm motility and morphology were determined. Abnormal forms of sperm from a differential count of 200 spermatozoa per animal (if possible) were recorded.

Necropsy: Macroscopic examination was completed for all toxicology animals at the scheduled euthanasia, as well as on animals that died or were euthanized *in extremis*. Necropsy included examination of the external surface, all orifices, the cranial cavity, the external surface of the brain and spinal cord and the thoracic, abdominal and pelvic cavities, including viscera. No tissue was preserved for histopathological examination in this dose range-finding study.

Organ weights: Weights of the following were obtained: adrenal gland, brain, epididymides (total and cauda), heart, kidneys, liver, ovaries, spleen, testes and thymus. Paired tested and epididymides were weighed separately. Absolute and organ-to-final-body-weight ratios were recorded.

Statistics: Statistical analysis was conducted using a two-tailed test for minimal significance level of 5% compared to treated control groups. The following statistical tests were used:

<u>STATISTICAL TEST</u>	<u>PARAMETER</u>
-Chi-Square test ⁶ with Yates' correction factor	Mating and Fertility Indices
-One-way ANOVA ⁷ with Dunnett's test ⁸	Corpora Lutea, Total Implantations, Viable Embryos, Parental Body Weights and Body Weight Changes, Parental Food Consumption, Testicular and Epididymal Sperm Numbers, Sperm Production Rate, Organ Weights (absolute and relative), Pre-Coital Intervals, Estrous Cycles
-Kruskal-Wallis test ⁹ with Mann-Whitney U-test ⁹	Mean Litter Proportions of Intrauterine Data (Considering the Litter, Rather than the Embryo, as the Experimental Unit), Sperm Motility and Sperm Morphology

Toxicokinetics: Toxicokinetic data were determined from a satellite toxicokinetic phase. Rats received the test article for 14 days. Blood samples were collected via retro-orbital sinus on the first and fourteenth day of dosing. Four animals per sex in the 5 and 25 mg/kg/day group were used for blood collection at 1 and 8 hours post dose.

Results

Mortality: Two male animals in the high dose group died (one on study day 12 and the other on study day 14). These deaths were considered to be test article related. There were no unscheduled deaths in the female animals in any group tested.

Disposition	Summary of Animal Disposition (N=25/sex/group)							
	Males (mg/kg)				Females (mg/kg)			
	0	5	10	25	0	5	10	25
Found Dead	0	0	0	2	0	0	0	0
Scheduled Necropsy	25	25	25	23	25	25	25	25

Clinical signs: Pharmacological effects were noted in the surviving male animals included repetitive chewing of the forelimbs, hyperactivity and excessive chewing and/or licking of the cage floor and/or walls one hour following dosing beginning on study day 6 and continuing through the scheduled necropsy. Other CNS effects included: exophthalmia, hypoactivity, repetitive chewing of the tail and/or hindlimbs, movement of the jaws, nodding of the head and/or excessive grooming and/or chewing. Occasional evidence of respiratory distress was noted in some male animals in the 10 and 25 mg/kg/day groups.

Daily Examinations Parameter	Incidence of Clinical Observations (n=25/sex/group)							
	Males (mg/kg)				Females (mg/kg)			
	0	5	10	25	0	5	10	25
Body Integument Dried red material right forelimb	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0

Scabbing distal end of tail	0/0	0/0	0/0	20/2	0/0	0/0	0/0	0/0
Unkempt appearance	0/0	0/0	0/0	0/0	0/0	0/0	0/0	3/1
Swelling left forelimb	0/0	0/0	0/0	0/0	0/0	0/0	0/0	6/2
Wet material urogenital area	0/0	0/0	0/0	1/1	0/0	0/0	0/0	3/2
Dried yellow material urogenital area	0/0	0/0	0/0	0/0	0/0	0/0	0/0	2/1
Cardio-Pulmonary								
Rales	0/0	0/0	2/1	5/4	0/0	0/0	0/0	0/0
Excreta								
Soft Stool	0/0	0/0	5/3	5/4	0/0	0/0	0/0	0/0
Oral Dental								
Dried red material around mouth	0/0	0/0	1/1	1/1	0/0	0/0	0/0	0/0
Eyes/ears/nose								
Dried red material around nose	0/0	1/1	19/2	0/0	0/0	3/3	9/4	11/5
Exophthalmos right eye	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/1

n/n = total occurrence/number of animals; -- = no data recorded

1 Hour Post-Dose Parameter	Incidence of Clinical Observations (n=25/group)							
	Males (mg/kg)				Females (mg/kg)			
	0	5	10	25	0	5	10	25
CNS/Behavior								
Repetitive chewing of left forelimb	0/0	122/22	207/24	91/17	0/0	73/19	85/22	67/22
Repetitive chewing of right forelimb	0/0	139/24	213/24	70/18	0/0	70/18	77/19	62/20
Hyperactive	0/0	152/25	165/24	96/23	0/0	85/24	73/24	40/21
Excessive chewing of cage floor/walls	0/0	215/19	240/21	179/23	0/0	129/21	107/18	90/18
Repetitive chewing of distal end of tail	0/0	4/4	0/0	11/3	0/0	0/0	0/0	4/1
Hypoactive	0/0	0/0	2/2	1/1	0/0	1/1	0/0	1/1
Repetitive chewing of right hindlimb	0/0	6/5	24/9	0/0	0/0	3/3	1/1	0/0
Excessive licking of cage floor/walls	0/0	2/2	16/7	91/22	0/0	2/2	12/5	46/15
Repetitive movement of head	0/0	0/0	0/0	8/3	0/0	0/0	0/0	0/0
Repetitive movement of left forelimb	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0
Repetitive chewing of left hindlimb	0/0	6/6	9/6	3/3	0/0	2/2	1/1	0/0
Repetitive movement of jaws	0/0	2/2	2/1	3/2	0/0	0/0	0/0	0/0
Excessive grooming	0/0	8/7	3/3	1/1	0/0	0/0	0/0	0/0
Excessive circling	0/0	2/2	3/1	5/4	0/0	0/0	0/0	0/0
Repetitive nodding of head	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0
Cardio/Pulmonary								
Rales	0/0	0/0	2/2	1/1	0/0	0/0	0/0	0/0
Eyes/Ears/Nose								
Exophthalmos right eye	0/0	0/0	9/5	45/19	0/0	4/3	7/5	20/12
Exophthalmos left eye	0/0	1/1	8/6	39/17	0/0	3/2	7/5	19/12
Oral/Dental								
Salivation	0/0	0/0	0/0	3/1	0/0	0/0	0/0	0/0
Clear wet material around mouth	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0

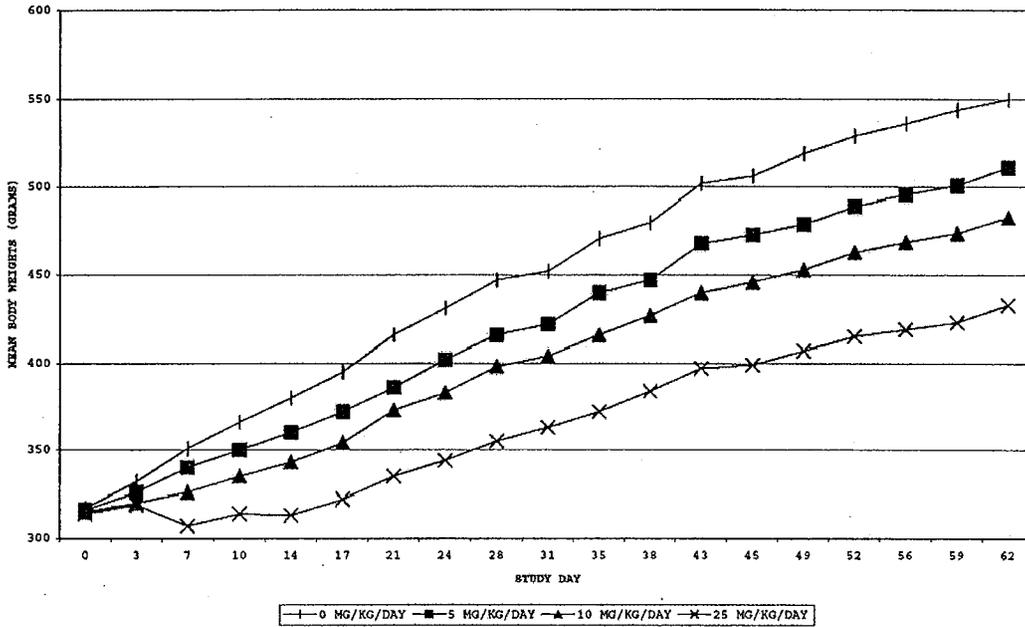
n/n = total occurrence/number of animals; -- = no data recorded

Body weight: Body weights of males were decreased at all doses in a dose-dependent fashion, as depicted in sponsor's figure 1 below. By the end of the study period (day 62) animals demonstrated an 8%, 12% and 27% reduction in body weights following 5, 10 and 25 mg/kg oxymorphone compared to control animals.

**Appears This Way
On Original**

PROJECT NO. 411004M
 SPONSOR: ENDO PHARM., INC.

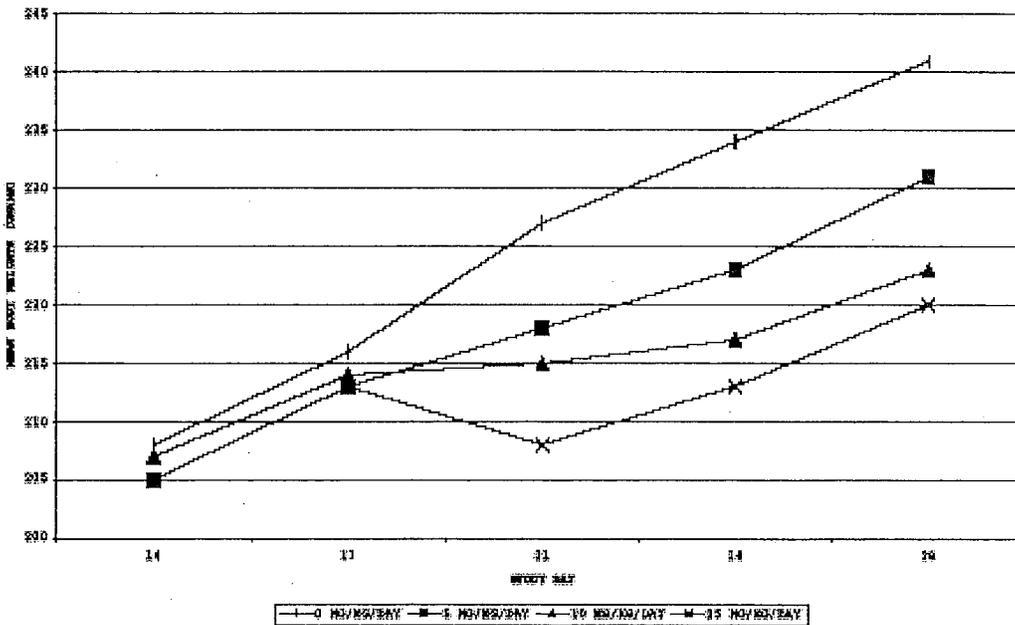
FIGURE 1 (MALES)
 FERT./EMBRYONIC DEV. STUDY OF OXYMORPHONE HYDROCHLORIDE IN RATS
 BODY WEIGHTS (GRAMS)



Best Possible Copy

PROJECT NO. 411004F
 SPONSOR: ENDO PHARM., INC.

FIGURE 4 (FEMALES)
 FERT./EMBRYONIC DEV. STUDY OF OXYMORPHONE HYDROCHLORIDE IN RATS
 BODY WEIGHT (GRAMS) - PRE-PARTING

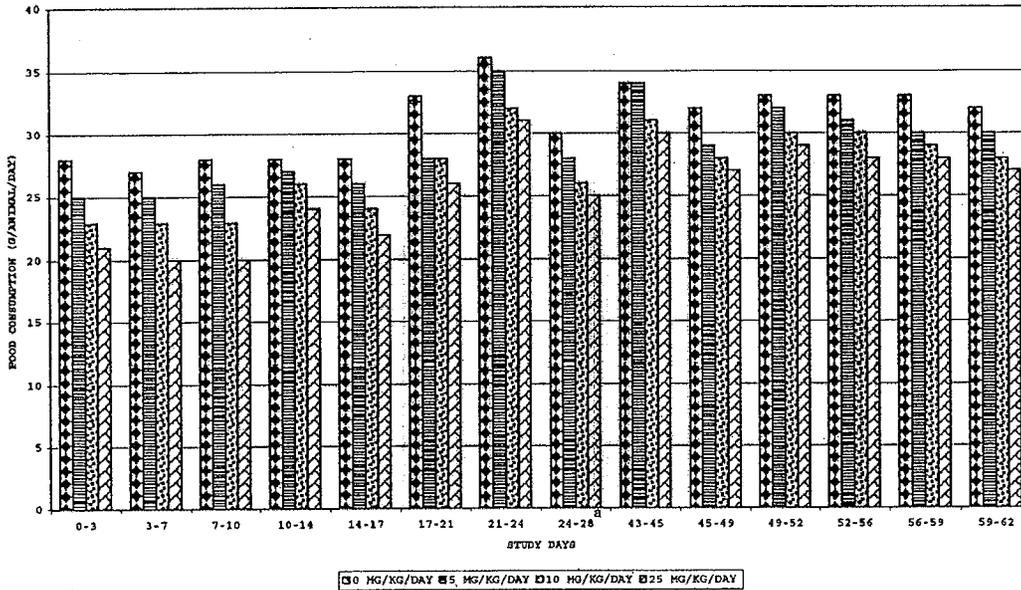


Food consumption: Food consumption in the male was decreased in a dose-dependent fashion as depicted in the figure below. The decrease was statistically significant in the

10 and 25 mg/kg groups at most days. A dose of 5 mg/kg also reduced food consumption which was significant on study days 0-3, 3-7, 7-10, 14-17, 17-21, 24-28 45-49, 56-59 and 59-62.

PROJECT NO. 411004M
SPONSOR: ENDO PHARM., INC.

FIGURE 3 (MALES)
PERT./EMBRYONIC DEV. STUDY OF OXYMORPHONE HYDROCHLORIDE IN RATS
FOOD CONSUMPTION (GRAMS/ANIMAL/DAY)

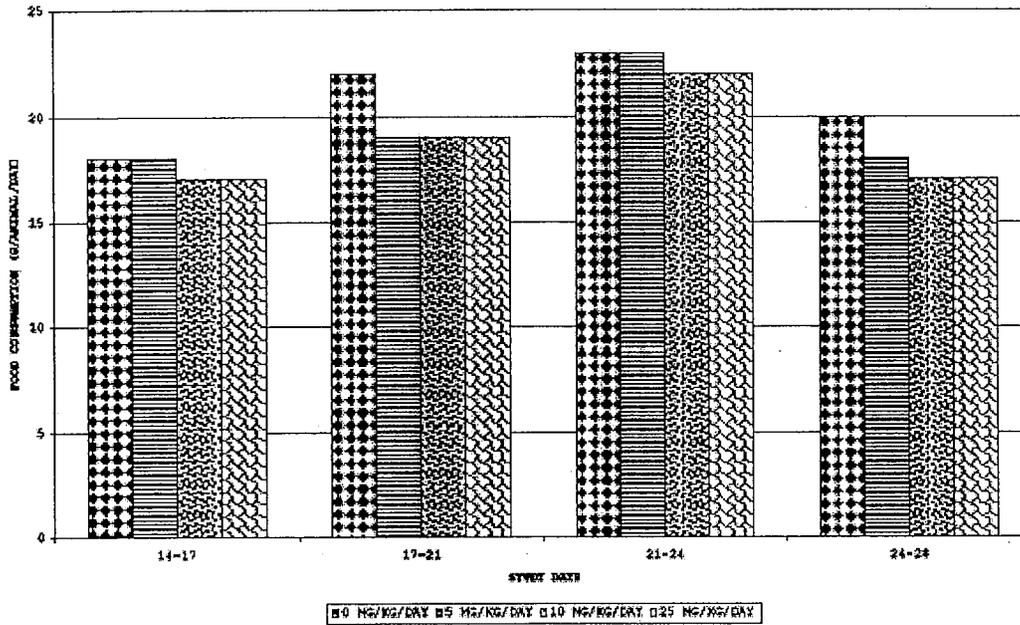


a - FOOD CONSUMPTION NOT RECORDED DURING THE BREEDING PERIOD (DAYS 28-43)

Appears This Way
On Original

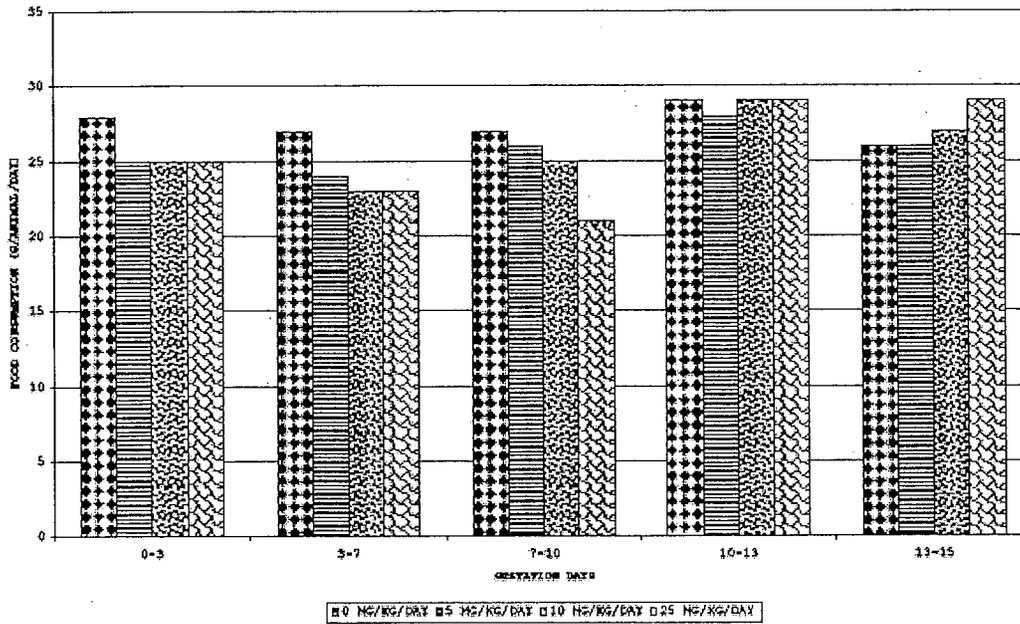
PROJECT NO. 411004F
 SPONSOR: ENDO PHARM., INC.

FIGURE 8 (FEMALES)
 FERT./EMBRYONIC DEV. STUDY OF OXYMORPHONE HYDROCHLORIDE IN RATS
 FOOD CONSUMPTION (GRAMS/ANIMAL/DAY) - PRE-NATAL



PROJECT NO. 411004F
 SPONSOR: ENDO PHARM., INC.

FIGURE 9 (FEMALES)
 FERT./EMBRYONIC DEV. STUDY OF OXYMORPHONE HYDROCHLORIDE IN RATS
 MEAN FOOD CONSUMPTION DURING GESTATION (GRAMS/ANIMAL/DAY)



Toxicokinetics: All of the parameters evaluated in the toxicokinetic phase (deaths, clinical observations, mean body weights, body weight gains and internal findings) were

representative to those observed in the main study. The sponsor analyzed plasma levels in both the range-finding study and the main study, and the results are reproduced in the table below:

Table 1: Comparison of 1 hour Plasma Levels from the Definitive Study versus that for the Range-finding Study.

Study Day	Sex	Dose Group (mg/kg/day)	Time (hr)	Plasma Concentration (ng/mL)	
				Range-finding Study (411001)	Definitive Study (411004)
1 st Day	M	1	1	0.78 (1.22*)	
1 st Day	M	5	1		4.86
1 st Day	F	1	1	1.04 (1.76*)	
1 st Day	F	5	1		5.90
1 st Day	M	10	1	7.24	
1 st Day	F	10	1	8.85	
1 st Day	M	25	1	16.63	11.85
1 st Day	F	25	1	21.96	15.97
1 st Day	M	50	1	25.63	
1 st Day	F	50	1	139.97	
14 th Day	M	1	1	18.33 (1.23*)	
14 th Day	M	5	1		6.10
14 th Day	F	1	1	2.14*	
14 th Day	F	5	1		6.17
14 th Day	M	10		69.52 (28.17*)	
14 th Day	F	10		18.16	
14 th Day	M	25	1	69.52	43.17
14 th Day	F	25	1	49.80	36.71
14 th Day	M	50	1	172.49	
14 th Day	F	50	1	184.63	

* - Method 2 value.

Necropsy: No internal abnormalities were noted in the two male animals found dead prior to scheduled necropsy. In males, minor changes were noted at necropsy, including one male in the high dose group demonstrating small seminal vesicles and dark red lungs or dark red patches on the lungs were noted which may be due to the

Parameter	Incidence of Necropsy Observations (n=25/group)							
	Males (mg/kg)				Females (mg/kg)			
	0	5	10	25	0	5	10	25
Lungs								
Dark red/dark red patches	1	1	1	3	0	0	0	1
Nodule	0	0	0	0	0	0	0	1
Seminal Vesicles								
Small	0	0	0	1	--	--	--	--
Thymus gland								
Small	0	0	0	0	0	0	0	1

Organ Weights:

In females with no evidence of mating, there were no significant changes in organ weights. The effect of oxymorphone on absolute and relative organ weights in males and females with evidence of mating is summarized in the table below:

Parameter	N	Summary of Organ Weight Changes (% Δ of control; Scheduled Necropsy)							
		Males (mg/kg/day)				Females (mg/kg/day)			
		0	5	10	25	0	5	10	25
		25	25	23	25	25	25	25	
Final Body Weight			-8*	-12*	-21*		-4*	-7*	-9*
Brain									
Absolute			+9*	+14*	+26*		+2	-1	-1
Relative to body weight			+8*	+8*	+33*		+7*	+6*	+10*
Liver									
Absolute			-15*	-19*	-32*		-4	-11*	-11*
Relative to body weight			-8*	-8*	-14*		0	-5*	-2
Kidney									
Absolute			-3	-8*	-14*		-2	-3	-4
Relative to body weight			+5*	+4	+8*		+3	+3	+6
Spleen									
Absolute			-3	-9*	-14*		-5	-5	-17*
Relative to body weight			+6	+4	+9		0	1	-8
Heart									
Absolute			-4	-6*	-12*		-9*	-8	-10*
Relative to body weight			+4	+7*	+11*		-6	-1	-1
Epididymis, Right									
Absolute			-1	-3	-7*		--	--	--
Relative to body weight			+5	+10*	+16*		--	--	--
Epididymis, Left									
Absolute			-1	-1	-9*		--	--	--
Relative to body weight			+6	+13*	+16*		--	--	--
Cauda Epididymis, Right									
Absolute			-4	-4	-12*		--	--	--
Relative to body weight			+2	+9	+11*		--	--	--
Cauda Epididymis, Left									
Absolute			-5	-4	-13*		--	--	--
Relative to body weight			+4	+9	+11*		--	--	--
Ovaries									
Absolute			--	--	--		-4	-9*	-11*
Relative to body weight			--	--	--		0	-2	0
Thymus									
Absolute			+6	0	-14*		-8	-10	-23*
Relative to body weight			+15	+13	+9		-3	-3	-14
Adrenal glands									
Absolute			+7	+1	+6		-4	-4	-3
Relative to body weight			+8*	+8*	+33*		0	4	8

n/n = total occurrence/number of animals

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

Reproductive performance of males were not detected at dose levels of 5, 10 or 25 mg/kg/day. Specifically, there were no changes in male mating index or male fertility index.

Parameter	Summary of Male Reproductive Performance (N=21-24)
-----------	--

	Males (mg/kg)			
	0	5	10	25
Male Mating Index (%)	96	96	96	91.3
Male Fertility Index (%)	87.5	91.7	88	91.3

n/n = total occurrence/number of animals

Spermatogenic Endpoints: There were no effects of oxymorphone at the doses tested on mean testicular and epididymal sperm numbers, sperm production rate, motility and morphology differential counts compared to control animals.

Female reproductive performance was not significantly altered by oxymorphone treatment. There was a significant but slight increase in the mean estrus cycle length in the high dose animals.

Parameter	Summary of Female Reproductive Performance (N=25)			
	Females (mg/kg)			
	0	5	10	25
Female Mating Index (%)	100	96	100	96
Female Fertility Index (%)	92	92	88	96
Mean estrus cycle length (days)	4.5	4.8	5.8	6.6*

* p < 0.05 compared to control

Parameter	Summary of Significant Embryonic Data			
	Females (mg/kg)			
	0	5	10	25
Number of gravid females	22	22	22	24
Viable embryos (mean)	16.1	14.9	13.9*	13.9*
Implantation sites (mean)	16.6	15.5	15.0*	14.5*
Corpora lutea (mean)	17.7	16.8	16.3	15.4*

* p < 0.05 compared to control

There were no significant changes in the number of dead embryos, early or late reabsorptions, post-implantation losses or pre-implantation losses at any dose of oxymorphone tested. In contrast, there were significant decreases in mean number of viable embryos and implantation sites at the two highest doses and a decrease in the mean number of corpora lutea at the highest dose tested (25 mg/kg/day).

Embryofetal development

Study title: A Study of the Effects of Oxymorphone Hydrochloride on Embryo/Fetal Development in Rats

Key study findings: Female rats were treated with oxymorphone (5, 10 and 25 mg/kg/day) from gestation day 6-17 in the definitive Segment II study in rats with the following key findings:

The following table presents the study group assignment:

<u>Group</u>	<u>Test Article</u>	<u>Dosage Level^a</u> <u>(mg/kg/day)</u>	<u>Dosage</u> <u>Concentration^a</u> <u>(mg/ml)</u>	<u>Dosage</u> <u>Volume</u> <u>(ml/kg)</u>	<u>Number</u> <u>Of</u> <u>Females</u>
1	Vehicle Control	0	0	10	25
2	Oxymorphone HCl	5	0.5	10	25
3	Oxymorphone HCl	10	1	10	25
4	Oxymorphone HCl	25	2.5	10	25

^a = Expressed in terms of the oxymorphone salt.

Parameters and endpoints evaluated: Animals were observed twice daily for mortality and moribundity. Clinical observations regarding general appearance and behavior were recorded from gestation days 0 through 20. Clinical observations included evaluation of changes in skin and fur appearance, eyes, mucous membranes, respiratory and circulatory systems, autonomic and central nervous systems, somatomotor activity and behavior. Animals were necropsied at scheduled euthanasia and the number and location of implantation sites, corpora lutea and the number of viable fetuses were recorded. Recognizable fetuses were examined externally and preserved in 10% neutral-buffered formalin. Body weights were recorded on gestation days 0, 6-18 and 20. Food consumption was recorded on gestation days 0, 6-18 and 20. Gravid uterine weights were recorded on at gestation day 20 at time of sacrifice. At necropsy, the thoracic abdominal and pelvic cavities were opened by a ventral midline incision and the contents were examined. The uterus and ovaries were removed. The number of corpora lutea on each ovary was recorded. Uteri with no macroscopic evidence of implantation were opened and subsequently analyzed for early implantation loss.

Fetal morphological examination. Each fetus was sexed, weighed and subjected to a detailed external examination including the eyes, palate and external orifices. Crown-rump measurements and degree of autolysis were recorded for late resorptions. Each fetus was examined visceraally and the sex was verified by internal examination. Fetal kidneys were examined and graded for renal papillae development. External, visceral and skeletal findings were recorded as developmental variations (alterations in anatomic structure that are considered to have no significant biological effect on animal health or body conformity) or malformations (structural anomalies that alter general body conformity, disrupt or interfere with body function or are generally thought to be incompatible with life).

Toxicokinetic analysis was conducted in a satellite group consisting of 20 animals dosed with 0.5 or 2.5 mg/kg/day. Blood samples were collected from 3 rats/group/time point via the retro-orbital sinus. Blood collection was according to the following schedule:

**Appears This Way
On Original**

<u>Subset</u>	<u>Targeted Time Point</u>	<u>Animals/Group</u>
A	Predose, 8 hr	3
B	0.5 and 10 hr	3
C	1 and 12 hr	3
D	2 and 16 hr	3
E	4 and 20 hr	3
F	6 and 24 hr	3

Results

Mortality (dams): One female in the high dose group (25 mg/kg/day) was found dead prior to dosing on gestation day 7. This death was considered to be related to treatment.

Mortality in Females	Incidence (n=25/group)			
	Dose oxymorphone (mg/kg)			
	0	5	10	25
Deaths prior to scheduled sacrifice	0	0	0	1

Clinical signs (dams): Pharmacological effects were noted 1 hour following dose administration and included hypoactivity, Straub tail, prostration, full body tetany and swaying from side to side.

Clinical Observations in dams (1 Hour Post-Dose)	Incidence (n=25/group)			
	Dose oxymorphone (mg/kg)			
	0	5	10	25
CNS/Behavior				
Straub tail	0/0	0/0	2/2	10/8
Rocks, lurches and sways as it walks	0/0	1/1	10/8	23/14
Prostrate	0/0	0/0	0/0	5/5
Hypoactive	0/0	1/1	13/8	25/13
Full body tetany	0/0	0/0	0/0	2/1
Head swaying side to side	0/0	3/2	2/2	0/0
Excessive chewing of limbs	0/0	0/0	9/7	7/5
Excessive chewing of caging	0/0	0/0	0/0	1/1
Excessive chewing of tail	0/0	0/0	1/1	0/0
Body / Integument				
Wet, yellow material urogenital area	0/0	0/0	0/0	1/1
Dried red material right forelimb	0/0	0/0	0/0	1/1
Wet red material right forelimb	0/0	0/0	0/0	3/1
Wet red material left forelimb	0/0	0/0	0/0	4/2
Wet red material right hindlimb	0/0	0/0	0/0	1/1
Cardio-Pulmonary				
Rales	0/0	0/0	0/0	1/1
Eyes/Ears/Nose				
Exophthalmus right eye	0/0	81/20	125/25	127/22
Exophthalmus left eye	0/0	81/20	125/25	129/22
Wet red material around nose	0/0	0/0	0/0	1/1
Oral/Dental				
Wet red material around nose	0/0	0/0	0/0	3/2

n/n = total occurrence/number of animals; -- = no data recorded

Body weight (dams): There was a dose-related decrease in body weight which became significant on gestation day 9-10 in the mid-dose and high-dose groups, respectively. Decreased body weight was also significant in the low dose group beginning on gestation day 13.

TABLE 4
STUDY OF OXYMORPHONE HYDROCHLORIDE ON EMBRYO/FETAL DEV. IN RATS
MEAN BODY WEIGHTS (GRAMS) DURING GESTATION

PROJECT NO. 411006
SPCNEOR:ENDO PHARM., INC. PAGE 1

GROUP :	1	2	3	4
DAY 0 MEAN	261.	264.	259.	259.
S.D./N	11.3/25	7.9/24	13.9/24	14.1/24
DAY 6 MEAN	299.	302.	294.	295.
S.D./N	13.5/25	11.0/24	15.4/24	14.0/24
DAY 7 MEAN	298.	303.	298.	301.
S.D./N	14.1/25	11.4/24	15.9/24	15.9/23
DAY 8 MEAN	304.	304.	300.	303.
S.D./N	14.8/25	12.3/24	17.1/24	19.5/23
DAY 9 MEAN	309.	306.	295.**	299.
S.D./N	15.0/25	13.1/24	16.7/24	17.9/23
DAY 10 MEAN	315.	308.	295.**	291.**
S.D./N	15.7/25	15.0/24	17.0/23	13.9/23
DAY 11 MEAN	323.	315.	300.**	293.**
S.D./N	15.8/25	14.6/24	15.6/24	13.3/23
DAY 12 MEAN	330.	320.	305.**	296.**
S.D./N	16.6/25	13.5/24	18.5/24	15.2/23
DAY 13 MEAN	335.	322.*	309.**	304.**
S.D./N	16.8/25	14.9/24	18.0/24	14.7/23
DAY 14 MEAN	339.	328.*	314.**	309.**
S.D./N	15.3/25	15.9/24	17.8/24	14.2/23
DAY 15 MEAN	348.	335.*	320.**	314.**
S.D./N	15.2/25	17.2/24	18.0/24	14.5/23
DAY 16 MEAN	360.	347.*	327.**	317.**
S.D./N	16.0/25	18.1/24	19.4/24	21.3/23
DAY 17 MEAN	376.	360.**	341.**	330.**
S.D./N	17.0/25	18.3/24	18.9/24	18.5/23
DAY 18 MEAN	393.	377.**	359.**	349.**
S.D./N	18.7/25	18.4/24	20.1/24	19.8/23
DAY 20 MEAN	432.	411.**	380.**	359.**
S.D./N	21.4/25	21.5/24	22.4/24	22.9/23

1- 0 MG/KG/DAY 2- 5 MG/KG/DAY 3- 10 MG/KG/DAY 4- 25 MG/KG/DAY

* - Significantly different from the control group at 0.05 using Dunnett's test
 ** - Significantly different from the control group at 0.01 using Dunnett's test
 NONGRAVID WEIGHT(S) NOT INCLUDED IN CALCULATION OF MEAN

RGSWS/Dvt
06/29/21

Initial body weight, terminal body weight, gravid uterine weight, net body weight and net body weight change are presented in the table below.

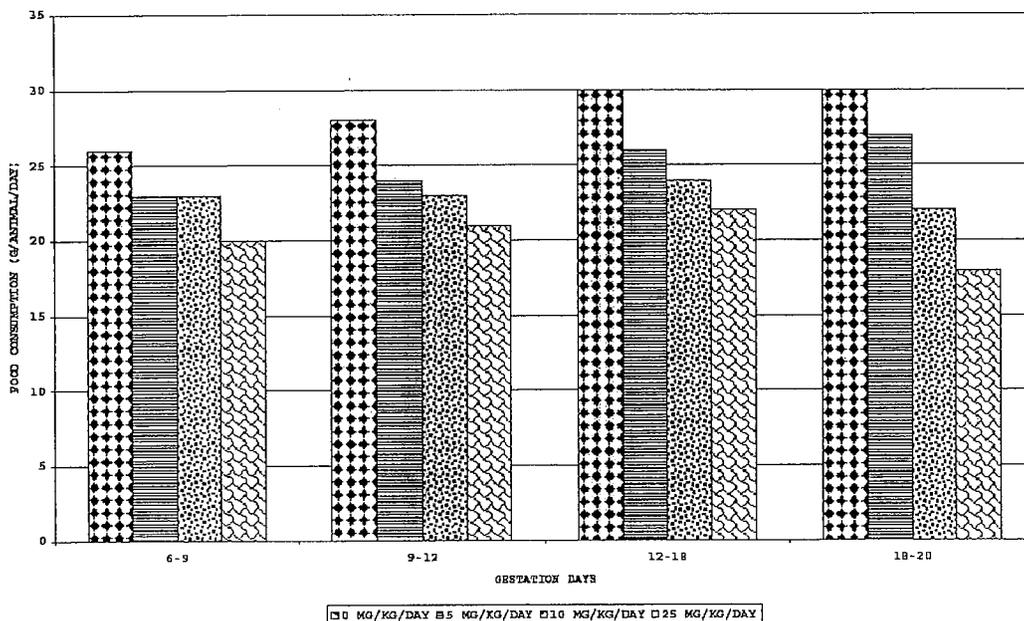
Parameter (% Δ of control)	Summary of body weights of dams (n=23-25/group)		
	Dose oxymorphone (mg/kg/day)		
	5	10	25
Initial body weight	+1	-1	-1
Terminal body weight	-5*	-12*	-17*
Gravid uterine weight	-1	-8*	-11*
Net body weight	-6*	-13*	-19*
Net body weight change	-30*	-56*	-80*

Food consumption (dams): Food consumption was significantly reduced by all doses of oxymorphone tested. Doses of either 10 or 25 produced a significant decrease in food consumption beginning with the first day of treatment (day 6-7 gestation). The low dose

of 5 mg produced a slight decrease in food consumption with statistical significance beginning at study day 8-9. The sponsor's figure below illustrates the dose-dependent decrease in food consumption in the dams during gestation.

PROJECT NO. -411006
 SPONSOR: ENDO PHARM., INC.

FIGURE 3
 STUDY OF OXYMORPHONE HYDROCHLORIDE ON EMBRYO/FETAL DEV. IN RATS
 MEAN FOOD CONSUMPTION DURING GESTATION (GRAMS/ANIMAL/DAY)



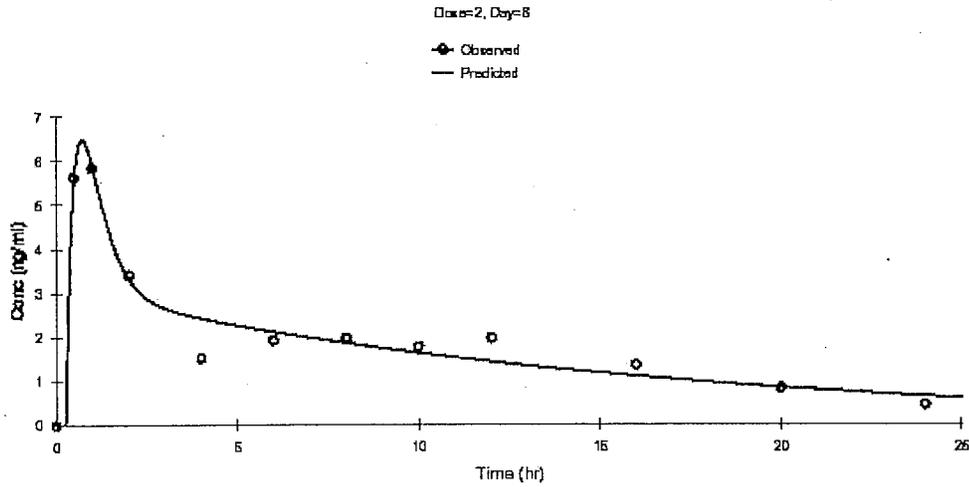
Toxicokinetics: Toxicokinetic analysis demonstrated that females dosed with either 5 or 25 mg/kg showed a dose related increase in the C_{max} and AUC_{24} values. There was no clear evidence that the AUC_{24} was altered from gestation day 6 to day 17. Initially, the high dose of oxymorphone increased the T_{max} and the $t_{1/2}$ compared to the low dose, however, by day 17, there was not clear difference between these parameters. Overall, the study clearly indicates that the female animals were exposed to significant amounts of oxymorphone. The table and charts below, taken from the sponsor's analysis summarized the toxicokinetic results for this study.

Toxicokinetic Parameters for All Data

Gestation Day	Dose mg/kg	T_{max} hr	C_{max} ng/mL	C_{24} ng/mL	AUC_{24} hr*ng/mL	k l/hr	$t_{1/2}$ hr	AUC_{∞} hr*ng/mL	Cl/F L/hr*kg
6	5	1	5.84	0.30	42.01	0.19	3.67	43.60	114.68
6	25	8	38.44	5.03	311.46	0.04	16.78	433.10	57.72
17	5	0.5	11.05	0.31	52.59	0.17	3.99	54.39	91.94
17	25	0.5	104.53	3.16	350.05	0.12	5.55	375.34	66.61

Plasma concentrations for the 5 and 25 mg/kg/day groups (a subset of 3 animals for each data point was used) on gestation day 6 were plotted and presented below:

Figure 1: Plasma Concentration versus Time – GD 6; 5 mg/kg

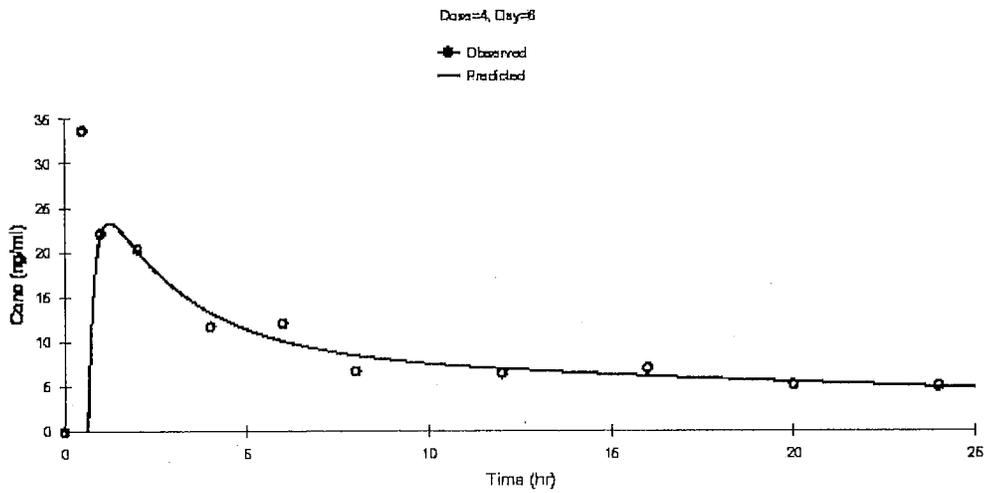


2-compartment model (Model 14 in WinNonlin)

Dose = Dose Group

Day = Gestation Day = GD

Figure 3: Plasma Concentration versus Time – GD 6; 25 mg/kg



2-compartment model (Model 14 in WinNonlin)

Dose = Dose Group

Day = Gestation Day = GD

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

Maternal necropsy data. The female animal in the high dose group that was found dead on gestation day 7 did not present with any internal abnormalities. Likewise all animals that survived to the scheduled sacrifice failed to demonstrate abnormalities upon necropsy. One female in the control group demonstrated fused placenta.

Gestation Day 20 Laparohysterectomy Data. There were no differences noted in the mean viable fetuses, % males, % females, dead fetuses, early resorptions, late resorptions, post implantation losses, implantation sites, corpora lutea, preimplantation losses or the number of gravid females. There was a slight but significant decrease in the mean fetal weights in the mid-dose and high-dose animals (5-6%) compared to control animals. This has been reported for other opioids and is likely treatment-related (Fujinaga, et al., 1986; Fujinaga and Mazze, 1988).

Offspring (malformations, variations, etc.): There were no significant differences in the number of malformations (external, soft tissue or skeletal) between treatment groups.

Examination of the fetuses for variations demonstrated an increase in the incidence of several skeletal variations. Although there was a slight increase in the incidence of fetuses with unossified pubis, accessory skull bone(s), 27 presacral vertebrae and bent rib(s), these changes were not statistically significant. All doses increased the mean number of animals with 14th rudimentary rib(s). The response, however, does not appear to be dose-dependent is within the historical control values, and the control values are below the historical control values. Therefore, the response does not appear to be clearly related to the drug treatment. Historical control data for the other findings suggest that they are within historical values with the exception of the one animal demonstrating accessory skulls bone(s) out of 370 fetuses in this study compared to no observations in the 403 historical litters.

Parameter	Dose (mg/kg) →	% per litter of fetal variations (n=23-25/group)				
		0	5	10	25	Historical Control
Number litters examined skeletally		25	24	24	23	403
% with 14 th Rudimentary rib(s)		1.4%	9.2%	6.2%	11.9%	2.5-12.0%
% with Pubis unossified		0	0	0	0.5%	0.0-0.5%
% with Accessory skull bone(s)		0	0	0	0.3%	0%
% with 27 presacral vertebrae		0	0	0	0.3%	0.0-1.8%
% with Bent rib(s)		0	0	0	0.3%	0.0-0.6%

Appears This Way
On Original

Doses: 0, 10, 25 and 50 mg/kg/day
from gestation day 7-20.

Species/strain: —(NZW)SPF rabbits

Number/sex/group: 25/group. The following groups were employed for the main study:

The following table presents the study group assignment:

<u>Group</u>	<u>Test Article</u>	<u>Dosage Level^a</u> <u>(mg/kg/day)</u>	<u>Dosage</u> <u>Concentration^a</u> <u>(mg/ml)</u>	<u>Dosage</u> <u>Volume</u> <u>(ml/kg)</u>	<u>Number</u> <u>of</u> <u>Females</u>
1	Vehicle Control	0	0.0	1.5	25
2	Oxymorphone Hydrochloride	10	6.67	1.5	25
3	Oxymorphone Hydrochloride	25	16.67	1.5	25
4	Oxymorphone Hydrochloride	50	33.33	1.5	25

^a = Expressed in terms of the oxymorphone salt.

Dosing was based upon the results of a dose range-finding study conducted in pregnant female rabbits (—-411007).

Route, formulation, volume, and infusion rate: Oral gavage, single daily dose.

Satellite groups used for toxicokinetics: 0/group

Study design: Animals were dosed from day 7-20 of gestation. The study design consisted on one vehicle control group and three treatment groups as indicated in the table above. Rabbits were artificially inseminated. The day of insemination was designated as gestation day 0.

Parameters and endpoints evaluated: Maternal observations during gestation included clinical signs and survival. In addition, body weights were recorded on gestation days 7, 7-21, 24 and 29. Gravid uterine weights were collected at gestation day 29 laparohysterectomy. Food consumption was recorded daily from gestation days 0-29. Blood samples were collected on the first and last days of dosing (gestation day 7 and 20, respectively at one and 24 hours following dosing. Necropsy was conducted at day 29 following laparohysterectomy. The thoracic, abdominal and pelvic cavities were opened and the contents were examined. The uterus and ovaries were excised, weighed and opened. The number of fetuses, early and late resorptions, if present, and the total number of implantation sites were recorded. The number of corpora lutea on each ovary was recorded and the individual uterine distribution of implantation sites was documented. Uteri with no macroscopic evidence of implantations were opened and analyzed for early implantation loss.

Each fetus was weighed, sexed and examined for external abnormalities. Crown-rump measurements were recorded for late resorptions. The sex of each fetus was determined internally and the viscera examined. Fetal kidneys were examined for renal papillae development. Carcasses were fixed and stained for skeletal examination. External, visceral and skeletal findings were recorded for developmental variations (alterations in

anatomic structure that are considered to have no significant biological effects on animals health or conformity, representing slight deviations from normal) or malformations (structural anomalies that alter general body conformity, disrupt or interfere with body function or may be incompatible with life).

Results

Mortality (dams): One control female died on gestation day 13 of unapparent causes. One female in the low dose group aborted on gestation day 21. This animal was hypoactive on gestation day 16 and 17, had hair loss on the inguinal areas and/or urogenital area during gestation days 15-21. As no abortions or deaths were noted in the mid-dose or high-dose group, these findings do not appear to be attributed to the oxymorphone treatment.

Parameter	Dose (mg/kg) →	Summary of material survival and pregnancy status (n=25/group)			
		0	10	25	50
Females in study		25	25	25	25
Females that aborted or delivered		0	1	0	0
Females that died		1	0	0	0
Females that were euthanized		0	0	0	0
Females examined at scheduled necropsy		24	24	25	25
Total females gravid		24	23	23	20

Clinical signs (dams): Clinical signs were recorded during daily examinations as well as 1-hour post dose. The table below summarized the incidence of clinical findings during the daily examinations.

Parameter	Dose (mg/kg) →	Incidence of treatment-related clinical signs (n=24-25/group)			
		0	10	25	50
Body/integument					
Dried brown material base of tail		0/0	4/3	2/1	11/5
Hair loss dorsal posterior area		0/0	0/0	1/1	7/2
Eyes/Ears/Nose					
Lacrimation right eye		0/0	0/0	0/0	8/1
Lacrimation left eye		0/0	0/0	0/0	6/2
Excreta					
Decreased defecation		0/0	3/2	76/17	52/19
Oral/Dental					
Upper left incisor missing		0/0	0/0	0/0	9/1
Upper right incisor missing		0/0	0/0	0/0	6/1

n/n = total occurrences/number of animals

1-hour post dose		Incidence of treatment-related clinical signs (n=24-25/group)			
Parameter	Dose (mg/kg) →	0	10	25	50
Behavior/CNS					
Hypoactive		2/2	19/15	48/21	61/22
Excessive chewing on cage bottom		0/0	0/0	1/1	1/1
Eyes/Ears/Nose					
Lacrimation of right eye		0/0	0/0	0/0	2/2
Mydriasis right eye		0/0	0/0	1/1	2/2
Mydriasis left eye		0/0	0/0	1/1	2/2
Lacrimation left eye		0/0	0/0	0/0	2/2
Exophthalmus right eye		0/0	0/0	1/1	2/2
Exophthalmus left eye		0/0	0/0	1/1	3/2
Excretia					
Soft stool		0/0	0/0	0/0	1/1

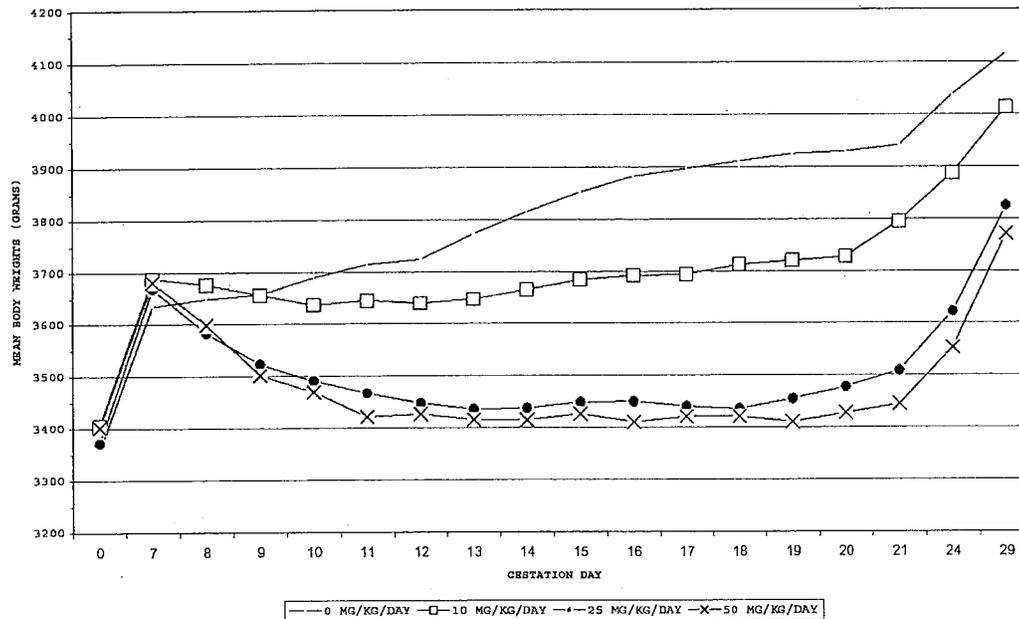
n/n = total occurrences/number of animals

Body weight (dams): Maternal body weight losses were noted during the first 3 days of dosing (gestation days 7-10) for all doses of oxymorphone tested. The body weights of dams in the 25 mg/kg and the 50 mg/kg group remained low throughout the dosing period. The dams treated with 10 mg/kg/day demonstrated reduced body weight gain compared to control animals throughout the dosing period. These observations are depicted in sponsor's figure 1 below. During the post-treatment period, all animals demonstrated an increase in body weights with the mid-dose and high-dose group appearing to gain faster than the 10 mg/kg and control groups, suggesting recovery. This decrease in body weight is considered to be related to the drug-treatment. Mean gravid uterine weights were similar across treatment groups.

Appears This Way
On Original

PROJECT NO. 411008
 SPONSOR: ENDO PHARMACEUTICALS

FIGURE 1
 STUDY OF OXYMORPHONE HYDROCHLORIDE
 ON DEVELOPMENT IN RABBITS
 MEAN BODY WEIGHTS (GRAMS)



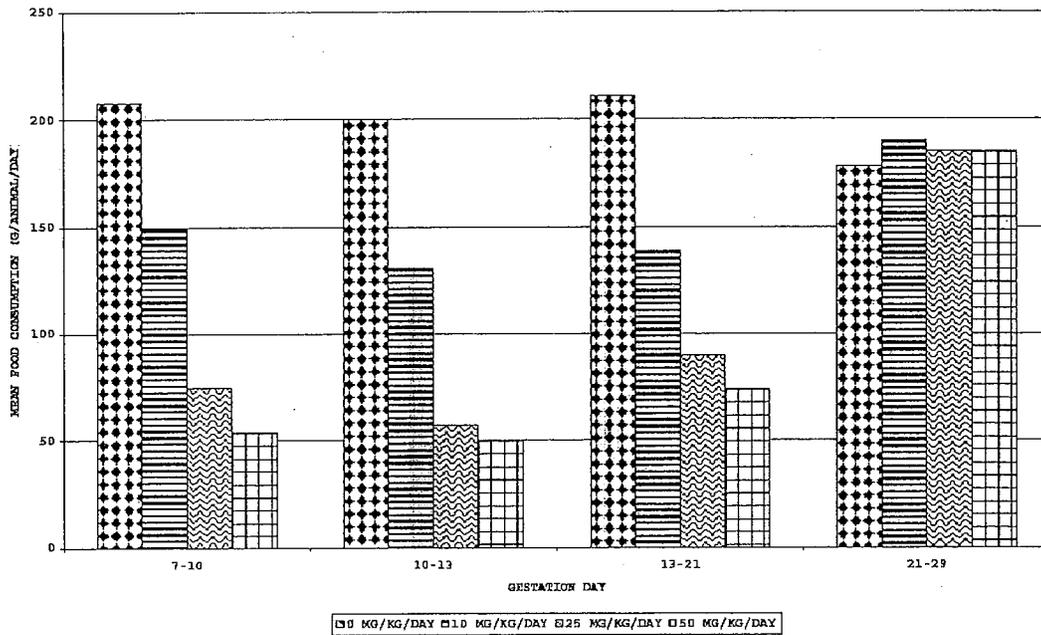
Food consumption (dams): Food consumption in the dams was significantly reduced in a dose-related manner throughout the treatment period. Mean food consumption during gestation (days 7-21) was reduced by 33, 62 and 69% following oxymorphone treatments of 10, 25 and 50 mg/kg, respectively. These reductions were considered to be treatment-related. Food consumption in all groups returned to normal following cessation of the drug treatment. These observations are depicted in the table and figure below:

Interval	Dose (mg/kg) →	Food Consumption (n=20-23/group) (% of Control, grams/animal/day)		
		10	25	50
Day 7-21		-33*	-62*	-69*
Day 21-29		+7	+4	+4
Day 0-29		-13*	-30*	-34*

Appears This Way
 On Original

PROJECT NO. 411008
 SPONSOR: ENDO PHARMACEUTICALS

FIGURE 3
 STUDY OF OXYMORPHONE HYDROCHLORIDE
 ON DEVELOPMENT IN RABBITS
 MEAN FOOD CONSUMPTION (GRAMS/ANIMAL/DAY)



Toxicokinetics: Toxicokinetic analysis in the range-finding study suggested that the peak plasma concentration of oxymorphone were obtained approximately 0.5 to 1 hour post dose. Therefore blood levels at 1 hour post-dose were compared. Increasing the dose of oxymorphone produced an increase in the plasma concentration of drug. There were no apparent differences in blood plasma levels between the first dose and the last dose.

Table 1: Comparison of the results from the analysis from the range-finding study with those obtained in the definitive study.

Dose Group (mg/kg)	Study Day†	1 hour (ng/mL) Range-Finding Study (—411007)	1 hour (ng/mL) Definitive Study (—411008)
1	7	1.74	
10	7		9.7
25	7	11.87	19.00
50	7		45.88
75	7	135.7	
1	20	1.44*	
10	20		9.03
25	20	15.0	14.88
50	20		21.92
75	20	32.47	

† - Study day is the same as gestation day.

* - Method 2 value