intravenous preparations of oxymorphone, there are no safety concerns for orally administered dosage forms. However, the drug product appears to contain [redacted] at levels that exceed ICH specifications for qualification.

The potential for dermal sensitization in guinea pigs was assessed via a modified Magnusson-Kligman Guinea Pig Maximization Test. The results suggested that oxymorphone is a dermal sensitizer under the conditions of the test.

Skin irritation studies in rabbits were also conducted to determine if oxymorphone produced skin irritation when administered via parenteral routes including intravenous, subcutaneous and intramuscular. In these studies 2 rabbits were administered 10 mg (about 4 mg/kg) of oxymorphone by the appropriate route. One animal was sacrificed 24 hours post dose and the other animal was sacrificed 7 days post dose. Histological examination of the injection sites failed to detect any oxymorphone-related irritation.

Study 51062 examined the hemocompatibility and hemolytic potential of oxymorphone. The results indicated that serum is compatible with oxymorphone at 10 mg/ml. The results indicated that a ratio of 1:3 (oxymorphone:plasma) was compatible, but anything greater resulted in precipitation. Regarding hemolytic potential, oxymorphone blood ratios of 1:1, 1:2, and 1:3 did not result in hemolysis.

3.4.2 Single-dose toxicity

The following acute and subacute toxicology studies were previously reviewed by Dr. Kathleen Haberny. The following summaries are reproduced verbatim from that review.

> **Single intravenous exposure in mice**: In the single dose intravenous toxicity study in mice, [redacted] of the oxymorphone doses administered at up to 4 mg/kg IV), did not significantly alter mortality and toxicity induced by acute oxymorphone at doses up to 200 mg/kg IV. The highest non-lethal oxymorphone doses in male and female mice were 100 mg/kg without [redacted] and 30 mg/kg in combination with [redacted]. However, mortality was increased in the lower dose by 1 of 10 males and 2 of the 10 females administered oxymorphone + [redacted]. The minimal lethal doses were 200 mg/kg/d in the absence of [redacted], and 100 mg/kg/d oxymorphone in combination with [redacted] in both males and females. The causes of death were not established. Toxicity was measured by mortality, clinical signs, changes in body weights and ophthalmoscopic examination. Necropsy was not performed. Clinical signs characteristic of mu opioid effects were observed at oxymorphone doses at and above 3 mg/kg IV, and included ataxia, increased motor activity and straub tail. At higher doses, tremors, gasping, loss of righting reflex and deaths were also observed. Corneal opacities were attributed to oxymorphone-induced loss of blinking reflex. There appeared to be an increase in some clinical signs, with [redacted] administration, although these changes were not consistently observed across doses of oxymorphone or across sexes.

Single intravenous exposure in Sprague-Dawley rats: There were no increases in oxymorphone-induced mortality, clinical signs, ophthalmic abnormalities and body weight changes at up to 100 mg/kg IV when combined with [redacted] of the oxymorphone doses at up to 2 mg/kg). The highest non-lethal oxymorphone dose was 30 mg/kg/d in the absence of, and in combination with [redacted] in both male and female rats. The minimal lethal dose with and without [redacted] in both males and females was 100 mg/kg/d. Clinical signs were consistent with known mu-opioid effects and included cloudy eyes, loss of righting reflex, red urine, lacrimation, hair loss, and noisy respiration at doses of 0.3 mg/kg IV and above. At the highest dose (100 mg/kg IV), convulsions, twitching and deaths were observed. Ophthalmic abnormalities were attributed to mu-opioid related depression of the blinking reflex.

Subacute intravenous exposure (2 weeks) in mice: The doses of oxymorphone administered to mice in this study (0.3, 3.0 and 30.0 mg/kg/d) represent approximately 0.5-45 times the human dose (3 mg/d, 0.05 mg/kg IV in a 60 kg patient) on a body surface area basis. There were no treatment-related deaths, differences in body weight changes, or ophthalmic abnormalities in mice administered oxymorphone with or without [redacted] for 14 days. Oxymorphone alone and in combination with [redacted] was associated with increased or decreased motor activity and straub tail. Motor activity was increased to a greater extent in male mice that received mid- and high-dose oxymorphone with [redacted] than without the degredant. There were no other differences in the severity of effects of oxymorphone when combined with [redacted]. Increased BUN and decreased calcium were observed in high dose males, but not females co-exposed to [redacted], and these effects were attributed to stress and hyperexcitability. Stress-related thymic necrosis was observed in males and females in the mid- and high-dose oxymorphone + [redacted] groups.

Subacute intravenous exposure (2 weeks) in rats: The oxymorphone doses administered in this study (0.3, 0.9, 3.0 and 30.0 mg/kg/d) represented approximately 1-100 times the human dose (3 mg/d IV, 0.05 mg/kg in a 60 kg patient) on a mg/m^2 basis. There was no effect of [redacted] co-administration when given at [redacted] on oxymorphone-induced mortality, clinical signs, body weight, food consumption, and ophthalmologic toxicity. Oxymorphone-related clinical signs were catalepsy, decreased motor activity, cage biting, salivation, noisy respiration, and cloudy eyes. The cloudy eyes, identified as corneal opacities are attributed to mu-opioid-induced decreased blinking reflex. Hematologic, serum chemistry, urinalysis, and organ weight alterations, gross pathology and histopathology at necropsy were attributed to oxymorphone administration and not related to co-treatment with [redacted]. The oxymorphone-induced changes included elevated serum aspartate aminotransferase activity at 0.9 mg/kg/day and above, reduced serum protein concentrations (male rats at 0.9 mg/kg/d and above), decreased mean serum triglyceride concentrations (male rats only at 30 mg/kg/d), and blood in urine at 0.9 mg/kg/day. Histopathology examination showed corneal mineralization and degeneration of the corneal epithelium at 0.9 mg/kg/day with and without [redacted] resulting from decreased blinking reflex. Drug

related atrophy of the uterine horns, reduced thickness of the vaginal mucosa, and decreased thymus weight were observed.

CONCLUSIONS

[redacted] of the oxymorphone doses did not potentiate the acute and subacute (14-day) intravenous toxicity of oxymorphone in mice and rats, with the exception that [redacted] appeared to potentiate oxymorphone-induced necrosis of the thymus in mice. The highest doses tested in the mice and rats were approximately 45 and 100 times respectively the human dose of 3 mg/day on a body surface area basis. The results of these studies suggest that daily exposure, for up to two weeks, to [redacted] in oxymorphone hydrochloride solution for intravenous administration, at therapeutic doses in humans, is reasonably safe from a pharmacology and toxicology viewpoint.

3.4.3 Repeat-dose toxicity: Thirteen week repeat-dose toxicology studies were conducted in rats, mice and dogs. In addition, an oral dose-range finding study was conducted in dogs to establish the definitive dosing for the 13-week oral toxicology study in dogs. Only the 13-week oral toxicology study will be reviewed for this NDA review. The 13-week repeat-dose studies in mice and rats have been previously reviewed by Dr. Kathleen Haberny (January 30, 2002). Her report is basically reproduced verbatim below (fit to the NDA format).

Study title: A 13-Week Oral Study of Oxymorphone Hydrochloride in Rats

Key study findings: Sprague-Dawley rats were treated with gradually escalating doses of oxymorphone (0, 10, 25, 50, 75 mg/kg/day from Day 14-91) via oral gavage. The following key findings were obtained:

1. Possible treatment-related death in a female rat given 75 mg/kg/d on treatment day 48.
2. Subacute liver inflammation, dark red lungs with hemorrhage, thymus hemorrhage observed in female given 75 mg/kg/d that died on day 48.
3. Treatment-related clinical signs typical of opioid-induced effects: GI, behavioral and CNS in nature including self-mutilation.
4. Body weights and body weight gains were significantly decreased in male rats at all doses (10-75 mg/kg/d), to 78%-66% of the control male weight gain; no treatment-related effects on weight or weight gains in female rats.
5. Decreased food consumption in male rats at all doses.
6. Hematology changes indicating mild anemia (↓6-9%).
7. Treatment-related decrease in mean total protein, albumin, calcium, and globulin in male rats, treatment-related decrease in cholesterol in female rats
8. Toxicokinetic evaluation demonstrated dose-related systemic exposure to oxymorphone, T_{max} generally 0.5-1 hour, AUC (0-24h) doubled from day 0 to day 88 in all dose groups, C_{max} and AUC dose-proportional.

9. The MTD was identified as 10 mg/kg/d in the males and 25 mg/kg/d in the females.
10. Based upon clinical signs, decreased body weights and food consumption in males a NOAEL was not obtained in this study (< 10 mg/kg). Although female rats were not as affected by oxymorphone as male rats, a NOAEL for female rats is also below 10 mg/kg/day based upon adverse clinical signs.

Study no.: [redacted]-411011
Volume #, and page #: Electronic Document Room
Conducting laboratory and location: [redacted]

Date of study initiation: January 28, 2001
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Oxymorphone, Lot 99193, [redacted] purity

Methods

Doses: The doses were administered in a graduated manner as follows:

Oxymorphone HCl (mg/kg/d)			
Target Dose	Days 0-6	Days 7-13	Days 14-91
0	0	0	0
10	10	10	10
25	25	25	25
50	25	50	50
75	25	50	75

Species/strain: [redacted] CD®(SD)IGS BR rats [redacted]

Number/sex/group or time point (main study): 10/sex/dose group
Route, formulation, volume, and infusion rate: Oral gavage at 10 ml/kg, as a single daily dose
Satellite groups used for toxicokinetics or recovery: 12/sex/dose group
Age: 6 weeks at initiation of dosing
Weight (nonrodents only): 181-247 g males, 141-193 g females
Unique study design or methodology (if any): Dosing was increased over the duration of the study to overcome tolerance and establish adequate toxicity.

Observation times and results

Mortality: Animals were observed for mortality pretest and twice daily. The following deaths were observed.* Only one death, a high-dose female on day 48, is considered to be drug-related.

Dose group (mg/kg/d)	No. of Deaths (dose at time of death)	Study Day	Probable Cause#
0	1 tk M (0 mg/kg/d)	84	Euthanized, urinary obstruction
10	1 tk F (10 mg/kg/d)	42	Euthanized, urinary obstruction
	1 tk M (10 mg/kg/d)	78	Undetermined
25	1 F (25 mg/kg/d)	5	Trapped in food jar, suffocation
50	1 tk M (50 mg/kg/d)	7	Trapped in food jar, suffocation
75	1M (25 mg/kg/d)	6	Trapped in food jar, suffocation
	1 tk M (25 mg/kg/d)	5	Trapped in food jar, suffocation
	1 tk F (25 mg/kg/d)	4	Trapped in food jar, suffocation
	1F (75 mg/kg/d)	48	Possibly Drug Related
	2 tk M (75 mg/kg/d)	14, 21	Mechanical injury during blood collection

*M: male; F: female; tk: toxicokinetic group animal

#Food jars removed after day 7, replaced with solid pellet food from hanging feeders

Clinical signs: Clinical signs were recorded both pretest and twice daily. Clinical signs were observed in all oxymorphone treated groups. The observations were primarily related to gastrointestinal, behavioral and central nervous system changes. Soft feces and vocalization upon handling were observed at a higher incidence at the time of dosing. The incidence of the remaining observed clinical signs was highest at 1 hour after dosing, and higher after the dose adjustments, during weeks 3-13. Inspection of the individual animal data revealed that the clinical signs persisted throughout the dosing period to week 13. The clinical signs observed during study days 14-91 are presented in the following tables:

Incidence of Clinical Signs at Time of Dosing (Total Occurrence/# Animals)

	Males					Females				
Dose (mg/kg/d)	**0**	**10**	**25**	**50**	**75**	**0**	**10**	**25**	**50**	**75**
Number of Animals	**10**	**10**	**10**	**10**	**9**	**10**	**10**	**9**	**10**	**10**
Soft Feces	0/0	3/3	2/1	12/4	16/5	0/0	0/0	2/2	6/4	9/6
Vocalization upon Handling	0/0	6/2	8/3	13/4	21/4	0/0	8/4	1/1	25/4	3/3
Chewing on Cage Bottom	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0
Hyperactive	0/0	0/0	0/0	2/2	0/0	2/1	0/0	0/0	1/1	0/0
Hyperactive to touch	0/0	1/1	1/1	4/2	5/3	0/0	0/0	0/0	6/1	2/2

Incidence of Clinical Signs at 1-Hour Past Dosing (Total Occurrence/# Animals)

	Males					Females				
Dose (mg/kg/d)	**0**	**10**	**25**	**50**	**75**	**0**	**10**	**25**	**50**	**75**
Number of Animals	**10**	**10**	**10**	**10**	**9**	**10**	**10**	**9**	**10**	**10**
Soft Feces	0/0	1/1	1/1	0/0	0/0	0/0	0/0	0/0	0/0	1/1
Vocalization upon Handling	0/0	1/1	0/0	0/0	5/2	0/0	1/1	0/0	8/1	0/0
Abnormal Stance	0/0	10/4	13/8	28/9	33/9	0/0	12/5	14/6	25/7	15/6
Excessive Chewing	0/0	20/5	41/7	38/10	44/8	0/0	31/9	31/8	43/9	30/10
Chewing on Forelimb(s)	0/0	45/7	59/10	18/7	22/7	1/1	57/10	67/7	33/8	34/7
Chewing on Cage Bottom	0/0	13/4	32/7	38/8	29/8	0/0	16/6	16/7	48/9	32/8
Chewing on Hindlimb(s)	0/0	0/0	0/0	0/0	0/0	0/0	1/1	1/1	1/1	0/0
Chewing on Tail	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0	1/1	0/0
Hyperactive	0/0	23/7	8/4	14/5	21/6	0/0	25/7	14/3	25/8	20/8
Hyperactive to Touch	0/0	0/0	1/1	1/1	6/3	0/0	1/1	0/0	2/1	4/4
Circling	0/0	1/1	0/0	1/1	1/1	0/0	3/2	1/1	0/0	0/0
Licking Cage	0/0	2/2	5/5	25/5	7/4	0/0	0/0	3/2	10/5	11/5
Excessive Grooming	0/0	2/1	1/1	1/1	0/0	1/1	5/3	4/3	1/1	2/2

Body weights: Body weights were recorded both pretest and weekly. The mean body weights and body weight gains at the end of the study are presented in the following table:

Body Weight Observations

	0 mg/kg/d	10 mg/kg/d	25 mg/kg/d	50 mg/kg/d	75 mg/kg/d
Males					
Weight in g, mean ± SD wk 13 (%control)	553 ± 80.6	481 ± 44.0* (-13%)	446 ± 25.3** (-19%)	440 ± 46.5** (-20%)	438 ± 33.9** (-21%)
Total Body weight gain in g, mean ± SD, wk 0-13 (%control)	338 ± 69.2	265 ± 40.6** (-22%)	230 ± 30.5 (-32%)	223 ± 52.3** (-34%)	225 ± 33.0** (-34%)
% Control bodyweight gain	100	78	68	66	66
Females					
Weight in g, mean ± SD wk 13 (%control)	307 ± 30.0	305 ± 38.1 (-1%)	288 ± 13.4 (-6%)	304 ± 23.7 (-1%)	289 ± 17.7 (-6%)
Body weight gain in g, mean ± SD wk 0-13 (%control)	136 ± 23.4	134 ± 24.4 (-2%)	118 ± 10.3 (-13%)	133 ± 17.4 (-2%)	118 ± 8.7 (-13%)
% Control bodyweight gain	100	98	87	98	87

*p<0.05; **p<0.01

Body weight gains were decreased by 22%, 32%, 34%, and 34% compared to control body weight gains in the male rats given 10, 25, 50 and 75 mg/kg/d oxymorphone, respectively, at the end of the study. The mean body weights and body weight gains were comparable to control body weights in the female rats in all groups at the end of the study.

Food consumption: Food consumption was recorded both pretest and weekly. Food consumption was lower in the male rats at all doses, during dosing periods from weeks 0-1, 2-9, and 12-13. There were no treatment-related effects on food consumption in the female rats. The results of food consumption measurements in the male rats are presented in the following table:

Food Consumption (g/animal/d) in Male Rats Given Oral Oxymorphone HCl for 13 Weeks

	0 mg/kg/d	10 mg/kg/d	25 mg/kg/d	50 mg/kg/d	75 mg/kg/d
Mean Food Consumption Baseline	24 ± 2.4	24 ± 1.8	24 ± 1.5	24 ± 1.5	23 ± 1.5
Mean Food Consumption Week 13	33 ± 5.2	30 ± 4.6	29 ± 2.1	27 ± 6.7	28 ± 3.7
Difference from Baseline (Week 13) (%)	+9 (137%)	+6 (125%)	+5 (121%)	+3 (112%)	+5 (122%)
Difference from Controls (Week 13) (%)	0	-9%	-12%	-18%	-15%

Ophthalmoscopy: Ocular exams were conducted both pretest and once during week 12. There were no treatment-related effects.

EKG: Not done.

Hematology: Blood samples were collected during study week 13. Minor changes in red blood cells and red blood cell indices along with larger increases in reticulocytes were observed in all groups of male rats that received oxymorphone, and in the female rat groups that received 50 and 75 mg/kg/day oxymorphone. MCV values were slightly increased at all doses in the male rats, and in the female rats that received 50 and 75 mg/kg/day. The hematology effects suggest mild anemia. The hematology parameters with statistically significant changes are presented in the following table.

Hematology Values: Week 13 (Means ± SD)

		0 mg/kg/d	10 mg/kg/d	25 mg/kg/d	50 mg/kg/d	75 mg/kg/d
Red Cells (mil/uL) (%control)	Males	7.95 ± 0.445	7.44 ± 0.671 (-6%)	7.50 ± 0.204 (-6%)	7.32 ± 0.344* (-8%)	7.26 ± 0.720* (-9%)
MCV (fL) (%control)	Males	50.6 ± 1.28	53.7 ± 1.79** (+6%)	54.2 ± 1.39** (+7%)	54.1 ± 1.12** (+7%)	54.6 ± 1.97** (+7%)
	Females	52.8 ± 1.45	53.9 ± 1.53 (+2%)	54.0 ± 1.61 (+2%)	55.7 ± 3.04** (+5%)	55.1 ± 1.89 (+5%)
MCH (uug) (%control)	Males	18.3 ± 0.52	19.7 ± 0.68** (+8%)	19.8 ± 0.55** (+8%)	19.7 ± 0.51** (+8%)	19.9 ± 0.71** (+9%)
Reticulocyte (%) (%control)	Males	0.5 ± 0.16	0.8 ± 0.21 (+60%)	1.2 ± 0.16** (+140%)	2.0 ± 0.57** (+300%)	1.4 ± 0.31** (+180%)
	Females	1.5 ± 0.25	1.2 ± 0.20* (-20%)	1.0 ± 0.21** (-33%)	1.6 ± 0.24 (+7%)	2.6 ± 0.23** (+73%)
Retic Absolute (mil/uL) (%control)	Males	0.039±0.0123	0.058±0.0141 (+49%)	0.087±0.0122** (+123%)	0.149±0.0427** (+280%)	0.104±0.0214** (+167%)
	Females	0.108±0.0232	0.088±0.0161 (-29%)	0.073±0.0192** (-32%)	0.109±0.0186 (+1%)	0.182±0.0151** (+68%)

*p<0.05; **p<0.01

Clinical chemistry: Blood samples were obtained during study week 13. Minor treatment-related decreases in mean total protein, albumin, calcium, and globulin levels were observed in the male rats. These parameters were also decreased in the female rats,

but the decreases were not statistically significant. Cholesterol was decreased in the female rats given 25, 50 and 75 mg/kg/day oxymorphone, although the decrease was not dose-related. The changes in clinical chemistry values are presented in the following table:

Clinical Chemistry Values: Week 13 (Means ± SD)

	0 mg/kg/d	10 mg/kg/d	25 mg/kg/d	50 mg/kg/d	75 mg/kg/d
Albumin (g/dL)					
Males	3.9 ± 0.19	3.6 ± 0.26* (-8%)	3.6 ± 0.16* (-8%)	3.5 ± 0.10** (-10%)	3.5 ± 0.23** (-10%)
Females	4.3 ± 0.62	4.3 ± 0.24 No change	4.2 ± 0.22 (-2%)	4.0 ± 0.31 (-7%)	3.9 ± 0.46 (-9%)
Total Protein (g/dL)					
Males	6.6 ± 0.21	6.1 ± 0.27** (-8%)	5.9 ± 0.29** (-11%)	5.8 ± 0.25** (-12%)	6.0 ± 0.50** (-9%)
Females	7.0 ± 0.69	6.8 ± 0.44 (-3%)	6.6 ± 0.26 (-6%)	6.5 ± 0.37 (-7%)	6.5 ± 0.51 (-7%)
Globulin (g/dL)					
Males	2.7 ± 0.14	2.5 ± 0.18 (-7%)	2.4 ± 0.26** (-11%)	2.3 ± 0.27** (-15%)	2.5 ± 0.31 (-7%)
Females	2.6 ± 0.45	2.4 ± 0.22 (-8%)	2.4 ± 0.14 (-8%)	2.5 ± 0.22 (-4%)	2.6 ± 0.37 no change
Calcium (mg/dL)					
Males	10.7 ± 0.35	10.3 ± 0.31* (-4%)	9.9 ± 0.47** (-8%)	10.0 ± 0.48** (-7%)	10.2 ± 0.40* (-5%)
Females	10.8 ± 0.58	10.7 ± 0.42 (-1%)	10.3 ± 0.28 (-5%)	10.3 ± 0.59 (-5%)	10.5 ± 0.70 (--3%)
Cholesterol (mg/dL)					
Females	85 ± 24.6	71 ± 10.0 (-16%)	54 ± 10.1** (-36%)	63 ± 11.6* (-26%)	60 ± 18.3** (-30%)

*p<0.05; **p<0.01

Urinalysis: Urine was collected during study week 13. Urinary pH was increased in the female rats to 6.3, 6.5 and 6.4 at 10, 50 and 75 mg/kg/day, respectively, compared to the control value (5.7). However, the mean pH in the control group was abnormally low.

Gross pathology: All animals found dead, euthanized *in extremis* or at scheduled necropsy (week 13). The macroscopic findings in the animals that died and were euthanized *in extremis* included red fluid contents in duodenum, white areas on liver, raised red area on lung, firm thymus, dark red lungs, clear fluid contents in the pericardial sac, dilated renal pelvis, distended ureter, reddened urethra, enlarged lymph nodes, calculi in the urinary bladder, reddened mucosa of the urinary bladder and thickened urinary bladder. Subacute liver inflammation, dark red lungs with hemorrhage, thymus hemorrhage observed in female given 75 mg/kg/d that died on day 48. There were no treatment-related effects in the macroscopic examination in the rats that were sacrificed at scheduled necropsy.

Organ weights (specify organs weighed if not in histopathology table): The organ weight changes were without relationship to dose and without correlating morphological findings, and were attributed to the changes in final total body weights.

Histopathology: Adequate Battery: yes (X), no ( )—explain

Peer review: yes ( ), no (X)
See histopathology summary table.

Urinary tract calculi were observed in 2 rats that were euthanized *in extremis*. Granulomatous inflammation in the lungs was observed in male rats in all treatment groups and in the females that were given 25, 50, and 75 mg/kg/d oxymorphone, without dose-related differences in severity or incidence. The inflammation was attributed to aspiration of test article. In the high-dose female that died on day 48, subacute liver inflammation, dark red lungs with hemorrhage, and thymus hemorrhage were observed.

Toxicokinetics: 0.5 ml blood collected from 3 rats/sex/group/timepoint at 0.5, 1, 2, 3, 4, 8, 12, 16, 20, and 24 hours after dosing on study days 0 (10 and 25 mg/kg/d groups), 7 (50 mg/kg/d group), 14 (75 mg/kg/d group), and 88 (all groups). The C_{max} and AUC values increased proportionally to dose, were higher on day 88 than on day 0, and were similar in males and females.

The results of the toxicokinetic evaluation are presented in the following table:

Oxymorphone Toxicokinetic Parameters Following Oral Administration for 89 Days in Rats*

Dose (mg/kg/d)	Study Day	Sex	Cmax (ng/ml)	Tmax (h)	AUC_{0-24h} (ng.h/ml)
10	0	M	8.34	0.5	47.4 (50.0)[a]
		F	6.68	0.5	41.0 (44.2)[a]
10	88	M	16.1	1	106
		F	18.1	1	101
25	0	M	13.2	0.5	98.2 (130)[a]
		F	9.24	4	113 (152)[a]
25	88	M	46.2	0.5	262
		F	94.9	0.5	367
50	0	M	70.7	2	356
		F	90	1	306
50	88	M	168	0.5	683
		F	156	0.5	656
75	0	M	236	1	703
		F	175	0.5	700
75	88	M	271	0.5	1093
		F	556	0.5	1428

*M:male; F:female
[a]Estimated AUC zero to infinity using terminal half-life of 8.9 h (Kel = 0.0779)

Other: None

Study title: A 13-Week Oral Study of Oxymorphone Hydrochloride in Mice

Key study findings:

1. Treatment-related deaths at 300 mg/kg/d (3M and 1F) and 600 mg/kg/d (1M and 1F).
2. Necroscopic observations in the animals that died: distended stomach and duodenum (1 M at 600); in the 300 mg/kg/d animals that died: enlarged vas deferens and prolapsed penis (all M), swollen green preputial gland (1M),

distended/thickened urinary bladder (1M), distended stomach and urinary bladder (1F), impacted caecum (1F), distended gallbladder (1F)

3. Treatment-related clinical signs typical of opioid-induced in rodents and were primarily CNS-related
4. Body weights decreased in all males (-5% to –14% without dose-relationship), body weight gains decreased in all males (–86%, -28%, -88%, and –107% at 300, 600, 50 and 75 mg/kg/d respectively); body weights decreased –6% to –10% in treated females, body weight gains decreased –39% to –51% in all treated females
5. Food consumption decreased in males and females during weeks 1-2 and 6-7
6. Toxicokinetic evaluation demonstrated dose-related exposure to oxymorphone; C_{max} and AUC dose proportional, T_{max} 0.5-1 hour
7. MTD less than 300 mg/kg/day due to deaths observed at doses of 300 mg/kg/day or greater.

Study no.: [redacted]-411012
Volume #, and page #: Electronic Document Room
Conducting laboratory and location: [redacted]

Date of study initiation: January 22, 2001
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Oxymorphone, Lot # 99193; [redacted] purity

Methods

Doses: The doses were administered in a graduated manner as follows:

Oxymorphone HCl (mg/kg/d)						
Target Dose	Days 0-6	Days 7-13	Days 14-41	Days 42-49	Days 50-62	Days 63-91
0	0	0	0	0	0	0
300	10	10	10	150	300	300
600	25	25	25	25	25	600
50	25	50	50	50	50	50
75	25	50	75	75	75	75

The doses were adjusted for body weights weekly, throughout the study.

Species/strain: [redacted] CD-1®(ICR)BR mice
Number/sex/group or time point (main study): 10/sex/group
Route, formulation, volume, and infusion rate: Oral by gavage at 10 ml/kg, once daily for 13 weeks
Satellite groups used for toxicokinetics or recovery: 3/sex/dose/timepoint
Age: 9 weeks
Weight (nonrodents only): 27.0-34.6 g males and 21.3-27.5 g females
Unique study design or methodology (if any):

Observation times and results

Mortality: Mice were monitored both pretest and twice daily during the study. Treatment-related deaths were observed in 3 males (2 found dead in weeks 9 and 12, 1 euthanized week 9) and 1 female (found dead week 9) at 300 mg/kg/day, and in 1 male (found dead week 9) and 1 female (found dead week 9) at 600 mg/kg/day. The deaths occurred after the last increase in dose to the 300 and 600 mg/kg/d levels.

Clinical signs: Clinical signs were recorded pretest and twice daily.

Incidence of Treatment-Related Clinical Signs: Time of Dosing (Total Occurrence/# Mice)

	Males					Females				
Dose (mg/kg/d)	0	10/ 150/ 300	25/ 600	50	75	0	10/ 150/ 300	25/ 600	50	75
Number of Animals	10	10	10	10	10	9	9	9	10	10
Swollen Abdomen	0/0	1/1	6/3	0/0	0/0	0/0	1/1	0/0	0/0	0/0
Unkempt Appearance	0/0	143/5	69/4	32/2	0/0	0/0	0/0	17/3	0/0	0/0
Abnormal Stance	0/0	0/0	4/3	0/0	0/0	0/0	0/0	1/1	0/0	0/0
Hypoactivity	0/0	3/3	3/2	0/0	0/0	0/0	1/1	1/1	0/0	0/0
Straub Tail	0/0	0/0	14/6	0/0	0/0	0/0	0/0	4/3	0/0	0/0
Rigid Muscle Tone	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Tremors	0/0	0/0	1/1	0/0	0/0	0/0	1/1	1/1	0/0	0/0

Incidence of Treatment-Related Clinical Signs: 1-H Past Dosing (Total Occurrence/# Mice)

	Males					Females				
Dose (mg/kg/d)	0	10/ 150/ 300	25/ 600	50	75	0	10/ 150/ 300	25/ 600	50	75
Number of Animals	10	10	10	10	10	9	9	9	10	10
Hypothermia	0/0	2/2	1/1	0/0	0/0	0/0	1/1	0/0	0/0	0/0
Swollen Abdomen	0/0	1/1	8/4	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Unkempt Appearance	0/0	129/5	67/6	33/2	0/0	0/0	3/3	15/3	0/0	0/0
Abnormal Stance	0/0	0/0	9/4	0/0	0/0	0/0	1/1	5/4	0/0	0/0
Hyperactivity	0/0	6/3	9/5	1/1	0/0	0/0	2/2	11/7	3/1	0/0
Hypoactivity	0/0	4/4	4/3	0/0	0/0	0/0	1/1	5/4	1/1	3/3
Straub Tail	0/0	45/8	81/10	41/9	53/8	1/1	46/7	50/9	57/6	50/8
Rigid Muscle Tone	0/0	03/3	7/4	0/0	0/0	0/0	0/0	3/3	0/0	1/1
Tremors	0/0	1/1	3/2	0/0	0/0	0/0	2/2	1/1	0/0	0/0

Body weights: Body weights were recorded pretest and weekly thereafter. The mean body weights and body weight gains at the end of the study are presented in the following table. Although body weight gains were decreased by up to 107%, the overall changes in body weight compared to control animals (3-5 g) is not considered to be significant.

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Body Weight Observations

	0 mg/kg/d	10/150/300 mg/kg/d	25/600 mg/kg/d	50 mg/kg/d	75 mg/kg/d
Males					
Weight (g, mean ± SD) wk 13	34.8 ± 3.19	30.5 ± 2.36*	33.0 ± 3.60	31.0 ± 1.63**	29.9 ± 2.16**
Percent control Weight	-	-12%	-5%	-11%	-14%
Total Body weight gain (g, mean ± SD) wk 0-13	4.3 ± 2.64	0.9 ± 1.60	3.1 ± 2.45	0.5 ± 2.41**	-0.3 ± 1.31
Difference in % Control bodyweight gain	100	-86%	-28%	-88%	-107%
Females					
Weight (g, mean ± SD) wk 13	30.0 ± 2.30	28.4 ± 1.47	27.5 ± 1.30	27.1 ± 1.98*	27.0 ± 2.23*
Percent Control Weight	-	-6%	-8%	-10%	-10%
Body weight gain (g, mean ± SD) wk 0-13	5.9 ± 1.11	3.6 ± 1.06*	2.9 ± 0.69	3.2 ± 1.81**	2.9 ± 1.70**
% Control bodyweight gain	100	-39%	-51%	-46%	-51%

*p<0.05; **p<0.01

Food consumption: Food consumption was recorded pretest and weekly. Statistically significant treatment-related decreases in food consumption were observed during weeks 1-2 and 6-7 only.

Changes in Food Consumption (g/animal/d) in Male Mice Given Oral Oxymorphone HCl for 13 Weeks

	0 mg/kg/d	10/150/300 mg/kg/d	25/600 mg/kg/d	50 mg/kg/d	75 mg/kg/d
Baseline Food Consumption	5.9 ± 1.02	5.9 ± 0.46	5.8 ± 0.52	6.8 ± 2.38	5.9 ± 0.70
Food Consumption Week 1-2	5.9 ± 1.47	4.6 ± 0.46**	4.3 ± 0.67**	4.7 ± 0.53**	4.4 ± 0.63**
Change from Baseline (%)	0	-22%	-26%	-31%	-25%
Difference from Control (%)	-	-22%	-27%	-20%	-25%
Food Consumption Week 6-7	5.7 ± 1.27	4.1 ± 0.35**	4.5 ± 0.60**	4.5 ± 0.43**	4.6 ± 0.49**
Change from Baseline (%)	-3%	-31%	-22%	-34%	-22%
Difference from Control (%)	-	-28%	-30%	-21%	-19%

*p<0.05; **p<0.01

Changes in Food Consumption (g/animal/d) in Female Mice Given Oral Oxymorphone HCl for 13 Weeks

	0 mg/kg/d	10/150/300 mg/kg/d	25/600 mg/kg/d	50 mg/kg/d	75 mg/kg/d
Baseline Food Consumption	5.4 ± 0.50	6.2 ± 2.03	7.5 ± 2.38**	5.4 ± 0.71	5.6 ± 0.41
Food Consumption Week 1-2	5.7 ± 1.05	5.7 ± 1.47	5.0 ± 1.26	4.2 ± 0.59*	4.3 ± 0.79*
Change from Baseline	+6%	-8%	-33%	-22%	-23%
Difference from Control	-	0	-12%	-26%	-25%
Food Consumption Week 6-7	6.2 ± 1.73	4.3 ± 0.32**	5.3 ± 1.86	4.6 ± 1.05*	4.6 ± 0.46*
Change from Baseline	+15%	-31%	-29%	-15%	-18%
Difference from Control	-	-31%	-15%	-26%	-26%

Ophthalmoscopy: Ocular exam was conducted both pretest and during study week 12. There were no treatment-related effects noted.

EKG: Not done.

Hematology: Blood samples were collected at the scheduled necropsy (study week 13). There were no treatment-related effects.

Clinical chemistry: Blood samples were collected at the scheduled necropsy (study week 13). There were no treatment-related effects.

Urinalysis: Not done.

Gross pathology: Gross pathological changes were recorded at the scheduled necropsy (study week 13). There were no treatment-related effects.

Necroscopic observations in the animals that died during administration of 600 mg/kg/d included distended stomach (1M) and duodenum (1 M). In the 300 mg/kg/d animals that died, enlarged vas deferens and prolapsed penis (all M), swollen green preputial gland (1M), distended/thickened urinary bladder (1M), distended stomach and urinary bladder (1F), impacted caecum (1F), and distended gallbladder (1F) were observed.

Organ weights (specify organs weighed if not in histopathology table): Organ weights were recorded at scheduled necropsy (study week 13). There were no treatment-related effects.

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no (X)
At scheduled necropsy (study week 13)

Toxicokinetics: 1.0 ml blood on study days 0, 7, 14, and 88 at 0.5, 1, 2, 3, 4, 8, and 24 hours after dosing. The C_{max} and AUC values increased proportionally to dose, and were similar in male and female mice.

Oxymorphone Toxicokinetic Parameters Following Oral Administration for 13 Weeks in Mice*

Dose (mg/kg/d)	Study Day	Sex	Cmax (ng/ml)	Tmax (h)	$AUC_{0\text{-}24h}$ (ng.h/ml)
300	88	M	2797	0.5	3590
		F	2757	0.5	3957
600	88	M	4493	0.5	6288
		F	3037	1	6557
50	7	M	224	0.5	730
		F	279	0.5	698
	88	M	262	0.5	405
		F	255	1	603
75	14	M	438	0.5	895
		F	354	1	886
	88	M	544	0.1	901
		F	351	0.1	682

*M:male; F:female

Other: None

Study title: A 13-Week Oral (Capsule) Study of Oxymorphone hydrochloride in dogs

Key study findings: Oxymorphone (0, 2, 10 or 40 mg/kg/day) was administered via oral gavage to dogs for a total of 13 weeks with the following results:

1. There were no mortalities.
2. Clinical signs were dose-dependent and characteristic of opioids. Animals in the 10 and 40 mg/kg groups demonstrated excessive salivation and foamy/wet material around the mouth. The high dose produced excessive head shaking and decreased mobility in one female and decreased defecation in 2 males and 1 female.
3. Body weight gain and food consumption decreased in the 10 and 40 mg/kg/day groups during the first two weeks of treatment, however, these effects demonstrated tolerance with time.
4. There were no biologically significant changes in hematology or clinical chemistry.
5. Females treated with the high dose had significantly lower spleen weights.
6. Histological analysis indicated that a single male and female animal in the high dose group demonstrated increased bile ducts in the liver with no other histological correlate for toxicity.
7. Based upon the bile duct increases, spleen weight changes and excessive head shaking at the high dose, a NOAEL of 10 mg/kg was proposed by the sponsor for both males and females. The reviewer concurs, as the changes in clinical signs and body weight/food consumption do not indicate safety issues. In the dog, this dose corresponds to an $AUC_{0\text{-}24h}$ of 186.72 and 177.62 ng•h/ml in males and females, respectively. C_{max} values at this dose on day 89 were 28.89 and 31.50 ng/ml in males and females, respectively.

Study no.: -411003
Volume #, and page #: N/A Electronic Submission
Conducting laboratory and location: [redacted]

Date of study initiation: November 29, 2000
GLP compliance: Yes
QA report: yes ( ) no ( )
Drug, lot #, and % purity: Oxymorphone hydrochloride, Lot A 4297A, purity was between [redacted] via HPLC analysis.
NOTE: Drug substance contained [redacted] as impurities and [redacted] as degradation products. The drug was administered orally via gelatin capsules and doses were individualized based on most recent body weights.

Methods:

Doses: 0, 2, 10 and 40 mg/kg.

Final Dose Levels

Group Number	Test Article	Dose Level (mg/kg/day)	Number of Animals	
			Males	Females
1[a]	Control	0	6	6
2	Low Dose	2	4	4
3	Mid Dose	10	4	4
4[a]	High Dose	40	6	6

[a] = At the end of the dosing period, two dogs/sex/group were assigned to the 28-day recovery period.

Species/strain: Beagle dogs
Number/sex/group or time point (main study): 4/sex/group.
Route, formulation, volume, and infusion rate: Oral gavage, capsule
Satellite groups used for toxicokinetics or recovery: 2/sex in Groups 1 and 4
Age: approximately 6 months
Weight (nonrodents only): Males 7.5-11.2 kg
Females 6.2-9.0 kg

Unique study design or methodology (if any): An escalating dosing regimen was employed for this study with the intention of achieving the maximum dose level for each group within 14 days. The schedule was reproduced from the study report below:

Group Number	Dose Oxymorphone mg/kg				
	Days 0-2	Days 3-6	Days 7-29	Days 30-40	Days 41-91
1	0	0	0	0	0
2	0.1	1	2	2	2
3	0.1	2	10	10	10
4	0.1	2	20	30	40

Observation times and results

Mortality: Mortality was examined twice daily. There were no unscheduled deaths.

Clinical signs: Clinical signs were monitored twice daily (at time of dosing and 1-2 hours post dosing). Detailed physical exams were conducted weekly. Clinical signs were recorded once weekly during the recovery period an on the day of necropsy. Clinical signs were noted at all dose levels.

Clinical Signs	Incidence of Clinical Signs							
	Males (mg/kg)				Females (mg/kg)			
	0	2	10	40	0	2	10	40
Clear discharge left eye	3/1	1/1	4/1	6/3	5/2	3/2	0/0	6/1
Injected Sclerea right eye	3/1	0/0	6/1	12/4	0/0	8/2	1/1	1/1
Injected sclera left eye	3/1	0/0	4/1	10/4	0/0	3/3	1/1	1/1

Defecation decreased	0/0	0/0	0/0	4/2	0/0	0/0	0/0	1/1
Wet clear material around mouth	3/2	0/0	17/4	21/6	1/1	5/3	7/2	22/6
Excessive salivation	0/0	0/0	8/2	19/6	0/0	0/0	1/1	39/6
White foamy material round mouth	0/0	0/0	6/2	11/5	0/0	0/0	3/1	12/5
Hypoactive	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/1
Excessive head shaking	0/0	0/0	0/0	0/0	0/0	0/0	0/0	2/1

n/n = total occurrence/number of animals

Body weights: Body weights were recorded twice weekly. There were no significant treatment-related body weight differences in males or females; however, there was a trend toward a decrease in body weights in both sexes at doses of 10 and 40 mg/kg/day. There was a significantly lower body weight gain in male dogs treated with 10 or 40 mg/kg/day during the first two weeks of treatment and on sporadic weeks thereafter. There was a significantly lower body weight gain in female animals treated with 40 mg/kg during weeks 1 and 2. The initial decreases were likely due to drug treatment.

Food consumption: Food consumption was recorded weekly and calculated as g/animal/day. Males treated with 10 mg/kg demonstrated significantly decreased food consumption from weeks 0 to 1. Males treated with 40 mg/kg demonstrated significantly decreased food consumption from weeks 1 to 2. Females treated with the 40 mg/kg/day dose of oxymorphone consumed less food than the other groups (46% reduction).

Ophthalmoscopy: Ophthalmic examinations were conducted prior to randomization and during study week 12. There were no test article related changes in ophthalmic parameters tested.

EKG: A modified lead 1, 2, 3 electrocardiogram for heart rate was conducted prior to randomization and during study week 12 approximately 2 hours after the last dose. All animals fell within the normal limits both pretest and at week 12.

Hematology: Completed during weeks -2, 4, 8 and prior to necropsy (study weeks 12 for main groups and 16 for recovery groups). The following parameters were evaluated:

Total Leukocyte Count (White Cell)
Erythrocyte Count (Red Cells)
Hemoglobin
Hematocrit
Mean Corpuscular Volume (MCV)
Mean Corpuscular Hemoglobin (MCH)
Mean Corpuscular Hemoglobin Concentration (MCHC)
Platelet Count (Platelet)
Prothrombin Time (Pro Time)
Activated Partial Thromboplastin Time (APTT)
Differential Leukocyte Count-Percent and Absolute
-Neutrophil
-Lymphocyte
-Monocyte
-Eosinophil
-Basophil

( ) = Designates tabular abbreviation.

The results indicated some minor changes in hematology parameters when examined as week 12, as summarized in the table below:

Parameter	Percent Change in Hematology (week 12)					
	Males (mg/kg)			Females (mg/kg)		
	2	10	40	2	10	40
Red blood cells	+11	+11	+13*	-4	+4	+10
Platelets	-11	+13	+11	-2	+6	+26
APTT	-7	-6	-3	-1	-5	-11*
% Neutrophils	-3	-2	-2	+13	+32*	+10

There was a slight decrease in lymphocyte numbers in females from weeks 4 and 8, which was no longer significant by week 12. None of these changes should have biological significance. There was a significant increase in neutrophils numbers at weeks 8 and 12 in the 10 mg/kg female animals only and therefore not dose-dependent. Overall, the results indicated that there were no apparent biologically significant test article effects on hematological parameters under the conditions tested.

Clinical chemistry: Completed during weeks -2, 4 and 8 prior to the scheduled necropsy. The following parameters were examined:

Albumin
Total Protein
Globulin [by calculation]
Albumin/Globulin Ratio
(A/G Ratio)
Total Bilirubin (Total Bili)
Urea Nitrogen
Creatinine
Alkaline Phosphatase
(Alkaline Phos'tse)
Alanine Aminotransferase
(Alanine Transfer)
Aspartate Aminotransferase
(Aspartat Transfer)
Gamma Glutamyltransferase
(Glutamyl Transfer)
Glucose
Total Cholesterol
(Cholesterol)
Calcium
Chloride
Phosphorus
Potassium
Sodium
Triglycerides
(Triglyceride)

Significant changes noted at the week 12 blood draws are summarized in the table below. There appeared to be a 16% increase in serum potassium in the 10 and 40 mg/kg dose group in males but not females. In contrast, females, but not males, demonstrated an elevation of glucose levels following does of 10 and 40 mg/kg. A significant increase in phosphorus levels in female dogs treated with 40 mg/kg was also noted. These changes do not appear to suggest significant toxicity at the doses tested.

Summary of Clinical Chemistry Changes (Week 12):

Parameter	Percent Control Changes in Clinical Chemistry					
	Males (mg/kg)			Females (mg/kg)		
	2	10	40	2	10	40
Potassium	-5	-16*	-16*	-6	-4	-1
Glucose	+8	+9	+9	+4	+22*	+17*
Phosphorus	-2	-4	--	-10	+4	+22*

-- No change

Urinalysis: Completed during weeks -2, 4 and 8 prior to the scheduled necropsy (weeks 12 and 16). The following parameters were evaluated:

Specific gravity (SG)
pH
Urobilinogen (URO)
Total Volume (TVOL)
Sodium (Urine Sodium)[a]
Potassium (Urine K)[a]
Calcium (Urine Calcium)[a]
Chloride (Urine Chloride)[a]
Phosphorus (Urine Phos)[a]
Color (CLOR)
Appearance (APP)
Protein (PRO)
Glucose (GLU)
Ketones (KET)
Bilirubin (BIL)
Occult blood (BLD)
Leukocytes (LEU)
Nitrites (NIT)
Microscopy of Sediment
[Tabular abbreviations appear on individual tables]

Female dogs treated with the 40 mg/kg dose of oxymorphone demonstrated a significant increase in urinary phosphorus at week 12. This effect was not evident in the recovery animals at week 16 or in the male animals treated with oxymorphone, which if anything suggest a dose-dependent decrease in urinary phosphorus levels.

	Percent Control Changes in Urinanalysis Parameters					
	Males (mg/kg)			**Females (mg/kg)**		
Parameter	**2**	**10**	**40**	**2**	**20**	**40**
Phosphorus	-12	-24	-66	+5	-28	+82*

-- No change

Gross pathology: Complete necropsy was performed on all animals. Dogs were euthanized via an intravenous injection of sodium pentobarbital followed by exsanguination. Necropsy consisted of examination of the externals surfaces, all orifices, cranial cavity, external and cut surface of the brain and spinal cord and thoracic and abdominal cavities including contents. Most collected tissues were preserved in 10% neutral buffered formalin. Bone marrow smears were fixed in alcohol, epididymides and testes were fixed in Bouin's solution and eyes with optic nerve were fixed in Davidson's solution. There were no test-article related microscopic findings noted upon necropsy in any animal. Sporadic or isolated changes were noted in some animals; however, there did not appear to be a clear-cut pattern suggesting macroscopic pathology associated with test-article administration (see below).

	Incidence of Macroscopic Findings (week 12)							
	Males (mg/kg)				**Females (mg/kg)**			
Parameter	**0**	**2**	**10**	**40**	**0**	**2**	**20**	**40**
Colon: Depressed area	0/6	0/4	0/4	1/6	0/6	0/4	0/4	0/6
Lymph Node, Med, Reddened	0/6	0/4	1/4	1/6	0/6	2/4	0/4	1/6
Spleen: accessory	0/6	0/4	0/4	1/6	0/6	0/4	0/4	0/6
Stomach: dark red area	0/6	0/4	0/4	0/6	0/6	0/4	0/4	1/6

n/n = total occurrence/number of animals

Organ weights (specify organs weighed if not in histopathology inventory table): See histopathology table.

The sponsor indicates that there were no test-article-related changes in organ weights at any dose tested. Although not present in males, oxymorphone administration significantly and dose-dependently decreased absolute and relative spleen weights in female dogs at the high dose. There were no histological correlates to these changes in the spleen.

Parameter	Percent Control Changes in Organ Weights					
	Males (mg/kg)			Females (mg/kg)		
	2	10	40	2	20	40
Spleen						
Absolute	+31	+3	+9	-22	-29	-46*
Relative to body weight	+20	-1	+11	-13	-22	-43*
Relative to brain weight	+24	-4	-3	-20	-21	-47*

-- No change

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no (X)

Parameter	Incidence of Microscopic Histological Changes							
	Males (mg/kg)				Females (mg/kg)			
	0	2	10	40	0	2	20	40
Colon								
Mild necrosis	0/6	0/4	0/4	1/6	0/4	0/4	0/4	0/4
Epididymides: Hypospermia								
Mild	0/4	0/4	1/4	1/4	NA	NA	NA	NA
Severe	0/4	1/4	0/4	0/4	NA	NA	NA	NA
Eyes/Optic Nerve								
Mild Retinal dysplasia	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
Esophagus								
Minimal mineralization	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
Gallbladder								
cytoplasmic vacuolization	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
Larynx								
Min-Mild lymphocyte infiltrate	0/4	0/4	0/4	1/4	4/4	2/4	3/4	1/4
Liver								
Increased bile ducts, mild	0/4	0/4	0/4	1/4	0/4	0/4	0/4	1/4
Lung								
Minimal histocytosis alveolar	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
Parathyroid								
Minimal to mild cyst	1/4	0/4	0/4	2/4	1/4	0/4	0/4	0/4
Salivary gland, mandibular								
Infiltrate, lymphocyte, min.	1/4	0/4	1/4	0/4	0/4	1/4	1/4	1/4
Skin								
Inflammation, granulomatous, minimal	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4

n/n = total occurrence/number of animals
NA = not applicable

There were no histological changes noted in the recovery animals.

Toxicokinetics: Blood samples for Groups 2, 3 and 4 were collected for toxicokinetic evaluation at 0, 1, 2, 4, 6, 8, 10, 12, 16, 20 and 24 h post dose on the first day each dose level reached its final dose level (study day 7 and 41 and on study days 30 and 89). The results are presented in the sponsor's table below. Peak plasma levels were generally obtained between 1 and 4 hours. There does not appear to be any differences between males and females. Exposure appears to increase with dose in the range tested. There was no evidence for drug accumulation over time.

Table1: Pharmacokinetic Parameters

Study Day	Dose mg/kg	Sex	T_{max} (hr)	C_{max} (ng/mL)	C_{last} (ng/mL)	AUC_{24} (hr*ng/mL)	k (1/hr)	$t_{1/2}$ (hr)	AUC_{∞} (hr*ng/mL)	AUC_{∞}/D (hr*ng/mL/mg)	CL/F (L/hr*kg)
7	2	F	1	3.54	0.27	28.85	0.13	5.16	30.86	15.43	64.80
7	2	M	2	4.27	0.75	18.60	0.11	6.59	26.08	13.04	76.67
7	10	F	6	10.63	3.86	141.09	0.02	31.89	318.57	31.86	31.39
7	10	M	1	9.94	1.98	100.62	0.06	11.06	132.29	13.23	75.59
30	2	F	1	4.16	0.57	16.66	0.25	2.78	18.94	9.47	105.58
30	2	M	6	4.07	0.27	23.72	0.25	2.80	24.82	12.41	80.57
30	10	F	2	20.65	1.43	197.56	0.16	4.29	206.40	20.64	48.45
30	10	M	4	13.71	1.39	158.04	0.15	4.49	167.04	16.70	59.87
41	40	F	2	110.84	5.99	710.37	0.15	4.69	750.92	18.77	53.27
41	40	M	2	89.99	4.26	608.11	0.16	4.33	634.72	15.87	63.02
89	2	F	1	9.33	0.26	33.11	0.22	3.22	34.30	17.15	58.31
89	2	M	2	3.73	0.58	24.02	0.13	5.27	28.43	14.22	70.35
89	10	F	2	28.89	1.45	186.72	0.14	5.11	197.39	19.74	50.66
89	10	M	1	31.50	0.82	177.62	0.21	3.37	181.63	18.16	55.06
89	40	F	2	158.88	7.35	992.56	0.12	5.95	1055.65	26.39	37.89
89	40	M	2	138.16	4.34	642.98	0.12	5.69	678.62	16.97	58.94

Histopathology inventory (optional)

Study	[redacted]-411011 13-week PO	[redacted]-411012 13-week, PO	[redacted]-411003 13-week PO	
Species	Rat	Mouse	Dog	
Adrenals	X*	X*	X*	
Aorta	X	X	X	
Bone Marrow smear	X	X	X	
Bone (femur)	X	X	X	
Brain	X*	X*	X*	
Cecum	X	X	X	
Cervix				
Colon	X	X	X	
Duodenum	X	X	X	
Epididymis	X*	X*	X*	

Esophagus	X	X	X	
Eye	X	X	X	
Fallopian tube	X	X		
Gall bladder		X	X	
Gross lesions	X	X	X	
Harderian gland				
Heart	X*	X*	X*	
Ileum	X	X	X	
Injection site				
Jejunum	X	X	X	
Kidneys	X*	X*	X*	
Lachrymal gland			X	
Larynx			X	
Liver	X*	X*	X*	
Lungs	X	X	X	
Lymph nodes, cervical				
Lymph nodes mandibular			X	
Lymph nodes, mesenteric	X	X	X	
Mammary Gland	X	X	X	
Nasal cavity				
Optic nerves	X	X	X	
Ovaries	X*	X*	X*	
Pancreas	X	X	X	
Parathyroid	X*	X*	X*	
Peripheral nerve	X	X	X	
Pharynx				
Pituitary	X	X	X	
Prostate	X*	X*	X*	
Rectum	X	X	X	
Salivary gland	X	X	X	
Sciatic nerve	X	X	X	
Seminal vesicles	X	X		
Skeletal muscle	X	X	X	
Skin	X	X	X	
Spinal cord	X	X	X	
Spleen	X*	X*	X*	
Sternum			X	
Stomach	X	X	X	
Testes	X*	X*	X*	
Thymus	X*	X*	X*	
Thyroid	X*	X*	X*	
Tongue			X	
Trachea	X	X	X	
Urinary bladder	X	X	X	
Uterus	X*	X*	X*	
Vagina	X	X	X	
Zymbal gland				

X, histopathology performed
*, organ weight obtained

3.4.4. Genetic toxicology

Genetic toxicology studies on oxymorphone were previously reviewed by this reviewer and reproduced below. Additional genetic toxicology studies on the impurity [redacted] were conducted and submitted during the NDA review process. These studies are reviewed following the studies on oxymorphone itself.

Study title: Bacterial Reverse Mutation Assay [for oxymorphone HCl and morphine sulfate]

Key findings: Oxymorphone HCl was tested in the Ames Reverse Mutation Assay at concentrations of 79.1, 211, 632, 1897 and 5270 µg/plate. There was no evidence for cytotoxicity or precipitate at any concentration. Under the conditions of the study, oxymorphone HCl was concluded to be negative in the bacterial reverse mutation assay.

Study no:	AA46XC.503.BTL and AA46XD.503.BTL
Study type (if not reflected in title):	Bacterial Reverse Mutation Assay (Ames Test)
Volume #, and page #:	Volume 2, page 103
Conducting laboratory and location:	[redacted]
Date of study initiation:	July 19, 2001
GLP compliance:	Yes, with some exceptions (analysis of the test article concentrations of oxymorphone and morphine were limited to stock solutions only and the stability of the morphine sulfate and morphine sulfate solutions were not determined by the testing facility).
QA reports:	yes (X) no ( )
Drug, lot #, radiolabel, and % purity:	Oxymorphone HCl, Mallinckrodt B14802 [redacted] Morphine Sulfate, [redacted] B05979 [redacted]
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 *uvr*A.

Dose selection criteria: Toxicity and formation of a precipitate.

Basis of dose selection: Doses used in initial toxicity-mutation assay were 2.64, 7.91, 26.4, 79.1, 211, 632, 1897 and 5270 µg/plate for oxymorphone and 2.5, 7.5, 25, 75, 200, 600, 1800 and 5000 µg/plate for morphine. The test article was soluble and clear in water at the highest concentrations tested. Neither precipitate nor appreciable toxicity was observed. 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: Test agent had an expiration date of November 2, 2010. Stability out to [redacted] days was determined by sponsor. Results indicated that drug solutions of 0.01 mg/ml, 1 mg/ml and 50 mg/ml both in water and pH 4.5 phosphate buffer was stable up to at least [redacted] days under ambient conditions. At [redacted] days, individual known and unknown degradants as well as total degradants were less than [redacted].

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 *Salmonella* strains	Rat	2-aminoanthracene	1.0
WP2*uvr*A			10
TA98	None	2-nitrofluorene	1.0
TA100, TA1535		Sodium azide	1.0
TA1537		9-aminoacridine	75
WP2 *uvr*A		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 79.1, 211, 632, 1897 and 5270 μg/plate for oxymorphone were tested. For morphine, concentrations of 75, 200, 600, 1800 and 5000 μg/plate were tested.

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 WP2uvrA, 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 oxymorphone tested reached the maximum recommended concentration was the molar equivalent of 5,000 μg/plate of morphine sulfate and 5) there was no evidence for a dose dependent increase in revertants following drug treatment.

Study outcome: Oxymorphone HCl did not produce any increases in the number of revertants in any tester stain under the conditions tested. Likewise, morphine sulfate did not produce any increase in the number of revertants in any tester strain either (data not shown). This is in concurrence with the Sponsor's conclusions.

Bacterial Mutation Assay
Summary of Results

Table 22

Test Article Id : Oxymorphone HCl
Study Number : AA46XC.503.BTL Experiment No : B2

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	25 ± 3	194 ± 12	34 ± 2	6 ± 2	15 ± 3
79.1	18 ± 7	190 ± 13	24 ± 5	11 ± 3	11 ± 4
211	23 ± 4	209 ± 30	36 ± 4	11 ± 1	13 ± 3
632	26 ± 2	179 ± 8	23 ± 4	10 ± 3	12 ± 3
1897	19 ± 2	173 ± 6	28 ± 3	10 ± 1	11 ± 3
5270	20 ± 1	165 ± 18	22 ± 1	7 ± 2	11 ± 2
Positive	93 ± 6	1083 ± 50	226 ± 14	430 ± 56	141 ± 11

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	17 ± 6	154 ± 6	22 ± 4	11 ± 4	10 ± 1
79.1	22 ± 4	193 ± 5	24 ± 6	9 ± 3	12 ± 2
211	20 ± 4	202 ± 14	20 ± 1	8 ± 1	15 ± 1
632	23 ± 6	182 ± 6	24 ± 4	10 ± 6	13 ± 1
1897	26 ± 7	202 ± 19	27 ± 6	9 ± 2	14 ± 1
5270	27 ± 6	185 ± 7	22 ± 3	11 ± 5	13 ± 5
Positive	483 ± 229	468 ± 30	179 ± 20	1189 ± 180	1003 ± 77

Vehicle = Vehicle Control
Positive = Positive Control
Plating aliquot: 100 µL

Study title: *In vitro* mammalian chromosome aberration test [oxymorphone HCl and morphine sulfate]

Key findings: Both oxymorphone HCl and morphine sulfate were considered to be negative for the induction of structural and numerical chromosome aberrations in the *in vitro* mammalian chromosome aberrations test using human peripheral blood lymphocytes under the conditions tested.

Study no: AA46XC-XD.341-BTL
Study type (if not reflected in title): *in vitro* mammalian chromosomal aberrations test using human peripheral blood lymphocytes
Volume #, and page #: Volume 2, Page 103
Conducting laboratory and location: [redacted]
Date of study initiation: July 17, 2001
GLP compliance: Yes, with some exceptions (analysis of the test article concentrations of oxymorphone and morphine were limited to stock solutions only and the stability of the morphine sulfate and morphine sulfate solutions were not determined by the testing facility).
QA reports: Yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Oxymorphone HCl, Mallinckrodt B14802, [redacted]
Morphine sulfate, [redacted] B05979, [redacted]
Formulation/vehicle: Water

Methods:

Strains/species/cell line: Human peripheral blood lymphocytes (HPBL)

Dose selection criteria: Preliminary toxicology assay and solubility

Basis of dose selection: Test articles were soluble in water at all concentrations tested (maximum concentration was 5,270 µg/ml oxymorphone and 5,000 µg/ml morphine sulfate). The preliminary toxicology test was performed to select final concentrations via test article effects on mitotic index. Cells were incubated with test article with or without S9 for 4 hours and for 20 hours in the absence of S9. The number of cells in mitosis per 500 cells scored was determined.

Range finding studies: HPBL were exposed to a total of 9 concentrations of oxymorphone ranging from 0.527 to 5270 µg/ml with and without S9 for 4 hours and without S9 for 20 hours. Toxicity (mitotic inhibition) in excess of 50% was not observed in any of the concentrations tested in the presence or absence of S9 during the 4 hour exposure. During the 20 hour continuous exposure conditions, the mitotic index in the presence of oxymorphone at 527 µg/ml was 31% below control, whereas the mitotic index at 1581 µg/ml was 93% below control.

Test agent stability: Stability of the test article was determined by the sponsor. Results indicated that drug solutions of 0.01 mg/ml, 1 mg/ml and 50 mg/ml both in water and pH 4.5 phosphate buffer was stable up to at least [redacted] days under ambient conditions. At [redacted] days, individual known and unknown degradants as well as total degradants were less than [redacted].

Metabolic activation system: Aroclor 1254-induced rat liver S9 was prepared from male Sprague-Dawley rats. Bulk preparations were assayed for their ability to metabolize 2-aminoanthracene and 7,12-dimethyl-benz(α)anthracene to forms mutagenic to *Salmonella typhimurium* TA100. S9 was tested at 20 µl per ml of medium (2%).

Controls:

Vehicle: Water

Negative controls: Water.

Positive controls: Mitomycin C (MMC) was used as a positive control in the non-activated study at a final concentration of 0.3 and 0.6 µg/ml. Cyclophosphamide (CP) was used as the positive control in the S9 activated study at a final concentration of 20 and 40 µg/ml.

Comments: The controls are consistent with OECD recommendations.

Exposure conditions:

Incubation and sampling times: 37°C, 5% CO_2.

Treatment Condition	Treatment Time	Recovery Time	Oxymorphone HCl Concentrations (µg/ml)
Non-activated	4 hr	16 hr	659, 1318*, 2635*, 5270*
	20 hr	0 hr	250*, 500, 700*, 900, 1100*, 1300, 1500
S9-activated	4 hr	16 hr	659, 1318*, 2635*, 5270*

* Concentrations scored for definitive assay.

Doses used in definitive study: See table above. The concentrations scored for the assay were based upon the mitotic index for the cells.

Study design: Chromosomal aberration was assessed via standard procedures by exposing duplicate cultures of human peripheral blood lymphocytes to at least 4 concentrations of test article as well as positive and negative controls. Dividing cells were harvested approximately 20 hours from the initiation of treatment. For chromosome aberration assays, 0.6 ml of heparinized blood was inoculated into 9.4 ml of complete medium supplemented with 1% PHA. The tubes were incubated at 37°C and 5% CO_2 in air for 44-48 hours. Treatment was completed by media replacement with fresh complete media or S9 reaction mixture to which 1 ml of dosing solution of test or control article was added. Cells were then incubated for 4 or 20 hours with in the incubator. For the 4 hour incubation, the treatment media was removed, cells were washed, complete medium containing PHA was added, and returned to the incubator for an additional 16 hours. Two hours prior to the scheduled cell harvest, cells were treated with Colcemed® at a final concentration of 0.1 µg/ml. Following treatment, the cells

were harvested via centrifugation and chromosomes were prepared via hypotonic treatment, fixation and staining.

Analysis:

No. of replicates: 2

Counting method: Metaphase cells with 46 centromeres were examined under oil immersion. Whenever possible, a minimum of 200 metaphase spreads (100 per duplicate treatment condition) were examined and scored for chromatid-type and chromosome-type aberrations.

Criteria for positive results: A test article was considered to induce a positive response when the percentage of cells with aberrations were increased in a dose-responsive manner with one or more concentrations being statistically elevated relative to the solvent control group. A reproducibly significant increase at the high dose only or one other dose only with no dose-response was considered positive.

Summary of individual study findings:

Study validity: The study appears to be valid for the following reasons: 1) the appropriate positive controls were employed according to OECD guidelines, 2) The appropriate number of cells were evaluated and 3) the conditions of the assay are appropriate based upon OECD guidelines. The dose selection based upon mitotic index was acceptable. The criteria for a positive result should not be based entirely upon statistical analysis; however, the use of these criteria did not affect the validity of the data.

Study outcome: For the 4-hour non-activated group, the highest test article concentration tested (5270 μg/ml) inhibited mitosis by 8% relative to controls. The concentrations selected for analysis were 1318, 2635 and 5270 μg/ml. The percentage of cells with structural or numerical aberrations was not significantly different than controls for any treatment concentration. In contrast, the positive control, Mitomycin C (0.6 μg/ml) significantly increased the percentage of cells with structural aberrations (14%).

For the 4-hour activated group, the highest test article concentration tested, (5270 μg/ml) produced 4% mitotic inhibition relative to controls. The concentrations selected for analysis were 1318, 2635 and 5270 μg/ml. The percentage of cells with structural or numerical aberrations was not significantly different than controls for any treatment concentration. In contrast, the positive control, cyclophosphamide (20 μg/ml) significantly increased the percentage of cells with structural aberrations (8%).

For the 20-hour non-activated group, the highest test article concentration tested (1100 μg/ml) inhibited mitosis by 52% relative to controls. The concentrations selected for analysis were 250, 700 and 1100 μg/ml. Additional concentrations of 500 and 900 were tested, but not scored. The percentage of cells with structural or numerical aberrations was not significantly different than controls for any scored concentration. In contrast, the positive control, Mitomycin C (0.3 μg/ml) significantly increased the percentage of cells with structural aberrations (14%).

The results of the study are summarized in Sponsor's Table 7, reproduced below:

Thus, oxymorphone tested negatively in the *in vitro* chromosome aberration assay under the conditions tested. This is in concurrence with the Sponsor's conclusion. Morphine sulfate also tested negative in this study (Data not shown).

TABLE 7
SUMMARY OF CYTOGENETIC ANALYSIS OF OXYMORPHONE HCl

Treatment (µg/mL)	S9 Activation	Treatment Time (hours)	Mean Mitotic Index	Cells Scored	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
Water	-	4	6.3	200	0.000	±0.000	0.0	0.0
Oxymorphone HCl								
1318	-	4	6.4	200	0.000	±0.000	0.0	0.0
2635	-	4	5.9	200	0.000	±0.000	0.5	0.0
5270	-	4	5.8	200	0.010	±0.100	0.0	1.0
MMC 0.6	-	4	3.1	200	0.175	±0.496	0.0	14.0**
Water	+	4	4.9	200	0.015	±0.122	0.5	1.5
Oxymorphone HCl								
1318	+	4	5.1	200	0.000	±0.000	0.0	0.0
2635	+	4	5.5	200	0.000	±0.000	0.5	0.0
5270	+	4	4.7	200	0.005	±0.071	0.0	0.5
CP 20	+	4	1.1	200	0.095	±0.341	0.0	8.0**
Water	-	20	6.4	200	0.000	±0.000	0.0	0.0
Oxymorphone HCl								
250	-	20	5.4	200	0.005	±0.071	0.0	0.5
700	-	20	4.8	200	0.000	±0.000	0.0	0.0
1100	-	20	3.1	200	0.005	±0.071	0.5	0.5
MMC 0.3	-	20	3.0	200	0.175	±0.464	0.0	14.0**

Treatment: Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, $p \leq 0.05$; **, $p \leq 0.01$; using the Fisher's exact test.

Study title: Mammalian Erythrocyte Micronucleus Test [oxymorphone HCl and morphine sulfate]

Key findings: A statistically significant increase in micronucleated polychromatic erythrocytes (MPCEs) was observed in male and female mice 24 hours after treatment with 500 mg oxymorphone/kg. The results indicate that oxymorphone HCl tested positive in the *in vivo* mouse micronucleus test under the conditions of the assay. In contrast, morphine sulfate was concluded to be negative in the *in vivo* mouse micronucleus test.

Study no: AA46XC.XD.123.BTL
Study type (if not reflected in title): *In vivo* mouse micronucleus assay
Volume #, and page #: Volume 2, page 168
Conducting laboratory and location: [redacted]
Date of study initiation: July 17, 2001
GLP compliance: Yes, with a few exceptions (stability of morphine sulfate and morphine sulfate solutions were not determined by the testing facility. Analysis of the

concentrations of oxymorphone and morphine were limited to the stock solutions, test solutions prepared by serial dilution were not analyzed.

QA reports: yes (X) no ( )

Drug, lot #, radiolabel, and % purity: Oxymorphone HCl, Mallinckrodt B14802, [redacted]
Morphine sulfate, [redacted] B05979, [redacted]

Formulation/vehicle: Water

Methods:

Strains/species/cell line: ICR Mice [redacted], male and female

Dose selection criteria: Pilot toxicology study

Basis of dose selection: Doses tested were 250, 500, 750 and 1054 mg oxymorphone HCl/kg body weight via oral gavage (20 ml/kg). Doses of morphine sulfate were 250, 500, 700, or 1000 mg/kg. A total of 5 males and 5 females per dose were tested (only 3 animals in the high dose groups).

Range finding studies: For the oxymorphone treatment groups, mortality was noted in3/3 males and 3/3 females in the 1054 mg/kg group and 4/5 males and 3/5 females at the 750 mg/kg group. For the morphine treatment groups, mortality was noted in 1/3 males and 2/3 females in the 1000 mg/kg group. Clinical signs are summarized in the table (below). Based upon these results, the high dose for the oxymorphone in the micronucleus assay was set at 500 mg/kg and the high dose for the morphine was set at 700 mg/kg. These doses correlate with the maximum tolerated non-lethal doses.

Table 1: Pilot Toxicity Study - Clinical Signs Following Dose Administration of Oxymorphone HCl and Morphine Sulfate in ICR Mice

Treatment	Clinical Observation	Number of Animals With Clinical Signs/Total Number of Animals Dosed		Number of Animals Died/Total Number of Animals Dosed	
		Males	Females	Males	Females
Oxymorphone HCL 250 mg/kg	Hyperactivity Erect tails Lethargy	5/5 5/5 1/5	5/5 5/5 0/5	0/5	0/5
500 mg/kg	Hyperactivity Erect tails Convulsions Prostration Lethargy Piloerection	5/5 5/5 1/5 1/5 5/5 5/5	5/5 5/5 1/5 0/5 5/5 0/5	0/5	0/5
750 mg/kg	Hyperactivity Erect tails Aggressiveness	5/5 5/5 0/5	5/5 5/5 1/5	4/5	3/5
1054 mg/kg	Convulsions Erect tails	3/3 3/3	3/3 3/3	3/3	3/3
Morphine Sulfate 250 mg/kg	Hyperactivity Erect tails	5/5 5/5	5/5 5/5	0/5	0/5
500 mg/kg	Hyperactivity Erect tails	5/5 5/5	5/5 5/5	0/5	0/5
700 mg/kg	Hyperactivity Erect tails Lethargy Piloerection	5/5 5/5 3/5 1/5	5/5 5/5 0/5 0/5	0/5	0/5
1000 mg/kg	Convulsions Erect tails Lethargy	3/3 3/3 2/3	3/3 3/3 0/3	1/3	2/3

Test agent stability: Expiration data of the oxymorphone powder was November 2, 2010. Stability of the test article was determined by the sponsor. Results indicated that drug solutions of 0.01 mg/ml, 1 mg/ml and 50 mg/ml both in water and pH 4.5 phosphate buffer was stable up to at least [redacted] days under ambient conditions. At [redacted] days, individual known and unknown degradants as well as total degradants were less than [redacted].

Metabolic activation system: N/A

Controls:

Vehicle: Water

Negative controls: Water

Positive controls: Cyclophosphamide (40 mg/kg)

Comments: Controls are acceptable in accordance with OECD guidelines.

Exposure conditions:

Incubation and sampling times: Animals were sacrificed either 24 or 48 hours after treatment.

Doses used in definitive study: 125, 250 and 500 mg/kg oxymorphone HCl via oral gavage (20 ml/kg). Morphine sulfate doses were 175, 350 and 700 mg/kg via oral gavage (20 ml/kg).

Study design: A total of 5 male and 5 female mice per treatment group were administered oxymorphone via oral gavage. An additional 5 males and 5 females for the high-dose group

were treated for replacement animals. A satellite group of 5 animals each were dosed for

	Number of Mice Per Sex Dosed	Number of Mice Per Sex Used for Bone Marrow Collection After Dose Administration 24 hr	48 hr
Vehicle Control			
Water	10	5	5
Test Article			
Oxymorphone HCl			
Low test dose (125 mg/kg)	5	5	0
Mid test dose (250 mg/kg)	5	5	0
High test dose (500 mg/kg)	15*	5	5
Test Article			
Morphine Sulfate			
Low test dose (175 mg/kg)	5	5	0
Mid test dose (350 mg/kg)	5	5	0
High test dose (700 mg/kg)	15*	5	5
Positive Control			
Cyclophosphamide	5	5	0

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

toxicokinetic analysis to be conducted by the sponsor. The study design was as follows:

At the scheduled time of sacrifice, bone marrow cells were collected 24 and 48 hours after treatment and examined microscopically for micronucleated polychromatic erythrocytes.

Analysis:

No. of replicates: 2000 polychromatic erythrocytes were scored for the presence of micronuclei for each mouse per group. The proportion of polychromatic erythrocytes to total erythrocytes was recorded per 1000 erythrocytes.

Counting method: Microscopy, under an oil immersion lens.

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

Summary of individual study findings:

Study validity: The study appears to be valid for the following reasons: 1) the positive control, cyclophosphamide produced a statistically significant increase in the number of micronucleated polychromatic erythrocytes compared to controls and 2) the sampling times, positive controls and study design were according to OECD guidelines. However, the highest dose tested for both oxymorphone and morphine appears to be beyond the maximum tolerated non-lethal dose for some animals. Given the use of replacement animals, the dose selection is acceptable as the dose clearly reaches the maximum tolerated dose in surviving animals.

Study outcome: Following dosing, mortality occurred in 2/15 males and 1/15 females treated with 500 mg/kg oxymorphone. Mortality also occurred in 3/15 males and 2/15 females treated with 700 mg/kg morphine sulfate. As extra animals were dosed at the high dose, a total of 5 animals in each group were evaluated for micronuclei. Clinical signs are presented in the sponsor's table 4 (below).

Table 4: Micronucleus Study - Clinical Signs Following Dose Administration of Oxymorphone HCl and Morphine Sulfate in ICR Mice

Treatment	Clinical Observation	Number of Animals With Clinical Signs/Total Number of Animals Dosed		Number of Animals Died/Total Number of Animals Dosed	
		Males	Females	Males	Females
Water 20 mL/kg	*	*/10	*/10	0/10	0/10
Oxymorphone HCl 125 mg/kg	Hyperactivity Erect tails	5/5 5/5	5/5 5/5	0/5	0/5
250 mg//kg	Hyperactivity Erect tails Lethargy	5/5 5/5 5/5	5/5 5/5 1/5	0/5	0/5
500 mg/kg	Hyperactivity Erect tails Lethargy Piloerection	15/15 15/15 10/15 1/15	15/15 15/15 1/15 0/15	2/15	1/15
Morphine Sulfate 175 mg/kg	Hyperactivity Erect tails	5/5 5/5	5/5 5/5	0/5	0/5
350 mg/kg	Hyperactivity Erect tails	5/5 5/5	5/5 5/5	0/5	0/5
700 mg/kg	Hyperactivity Erect tails Lethargy Piloerection	15/15 15/15 11/15 2/15	15/15 15/15 1/15 0/15	3/15	2/15
Cyclophosphamide 40 mg/kg	*	*/5	*/5	0/5	0/5

* No clinical signs observed, all dosed animals appeared normal

Toxicokinetic analysis suggested comparable exposures between males and females (table 2 below).

Table 2. Mean Plasma Concentrations (n=5) and Toxicokinetic Parameters of Oxymorphone in ICR Mice Following Single Oral Administration (QPS Project Number 52-0202PK)

Sex	Male			Female		
Dose, mg/kg	125	250	500	125	250	500
Plasma Conc, ng/mL						
1 h	737.400[a]	2436.408	2621.072	768.408	1746.480	5329.216
4 h	305.805	399.170	2605.552	343.010	574.454	1464.288
24 h	2.192	10.475	10.795	3.885	12.018	62.066
Toxicokinetic Parameter						
Cmax, ng/mL	737.400	2436.408	2621.072	768.408	1746.480	5329.216
Tmax, h	1.0	1.0	1.0	1.0	1.0	1.0
AUC_{0-24h}, ng/mL·h	5013.477	9568.024	35313.950	5520.278	10219.360	28118.400
T1/2, h	2.8	ND	ND	ND	2.8	3.9

[a]n=4
ND=not determined due to insufficient data points.

Results of the micronucleus assay indicated that in females, oxymorphone treatment at doses of 250 mg/kg significantly increased the number of micronucleated polychromatic erythrocytes per polychromatic erythrocytes scored (24 hours). Significant increases were also obtained in both males and females following 500 mg/kg doses. These increases appeared to be dose-related. Cyclophosphamide (50 mg/kg) also produced a significant response. When measured at 48 hours, oxymorphone treatment of 500 mg/kg significantly increased the number of MPCE/PCE scored in males only, although the incidence in drug treated females was increased. The results for oxymorphone are reproduced in sponsor's table 5 below: As noted in the table above, oxymorphone treatment reduced the ratio of polychromatic erythrocytes to total erythrocytes by 19 to 40% relative to controls. This suggests toxicity and that oxymorphone was

Table 5: Summary of Bone Marrow Micronucleus Analysis
Following Dose Administration of Oxymorphone HCl in ICR Mice

Treatment	Sex	Time (hr)	Number of Mice	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Micronucleated Polychromatic Erythrocytes: Number per 1000 PCEs (Mean +/- SD)	Micronucleated Polychromatic Erythrocytes: Number per PCEs Scored[1]
Water							
20 mL/kg	M	24	5	0.523 ± 0.02	—	0.5 ± 0.00	5 / 10000
	F	24	5	0.505 ± 0.04	—	0.4 ± 0.22	4 / 10000
Oxymorphone HCl							
125 mg/kg	M	24	5	0.413 ± 0.07	-21	0.5 ± 0.35	5 / 10000
	F	24	5	0.353 ± 0.03	-30	0.9 ± 0.22	9 / 10000
250 mg/kg	M	24	5	0.365 ± 0.02	-30	1.2 ± 0.76	12 / 10000
	F	24	5	0.381 ± 0.05	-25	1.3 ± 0.27	**13 / 10000
500 mg/kg	M	24	5	0.343 ± 0.06	-34	5.4 ± 1.29	*54 / 10000
	F	24	5	0.410 ± 0.08	-19	3.8 ± 3.27	*38 / 10000
CP							
50 mg/kg	M	24	5	0.271 ± 0.04	-48	28.8 ± 3.27	*288 / 10000
	F	24	5	0.357 ± 0.08	-29	27.8 ± 5.82	*278 / 10000
Water							
20 mL/kg	M	48	5	0.513 ± 0.05	—	0.4 ± 0.22	4 / 10000
	F	48	5	0.492 ± 0.05	—	0.4 ± 0.22	4 / 10000
Oxymorphone HCl							
500 mg/kg	M	48	5	0.307 ± 0.06	-40	1.4 ± 1.47	**14 / 10000
	F	48	5	0.356 ± 0.07	-28	0.8 ± 0.45	8 / 10000

[1]*, p≤0.05 (Kastenbaum-Bowman Tables)
** Statistically significant but not biologically relevant

bioavailable to the target bone marrow. The increases in MPCE/PCE scored noted above following a dose of 250 mg/kg at 24 hours and a dose of 500 mg/kg at 48 hours were not considered to be biologically relevant as they were within the historical control range for the controls (0-14 in males and 0-18 in females).

Results of the micronucleus assay for morphine indicate that morphine sulfate treatments did not significantly alter the number of micronucleated polychromatic erythrocytes per PCE scored 24 hours after treatment at any dose tested. In contrast, cyclophosphamide (50 mg/kg) produced significant increases. However, at 48 hours, treatment with the high dose morphine sulfate (700 mg/kg produced a significant increase in MPCE/PCE scored in female mice only. The results are reproduced in Sponsor's table 8 below: As noted above, morphine sulfate treatment produced a reduction of 25 to 36% in the ratio of polychromatic erythrocytes to total erythrocytes relative to controls. This indicates toxicity and that the

Table 8: Summary of Bone Marrow Micronucleus Analysis
Following Dose Administration of Morphine Sulfate in ICR Mice

				PCE/Total		Micronucleated Polychromatic Erythrocytes	
Treatment	Sex	Time (hr)	Number of Mice	Erythrocytes (Mean +/- SD)	Change from Control (%)	Number per 1000 PCEs (Mean +/- SD)	Number per PCEs Scored[1]
Water							
20 mL/kg	M	24	5	0.523 ± 0.02	—	0.5 ± 0.00	5 / 10000
	F	24	5	0.505 ± 0.04	—	0.4 ± 0.22	4 / 10000
Morphine Sulfate							
175 mg/kg	M	24	5	0.388 ± 0.04	-26	0.6 ± 0.55	6 / 10000
	F	24	5	0.380 ± 0.02	-25	0.9 ± 0.74	9 / 10000
350 mg/kg	M	24	5	0.377 ± 0.06	-28	0.8 ± 0.45	8 / 10000
	F	24	5	0.321 ± 0.02	-36	1.1 ± 0.42	11 / 10000
700 mg/kg	M	24	5	0.353 ± 0.06	-33	1.0 ± 0.35	10 / 10000
	F	24	5	0.347 ± 0.04	-31	1.1 ± 0.42	11 / 10000
CP							
50 mg/kg	M	24	5	0.271 ± 0.04	-48	28.8 ± 3.27	*288 / 10000
	F	24	5	0.357 ± 0.08	-29	27.8 ± 5.82	*278 / 10000
Water							
20 mL/kg	M	48	5	0.513 ± 0.05	—	0.4 ± 0.22	4 / 10000
	F	48	5	0.492 ± 0.05	—	0.4 ± 0.22	4 / 10000
Morphine Sulfate							
700 mg/kg	M	48	5	0.331 ± 0.04	-35	0.9 ± 0.42	9 / 10000
	F	48	5	0.340 ± 0.02	-31	1.2 ± 0.84	**12 / 10000

1*, p≤0.05 (Kastenbaum-Bowman Tables)
** Statistically significant but not biologically relevant

morphine sulfate was bioavailable to the target bone marrow. The statistically significant increase in the MPCE/PCE scored in females at 48 hours was not considered to be biologically relevant, as it is within the historical range off the controls (0-18).

Overall, the results indicated that oxymorphone, but not morphine, produced a significant increase in micronucleated polychromatic erythrocytes at a dose of 500 mg/kg under the conditions tested. This conclusion is in concurrence with the sponsor.

Study title: Mammalian erythrocyte micronucleus test [oxymorphone HCl]

Key findings: Oxymorphone at a dose of 20 mg/kg and 40 mg/kg produced a significant increase in the incidence of micronucleated polychromatic erythrocytes in bone marrow of the rat under the conditions tested. Thus, oxymorphone is considered positive in the rat micronucleus assay, an *in vivo* test for mammalian chromosomal damage.

Study no: AA46XC.125.BTL
Study type (if not reflected in title): *In vivo* rat micronucleus test
Volume #, and page #: Volume 2, Page 250
Conducting laboratory and location: [redacted]
Date of study initiation: October 18, 2001
GLP compliance: Yes, with the exception that analysis of the test article concentration was limited to the stock solution. Test articles prepared by serial dilution from the stock solution were not independently analyzed.
QA reports: Yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Oxymorphone HCL, Mallinckrodt B14802, [redacted]

Formulation/vehicle: Water

Methods:

Strains/species/cell line: [redacted] CD (SD) IGS BR rats, male and female
Dose selection criteria: Dose range-finding study for toxicity
Basis of dose selection: Tested doses of 50, 75, 100 or 150 mg/kg oxymorphone via oral gavage (10 ml/kg) in preliminary toxicity study (n=5/sex/group).
Range finding studies: Mortality was observed at all doses tested as indicated in the table below. Based on these findings, a dose of 40 mg/kg was set as the high dose for the micronucleus assay as an estimate of the maximum tolerated dose.

Table 1: Toxicity Study - Clinical Signs
Following Dose Administration of Oxymorphone HCl in [redacted]CD® (SD) IGS BR Rats

Treatment	Clinical Observation	Number of Animals With Clinical Signs/Total Number of Animals Dosed		Number of Animals Died/Total Number of Animals Dosed	
		Males	Females	Males	Females
Oxymorphone HCl 50 mg/kg	Lethargy Piloerection	5/5 3/5	5/5 1/5	2/5	2/5
75 mg/kg	Lethargy Piloerection Prostration Irregular breathing Crusty eyes	3/5 2/5 0/5 0/5 0/5	3/5 1/5 1/5 1/5 1/5	3/5	4/5
100 mg/kg	Lethargy Piloerection	5/5 1/5	5/5 2/5	4/5	3/5
150 mg/kg	Lethargy Piloerection	5/5 1/5	5/5 0/5	4/5	5/5

Test agent stability: Expiration date on the powdered drug is November 2, 2010. Stability of the test article was determined by the sponsor. Results indicated that drug solutions of 0.01 mg/ml, 1 mg/ml and 50 mg/ml both in water and pH 4.5 phosphate buffer was stable up to at least [redacted] days under ambient conditions. At [redacted] days, individual known and unknown degradants as well as total degradants were less than [redacted].

Metabolic activation system: N/A

Controls:

Vehicle: Water

Negative controls: Water

Positive controls: Cyclophosphamide

Comments: Positive control is acceptable in accordance with OECD guidelines.

Exposure conditions:

Incubation and sampling times: Animals were sacrificed at 24 and 48 hours.

Doses used in definitive study: 10, 20 and 40 mg/kg oxymorphone/kg via oral gavage (10 ml/kg)

Study design: A total of 5 male and 5 female rats per treatment group were administered oxymorphone via oral gavage. An additional 5 males and 5 females for the high-dose group were treated for replacement animals. A satellite group of 5 animals each were dosed for toxicokinetic analysis to be conducted by the sponsor. The study design was as follows:

	Number of Rats Per Sex Dosed	Number of Rats Per Sex Used for Bone Marrow Collection After Dose Administration - 24 hr	48 hr
Vehicle Control			
Water	10	5	5
Test Article			
Oxymorphone HCl			
Low test dose (10 mg/kg)	5	5	0
Mid test dose (20 mg/kg)	5	5	0
High test dose (40 mg/kg)	15*	5	5
Positive Control			
CP (40 mg/kg)	5	5	0

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

Analysis:

No. of replicates: A total of 2000 polychromatic erythrocytes were scored for the presence of micronuclei. The number of polychromatic erythrocytes to total erythrocytes was recorded per 1000 erythrocytes. 2 slides were prepared for each rat.

Counting method: Slides were analyzed microscopically using an oil immersion lens.

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

Summary of individual study findings:

Study validity: The study appears to be valid for the following reasons: 1) the positive control, cyclophosphamide produced a statistically significant increase in the number of micronucleated polychromatic erythrocytes compared to controls and 2) the sampling times, positive controls and study design were according to OECD guidelines. Dosing was adequate for the study.

Study outcome: Mortality and clinical signs following oxymorphone administration are presented in the sponsor's table 3 (below). As noted, 2/15 males and 1/15 females in the high dose group died. These animals were replaced for analysis of micronucleated erythrocytes.

Table 3: Definitive Micronucleus Study - Clinical Signs
Following Dose Administration of Oxymorphone HCl in [redacted]:CD® (SD) IGS BR Rats

Treatment	Clinical Observation	Number of Animals With Clinical Signs/Total Number of Animals Dosed		Number of Animals Died/Total Number of Animals Dosed	
		Males	Females	Males	Females
Water 10 mL/kg	*	*/10	*/10	0/10	0/10
Oxymorphone HCl 10 mg/kg	Lethargy	5/5	5/5	0/5	0/5
20 mg/kg	Lethargy	5/5	5/5	0/5	0/5
40 mg/kg	Lethargy Piloerection	15/15 14/15	15/15 14/15	2/15	1/15
CP 40 mg/kg	*	*/5	*/5	0/5	0/5

*No clinical signs observed, all animals appeared normal after dose administration

Analysis of the bone marrow cells collected at 24 and 48 hours after treatment indicated that at 24 hours oxymorphone at 20 mg/kg significantly increased the number of micronucleated polychromatic erythrocytes per PCE scored in both males and females. Males treated with the 40 mg/kg dose also demonstrated a significant increase in micronucleated erythrocytes, although MPCE/PCE in females at this dose was not significantly different than controls. At 48 hours, the high dose oxymorphone was not significantly different from controls. Cyclophosphamide treatment produced a significant increase in micronucleated erythrocytes. These results are summarized in sponsor's table 4, reproduced on the following page:

Table 4: Summary of Bone Marrow Micronucleus Analysis
After a Single Dose of Oxymorphone HCl in [redacted]CD® (SD) IGS BR Rats

Treatment	Sex	Time (hr)	Number of Rats	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Micronucleated Polychromatic Erythrocytes: Number per 1000 PCEs (Mean +/- SD)	Micronucleated Polychromatic Erythrocytes: Number per PCEs Scored[1]
Water							
10 ml/kg	M	24	5	0.663 ± 0.06	—	0.7 ± 0.45	7 / 10000
	F	24	5	0.662 ± 0.04	—	0.7 ± 0.67	7 / 10000
Oxymorphone HCl							
10 mg/kg	M	24	5	0.649 ± 0.02	-2	1.4 ± 0.42	14 / 10000
	F	24	5	0.663 ± 0.02	0	0.5 ± 0.61	5 / 10000
20 mg/kg	M	24	5	0.653 ± 0.04	-2	1.7 ± 0.57	*17 / 10000
	F	24	5	0.685 ± 0.05	3	1.6 ± 0.65	*16 / 10000
40 mg/kg	M	24	5	0.664 ± 0.04	0	2.6 ± 0.42	*26 / 10000
	F	24	5	0.693 ± 0.04	5	1.1 ± 0.42	11 / 10000
CP							
40 mg/kg	M	24	5	0.599 ± 0.03	-10	29.8 ± 9.26	*298 / 10000
	F	24	5	0.604 ± 0.03	-9	18.5 ± 6.20	*185 / 10000
Water							
10 ml/kg	M	48	5	0.667 ± 0.08	—	0.4 ± 0.42	4 / 10000
	F	48	5	0.675 ± 0.02	—	0.7 ± 0.76	7 / 10000
Oxymorphone HCl							
40 mg/kg	M	48	5	0.677 ± 0.03	1	0.7 ± 0.45	7 / 10000
	F	48	4**	0.769 ± 0.02	14	0.5 ± 0.41	4 / 8000

[1]*, p≤0.05 (Kastenbaum-Bowman Tables)
**One of the animals was considered to be outlier, number of MPCEs (15) for the animal was not used in statistical analysis

Toxicokinetic analysis indicated that both males and females demonstrated similar exposures (below):

Table 2. Mean Plasma Concentrations and Toxicokinetic Parameters of Oxymorphone in Sprague-Dawley Rats Following Single Oral Administration (QPS Project Number 52-0201PK)

Sex	Male			Female		
Dose, mg/kg	10	20	40	10	20	40
Plasma Conc, ng/mL						
1 h	15.675	20.994	26.482	13.646	34.919	22.425
4 h	6.763	9.598	26.410	7.806	14.933	23.777
24 h	0.222	3.993	8.107	1.084	5.128	10.244
Toxicokinetic Parameter						
Cmax, ng/mL	15.675	20.994	33.422	13.646	35.573	28.518
Tmax, h	1.0	1.0	2.8	1.0	1.6	2.2
AUC_{0-24h}, ng/mL·h	111.348	192.293	437.750	127.896	292.844	420.726

As noted in the table above, oxymorphone treatment produced only a slight (2%) reduction in the ratio of polychromatic erythrocytes to normal erythrocytes. This indicates that oxymorphone did not inhibit erythropoiesis in these animals.

Overall, these results suggest that oxymorphone at a dose of 20 mg/kg and 40 mg/kg produces a significant increase in the incidence of micronucleated polychromatic erythrocytes in bone marrow of the rat. Thus,

oxymorphone is considered positive in the rat micronucleus assay, an *in vivo* test for mammalian chromosomal damage, under the conditions tested. This conclusion is in concurrence with the Sponsor.

Study title: Mammalian erythrocyte micronucleus test with kinetochore analysis [oxymorphone HCl]

Key findings: The results of this study confirm that oxymorphone at doses of 250 and 500 mg/kg produces a significant increase in the incidence of micronucleated polychromatic erythrocytes and therefore is clastogenic. Kinetochore analysis indicated that oxymorphone does not increase the percentage of micronucleated erythrocytes with kinetochores, suggesting that the DNA damage is due to chromosomal breakage rather than loss of the entire chromosome.

Study no: AA46XC.126.BTL
Study type (if not reflected in title): *in vivo* mouse micronucleus test
Volume #, and page #: Volume 2, page 330
Conducting laboratory and location: [redacted]
Date of study initiation: October 18, 2001
GLP compliance: Yes, with the exception that the oxymorphone analysis was limited to the stock solution. Test solutions, prepared via serial dilution of the stock solution, were not analyzed.
QA reports: Yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Oxymorphone HCl, Mallickrodt B14802, [redacted]

Formulation/vehicle: Water

Methods:

Strains/species/cell line: ICR mice (male and female)
Dose selection criteria: Previous toxicity results
Basis of dose selection: Doses were based upon results of the previous study AA46XC_XD.123.BTL
Range finding studies: N/A.
Test agent stability: Test article had an expiration date (powder) of November 2, 2010. Stability was tested by the sponsor. Results indicated that drug solutions of 0.01 mg/ml, 1 mg/ml and 50 mg/ml both in water and pH 4.5 phosphate buffer was stable up to at least [redacted] days under ambient conditions. At [redacted] days, individual known and unknown degradants as well as total degradants were less than [redacted].
Metabolic activation system: N/A
Controls:
Vehicle: Water
Negative controls: Water
Positive controls: Cyclophosphamide monohydrate as a positive control for MPCE analysis. Vinblastine was used as a positive control for kinetochore analysis (i.p.)
Comments: These controls are adequate.
Exposure conditions:
Incubation and sampling times: 24 hour treatment
Doses used in definitive study: 250 and 500 mg/kg oxymorphone HCl via oral gavage (20 ml/kg).

Study design:

	Number of Mice Per Sex	Number of Mice Per Sex Used for Bone Marrow Collection 24 hours After Dose Administration
Vehicle Control: Water	5	5
Test Article: Oxymorphone HCl		
Mid test dose (250 mg/kg)	5	5
High test dose (500 mg/kg)	10*	5
Positive Control: CP 50 mg/kg	5	5
Positive Control: VB 6 mg/kg	5	5

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

Analysis:

No. of replicates: A total of 2000 polychromatic erythrocytes were scored for the presence of micronuclei. The number of polychromatic erythrocytes to total erythrocytes was recorded per 1000 erythrocytes. 2 slides were prepared for each rat. For kinetochore analysis, 25 micronucleated polychromatic erythrocytes per animal were analyzed for the presence or absence of kinetochores.

Counting method: Slides were analyzed microscopically using an oil immersion lens. For kinetochore analysis, a microscope with a blue excitation filter and barrier filter for 520 nm was used with an oil immersion lens.

Criteria for positive results: "The test article was considered to induce a positive response if a dose-responsive increase in micronucleated polychromatic erythrocytes was observed and one or more doses were statistically elevated relative to the vehicle control ($p \leq 0.05$, Kastenbaum-Bowman Tables)." "The test article was considered positive for induction of aneuploidy if the frequency of kinetochore-positive micronucleated PCEs in the test article-treated groups was statistically increased (Fisher's exact test, $p \leq 0.05$) above the vehicle (negative) control."

Summary of individual study findings:

Study validity: The study appears to be valid for the following reasons: 1) the positive control, cyclophosphamide, produced a statistically significant increase in the number of micronucleated polychromatic erythrocytes compared to controls and 2) the sampling times, positive controls and study design were according to OECD guidelines. The kinetochore analysis also appears valid as vinblastine produced a clear increase in the number of micronucleated polychromatic erythrocytes with kinetochores.

Study outcome: There was no mortality with either dose tested in this study. Clinical observations are summarized in the sponsor's table 1 reproduced below:

Table 1: Micronucleus Assay with Kinetochore Analyses - Clinical Signs Following Dose Administration of Oxymorphone HCl In ICR Mice

Treatment	Clinical Observation	Number of Animals With Clinical Signs/Total Number of Animals Dosed		Number of Animals Died/Total Number of Animals Dosed	
		Males	Females	Males	Females
Water 20 mL/kg	*	*/5	*/5	0/5	0/5
Oxymorphone HCl 250 mg/kg	Hyperactivity Erect tails	5/5 5/5	5/5 5/5	0/5	0/5
Oxymorphone HCl 500 mg/kg	Hyperactivity Erect tails Lethargy Crusty eyes	10/10 10/10 10/10 0/10	10/10 10/10 10/10 3/10	0/10	0/10
Cyclophosphamide 50 mg/kg	*	*/5	*/5	0/5	0/5
Vinblastine 6 mg/kg	*	*/5	*/5	0/5	0/5

*No clinical signs observed, all animals appeared normal after dose administration

Table 2: Summary of Bone Marrow Micronucleus Analysis After a Single Dose of Oxymorphone HCl In ICR Mice

Treatment	Sex	Time (hr)	Number of Mice	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Micronucleated Polychromatic Erythrocytes: Number per 1000 PCEs (Mean +/- SD)	Micronucleated Polychromatic Erythrocytes: Number per PCEs Scored[1]
Water							
20 mL/kg	M	24	5	0.458 ± 0.03	—	0.5 ± 0.00	5 / 10000
	F	24	5	0.479 ± 0.05	—	0.6 ± 0.22	6 / 10000
Oxymorphone HCl							
250 mg/kg	M	24	5	0.401 ± 0.02	-12	2.7 ± 0.91	*27 / 10000
	F	24	5	0.354 ± 0.08	-26	3.4 ± 1.29	*34 / 10000
500 mg/kg	M	24	5	0.343 ± 0.07	-25	2.1 ± 1.14	*21 / 10000
	F	24	5	0.348 ± 0.05	-27	3.3 ± 0.76	*33 / 10000
CP	M	24	5	0.276 ± 0.05	-40	29.4 ± 4.76	*294 / 10000
50 mg/kg	F	24	5	0.321 ± 0.01	-33	29.9 ± 2.84	*299 / 10000

[1]*, p≤0.05 (Kastenbaum-Bowman Tables)

As indicated in the sponsor's table 2 above, both the 250 and 500 mg/kg dose of oxymorphone produced a significant increase in the number of micronucleated polychromatic erythrocytes per number of PCEs scored. Likewise, cyclophosphamide also significantly increased the number of micronucleated polychromatic erythrocytes. Oxymorphone treatments produced a 12 to 27% decrease in the ratio of polychromatic erythrocytes to total erythrocytes, indicating that the drug was bioavailable to the bone marrow target tissue.

As outlined in the table below (data taken from sponsor's table 4), treatment of the animals with vinblastine produced a significant increase in the percentage of micronucleated polychromatic erythrocytes with kinetochores compared to water control. Treatment with either dose of oxymorphone did not significantly

alter the proportion of cells with kinetochores in either males or females treated with 250 mg/kg or males treated with 500 mg/kg. There was a significant increase in the proportion of cells with kinetochores in female animals treated with oxymorphone at 500 mg/kg, however, the sponsor did not interpret this to be biologically significant as it was only a 2 fold increase over the negative control. No historical control data was provided. These data indicate that, for the most part, the effects of oxymorphone on the incidence of micronucleated polychromatic arise primarily from chromosomal breaks rather than loss of whole chromosomes.

Treatment	% of total Micronucleated polychromatic erythrocytes scored			
	Males		Females	
	Without Kinetochores	With Kinetochores	Without Kinetochores	With Kinetochores
Water	89	11	94	6
Oxymorphone HCl 250 mg/kg	88	12	95	5
Oxymorphone HCl 500 mg/kg	87	13	86	14
Vinblastine 6 mg/kg	9	91	10	90

Overall, the results support the previous findings that oxymorphone is a positive clastogen and provide evidence that oxymorphone is a negative aneugen.

Study title: Bacterial Reverse Mutation Study with [redacted]

Key findings: [redacted] : was tested in the bacterial reverse mutation assay at concentrations up to 1000 µg/plate with and without metabolic activation. The results indicated that under the conditions of the study, [redacted] : was not mutagenic in any strain tested.

Study no.: Endo # SP0002-210-03
Volume #, and page #: Submission N 000 MR
Conducting laboratory and location: [redacted]

Date of study initiation: March 26, 2003
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: [redacted], Lot D14182, [redacted]

Methods

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

Doses used in definitive study: The concentrations used in the definitive assay (B-1) were 62.5, 125, 250, 500 and 1000 µg/plate. A confirmation assay was also completed (B-2).

Basis of dose selection: Concentrations for the definitive mutation assay were chosen based upon results of a range-finding test using strain TA100 and WP2 uvrA. Seven concentrations of test article ranging from 5 to 5000 µg/plate were evaluated with and

without rat liver S-9. Following 64 hour incubation period, plates with no interfering precipitate were counted by automatic colony counter (plates with precipitate were counted by hand).

Negative controls: As stock solutions of the test article were prepared in 0.16 N HCl, this was used as the solvent control.

Positive controls: The following positive controls were used (sponsor's table). All positive controls were dissolved in DMSO (except NaAz and MMS, which were dissolved in sterile water).

Strain	S-9	Chemical	Concentration (μg/plate)
TA98	-	2-NF (2-Nitrofluorene)	5.0
TA98	+	2-AA (2-Aminoanthracene)	1.25
TA100	-	NaAz (Sodium Azide)	1.0
TA100	+	2-AA (2-Aminoanthracene)	1.25
TA1535	-	NaAz (Sodium Azide)	1.0
TA1535	+	2-AA (2-Aminoanthracene)	1.25
TA1537	-	9-AA (9-Aminoacridine)	50
TA1537	+	2-AA (2-Aminoanthracene)	1.25
WP2 uvrA	-	MMS (Methyl Methanesulfonate)	4000
WP2 uvrA	+	2-AA (2-Aminoanthracene)	10

Incubation and sampling times: For the range finding test, plates were incubated for approximately 64 hours. For the definitive assay, plates were incubated for approximately 68 hours.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The criteria for a valid assay were met. These included mean reversion frequency of the test article solvent control plates falling into pre-specified ranges, the positive controls had a mean reversion frequency 3-times or more greater than the mean reversion frequency of the solvent controls and the tester strains were confirmed for the appropriate growth dependency. The criteria for a negative response are acceptable. Specifically a response was considered to be negative if all strains treated with the test article had a mean reversion frequency that was less than twice the mean reversion frequency of the corresponding solvent control plates in TA98 and TA100 and less than 3-times in TA1535, TA1537 and WP2 uvrA, and there was no evidence of a concentration-dependent response. A response was considered positive if either strain TA98 or TA100 exhibited a mean reversion frequency at least double the mean reversion frequency of the corresponding solvent control in at least one concentration, or if either strain TA1535, TA1537 or WP2 uvrA exhibited a 3-fold increase in mean reversion frequency compared

to solvent control. In addition, the response must be concentration-dependent. A response was considered equivocal if it did not fulfill criteria for a negative or a positive response. The study appears to be valid.

Study outcome: The results of the range-finding assays for strain TA100 and WP2 uvrA are summarized in the table below. Cell viability was expressed as the relative cloning efficiency (number of colonies in test plates divided by the number of colonies in the solvent control plates times 100).

Summary of the Viability of bacterial strains in the presence of [redacted] :

Concentration (µg/plate)	TA100		WP2 uvrA	
	- S9	+ S9	-S9	+S9
5.0	125%	103%	112%	103%
10	122%	110%	109%	95%
50	114%	109%	106%	98%
100	101%	107%	106%	101%
500	109%	103%	100%	103%
1000	57%	38%	51%	61%
5000	0%	0%	0%	0%
Solvent control	100%	100%	100%	100%

As noted in the table above, in the absence of S9 5000 µg/plate [redacted] lead to 0% viability, and 1000 µg/plate [redacted] produced 57% viability. For all studies, a greater than 50% decrease in viability was obtained at concentrations of either 500 or 1000 µg/plate. As such, the highest concentration chosen for the definitive assay was 1000 µg/plate.

The results of the definitive assay without metabolic activation are provided in the sponsor's table 3 reproduced below. The results indicate that under the conditions tested, [redacted] did not increase the average revertants per plate.

TABLE 3
SALMONELLA TYPHIMURIUM/ESCHERICHIA COLI PLATE INCORPORATION MUTATION ASSAY
MUTATION ASSAY RESULTS - WITHOUT ACTIVATION

SPONSOR: Mallinckrodt, Inc. STUDY NO.: 0795-2140
EXPERIMENT NO.: B-1 SOLVENT: 0.16 N HCl
TEST ARTICLE: [redacted] CONC. IN: µg/plate

S. typhimurium		Average No. of Revertants Per Plate						
		Positive Control	Solvent Control	Concentration per plate 62.5	125	250	500	1000
STRAIN: TA98	REVERTANTS	634	26	23	21	24	15	2
DATE PLATED:	STD. DEV.	68	3	4	3	3	2	3
04/04/03	LAWN	NL	NL	NL	NL	NL	NL	SR
CELLS SEEDED: 9.740E+07	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA100	REVERTANTS	301	42	41	38	24	25	7
DATE PLATED:	STD. DEV.	13	3	5	5	6	6	2
04/04/03	LAWN	NL	NL	NL	NL	NL	NL	SR
CELLS SEEDED: 1.068E+08	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA1535	REVERTANTS	309	9	8	14	11	6	4
DATE PLATED:	STD. DEV.	31	1	2	3	4	1	1
04/04/03	LAWN	NL	NL	NL	NL	NL	NL	SR
CELLS SEEDED: 5.800E+07	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA1537	REVERTANTS	248	10	5	8	5	4	3
DATE PLATED:	STD. DEV.	55	3	2	1	1	1	3
04/04/03	LAWN	NL	NL	NL	NL	NL	NL	SR
CELLS SEEDED: 5.540E+07	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP

E. coli		Positive Control	Solvent Control	Concentration per plate 62.5	125	250	500	1000
STRAIN: WP2 uvrA	REVERTANTS	471	18	13	15	16	10	5
DATE PLATED:	STD. DEV.	86	2	3	1	4	2	1
04/04/03	LAWN	NL	NL	NL	NL	NL	NL	SR
CELLS SEEDED: 7.820E+07	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP

NL = Normal, healthy microcolony lawn. NP = No precipitate.
SR = Slight reduction in the microcolony lawn.

The results of the definitive assay with metabolic activation are reproduced by the sponsor's table 4 below. The results indicate that under the conditions tested, [redacted] was not mutagenic in any bacterial strain tested.

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TABLE 4
SALMONELLA TYPHIMURIUM/ESCHERICHIA COLI PLATE INCORPORATION MUTATION ASSAY
MUTATION ASSAY RESULTS - WITH S-9 ACTIVATION

SPONSOR: Mallinckrodt, Inc. STUDY NO.: 0795-2140
EXPERIMENT NO.: R-1 SOLVENT: 0.16 N HCl
TEST ARTICLE: [redacted] CONC. IN: µg/plate

S. typhimurium		Average No. of Revertants Per Plate						
		Positive Control	Solvent Control	Concentration per plate 62.5	125	250	500	1000
STRAIN: TA98	REVERTANTS	683	31	24	27	26	18	15
DATE PLATED: 04/04/03	STD. DEV.	58	4	1	3	8	1	3
	LAWN	NL	NL	NL	NL	NL	NL	NL
CELLS SEEDED: 9.740E+07	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA100	REVERTANTS	493	45	44	45	34	27	18
DATE PLATED: 04/04/03	STD. DEV.	14	2	3	6	4	10	2
	LAWN	NL	NL	NL	NL	NL	NL	NL
CELLS SEEDED: 1.068E+08	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA1535	REVERTANTS	159	14	17	16	11	8	7
DATE PLATED: 04/04/03	STD. DEV.	11	2	4	7	2	2	0
	LAWN	NL	NL	NL	NL	NL	NL	NL
CELLS SEEDED: 5.800E+07	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA1537	REVERTANTS	82	13	9	11	8	5	3
DATE PLATED: 04/04/03	STD. DEV.	5	1	2	3	0	2	2
	LAWN	NL	NL	NL	NL	NL	NL	NL
CELLS SEEDED: 5.540E+07	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP

E. coli		Positive Control	Solvent Control	Concentration per plate 62.5	125	250	500	1000
STRAIN: WP2 uvrA	REVERTANTS	203	20	19	15	15	10	8
DATE PLATED: 04/04/03	STD. DEV.	4	4	2	5	1	3	1
	LAWN	NL	NL	NL	NL	NL	NL	NL
CELLS SEEDED: 7.620E+07	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP

NL = Normal, healthy microcolony lawn. NP = No precipitate.

Similar results were obtained in a confirmatory assay, suggesting that, under the conditions of this study, [redacted] was non-mutagenic in the bacterial reverse mutation assay.

Study title: In Vitro Chromosome Aberrations Study with [redacted] in Chinese Hamster Ovary Cells

Key findings: The impurity, [redacted] tested positive for its potential to induce chromosome aberrations in cultured CHO cells both with and without metabolic activation. Under the conditions of the study, [redacted] is therefore considered to be clastogenic.

Study no.: ENDO # SP0002-220-03
Volume #, and page #: Electronic Submission (7/22/2003)
Conducting laboratory and location: [redacted]

Date of study initiation: March 27, 2003
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: [redacted], Lot D14182, [redacted]

Methods: The ability for [redacted] 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 [redacted] 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 [redacted] of the CHO cell line was used in the study. The cells originated at [redacted]. 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 μg/ml for non-activated system and 0.1, 0.5, 1, 5 and 10 μg/ml for activated system. Based on the relative mitotic index results, the chromosome aberrations 1.0, 5.0 and 7.5 μg/ml for the non-activated system and 1.0, 5.0 and 10 μg/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 ere 0.05, 0.1, 0.5, 1, 5 and 7.5 μg/ml and 0.05, 0.1, 0.5, 1, 5 and 10 μg/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 μg/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,

10, 50, 100, 250, 500, 1000 and 2500 μg/ml under both activated and non-activated conditions. Untreated (water) and solvent (acidic water) controls were employed. 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. If possible, 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. If no cytotoxicity was observed, the maximum concentration tested with four decreasing concentration were scored. The sponsor's table 2 below summarizes the results of the mitotic index range-finding test. As indicated in table 2, the lowest concentration tested (5 μg/ml) without activation produced 78% relative mitotic index, whereas the next highest concentration produced 1% relative mitotic index, suggesting cytotoxicity. With metabolic activation, the relative mitotic index was 58% at 5 μg/ml, 94% at 10 μg/ml and 0% at 50 μg/ml.

TABLE 2
CHROMOSOME ABERRATION ASSAY IN CHO CELLS
MITOTIC INDEX - RANGE FINDING TEST

SPONSOR : Mallinckrodt, Inc. SOLVENT : Acidic water STUDY NO. : 0795-3110
TEST ARTICLE : [redacted] TRIAL NO.: A2

Without Activation - Treatment: 4 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	97	A	53	10.0	103%	Untreated	12	A	43	11.8	236%
		B	47					B	75		
Solvent	35	A	53	9.7	100%	Solvent	38	A	20	5.0	100%
		B	44					B	30		
5	34	A	36	7.6	78%	5	24	A	15	2.9	58%
		B	40					B	14		
10	89	A	1	0.1	1%	10	90	A	18	4.7	94%
		B	0					B	29		
50	4	A	0	0	0	50	39	A	0	0	0
		B	0					B	0		
100	11	A	0	0	0	100	46	A	0	0	0
		B	0					B	0		
250	82	A	0	0	0	250	31	A	0	0	0
		B	0					B	0		
500	52	A	0	0	0	500	44	A	0	0	0
		B	0					B	0		
1000	80	A	0	0	0	1000	56	A	0	0	0
		B	0					B	0		
2500	78	A	0	0	0	2500	75	A	0	0	0
		B	0					B	0		

All test article concentrations and untreated were compared to solvent.

$MI = \frac{\text{No. of dividing cells scored from 1000 cells}}{10}$ $RMI = \frac{\text{Test Dose MI}}{\text{Solvent Control MI}} \times 100$

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 Ca^{2+} and Mg^{2+} for an additional 16 hours prior to harvest. In the confirmatory assay, the cells were treated with [redacted] 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%.

TABLE 4
CHROMOSOME ABERRATION ASSAY IN CHO CELLS
MITOTIC INDEX - DEFINITIVE ASSAY

SPONSOR : Mallinckrodt, Inc. SOLVENT : Acidic water STUDY NO. : 0795-3110
TEST ARTICLE : [redacted] TRIAL NO.: B1

Without Activation - Treatment: 4 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)
		A	34					A	31		
Untreated	12	B	33	6.7	92%	Untreated	70	B	28	5.9	159%
		A	35					A	17		
Solvent	27	B	38	7.3	100%	Solvent	59	B	20	3.7	100%
		A	34					A	17		
0.1	65	B	33	6.7	92%	0.1	7	B	20	3.7	100%
		A	37					A	19		
0.5	14	B	28	6.5	89%	0.5	42	B	29	4.8	130%
		A	46					A	18		
1.0	5	B	28	7.4	101%	1.0	62	B	11	2.9	78%
		A	23					A	22		
5.0	33	B	23	4.6	63%	5.0	95	B	19	4.1	111%
		A	19					A	5		
7.5	21	B	15	3.4	47%	10	67	B	7	1.2	32%
		A	36					A	5		
* MMC 0.4	41	B	35	7.1	106%	** CP 7.5	8	B	6	1.1	19%
		A	13					A	0		
* MMC 0.8	15	B	19	3.2	48%	** CP 12.5	98	B	0	0	0

* MMC = Mitomycin-C ** CP = Cyclophosphamide

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

$MI = \frac{\text{No. of dividing cells scored from 1000 cells}}{10}$ $RMI = \frac{\text{Test Dose MI}}{\text{Solvent Control MI}} \times 100$

The results of the chromosomal aberrations test in the absence of metabolic activation are presented in sponsor's table 5 reproduced on the next page. As noted, the highest concentration tested (7.5 µg/ml), which produced a 47% relative mitotic index, significantly increased the % of cells with aberrations compared to controls. The slight increase in the % of cells with aberrations noted in the cells treated with 5 µg/ml [redacted] (which produced a relative mitotic index of 63%) was not significant.

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TABLE 5
CHROMOSOME ABERRATION ASSAY IN CHO-CELLS
CHROMOSOME ABERRATIONS - DEFINITIVE ASSAY

TEST ARTICLE: [redacted] STUDY NO.: 0795-3110
SPONSOR: Mallinckrodt, Inc. TREATMENT TIME: 4 Hours TRIAL NO.: B1
SOLVENT: Acidic water HARVEST TIME: 20 Hours 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								
						Simple		Complex					Simple	Complex			Others				
		tg	sg	% c	%pp	tb	isb	tr	qr	str	id	ctri	sb	d	r	dm	pu	ad*			
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		0.54	35.0	
MMC 0.4 A+B	200	2		0.0	0.0	27	12	7	16	17	5	10	11			1	1	1	0.585	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.

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

TEST ARTICLE: [redacted]
SPONSOR: Mallinckrodt, Inc.
SOLVENT: Acidic water
TREATMENT TIME: 4 Hours
HARVEST TIME: 20 Hours
[redacted] 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				Others			
						Simple		Complex					Simple	Complex						
		tg	ag	% c	%pp	tb	isb	tr	qr	cr	id	ci	sb	d	r	dm	pu	ad*			
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		6	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	9	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	19	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.

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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. SOLVENT : Acidic water STUDY NO. : 0795-3110

TEST ARTICLE : [redacted] 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	49	A	51	7.3	78%	Untreated	5	A	25	4.8	117%
		B	22					B	23		
Solvent	61	A	43	9.3	100%	Solvent	98	A	21	4.1	100%
		B	50					B	20		
0.05	22	A	43	7.4	80%	0.05	91	A	26	4.7	115%
		B	31					B	21		
0.1	90	A	21	5.0	54%	0.1	76	A	35	5.9	144%
		B	29					B	24		
0.5	72	A	21	4.9	53%	0.5	57	A	33	4.9	120%
		B	28					B	16		
1.0	8	A	28	5.6	60%	1.0	14	A	19	3.8	93%
		B	28					B	19		
5.0	42	A	0	0	0	5.0	69	A	28	5.6	137%
		B	0					B	28		
7.5	55	A	0	0	0	10	70	A	2	0.7	17%
		B	0					B	5		
MMC 0.2	80	A	31	5.1	70%	CP 7.5	95	A	4	0.9	19%
		B	20					B	5		
MMC 0.4	75	A	14	2.6	36%	CP 12.5	74	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.

$MI = \frac{\text{No. of dividing cells scored from 1000 cells}}{10}$ $RMI = \frac{\text{Test Dose MI}}{\text{Solvent Control MI}} \times 100$

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: [redacted] TREATMENT TIME: 20 Hours [redacted] STUDY NO.: 0795-3110
SPONSOR: Mallinckrodt, Inc. HARVEST TIME: 20 Hours TRIAL NO.: B2
SOLVENT: Acidic water 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								
						Simple		Complex					Simple	Complex			Others				
		tg	sg	% e	%pp	tb	isb	tr	qr	cr	id	ci	sb	d	r	dm	pu	ed*			
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	9	5	22	6	2	4	6				2	1	0.60	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

* ed = 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 [redacted] historical Untreated(water) control data(0.22%) since the solvent for test article was 1N HCl and water, and concurrent value is 0%
In the Chi-square test, MMC was compared to [redacted] 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, [redacted] 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

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

TEST ARTICLE: [redacted]
SPONSOR: Mallinckrodt, Inc.
SOLVENT: Acidic water
TREATMENT TIME: 4 Hours
HARVEST TIME: 20 Hours
[redacted] STUDY NO.: 0795-3110
TRIAL NO.: 82
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								
						Simple		Complex				Simple	Complex			Others				
		tg	sg	%s	%pp	sb	isb	te	qr	cr	id	ei	sb	d	r	dm	pu	ed*			
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										0.015	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									0.030	2.5	p=0.7210
5.0 A	100			0	0	4	1	1	4	1			1						0.12	9.0	-
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	16.0	
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	8				15	4	1.18	47.0	
CP 7.5 B	100			0	0	5	10	5	12	8	13	8	2				13		0.76	42.0	
CP 7.5 A+B	200			0.0	0.0	14	15	14	21	15	27	10	8				28	4	0.960	44.5	p<0.0001

CP = Cyclophosphamide
Trend test: P<0.0001

* ed = 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.

3.4.5. Carcinogenicity

Carcinogenicity studies for oxymorphone were not submitted with the NDA. Carcinogenicity studies in rats and mice were initiated in July and August of 2002, respectively. Study protocols were reviewed by the FDA CAC. In the rat study, doses of 5, 10 and 25 mg/kg/day are being tested in males and 10, 25 and 50 mg/kg/day in females. In the mouse study, doses of 10, 25, 75 and 150 mg/kg/day are being tested in both males and females. A gradual dose escalation over the first four weeks is being used to reach the higher doses in mice.

Based upon prior agreement with the Division, the carcinogenicity assessment for oxymorphone can be submitted as Phase 4 commitments.

3.4.6. 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.
2. Clinical signs associated with mortality were primarily associated with classical opioid-receptor mediated effects including whole body tetany, Straub tail and excessive chewing. Additional clinical signs include decreased defecation, absent feces, exophthalmia and lacrimation.
3. The mean number of days between pairing and coitus was increased in the high dose group compared to control values.
4. Mean estrus cycle length was increased in the females treated with 50 mg/kg/day.
5. Oxymorphone at a dose of 50 mg/kg/day produced decreased mean number of viable embryos and decrease the number of implantation sites and corpora lutea.

Study no.: [redacted]-411001
Volume #, and page #: EDR
Conducting laboratory and location: [redacted]

Date of study initiation: September 5, 2000
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Oxymorphone HCl USP, Lot 0881 A 42771, purity was [redacted](doses were corrected based on purity). Analysis of this lot provided the following impurity profile.

TEST	RESULTS	LIMITS
Related Substance: Impurities:		
[redacted]		
Degradation Products:		
[redacted]		
Other Impurities:	[redacted]	
Total Related Substances:	[redacted]	[redacted]
Residual Solvents(IPA)	[redacted]	[redacted]

Methods

Doses: 0, 0.1, 1, 10, 25 and 50 mg/kg/day (doses based on a 5-day preliminary study).

Species/strain: Rats [redacted]:CD(SD)IGS BR

Number/sex/group: 10/sex/group for toxicology

Route, formulation, volume, and infusion rate: oral gavage, deionized reverse osmosis treated water in a volume of 10 ml/kg.

Satellite groups used for toxicokinetics: 36 rats/sex/group.

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 CO_2 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 tested 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.

	Summary of Animal Disposition											
	Males (mg/kg)						Females (mg/kg)					
Disposition	**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:

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												
Gasping	--	--	--	--	--	--	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 [redacted] - 411001)	Definitive Study [redacted] - 411004)
1st Day	M	1	1	0.78 (1.22*)	
1st Day	M	5	1		4.86
1st Day	F	1	1	1.04 (1.76*)	
1st Day	F	5	1		5.90
1st Day	M	10	1	7.24	
1st Day	F	10	1	8.85	
1st Day	M	25	1	16.63	11.85
1st Day	F	25	1	21.96	15.97
1st Day	M	50	1	25.63	
1st Day	F	50	1	139.97	
14th Day	M	1	1	18.33 (1.23*)	
14th Day	M	5	1		6.10
14th Day	F	1	1	2.14*	
14th Day	F	5	1		6.17
14th Day	M	10		69.52 (28.17*)	
14th Day	F	10		18.16	
14th Day	M	25	1	69.52	43.17
14th Day	F	25	1	49.80	36.71
14th Day	M	50	1	172.49	
14th 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	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
N	10	10	10	10	10	9	10	10	10	10	10	10
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	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
N	10	10	10	10	9	9	10	8	10	9
Liver										

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).

	Summary of Male Reproductive Performance (N=9-10)					
	Males (mg/kg)					
Parameter	**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 [redacted] 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:

	Summary of Female Reproductive Performance (N=9-10)					
	Males (mg/kg)					
Parameter	**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.

TABLE 28 (TOXICOLOGY PHASE)

PROJECT NO [redacted] 411001F R-F STUDY OF OXYMORPHONE HYDROCHLORIDE IN RATS PAGE 1

SPONSOR: ENDO PHARM., INC. SUMMARY OF MEAN EMBRYONIC DATA AT THE SCHEDULED NECROPSY

GROUP		VIABLE EMBRYOS	DEAD EMBRYOS	RESORPTIONS EARLY	RESORPTIONS LATE	POST IMPLANTATION LOSS	IMPLANTATION SITES	CORPORA LUTEA	PRE IMPLANTATION LOSS	NO. OF GRAVID FEMALES
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

PLSUv4.02
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 were 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.: [redacted]-411004
Volume #, and page #: EDR
Conducting laboratory and location: [redacted]

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, [redacted] purity (note doses were calculated based on purity of the test article).

Methods

Doses: 0, 5, 10 and 25 mg/kg
Species/strain: [redacted]:CD®(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 CO_2 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:

STATISTICAL TEST	PARAMETER
-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.

	Summary of Animal Disposition (N=25/sex/group)							
	Males (mg/kg)				Females (mg/kg)			
Disposition	**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

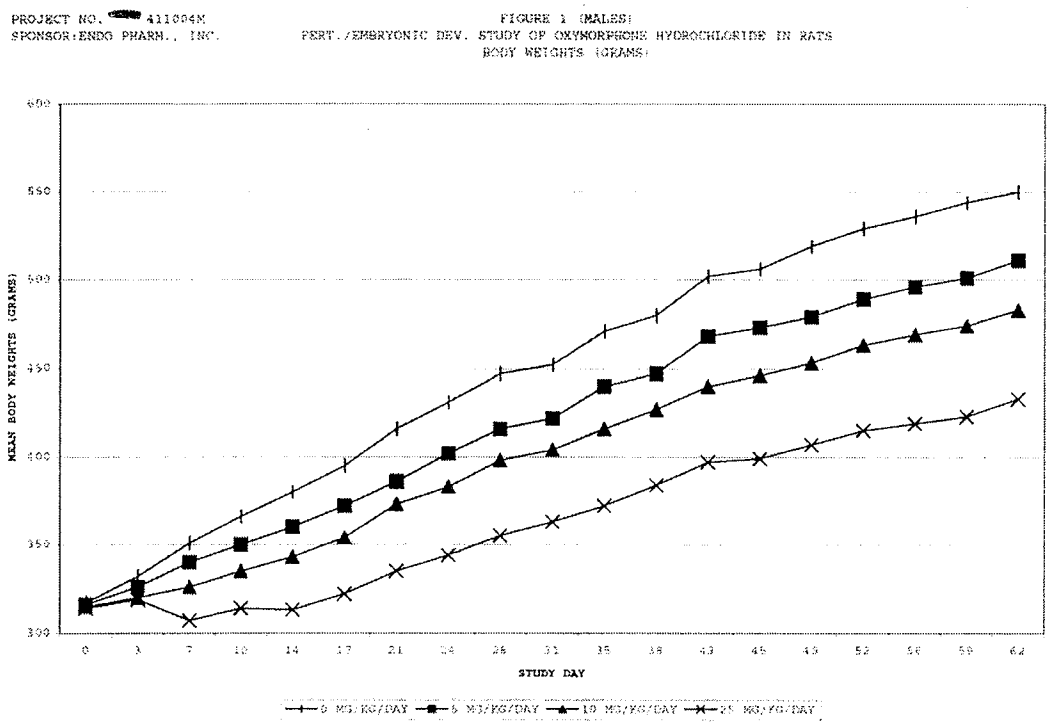
Scabbing distal end of tail	0/0	0/0	0/0	20/2	0/0	0/0	0/0	0/0
Unkempt appearance	0/0	0/0	0/0	0/0	0/0	0/0	0/0	3/1
Swelling left forelimb	0/0	0/0	0/0	0/0	0/0	0/0	0/0	6/2
Wet material urogenital area	0/0	0/0	0/0	1/1	0/0	0/0	0/0	3/2
Dried yellow material urogenital area	0/0	0/0	0/0	0/0	0/0	0/0	0/0	2/1
Cardio-Pulmonary								
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
Exophthalmus right eye	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/1

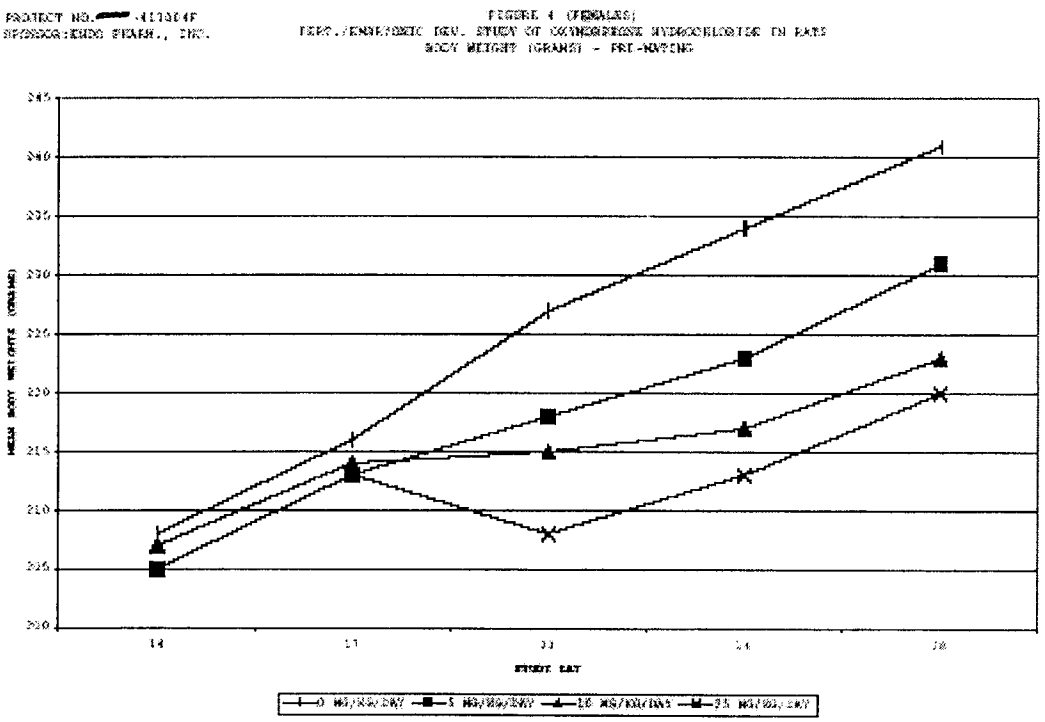
n/n = total occurrence/number of animals; -- = no data recorded

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								
Exophthalmus right eye	0/0	0/0	9/5	45/19	0/0	4/3	7/5	20/12
Exophthalmus 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.



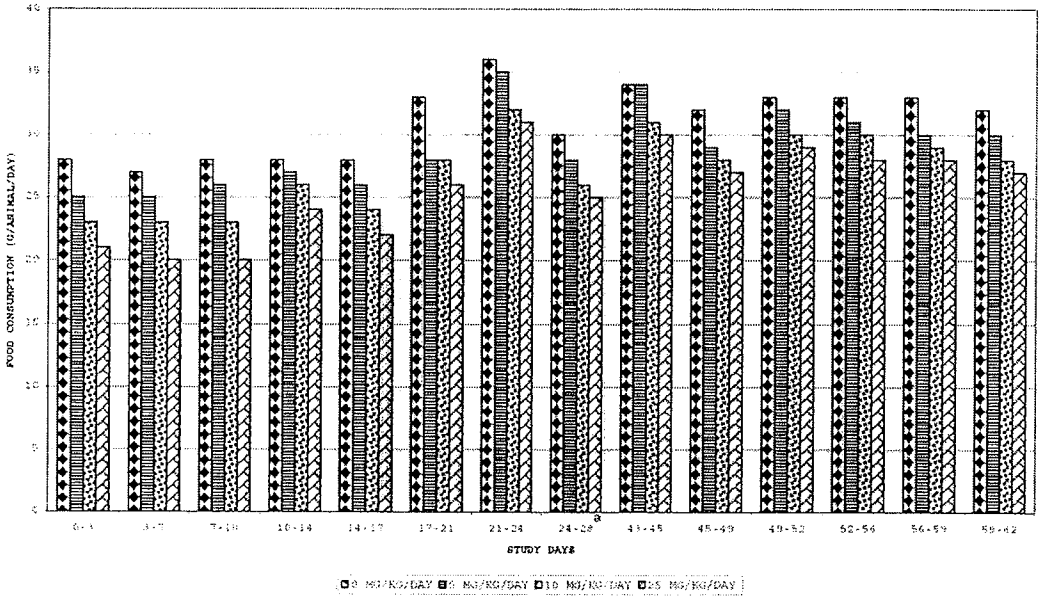


Food consumption: Food consumption in the male was decreased in a dose-dependent fashion as depicted in the figure below. The decrease was statistically significant in the

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. [redacted]-411(0)1M
SPONSOR:ENDO PHARM., INC.

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



a - FOOD CONSUMPTION NOT RECORDED DURING THE BREEDING PERIOD (DAYS 28-41)

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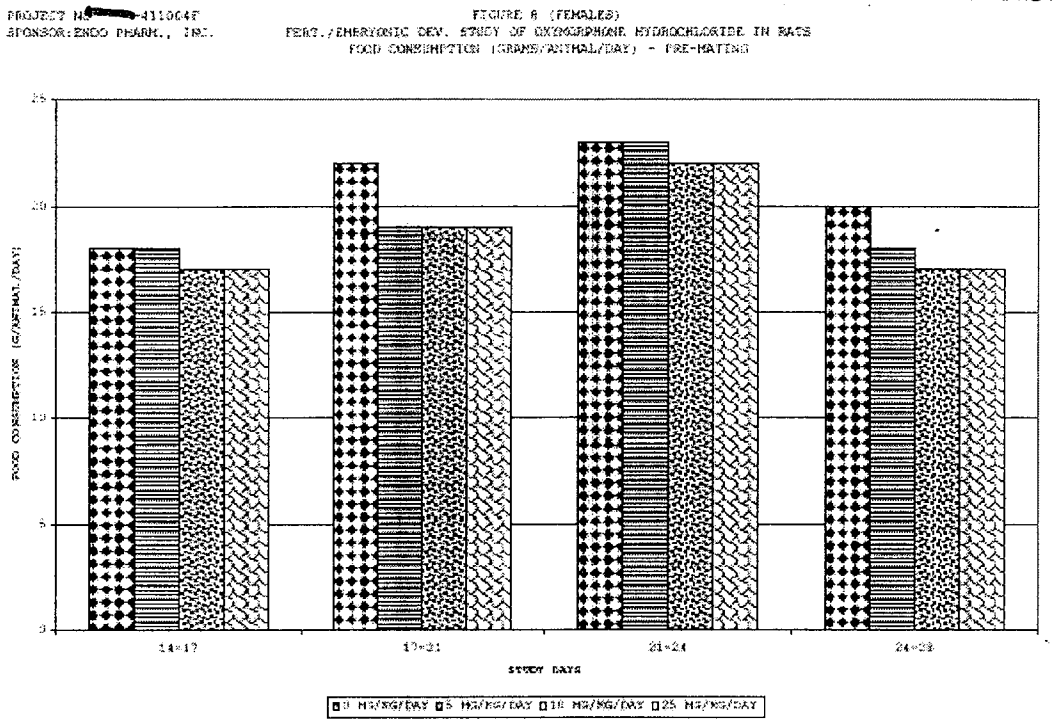

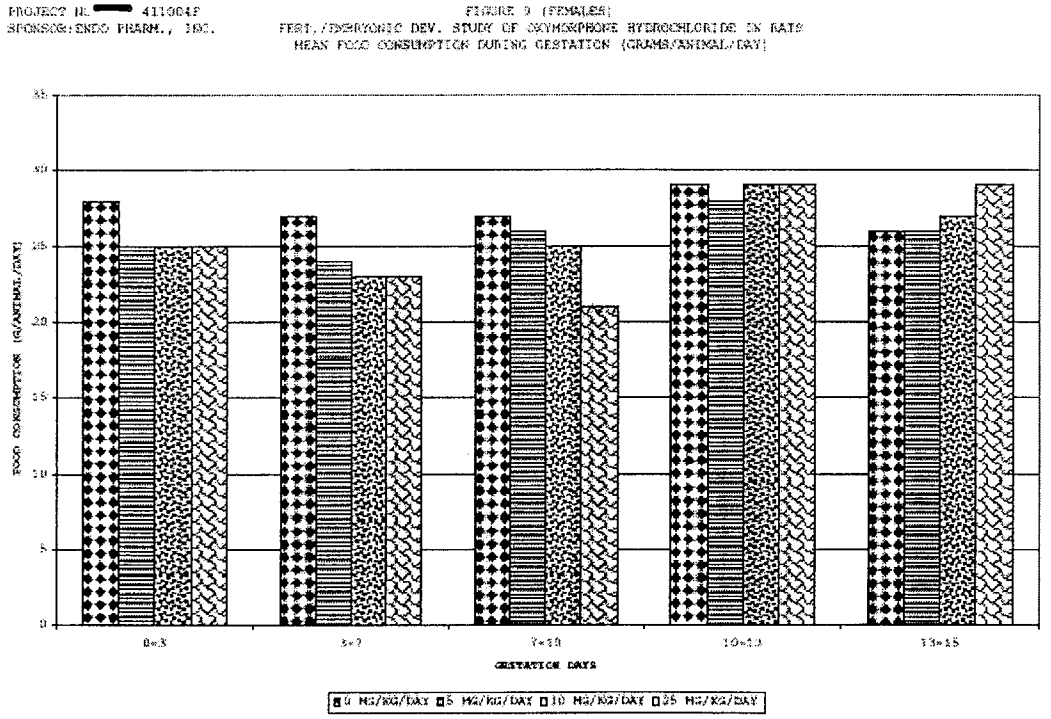

Toxicokinetics: All of the parameters evaluated in the toxicokinetic phase (deaths, clinical observations, mean body weights, body weight gains and internal findings) were

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)
1st Day	M	1	1	0.78 (1.22*)	
1st Day	M	5	1		4.86
1st Day	F	1	1	1.04 (1.76*)	
1st Day	F	5	1		5.90
1st Day	M	10	1	7.24	
1st Day	F	10	1	8.85	
1st Day	M	25	1	16.63	11.85
1st Day	F	25	1	21.96	15.97
1st Day	M	50	1	25.63	
1st Day	F	50	1	139.97	
14th Day	M	1	1	18.33 (1.23*)	
14th Day	M	5	1		6.10
14th Day	F	1	1	2.14*	
14th Day	F	5	1		6.17
14th Day	M	10		69.52 (28.17*)	
14th Day	F	10		18.16	
14th Day	M	25	1	69.52	43.17
14th Day	F	25	1	49.80	36.71
14th Day	M	50	1	172.49	
14th 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	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
N	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 leutea (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 leutea 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:

1. One female in the high dose group was found dead on gestation day 7.
2. Clinical signs were evident in all treatment groups and were consistent with a high dose opioid and included CNS behavior such as Straub tail, rocking and lurching, hypoactivity and exophthalmus.
3. There was a dose-related and time-related decrease in body weight and food consumption in all dose groups. A NOAEL for maternal toxicity was not established and should be considered to be < 5 mg/kg/day.
4. 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.
5. There were no significant malformations (external, soft tissue or skeletal) between treatment groups, indicating that oxymorphone was not teratogenic under the conditions tested.
6. There was a slight increase in the incidence of fetuses with variations (fetuses with unossified pubis, accessory skull bone(s), 27 presacral vertebra and bent ribs, however, these changes were not statistically significant and/or were within the historical control range.
7. Overall the results suggest that the NOAEL for maternal toxicity was < 5 mg/kg based on findings consistent with exaggerated pharmacology associated with the opioid class of compounds.
8. Developmental toxicity was noted at both the mid-dose and the high-dose groups based upon decreased mean fetal body weights. Therefore the NOAEL for developmental toxicity was 5 mg/kg/day (in agreement with the sponsor).

Study no.: [redacted]-411006
Volume #, and page #: Electronic Document Room
Conducting laboratory and location: [redacted]

Date of study initiation: March 2, 2001
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Oxymorphone hydrochloride USP, Lot 0881 A 42771, [redacted] purity (dose calculations were corrected based on the purity of the test article).

Methods

Doses: 5, 10 and 25 mg/kg/day
Species/strain: Female [redacted]:CD(SD)IGS BR rats from [redacted].
Number/sex/group: 25 females/group
Route, formulation, volume, and infusion rate: Oral gavage, dissolved in water, dose volume of 10 ml/kg based upon the most recently recorded body weights.
Satellite groups used for toxicokinetics:
Study design: Animals were dosed once a day on gestation days 6-17. Dosing was based on the results of a dose-range-finding study in pregnant female rats ([redacted]-411005).

The following table presents the study group assignment:

Group	Test Article	Dosage Level[a] (mg/kg/day)	Dosage Concentration[a] (mg/ml)	Dosage Volume (ml/kg)	Number Of Females
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 viscerally 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:

Subset	Targeted Time Point	Animals/Group
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

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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
PROJECT NO [redacted]411006 STUDY OF OXYMORPHONE HYDROCHLORIDE ON EMBRYO/FETAL DEV. IN RATS PAGE 1
SPONSOR:ENDO PHARM., INC. MEAN BODY WEIGHTS (GRAMS) DURING GESTATION

GROUP :		1	2	3	4
DAY 6	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	19.9/23
DAY 8	MEAN	304.	304.	300.	303.
	S.D./N	14.9/25	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	16.7/25	15.0/24	17.0/23	13.9/23
DAY 11	MEAN	323.	315.	300.**	298.**
	S.D./N	15.9/25	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	16.9/25	19.1/24	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	19.5/23
DAY 18	MEAN	392.	377.**	359.**	349.**
	S.D./N	18.7/25	19.4/24	20.1/24	19.9/23
DAY 20	MEAN	432.	411.**	390.**	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

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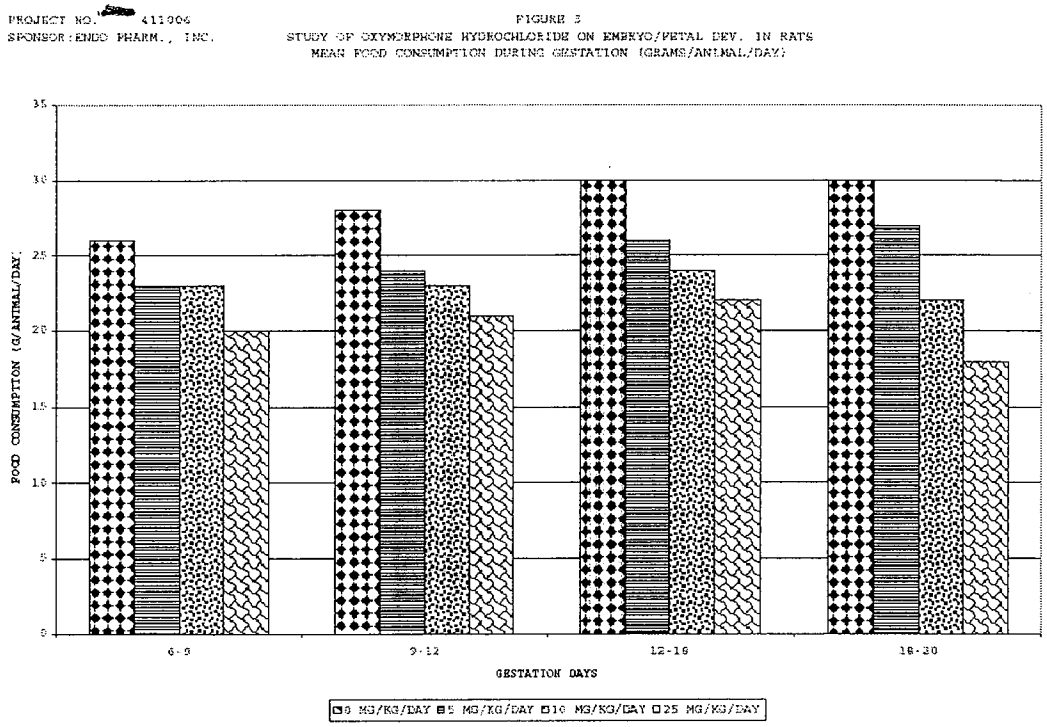
Initial body weight, terminal body weight, gravid uterine weight, net body weight and net body weight change are presented in the table below.

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.



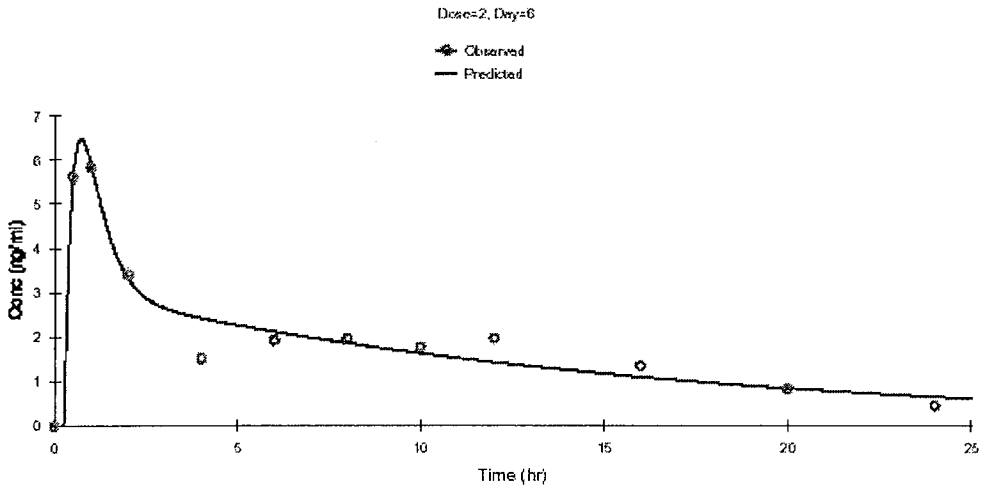
Toxicokinetics: Toxicokinetic analysis demonstrated that females dosed with either 5 or 25 mg/kg showed a dose related increase in the C_{max} and AUC_{24} values. There was no clear evidence that the AUC24 was altered from gestation day 6 to day 17. Initially, the high dose of oxymorphone increased the T_{max} and the t½ 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 1/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:

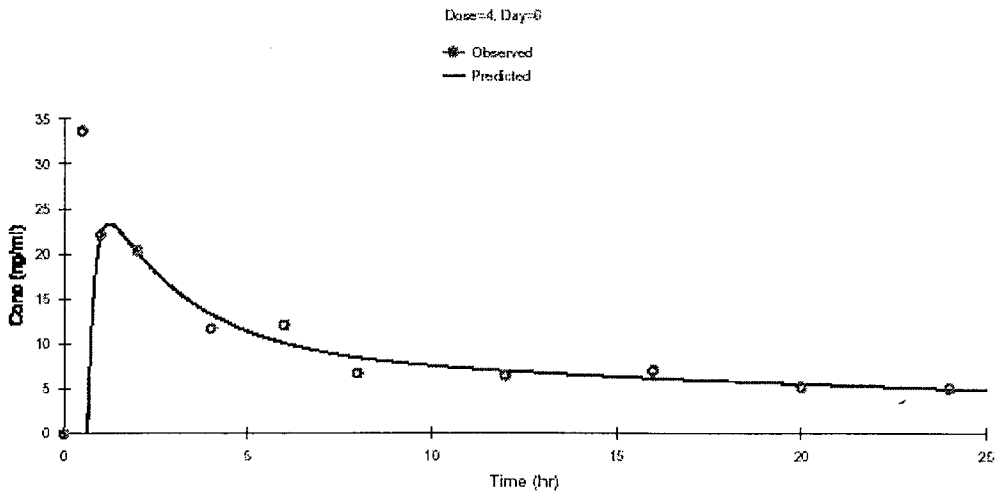
Figure 1: Plasma Concentration *versus* Time – GD 6; 5 mg/kg



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.

	% per litter of fetal variations (n=23-25/group)				
Parameter Dose (mg/kg) →	**0**	**5**	**10**	**25**	**Historical Control**
Number litters examined skeletally	25	24	24	23	403
% with 14th 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%

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

Key study findings: Female rabbits were treated with oxymorphone from gestation day 7 through gestation day 20 in a definitive segment II study with the following key findings:

1. There were no clear treatment-related mortalities or early abortions noted in the study.
2. Clinical signs such as hypoactivity and decreased defecation were noted in all treatment groups in a dose-related manner. The mid-dose and high-dose group also demonstrated a few incidences of lacrimation, excessive chewing, exophthalamus and mydriasis.
3. Maternal body weights were decreased in a dose-related manner at all doses tested during the first 3 days of treatment. Body weights of dams treated with 25 or 50 mg/kg/day remained reduced throughout the treatment period, while animals in the low dose group did not demonstrate an overall body weight gain during the treatment period. Food consumption followed a similar pattern.
4. There was a significant decrease in the mean fetal weight of male pups (↓16%) born to the high-dose dams, but this decrease was not noted in the females (↓7%) of the combined fetuses (↓10%).
5. There were no clear increases in the incidence of fetal malformations or variations. Some offspring malformations were noted (mandibular micrognathia, aglossia, lobular agenesis of the lung, sterneabrae fused or forked rib), however, these were either not statistically significant or were within the range of historical controls.
6. The NOAEL for maternal toxicity was < 10 mg/kg based upon the increased incidence of hypoactivity, decreased defecation, body weight and food consumption. Based upon the presence of maternal toxicity, the study is considered to be valid.
7. The NOAEL for fetal development was 25 mg/kg/day based upon a decrease in fetal body weights in males at the high dose (in agreement with the Sponsor).

Study no.: [redacted]-411008
Volume #, and page #: Electronic Document Room
Conducting laboratory and location: [redacted]

Date of study initiation: February 1, 2001
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Oxymorphone HCl USP, Lot 0881 A42771, [redacted] (doses were corrected based upon the purity of the test article.

Methods
Doses: 0, 10, 25 and 50 mg/kg/day from gestation day 7-20.
Species/strain: [redacted](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:

Group	Test Article	Dosage Level[a] (mg/kg/day)	Dosage Concentration[a] (mg/ml)	Dosage Volume (ml/kg)	Number of Females
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 ([redacted]-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 (gestatin 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.

	Summary of material survival and pregnancy status (n=25/group)			
Parameter Dose (mg/kg) →	**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.

Daily examinations	Incidence of treatment-related clinical signs (n=24-25/group)			
Parameter Dose (mg/kg) →	**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

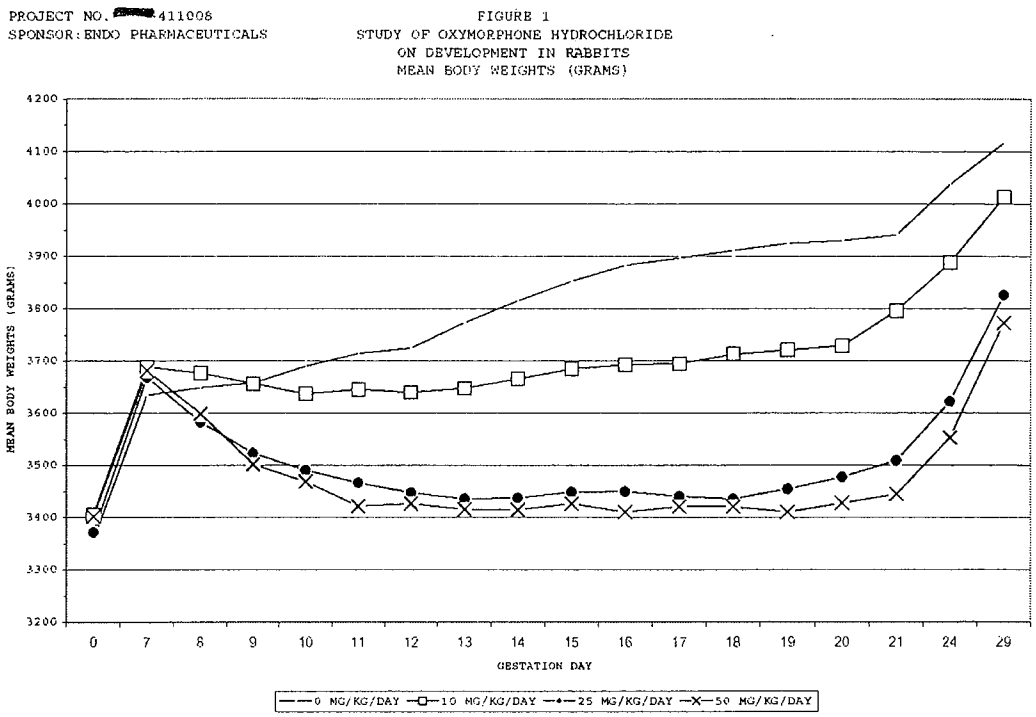
n/n = total occurrences/number of animals

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
Exretia				
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.



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:

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

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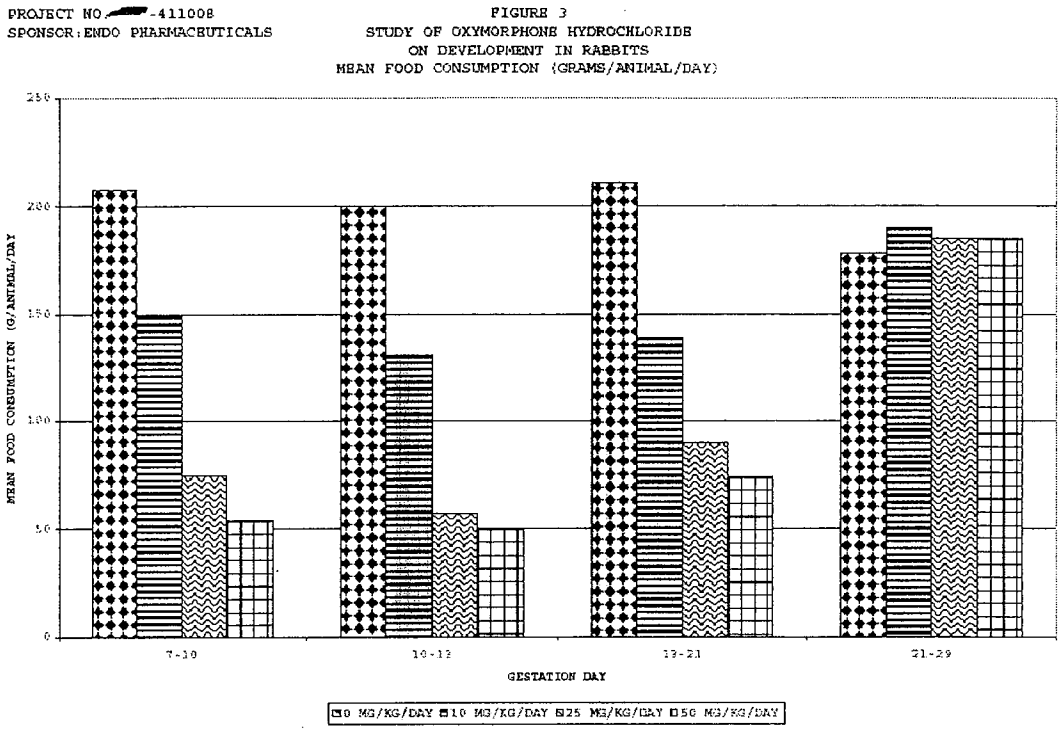

Toxicokinetics: Toxicokinetic analysis in the range-finding study suggested that the peak plasma concentration of oxymorphone were obtained approximately 0.5 to 1 hour post dose. Therefore blood levels at 1 hour post-dose were compared. Increasing the dose of oxymorphone produced an increase in the plasma 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 ([redacted]-411007)	1 hour (ng/mL) Definitive Study ([redacted]-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

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.): One female in the control group died on gestation day 13. There were no internal observations at necropsy to suggest the cause of death. In the 10 mg/kg group, one female aborted one normally developing implantation. At necropsy, this animal had dark red lungs and five normally developing implantations and two early resorptions *in utero*. All other animals that survive to the scheduled necropsy on gestation day 29 did not show any treatment related internal findings. Additional findings that are common to the laboratory rabbit and not thought to be related to drug treatment included: cystic oviducts, accessory spleens and blood vessel variation in the left carotid artery.

Gestation Day 29 Laparohysterectomy Data. There were no significant changes in the mean fetal data at scheduled necropsy (number of male fetuses, number of female fetuses, viable fetuses, dead fetuses, early resorptions, late resorptions, post implantation loss, implantation sites, corpora lutea, pre-implantation loss or fetal weights). There was a significant decrease in male fetal weights in the high-dose group compared to controls, however, there were no differences in female fetal weights or mean combined fetal weights. These data are expressed below in table format:

	Fetal Weights (n=17-21/group) (% of Control, grams)		
Dose (mg/kg) →	**10**	**25**	**50**
Male	-3	-7	-16*
Female	-1	-7	-7
Combined	-2	-8	-10

Offspring (malformations, variations, etc.): The table below summarizes the results of the analysis of fetuses for malformations:

	Percentage of fetuses with malformations (% per liter)				
Parameter Dose (mg/kg) →	**0**	**10**	**25**	**50**	**Histor. Range**
Number of fetuses (litters) examined	127 (22)	116 (22)	143 (22)	113 (22)	
External Malformations					
Mandibular micrognathia	0	0	0	0.6	
Aglossia	0	0	0	0.6	0-1
Visceral Malformations					
Lungs – lobular agenesis	0	0	0	0.6	0-0.9
Skeletal Malformations					
Sternebrae fused	0	0	0.6	0.6	0-1.9
Rib anomaly (forked rib)	0	0	0	1	0-7.1

n/n = total occurrences/number of animals

The incidence of aglossia, lung lobular agenesis, fused sternebrae and rib anomaly were within the historical control range for the laboratory and there was no clear dose-

dependent relationship noted under the conditions of the assay. There is no evident relationship to the drug treatment. The finding of mandibular migrognathia was not noted in the historical controls; however, this incidence is within the range of the more severe finding of mandibular agnathia (0-1). The low incidence, lack of statistical significance and lack of apparent dose-dependency with this finding (under the conditions tested) suggests that this finding is not clearly related to drug treatment.

Prenatal and postnatal development

Study title: Study of the Effects of Oxymorphone Hydrochloride on Pre- and Postnatal Development, Including Maternal Function in the Rat

Key study findings: Female rats (F_0) were treated with oxymorphone (0, 1, 5, 10 or 25 mg/kg/day) from gestation day 6 to lactation day 20 in a segment III study with the following key findings:

1. F_0 female mortality was noted in 5 of 25 in the 10 mg/kg/day group and 1/25 in the 25 mg/kg/day group. There were a significantly greater number of gravid females in the high dose group with total litter loss (13/25). Animals that died prior to scheduled necropsy exhibited typical clinical signs produced by high doses of opioids.
2. Clinical signs in surviving F_0 females were noted 1 hour post-dose in all treatment groups, including Straub tail and exophthalamus. Animals in the 5, 10 and 25 mg/kg/day group demonstrated hyperactivity as well as abnormal posture. Higher doses produced whole body tetany, hypoactivity, rales, piloerection and salivation.
3. Mean body weight losses in the F_0 females was significantly reduced early during treatment with the higher doses, however, as the treatment time progressed, a significant reduction in mean body weights were noted in all treatment groups. These effects were noted both during gestation and lactation.
4. In surviving F_0 females, there were not differences between the number of pups born and the number of implantation sites between groups.
5. Mean litter size in the F_1 generation born to the high dose F_0 females was 18% lower than controls (this reduction was not statistically significant, but is likely related to the drug treatment.
6. Post-natal survival of the F_1 pups was reduced in the 25 mg/kg/day treatment group.
7. Offspring mean body weights during the pre-weening period were significantly reduced in the 25 mg/kg/day treatment groups compared to controls, whereas pups in the low dose group 1 mg/kg/day demonstrated a significantly higher mean offspring weight compared to controls.
8. There were no treatment-related findings on PND 21 pups not selected for further study at necropsy.
9. Developmental landmarks in the F_1 males indicated that balanopreputial separation in males from the 25 mg/kg/day group was delayed compared to

controls, whereas males in the 1 mg/k/day group reached balanopreputial separation earlier than controls. There were no differences in the mean day of acquisition of vaginal patency in F_1 females between treatment groups.

10. There were no treatment-related effects of oxymorphone in behavioral evaluation (acoustic startle, locomotor and Biel Maze Swimming Trials).
11. Reproductive performance in the F_1 generation was not altered by F_0 generation oxymorphone treatment at any dose level tested.
12. Body weights in the F_1 generation during the post-weening period were significantly reduced in the 25 mg/kg/day groups on PND28, 35, 42, 49, 56, 62 and 70. Mean body weights were reduced in the 10 mg/kg/day groups on PND 49, 56, 63 and 70. Mean body weights in males in the 5 mg/kg/day group were also significantly reduced compared to controls on PND 63.
13. There were no findings in the F_2 fetuses which could be attributed to F_0 maternal treatment with oxymorphone.

Study no.: [redacted]-411009
Volume #, and page #: Electronic Document Room
Conducting laboratory and location: [redacted]

Date of study initiation: February 9, 2002
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Oxymorphone HCl, Lot 0881 A 42771, [redacted] purity (dose calculates were corrected based upon the purity of the drug).

Methods

Doses: 0, 1, 5, 10 and 25 mg/kg/day. F_0 females were dosed from gestation day 6 through lactation day 20.

Species/strain: [redacted] CD(SD)IGS BR female rats

Number/sex/group: 25 females per group as below:

Group	Test Article	Dosage Level[a] (mg/kg/day)	Dosage Concentration[a] (mg/ml)	Dosage Volume (ml/kg)	Number of Females
1	Vehicle	0	0	10	25
2	Oxymorphone HCl	1	0.1	10	25
3	Oxymorphone HCl	5	0.5	10	25
4	Oxymorphone HCl	10	1.0	10	25
5	Oxymorphone HCl	25	2.5	10	25

[a] - Expressed in terms of the oxymorphone salt

Route, formulation, volume, and infusion rate: Oral gavage as a single dose. Volume was 10 ml/kg in all groups.

Satellite groups used for toxicokinetics: None

Study design: F_0 female rats were dosed from gestation day 7 through lactation day 20 with oxymorphone or vehicle. Females were paired with an untreated resident male of the same strain. Once positive identification of mating was obtained (vaginal copulatory plug or presence of sperm in a vaginal smear), the females were transferred to plastic maternity cages. All females were allowed to deliver naturally and rear their young to weaning (PND 21). To reduce variability among the litters, eight pups per litter (4 per sex) were randomly selected on PND 4. Between PND 10 and 16, 25 males and females were randomly chosen for the F_1 generation. Offspring (F_1) were housed in housed until PND 28 then individually housed until euthanasia (with the exception of the mating period, when each F_1 female was cohabitated with F_1 makes in the home cage of the male).

Parameters and endpoints evaluated: Animals were observed twice daily for mortality and moribundity. Clinical observations included evaluations of skin and fur appearance, eyes, mucous membranes, respiratory and circulatory systems, autonomic and central nervous systems, somatomotor activity and behavior. In addition, females were observed during the treatment period at the time of dosing and one and three hours following dosing. Body weights were recorded on gestation days 0, 6, 9, 12, 15, 18 and 20 and on lactation days 1, 4, 7, 10, 14, 17 and 21. Food consumption was recorded on the corresponding gestation and lactation body weight days. During parturition, females were observed two times each day for initiation and completion of parturition and for signs of dystocia. The day at which parturition was first observed was designated PND 0. When parturition was complete, the numbers of stillborn and live pups in each litter was recorded. Pups were sexed and examined for gross malformations.

Gross necropsy was performed on F_0 animals which died prior to scheduled euthanasia. The number and location of corpora lutea and implantation sites were recorded. Females which failed to deliver were euthanized on post-mating day 25, their abdominal and thoracic cavities were opened, the contents examined and pregnancy status was determined. Females with total litter loss were examined for the number of former implantation sites. Females that survived to lactation day 21 were euthanized and necropsied. The number of former implantation sites was recorded.

Testing of the F1 litters included daily examination of general appearance, behavior and survival. Any pups that died were necropsied. Litters were examined daily for adverse changes in appearance or behavior. Each pup received a detailed physical examination on PND 1, 4, 7, 10, 14, 17 and 21 and weekly thereafter. Body weights of pups were recorded on PND 1, 4, 7, 10, 14, 17, 21 and weekly thereafter. Pups were sexed individually on PND 0, 4 and 21. A minimum of 1 male and 1 female per litter were selected for assessment of attainment of developmental landmarks, neurobehavioral evaluations and reproductive capacity.

Results