

TABLE 55: Significant changes in organ weights after IV or SC buprenorphine + naloxone (3:2) in dogs for 28 days (n=5/group)

Parameter	Sex	Control	0.15 mg/kg, IV	1.5 mg/kg, IV	15 mg/kg SC
Body weight, kg	M+F	10.6±1.2	N.S.	N.S.	8.7±0.8#
Heart, g	M+F	85.7±11.9	N.S.	N.S.	66.1±6.8#
Liver, g	M+F	404±65	N.S.	N.S.	319±45#
Lungs, g	M+F	122±16	N.S.	100±14#	95±14#
Kidney, left, g	M+F	30.6±3.4	N.S.	N.S.	26.1±2.0#
Thymus, g	M+F	11.0±2.2	N.S.	6.3±3.8#	6.2±3.0#

N.S.= not significant. #Conducting laboratory did not report p-values, only t-values.

Histopathology: Inflammatory reactions were apparent at the injection sites were apparent at histological examination.

KEY STUDY FINDINGS

Dogs received daily injections for 4 weeks containing 3:2 buprenorphine HCl plus naloxone HCl doses of 0.15 and 1.5 mg/kg, IV, or 15 mg/kg, SC (as buprenorphine). The dogs showed <25% as much body weight gain as the controls at all doses. Food consumption was significantly decreased at the two higher doses starting from the first week and 10-13% at the low dose during weeks 3 and 4. The SC route showed injection site intolerance, significantly elevated reticulocytes (+37%), platelets (+32%), cholesterol (+16%) and α₂-globulin (+52%). Dogs treated SC showed significant decreases in body weight and the weights of the heart, liver, lungs, left kidney and thymus. No apparent target organ toxicities were observed at 1.5 mg/kg, IV, and 15 mg/kg, SC. Overall, the NOAEL was <0.15 mg/kg and the LOAEL was 0.15 mg/kg, IV.

General Comments: All doses of the buprenorphine/naloxone (3:2) mixture cited in the following study refer only to the buprenorphine portion of the mixture.

All dogs were treated with mebendazole (100 mg/day, 5 days, PO) 4 weeks before treatment with test drugs.

Study Title: 4-Week Toxicity of the Substance Mixture Buprenorphine-HCl, Lot No. 21 and Naloxone-HCl, Batch No. 231 (Ratio 3:2) — Called for Short 'BUP + NAL' — by Intramuscular Administration to Beagle Dogs.

Study No.: RC84198 Volume #: 16 Tab#: 84198

Conducting Laboratory: []

Date of Study Initiation: August 30, 1984

GLP Compliance/QA Report: (X) Yes () No

Methods:

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Dosing: Daily, 7 days/week for 4 weeks

Species/strain: Dog / beagle

No./sex/group: 3/sex/dose (main study)

Age: 9-10 months

Weight: 8.58-9.02 kg (combined sex group means)

Satellite group for recovery: 1/sex from the control and high dose groups were retained for 4 weeks at the end of treatment.

Dosage groups: Buprenorphine + Naloxone (3:2): 0 (vehicle), 0.5, 2.0 and 8.0 mg/kg/day

Route, form, volume: Intramuscular, solution (0.2%) or suspension (0.25 ml/kg).

Drug lot nos.: Buprenorphine, lot #21 (pure); naloxone, lot #231

Formulation/vehicle: 5% aqueous glucose

Observation period: 4 weeks (+ 4-week recovery for some control and high dose dogs)

Clinical signs: Observed for external appearance and behavior, including general reflexes, once/day. Local tolerance (injection site) was monitored. Feces were monitored.

Body weights: Measured at initiation of treatment and weekly thereafter.

Food consumption: Food (50 g/kg) was offered for 2 hours post-treatment or longer (up to 8 hours) for animals with poor appetite. Daily consumption was estimated by weighing the residue. Monitoring of water consumption was done daily.

Ophthalmoscopy: Twenty-four hours after the last dosing at 4 weeks or at 8 weeks in the recovery dogs, the eyes were examined with a ophthalmoscope (with slit lamp), and pupillary reaction to light was examined. Auditory acuity and dentition were also examined.

EKG: Electrocardiographic examinations were conducted before, 5 minutes after dosing and, in the control and high-dose groups also at 30 minutes after dosing, on the first test day and in test week 4 in all dogs and in test week 8 in the recovery dogs. Limb lead II was evaluated. Systolic blood pressure was measured with an inflatable sleeve applied to the forelimb of conscious dogs after 4 weeks of treatment (24 hours after dosing) and after 8 weeks in the recovery dogs.

Hematology: Blood was drawn before the first drug administration and during week 4 in all dogs, as well as in all surviving recovery dogs at week 8, to measure hemoglobin, erythrocytes and leukocytes, differential blood count, hematocrit, thromboplastin time, erythrocyte sedimentation rate, blood clotting time, platelet count and reticulocyte count.

Clinical chemistry: Serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, creatinine, urea, uric acid, glucose, total bilirubin, sodium, potassium, calcium, chloride, total protein, albumin, globulin, total cholesterol and plasma lactate dehydrogenase were measured. Liver function was also measured with the bromsulphthalein test.

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Urinalysis: Urine was collected for 5 hours following administration of 50 ml of water per kg body weight via stomach tube, before the first drug treatment and during week 3 in all dogs and week 7 in the recovery dogs. Urine specimens were monitored for color, specific gravity, protein, glucose, bilirubin, hemoglobin, ketone bodies, pH, urobilinogen and identification of sediment.

Gross pathology: All surviving animals were euthanized with 0.3 ml IV of _____
 _____ exsanguinated by carotid dissection after 4 or 8 weeks for necropsy.

Organs weighed: Adrenals, brain, gonads, heart, kidneys, liver, lungs, pituitary, spleen, thymus and thyroid.

Histopathology: Samples from a number of tissues were preserved in buffered 10% formalin (see check list below) and stained with H & E after preparation of paraffin sections. In addition, frozen sections of heart, liver and kidney were stained with Sudan III.

Adrenals	X
Aorta	X
Bone marrow	X
Bone	X
Brain	X
Cecum	
Colon	X
Duodenum	X
Epididymes	
Esophagus	X
Eyes with optic nerve	X
Fallopian tube	
Gall bladder	X
Gross lesions	
Gonads	X
Harderian gland	
Heart	X
Ileum	X
Injection sites	X
Jejunum	X
Kidneys	X
Lachrymal gland	
Larynx	X
Liver	X
Lungs	X
Lymph nodes, cervical	
Lymph nodes, mandibular	
Lymph nodes, submaxillary	
Lymph nodes, mesenteric	X
Mammary glands	X
Nasal cavity	
Pancreas	X
Parathyroid	
Peripheral nerve	X

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Pharynx	
Pituitary	X
Prostate	X
Rectum	X
Salivary gland	X
Seminal vesicles	
Skeletal muscle	X
Skin	X
Spinal cord	X
Spleen	X
Stomach	X
Thymus	X
Thyroid	X
Tongue	X
Trachea	X
Urinary bladder	X
Uterus	X
Vagina	

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Results:

Clinical signs: The two higher dose caused sedation (decreased motility) during week 1 which lasted about a day. Dogs at all dose levels showed a dose-related incidence of vomiting (3/8, 4/6, 7/8) during the first 2 weeks, either shortly before or within 1.5 hours after dosing. Dogs at all dose levels also showed a dose-related incidence of pain reaction to the injection, particularly during the first 2 weeks.

Body weights: No significant differences in body weights between the treatment groups and corresponding control (combined male and female) group were reported. However, if body weight gains are calculated for the individual treatments, all doses of buprenorphine+naloxone caused a mean weight loss during the 4-week treatment period (Table 56). Unlike control dogs of the same age range and from the same supplier in other 4-week studies conducted by the same laboratory, which had mean weight gains of 0.59-1.19 kg, the controls in this study showed very little weight gain. The mean % body weight losses from the pretreatment weights in the low, mid and high dose groups were 3.26, 9.42 and 7.79%, respectively.

TABLE 56: Body weight gains/losses of dogs (male + female) receiving IM buprenorphine + naloxone (3:2) for 28 days.

Buprenorphine Dose (mg/kg/d)	Body weight gain in dogs (kg)	
	Mean ± S.D.	% of Control
Control	0.04 ± 0.27 (8)	
0.5	-0.28 ± 0.41 (6)	0%
2.0	-0.85 ± 0.50 (6)*	0%
8.0	-0.68 ± 0.64 (8)†	0%

*P=0.001 compared with the control group (calculated from body weights by reviewer).
†t-test not valid due to unequal variance between test group and control.

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Food Consumption: Sponsor reported food consumption in terms of grams/kg of body weight per day and found significant decreases relative to the control (combined male and female) group in dogs receiving low, middle and high doses during week 1 (62%, 50% and 56% of control, respectively).

Ophthalmoscopy: Unremarkable

EKG: There was a tendency for all treated groups to have an elevation of heart rate when measured at week 4 compared with the reading of the same dogs on Day 1, thirty minutes after dosing, which appeared to be dose-related, with mean incremental increases of 15, 24 and 45 beats/minute (Table 57). Peripheral mean arterial pressure was also significantly increased relative to the vehicle controls at the two higher doses, but this did not appear to be dose-related.

TABLE 57: Significant changes in cardiovascular parameters after IM buprenorphine + naloxone (3:2) in dogs for 4 weeks (n=6-8/group).

Parameter	Sex	Control	0.5 mg/kg/d	2 mg/kg/day	8 mg/kg/day
Heart rate on Day 1	M+F	103±14	109±23	103±13	102±18
Heart rate, Week 4	M+F	110±17	124±25 N.S.	127±13‡	147±27‡
Mean arterial pressure	M+F	101±7	103±7 N.S.	116±6**	112±9*

N.S.= not significant; ‡p<0.01, compared to within-group values on Day 1. *P<0.05, **p<0.01, compared with the vehicle-control group.

Hematology:

TABLE 58: Significant changes in hematological parameters after IM buprenorphine + naloxone (3:2) in dogs for 28 days (n=6-8/group).

Parameter	Sex	Control	0.5 mg/kg/d	2 mg/kg/day	8 mg/kg/day
Reticulocytes, %RBCs	M+F	3.38±0.52	N.S.	5.17±1.94†	4.25±1.91†
Platelets (x10 ³ /µl)	M+F	265±21	N.S.	N.S.	311±39*

N.S.= not significant; *P=0.0109. † t-test not valid due to unequal variance.

Clinical chemistry:

TABLE 59: Significant changes in blood chemistry parameters after IM buprenorphine + naloxone (3:2) in dogs for 28 days (n=6-8/group).

Parameter	Sex	Control	0.5 mg/kg/d	2 mg/kg/day	8 mg/kg/day
ALT, U/l	M+F	20.6±4.0	N.S.	31.0±8.7†	27.0±6.4*
AST, U/l	M+F	12.8±1.4	N.S.	23.8±4.8†	21.9±5.7†
LDH, U/l	M+F	19.5±4.1	N.S.	N.S.	27.6±7.5*
BSP, % retention	M+F	8.91±1.11	7.07±0.73**	6.68±0.74**	7.32±2.33†

N.S.= not significant; *P<0.05, **p<0.01, ***p<0.001. † t-test not valid due to unequal variance.

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Urinalysis: Unremarkable

Gross pathology: Unremarkable

Organ weights: Within normal limits for all organs weighed.

Histopathology: Other than the expected injection site reactions described as "phlegmonous and necrotising inflammation", the most common finding was the presence of microliths in the collecting tubules of the kidneys, but the latter appeared unrelated to dose and was also observed in control animals.

KEY STUDY FINDINGS

Dogs received daily injections for 4 weeks containing 3:2 buprenorphine HCl plus naloxone HCl doses of 0.5, 2.0 and 8.0 mg/kg, IM (as buprenorphine). All treated groups had weight loss, which was significant for the mid dose. Food consumption was significantly decreased by all three doses during the first week. Incidence of emesis was dose-related, and the two higher doses caused sedation. There was a dose-related mean incremental increase in heart rate of 15, 24 and 45 beats/minute, compared with heart rates of the same groups at 30 minutes after treatment on Day 1. Mean arterial pressure was also significantly greater in the mid and high dose groups compared with the control group during Week 4. Dogs receiving 2.0 mg/kg/day showed a 53% increase in reticulocytes and those receiving 8 mg/kg/day showed a small, but significant, 17% increase in platelets. Significant elevations of ALT (but still within the normal range) and LDH occurred at the high dose, whereas the % BSP retention was significantly decreased at all doses. At the two higher doses, inflammatory reactions were observed histologically at the injection sites.

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OVERALL TOXICOLOGY SUMMARY

Lethality. The single-dose LD₅₀s for buprenorphine alone, naloxone alone and buprenorphine plus naloxone in various ratios were determined in mice and rats after the oral, IV and SC routes of administration. In general, the lethality of the combination did not occur at lower doses than lethality of the individual drugs and, in a few studies, the combination appeared to be less toxic than the individual constituents for causing death. Death was usually preceded by convulsions, and necropsy of these animals often showed lung congestion and pale patches or pallor of the liver.

Multiple-dose toxicity studies were conducted with rats and dogs by oral, IV, IM and SC routes. Deaths occurring in these studies are listed in the table below:

TABLE 60: Subacute toxicity studies of buprenorphine and naloxone in combination.

Species	Route	Duration	Bup:Nal Ratio	Doses, mg/kg/d	Deaths or Kills*
Rat	Oral	4 Weeks	1:1	0, 6 ² , 30 ³ and 150	1/10 high dose
Rat	Oral	4 Weeks	1:1	0, 10 ² , 80 ³ & 640	2/60 high dose
Rat	IV	2 Weeks	3:2	0, 0.4 ³ , 2 and 10	1/10 at mid dose 10/10 high dose*
Rat	IV	4 Weeks	3:2	0, 0.45 ¹ and 4.5 ³	None
Rat	IM	4 Weeks	3:2	0, 0.9 ¹ , 9 ³ and 90	2/60 high dose**
Rat	SC	2 Weeks	3:2	0, 0.4 ³ , 2 and 10	None
Rat	SC	4 Weeks	3:2	90 ¹	None
Dog	Oral	4 Weeks	1:1	0, 150 ³ and 250	1:2 at high dose
Dog	Oral	4 Weeks	1:1	0, 2.5 ² , 15 ³ and 90	None
Dog	IV	≤ 10 days	3:2	6.67 ¹ – 43.33	None (but toxic)
Dog	IV	4 Weeks	3:2	0.15 ³ and 1.5	None
Dog	IM	4 Weeks	3:2	0, 0.5 ² , 2 ³ and 8	None
Dog	SC	4 Weeks	3:2	0, 15 ³	None

¹Lowest dose had statistically significant adverse effects compared with controls.

²NOAEL dose. ³LOAEL dose.

*Euthanized prematurely. **Plus one high-dose rat during early recovery phase.

Except for possible adverse effects on food consumption and body weight gain (see below), the oral NOAEL doses for rats and dogs are 10 and 2.5 mg/kg/day for 4 weeks, respectively, or approximately 4 times and 3 times, respectively, the maximum recommended daily human dose of 0.4 mg/kg (24 mg/60 kg on a mg/m² basis).

Clinical signs. Clinical signs of acute toxicity in mice and rats included hunched posture, lethargy, piloerection, ptosis, ataxia and decreased respiration. Multi-dose

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toxicity in rats and dogs included injection site reactions, sedation, excessive salivation and convulsions in some animals at high dose in both species, localized hair loss in rats and vomiting in dogs.

Body weights. Male rats were more sensitive than females to the effects of the drug combination on body weight gain, such that significant decreases from the controls were observed following the lowest doses of buprenorphine tested: 10 mg/kg/day, PO, for 28 days, 0.27 mg/kg/day, IV, for 14 days, 0.5 mg/kg/day, IV, for 28 days, 0.9 mg/kg, IM, for 28 days and 0.27 mg/kg/day, SC, for 14 days. The lowest doses tested orally (1:1) or IV (3:2) in dogs, 2.5 or 0.15 mg/kg/day (as buprenorphine HCl), respectively, also significantly blocked weight gain over a 28-day period, as did IM (3:2) and SC (3:2) doses of 2.0 and 15 mg/kg/day, respectively (as buprenorphine HCl).

Food consumption. One of the conducting laboratories expressed food consumption by rats as grams per kg of body weight per day on a weekly basis. This method would tend to minimize (i.e., conceal) decreases in food consumption coinciding with weight loss, as long as the food consumption per unit of body weight remained relatively constant. On this basis, however, most drug-treated groups showed significant decreases in food consumption relative to body weight at least during the first week, relative to controls. In many studies, males were affected to a greater extent than females. Decreased food consumption also occurred in dogs receiving the 1:1 combination in a 4-week study. The oral NOAEL dose in dogs was 2.5 mg/kg/day, but significant decreases in food consumption were observed for at least one week during treatment with the lowest doses used by the IV (0.15 mg/kg/day), IM (0.5 mg/kg/day) and SC (15 mg/kg/day) routes.

Cardiovascular parameters. With treatment of dogs by the IM route for 4 weeks, doses of 0.5, 2 and 8 mg/kg/day were accompanied with dose-related mean incremental increases in heart rate of 15, 24 and 45 beats/minute, compared with heart rates measured in the same groups at 30 minutes after treatment on Day 1. Mean arterial pressure was also significantly greater in the mid and high dose groups compared with the control group during Week 4.

Ophthalmoscopy. None of the studies with rats or dogs reported any treatment-related effects.

Hematology. Dosing rats for 4 weeks with the combination caused a dose-related increase in reticulocytes in both sexes after oral administration. Increases of about 50%, >100% and 200% occurred after IV (4.5 mg/kg), SC (90 mg/kg) and IM (90 mg/kg) dosing, respectively. Reticulocytes also more than doubled in female dogs after oral administration (90 mg/kg/day) for 4 weeks, but no significant change occurred in

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males. Dogs (combined sexes) treated SC with 15 mg/kg/day in a 3:2 combination with naloxone for 4 weeks also showed a significant increase (37%) in reticulocytes, as well as an increase (32%) in platelets. Similar results were obtained in dogs treated IM with 2.0 mg/kg/day, which showed a 53% increase in reticulocytes, or with 8 mg/kg/day, which showed a significant 17% increase in platelets.

Clinical chemistry. Subacute dosing with the combination in the lowest doses tested increased blood glucose in female rats and dogs with oral administration (10 mg/kg and 2.5 mg/kg, respectively) and in both sexes after IV (0.5 mg/kg) and IM (0.9 mg/kg) administration (Tables 34, 42, 47 and 53). The resulting means, however, were still within the normal range. Another common finding in rats regardless of sex and route of administration is a slight, statistically significant, elevation of sodium, but within the normal range. This was not observed in dogs, and thus is not biologically significant.

Urinalysis. None of the studies with rats reported any treatment-related effects, except for an increase in volume production by rats receiving an oral dose of 640 mg/kg/day (as buprenorphine) from week 2 onwards.

Gross pathology. The major finding in rats and dogs was injection site reaction when the combination was administered parenterally (IV, IM and SC).

Organ weights. Dosing with the combination in the lowest doses tested decreased liver weights in rats after oral (10 mg/kg), IV (0.5 mg/kg) and IM (0.9 mg/kg) administration. High parenteral doses increased spleen and adrenal weights.

Histopathology. Drug-related histopathology was generally confined to injection site intolerance of the combination and included muscle induration and swelling, hemorrhage, degeneration, necrosis, inflammatory cell infiltration and fibroblast proliferation in rats and dogs.

REPRODUCTIVE TOXICITY

General Comments: All doses of the buprenorphine/naloxone (1:1) mixture cited in the following study refer only to the buprenorphine portion of the mixture.

Study Title: Influence of the Substance Mixture Buprenorphine-HCl, Lot No. 21 and Naloxone-HCl, Batch No. 231 (Ratio 1:1) — Called for Short 'BUP + NAL' — on the Pregnant Rat, the Embryo and the Foetus by Oral Administration

Study No.: 38278 Volume #: 17 Tab#: 38278

Conducting Laboratory. [

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Date of Study Initiation: September 18, 1984

GLP Compliance/QA Report: (X) Yes () No

Drug lot nos.: Buprenorphine, lot #21; naloxone, lot #231

Protocol reviewed by Division: () Yes (X) No

Methods:

Species/strain: Rat / Sprague-Dawley

Doses employed: Buprenorphine + Naloxone (1:1): 0 (vehicle), 10, 50 and 250 mg/kg/day (each drug)

Route of administration: Oral gavage (suspension in 0.8% hydroxypropyl-cellulose, 5 ml/kg)

Study design: Dosing occurred daily, from gestation days 6 through 15. Euthanasia and examination on gestation day 20. Rats were observed for external appearance, behavior and production of feces daily. Aborting rats were euthanized and any recovered fetuses were examined for abnormalities. Body weights were measured daily and used for daily dosing. Food consumption was determined daily by weighing the residue (rats housed individually). Water consumption was monitored.

No./sex/group: 24/females/dose

Parameters and endpoints evaluated:

Maternal: clinical signs, body weights, food consumption, histopathology of abnormal organs.

Fetal: numbers of fetuses and placentae; determination of sex and viability of fetuses; number and size of resorptions; number of implantations and location of fetuses in uterus; number of corpora lutea in ovaries; weight of fetuses and placentae; external examination for malformations; examination of the eyes; determination of head size; examination of internal organs, staining and examination of the skeletal system in half of the fetuses.

Calculations: % distribution of corpora lutea, implantations and fetuses; % malformation rate; % resorption rate; % fetal variation rate; % pre-implantation loss; and % post-implantation loss.

Statistical evaluations: For comparison of values with standard deviations, ANOVA and Student's t-test were used. For comparison of values without standard deviations, Fisher's exact test was employed.

Results:

Clinical signs: No changes in appearance, behavior or feces were noted in the low and middle dose groups. Some of the high-dose animals displayed dyspnea, hemochromodacryorrhea, hemorrhagic rhinitis and vaginal bleeding.

TABLE 61: Parameters related to oral treatment of pregnant rats with buprenorphine + naloxone (1:1)

Parameter Measured or Derived	Treatment (mg/kg/day, PO)			
	Vehicle	10	50	250
Mortality incidence during treatment	0/24	0/24	1/24	4/24
Mean body weights on gestation day 0 (g)	214	215	210	216
Mean body weights on gestation day 15 (g)	290	265**	256**	250**
Mean body weights on gestation day 20 (g)	350	294**	284**	283**
Corpora lutea (mean/dam)	13.6	13.8	14.1	14.0
Implantations (mean/dam)	13.6	13.8	14.1	14.0
Fetuses and placentae (mean/dam)	12.8	11.7	9.5	6.2**
Sex distribution of fetuses (% male)	54	49	50	54
Dams with no fetuses on day 20 (incidence)	0/24	3/24	6/23	10/21
Resorptions (mean/dam)	0.8	2.0	4.6*	7.8**
Resorption rate (%)	5.8	14.8	32.6	55.9
Early resorptions (% of all resorptions)	0	85.7	87.7	92.9
Dead fetuses (total/group)	0	0	0	0
Runts (total/group)	0	0	1	1
Malformations (total/group)	0	0	0	0
Fetuses with skeletal variations (total/group)	40	37	19	17
Skeletal variation (cf. Dawson) rate (%)	25.6	25.7	17.1	27.0
Fetuses with visceral variations (total/group)	9	12	17	9
Visceral variation (cf. Wilson) rate (%)	5.9	8.8	15.7	15.0
Macroscopic variations (total/group)	0	0	0	0
Pre-implantation loss (%)	0	0.3	0	0.4
Post-implantation loss (%)	5.8	14.8	32.6	55.9
Mean weight of all viable fetuses (grams)	3.79	3.64	3.71	3.74
No. of dams with gross lesions at necropsy	0	0	1	4

*P<0.05, **p<0.001, compared with vehicle-treated control group.

Mortality: The one mid-dose and four high-dose rats that died during treatment (between days 9 and 12 of gestation) all showed hemorrhagic gastric wall lesions.
Body weights: Weight loss in all three drug-treated groups was readily discernible by gestation day 8 and body weights remained below the controls to the time of termination, at which they were decreased by 16-19%.

Food consumption: Data for food consumption was presented only in a figure (Vol. 17, tab 38278, p. 56), which showed a marked decrease from about 90 g of food per kg of body weight on gestation day 6 to 40 or less g/kg on days 7 and 8, with

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gradual recovery to near (but still somewhat below) the control level of food consumption by day 13.

Toxicokinetics: No plasma samples were taken for measurement of drug concentrations.

Gross pathology: There was no report on the dams other than those dying prematurely.

KEY STUDY FINDINGS

Oral treatment of pregnant rats from day 6 through day 15 of gestation with 1:1 buprenorphine/naloxone doses of 10/10, 50/50 and 250/250 mg/kg/day showed dose-related mortality (0, 1 and 4), decreased food consumption and body weight loss in the dams and dose-related increases in post-implantation losses, including total litter resorptions. High-dose dams showed evidence of bleeding disorder, but prothrombin times and platelet counts were not assessed. For dams, the oral NOAEL was <10 mg/kg and the LOAEL was 10 mg/kg. For embryotoxicity, the oral NOAEL was 10 mg/kg (each drug). No teratogenicity was observed.

General Comments: All doses of the buprenorphine/naloxone (3:2) mixture cited in the following study refer only to the buprenorphine portion of the mixture.

Study Title: Influence of the Substance Mixture Buprenorphine-HCl, Lot No. 21 and Naloxone-HCl, Batch No. 231 (Ratio 3:2) — Called for Short 'BUP + NAL' — on the Pregnant Rat, the Embryo and the Foetus by Intramuscular Administration

Study No.: 38279 **Volume #:** 17 **Tab#:** 38279

Conducting Laboratory: []

Date of Study Initiation: September 4, 1984

GLP Compliance/QA Report: (X) Yes () No

Drug lot nos.: Buprenorphine, lot #21; naloxone, lot #231

Protocol reviewed by Division: () Yes (X) No

Methods:

Species/strain: Rat / Sprague-Dawley

Doses employed: Buprenorphine + Naloxone (3:2): 0 (vehicle), 0.3, 3.0 and 30 mg/kg/day

Route of administration: Intramuscular (suspension in 5% glucose, 5 ml/kg)

Study design: Dosing occurred daily, from gestation days 6 through 15. Euthanasia and examination were done on gestation day 20. Rats were observed for external appearance, behavior and production of feces daily. Aborting rats were to be euthanized and any recovered fetuses were examined for abnormalities. Body weights were measured daily and used for daily dosing. Food consumption was

determined daily by weighing the residue (rats housed individually). Water consumption was monitored.

No./sex/group: 24/females/dose

Parameters and endpoints evaluated:

Maternal: clinical signs, body weights, food consumption, histopathology of abnormal organs.

Fetal: numbers of fetuses and placentae; determination of sex and viability of fetuses; number and size of resorptions; number of implantations and location of fetuses in uterus; number of corpora lutea in ovaries; weight of fetuses and placentae; external examination for malformations; examination of the eyes; determination of head size; examination of internal organs, staining and examination of the skeletal system in half of the fetuses.

Calculations: % distribution of corpora lutea, implantations and fetuses; % malformation rate; % resorption rate; % fetal variation rate; % pre-implantation loss; and % post-implantation loss.

Statistical evaluations: For comparison of values with standard deviations, ANOVA and Student's t-test were used. For comparison of values without standard deviations, the chi² test was employed.

Results:

Clinical signs: No changes in appearance, behavior, feces or injection site reactions were noted in the low and middle dose groups, except for the mid-dose rat that died, which showed hemorrhagic rhinitis and dyspnea 3 days before death. The high-dose rats had ataxia lasting >24 hours after the first injection and for shorter periods after subsequent injections. They also had swollen thighs during the latter half of treatment. Some of the high-dose animals displayed dyspnea sporadically.

Mortality: The one mid-dose and two high-dose rats that died during treatment (between days 11 and 15 of gestation) all had stomachs and esophagi filled tightly with bedding material. The mid-dose rat had an edematous lung lobe, a small spleen and hemorrhagic rhinitis, one high-dose rat had a small spleen and hemorrhagic rhinitis, and both high-dose rats that died had multiple stomach ulcers and hemorrhagic foci.

Body weights: Decreased body weight gain in all three drug-treated groups was readily discernible by gestation day 9 and body weights remained below the controls to the time of termination, at which they were decreased from the control mean in a dose-related manner by 11, 15 and 29%.

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TABLE 62: Parameters related to intramuscular treatment of pregnant rats with buprenorphine + naloxone (3:2)

Parameter Measured or Derived	Treatment (mg/kg/day, IM)			
	Vehicle	0.3	3.0	30
Mortality incidence during treatment	0/24	0/24	1/24	2/24
Mean body weights on gestation day 0 (g)	211	212	208	209
Mean body weights on gestation day 15 (g)	288	269*	262*	219*
Mean body weights on gestation day 20 (g)	342	305*	289*	243*
Corpora lutea (mean/dam)	13.3	13.1	12.8	14.0
Implantations (mean/dam)	13.2	13.1	12.8	13.7
Fetuses and placentae (mean/dam)	12.9	12.4	12.0	3.3*
Sex distribution of fetuses (% male)	53	55	55	60
Dams with no fetuses on day 20 (incidence)	0/24	1/24	1/23	16/22
Resorptions (mean/dam)	0.3	0.8	0.9	10.4*
Resorption rate (%)	2.5	5.7	6.8	76.1*
Early resorptions (% of total resorptions)	12.5	94.4	65.0	97.4
Dead fetuses (total/group)	0	0	0	0
Runts (total/group)	0	0	3	0
Malformations (total/group)	0	1	0	0
Fetuses with skeletal variations (total/group)	45	43	36	18
Skeletal variation (cf. Dawson) rate (%)	28.1	28.9	25.5	48.6
Fetuses with visceral variations (total/group)	18	12	17	4
Visceral variation (cf. Wilson) rate (%)	12.1	8.1	12.7	11.4
Macroscopic variations (total/group)	0	0	0	0
Pre-implantation loss (%)	0.3	0	0	2.0
Post-implantation loss (%)	2.5	5.7	6.8	76.1*
Mean weight of all viable fetuses (grams)	3.71	3.60	3.82	3.72
No. of dams with gross lesions at necropsy	0	0	1	2

*P<0.01, compared with vehicle control.

Food consumption: Data for food consumption was presented only in a figure (Vol. 17, tab 38279, p. 59), which showed a dose-related decrease from about 85 g of food per kg of body weight on gestation day 6 to about 25 g/kg on days 7 and 8 for the high-dose group, with gradual recovery to near (but still somewhat below) the control level of food consumption by day 16.

Toxicokinetics: No plasma samples were taken for drug analysis.

Gross pathology: There was no report on the dams other than those dying prematurely.

KEY STUDY FINDINGS

Intramuscular treatment of pregnant rats from day 6 through day 15 of gestation with 3:2 buprenorphine/naloxone doses of 0.3/0.2, 3/2 and 30/20 mg/kg/day showed a dose-related mortality (0, 1 and 2), a decreased food consumption and body weight loss in the dams and dose-related increases in post-implantation losses, including total litter resorptions. High-dose rats showed ataxia and occasional dyspnea. For dams, the IM NOAEL was <0.3 mg/kg buprenorphine + 0.2 mg/kg naloxone, and the LOAEL was 0.3 mg/kg buprenorphine + 0.2 mg/kg naloxone. For embryotoxicity, the IM NOAEL was 3 mg/kg buprenorphine + 2 mg/kg naloxone. No treatment-related teratogenicity was reported.

General Comments: All doses of the buprenorphine/naloxone (1:1) mixture cited in the following study refer only to the buprenorphine portion of the mixture.

Study Title: Influence of the Substance Mixture Buprenorphine-HCl, Lot No. 21 and Naloxone-HCl, Batch No. 231 (Ratio 1:1) — Called for Short 'BUP + NAL' — on the Pregnant Rabbit, the Embryo and the Foetus by Oral Administration

Study No.: 38282 **Volume #:** 17 **Tab#:** 38282

Conducting Laboratory: []

Date of Study Initiation: September 10, 1984

GLP Compliance/QA Report: (X) Yes () No

Drug lot nos.: Buprenorphine, lot #21; naloxone, lot #231

Protocol reviewed by Division: () Yes (X) No

Methods:

Species/strain: Rabbit/White Russian

Doses employed: Buprenorphine + Naloxone (1:1): 0 (vehicle), 0.4, 4.0 and 40 mg/kg/day (each drug)

Route: Oral gavage (suspension in 0.8% hydroxypropyl-cellulose, 10 ml/kg).

Study design: Dosing occurred daily, from gestation days 6 through 18. Euthanasia and examination occurred on gestation day 29. Rabbits were observed for external appearance, behavior and production of feces daily. Aborting rabbits were euthanized and any recovered fetuses were examined for abnormalities. Body weights were measured daily and used for daily dosing. Food consumption was determined daily by weighing the residue. Water consumption was monitored.

No./sex/group: 12 females/dose

Parameters and endpoints evaluated:

Maternal: clinical signs, body weights, food consumption, histopathology of abnormal organs.

Fetal: numbers of fetuses and placentae; determination of sex and viability of fetuses; number and size of resorptions; number of implantations and location

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of fetuses in uterus; number of corpora lutea in ovaries; weight of fetuses and placentae; external examination for malformations; examination of the eyes; determination of head size; viability of the fetuses for 0-7 hours and for 7-24 hours examination of internal organs, staining and examination of the skeleton. Calculations: % distribution of corpora lutea, implantations and fetuses; % malformation rate; % resorption rate; % fetal variation rate; % pre-implantation loss; and % post-implantation loss.

Statistical evaluations: For comparison of values with standard deviations, ANOVA and Student's t-test were used. For comparison of values without standard deviations, Fisher's exact test was employed.

Results:

TABLE 63: Parameters related to oral treatment of pregnant rabbits with buprenorphine + naloxone (1:1)

Parameter Measured or Derived	Treatment (mg/kg/day, PO)			
	Vehicle	0.4	4.0	40
Mortality incidence during treatment	0/12	0/12	0/12	0/12
Mean body weights on gestation day 0 (kg)	2.41	2.47	2.33	2.43
Mean body weights on gestation day 18 (kg)	2.59	2.65	2.57	2.62
Mean body weights on gestation day 29 (kg)	2.71	2.78	2.72	2.76
Corpora lutea (mean/does)	8.6	7.3	8.3	8.7
Implantations (mean/does)	6.8	6.7	7.0	7.5
Fetuses and placentae (mean/does)	6.6	5.9	6.7	7.1
Sex distribution of fetuses (% male)	51	48	50	48
Does with no fetuses on day 29 (incidence)	0	0	0	2
Resorptions (mean/does)	0.3	0.8	0.3	0.5
Resorption rate (%)	3.7	11.3	4.8	6.7
Early resorptions (% of total resorptions)	66.7	100	100	60.0
Dead fetuses, 0-6 hours (total/group)	0	0	0	1
Dead fetuses, 7-24 hours (total/group)	5	2	1	2
Runts (total/group)	1	1	0	1
Malformations (total/group)	0	0	0	0
Fetuses with skeletal variations (total/group)	22	17	30	20
Skeletal variation (cf. Dawson) rate (%)	27.8	23.9	37.5	28.2
Macroscopic variations (total/group)	0	0	0	0
Pre-implantation loss (%)	20.4	9.1	15.2	13.8
Post-implantation loss (%)	9.8	13.8	6.0	9.3
Mean weight of all viable fetuses (grams)	41.0	41.7	44.3	41.7
No. of does with gross lesions at necropsy	0	0	0	0

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Clinical signs: No changes in appearance, behavior or feces were noted in the low and middle dose groups.

Mortality: None of the does died prematurely. The incidence of dead fetuses (i.e., viability through the first 24 hours postpartum) did not appear to be related to treatment. However, two of the high dose rabbits underwent spontaneous abortions (gestation days 21 and 28).

Body weight: No significant differences from the control group were observed on days 6, 18 and 29 of gestation (the latter two gestation days shown in Table 63).

Food consumption: Data for food consumption were presented only in a figure (Vol. 17, tab #38282, p. 31), which showed a slight decrease from controls on days 8-14 in the low dose group and a somewhat greater decrease from the control level in the high-dose group on days 7-10. These decreases were transient.

Toxicokinetics: No plasma samples were taken for measurement of drug concentrations.

Gross pathology: Macroscopic inspection during necropsy did not reveal any treatment-related pathological changes.

KEY STUDY FINDINGS

Oral treatment of pregnant rabbits from day 6 through day 18 of gestation with 1:1 buprenorphine/naloxone doses of 0.4/0.4, 4.0/4.0 and 40/40 mg/kg/day showed no signs of toxicity, other than a transient decrease in food consumption, and two of the high-dose rabbits had spontaneous abortions. The oral NOEL for maternal toxicity was 4.0 mg/kg/day and the NOEL for embryotoxicity or fetotoxicity was 40 mg/kg/day. No teratogenicity was observed at doses up to 40 mg/kg/day (each drug).

General Comments: All doses of the buprenorphine/naloxone (3:2) mixture cited in the following study refer only to the buprenorphine portion of the mixture.

Study Title: Influence of the Substance Mixture Buprenorphine-HCl, Lot No. 21 and Naloxone-HCl, Analysis 231 (Ratio 3:2) — Called for Short 'BUP + NAL' — on the Pregnant Rabbit, the Embryo and the Foetus by Intramuscular Administration

Study No.: 38283 Volume #: 17 Tab#: 38283

Conducting Laboratory: []

Date of Study Initiation: August 27, 1984

GLP Compliance/QA Report: (X) Yes () No

Drug lot nos.: Buprenorphine, lot #21; naloxone, lot #231

Protocol reviewed by Division: () Yes (X) No

Methods:

Species/strain: Rabbit / White Russian

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Doses employed: Buprenorphine + Naloxone (3:2): 0 (vehicle), 0.3, 3.0 and 30 mg/kg/day

Route of administration: Intramuscular (suspension in 5% glucose, 2.5 ml/kg)

Study design: Dosing occurred daily, from gestation days 6 through 18. Euthanasia and examination were done on gestation day 29. Rabbits were observed for external appearance, behavior and production of feces daily. Aborting rabbits were to be euthanized and any recovered fetuses were examined for abnormalities.

Body weights were measured daily and used for daily dosing. Food consumption was determined daily by weighing the residue. Water consumption was monitored.

No./sex/group: 12 females/dose

Parameters and endpoints evaluated:

Maternal: clinical signs, body weights, food consumption, histopathology of abnormal organs.

Fetal: numbers of fetuses and placentae; determination of sex and viability of fetuses; number and size of resorptions; number of implantations and location of fetuses in uterus; number of corpora lutea in ovaries; weight of fetuses and placentae; external examination for malformations; examination of the eyes; determination of head size; examination of internal organs, staining and examination of the skeletal system.

Calculations: % distribution of corpora lutea, implantations and fetuses; % malformation rate; % resorption rate; % fetal variation rate; % pre-implantation loss; and % post-implantation loss.

Statistical evaluations: For comparison of values with standard deviations, ANOVA and Student's t-test were used. For comparison of values without standard deviations, Fisher's exact test was employed.

Results:

Clinical signs: No changes in appearance, behavior or injection site reactions were noted in the low-dose group. The middle and high dose groups displayed local intolerance to the injections, including swelling of the thighs up to 4 times the diameter of the controls and lacerated discharging wounds in some of the high dose rabbits, which began to heal after the injections stopped.

Mortality: Two high-dose rabbits had tonic-clonic seizures a few minutes after drug injection on day 17 of gestation and died. Necropsy did not reveal any substance-related pathological changes.

Body weights: Decreased body weight gain was exhibited in the low-dose group from days 7 through 14. The mid-dose group remained similar to the controls, whereas the high-dose group had body weights exceeding the controls from day 8 through day 20. However, none of these changes was marked enough for significant differences between the control and drug-treated groups on gestation days 6, 18 and 29 (the only days during treatment for which individual body weights were reported). The study laboratory attributed the lack of correlation between

body weight and food consumption changes to weight gain caused by marked swelling at the injection site in the middle and high dose rabbits.

TABLE 64: Parameters related to intramuscular treatment of pregnant rabbits with buprenorphine + naloxone (3:2)

Parameter Measured or Derived	Treatment (mg/kg/day, IM)			
	Vehicle	0.3	3.0	30
Mortality incidence during treatment	0/12	0/12	0/12	2/12
Mean body weights on gestation day 0 (kg)	2.25	2.21	2.24	2.30
Mean body weights on gestation day 18 (kg)	2.45	2.41	2.52	2.65
Mean body weights on gestation day 29 (kg)	2.53	2.47	2.49	2.48
Corpora lutea (mean/doe)	8.2	8.6	7.8	7.7
Implantations (mean/doe)	6.8	6.8	6.5	6.5
Fetuses and placentae (mean/doe)	5.9	6.1	5.4	5.4
Sex distribution of fetuses (% male)	51	52	42	56
Does with no fetuses on day 29 (incidence)	0/12	0/12	0/12	1/10
Resorptions (mean/doe)	0.8	0.7	1.1	1.1
Resorption rate (%)	12.3	9.9	16.7	16.9
Early resorptions (% of total resorptions)	90	87.5	84.6	100
Dead fetuses, 0-6 hours (total/group)	0	3	1	2
Dead fetuses, 7-24 hours (total/group)	1	0	4	0
Runts (total/group)	1	2	5	3
Malformations (total/group)	0	1	2	0
Malformation rate (%)	0	1.4	3.1	0
Litters w/ skeletal variations (incidence/grp.)	11/12	11/12	9/12	8/9
Fetuses with skeletal variations (total/group)	26	24	16	29
Skeletal variation (cf. Dawson) rate (%)	36.6	32.9	24.6	53.7*
Macroscopic variations (total/group)	0	0	0	0
Pre-implantation loss (%)	17.3	21.4	17.0	15.9
Post-implantation loss (%)	13.6	13.6	23.1	20.0
Mean weight of all viable fetuses (grams)	40.6	42.3	43.1	40.1
No. of does with gross lesions at necropsy	N.R.	N.R.	N.R.	N.R.

*Significantly different from control (Fisher's exact test). N.R.=Not reported.

Food consumption: Data for food consumption was presented only in a figure (Vol. 17, tab 38283, p. 36), which showed a transient, inverse dose-related decrease from about 50-60 g of food per kg of body weight on gestation day 6 to a minimum of >20 g/kg on day 7 for the low-dose group, with recovery to near (but still somewhat below) the control level of food consumption by day 8.

Toxicokinetics: No samples were taken for measurement of drug concentrations. Gross pathology: There was no report on the individual does. Local tolerance reactions were not reported for individual does. At termination, two of the mid-dose does and five of the high-dose does showed fibrous indurations in the musculature up to 10 mm in diameter, but no necrosis was observed. An edematous, diffuse indurated femoral musculature was observed in the two does that died prematurely. All 5 dead fetuses and 4/5 of the runts came from the same mid-dose doe. Malformations were acephalus in one runt from the low-dose group and two cases of omphalocele in two runts from the same litter in the mid-dose group. Some of the historical control data for white Russian rabbits, including thalidomide as a positive control, from the testing laboratory are shown in the table below:

TABLE 65: Historical control data from segment II studies in white Russian rabbits

Parameter Measured or Derived	Control rabbits	Normal test rabbits	Thalidomide, 180 mg/kg/d, PO
Number of pregnant rabbits/fetuses studied	180/1404	532/4202	24/125
Abortion rate (%)	0.07	0.09	12.5
Corpora lutea (mean/doe)	9.4	9.5	9.5
Living fetuses (mean/doe)	7.80	7.89	5.2
Dead fetuses (mean/doe)	0.25	0.28	1.6
Resorptions (mean/doe)	0.24	0.77	3.1
Malformation rate (%)	0.21	0.30	10.4
Pre-implantation loss (%)	12.5	16.2	11.9
Post-implantation loss (%)	8.2	12.1	43.7
Mean weight of all viable fetuses	35.4 g	35.1 g	-8.2%

KEY STUDY FINDINGS

Intramuscular treatment of pregnant rabbits from day 6 through day 18 of gestation with 3:2 buprenorphine/naloxone doses of 0.3/0.2, 3/2 and 30/20 mg/kg/day showed moderate to severe injection site reactions, transiently decreased food consumption and body weight changes in the dams that were inversely related to dose because of injection site swelling. Two high-dose does died of seizures. One low-dose and two mid-dose fetal malformations were observed, but none were found at the high-dose. The % malformation rates for these observations exceed the historical controls for normal test rabbits by 5- to 10-fold. In addition, a statistically significant increase in the skeletal variation rate was observed at the high dose (53.7% vs. 36.6% in the controls). The maternal NOAEL for local tolerance was 0.3 mg/kg, and 30 mg/kg was lethal. An NOAEL for developmental toxicity was not clearly demonstrated because of a malformation at the lowest dose tested.

Summary and Evaluation:

Teratogenicity studies were carried out in rats and rabbits with the combinations of buprenorphine and naloxone at the buprenorphine HCl doses indicated in Table 66.

TABLE 66: Reproductive toxicity studies of buprenorphine + naloxone in combination.

Species	Route	Duration	Bup:Nal Ratio	Dose, mg/kg/d*	Dose, mg/m ² /d*
Rat	Oral	GD 6-15	1:1	0, 10, 50 and 250	60, 300 and 1500
Rat	IM	GD 6-15	3:2	0, 0.3, 3 and 30	1.8, 18 and 180
Rabbit	Oral	GD 6-18	1:1	0, 0.4, 4 and 40	4.8, 48 and 480
Rabbit	IM	GD 6-18	3:2	0, 0.3, 3 and 30	3.6, 36 and 360

*Dose expressed as buprenorphine HCl content. GD = Inclusive gestational days of dosing.

Pregnant rats receiving combination treatment showed dose-related mortality (0, 1 and 4 after oral; 0, 1 and 2 after IM), decreased food consumption and body weight loss, as well as dose-related increases in post-implantation losses, including total litter resorptions. High-dose dams showed ataxia (IM), occasional dyspnea, and evidence of bleeding disorder, but prothrombin times and platelet counts were not assessed. One IM low-dose fetus had hydrocephalus. No teratogenicity was reported by either route.

Pregnant rabbits receiving oral treatment showed no signs of toxicity, other than a transient decrease in food consumption, but two of the high-dose rabbits had spontaneous abortions. The oral NOEL for maternal toxicity was 4.0 mg/kg/day and the NOEL for embryotoxicity or fetotoxicity was 40 mg/kg/day. No teratogenicity was observed. Pregnant rabbits receiving intramuscular treatment with the combination showed moderate to severe injection site reactions, transiently decreased food consumption and body weight changes in the dams that were inversely related to dose because of injection site swelling. Two high-dose does died of seizures. One low-dose and two mid-dose fetal malformations (acephalus, 2 omphalocele, respectively) were observed, but no malformations were observed at the high-dose. A statistically significant increase in skeletal variations occurred at the high dose (53.7% rate vs. 36.6% rate in the controls). The maternal NOAEL for local tolerance was 0.3 mg/kg, and 30 mg/kg was lethal. An NOAEL for developmental toxicity was not clearly demonstrated, because of a malformation at the low dose.

Labeling Recommendations: (Please see pp. 116-117)

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GENETIC TOXICOLOGY:

Study Title: Study of buprenorphine / naloxone (4:1 mixture) in bacterial mutations assays using *Salmonella typhimurium* and *Escherichia Coli*.

Study No.: Protocol #YV4254

Study Type: In vitro (Ames test)

Volume #18, Tab #: RC980112

Conducting Laboratory: [

Date of Study Initiation/completion: July 17 through September 24, 1998.

GLP Compliance/QA Reports: (X) Yes () No

Drug Lot Number: Buprenorphine HCl, V06101 (pure); naloxone HCl, V04434 (pure).

Study Endpoint: reversion of bacteria to amino acid independence for colony growth

Methodology:

Species/Strains: *S. typhimurium* strains TA1535, TA1537, TA98 and TA100; *E. coli* strains WP2P and WP2PuvrA

Dose Selection Criteria:

Basis of dose selection: Toxicity and precipitation in range finding studies.

Range finding studies: Doses of 100-5000 µg/plate were tested.

Test Agent Stability: Not reported by contract lab.

Metabolic Activation System: S9 fraction of liver from Sprague-Dawley rats pretreated 3 days with phenobarbital (80 mg/kg) and β-naphthoflavone (100 mg/kg) in corn oil.

Controls:

Vehicle: DMSO, 100 µl (5 plates/strain)

Positive Controls: In the absence of S9, daunomycin HCl for TA98, sodium azide for TA100 and TA1535, acridine mutagen ICR191 for TA1537, mitomycin C for WP2P and N-ethyl-N'-nitro-N-nitrosoguanidine were used as positive controls. 2-Aminoanthracene served as positive control for all incubations done in the presence of S9. All compounds were dissolved in DMSO except mitomycin C and sodium azide, which were dissolved in water.

Comments:

Exposure Conditions:

Incubation and sampling times: The incubation period for each experiment was 3 days at 37°C.

Doses used in definitive study: 50-2500 µg of buprenorphine + naloxone, 4:1 ratio as the hydrochloride salts (4.5:1 as the bases), ±S9.

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Study design: Pre-incubation protocol used for experiments that included S9 involved placing each compound/strain group of bijoux on an orbital shaker (~140 rpm) for 60 minutes at 37°C, before adding the top agar.

Analysis:

No. slides/plates/replicates/animals analyzed: Five plates for control solvent (DMSO); three plates/concentration of test drug; two plates/concentration of positive control substance.

Counting method: Automated counter adjusted to optimize counting of mutants. Cytotoxic endpoints: Not described.

Genetic toxicity endpoints/results: Test is positive when the number of revertant colonies is >3x (for TA1535 and TA1537) or >2x (all other strains) background control values.

Statistical methods: Although carried out automatically by ARTEMIS computer programs (Student's t-test), the outcome of statistical analysis was not used in the evaluation of the data.

Criteria for Positive Results: Endpoints described above are reached under conditions where the solvent control data are acceptable and the positive control data show unequivocal positive responses.

RESULTS:

Study Validity: Validity was determined by positive responses from the positive control substances tested in parallel incubations. In the definitive study, the positive control used with the TA1537 strain of *S. typhimurium* failed to test positive in the absence of S9, which necessitated a repeat assay of this strain. Study Outcome: In the initial dose range finding study using six concentrations over the range of 100-5000 µg/plate, the test compound precipitated at doses of 2500-5000 µg/plate, and the background lawn was sparse or absent at doses of 1000-5000 µg/plate. Consequently, the second (definitive) study was conducted using six concentrations over the range of 50-2500 µg/plate, as well as a preincubation step for the plates containing S9. In the second study, the test compound precipitated at 2500 µg/plate and the background lawn was sparse at 1000 µg/plate in the absence of S9. In the presence of S9, the test compound precipitated at 1000-2500 µg/plate and the background lawn was sparse at 500 µg/plate. Also, in the second (definitive) study, the positive control used with the TA1537 strain of *S. typhimurium* in the absence of S9 failed to test positive. The summary data are presented in the following table:

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Species/Strains: *S. typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100.

Dose Selection Criteria:

Basis of dose selection: Toxicity in range-finder experiment with TA100 strain.

Range finding studies: Doses of 8-5000 µg/plate were tested.

Test Agent Stability: Not reported by contract lab.

Metabolic Activation System: S9 fraction of liver from male Wistar rats pretreated 5 days earlier with Aroclor-1254 (500 mg/kg) in corn oil (200 mg/ml).

Controls:

Vehicle: DMSO

Positive Controls: In the absence of S9, 2-nitrofluorene (50 µg/plate) for TA98 and TA1538, sodium azide (2 µg/plate) for TA100 and TA1535, 9-amino-acridine (50 µg/plate) for TA1537, mitomycin C for WP2P and N-ethyl-N'-nitro-N-nitrosoguanidine were used as positive controls. 2-Aminoanthracene (5 µg/plate, except for 10 µg/plate with TA1537) served as positive control for all incubations done in presence of S9.

Exposure Conditions:

Incubation and sampling times: The incubation period for each experiment was 2 days at 37°C (in the dark).

Doses used in definitive study: In the absence of S9, 4-2500 µg of naloxone hydrochloride; in the presence of S9, 0.32-200 µg of naloxone hydrochloride. Each dose was tested in triplicate.

Study design: The following components were added sequentially to tubes containing 2.5 ml of molten soft agar at 46°C: 0.1 ml overnight bacterial culture; 0.1 ml dilution of test agent or DMSO; 1.0 ml of appropriate cofactor solution; and 0.1 ml of S9 fraction for plates requiring activation. The mixture was then poured on to minimal Davis agar plates.

Analysis:

No. slides/plates/replicates/animals analyzed: Three plates for control solvent (DMSO), each concentration of naloxone and each concentration of positive control substance were run per experiment. Two experiments were conducted.

Counting method: Automated electronic colony counter

Cytotoxic endpoints: Background lawn growth was inspected for toxicity signs.

Genetic toxicity endpoints/results: Test is positive when the number of revertant colonies exceeded the normal historical range, were significantly different from negative controls, and a significant dose-response relationship is established.

Statistical methods: If the mean of 6 plates (3 from each experiment) fell outside the normal range (i.e., the laboratory's historical control mean with 95% confidence limits), plate counts were compared with the controls using

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Student's t-test. Linear regression analysis was performed to establish whether a significant dose-response relationship existed.

Other:

Criteria for Positive Results: Endpoints described above are reached under conditions where the solvent control data are acceptable and the positive control data show unequivocal positive responses above the historical negative control (normal) range.

RESULTS:

Study Validity: All mean negative control counts were within the normal range, and all mean positive control counts exceeded the normal range.

Study Outcome: In the initial dose range finding study using six concentrations over the range of 8-5000 µg/plate, the background lawn for TA100 was sparse or absent at doses of 1000 and 5000 µg/plate in the presence of S9 and 5000 µg/plate in the absence of S9.

TABLE 68: Definitive mutagenicity study of naloxone HCl in five *S. typhimurium* strains (from NDA, Vol. 18, tab 105983, pp. 16-19)

Test strain	S9	Mean revertants/plate from both expts. (µg/plate)						Positive Control	Normal Mean
		DMSO	4/0.32	20/1.6	100/8	500/40	2500/200		
TA98	-	8.5	8.5	8.3	7.0	8.0	6.5	378.3	23
	+	28.2	22.2	21.5	22.8	21.0	19.2	351.0	36
TA100	-	88.7	96.0	84.8	92.0	97.7	86.5	349.2	113
	+	112.2	103.5	109.0	121.7	132.7	140.3	579.5	132
TA1535	-	18.8	17.7	14.5	14.8	12.7	11.2	111.0	16
	+	17.0	13.6	13.5	13.7	14.0	39.0	145.0	16
TA1537	-	5.2	4.0	5.7	5.3	3.7	4.2	269.6	8
	+	6.5	5.8	6.8	4.5	5.2	5.0	169.0	8
TA1538	-	6.5	7.5	7.2	5.7	7.3	4.4	336.5	12
	+	18.8	18.2	19.8	18.5	18.3	18.8	241.8	21

At the concentrations tested, naloxone was negative for mutagenicity in the absence or presence of S9 for strains TA98, TA1537 and TA1538. A dose-related increase in number of revertants was observed with TA100 in the presence of S9, but all counts were within the 95% confidence limits of the testing laboratory's historical control range (85 – 178 colonies/plate). The high dose of TA1535 (200 µg/plate) in the presence of S9 showed a statistically significant increase in revertants beyond the historical control range (0 – 32 colonies/plate), but showed no dose-response relationship. All of the positive control compounds produced statistically significant increases in revertant colonies compared to the solvent controls and produced numbers of colonies beyond the laboratory's historical control range.

NDA 20-733 (Suboxone®)

SUMMARY:

When naloxone HCl alone was tested for mutagenicity in 5 strains of *S. typhimurium* in the dose range of 4-2500 µg/plate in the absence of S9 and 0.32-200 µg/plate in the presence of S9, strains TA98, TA1537 and TA1538 were negative. TA100 showed a dose-related increase in number of revertants in the presence of S9, but all counts were within the historical control range. This is considered negative. The high dose of TA1535 in the presence of S9 gave a statistically significant increase in revertants beyond the historical control range, but showed no dose-response relationship. It was concluded by the sponsor that naloxone has weak mutagenic activity in the base-pair substitution strains in the presence of metabolic activation.

Study Title: Study of buprenorphine / naloxone (4:1 mixture) in an *in vitro* cytogenetic assay in human lymphocytes.

Study No.: Protocol #SV0959; Report No. — P/6052

Study Type: In vitro genotoxicity assay with cultured human lymphocytes.

Volume #18, Tab #: RC980113

Conducting Laboratory: [

Date of Study Initiation/completion: July 17 through September 8, 1998

GLP Compliance/QA Reports: (X) Yes () No

Drug Lot Number: Buprenorphine HCl, V06101 (— pure); naloxone HCl, V04434 (— pure).

Study Endpoint: Chemically induced changes in metaphase chromosomes of human lymphocytes – increased incidence above that in solvent controls.

Methodology:

Species/Strains: Human (two blood donors)

Dose Selection Criteria:

Basis of dose selection: Toxicity at upper end of tested range.

Range finding studies: None.

Test Agent Stability: All dosing solutions in DMSO were within 3% of target by HPLC analysis.

Metabolic Activation System: S9 fraction of liver from Sprague-Dawley rats pretreated 3 days with phenobarbital (80 mg/kg) and β-naphthoflavone (100 mg/kg) in corn oil.

Controls:

Vehicle: DMSO, 100 µl/10 ml of culture

Positive Controls: Mitomycin C (0.75 µg/ml) in the absence of S9 and cyclophosphamide (50 µg/ml) were used as positive controls.

RESULTS:

Study Validity: Both of the positive controls, mitomycin C and cyclophosphamide, came out positive in these studies, and the highest concentrations of 4.5:1 buprenorphine:naloxone used produced an adequate degree of cytotoxicity.

Study Outcome: Reductions in mean mitotic activity, compared with solvent controls, occurred in cultures from Donor #1 in the absence of S9 (-35%) and from Donor #2 in the absence (-57%) or presence (-51%) of S9 at the highest concentrations of test drug selected for chromosomal aberration analysis.

Except for cultures from Donor #2 in the presence of S9, a concentration of 100 µg/ml caused excessive cytotoxicity resulting in the absence of sufficient metaphases for analysis.

TABLE 69: Chromosomal aberration analysis in human lymphocytes from two donors. (from NDA, Vol. 18, tab RC980113, pp. 25-26)

Drug/ Conc., µg/ml	Mean % Mitotic Index				Mean % aberrant cells excluding gaps			
	Donor #1		Donor #2		Donor #1		Donor #2	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Vehicle ¹	9.3	7.6	10.1	6.3	0.00	0.00	0.50	0.00
5			11.1				1.00	
10	10.8	9.9		6.0	0.50	0.50		0.00
20			8.9				1.00	
40	9.7	9.5	4.3		0.00	1.00	0.50	
45				5.4				0.00
80	6.0	8.1			0.00	1.00		
100				3.1				0.50
Mit. C ²	6.1		4.8		32.00**		28.00**	
Cyclo. ³		6.2		4.5		24.00**		24.00**

¹DMSO, 10 µl/ml; ²Mitomycin C, 0.75 µg/ml; ³Cyclophosphamide, 50 µg/ml.

**Significantly different from vehicle, p<0.01 (Fisher's Exact Test, one-sided)

SUMMARY:

In the human lymphocyte test using two donors, the buprenorphine + naloxone (4.5:1) combination was negative over the dose range of 5-80 µg/ml in the absence of S9 and negative at 10-100 µg/ml in the presence of S9, whereas the positive controls, mitomycin C and cyclophosphamide, caused significant aberrations. Thus, the buprenorphine-naloxone combination is not clastogenic in this test.

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NDA 20-733 (Suboxone®)

Study Title: Study to evaluate the chromosome damaging potential of a test sample of naloxone hydrochloride by its effect on cultured human lymphocytes using an in vitro cytogenetics assay.

Study No.: RCP 4/HLC/AR/KF6

Study Type: In vitro genotoxicity assay with cultured human lymphocytes.

Volume #18, Tab #: 38746

Conducting Laboratory: _____

Date of Study Initiation/completion: September 3, through October 9, 1984.

GLP Compliance/QA Reports: (X) Yes () No

Drug Lot Number: Analysis batch #231

Study Endpoint: Chemically induced changes in metaphase chromosomes of human lymphocytes – increased incidence above that in solvent controls.

Methodology:

Species/Strains: Human (one male and one female blood donor)

Dose Selection Criteria:

Basis of dose selection: Toxicity in preliminary range finding study.

Range finding studies: Concentrations of 10-5000 µg/ml were tested.

Test Agent Stability: Not reported by contract lab.

Metabolic Activation System: S9 fraction of liver from male Wistar rats pretreated 5 days earlier with Aroclor-1254 (500 mg/kg) in corn oil (200 mg/ml).

Controls:

Vehicle: DMSO, 100 µl/10 ml of culture

Positive Controls: Methylmethanesulfonate (10 µg/ml) in the absence of S9 and cyclophosphamide (100 µg/ml) in the presence of S9 were used as positive controls.

Exposure Conditions:

Incubation and sampling times: Cultured cells from the donors were incubated for 3 hours with drug or vehicle ±S9 fraction, washed twice with sterile saline and then incubated for another 24 hours before harvesting the cells.

Doses used in definitive study: Naloxone HCl concentrations of 1250, 2500 and 5000 µg/ml were used for incubations with or without S9.

Study design: For each donor, duplicate cultures treated with vehicle, test substance (3 concentrations) or positive control substance were selected for chromosomal aberration analysis. Cells were cultured in 20 ml — buffered — medium containing 20% fetal calf serum, 50 µg/ml gentamycin and 0.1 ml phytohemagglutinin for 44 hours before drug exposure. About 1 hour prior to harvesting, colchicine (1 µg/ml, final concentration) was added to each culture. After centrifugation, incubation/washing with 10 ml of 0.075 M KCl, and re-centrifugation, the remaining cells were fixed with methanol/glacial acetic acid

(3:1), mounted on slides, air-dried, stained with 4% Gurr's Giemsa R66 stain in pH 6.8 buffer, rinsed with water, blotted dry and mounted with coverslips.

Analysis:

No. slides/plates/replicates/animals analyzed: The mitotic index was determined by examining at least 500 lymphocytes per culture and calculating the percentage of cells in metaphase. Where possible, 100 cells in metaphase were analyzed from each selected culture for the incidence of structural chromosomal damage.

Selection endpoints: Highest concentration selected had significant reduction of mean mitotic activity or excessive cytotoxicity at the concentration above or was 5000 µg/ml.

Genetic toxicity endpoints/results: Significant increase in the percentage of aberrant cells.

Statistical methods: The chi-squared test was used to compare numbers of aberrations between treated cultures and appropriate controls.

Other:

Criteria for Positive Results: An increase in the mean percentage of aberrant cells, relative to the vehicle control values, by the chi-squared test or a significant dose-related increase in the mean percentage of aberrant cells or frequency of aberrations/100 cells, when analyzed by linear regression.

RESULTS:

Study Validity: The positive control for incubations with S9, cyclophosphamide, came out positive in these studies, and the highest concentration of naloxone used produced an adequate degree of cytotoxicity.

Study Outcome: In the preliminary toxicity study, 5000 µg/ml caused only slight mitotic inhibition in the absence of S9, but there was about an 80% reduction in mean mitotic activity, compared with solvent controls, in the presence of S9. In the main study, the positive control for incubations in the absence of S9, methyl-methanesulfonate, failed to be significantly positive. In the presence of S9, the frequency of aberrations/100 cells increased linearly with dose in a significant manner ($r = 0.948$; $p < 0.05$). As shown in the table below, the increase in the mean % of cells with aberrations (not including gaps) was significant at the high dose in the absence of S9 and significant at the middle and high doses in the presence of S9. Similar statistical results are obtained if gaps are included.

TABLE 70: Chromosomal aberration analysis in human lymphocytes from two donors.
(from NDA, Vol. 18, tab 38746, pp. 16-17 and 20-21)

Drug/ Conc., µg/ml	Mean % Mitotic Index		Mean % aberrant cells excluding gaps			
	-S9	+S9	-S9		+S9	
	Both donors	Both donors	Donor-1	Donor-2	Donor-1	Donor-2
Vehicle ¹	2.9	4.4	4	5	4	6
1250	2.2	1.8	3	3	4	8
2500	1.7	1.7	1	9	21***	4***
5000	3.5	0.9	12***	21***	100***	41***
MMS ²	Not reported		6	6		
Cyclo. ³		Not reported			30***	75***

¹DMSO, 10 µl/ml; ²Methylmethanesulfonate, 10 µg/ml; ³Cyclophosphamide, 100 µg/ml.
***P<0.001, when donor #1 (male) and #2 (female) data are combined (chi²-test).

SUMMARY:

In the human lymphocyte test using two donors, naloxone HCl was positive for clastogenicity at 5000 µg/ml in the absence of S9 (although the positive control methylmethanesulfonate was not significantly higher than DMSO) and positive at 2500 and 5000 µg/ml (as was cyclophosphamide) in the presence of S9, where naloxone showed a positive dose-dependent relationship. Thus, naloxone is clastogenic in this test.

[Naloxone also was reported by the sponsor to be weakly (sporadically) positive in the mouse L5178Y lymphoma fluctuation assay in the presence, but not the absence, of S9 in a study (Vol. 18, tab 38745) which is not reviewed herein, as no comparable study report with the buprenorphine/naloxone combination was submitted in this NDA.]

Study Title: Study of buprenorphine / naloxone (4:1 mixture) in the rat bone marrow micronucleus test.

Study No.: Protocol #SR0958; Report No. — P/6063

Study Type: In vivo genotoxicity assay.

Volume #18, Tab #: RC980114

Conducting Laboratory: [_____]

Date of Study Initiation/completion: July 17 through October 9, 1998

GLP Compliance/QA Reports: (X) Yes () No

Drug Lot Number: Buprenorphine HCl, V06101 (_____pure); naloxone HCl, V04434 (_____pure).

Study Endpoint: Induction of micronucleated polychromatic erythrocytes in rat bone marrow.

Methodology:

Species/Strains: Rat / _____ (—AP,SD)

Dose Selection Criteria:

Basis of dose selection: Toxicity of IV preparations in acidified water (pH 4) at doses ranging from 32 to 80 mg/kg.

Range finding studies: A total of 5 single-dose experiments were conducted with a total of 13 rats using drug dissolved in acidified water at various volumes (2.5, 3.3 and 5.0 ml/kg) until 32 mg/kg was chosen as the maximum tolerable dose. The main study was conducted with a different vehicle, however, which contained hydroxypropyl- β -cyclodextrin (concentration not given). Because of differences in specific gravity for the new vehicle, the high dose actually given was calculated to be 37 mg/kg.

Test Agent Stability: Analysis of the dosing solutions by HPLC indicated that all batches of dosing formulation analyzed were within 3.5% of the intended concentrations.

Metabolic Activation System: *In vivo*.

Controls:

Vehicle: Hydroxypropyl- β -cyclodextrin (concentration not given), 3 ml/kg, IV.

Positive Controls: Cyclophosphamide, 20 mg/kg, PO.

Exposure Conditions:

Incubation and sampling times: Rats were euthanized with halothane plus cervical dislocation at 24 hours (all groups) and 48 hours (vehicle and high dose only) after drug administration, and marrow was harvested from the iliac end of the femur.

Doses used in definitive study: 9.5, 18.5 and 37 mg/kg, IV.

Study design: Five rats of each sex/dose were used for marrow harvesting at 24 hours and 5/sex in the vehicle and high dose groups for marrow harvesting at 48 hours after drug administration.

Analysis:

No. slides/plates/replicates/animals analyzed: The ratio of polychromatic to normochromatic erythrocytes was determined by examining a sample size of 1000 erythrocytes per animal. A total of 2000 polychromatic erythrocytes from each animal were examined for the presence of micronuclei. This was extended to another 2000 for the 24-hour time point in males given vehicle and high dose.

Selection endpoints: Doses above 32 mg/kg (in acidic vehicle) caused excessive clinical signs (e.g., clonic convulsions, extreme decrease in activity, sedation or death) and/or tail-biting behavior.

Genetic toxicity endpoints/results: Significant increase in the percentage of polychromatic erythrocytes with micronuclei compared with the controls.

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Statistical methods: Fisher Exact Probability Test (one-sided) was used to evaluate the percentage of metaphases showing abnormalities (excluding cells with only gap-type aberrations).

Other:

Criteria for Positive Results: A statistically and biologically significant increase in the incidence of micronucleated polychromatic erythrocytes when compared with both historical and concurrent vehicle control incidences.

RESULTS:

Study Validity: The positive control, cyclophosphamide, caused marked increases in the frequencies of micronucleated polychromatic erythrocytes (PE).

Study Outcome: None of the buprenorphine/naloxone-treated groups showed significant changes from the vehicle-treated controls, except for the high dose males at 24 hours after dosing, who had a significant decrease in % of erythrocytes that were PE and a significant increase in micronucleated PE. The second 2000 PE that were examined from the high dose males, however, had one order of magnitude lower incidence of micronucleated PE than did the first 2000 examined, and the second 2000 PE from the controls had half the incidence of micronucleated PE as the first 2000 PE from the controls, such that the difference between the two male groups was not statistically significant.

TABLE 71: Mean % of polychromatic erythrocytes and micronuclei incidence therein from bone marrow of rats given an intravenous dose of buprenorphine + naloxone (4.5:1) (from NDA, Vol. 18, tab RC980114, pp. 26-31)

Drug dose, mg/kg	Mean % Polychromatic RBCs				Mean incidence of micronuclei/1000 PE			
	Males		Females		Males		Females	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Vehicle ¹	23.5	33.8	21.7	23.5	1.4 (1.1)	2.0 (0.9)	1.0 (0.7)	1.0
1 st count	(7.5)	(20.2)	(6.9)	(6.2)			(0.7)	(0.7)
2 nd count					0.7 (0.6)			
9.5	19.9	--	28.2	--	1.9 (1.1)	--	1.0 (0.7)	--
18.5	23.9	--	25.4	--	1.2 (1.3)	--	1.6 (0.9)	--
37	16.1*	32.6	19.2	38.5	3.0*(1.2)	0.5 (0.0)	1.9 (1.5)	1.2
1 st count	(4.6)	(29.1)	(4.7)	(13.3)		n=4		(1.4)
2 nd count					0.3 (0.3)			
Cyclo. ²	24.1	--	29.5	--	28**(8.2)	--	35.7**(9.7)	--

¹Volume of vehicle, 3 ml/kg, IV.

²Cyclophosphamide, 20 mg/kg, PO

*P<0.05, **p<0.01, compared with the corresponding vehicle-treated controls.

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SUMMARY:

In the rat bone marrow micronucleus test, IV administration of buprenorphine: naloxone (4.5:1) in single doses of 9.5-37 mg/kg (in a hydroxypropyl- β -cyclodextrin-containing vehicle) were negative for increasing the incidence of micronucleated polychromatic erythrocytes (PE), although the high-dose males showed a significantly decreased % of PE 24 hours after dosing, compared with the vehicle-treated controls.

SPECIAL TOXICOLOGY STUDIES:

General Comments: The vehicle formulation of "BUP+NAL injection solution, batch no. 835" tested in the following *in vitro* blood compatibility study was not identified.

Study Title: Examination of the substance mixture buprenorphine-HCl, lot no. 21 and Naloxone-HCl, batch no. 231 (ratio 3:2) as well as BUP + NAL injection solution, batch no. 835 on hemolytic and protein precipitating properties *in vitro*.

Study No.: 84204 **Volume #:** 11 **Tab#:** 84204

Conducting Laboratory: []

Date of Study Initiation: May 8, 1984

GLP Compliance/QA Report: (X) Yes () No

Methods: Blood (citrate) was taken from 5 healthy beagle dogs (breeder, _____) Aliquots of 0.05 ml blood were used in incubations (total volume, 2.0 ml) with various concentrations of test substance dissolved in 5% glucose solution. Hemolysis was judged to be none, slight, moderate or marked (score, 0 to 3+) by the _____

Drug lot nos.: Buprenorphine, lot #21; naloxone, lot #231

Observations and times: Examination for hemolytic and protein-precipitating properties was done immediately after mixing, as well as 1, 2, 4, 6, 24 and 48 hours after mixing.

Results: The 5% glucose control vehicle itself showed a mild to moderate degree of hemolysis, the progression of which over time appeared to be delayed somewhat by the two lowest concentrations of the buprenorphine-naloxone combination tested, 0.01% and 0.05%. Higher concentrations of the combination caused protein precipitation throughout the 48-hr time period studied, as well as a progression over time from mild hemolysis (+) at 2 hours to marked hemolysis (+++) by 48 hours.

TABLE 72: Summary of compatibility (hemolysis and precipitation) studies with dog blood.

Substance Tested	Conc., %	HEMOLYSIS/PRECIPITATION (hours after mixing with blood)						
		0 hr	1 hr	2 hr	4 hr	6 hr	24 hr	48 hr
Saline	0.9	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Glucose	5.0	0/0	+/0	+/0	+/0	++/0	++/0	++/0
Bup+Nal ¹ (mixture)	0.01	0/0	0/0	0/0	+/0	+/0	+/0	++/0
	0.05	0/0	0/0	0/0	0/0	0/0	0/0	++/0
	0.1	0/P	0/P	+/P	+/P	+/P	++/P	+++/P
	0.5	0/P	0/P	+/P	+/P	+/P	++/P	+++/P
	1.0	0/P	0/P	+/P	+/P	++/P	++/P	+++/P
Bup+Nal (ampoule) ²	0.01	0/0	0/0	0/0	0/0	+/0	+/0	++/0
	0.05	0/0	0/0	0/0	0/0	0/0	+/0	++/0
Saponin	0.0033	0/0	+/0	+/0	+/0	+/0	+/0	+/0

¹Mixture (3:2) prepared by the contract laboratory in 5% glucose solution.

²Mixture (3:2; vehicle unspecified) supplied to the testing laboratory in ampoules by the sponsor.

Summary:

Buprenorphine:naloxone (3:2) combination in 5.0% glucose solution was incompatible with dog blood at concentrations in excess of 0.05% (0.5 mg/ml), causing protein precipitation at all time points tested (0-48 hours), and causing greater hemolysis than 5% glucose alone by 48 hours. The vehicle for the drug combination, 5% glucose, caused a time-dependent mild to moderate hemolysis relative to isotonic saline. The protocol for this test differs from those more commonly used, which use human blood for hemolysis and plasma for protein flocculation, as well as shorter time periods of incubation.

General Comments: The formulation vehicle for "BUP+NAL injection solution, batch no. 835" was not identified in the following study report.

Study Title: Acute local tolerance study in beagle dogs of the substance mixture buprenorphine-HCl and naloxone-HCl injection solution (Ratio 3:2) after single intravenous, intramuscular, intraarterial and perivenous administration.

Study No.: 84193 Volume #: 11 Tab#: 84193

Conducting Laboratory: [

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Date of Study Initiation: April 13, 1984

GLP Compliance/QA Report: (X) Yes () No

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Methods: Six beagle dogs, 3/sex (breeder, the conducting laboratory), 1-2 years of age and weighing 8.2-14.0 kg were used. Each dog received 0.3 mg buprenorphine with 0.2 mg naloxone (presumably in 1 ml) at each of four sites on the right side: vena saphena parva (IV), arteria femoralis (IA), hind limb muscle (IM) and beside the vena saphena para (paravenous). Corresponding injections of 0.9% saline (presumably 1 ml, but volume not stated in report) on the left side served as control.

Drug lot no.: 835

Observations and times: Local reactions were inspected macroscopically in surviving dogs at 2, 24, 48 and 96 hours after dosing. Autopsy was performed on 1 dog/sex at 24, 48 and 96 hours after dosing. Sections of the affected tissues were fixed in 10% buffered formalin, stained with hematoxylin-eosin and, after preparation of paraffin sections, were examined histologically and were scored as 0 (no pathological findings), B (perivascular hemorrhage), I (perivascular inflammation), and N (necrotic areas), ranging in intensity from (+) = minimal to + = slight to ++ = moderate to +++ = marked. Results:

Macroscopic examination did not distinguish any differences between saline and drug injection sites. Histopathologic evaluations of the IV and paravenous sites were identical for each of the six dogs and indicated minimal to slight perivascular hemorrhages in more of the saline sites than at the drug sites. The only route that appeared to show some drug-related site reactions was the IM route, which had reports of necrosis (slight or moderate) and moderate inflammation in one female at 24 hours and one male at 96 hours after drug injection.

TABLE 73: Summary of injection site reactions in dogs given BUP + NAL (0.3+0.2 mg)

Injection Route	Saline/Drug Histopathology Scores and Intensities at Times After Dosing					
	24 Hours		48 Hours		96 Hours	
	Male	Female	Male	Female	Male	Female
IV	B+/B+,I+	0/0	B+/0	B+,I+/0	B(+)/0	0/B(+)
IM	0/0	0/I++,N+	I(+)/I+	0/0	0/I++,N++	0/0
IA	0/0	0/0	0/0	0/I(+)	0/0	0/0
Paravenous	B+/B+,I+	0/0	B+/0	B+,I+/0	B(+)/0	0/B(+)

Sponsor reported that none of the six dogs showed any influence of the test substance on behavior, external appearance, food or water consumption, body weight gain or feces.

Summary:

In dogs injected with saline (left side) or 0.3 mg buprenorphine + 0.2 mg naloxone (right side) at each of four different routes (IV, IM, IA and paravenous) and examined 24, 48 or 96 hours later (1/sex/time point), little difference was observed between test

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and saline control injection sites, except for the appearance of moderate irritation and mild to moderate necrosis in two of the six dogs tested after IM administration.

OVERALL SUMMARY AND EVALUATION:

Introduction:

Sponsor wishes to market sublingual tablets under the trade name Suboxone that contain the opioid partial *mu* agonist buprenorphine HCl and the opioid full antagonist naloxone HCl in a 4:1 ratio (as bases) for the treatment of opioid addiction in daily doses of 4 to 24 mg (as buprenorphine), equivalent to a maximum daily buprenorphine dose of 0.4 mg/kg (14.8 mg/m² body surface area) for a 60-kg person. The tablets, weighing 100 or 400 mg, will contain 2 or 8 mg of buprenorphine, as well as 0.5 or 2 mg of naloxone, respectively, and the inactive ingredients lactose, mannitol, starch, lemon & lime flavor, povidone K30, citric acid, magnesium stearate, acesulfame K, sodium citrate and ~~_____~~. The purpose of combining naloxone with the buprenorphine is to discourage intravenous abuse of this sublingual formulation of buprenorphine, since naloxone has poorer bioavailability by the sublingual route than does buprenorphine; but taken parenterally, the naloxone should antagonize the agonistic effects of the buprenorphine.

The present non-clinical review has concentrated upon studies of mixtures of naloxone with buprenorphine, since most non-clinical studies of buprenorphine as a single entity that were included in this submission have been reviewed previously under the sponsor's NDAs for Buprenex® (NDA 18-401) and Subutex® (NDA 20-732). Buprenex® has been marketed in the U.S. since 1982 as a parenteral opioid analgesic at recommended doses of 0.3 mg, IV, or 0.6 mg, IM. Subutex® is the naloxone-free sublingual form of buprenorphine intended for in-patient treatment of opioid abuse at the same doses of buprenorphine as are in Suboxone. Naloxone is available in injectable form (Narcan® and generics) for reversing the effects of opioid agonists, generally in incremental doses of 0.01 mg/kg of body weight, IV, SC or IM.

Primary pharmacological interactions between buprenorphine and naloxone:

Antinociception. In the rat tail-pressure test naloxone, when co-administered SC with morphine (3 mg/kg) or buprenorphine (0.03 mg/kg), appeared to cause a more extensive antagonism of morphine than antagonism of buprenorphine, in terms of both maximum inhibition and duration. In a 3:2 ratio, buprenorphine (0.03 mg/kg) with naloxone (0.02 mg/kg) did not show less antinociceptive effect than buprenorphine (0.03 mg/kg) alone.

Drug discrimination. In male rats trained to discriminate between buprenorphine (0.03 mg/kg, SC) and saline (1 ml/kg, SC), the co-administration of naloxone in SC doses of 0.002, 0.01 and 0.02 mg/kg decreased buprenorphine-appropriate lever responding during a 10-min testing period from 97% after buprenorphine alone (0.03 mg/kg, SC) to 93, 59 and 23%, respectively. In male rats rendered physically dependent upon morphine by constant IP infusion of 100 mg/kg/day for 2 days, buprenorphine alone (0.03 and 0.3 mg/kg, IV) elicited a limited number of withdrawal signs (weight loss and wet-dog shakes), whereas naloxone (0.02 and 0.2 mg/kg, IV) elicited a wider spectrum of signs in a dose-related manner, which was not significantly affected by the addition of buprenorphine at 1.5X the naloxone dose.

ADME. The disposition of [³H]-buprenorphine, with or without unlabeled naloxone, and [³H]-naloxone, with or without unlabeled buprenorphine, was studied in rats and dogs after oral (1:1 ratio), IV (3:2 ratio) and IM (3:2 ratio) routes. Plasma concentrations of unchanged [³H]-drug were measured in rats from 5 minutes to 8 hours after administration and, in dogs, up to 96 hours after administration. Elimination half-lives or clearances were not calculated from these data, but in general, buprenorphine disappeared from plasma at a slower rate than did naloxone in both species. After oral administration of 80 mg/kg of each drug in combination to rats, T_{max} , C_{max} and AUC_{0-8hr} for buprenorphine were 30 minutes, 915 ng/g of plasma, and 243 ng•hr/g of plasma, respectively, and the same parameters for naloxone were 15 minutes, 134 ng/g of plasma and 26 ng•hr/g of plasma, respectively. Excretion of radioactivity in feces and urine was measured for 96 hours in rats and 7 days in dogs. In both species, the major route of excretion of radioactivity from buprenorphine was in the feces (50-74%), whereas the major route of excretion of radioactivity from naloxone was in the urine (35-65%). Following oral administration of the 1:1 combination in rats and dogs, 55-64% of the administered buprenorphine radioactivity was excreted in feces and 6-12% in urine, whereas 39-50% of the naloxone radioactivity was excreted in urine and 12-22% in feces. This difference in major route of excretion between the two drugs was not substantially affected by co-administration of the two drugs by the oral, intravenous or intramuscular routes.

The distribution of excreted radioactivity among polar and non-polar metabolites, as well as unchanged parent drug, was studied with thin-layer chromatography, but discrete bands were not always obtained with the solvent systems used. In general, most of the radioactivity in feces from [³H]-buprenorphine was in the form of parent drug (42-83%), whereas most of the radioactivity in urine from [³H]-naloxone was in the form of polar metabolites. One of the major differences between rats and dogs is that after [³H]-naloxone, a higher percentage of the radioactivity in rat urine was labile (i.e., removed by freeze-drying), indicating a greater degree of metabolism of the naloxone molecule in the area of the radiolabel. In humans, the N-dealkylation of buprenorphine

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to nor-buprenorphine is catalyzed by cytochrome P450 3A4 (Iribarne *et al.*, 1997; Kobayashi *et al.*, 1998).

Safety evaluation:

Toxicity studies. Aside from the obvious adverse effects of overdose on the CNS, such as sedation, lethargy, ataxia, vomiting, respiratory depression, tremor and seizures, no peripheral target organ was consistently identified in toxicological studies of buprenorphine with naloxone at various combination ratios, when administered by the oral, IV, IM or SC routes to mice, rats and dogs for periods of time up to 28 days.

In single-dose toxicity studies using mice and rats, the IV route was the most toxic for either drug alone or for the combination. The combination did not exacerbate the toxicity beyond that exhibited by either drug alone by the oral route, and in fact, was significantly less toxic than the individual drugs by the IV route in the mouse and by the SC route in the rat. Given orally, naloxone did not affect the lethality of buprenorphine in the mouse, and buprenorphine (1000 mg/kg) did not affect the lethality of naloxone in the rat. Common clinical signs preceding early deaths were convulsions and depressed respiration. Common findings at necropsy of animals dying shortly after dosing included congestion or hemorrhage of the lungs, pale patches or pallor of the liver and sometimes pallor of the kidneys.

None of the multiple-dose toxicity studies in rats and dogs involved daily dosing of the buprenorphine-naloxone combination for a period of time longer than 28 days, none were carried out with the ratio (4:1) intended for clinical use and none were conducted using the sublingual route of administration. In most studies, oral dosing was with a 1:1 ratio and parenteral dosing used a 3:2 ratio of buprenorphine HCl to naloxone HCl. The most common observations across studies and across species were local site reactions (hemorrhage, inflammation, swelling, necrosis) when the combination was administered parenterally, and some systemic observations. The latter included decreased food consumption, decreased body weight gain, and increases in reticulocytes as a percentage of total erythrocytes, as well as increases in platelet count, by any route of administration. In 4-week studies using the oral route, the NOAEL was 6 and 10 mg/kg (each drug) in two studies with rats, and 2.5 mg/kg (each drug) in one dog study, except for effects of these low doses on food consumption and body weight gains. Deaths occurred in 1 of 10 rats given 150 mg/kg and 2 of 60 rats given 640 mg/kg (each drug) orally, and in 1 of 2 dogs given 250 mg/kg (each drug) orally.

Carcinogenicity. Carcinogenicity studies for buprenorphine alone were reviewed under NDA 20-732. Carcinogenicity studies of the combination of buprenorphine with naloxone have not been carried out, but the sponsor is in the process of toxicokinetics method development and dose range finding studies for a life-time dietary study.

Immunotoxicity. No studies of the combination were conducted.

Reproductive toxicity. Teratogenicity studies were carried out with buprenorphine and naloxone combinations (1:1 for oral and 3:2 for IM) in rats (during gestation days 6-15) and rabbits (during gestation days 6-18). Pregnant rats receiving combination treatment (10, 50 and 250 mg/kg/day, PO, of each drug or 0.3, 3 and 30 mg/kg/day, IM, as buprenorphine HCl) showed dose-related mortality (0, 1 and 4 after oral; 0, 1 and 2 after IM), decreased food consumption and body weight loss, as well as dose-related increases in post-implantation losses, including total litter resorptions. High-dose dams showed ataxia (IM), occasional dyspnea, and evidence of bleeding disorder. One IM low-dose fetus had hydrocephalus, which is considered incidental. No teratogenicity was reported at doses up to 100 times (PO) or 12 times (IM) the maximum daily human sublingual dose of 24 mg on a mg/m² basis.

Pregnant rabbits receiving oral treatment (0.4, 4 and 40 mg/kg/day of each drug) showed no signs of toxicity, other than a transient decrease in food consumption, but two of the high-dose rabbits had spontaneous abortions. There was no maternal toxicity at 4.0 mg/kg/day. The NOAEL for embryotoxicity or fetotoxicity was 40 mg/kg/day. No teratogenicity was observed at oral doses up to 32 times the maximum daily human sublingual dose of 24 mg on a mg/m² basis. Pregnant rabbits receiving intramuscular treatment with the combination (0.3, 3 and 30 mg/kg/day, as buprenorphine HCl) showed moderate to severe injection site reactions, transiently decreased food consumption and body weight changes in the dams that were inversely related to dose because of injection site swelling. Two high-dose does died of seizures. One low-dose and two mid-dose fetal malformations (acephalus, 2 omphalocele, respectively) were observed, but no malformations were observed at the high-dose or in the vehicle control group. A statistically significant increase in skeletal variations occurred at the high dose (53.7% rate vs. 36.6% rate in the controls). Local intolerance (injection site reactions) occurred at doses above 0.3 mg/kg, and 30 mg/kg was lethal. An NOAEL for developmental toxicity was not clearly demonstrated.

The effects of combinations of buprenorphine and naloxone on fertility and post-natal development have not been studied. Such studies have been conducted with rats using buprenorphine HCl alone in doses up to 80 mg/kg/day (see pharm/tox review of NDA 20-732, Dec. 5, 1997).

Genotoxicity. The genotoxic potential of buprenorphine and naloxone in combination at a 4:1 ratio (by weight as the hydrochlorides) was investigated in two *in vitro* tests (Ames and human lymphocyte) and in one *in vivo* test (rat micronucleus). Naloxone alone was also studied in three *in vitro* tests (Ames, mouse lymphoma cells and human lymphocytes). The studies of the combination are summarized in the table below. As

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indicated previously in the pharm/tox review of NDA 20-732, buprenorphine alone yielded equivocal results in the Ames test, being negative in studies by two laboratories, but positive for a frame shift mutation at high dose (5 mg/plate) in a third study. Buprenorphine alone has not been tested in the other two tests used for the combination (human lymphocyte and rat micronucleus), but was negative in the mouse lymphoma L5178Y cell fluctuation assay.

TABLE 74: Mutagenicity studies of buprenorphine with naloxone in a 4:1 combination (as salts or 4.5:1 as bases) or naloxone alone.

Test System	S9	Dose range tested	Outcome
Ames ^a Phase 1 (range finding)	-	100 - 5000 µg/plate	Precipitation 2.5-5 mg/plate & sparse lawn 1-5 mg/plate.
	+		
Ames ^a Phase 2	-	50 – 2500 µg/plate	NEGATIVE (Not Mutagenic)
	+	50 – 2500 µg/plate liquid preincubation	NEGATIVE (Not Mutagenic)
Cultured human lymphocytes ¹	-	10, 40 or 80 µg/ml	Dose-related cytotoxicity but not clastogenic
	+	10, 40 or 80 µg/ml	
Cultured human lymphocytes ²	-	5, 20 or 40 µg/ml	Dose-related cytotoxicity but not clastogenic
	+	10, 45 or 100 µg/ml	
<i>In vivo</i> rat micronucleus		Tested at MTD = 37 mg/kg, i.v. (n = 5/sex)	Not clastogenic, but sig. decrease in % of polychromatic erythrocytes in males at 24 hrs
<i>S. typhimurium</i> 5 Ames strains*	-	4 – 2500 µg/ml	NEGATIVE
	+	0.32 – 200 µg/ml	POSITIVE (weakly) in TA100 & TA1535
Cultured human lymphocytes*	-	1250, 2500 and	CLASTOGENIC
	+	5000 µg/ml	CLASTOGENIC

^aStrains tested: *S. typhimurium* TA1535, TA1537, TA98, TA100 and *E. coli* WP2P and WP2P *uvrA*

¹For *in vitro* human lymphocyte cytogenetic assay, blood was obtained from Donor 1.

²For *in vitro* human lymphocyte cytogenetic assay, blood was obtained from Donor 2.

*Study conducted with naloxone HCl alone.

Naloxone alone was reported to be a weak mutagen in the mouse lymphoma L5178Y cell fluctuation assay when cytotoxicity was only 18% survival at 100 µg/ml (not reviewed herein; summary, Vol. 1.6, p. 118), but to be negative in this assay when cytotoxicity was 44% survival at 100 µg/ml (not reviewed herein; summary, Vol. 1.6, p. 120). Naloxone alone was also found to be positive for chromosomal aberrations in two human lymphocyte tests (one not reviewed herein: summary, Vol. 1.6, p. 124). The combination has not been tested in other non-ICH tests in which buprenorphine alone was reported to be positive in NDA 20-732: the DNA synthesis inhibition test with

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testicular tissue from mice and the unscheduled DNA synthesis test using mouse testicular cells.

Tissue tolerance. As mentioned above for those subacute and reproductive toxicity studies which used parenteral routes of administration, the combination of buprenorphine and naloxone in high doses (i.e., high concentrations) caused very marked injection site reactions, which in some cases such as the IV route, precluded the ability to give more than a few multiple injections at the same site, even on an intermittent basis. Combinations containing buprenorphine concentrations as low as about 1 mg/ml caused marked swelling in rabbit muscle with alternate day dosing per thigh. An *in vitro* study with dog blood showed that 1 mg/ml of the 3:2 combination caused protein precipitation at all exposure times tested and greater hemolysis at 48 hr than that occurring with vehicle alone. The only local tolerance study, which used the 3:2 combination in dogs, appears to have used a single 1-ml injection at each site that contained lower concentrations of buprenorphine and naloxone (0.3 and 0.2 mg/ml) than the concentrations likely to be used if an attempt were made to dissolve and inject a tablet containing 2 or 8 mg of buprenorphine plus 0.5 or 2 mg of naloxone, respectively. Thus, the results of the single-dose local tolerance study in dogs may not be predictive of local adverse consequences of attempts to abuse Suboxone by parenteral administration.

Clinical Relevance of Safety Issues:

Although nearly all of the *in vivo* non-clinical toxicology studies used the buprenorphine-naloxone combination in a 1:1 ratio orally or a 3:2 ratio parenterally, they are considered adequate for supporting the safety of clinical use of the combination in a 4:1 ratio by the sublingual route, because no target organ or synergistic toxicities were observed.

Other Clinically Relevant Issues:

Since the target population for this product consists of opioid-dependent individuals, many of whom are experienced intravenous opioid abusers, the marked local site reactions caused by parenteral routes in the non-clinical studies make it imperative that the target population be warned against attempting to self-administer this product by injection.

Conclusions:

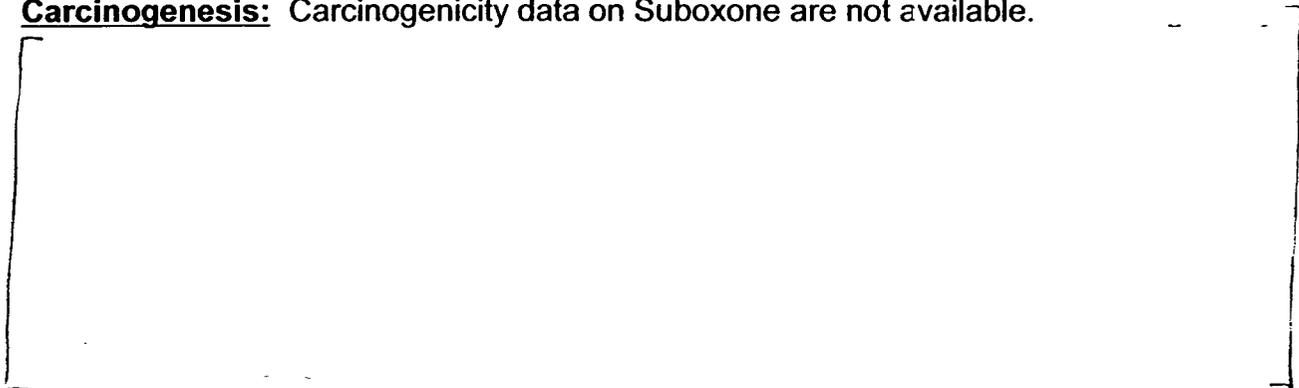
Despite the extensive number of studies employing several different routes of administration, no target organ or synergistic toxicities were readily apparent in rats or dogs. A sufficient number of preclinical toxicology studies have been conducted to support the safety of Suboxone as a treatment of individuals who are addicted to opioid drugs.

Communication Review – Labeling Review (for the label starting Vol. 1.1, p. 133)

- 1) The following format and content are recommended for the pertinent non-clinical sections of the label:

Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis: Carcinogenicity data on Suboxone are not available.



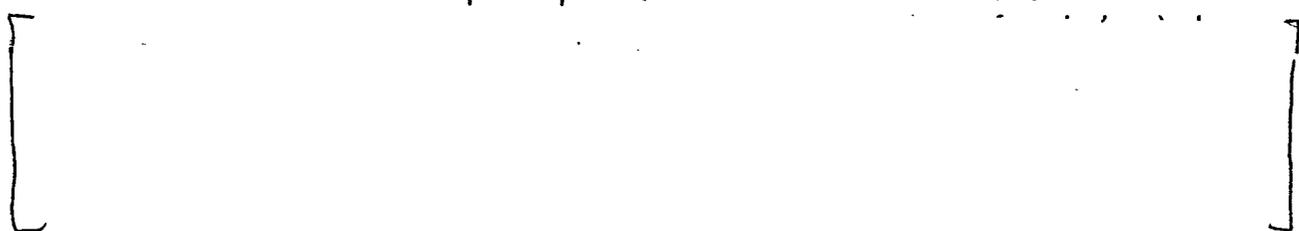
_____ Combinations (4:1) of buprenorphine _____ and naloxone _____ were not mutagenic in the bacterial mutation assay using four strains of *S. typhimurium* and two strains of *E. coli*. The combinations were not clastogenic in an *in vitro* cytogenetic assay in human lymphocytes or in an intravenous micronucleus _____ in the rat.

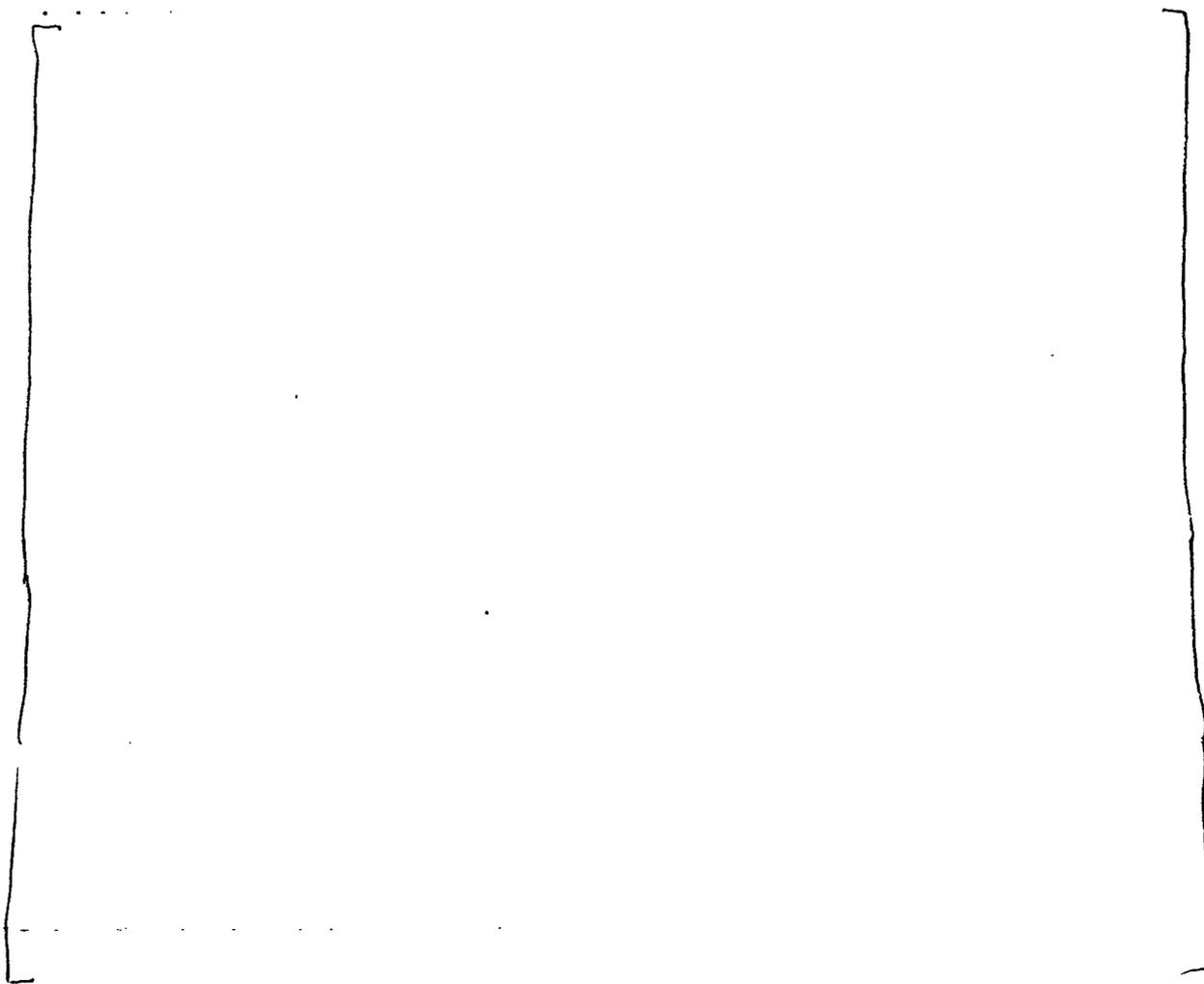
Impairment of Fertility:



Pregnancy Category C:

Teratogenic effects: Effects on embryo-fetal development were studied in Sprague-Dawley rats and Russian white rabbits following oral (1:1) and intramuscular (3:2) administration of mixtures of buprenorphine _____ and naloxone _____





- 2) The label should contain a warning against attempting to dissolve the Suboxone tablet and injecting it parenterally, because this could result in severe injection site reactions. For example,



RECOMMENDATIONS:

Internal comments: The pharmacological and toxicological profiles observed in laboratory animals have demonstrated a reasonable safety for support of the proposed

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labeled use in humans. The application is approvable on the basis of the non-clinical pharmacology, as Suboxone appears to have a favorable risk/benefit ratio. Before the application can be approved, however, the label should be amended as recommended under "Labeling Review."

To Applicant: The label should be amended as recommended under "Labeling Review," (subject to modification by the Agency's team in charge of reviewing the label, which is scheduled to meet at a later date).

[/S/]
David A. Brase, Ph.D. (reviewer)

October 26, 1999
Date completed

[/S/]
Dou Huey Jean, Ph.D. (peer reviewer)

October 26, 1999
Date completed

References

Iribarne, C., *et al.*: Involvement of cytochrome CYP3A4 in N-dealkylation of buprenorphine in human liver microsomes. *Life Sci.* 60:1953-1964, 1997.

Kobayashi, K., *et al.*: Human buprenorphine N-dealkylation is catalyzed by cytochrome CYP 3A4. *Drug Metab. Dispo.* 26: 818-821, 1998.

**APPEARS THIS WAY
ON ORIGINAL**

1.1
January 12, 1998

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
(Review of Carcinogenicity Data in Attachment 1)

Division of Anesthetic, Critical Care, and Addiction Drug Products, HFD-170

NDA 20-732

Type and Date of Submission: Original, March 31, 1997

Date Received by Reviewer: August 21, 1997 (by reviewer)

Date Completed: Draft, December 5, 1997; Revision, January 12, 1998

Reviewer: David Brase, Ph.D., Pharmacologist

Team Leader: Lucy Jean, Ph.D., Pharmacologist

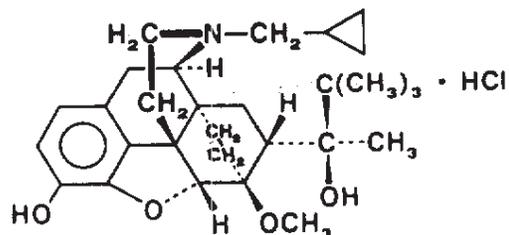
Sponsor: Reckitt & Colman Pharmaceuticals, Inc., 1909 Huguenot Road,
Richmond, VA

Drug: Buprenorphine HCl (sublingual tablets); Subutex®

Chemical name: [5 α ,7 α (S)]-17-(cyclopropylmethyl)- α -(1,1-dimethylethyl)-4,5-
epoxy-18,19-dihydro-3-hydroxy-6-methoxy- α -methyl-6,14-
ethenomorphinan-7-methanol hydrochloride

CAS number: 53152-21-9
52485-79-7 (free base)

Structure:



Molecular weight: 504.09

Relevant INDs: IND 35,877 Buprenorphine hydrochloride
IND 45,219 Buprenorphine hydrochloride (Buprenex®)

Relevant NDA: NDA 18-401. Many of the older preclinical pharmacology and

toxicology studies submitted with this NDA were submitted previously under NDA 18-401 for buprenorphine hydrochloride injectable by Eaton-Reccol, Inc., subsidiary company of Norwich-Eaton Pharmaceuticals (Norwich, NY) and were reviewed in 1980 or 1981 by former FDA pharmacologist, Dr. Frank Vocci. Any information from his reviews that is mentioned in this one will be cited as being from Vocci, 1980, or Vocci, 1981.

Drug class: Opioid partial μ -agonist (Schedule ^{(b) (4)} controlled substance)

Indication: Treatment of opioid addiction (Orphan drug status)

Dose: Maximum recommended daily dose is 32 mg/day, sublingually. However, the recommended dosage range of daily dosage is dependent upon the therapeutic endpoint:

1. Induction (initial dosing): 2-4 mg/day.
2. Maintenance:
 - a. Suppression of withdrawal symptoms: 2-8 mg/day.
 - b. Blockade of exogenously administered opiates: 4-16 mg/day.
 - c. Dosing interval of > 24 up to 72 hours: 8-32 mg/dose.

Recommended duration of drug administration is up to one year.

Table 1: Clinical formulation (Composition)

Ingredients	Amount/tablet (mg)	
	Dose of buprenorphine base/tablet:	^{(b) (4)} 2.0 mg
Buprenorphine hydrochloride	^{(b) (4)}	
Lactose	^{(b) (4)}	
Mannitol	^{(b) (4)}	
^{(b) (4)}	^{(b) (4)}	
Povidone K30	^{(b) (4)}	
Citric Acid, ^{(b) (4)}	^{(b) (4)}	
Sodium citrate	^{(b) (4)}	
Magnesium stearate	^{(b) (4)}	
TABLET WEIGHT	^{(b) (4)}	

Route of administration: Sublingual

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Studies reviewed within this submission:

Acute intravenous toxicity study in the mouse: buprenorphine and identified impurities, Report No. RC 80218 (March 1980), Vol. 1.7 (Tab #6) {quality assurance signatures of (b)(4)}

Primary skin irritation study: Buprenorphine hydrochloride batch no. PP1121/16, Report No. RC 79189 (December 1979), Vol. 1.7 (Tab #7) {no GLP or quality assurance statements or signatures}

Primary skin irritation study: Buprenorphine hydrochloride batch no. 764, Report No. RC 79190 (December 1979), Vol. 1.7 (Tab #8) {no GLP or quality assurance statements or signatures}

Eye irritation study: Buprenorphine hydrochloride batch no. PP1121/16, Report No. RC 79191 (December 1979), Vol. 1.7 (Tab #9) {no GLP or quality assurance statements or signatures}

Eye irritation study: Buprenorphine injection, batch no. 764, Report No. RC 79192 (December 1979), Vol. 1.7 (Tab #10) {no GLP or quality assurance statements or signatures}

Acute toxicity by inhalation of buprenorphine, Report No. RC 80219 (February 1980), Vol. 1.7 (Tab #11) {quality assurance signatures of (b)(4)}

Buprenorphine oral toxicity study in beagle dogs, Report No. RC 80208 (14 November 1980), Vol. 1.9 (Tab #27) {quality assurance signatures of (b)(4)}

Chronic (fifty-two week) oral toxicity study of buprenorphine in dogs, Project No. RC 97020 (September 14, 1989), Vol. 1.10 (Tab #28) {Approved by (b)(4)}

Effect of buprenorphine hydrochloride on pregnancy of the rat (oral administration), Report No. RC 82211 (Oct. 1, 1982), Vol. 1.11 (Tab #35) {GLP statement signed by (b)(4) and quality assurance statement signed by (b)(4)}

Effect of buprenorphine hydrochloride on pregnancy of the rabbit (oral administration), Report No. RC 82212 (December 1982), Vol. 1.11 (Tab #36) {GLP statement signed by (b)(4) and quality assurance statement signed by (b)(4)}

Effect of buprenorphine hydrochloride on fertility and general reproductive performance of the rat (oral administration), Report No. CSR R07206 (17 June 1986), Vol. 1.11 (Tab #39) {GLP statement signed by (b)(4) and quality assurance audit statement signed by (b)(4)}

Effect of buprenorphine hydrochloride on peri- and post-natal development of the rat (oral administration), Report No. CSR r07067 (17 April 1986), Vol. 1.12 (Tab #41) {GLP statement signed by (b)(4) and quality assurance statement signed by (b)(4)}

Mutagenicity studies on buprenorphine hydrochloride, Report No. RC 84145 (September 1984), Vol. 1.12 (Tab #45) {GLP statement signed by Prof. Dr. (b)(4) and quality assurance statement signed by Dr. (b)(4)}

Mutagenicity studies of buprenorphine hydrochloride in in vitro bacterial systems, Report No. RC 8532 (March 1985), Vol. 1.12 (Tab. #46) {Approved by (b)(4); no GLP or quality assurance statements or signatures}

Study to evaluate the chromosome damaging potential of buprenorphine hydrochloride by its effects on cultured Chinese hamster ovary (CHO) cells using an in vitro cytogenetics assay, Study No. RC 8582 (11 October 1985), Vol. 1.12 (Tab #47) {GLP statement signed by (b)(4) quality assurance audit statement signed by (b)(4)}

Study to determine the ability of buprenorphine hydrochloride to induce mutations in 6-thioguanine resistance in mouse lymphoma L5178Y cells using a fluctuation assay, Report No. RC 8583 (October 1985), Vol. 1.12 (Tab. #48) {GLP statement signed by (b)(4) and quality assurance statement signed by (b)(4)}

Studies not reviewed within this submission:

- 1. The following studies were described previously in Frank Vocci's review of NDA 18-401 (1980).**

Buprenorphine acute intramuscular toxicity in rats, Report No. RC 79126 (5 July 1979), Vol. 1.7 (Tab #1) {no GLP or quality assurance statements or signatures}

Buprenorphine acute intravenous toxicity in rats, Report No. RC 79127 (14 May 1979), Vol. 1.7 (Tab #2) {no GLP or quality assurance statements or signatures}

Buprenorphine acute intravenous toxicity in cross-bred dogs, Report No. RC 79128 (2 April 1979), Vol. 1.7 (Tab #3) {no GLP or quality assurance statements or signatures}

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Buprenorphine acute intravenous toxicity in baboons, Report No. RC 79129 (11 June 1979), Vol. 1.7 (Tab #4) {no GLP or quality assurance statements or signatures}

Observations on the acute toxicity of buprenorphine in rodents, Report No. RC 7668 (29 April 1976), Vol. 1.7 (Tab #5) {Authorized by [REDACTED] (b)(4)}

Investigation of the phototoxic potential of buprenorphine hydrochloride in the albino guinea pig, Report No. RC 7732 (February 1977), Vol. 1.7 (Tab #12) {quality assurance signatures of [REDACTED] (b)(4)}

Tolerance of buprenorphine injectable solution following parenteral administration in cross-bred dogs (with special reference to local reactions), Report No. RC 79125, Vol. 1.7 (Tab #13) {no GLP or quality assurance statements or signatures}

In vitro haemolytic properties of buprenorphine injection solution, Report No. RC 79124 (29 May 1979), Vol. 1.7 (Tab #14) {no GLP or quality assurance statements or signatures}

Evaluation of haemolytic activity of buprenorphine in human blood, Report No. RC 8440 (March, 1984)[study done at Lederle in 1975], Vol. 1.7 (Tab #15) {Approved by [REDACTED] (b)(4)}

Buprenorphine 30 day subcutaneous toxicity study in rats, Report No. RC 7203 (June 1972), Vol. 1.7 (Tab #16) {no GLP or quality assurance statements or signatures}

A 30-day [s.c.] toxicity study in dogs, Report No. RC 7202 (June 1972), Vol. 1.8 (Tab #17) {no GLP or quality assurance statements or signatures}.

Buprenorphine four-week intravenous toxicity in beagle dogs, Report No. RC 79132 (21 May 1979), Vol. 1.8 (Tab #18) {quality assurance signature of [REDACTED] (b)(4)}

Buprenorphine four-week intravenous toxicity in baboons, Report No. RC 79133 (13 June 1979), Vol. 1.8 (Tab #19) {quality assurance signature of [REDACTED] (b)(4)}

One-month oral toxicity study in Wistar rats, Report No. CSR 105817 (September 1973), Vol. 1.8 (Tab #20) {no GLP statement or signature}

One-month oral toxicity study in rhesus monkeys, Report No. CSR 105816 (September 1973), Vol. 1.8 (Tab #21) {no GLP or quality assurance statements or signatures}.

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One month sublingual toxicity study of buprenorphine in [cynomolgus] monkeys, Report No. RC 77138 (August 1977), Vol. 1.8 (Tab #22) {no GLP or quality assurance statements or signatures}

Six-month intramuscular toxicity study in Wistar rats, Report No. CSR 105815 (August 1974), Vol. 1.9 (Tab #25) {no GLP or quality assurance statements or signatures}

Six-month intramuscular toxicity study in olive baboons, Report No. CSR 105812 (July 1974), Vol. 1.9 (Tab #26) {no GLP or quality assurance statements or signatures}

Teratogenicity studies in the rabbit and rat, Report No. CSR 103401 (February 1974), Vol. 1.10 (Tab #29) {no GLP or quality assurance statements or signatures}

Buprenorphine teratological studies in rats and rabbits, Report No. RC 7205 (June 1972), Vol. 1.10 (Tab #30) {no GLP or quality assurance statements or signatures}

Buprenorphine: the effect of intravenous administration on the pregnancy of the rat, Report No. RC 79130 (5 July 1979), Vol. 1.10 (Tab #31) {quality assurance signature of [REDACTED] (b) (4)}

Buprenorphine: the effect of intravenous administration on the pregnancy of the rabbit, Report No. RC 79131 (2 July 1979), Vol. 1.10 (Tab #34) {no GLP or quality assurance statements or signatures}

Fertility study in the rat, Report No. CSR 103391 (December, 1975), Vol. 1.11 (Tab #37) {no GLP or quality assurance statements or signatures}

Buprenorphine: Peri- and Postnatal Toxicity Study in Rats, Report No. CSR 103399 (January 1976), Vol. 1.11 (Tab #38) {no GLP or quality assurance statements or signatures}

[Same report (RC 7732) as in Vol. 1.7. Tab #12] Investigation of the phototoxic potential of buprenorphine hydrochloride in the albino guinea pig, Vol. 1.12 (Tab #42).

Delayed Dermal Sensitization Study in the Guinea Pig, buprenorphine batch no. PP.1101/7, Report No. RC 79100 (November, 1978), Vol. 1.12 (Tab #43) {no GLP or quality assurance statements or signatures}

Mutagenicity testing of compound RX 6029, Report No. RC 7999 (November 1978), Vol. 1.12 (Tab #44) {no GLP or quality assurance statements or signatures}

2. The following studies were reviewed previously by Frank Vocci under NDA 18-401 (Supplement number not identified; 1981).

Subacute (3-month) subcutaneous toxicopathologic study of buprenorphine (EU-4764) in rats, Report No. RC 8315 (March 19, 1980), Vol. 1.8 (Tab #23) {Approved by (b)(4); audited by (b)(4); no GLP}

Subacute (3 month) intramuscular toxipathologic study of buprenorphine (EU-4764) in dogs, Report No. RC 8317(August 29, 1980), Vol. 1.9 (Tab #24) {Approved by (b)(4); GLP statement signed by (b)(4),

General reproduction studies of buprenorphine (EU-4764) in Sprague-Dawley rats, Report No. RC 8439 (March 14, 1980), Vol. 1.10 (Tab #32) {Approved by (b)(4)
(b)(4)

Effect of buprenorphine hydrochloride on pregnancy of the New Zealand white rabbit, Report No. RC 80216 (12 May 1980), Vol. 1.10 (Tab #33) {GLP statement signed by (b)(4); and (b)(4) quality assurance statement signed by (b)(4)

Effect of buprenorphine hydrochloride on peri- and post-natal development of the rat, Report No. RC 80217 (5 June 1980), Vol. 1.12 (Tab #40) {GLP statement signed by (b)(4) and quality assurance statement signed by (b)(4)
(b)(4)

Disclaimer: Portions of this review may contain data and/or text directly excerpted from the sponsor's submission or from previous FDA reviews of this drug.

Previous clinical experience:

Buprenorphine is a partial agonist of opioid μ receptors which is indicated for the relief of moderate to severe pain. It is used mainly in the management of postoperative pain. It has been marketed since December of 1981 by Reckitt & Colman Pharmaceuticals as buprenorphine HCl injectable (Buprenex[®]) containing 0.3 mg buprenorphine (base)/ml. At a parenteral dose of 0.3 mg, buprenorphine is capable of producing analgesia equivalent to that produced by a 10-mg dose of morphine with no significant differences in the incidences of respiratory depression, nausea, vomiting, sweating or drowsiness in adults (Edge *et al.*, 1979; Tigerstedt and Tammisto, 1980). The label indicates that after an intravenous dose of 0.3 mg, buprenorphine exhibits a mean half-life of 2.2 hours (range, 1.2-7.2 hours), but its pharmacodynamic effects may last 6-8 hours in adults, possibly due to a slow dissociation rate from opioid μ receptors. However, a recent pharmacokinetic study of buprenorphine (5 μ g/kg, s.c.) given to six patients for postoperative pain

indicated a much longer terminal half-life in plasma of 23 hours (Gralow *et al.*, 1995). The difference was attributed to the development of a more sensitive assay, capable of measuring plasma levels as low as 40 pg/ml. After i.v. administration of 1 mg to six healthy volunteers, the mean (\pm S.D.) $t_{1/2}$ was reported to be 16.2 ± 20.2 hours in a more recent study (Mendelson *et al.*, 1997). Buprenorphine has poor oral bioavailability (Bullingham *et al.*, 1980), and thus, it is generally administered parenterally. However, it may display greater bioavailability after sublingual administration (Bullingham *et al.*, 1981; Mendelson *et al.*, 1997), perhaps comparable to that of fentanyl but greater than that of methadone, heroin, hydromorphone, levorphanol, oxycodone, morphine and naloxone (Weinberg *et al.*, 1988), and has been marketed abroad for a number of years in a sublingual formulation. The sublingual formulation marketed in New Zealand also contains the opioid antagonist naloxone. Buprenorphine is capable of producing a morphine-like euphoria (e.g., Pickworth *et al.*, 1993), and thus, its use can result in the development of psychic dependence. It can also produce physical dependence, but abstinence may result in a relatively mild withdrawal syndrome due to its slow dissociation from opioid receptors. Because of its ability to produce psychic and physical dependence, buprenorphine is a controlled narcotic drug (Schedule ^{(b)(4)}). It is also capable of precipitating withdrawal symptoms in individuals who are highly physically dependent upon other μ opioids, but may attenuate withdrawal in individuals who have a relatively low level of physical dependence.

Potential adverse effects of buprenorphine include many of the same adverse effects that are typical of other μ agonists. Adverse effects with an incidence of $>1\%$ that are associated with the administration of buprenorphine include nausea, dizziness, drowsiness, hypoventilation, miosis, vomiting, hypotension, sweating and headache (PDR, 1995). Some of these, such as respiratory depression, may display a ceiling effect (attenuated dose-response relationship) at supratherapeutic doses of buprenorphine.

PHARMACOLOGY:

I. Brief summary of preclinical pharmacology of buprenorphine

The older preclinical pharmacology and toxicology literature on buprenorphine has been reviewed (Cowan *et al.*, 1977a; Heel *et al.*, 1979; Rance, 1979), and unpublished preclinical studies submitted to the FDA under an earlier NDA (#18-401) were summarized by Frank Vocci in reviews dated May 1, 1980, and March 12, 1981.

Buprenorphine is an opioid analgesic derived from thebaine that is regarded as a partial agonist of opioid μ receptors (Martin *et al.*, 1976), with the result that it displays both agonistic and antagonistic activities (Cowan *et al.*, 1977b). Compared with morphine and most other μ agonists (except lofentanil), buprenorphine displays a slower dissociation rate from μ receptors, thus rendering

the agonistic effects of this drug difficult to antagonize with the subsequent administration of a narcotic antagonist, such as naloxone.

As a partial agonist, buprenorphine can also exert antagonistic effects against high doses of full agonists of μ receptors. A study comparing the abilities of several opioid agonist-antagonist and full antagonist drugs, administered i.p., to antagonize a maximally antinociceptive dose of morphine (21.5 mg/kg, i.p.) in mice was carried out by Friderichs (1992). Buprenorphine exhibited full antagonism of morphine in the mouse tail-flick test. Listed in order of decreasing relative antagonistic potencies (most potent = 1), the drugs tested against morphine were naltrexone (1), naloxone (4.5), levallorphan (16), **buprenorphine** (26), nalorphine (35), nalbuphine (316), and pentazocine (461). Thus, buprenorphine has approximately 1/6 the antagonistic potency of naloxone against a high dose of morphine, which acts predominantly at the μ opioid receptor. Several animal studies have indicated that buprenorphine may also act as a pure antagonist of opioid *kappa* receptors (Leander, 1988; Negus *et al.*, 1989, 1991; Richards and Sadee, 1985), including a study in which buprenorphine precipitated withdrawal signs in rhesus monkeys made dependent on the *kappa* agonist U-50,488 (Gmerek *et al.*, 1987).

Receptor binding pharmacology. Regarding the ability to inhibit the binding of [³H]-DAMGO (a specific and potent μ agonist synthetic opioid peptide) to opioid μ receptors in rat brain membranes, the relative potencies of naltrexone, naloxone, levallorphan and buprenorphine are approximately 1:7:3:51, respectively (Codd *et al.*, 1995). Thus, buprenorphine has approximately 1/7 the affinity of naloxone at the μ opioid receptor.

Dissociation studies from rat brain homogenates indicated that the $t_{1/2}$ for buprenorphine dissociation (45 min) was about 15 times longer than the $t_{1/2}$ for naloxone dissociation (3 min) at 37°C (Hambrook and Rance, 1976). This relatively slow dissociation rate could account for buprenorphine's relatively long duration of action, the limited ability of naloxone to reverse its effects in naive animals and to precipitate withdrawal signs in buprenorphine-dependent animals, and a relatively mild, sometimes not readily apparent, withdrawal syndrome upon abstinence after chronic administration. Possibly because of the ability of fat to serve as a reservoir for buprenorphine, the apparent dissociation of buprenorphine from opioid receptors *in vivo* may be much longer than that observed *in vitro*, as tightly bound tritiated buprenorphine in rat brain was observed in one study to have a decay half-life *in vivo* of approximately 69 hours (Pontani *et al.*, 1985). This feature has made *in vivo* receptor PET imaging and brain distribution studies possible with the use of an [¹¹C]-labeled buprenorphine (Galynker *et al.*, 1996).

SAFETY PHARMACOLOGY:

Respiration. In a recent comparative study of 9 volunteers with histories of opioid

abuse given sublingual buprenorphine (0, 0.5, 2, 8, 16 and 32 mg) or oral methadone (3.75, 15 and 60 mg) once/week in a Latin-square design, both drugs produced typical opioid effects of long duration, including respiratory depression, pupillary constriction, euphoria and sedation (Walsh *et al.*, 1995). The methadone, showed linear dose-related effects, whereas buprenorphine had non-linear dose-effect curves with maxima for most physiological and subjective measures occurring between 4 and 8 mg, with no further increases at the 16-mg and 32-mg doses. The authors suggested that this "ceiling effect" for buprenorphine, which had been observed in many previous animal studies, may reduce the abuse liability and increase the safety of buprenorphine (Walsh *et al.*, 1995).

Cardiovascular system. The cardiovascular effects of buprenorphine have been studied in the rat, guinea pig, cat and dog. In all of these species, buprenorphine produced bradycardia in the absence of any marked effect on blood pressure. In rats, isoproterenol countered the bradycardia, but in dogs, the bradycardia was exacerbated by epinephrine and norepinephrine, which also facilitated the development of arrhythmias (Vocci, 1980).

Abuse/dependence liability. Although studies with human volunteers indicated a low abuse potential for buprenorphine (Jasinski *et al.*, 1978), a sublingual preparation that has been available in Europe for several years has become highly abused in some countries by intravenous drug abusers. In a survey of over 1,000 i.v. drug abusers in Glasgow, Scotland, that was conducted in 1990 and 1991, the three most commonly injected drugs were buprenorphine, heroin and temazepam (Possilpark Group, 1993), although other studies suggested that buprenorphine, temazepam and amphetamines, rather than heroin, were the three most commonly injected drugs (Frischer, 1992; Lavelle *et al.*, 1991). Some abusers injected buprenorphine in a "cocktail" with temazepam (Forsyth *et al.*, 1993). Similarly, high incidences of buprenorphine abuse among opiate abusers have been reported from Belgium (Debrabandere *et al.*, 1992), France (Arditti *et al.*, 1992), Ireland (Hand *et al.*, 1989; O'Connor *et al.*, 1988), Spain (San *et al.*, 1993; Segui *et al.*, 1991), New Zealand (Rainey, 1986; Robinson *et al.*, 1993; Dore *et al.*, 1997) and India (Chowdhury and Chowdhury, 1990), often in a "cocktail" with benzodiazepines or cyclizine (Singh *et al.*, 1992). Intranasal abuse of buprenorphine has also been reported, in which the sublingual tablets are crushed into a fine powder and then snorted, which reputedly results in a more rapid psychoactive effect than the sublingual route (Strang, 1991). Recent reports from southern France indicate that buprenorphine is also a popular choice on forged prescriptions (Baumevielle *et al.*, 1997; Lapeyre-Mestre *et al.*, 1997). These clinical reports on abuse are consistent with earlier studies with non-human primates, which demonstrated self-administration properties of buprenorphine in rhesus monkeys (Mello *et al.*, 1981; Mello and Mendelson, 1985; Woods, 1977; Yanagita *et al.*, 1982; Young *et al.*, 1984).

An attempt in Scotland to counter buprenorphine abuse by scheduling the drug as a controlled substance caused only a temporary decline of a few months in positive urine specimens analyzed in a Glasgow laboratory (Stewart, 1991). An attempt to discourage i.v. abuse of buprenorphine in New Zealand by the introduction of a combination tablet containing 0.2 mg of buprenorphine and 0.17 mg of naloxone, led to some decrease in the incidence of abuse, but the combination tablet still remained an agent of i.v. misuse, despite one-third of the i.v. users reporting instances of withdrawal symptoms (Robinson *et al.*, 1993). In addition, the maintenance of opioid addicts on sublingual buprenorphine (8 mg/day) was found in one study of eight subjects to not prevent opioid agonist-like effects of a supplemental i.m. injection of buprenorphine, which was interpreted by the authors as indicating some abuse potential of parenteral buprenorphine in buprenorphine-maintained patients (Strain *et al.*, 1997).

Buprenorphine has the capability of producing physical dependence within one to two months of continuous use (Heel *et al.*, 1979). Abrupt withdrawal from buprenorphine has been described as being of the μ opioid type, though beginning somewhat more slowly and being of milder intensity than withdrawal from most other opioids and not showing a peak in adults until day 5 of abstinence (San *et al.*, 1992). When withdrawal induced by naloxone (1.2 mg, i.v.) was studied in seven buprenorphine-dependent patients 3 to 6 hours after their last i.v. dose of buprenorphine, the most frequently observed signs were mydriasis, systolic hypertension, tachypnea, muscle pains, yawning, anxiety, restlessness and craving (Nigam *et al.*, 1994). In contrast, abrupt withdrawal for 72 hours in eight individuals dependent upon buprenorphine, 8 mg/day (sublingual), was recently reported to result in no subjective symptoms or physiological signs of opioid withdrawal (Eissenberg *et al.*, 1997). However, a weak withdrawal syndrome was reported to occur in a neonate born to a heroin addict maintained on buprenorphine approximately 48 hours after birth, characterized by agitation, sleep disorders, tremor, yawning, noisy breathing and a slight fever (Marquet *et al.*, 1997).

PHARMACOKINETICS/TOXICOKINETICS:

In Vitro Studies. Comparative studies of partition coefficients (heptane : pH 7.4 buffer) indicated that buprenorphine was about 40 times as lipophilic as etorphine, about 3500 times as lipophilic as naloxone and six million times as lipophilic as dihydromorphine (Hambrook and Rance, 1976). The pKa of the amino group in buprenorphine is 8.4, and the log partition coefficient for buprenorphine in an octanol/pH 6.6 buffer system is 3.37 (NDA, Vol. 1.1, p. 224). Thus, increasing pH would be expected to increase sublingual absorption by increasing the portion of the drug in the unionized form, but this would be countered by a decreased solubility, since the aqueous solubility of buprenorphine is 1.1 mg/ml at pH 6, but only 0.11 mg/ml at pH 7 (NDA, Vol. 1.1, p. 224). Equilibrium dialysis studies indicate that buprenorphine is about 96% bound to human plasma proteins, particularly to α - and β -globulins, and may compete with lipids for binding to

albumin (Vocci, 1980). Buprenorphine shows somewhat less binding to rat (85-87%) and dog (78-81%) plasma proteins (Walter and Inturrisi, 1995).

Preclinical *in vivo* studies. Absorption, distribution, metabolism and excretion studies with buprenorphine have been recently reviewed (Walter and Inturrisi, 1995). Tissue distribution studies using [³H]-buprenorphine have been conducted in rats at single doses of 200 µg/kg, i.v. (Table 2), and 20 µg/kg, i.m. (Table 3).

After i.v. administration of the 200 µg/kg dose, the highest tissue levels were observed at the first time of sampling (15 minutes) and were greatest in fat, lung, kidney, heart, spleen and brain tissue. By 24-48 hours after administration, the highest levels were observed in fat, spleen, kidney and liver.

Table 2: Distribution of [15,16(n)-³H]buprenorphine in rats after a dose of 200 µg/kg, i.v. (from Pontani *et al.*, 1985, via Walter and Inturrisi, 1995).

Tissue ^a	Buprenorphine concentration (ng/g of tissue or ng/ml of fluid)							
	0.25	0.5	1.0	2.0	4.0	6.0	24	48
Plasma	46	19	11	4.3	1.5	0.6	0.6	0.6
Brain	117	57	51	21	11	6.3	0.9	0.1
Liver	80	41	25	12	7.7	6	3.2	1.8
Heart	126	63	38	13	4.5	1.8	1.1	1.1
Lung	225	123	85	27	11	6	6.5	3.5
Kidney	182	98	66	25	9	4	3.3	2.5
Spleen	124	66	42	17	5.2	3	6.3	4.0
Testes	61	60	45	20	5.8	2.5	1.0	0.4
Muscle	77	41	31	17	5	0.8	1.3	1.2
Fat	239	241	262	206	89	39	19.3	13.7

^aTissue levels are expressed as the mean of 3 male Wistar rats.

A somewhat different time course of tissue distribution was observed with the i.m. administration of a 10-fold lower dose of buprenorphine (20 µg/kg), with brain and spleen levels peaking at 30 minutes and fat levels peaking at 4 hours. By 24 hours after administration, the highest levels of buprenorphine were found in liver, kidney and fat (Table 3).

The effect of route of administration on the bioavailability of [³H]buprenorphine (200 µg/kg) was studied in rats by Brewster *et al.* (1981a). The difference in bioavailability between the intraduodenal and intrahepatoportal

routes provides a direct demonstration of the impact of metabolism of the drug by the gut wall on bioavailability (Table 4). Such metabolism, primarily involving conjugation with glucuronic acid, has been demonstrated in the rat by other investigators (Castle *et al.*, 1985; Rance and Shillingford, 1977). The glucuronide conjugate of buprenorphine is excreted in the bile and undergoes extensive

Table 3: Distribution of [15,16(n)-³H]buprenorphine in male Sprague-Dawley rats (n = 2) after a dose of 20 µg/kg, i.m. (Walter and Inturrisi, 1995).

Time, hr	Buprenorphine concentration (ng/g of tissue or ng/ml of fluid)						
	0.25	0.5	1.0	2.0	4.0	6.0	24
Plasma	5.5	3.0	2.1	1.3	0.4	0.6	0.1
Brain	9.4	11.9	8.7	6.6	5.3	3.5	0.4
Liver	25.8	24.5	17.8	13.7	8.2	9.8	3.4
Heart	21.1	12.7	6.4	4.3	1.6	1.6	0.3
Lung	34.6	21.4	11.0	7.8	3.4	3.5	0.8
Kidney	29.3	19.4	10.6	7.7	3.3	4.2	2.3
Spleen	5.7	12.3	7.1	4.1	2.9	3.3	0.9
Testes	2.7	3.8	4.0	3.1	0.8	0.7	0.1
Diaphragm	15.3	10.4	5.7	3.9	1.3	1.2	0.2
Fat	12.3	24.5	28.9	29.5	33.0	15.7	1.6

Table 4: Route-dependence of buprenorphine bioavailability in the rat.

Route	AUC _{0-4hr} , ng•min/ml ^a	Relative bioavailability, 0-4 hr ^{a,b}
Intraarterial	1852 ± 189	100
Intravenous	1807 ± 242	98 ± 13
Intrarectal	1000 ± 267	54 ± 14
Intrahepatoportal	900 ± 161	49 ± 9
Sublingual	249 ± 39	13 ± 2 ^c
Intraduodenal	180 ± 71	10 ± 4

^aValues represent the mean (±SEM) for 4 rats.

^bRelative to the intraarterial route.

^cIt was noted that the slow absorption profile for this route in the rat likely resulted in an underestimation of bioavailability over the 4-hour period of measurement.

enterohepatic cycling in the rat (Brewster *et al.*, 1981b). As this cycling progresses, the proportion of buprenorphine to N-dealkylbuprenorphine (norbuprenorphine) excreted in bile as glucuronides increases in favor of the N-dealkyl metabolite. Excretion of norbuprenorphine in bile may occur more extensively in male rats than in female rats (Brewster *et al.*, 1981b). As might be expected from a drug excreted in bile, the major route of elimination of buprenorphine was found in rats, dogs and rhesus monkeys to be through the feces (Walter and Inturrisi, 1995).

Table 5: Peak concentrations measured after administration of various doses of buprenorphine by various routes to several species (Walter and Inturrisi, 1995).

SPECIES	ROUTE	DOSE, $\mu\text{g}/\text{kg}$	T_{max} , min.	Plasma C_{max} ^a	Brain C_{max} ^a
Rat	oral	100	10	5	2
	oral	20,000	10	66	249
	i.m.	20	10-15	4	6 or 14
	i.m.	5,000	15	370	
	s.l.	20	30	0.4	5
	s.l.	200	60	1.1	
Rabbit	i.m.	5,000	15	132	
Dog	oral	100	60-120	2-3	
	oral	800	30-60	10-14	
	i.m.	20	~ 15	3	
Baboon	oral	40	120	0.4	
	i.v.	5,000	4	2,290	
	i.m.	2	~ 15	0.8	
	i.m.	5,000	30	805	
Rhesus monkey	oral	15	60	0.8-3	0.3
	i.m.	2	~ 15	0.7	4
Cynomolgus monkey	i.m.	38	15	20	
	s.l.	38	120	4	
	b.u.	38	120	4	

^aExpressed as ng/ml or ng/g.

Dose-concentration relationships. The relationship between dose, route and maximum plasma levels of buprenorphine has been investigated in several species, and some studies with rats and rhesus monkeys have also measured brain levels of buprenorphine (Table 5). In general, these studies demonstrated relatively rapid achievement of peak plasma concentrations (approximately 15 minutes) after i.m. injection, with slower achievement of peak concentrations (up to 2 hours) after sublingual or oral administration.

Clinical *in vitro* studies (literature report): Recent studies with 18 human liver microsomal preparations and 10 heterologously expressed cytochrome P450 enzymes have identified cytochrome P450 3A4 as the major P450 enzyme responsible for the N-dealkylation of buprenorphine to norbuprenorphine (Iribarne *et al.*, 1997).

Clinical *in vivo* studies (literature reports): The volume of distribution of buprenorphine in adults after i.v. administration was reported to be 97 to 188 liters (Bullingham *et al.*, 1983). A comparison of oral with i.v. administration indicated a mean oral bioavailability of about 15% (Bullingham *et al.*, 1980). The mean bioavailability after s.l. administration is better (29-55%), but there is wide variation (16-94%) among subjects (Bullingham *et al.*, 1982; Kuhlman *et al.*, 1996; Mendelson *et al.*, 1997). The systemic clearance of buprenorphine is high enough (> 1000 ml/minute) to be suggestive that it depends upon hepatic blood flow, and thus, is largely dependent upon hepatic clearance. An anesthesia-induced decrease in hepatic blood flow, for example, could be expected to result in increased plasma concentration of buprenorphine (Bullingham *et al.*, 1980). Trough serum concentrations of buprenorphine and norbuprenorphine were measured in a pregnant woman maintained on 4 mg/day a few days before giving birth and in the newborn's serum about 20 hours after birth (Marquet *et al.*, 1997). Serum buprenorphine was about six times greater in the newborn (1.9 ng/ml) than in the mother (0.3 ng/ml), whereas the norbuprenorphine concentrations were comparable, being 1.7 ng/ml in the newborn and 2.3 ng/ml in the mother. The higher serum buprenorphine:norbuprenorphine ratio in the newborn (1.1) than in the mother (0.13) was attributed to immaturity of hepatic drug metabolism in the newborn (Marquet *et al.*, 1997).

TOXICOLOGY:

Single dose toxicity in rodents. Since buprenorphine has been marketed for over 15 years, its (historical) preclinical acute toxicity has been defined in terms of LD₅₀'s, as exemplified in Table 6 (Vocci, 1980, p. 30).

Table 6: Route dependence of LD₅₀'s for buprenorphine in rodents.

SPECIES	ROUTE	Mean LD ₅₀ , mg/kg	Range, mg/kg
Mice (female)	Oral	260	223-304
	Intraperitoneal	90	64-126
	Intravenous	28	26-31
	Subcutaneous	>600	
Rats (female)	Oral	>600	
	Intraperitoneal	288	214-387
	Intravenous	31	26-37
	Subcutaneous	>600	
	Intramuscular	>100	

TITLE: Acute intravenous toxicity study in the mouse: buprenorphine and identified impurities

REPORT NO.: 2204-46/31 (b)(4)

STUDY DATES: September to December, 1979

SPECIES/STRAIN: Mouse, Swiss Albino (b)(4)

Acute i.v. toxicities of buprenorphine and several identified impurities were assessed in mice, about 5 weeks old at the time of receipt from (b)(4), the males weighing 26-38 grams and the females weighing 21-26 grams at the time of drug administration. Preliminary dose-finding experiments were conducted with buprenorphine and six identified impurities, during which mice were observed for mortality for 7 days. Doses of four of the impurities were limited by their solubilities in the vehicle. No deaths occurred in mice treated with the impurities, (b)(4) or the vehicle control. In the main experiments, mice (5 male and 5 female per dose) were observed for mortality for 14 days, after which survivors were euthanized with pentobarbital and necropsied. The results are summarized in Table 7.

At necropsy, the observation of lesions were infrequent and most involved the lungs, particularly in the mice receiving buprenorphine. Two female mice that received (b)(4) or highest doses of (b)(4) 42 or 60 mg/kg, had opacity of one eye. One mouse that received (b)(4), 21 mg/kg (second highest dose) showed "accentuated liver lobular patterns." One male mouse in each group receiving the 70.8 mg/kg (second highest) dose of buprenorphine and the 60 mg/kg (high) dose of (b)(4) had enlarged spleen. None of the vehicle-treated mice showed any of these lesions.

Conclusion: Both of the impurities, (b)(4) (which is also a metabolite of

buprenorphine) and its (b)(4), are more potent than buprenorphine for causing lethality in mice.

Table 7: Acute i.v. toxicity of buprenorphine and congeners in mice (NDA, Vol. 1.7, tab 6).

COMPOUND ^a	Relationship to Buprenorphine	Lot No.	LD ₅₀ (95% C.I.)
Buprenorphine	Main drug	PP 1101/7	56 mg/kg (42-74)
(b)(4)	(b)(4)	BI 641/1	16 mg/kg (13-19)
		MR 1576/1	34 mg/kg (28-42)
		BI 640/1	> 40 mg/kg
		BI 645/2	> 40 mg/kg
		BI 729/7	> 40 mg/kg
		BI 735/3	> 20 mg/kg
Vehicle ^b	Control		> 20 ml/kg

^aChemical structures given in DMF 12,412 (Reckitt & Colman, 3/18/97).

^b(b)(4) citric acid in (b)(4) dextrose adjusted to pH 4.0 with 0.1M NaOH.

TITLE: Buprenorphine oral toxicity study in beagle dogs (final report: repeated daily dosage for 52 weeks)

REPORT NO.: RKT 77/80367 (b)(4)

STUDY DATES: 22 February 1979 to 25 February 1980.

SPECIES/STRAIN: Dog, pure-bred beagle (b)(4)

Beagle dogs between 16 and 19 weeks of age and weighing 7-9 kg were allocated to four treatment groups to yield similar mean weights/group and to disperse litter mates evenly among the groups, which contained four dogs of each sex per group. Thus, the dogs were not randomized. In addition, some switching of dogs between groups occurred after clinical chemistry measurements, but before commencement of treatment, and one such switch was discovered on day 5 of treatment to have been erroneous, resulting in another switch. Each group of dogs received daily oral doses of buprenorphine HCl, 0 (empty gelatin capsule), 0.2, 3.5 or 75 mg/kg, seven days/week for 52 weeks (NDA, Vol. 1.9, tab 27).

Two dogs were euthanized for humane reasons prior to the end of treatment. One that had received buprenorphine, 75 mg/kg/day, was euthanized on day 58 of treatment due to marked deterioration of clinical condition, including subdued behavior, inability to stand and development of convulsions. Autopsy indicated a GI tract full of dark green/black-stained sawdust, a pale and mottled surface of the liver, minimal number of papilliform projections of the mucosal surface in the gall bladder, and moderate congestion of all lobes of the lungs. Histological

examination revealed minimal bile duct proliferation, finely vacuolated hepatocytes, trace fat deposition and decreased glycogen in the liver, which were considered to be related to treatment. The second dog had received buprenorphine, 0.2 mg/kg/day, and was euthanized on day 341 (week 49) of treatment, after undergoing a precipitous 2-kg body weight loss during week 45 and further loss during week 48. Autopsy indicated multiple small dark foci in the mucosa of the stomach antrum, moderate congestion of the left A-V valve in the heart, and marked atrophy of the acinar portion of the pancreas. This atrophic pancreatitis was not considered to be drug-related. Aside from the two dogs that were euthanized, various clinical signs during treatment and findings from terminal studies in beagle dogs (male and female combined) are summarized in Table 8.

Table 8: Clinical signs during one-year oral toxicity study in beagle dogs.

Time(s) observed	Observation	Treatment, mg/kg/day			
		0	0.2	3.5	75
Day 1	Vomiting after dose administration		+	+	+
Week 1	Excessive salivation on isolated occasions		+	+	+
Week 1	Transient body weight loss		+	++	+++
Weeks 1-2	Dose-related decrease in appetite		+	++	+++
Weeks 1-2	Muscular tremors seen occasionally		+	+	+
Weeks 1-3	Dose-related abnormal quietness		+	++	+++
by Week 8	Red rash on skin of abdomen/inner limbs	+	+	+	+
Various	Decreased water consumption				+
Various	Elevated group mean urinary pH			+	+
Various	Occasional failure to drink daily milk		+	+	++
Pathology	Enlarged prostate			+	+
Histology	Mild to moderate bile duct hyperplasia with associated peribiliary fibrosis			1 dog	all dogs

Although the buprenorphine-treated dogs recovered from the weight loss that occurred during the first week of treatment ($p < 0.01$ for the middle and high doses), the control groups remained the heaviest during the year, particularly the control females, which continued to gain weight at a higher rate than the buprenorphine-treated females.

Significant elevations in urinary pH from the corresponding control values

were seen in groups receiving the 0.2 mg/kg/day dose of buprenorphine at week 12, the 3.5 mg/kg/day dose of buprenorphine at weeks 25 ($p < 0.01$), 40 ($p < 0.001$) and 51 ($p < 0.001$) and the 75 mg/kg/day dose of buprenorphine at weeks 25 ($p < 0.05$), 40 ($p < 0.001$) and 51 ($p < 0.001$). The increases in urinary pH were considered to be treatment-related.

There were no general treatment-related changes in organ weights, but certain statistically significant sex-specific differences were observed, as indicated in Table 9.

Table 9: Significant effects of oral buprenorphine on organ weights or relative organ weights in a one-year chronic toxicity study in beagle dogs.

SEX	ORGAN	Dose ^a	Parameter, grams or % of Body Wt.	#/dose	P-value
M	Prostate	0	5.72 ± 1.46 g	4	---
		0.2	6.04 ± 2.13 g	4	NS
		3.5	11.46 ± 2.90 g	4	0.0124
		75	9.23 ± 2.12 g	3	0.0477
F	Kidneys	0	0.42 ± 0.06 %	4	---
		0.2	0.53 ± 0.07 %	3	NS
		3.5	0.56 ± 0.04 %	4	0.0099
		75	0.51 ± 0.04 %	4	0.0498
F	Adrenals	0	0.0122 ± 0.0032 %	4	---
		0.2	0.0149 ± 0.0015 %	3	0.0147
		3.5	0.0153 ± 0.0026 %	4	0.0254
		75	0.0152 ± 0.0026 %	4	0.0268

^aExpressed as mg/kg/day, p.o. Each value is expressed as the mean ± S.D.

The prostate weights in the 3.5 and 75 mg/kg/day dosed males were significantly increased compared with the control mean at the $p = 0.0124$ and $p = 0.0477$ levels, respectively (calculated by FDA statistician, Jonathan Ma). This finding was reported to be "fortuitous", "as histological examination revealed no toxicologically significant changes in the prostate." Reports of microscopic findings mentioned only four prostates: two with small areas of subepithelial mononuclear aggregates in prostatic urethra (from 0.2 and 75 mg/kg/day dogs); one "enlarged with multiple small and large areas of follicular prostatitis" and one "apparently enlarged with prominent parenchymal structure" (from two dogs receiving 3.5 mg/kg/day).

Although not mentioned by the sponsor, this reviewer calculated significant changes in kidney and adrenal weights for the buprenorphine-treated female dogs, compared with placebo-treated females, when expressed as a percentage of body weight. Significant differences in these parameters were not observed in the males. Mild kidney pathology (e.g., foci dystrophic mineralization in the papillae; fat deposition in cortical tubules) was observed in both sexes from all groups, which did not appear to be related to treatment. Adrenals were only mentioned among the microscopic findings for three dogs: one control female had small areas of extracapsular cortical tissue; one female treated with 3.5 mg/kg/day had a few small areas of cortical "nodular hyperplasia" in one gland; and one male treated with 75 mg/kg/day had a small area of intracapsular cortical tissue in one gland. *Conclusion:* The most biologically significant drug-related finding was the mild to moderate bile duct hyperplasia with associated peribiliary fibrosis in all dogs receiving the high dose of buprenorphine (75 mg/kg/day, p.o.), indicating a hepatotoxic effect of this dose. As one dog receiving the middle dose (3.5 mg/kg/day) also showed this effect, the NOAEL for this effect appeared to be the low dose, 0.2 mg/kg/day. However, other adverse effects were occasionally noted at the low dose (Table 8). No mechanisms were suggested for the significant elevations in urinary pH or enlarged prostates.

TITLE: Chronic (fifty two week) oral toxicity study of buprenorphine in dogs.
PROJECT NO.: 758.09.00-BD [REDACTED] (b) (4)

STUDY DATES: March, 1987, to May, 1988.

SPECIES/STRAIN: Dog, pure-bred beagle [REDACTED] (b) (4)

LOT NO.: 39, 39C and 39D.

Beagle dogs between 18 and 25 weeks of age and weighing 7.1-10.2 kg (on arrival) were acclimatized for 56 days before being allocated to four treatment groups to yield similar mean weights/group and to distribute litter mates evenly among the groups, which contained four dogs of each sex per group. Thus, the dogs were not randomized. Each group of dogs received daily oral doses of buprenorphine HCl, 0 (gelatin capsule containing lactose), 0.2, 3.5 or 75 mg/kg, seven days/week for 52 weeks. The parameters measured included mortality, clinical observations (daily), food consumption (daily), body weight (weekly), hematological parameters, coagulation test, blood chemistry and urine composition/cellularity (two baselines and weeks 4, 12, 25, 38 and 51), electrocardiograms (weeks 15, 26, 39 and 52), ocular examinations (every 13 weeks), organ weights and histology (at necropsy). Individual doses were adjusted weekly (and weighed into gelatin capsules separately for each animal) according to the most recent body weight (NDA, Vol. 1.10, tab 28).

Two of the dogs receiving the 75 mg/kg/day dose were euthanized, one

female during week 31 and one male during week 36 of the study. The female's body weight had decreased from 12.7 kg at week 21 to 10.0 kg, during which it had elevated liver enzymes in plasma, decreased plasma protein and anemia, and it exhibited diminished physical activity, with stiffness of limb movement. Necropsy of the female showed evidence of hepatic injury (marked bile duct proliferation, minimal fibrosis, and capillary proliferation confined to the portal tracts) as a primary abnormality. This dog also showed necrosis and ulceration of the small intestinal mucosa. Although the male dog also had weight loss, elevated liver enzymes and bile duct proliferation (moderate) with minimal fibrosis, the primary cause of illness was determined at necropsy to be bronchial pneumonia with abscess formation in ribs, bronchial lymph nodes and thymus.

After the treatment period, all surviving dogs were fasted overnight and euthanized by exsanguination under deep anesthesia with sodium pentobarbital. Among the organs that were weighed at necropsy (adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostate, testes, thyroid, uterus), the only significant difference observed was in testes from the males treated with the 3.5 mg/kg/day dose. Mean weights (\pm S.D., $n=4$) of testes from the control, 0.2, 3.5 and 75 mg/kg/day treatment groups were 19.0 ± 2.78 , 17.6 ± 3.87 , 27.0 ± 1.48 and 21.3 ± 1.48 grams, respectively (NDA, Vol. 10, tab 28, Table 23). This difference remained significant at the $p < 0.01$ level when the testes weights were expressed as ratios to body weight or brain weight. In contrast to the significant changes in prostate weight observed in the earlier one-year oral chronic toxicity studies using the same doses in pure-bred beagles, no significant increases in prostate weights were observed in this study. Mean (\pm S.D.) weights of the prostate for the control, low, medium and high dose groups ($n=4$ /group) were 9.03 ± 2.53 , 7.32 ± 3.04 , 9.93 ± 3.22 and 8.73 ± 1.85 , respectively. In the female dogs, significant and dose-related increases in mean kidney weights were noted, but only when expressed relative to body weights. Similarly, a significant increase in mean adrenal weight, relative to body weight, was noted in the 75 mg/kg/day females. Although not statistically significant, the increases in absolute adrenal weights in females appeared to be dose-related, averaging 1.72, 1.80, 1.92 and 2.07 grams per pair in the control, 0.2, 3.5 and 75 mg/kg/day treatment groups, respectively. Similar kidney and adrenal weight differences were not observed in the males.

In the dogs that were not euthanized prematurely, no treatment-related changes were noted in food consumption, hematological parameters, urine composition or cellularity, EKG indices of cardiac function, during ocular examinations, and blood chemistry, except for two dogs receiving the 75 mg/kg/day dose that displayed elevations in plasma alkaline phosphatase and/or ALT activities at 3 or 4 different testing periods. These increases are consistent with the post mortem finding of the treatment-related histopathology of bile duct proliferation observed in all dogs treated with the 75 mg/kg/dose, some of which also showed hemosiderosis, fibrosis and capillary proliferation in the portal tracts.

Unlike the significant increases in urine pH observed in the earlier one-year oral chronic toxicity studies using the same doses in pure-bred beagles, no significant increases were observed in urine pH when measured at 12, 25, 38 or 51 weeks of treatment. Significant elevations in urinary pH from the corresponding control values were observed only at the 4-week testing period in groups receiving the 3.5 and 75 mg/kg/day doses of buprenorphine ($p < 0.01$). At this time period, there was a dose-related increase in the mean (\pm S.D., $n=8$) urine pH's for the 0.2, 3.5 and 75 mg/kg/day treatment groups of 6.94 ± 0.82 , 7.19 ± 0.53 and 7.31 ± 0.65 , respectively, compared with mean urine pH of the control group 6.44 ± 0.32 .

Conclusion: Identification of the target organ of toxicity as the hepatobiliary system (bile duct proliferation, fibrosis and hemosiderosis), as observed in the first one-year chronic toxicity study with the high dose (75 mg/kg/day, p.o.), was confirmed in this study, as were significant changes in adrenal and kidney weights, relative to body weight of females. However, chronic elevation of urine pH and increased prostate weights reported for the middle and high buprenorphine doses in the first study were not confirmed. The NOEL of oral buprenorphine hydrochloride in this study was indicated by [REDACTED] ^{(b) (4)} to be 0.2 mg/kg/day.

Reprotoxicity in rats. In a study reported in the literature, prenatal administration of buprenorphine to rats via osmotic minipumps was carried out at three doses (0.3, 1.0 and 3.0 mg/kg/day) from day 8 of gestation to parturition (Hutchings *et al.*, 1995). Buprenorphine produced a dose-related decrease in maternal water intake, but had no effect on maternal weight gain, the frequency of fetal resorption, or on birth weight of the pups. It had no teratogenic effect, no effect on perinatal mortality, and effects on postnatal growth were inconsistent.

TITLE: Effect of buprenorphine hydrochloride on pregnancy of the rat (oral administration)

REPORT NO.: RC 82211 [REDACTED] ^{(b) (4)}

STUDY DATES: April 7, 1982 to April 26, 1982 (period of animal treatment)

SPECIES/STRAIN: Rat, [REDACTED] ^{(b) (4)} (CrL: [REDACTED] ^{(b) (4)} CD(SD) BR strain)

Buprenorphine HCl (lot no. 11) was given to groups of 25 rats, weighing 169-207 grams, in single daily doses of 0, 40, 80 or 160 mg(base)/kg, on Days 6 through 15 of gestation. The drug was administered by gastric intubation as a suspension in 1% aqueous methylcellulose. All rats were weighed on Days 1, 3, 6, 8, 10, 14, 17 and 20 of gestation. On Day 20 of gestation, the rats were euthanized by CO₂ asphyxiation, following which they were dissected and examined for congenital abnormalities and macroscopic pathological changes in maternal organs. Corpora lutea were counted, litter numbers and weights were determined and fetuses were examined for skeletal and visceral abnormalities. At all test doses of buprenorphine, dams had swelling and reddening of the fore-feet and tail, showed transient salivation, brown discoloration of the coat, hair loss and red discoloration of the urine. Transient darkening of the eyes occurred after the

second and third doses of 80 and 160 mg/kg/day. Dams also had retardation of body weight gain of similar magnitude at all doses (23-34% of control on day 10, 53-55% of control on day 14, 67-70% of control on day 17 and 71-73% of control on day 20 of gestation). Fetal weights were significantly higher ($p < 0.01$) in groups receiving 40 and 160 mg/kg/day than in the control group. However, no significant effects of this treatment were observed on litter size, *in utero* deaths, skeletal malformations or visceral anomalies, indicating a lack of embryotoxicity and teratogenicity in oral buprenorphine doses up to 160 mg/kg/day, which can be considered the NOAEL for genotoxicity in rats.

TITLE: Effect of buprenorphine hydrochloride on fertility and general reproductive performance of the rat (oral administration)

REPORT NO.: CSR 07206 (b)(4)

STUDY DATES: November 28, 1984 to July 31, 1985

SPECIES/STRAIN: Rat, (b)(4) (CrL: (b)(4) CD(SD) BR strain)

Buprenorphine HCl (lot no. 22) was given to groups of 15 male rats weighing 171-217 grams, and groups of 30 female rats (same strain), weighing 164-209 grams, in single daily doses of 0, 0.67, 7.2 or 80 mg/kg. The drug was administered by gastric intubation (10 ml/kg body weight) as a suspension in 1% aqueous methylcellulose. The males were treated for 9 weeks prior to pairing, during mating, and up to euthanasia. Females were treated for 2 weeks prior to pairing, during mating and through euthanasia either on gestational day 20 or post partum day 21. Experimental parameters measured in parent rats included clinical signs (daily), mortality, food consumption (weekly), water consumption (during two weeks prior to mating), body weight in males (weekly), body weight in females (daily during gestation and weekly post partum), pregnancy rate, mating performance and gestation period. In dams euthanized on Day 20 of gestation, the number of corpora lutea, number and distribution of live young and of embryonic/fetal deaths, individual fetal weights, and gross fetal abnormalities were determined. Term litters were examined for the following developmental stages: surface righting reflex, startle reflex, air righting reflex and pupillary reflex. After weaning, they were also examined for body weight gain (weekly), general (Actimat test) and coordinated (accelerating rotorod test) motor activity (at 6 weeks of age), passive avoidance learning (at 7 weeks of age) and reproductive capacity (about 12 weeks of age), except for those from parents treated with buprenorphine, 80 mg/kg/day, which were euthanized at about 6 weeks of age. Around the time of weaning, all F2 pups and F1 parents were euthanized and examined for external and internal abnormalities.

Dose-related signs of treatment of the F0 parents included salivation, dry tails, and erythema and swelling of the paws; decreased water consumption; and reduced food consumption and weight gain, especially among the males. Females showed an increase in perinatal weight loss, and one female dosed at 80 mg/kg/day died two days after parturition.

Among the dams euthanized on day 20 of gestation, the litter data showed no significant effects of the treatments. Among the dams allowed to complete gestation, there were no significant differences in litter sizes at birth, but there was a dose-related increase in the incidence of total litter losses post partum which, in order of increasing dosage, was 0/16, 2/16, 3/16 and 7/15 (not including the dam that died two days after parturition, which had a total litter loss one day after parturition). Thus, in terms of postnatal viability, no NOAEL was established by this study.

During the pre-weaning period, all offspring in all litters were examined to determine the age at which the following behaviors developed, and no significant differences among treatment groups were observed when age was expressed as days post-coitum (NDA, Vol. 1.11, tab 39, Table 13): 1) Surface righting reflex (25.1-25.6 days); 2) startle reflex (34.9-35.5 days); 3) air righting reflex (37.5-38.1 days; and 4) pupil reflex (100% on Day 20). On Day 21 after birth, 12 male and 12 female pups were selected from each treatment group for further study. Citing the low number of litters from the 80 mg/kg/day treatment group to chose pups from, however, the contract laboratory abandoned further study of this F1 group at about Week 6. Consequently, post-weaning group mean weights were only presented (NDA, Vol. 1.11, tab 39, Table 15) for weeks 4, 5 and 6 of age, as shown in Table 10.

Table 10: Body weights of F1 male and female offspring post weaning from parents treated with buprenorphine prior to mating and dams through post partum day 21.

WEEK	Parental buprenorphine dosing history (mg/kg/d) for F1 groups							
	Males (mean body weight in g)				Females (mean body weight in g)			
	0	0.8	8	80	0	0.8	8	80
4	84	86	93	83	79	77	86	72
5	138	139	151	134	120	115	126	111
6	199	199	216	197	152	145	158	143

The remaining F1 generations, i.e., those derived from the 0, nominal 0.8 (actual, 0.67) and nominal 8 (actual, 7.2) mg/kg/day treated F0 parents, were examined with regard to 1) weekly weight gain; 2) onset of vaginal opening in the F1 females; 3) activity (low, high and total) measured over a 5-minute test period with an Actimat (ARS Electronic Equipment; Marsden and King, 1979) at the age of 6 weeks; 4) performance on the accelerating rotarod test at the age of 6 weeks (Jones and Roberts, 1968); 5) performance on the one-trial passive avoidance test at the age of 7 weeks (Jarvik and Kopp, 1967); and 7) assessment of reproductive capacity.

There were no significant differences among the F1 of treatment groups with respect to weight gain, onset time for vaginal opening, rotarod performance, passive avoidance latency, pregnancy rate of F1 females (100% for all groups), duration of gestation in F1 females (21.8-22.0 days) or sex ratio in offspring of F1 rats at birth. There was no obvious adverse effect of drug treatment on macroscopic abnormalities among the offspring at terminal autopsy. F1 females from buprenorphine-treated groups exhibited a statistically significant ($p < 0.05$) increase in low level activity above controls, but the differences between groups were not large enough ($< 4\%$) to be of biological significance. The mean number of live pups per litter was slightly smaller in both buprenorphine treatment F1 groups, but the differences were not supported by a significant "H" statistic. The mean % male/litter at weaning was higher ($p < 0.05$) in the 8 mg/kg/day treatment F2 offspring (55.4%) than in the 0.8 mg/kg/day (44.1%) or control (46.5%) F2 offspring.

Conclusion: The treatment of rats with buprenorphine doses of 0.67 or 7.2 mg/kg/day did not result in ~~reproductive toxicity~~ in the F1 generation. It is not clear why the study of F1 rats from the 80 mg/kg/day treatment group was aborted when the F1 rats were about ready for activity and rotarod testing at six weeks of age. In terms of postnatal viability, no NOAEL was established by this study, as even the low dose (0.67) resulted in a decrease in postnatal viability.

TITLE: Effect of buprenorphine hydrochloride on peri- and post natal development of the rat (oral administration)

REPORT NO.: RKT 230/85521 (b)(4)

STUDY DATES: December 21, 1984 to February 3, 1985

SPECIES/STRAIN: Rat, (b)(4) (CrL: (b)(4) CD(SD) BR strain)

Buprenorphine HCl (lot no. 22) was given to groups of 25 gravid female rats 8 to 9 weeks of age in single daily oral doses of 0, 0.8, 8, and 80 mg/kg (based on body weights at day 15), from Day 15 of pregnancy to Day 21 post partum. On or shortly after post partum Day 21 (weaning), dams and their litters were euthanized and examined for external and internal abnormalities.

Dose-related signs of treatment in the dams included erythema and swelling of the paws and reddened tails during the first 6 days of treatment, associated with later scab formation on the tails; post-dosing salivation; decreased water consumption, decreased food consumption, and reduction in weight gain.

The incidence of total litter losses, which occurred within the first 4 days post partum, was 1/21, 0/23, 5/24 and 5/25 at buprenorphine doses of 0, 0.8, 8 and 80 mg/kg/day, respectively. The one affected control dam, however, was found to have a uterine abnormality and thus was eliminated from control group calculations. Dose-related effects of treatment included increase in pup mortality up to post partum Day 4, even with exclusion of total litter losses; decrease in litter size; and reduction in litter weight, significant at all doses by Day 4. Pups from

dams treated with the 80 mg/kg/day dose displayed significant ($p < 0.05$) delays in the occurrence of the surface righting reflex and startle response. Three pups from two litters in this treatment group showed slight swelling of the forepaws and one from a different litter showed swelling of one hind limb. Other abnormalities observed in the offspring of drug-treated groups that were not observed in the control group were two pups with tip of tail discolored black on day 1 and tail foreshortened at autopsy (0.8 and 80 mg/kg/day) and one pup with tail missing at birth (8 mg/kg/day); and total situs inversus in two pups (0.8 and 80 mg/kg/day).

TITLE: Effect of buprenorphine hydrochloride on pregnancy of the rabbit (oral administration)

REPORT NO.: RKT 200-R/821016 (b) (4)

STUDY DATES: April 20, 1982 to May 18, 1982 (preliminary study)
May 18, 1982 to June 25, 1982 (teratology study)

SPECIES/STRAIN: Rabbit, New Zealand white (b) (4)

Buprenorphine HCl (code no. M60029A, lot no. 11) was given to groups of 16 rabbits weighing 2.8-4.4 kg, in single daily doses of 0, 1, 5 or 25 mg(base)/kg, on Days 6 through 18 of gestation. The drug was administered by gastric intubation (5 ml/kg) as a suspension in 1% aqueous methylcellulose. All rabbits were weighed on Days 1, 6, 8, 10, 14, 19, 23 and 29 of gestation. On Day 29 of gestation, the rabbits were euthanized by cervical dislocation, following which they were dissected and examined for congenital abnormalities and macroscopic pathological changes in maternal organs. After external examination, offspring were euthanized by intrathoracic pentobarbital injection, weighed, sexed and examined for visceral abnormalities. Suspected abnormalities were confirmed by further microdissection or histopathology. Rabbits given 5 and 25 mg/kg doses showed anorexia and/or reduced fecal output. A decrease in body weight gain occurred during the first two days of 5 mg/kg dosing and weight loss occurred during the first four days of 25 mg/kg dosing. Decreased body weight gain also occurred in does receiving the two higher doses during the period (days 19-23) following withdrawal of buprenorphine. There was a trend for lower implantation rates in the buprenorphine-treated groups, reported to be significant by the Jonckere test ($p < 0.05$), which in turn, led to significantly lower live litter size, litter weights, but higher mean weights of live pups.

Table 11 summarizes the individual litter data regarding total embryonic deaths relative to the number of implants for each treatment group. These two parameters were analyzed statistically (by Jonathan Ma, FDA statistician) in a 2x4 table, and the resulting Chi-square statistic (with 3 degrees of freedom) resulted in a probability value of 0.056, indicating borderline significance.

Table 11: Embryotoxicity in rabbits treated with buprenorphine during days six through 18 of gestation (from NDA, Vol. 1.11, tab #36, Appendix 5, pp. 46-49).

Buprenorphine dose, mg/kg/day	No. of gravid rabbits*	Embryonic deaths	No. of implants	% Loss
0	13	8	135	5.9%
1	15	6	130	4.6%
5	14	16†	122	13.1%
25	12	9‡	87	10.3%

*Two rabbits were nongravid in the 0, 5 and 25 mg/kg treatment groups. Does not include rabbits killed prior to end of study.

†Includes total abortion (5/5 implants) in rabbit #314.

‡Includes total resorption (2/2 implants) in rabbit #411.

The number and percentage of skeletal anomalies tended to be higher among all drug-treated groups (Table 12), but differences from the control group were reportedly not significant ($p > 0.05$) by the Kruskal-Wallis test. A 2X4 Chi-square test (performed by Jonathan Ma) resulted in a p-value of 0.059. It was also reported that the mean incidence of fetuses with extra (13th) thoraco-lumbar ribs or variant sternbrae were not associated with treatment.

Table 12: Teratogenicity study of buprenorphine treatment of rabbits during gestational days six through 18 (from NDA, Vol. 1.11, tab. #36, Table 7).

Oral dose of Buprenorphine (mg/kg/day)	No. of Rabbit Litters	No. of Fetuses Examined	% of Examined Fetuses Showing:		
			Malformations	Anomalies at:†	
				Gross Autopsy	Skeletal examination
0	13	126	2.5%	1.5%	11.9%
1	15	124	1.9%	8.6%	18.5%*
5	13	106	1.7%	2.2%	25.5%*
25	11	78	3.3%	2.3%	21.8%*

†Excludes fetuses showing malformation (in sponsor's calculations only).

*Reviewer's calculation of %, which does not match that of sponsor's in NDA.

Conclusion: There was no significant teratogenicity of oral buprenorphine in doses up to 25 mg/kg/day. There were tendencies toward increased embryotoxicity and skeletal abnormalities in the drug-treated groups, but both observations were only of borderline significance ($0.05 < p < 0.06$).

NDA 20-732

Genotoxicity:

TITLE: Mutagenicity studies on buprenorphine hydrochloride.
REPORT NO.: RPEX 30000079 (b) (4)
STUDY DATES: May 25, 1983 to June 1, 1983
SPECIES/STRAIN: *Saccharomyces cerevisiae* MP-1

Concentrations of buprenorphine HCl (lot no. 13) ranging from 1 µg/ml to 10 mg/ml were compared with vehicle (DMSO) in this strain of yeast for recombinant, gene convertant and forward mutation-inducing properties in the absence and presence of metabolic activation (±S9). Buprenorphine inhibited yeast growth in the S9-free medium at concentrations above 1 mg/ml, but did not induce any recombinants (complete agar medium), gene convertants (tryptophan agar medium) or forward mutants (actidione agar medium).

TITLE: Mutagenicity studies on buprenorphine hydrochloride.
REPORT NO.: RPEX 30000079 (b) (4)
STUDY DATES: March 21, 1983 to March 28, 1983
SPECIES/STRAIN: *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538.

The Ames test was conducted with buprenorphine (lot no. 13) concentrations ranging from 10 µg/plate to 1 or 5 mg/plate, in the absence and presence of metabolic activation (±S9). The TA100 and TA 1535 strains are capable of detecting base-pair type mutagens and the other three strains are capable of detecting frame-shift type mutagens. The incubation time was 72 hours, and the positive control consisted of 100 µg per plate of 2-aminofluorene with 8% or 16% S9 mixture. As shown in Table 13, there appeared to be a stimulation of revertants in the TA98 strain and, to a lesser extent, in the TA1538 strain by the 5 mg/plate concentration of buprenorphine. This concentration was not tested in the TA100 and TA1537 strains, the highest concentration tested in the latter two strains being 1 mg/plate. Dr. (b) (4) concluded that the results from test strains TA98 and TA1538 indicate a mutagenic activity for buprenorphine HCl at and above its threshold for cytotoxicity and that this mutagenic activity is likely caused by a frame shift.

Table 13: Mutagenicity study of buprenorphine in five Salmonella TA strains.

Test strain	%S9 used	Mean Number of Revertants per Plate*					SIG/ NS †	+ cont. 100 µg
		Vehicle	10 µg	100 µg	1 mg	5 mg		
TA98	0	37.0	32.7	36.3	30.7	902.7	NS	N.T.
	0	34.0	25.3	34.7	27.0	533.3	NS	N.T.
	8	50.4	60.3	58.7	52.3	733.3	SIG	>500
	16	52.2	44.0	77.7	64.0	454.7	SIG	>500
TA100	0	131.2	128.7	119.3	155.3	N.T.	NS	N.T.
	0	124.6	135.3	138.3	155.3	N.T.	SIG	N.T.
	8	125.4	142.0	134.3	145.0	N.T.	NS	>500
	16	160.2	141.7	163.3	160.7	N.T.	NS	>500
TA1535	0	56.6	47.0	53.7	41.7	N.T.	NS	N.T.
	0	50.8	47.7	43.3	42.0	N.T.	NS	N.T.
	8	22.2	17.3	22.3	18.0	19.3	NS	58.0
	16	21.6	26.7	20.3	14.3	32.3	NS	53.0
TA1537	0	12.8	12.7	15.7	N.T.	N.T.	NS	N.T.
	0	18.0	16.7	18.7	9.0	N.T.	NS	N.T.
	8	21.2	25.3	30.0	N.T.	N.T.	NS	115.0
	16	35.2	29.7	36.0	19.7	N.T.	NS	178.3
TA1538	0	26.6	33.3	33.7	26.0	127.0	SIG	N.T.
	0	33.2	31.7	23.0	20.3	94.0	NS	N.T.
	8	45.0	64.7	50.3	40.7	122.7	NS	>500
	16	47.2	53.3	56.0	44.3	48.7	NS	>500

*Means were calculated from individual plate data for vehicle (n=5) and buprenorphine (n=3/conc.) treatments cited in NDA, Vol. 1.12, tab 45, Tables 5-26.

†Significance (SIG) or nonsignificance (NS) were determined by the Jonckheere Test (one-way ANOVA with ordered alternatives). Shaded values indicate significant differences ($p < 0.050$) from vehicle control by the Wilcoxon multiple comparisons test. N.T. = Not Tested.

TITLE: Mutagenicity studies of buprenorphine hydrochloride in in vitro bacterial systems

REPORT NO.: RC 8532

(b) (4)

preliminary study Report #002275; main study Report #000558)

STUDY DATES: January 21, 1981 to March 14, 1981

SPECIES/STRAIN: *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538; *E. coli* strain WP2uvrA; *Bacillus subtilis* strains M45Rec⁻ and H17Rec⁺.

In a preliminary study of reverse mutation (Ames test) conducted by the Japanese testing facility, buprenorphine was tested at concentrations ranging from 0.5 to 5000 $\mu\text{g}/\text{plate}$ in five strains of *Salmonella typhimurium*, as well as *E. coli* strain WP2uvrA, in the absence or presence of the S-9 fraction from rat liver. The results of this preliminary study, shown in Table 14, were that buprenorphine was

Table 14: Preliminary mutagenicity study of buprenorphine in 5 *Salmonella* strains and one *E. coli* strain (from NDA, Vol. 1.12, tab 46, pp. 11-16)

Test strain	S-9	Revertants per Plate by Buprenorphine, μg						+ cont	+ cont (2AA) ^f
		Vehicle	0.5	5	50	500	5000		
TA98	-	2*	9	12	11	8	2	86 ^a	9(0.5)
	+	14	26	12	16	13	12	N.T.	210(0.5)
TA100	-	88	74	79	66	34	0	563 ^b	78(0.5)
	+	88	90	77	101	69	89	N.T.	365(0.5)
TA1535	-	12	14	12	4	3	6	138 ^c	12(2)
	+	13	13	14	9	5	4	N.T.	174(2)
TA1537	-	9	8	7	2	0	0	658 ^d	11(2)
	+	8	24	9	14	0	2	N.T.	239(2)
TA1538	-	4	7	4	4	0	0	172 ^e	6(0.5)
	+	29	27	29	21	15	10	N.T.	354(0.5)
WP2uvra	-	9	4	9	6	6	0	1009 ^g	6(80)
	+	9	10	10	14	7	11	N.T.	101(80)

*Reported as unusually low, with normal values in the 12-20 range. N.T. = Not tested. ^a2-Nitrofluorene, 1 $\mu\text{g}/\text{plate}$. ^bMethymethane sulfonate, 200 $\mu\text{g}/\text{plate}$.

^cN-ethyl-N'-nitrosoguanidine, 5 $\mu\text{g}/\text{plate}$. ^d9-Aminoacridine, 80 $\mu\text{g}/\text{plate}$.

^e2-Nitrofluorene, 2 $\mu\text{g}/\text{plate}$. ^f2-Aminoanthracene, with $\mu\text{g}/\text{plate}$ doses indicated in parentheses (0.5) or (2). ^gN-ethyl-N'-nitrosoguanidine, 2 $\mu\text{g}/\text{plate}$.

cytotoxic at the higher concentrations, with the 5000 $\mu\text{g}/\text{plate}$ dose killing most of the bacteria in the absence of metabolic activation, that buprenorphine precipitated on the agar plate at the higher doses, and that it did not show any mutagenic potential.

In the main reverse mutation (Ames test) study, buprenorphine was tested at concentrations ranging from 0.5 to 500 $\mu\text{g}/\text{plate}$ in triplicate, using the same five strains of *Salmonella typhimurium* used in the preliminary study (Table 15).

Table 15: Ames test mutagenicity study of buprenorphine in 5 *Salmonella* strains and one *E. coli* strain (from NDA, Vol. 1.12, tab 46, pp. 32-37)

Test strain	S-9	Mean Revertants/Plate by Buprenorphine, μg								+ cont	+ cont (2AA) ^f
		0.0	0.5	1	5	10	50	100	500		
TA98	-	17	16	15	13	14	10	10	11	109 ^a	16(0.5)
	+	10	11	11	8	13	10	14	10	N.T.	370(0.5)
TA100	-	91	86	87	93	92	76	4	14	713 ^b	98(0.5)
	+	77	69	74	79	76	87	83	66	N.T.	451(0.5)
TA1535	-	7	7	5	5	6	3	2	2	338 ^c	7(2)
	+	5	5	4	6	6	7	4	3	N.T.	174(2)
TA1537	-	6	1	5	4	4	2	2	0	2940 ^d	8(2)
	+	3	3	4	4	3	2	4	0	N.T.	239(2)
TA1538	-	7	7	8	6	5	2	1	2	336 ^e	5(0.5)
	+	4	4	7	5	5	5	4	0	N.T.	354(0.5)
WP2uvra	-	21	13	19	13	15	15	13	6	1309 ^g	16(80)
	+	13	18	13	18	12	13	10	13	N.T.	348(80)

N.T. = Not tested. ^a2-Nitrofluorene, 1 $\mu\text{g}/\text{plate}$. ^bMethymethane sulfonate, 200 $\mu\text{g}/\text{plate}$. ^cN-ethyl-N'-nitrosoguanidine, 5 $\mu\text{g}/\text{plate}$. ^d9-Aminoacridine, 80 $\mu\text{g}/\text{plate}$. ^e2-Nitrofluorene, 2 $\mu\text{g}/\text{plate}$. ^f2-Aminoanthracene, with $\mu\text{g}/\text{plate}$ doses indicated in parentheses (0.5), (2) or (80). ^gN-ethyl-N'-nitrosoguanidine, 2 $\mu\text{g}/\text{plate}$.

Conclusion: It was concluded from this (non-GLP) study that buprenorphine did not exhibit any mutagenicity in the Ames test using various *Salmonella typhimurium* TA strains and an *E. coli* WP2uvra strain (NDA, Vol. 1.12, tab 46).

In a preliminary study of the bacterial DNA repair test (rec-assay), buprenorphine was tested at concentrations ranging from 0.8 to 8000 $\mu\text{g}/\text{plate}$ in

two strains of *Bacillus subtilis*. One is a repair-deficient strain (M45Rec⁻), and the other is a repair-proficient strain (H17Rec⁺). Mutagens causing DNA damage (e.g., mitomycin C) cause a differential inhibition of growth of the two strains, whereas protein synthesis inhibitors that do not cause DNA damage (e.g., kanamycin) cause equal inhibition of growth. Buprenorphine in amounts of 0.8, 8 and 80 µg/disc did not affect growth of either strain, whereas 800 µg buprenorphine, 20 µg kanamycin and 8000 µg buprenorphine caused equal growth inhibitions in both strains of 3, 7.5 and 9 mm, respectively, in the regular incubation method and equal growth inhibitions in both strains of 9, 14 and 15 mm, respectively, in a cold incubation method to enhance drug diffusion. In a subsequent study (Report #000558), buprenorphine doses of 25, 50 and 100 (regular method) µg/disc did not have a lethal effect on the strains, whereas doses of 100 (cold incubation method), 200 and 400 µg/disc showed a dose-related lethal effect to a similar extent in both strains. In contrast, the positive mutagenic control, mitomycin C (0.2 µg) caused 3.6 times (regular method) or 5.7 times (cold incubation method) more lethality in the M45Rec⁻ strain as in the H17Rec⁺ strain.

Conclusion: It was concluded that buprenorphine did not exhibit any mutagenicity in the *Bacillus subtilis* rec-assay (NDA, Vol. 1.12, tab 46).

TITLE: Mutagenicity studies on buprenorphine hydrochloride.

REPORT NO.: RPEX 30000079 (b) (4)

STUDY DATES: April 5, 1983 to April 7, 1983

SPECIES/STRAIN: *E. coli* strains, WP2 (Trp⁻), WP67 and CM871

The Green-Tweats survival test was conducted with buprenorphine concentrations of 5, 10 and 20 mg/ml in three *E. coli* strains, in the absence and presence of metabolic activation (±S9). The WP2 (trp⁻) strain has an intact DNA repair system, whereas the other WP67 and CM871 mutant strains have defective repair systems, and therefore, are more sensitive to DNA injury, resulting in a higher number of dead bacteria than the WP2 strain when exposed to injurious substances (or ultraviolet light). Visible precipitation or turbidity indicated that the concentrations tested were likely too high; nevertheless, the results indicated an ability of buprenorphine to inactivate the WP67 and CM871 mutants more extensively than the WP2 (trp⁻) strain.

TITLE: Study to evaluate the chromosome damaging potential of buprenorphine hydrochloride by its effects on cultured Chinese hamster ovary (CHO) cells using an in vitro cytogenetics assay

STUDY NO.: RCP 8/CHO/KF19/CH3 (b) (4)

STUDY DATES: June 25, 1985 to September 30, 1985

SPECIES/STRAIN: Chinese hamster, cultured ovary cells (b) (4)

Studies to evaluate the clastogenicity potential of buprenorphine HCl by incubation with cultured Chinese hamster ovary (CHO) cells included a preliminary

concentration range-finding study with single cultures that used buprenorphine concentrations ranging from 5 to 2000 $\mu\text{g/ml}$, in the absence and presence of the S-9 liver fraction from rats pretreated with Aroclor-1254. The 100 $\mu\text{g/ml}$ and 200 $\mu\text{g/ml}$ concentration were toxic in the absence and presence of S-9, respectively. Thus, concentrations of 12.5 to 75 $\mu\text{g/ml}$, in the absence of S-9, and 25 to 150 $\mu\text{g/ml}$ in the presence of S-9 were chosen for the main (repeated) study. However, the highest concentrations, \pm S-9, proved to be too toxic to score (Table 16).

Table 16: Clastogenic studies with buprenorphine in cultured CHO cells (from NDA, Vol. 1.12, tab 47, Tables 1-4).

Parameter measured	Buprenorphine concentration, $\mu\text{g/ml}$						Positive control*
	S-9	Vehicle	12.5	25	50	100	
<i>Experiment 1</i>							
Frequency of aberrations per 100 cells, including gaps	-	14	8	14	14	N.T.	44
	+	4.5	N.T.	5.5	6	4.5	366
% of Cells with aberrations, including gaps	-	11	7.5	13	12	N.T.	32
	+	4.5	N.T.	3.5	6	4.5	90
Mitotic Index	-	1.4	1.8	0.8	1.8	N.T.	
	+	0.8	N.T.	0.8	2.2	7.0	
<i>Experiment 2</i>	S-9						
Frequency of aberrations per 100 cells, including gaps	-	12	5.5	12	8	N.T.	74
	+	16	N.T.	12	14	7.5	563
% of Cells with aberrations, including gaps	-	11	4.5	10	5.5	N.T.	46
	+	12	N.T.	11	13	7.5	96
Mitotic Index	-	1.8	3.0	2.0	17.8	N.T.	
	+	1.6	N.T.	1.8	2.2	2.0	

*Methylmethane sulfonate (50 $\mu\text{g/ml}$) in the absence of S-9 and cyclophosphamide in the presence of S-9. N.T. = Not tested.

Although there were marked increases in mitotic index at 100 $\mu\text{g/ml}$ in the presence of S-9 in Experiment 1 and at 50 $\mu\text{g/ml}$ in the absence of S-9 in Experiment 2, this was considered by the contract lab to be a possible artefact. Apart from a significant decrease in aberrations, including gaps, at 100 $\mu\text{g/ml}$ in the presence of S-9, which does not have any biological significance, the variations from controls were not significant.

Conclusion: Buprenorphine, in concentrations up to 50 µg/ml (minus S-9) or 100 µg/ml (plus S-9), did not exhibit significant clastogenic activity, although higher concentrations were cytotoxic in cultured CHO cells.

TITLE: Study to determine the ability of buprenorphine hydrochloride to induce mutations in 6-thioguanine resistance in mouse lymphoma L5178Y cells using a fluctuation assay

STUDY NO.: RCP 8/(b)(4)/KF15 (b)(4) 3 (b)(4)

STUDY DATES: June 26, 1985 to October 13, 1985

SPECIES/STRAIN: Mouse, cultured L5178Y cells

Mutagenicity studies in mouse lymphoma L5178Y cells were conducted to evaluate the potential of buprenorphine HCl to induce mutation to 6-thioguanine resistance. In a preliminary concentration range-finding study with single flasks of cells incubated for 2 hours at 37°C with buprenorphine concentrations ranging from 5 to 2000 µg/ml, in the absence and presence of the S-9 liver fraction from rats pretreated with Aroclor-1254, it was found that the 100 µg/ml concentration killed 97% and 75% of the lymphoma cells in the absence and presence of S-9, respectively, and higher concentrations allowed zero survival. Subsequent experiments were conducted with duplicate 12.5, 25, 50 and 100 µg/ml concentrations in the absence and presence of S-9; and with 6.3, 1.25, 25 and 50 µg/ml in the absence of S-9 and 12.5, 25, 50 and 100 µg/ml in the presence of S-9. Additional duplicate cultures were treated with 4-nitroquinoline-1-oxide, 0.19 and 0.38 µg/ml, in the absence of S-9 or with benzo(a)pyrene, 2.0 and 3.0 µg/ml, in the presence of S-9, as positive controls.

One replicate treated with a buprenorphine concentration of 12.5 µg/ml showed 10 6TG^R mutants/10⁶ cells, relative to a vehicle control value of 2/10⁶ cells, at day 7, but the other replicate, as well as cells treated with the other

Table 17: Mutagenicity study of buprenorphine in cultured mouse lymphoma L5178Y cells (from NDA, Vol. 1.12, tab 48, Tables 4-7).

Mutagen	Conc. µg/ml	S-9	Relative survival, %		6TG ^R mutants/10 ⁶ cells	
			Expt. 1	Expt. 2	Expt. 1	Expt. 2
Vehicle control		-	100	100	1	0.4
		+	100	100	2	1
4-Nitroquinoline-1-oxide	0.19	-	22, 31	19, 15	75, 72	17, 27
	0.38	-	1, 4	2, 1	237, 274	24, 28
Benzo(a)pyrene	2.0	+	77, 91	71, 67	28, 30	3, 1
	3.0	+	80, 79	78, 71	49, 40	7, 5

buprenorphine concentrations all displayed mutation rates below the vehicle control value. No significant mutations were observed at 12.5 µg/ml or any other buprenorphine concentration in the second experiment. Therefore, no statistically significant, dose-related increases in the mutation rate of mouse lymphoma L5178Y cells were observed in these studies, except with the positive control drug treatments, as shown in Table 17.

TITLE: Mutagenicity studies on buprenorphine hydrochloride.
REPORT NO.: RPEX 30000079 (b) (4)
STUDY DATES: April 11, 1983 to June 6, 1983 (bone marrow study)
June 6, 1983 to June 28, 1983 (spermatogonia study)
SPECIES/STRAIN: Chinese hamster (*Cricetus griseus*)

In vivo chromosome aberration tests used Chinese hamsters at least 10 weeks old. They were given the highest tolerable oral dose of buprenorphine HCl, 400 mg/kg. Bone marrow samples from hamsters of both sexes with a mean weight of 30 grams were examined for chromosomal aberrations at 6, 24 and 48 hours after a single oral dose. Spermatogonia from hamsters with a mean weight of 36 grams were examined for chromosomal aberrations at 24 and 48 hours, after a single oral dose. For the bone marrow study, each animal was administered demecolcine (5 mg/kg, i.p.) 2 hours before completion of the testing period. At least 50 complete and evenly stained metaphase nuclei were evaluated per animal. Results of the microscopic examination indicated no chromosome-breaking activity of this oral dose for either bone marrow or spermatogonia cells. However, no positive controls were run in this study.

TITLE: Mutagenicity studies on buprenorphine hydrochloride.
REPORT NO.: RPEX 30000079 (b) (4)
(b) (4)
STUDY DATES: April 11, 1983 to June 15, 1983
SPECIES/STRAIN: Mouse, ICR

The DNA synthesis inhibition (DSI) test *in vivo* was conducted with male ICR mice (b) (4) weighing 25-30 grams. They were prelabeled with [methyl-¹⁴C]thymidine (0.5 µCi, s.c.). Three hours after administration of vehicle or buprenorphine (lot no. 13), each mouse received [methyl-³H]thymidine (10 µCi, s.c.), followed 45 minutes later by unlabeled thymidine (3 mg, s.c.). After euthanasia, the testes were removed and homogenized in 5% TCA, the ³H and ¹⁴C that had been incorporated into DNA *in vivo* were measured by dual channel liquid scintillation spectrometry, and DNA concentration was determined by a diphenylamine color reaction. The results are summarized in Table 18.

Table 18: Inhibition (%DSI) of *in vivo* thymidine incorporation into testicular DNA in mice given a single dose of buprenorphine (NDA, Vol. 1.12, tab 45, Appendix 10).

	Dose of Buprenorphine, mg/kg, s.c.							
	Control	10	20	50	100	200	500	1000
% DSI	0.0	7.7	8.3	6.9	19.3*	12.4	15.0	13.5*
					--- †	14.9	32.5*	26.0*
					15.5	16.5		

* $p=0.05$ (Wilcoxon) † Thymidine incorporation was 115% of control value.

Comment: It was not clear from the report whether each experimental value represented one mouse or the mean (without the S.D. or S.E.M.) from one group of mice, although the general description of the protocol indicated that usually 4 mice were used per dose.

For the combination DSI test *in vivo/in vitro*, male ICR mice were administered vehicle (negative control), methylnitroso-urea (positive control) or buprenorphine and euthanized three hours later. After removal of the tunica albuginea, the remaining testicular tissue was incubated at 32°C with collagenase to dissociate the tubules. After thorough washing, the tubules were incubated for 1 hour at 33°C in Eagle's minimal essential medium (MEM⁺) containing Hank's salts, 6 mM DL-lactic acid, 2 mM sodium pyruvate and 1.25 μ Ci of [methyl-³H]thymidine. After replacement of the radioactive solution with 2.5 ml of Dispase/DNase/thymidine solution in MEM⁺, the tubules were incubated for another hour, during which cell aggregates were dissociated. Cells were then isolated by density gradient centrifugation (using ^{(b)(4)}), four fractions were harvested and lysed, and the DNA from each fraction was isolated and quantified by UV absorption at 260 nM and its radioactivity was determined by scintillation spectroscopy. The results of this study are summarized in Table 19.

Table 19: Inhibition (% DSI) of thymidine incorporation into testicular cells isolated from mice given a single dose of buprenorphine (from NDA, Vol. 1.12, tab. 45, Table 28).

% DSI for Percoll frac.#	Dose of Buprenorphine, mg/kg, s.c.				Methylnitroso-urea 100 mg/kg, i.p.
	Control	50	200	1000	
Fraction #1	0.0	23.6*	28.8*	37.7*	58.9*
Fraction #2	0.0	22.3	19.5	--- †	34.5
Fraction #3	0.0	28.1	3.4	--- †	29.2
Fraction #4	0.0	33.1	25.9	0.6	68.3

* $p \leq 0.05$ (Wilcoxon); each value is derived from $n=4$.

† Thymidine incorporation was > 100% of control mean.

For the unscheduled DNA synthesis (UDS) test *in vitro*, methods analogous to the *in vivo/in vitro* DSI test were conducted with buprenorphine, except that N-hydroxyurea was added to the 90-minute incubation with [methyl-³H]thymidine (3 μ Ci) and there was no additional incubation with Dispase/DNase/thymidine solution. Instead, the tubules were lysed in 0.3 M KOH and the DNA was isolated, quantified by UV absorption and counted for radioactivity. Thymidine incorporation that is not inhibited by N-hydroxylurea is regarded as an index of unscheduled DNA synthesis to repair DNA damaged by the drug treatment. The results are summarized in Table 20.

Table 20: Unscheduled DNA synthesis (% UDS) by testicular cells isolated from mice given a single dose of buprenorphine (from NDA, Vol. 1.12, tab 45, Appendix 10 and Tables 28 & 29).

	Dose of Buprenorphine, mg/kg, s.c.					Methylnitrosourea, mg/kg		
	Control	50	100	200	1000	60	100	120
% UDS	0.0	(---)†		(---)†	(---)†		(7.6)†	
	0.0	---‡	6.2	53.7*		74.0*		194.2*
	0.0	65.3		117.2*			138.8*	

* $p \leq 0.05$ (Wilcoxon; each value is derived from $n=4$, except 2nd control ($n=11$)).

† Experiment not considered valid because positive control did not work.

‡ N-Hydroxylurea-resistant thymidine incorporation was only 92.4% of control.

Conclusion: On the basis of the results of his DSI and UDS tests, Dr. (b)(4) concluded that buprenorphine should be classified as active in these tests, and therefore, it should be regarded as a suspected carcinogen. However, these tests are not included among standard mutagenicity tests recommended in the ICH guidelines, whereas buprenorphine was not mutagenic in other tests included in the ICH guidelines, such as tests with CHO cells and mouse lymphoma cells.

Special Toxicology Studies (Topical administration):

TITLE: Primary skin irritation study.

REPORT NO.: 3/7912 (b)(4)

STUDY DATES: December 3-6, 1979.

LOT NO. PP1121/16 (buprenorphine HCl powder); Batch 764 (buprenorphine injection).

SPECIES/STRAIN: Rabbit, New Zealand White

A skin irritation study with rabbits was conducted as described in *FR 38*(No. 187, §1500): 41, 1973. Buprenorphine HCl powder (0.5 g) was moistened with 0.5 ml sterile water, 0.5 ml of the test sample was applied to each of two 2.5 cm² surgical lint pads attached to adhesive wrapping, and then applied for 24 hours to

the shaved skin of rabbits. Skin irritation was assessed 1 hour and 48 hours after removal of the patches. The average scores for the 6 rabbits tested in this manner were 2.3 for erythema and 0.0 for edema, giving a primary irritation score of 0.6. *Conclusion:* According to the Draize system of classification, buprenorphine HCl powder (or slurry) would be considered a "mild irritant" (primary irritation score <2). (NDA, Vol. 1.7, tab 7)

A similar study (NDA, Vol. 1.7, tab 8) was conducted with six rabbits using buprenorphine injection solution (0.3 mg/ml), in which 0.5 ml of this solution was applied directly to the 2.5 cm² patches. The average scores for the 6 rabbits tested in this manner were 0.0 for erythema and 0.0 for edema, giving a primary irritation score of 0.0.

Conclusion: According to the Draize system of classification, Buprenorphine Injection (batch #764) would be considered a non-irritant.

TITLE: Eye irritation study.

REPORT NO.: 3/7912

(b) (4)

STUDY DATES: December 3-6, 1979.

LOT NO. PP1121/16 (buprenorphine HCl powder); Batch 764 (buprenorphine injection).

SPECIES/STRAIN: Rabbit, New Zealand White

An eye irritation study with rabbits was conducted as described in *FR 38*(No. 187, §1500): 42, 1973. Buprenorphine HCl powder (0.1 g) was instilled into one eye of each of six rabbits, and without any eye washing, the eyes were examined at 24, 48 and 72 hours after instillation of the powder. The eyes were scored according to the method of Draize for damage or irritation to the cornea (maximum score = 80), iris (maximum = 10) and conjunctivae (maximum = 20), using the untreated eye as a control. The average scores for the rabbits in this study were cornea = 0.0, iris = 0.0 and conjunctivae = 6.0, with four of the six rabbits exhibiting reactions to the powder (NDA, Vol. 1.7, tab 9).

Conclusion: Buprenorphine HCl powder would be classified as an irritant to the eye.

A similar study (NDA, Vol. 1.7, tab 10) was conducted with six rabbits using buprenorphine injection solution (0.3 mg/ml), in which 0.1 ml of this solution was instilled into one eye of each rabbit. The average scores for the rabbits in this study were cornea = 0.0, iris = 0.0 and conjunctivae = 0.0, with none of the six rabbits exhibiting reactions to the solution.

Conclusion: According to the Draize system of classification, Buprenorphine Injection (batch #764) was not an irritant to the eye.

Special Toxicology Studies (Inhalational administration):

TITLE: Acute toxicity by inhalation of buprenorphine.
 REPORT NO.: 2150-46/32 (b)(4)

STUDY DATES: November, 1979 to January, 1980.
 LOT NO. PP1121/16 (buprenorphine HCl powder).
 SPECIES/STRAIN: Rat, (b)(4) (Sprague-Dawley origin)

Groups of 12 rats (6/sex) were exposed for four hours to one of four atmospheres containing 0 (control), 0.10, 0.31 or 0.92 mg of buprenorphine dust/liter of air produced by a (b)(4) dust generator and flowing through a "nose only" 10-liter clear plastic cylinder at a flow rate of approximately 15 liters/minute. Oxygen inside the chamber was monitored continuously using a remote sensor, the concentration of buprenorphine was determined gravimetrically at 30-minute intervals, and the distribution of buprenorphine particle sizes was also measured at 30-minute intervals using a (b)(4) covering the particle aerodynamic mass median diameter range of (b)(4). All rats were weighed immediately before exposure, immediately after exposure and on days 1, 2, 3, 4, 10 and 14 of a 14-day post-exposure observation period. One objective of the study, i.e., to determine the LC₅₀ of buprenorphine by inhalation, was not achieved, due to the lack of lethality. Another part of the protocol, i.e., to do histological examination of tissues of the respiratory tracts of rats exposed to the buprenorphine concentration causing 25-50% mortality was not conducted. Immediately following exposure, one male and one female from each group had blood taken from the retro-orbital sinus for the determination of plasma concentrations of buprenorphine, but the results were not included in this report. Also immediately following exposure, another male and female were euthanized with pentobarbital and necropsied, as were the surviving rats at the end of the 14-day observation period. Acute parameters and clinical signs are summarized in Table 21.

Table 21: Effects of buprenorphine dust inhalation for 4 hours on rats (n = 12/grp).

Buprenorphine exposure (mg/L air)	(b)(4) Percentile particle diameter (mean ± SD, μm)	Mortality (%)	Mean weight loss during exposure (g)	% Incidence of semi- or unconsciousness
0	(b)(4)	0	12.0 ± 2.7	0
0.10	(b)(4)	0	5.1 ± 1.2	0
0.31	(b)(4)	0	8.8 ± 3.3	58
0.92	(b)(4)	0	7.0 ± 3.8	25

The buprenorphine-treated rats lost less weight during the 4 hours of exposure,

possibly due to an attenuation of immobilization stress, compared with the controls. The controls, however recovered their pre-exposure body weights more rapidly than did the buprenorphine-exposed groups. Food consumption was not measured in this study. Macroscopic pathology observed in the drug-treated, but not control, groups involved mainly bladder abnormalities in the low (1 male, 1 female) and middle (1 male, 1 female) exposure groups, but "were thought not to be related to exposure of the test article" (NDA, Vol. 1.7, tab 11).

Conclusion: The NOAEL of atmospheric buprenorphine dust is 0.10 mg/liter of air in rats, and all rats survived a 4-hour exposure to an atmosphere containing nine times the NOAEL, without exhibiting any target organ toxicity.

OVERALL SUMMARY and EVALUATION

Buprenorphine is a potent, but partial, agonist of opioid μ receptors which has been marketed in a parenteral formulation (Buprenex[®]) since 1981 for the relief of moderate to severe pain. The sponsor proposes to market sublingual (s.l.) tablet formulations of buprenorphine hydrochloride (Subutex[®]) containing (b) (4) 2.0 and 8.0 mg for the treatment of opiate addiction, with recommended daily doses of 2-4 mg for induction, (b) (4) mg for suppression of withdrawal symptoms and (b) (4) mg for blocking the effects of heroin or exogenously administered opiates.

Efficacy: Animal studies have indicated that buprenorphine is a partial agonist of μ -opioid receptors and has characteristics consistent with potential use for treatment of opiate addiction. The medical officer's review on clinical data will provide a conclusion relevant to this indication for use. The drug is marketed for this indication in France.

Safety Evaluation: Unlike full μ -opioid agonists, buprenorphine exhibits a "ceiling effect" for respiratory depression in animals and humans. The cardiovascular effects of buprenorphine have been studied in the rat, guinea pig, cat and dog. In all of these species, buprenorphine produced bradycardia without any marked effect on blood pressure.

Regarding abuse liability, buprenorphine is considered to possess lower abuse liability than most of the other available opioids with μ -agonist activity and is currently a schedule (b) (4) controlled substance in the United States. The availability of a sublingual tablet in several other countries, however, has led to extensive i.v. abuse, as well as some intranasal abuse, of the crushed tablets by heroin addicts. Even before these reports appeared, control of buprenorphine under Schedule (b) (4) of the CSA was being considered by the FDA for NDA 18-401 (Vocci, 1981).

Pharmacokinetics. Despite being highly lipophilic, buprenorphine has poor oral bioavailability (about 15%) because of extensive metabolism (mostly glucuronidation) by the intestinal wall and liver, but undergoes enterohepatic cycling before elimination mainly through the feces. Greater bioavailability is

achieved by the parenteral and s.l. routes, although the latter is subject to wide interindividual variability ($29 \pm 10\%$ in humans, Mendelson *et al.*, 1997). A small portion of the drug in brain, possibly bound to opioid μ receptors, disappears very slowly. The 4-hour AUC in rats after s.l. administration of a 0.2 mg/kg dose is approximately 250 ng•min/ml. Following parenteral administration in rats, the highest tissue levels were reached within 10 minutes in fat, lung, kidney, liver and spleen. By 24 hours, the tissue levels were very low. Refer to "Overall Summary" in the carcinogenicity review (Attachment 1) for additional summary of the PK/TK/ADME data.

Toxicology. Single-dose studies were performed in both rodents and non-rodents, utilizing a variety of routes of administration. These studies have indicated a wide margin of safety of buprenorphine, relative to its antinociceptive effects.

One-month toxicity studies were conducted in rats by the s.c. (high dose = 5 mg/kg/day) and oral (high dose = 80 mg/kg/day) routes, in dogs by the s.c. (high dose = 5 mg/kg/day) and i.v. (high dose = 32 mg/kg/day) routes, in baboons by the i.v. route (high dose = 18 mg/kg/day), in rhesus monkeys by the oral route (high dose = 80 mg/kg/day), and in cynomolgus monkeys by the s.l. route (high dose = 3.2→1.6 mg/kg/day) of administration. In general, these studies indicated decreased body weight gains often accompanied by reduction in food consumption in comparison with control animals and local irritation at the injection sites in those studies involving parenteral administration. Postmortem gross and microscopic examination confirmed the inflammation at the i.v. injection sites. After i.v. administration, histological changes were seen in the adrenals (dilation of zona glomerulosa), spleen (cell depletion) and testes (diffuse or localized atrophy) of dogs and in lungs (acute edema) of baboons, which might have been associated with treatment. In the s.l. study, there were no adverse local effects, nor any clinical, biochemical or pathological findings that were attributable to buprenorphine. Three month studies in dogs (high dose = 2.5 mg/kg/day, i.m.) and rats (high dose = 5 mg/kg/day, s.c.) confirmed the intolerance to the drug around the injection sites and also the relative lack of systemic toxicity, other than that attributed to local tissue damage, such as elevation of serum aspartate aminotransferase activity.

In six-month toxicity studies with rats and baboons, i.m. buprenorphine exhibited low levels of tissue and biochemical toxicity, even when parenteral doses up to 5 mg/kg/day were administered. The major significant observation in both species in these studies was a local inflammatory effect at the injection site, which limited the high dose administered. Dose-related behavioral aggressiveness occurred in rats, including attempts to bite the handler and violent fighting among themselves after dosing, which may have contributed to some deaths (raticide).

In two one-year oral studies in beagle dogs, the only significant findings at post mortem histological examination were mild to moderate hyperplasia of the bile

duct with associated peribiliary fibrosis, which occurred in all dogs receiving the high dose (75 mg/kg/day) and possibly also in one dog receiving the mid-dose (3.5 mg/kg/day). The first study indicated significant increases in prostate weight in the mid- and high-dose males, but this was not confirmed in the second study. The first study indicated significant increases in the adrenal weights of females only, relative to body weights, for all three doses of buprenorphine (0.2, 3.5 and 75 mg/kg/day, p.o.), and a significant increase was confirmed in the second study for the dose of 75 mg/kg/day. The first study indicated significant increases in the kidney weights of females only, relative to body weights, for the middle and high doses of buprenorphine (3.5 and 75 mg/kg/day, p.o.), and a significant increase was confirmed in the second study for these doses, as well as for the low dose.

Reproductive toxicity. Segment I, II and III reproduction studies were conducted with rats, and segment II teratology studies were conducted with rabbits. Buprenorphine had no effect on fertility, length of gestation or parturition, except at the highest i.m. dose, where dams experienced some difficulty in parturition and neonatal mortality was high. Buprenorphine produced statistically significant ($p < 0.05$), post-implantation losses and early fetal deaths with parenteral (i.m.), but not oral (up to 160 mg/kg/day), administration in rats in doses as low as 0.05 mg/kg/day (0.0133 times the MRHD of 32 mg = 0.533 mg/kg, after correcting for surface area equivalence). No obvious fetal malformations were noted in rats when buprenorphine was administered by oral (up to 160 mg/kg/day), intramuscular (up to 5 mg/kg/day) or intravenous (up to 0.8 mg/kg/day) routes, although significant increases in skeletal abnormalities were observed after subcutaneous administration of 1 and 5 mg/kg doses. In rabbits, buprenorphine treatment (up to 25 mg/kg/day) during gestational days 6-18 produced a dose-related trend ($p < 0.05$) for extra rib formation after intramuscular administration. Similar trends with low (1 mg/kg/day) and high (25 mg/kg/day) oral doses were not statistically significant. No such trend occurred with intravenous administration (up to 0.8 mg/kg/day). Post partum losses in rats, largely involving entire litters, may have resulted from nursing failures by the dams. With oral administration, the NOAEL for teratogenicity in rats was 160 mg/kg/day, but the NOAEL for viability of offspring was not established.

Carcinogenicity. Reviewed by BeLinda Hayes (See Attachment 1).

Genotoxicity. In several tests including clastogenic studies with CHO cells *in vitro* and Chinese hamster bone marrow and spermatogonia cells *in vivo*, mutagenicity studies with cultured mouse lymphoma cells and yeast *Saccharomyces cerevisiae* MP-1 cells, buprenorphine was found to have no mutagenic action. Three studies had some positive results, including the highest concentrations tested in the TA98, TA100 and TA1538 *Salmonella* strains in one of the Ames test batteries (Table 13); the Green-Tweats bacterial survival test; and the DNA synthesis inhibition (DSI)/unscheduled DNA synthesis (UDS) tests involving [³H]thymidine incorporation into DNA of mouse testicular cells. The one positive Ames test contradicted negative results from two previous tests by other investigators.

Although the sponsor had concluded from genotoxicity testing that there is no convincing evidence that buprenorphine is mutagenic in these tests or that it constitutes a genetic hazard for man, mixed results from genotoxicity testing were forwarded along with carcinogenicity data (reviewed by BeLinda Hayes) to the Executive Carcinogenic Assessment Committee (chaired by Ron Steigerwalt) for further consideration. The CAC recommended consultation with the Genotoxicity Committee, which was requested through its co-chair, Anita Bigger. Dr. Bigger concluded that: 1) Studies submitted by the sponsor did include the current tests required for a standard battery and meet current validity standards; 2) the DSI and UDS studies with mouse testicular tissue do appear valid and clearly show genotoxic activity, possibly indicating an interaction with DNA that could lead to point mutations, perhaps through a frameshift mechanism [as suggested by the one positive Ames assay]; 3) Mutagenicity studies with mammalian cells might not have picked up this type of genotoxicity because of relative cell survival greater than 10-20% in the sponsor's studies, and 4) concern regarding potential genotoxic activity should be included in the label.

Special Toxicology - Topical and Respiratory Exposure. Studies in rabbits indicated that buprenorphine HCl powder is a mild irritant to the skin and irritant to the eye, whereas buprenorphine HCl injectable (Buprenex®) is not an irritant. Inhalational studies in the rat using a dust generator indicated that the NOAEL of atmospheric buprenorphine dust is (b) (4) of air, and all rats survived a 4-hour exposure to an atmosphere containing nine times the NOAEL.

Conclusions:

Buprenorphine exhibits a higher margin of safety than full agonists of opioid μ receptors. However, due to the substantial number of reports of abuse in the biomedical literature from foreign countries where buprenorphine sublingual tablets have been available for outpatient treatment for a number of years, it is recommended that the Controlled Substances Evaluation Team give serious consideration to the rescheduling of buprenorphine to a more restrictive schedule (b) (4) of any sublingual tablet product containing this drug.

Because of high partitioning in fat, enterohepatic cycling and slow dissociation from opioid μ receptors, treatment of opioid addiction with buprenorphine may require not more than once/day dosing.

Buprenorphine has intrinsic subcutaneous and muscle irritating properties that likely are concentration-related and are associated with secondary hematologic and serum chemistry changes. Significant effects of chronic buprenorphine on the adrenals have been noted in several studies: in both one-year oral administration studies in dogs (increased % of body weight in females); in a one-month study of i.v. administration (4 or 32 mg/kg/day) in beagle dogs (dilation of zona

glomerulosa); and in a one-month study of i.v. administration (0.6 mg/kg/day) in olive baboons (increased weight). However, the physiological significance of these changes in the adrenal cortex is unknown. Chronic administration of 75 mg/kg/day produced target organ toxicities of proliferation, fibrosis and hemosiderosis in the hepatobiliary system of dogs; the NOAEL was (b)(4) mg/kg/day.

The available data showed that buprenorphine interacted with DNA in three tests and should be considered as genotoxic.

Buprenorphine does not affect fertility and no fetal malformations were statistically attributable to oral buprenorphine treatment in rats (up to 160 mg/kg/day) and rabbits (up to 25 mg/kg/day). Therefore, buprenorphine is not a teratogen.

In general, animals and humans showed qualitatively similar metabolic profiles. Buprenorphine is significantly metabolized in the liver via conjugation and N-dealkylation. Major excretion is via the feces. The extent of plasma protein binding is higher in humans than that observed in animals.

RECOMMENDATIONS

Internal: The pharmacological and toxicological profiles observed in laboratory animals have demonstrated a reasonable safety for support of the proposed labeled use in humans. The application is approvable based upon the pharmacology. Before the application can be approved, however, the label should be amended as recommended under "Labeling Review."

Since buprenorphine is metabolized by CYP 3A4, perhaps the label should indicate that there is a potential for drug interactions with other drugs or substances known to inhibit this enzyme, including ketoconazole, itraconazole, fluconazole, erythromycin, clarithromycin, naringenin, cimetidine, amiodarone, ciprofloxacin, troleandomycin, fluvoxamine and norfluoxetine. This information has been transmitted to the medical officer.

It is recommended that serious consideration be given to the rescheduling of buprenorphine to a more restrictive schedule (b)(4) at the time of marketing approval of any sublingual tablet formulation of this drug. This has been discussed with the Controlled Substances Evaluation Team (CSET) leader, and a review of the scheduling of buprenorphine hydrochloride sublingual tablets will be conducted by the CSET.

External: (see Labeling Review)

Labeling Review

The proposed label is deficient under the "Carcinogenesis, Mutagenesis, Impairment of Fertility" and "Pregnancy" subsections of **PRECAUTIONS**. The following labeling is recommended:

Carcinogenesis: Carcinogenicity studies were conducted in Sprague-Dawley rats and CD-1 mice. Buprenorphine was administered in the diet at doses of 0.6, 5.6, and 56.2 mg/kg/day for 27 months in rats. These doses were approximately equivalent to 0.2, 2, and 17 times the maximum human dose (32 mg) on a mg/m² basis. There were statistically significant increases in testicular interstitial (Leydig's) cell tumors based on the trend test unadjusted for survival. Pairwise comparison against control also showed significance at the high dose. In the mouse study, buprenorphine was administered in the diet at doses of 8, 50, and 100 mg/kg/day for 86 weeks. The high dose was approximately equivalent to 15 times the maximum human dose (32 mg) on a mg/m² basis. Buprenorphine was not carcinogenic in mice.

Mutagenesis: Buprenorphine was studied in a series of tests utilizing gene, chromosome and DNA interactions in both prokaryotic and eukaryotic systems. Results were negative in yeast (*Saccharomyces cerevisiae*) for recombinant, gene convertant, or forward mutations; negative in *Bacillus subtilis* "rec" assay; negative for clastogenicity in CHO cells, Chinese hamster bone marrow and spermatogonia cells; negative in mouse lymphoma L5178Y cell fluctuation assay. Results were equivocal in the Ames test: negative in studies by two laboratories, but positive for frame shift mutation at high dose (5 mg/plate) in a third study. Results were positive in the Green-Tweats (*E. coli*) survival test, positive in a DNA synthesis inhibition (DSI) test with testicular tissue from mice, for both *in vivo* and *in vitro* incorporation of [³H]thymidine; and positive in unscheduled DNA synthesis (UDS) test using mouse testicular cells.

Pregnancy: Pregnancy Category C. Buprenorphine has been shown to have an embryocidal effect in rats when given during gestational days 6-15 in intramuscular doses of 0.05 to 5.0 mg/kg/day. There are no adequate and well-controlled studies in pregnant women. Buprenorphine should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Buprenorphine produced statistically significant pre-implantation losses, post-implantation losses and early fetal deaths in rats with intramuscular administration of 0.05 mg/kg/day, but not with oral administration of daily doses up to 160 mg/kg. No drug-related fetal malformations were noted in rats when buprenorphine was administered by oral (≤ 160 mg/kg/day), intramuscular (≤ 5.0 mg/kg/day) or intravenous (≤ 0.8 mg/kg/day) routes, although significant increases in skeletal abnormalities were observed after administration of 1 and 5 mg/kg/day subcutaneously. In rabbits, buprenorphine treatment during gestational days 6-18 produced a significant dose-

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related trend for extra rib formation in fetuses after intramuscular (0.05 to 5 mg/kg/day), but not intravenous (0.05 to 0.8 mg/kg/day), administration. An increased incidence of skeletal anomalies in rabbit fetuses after oral administration (1 to 25 mg/kg/day) did not reach statistical significance, but there was a significant increase in pre-implantation losses in the oral study.

The following are suggested changes in, or questions of, the proposed draft label dated March 28, 1997, located in Vol 1.1, pp. 47-77 (☐ = insert space):

Line Change

(b) (4)

Daniel A. Brase
David A. Brase, Ph.D. (reviewer)

January 16, 1998
Date completed

Dou Huey Jean
Dou Huey Jean, Ph.D. (peer-reviewer)

January 16, 1998
Date completed

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