

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 esophogi 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/doe)	8.6	7.3	8.3	8.7
Implantations (mean/doe)	6.8	6.7	7.0	7.5
Fetuses and placentae (mean/doe)	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/doe)	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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NDA 20-733 (Suboxone®)

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: [_____]

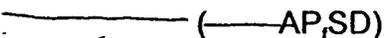
Date of Study Initiation/completion: July 17 through October 9, 1998

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:

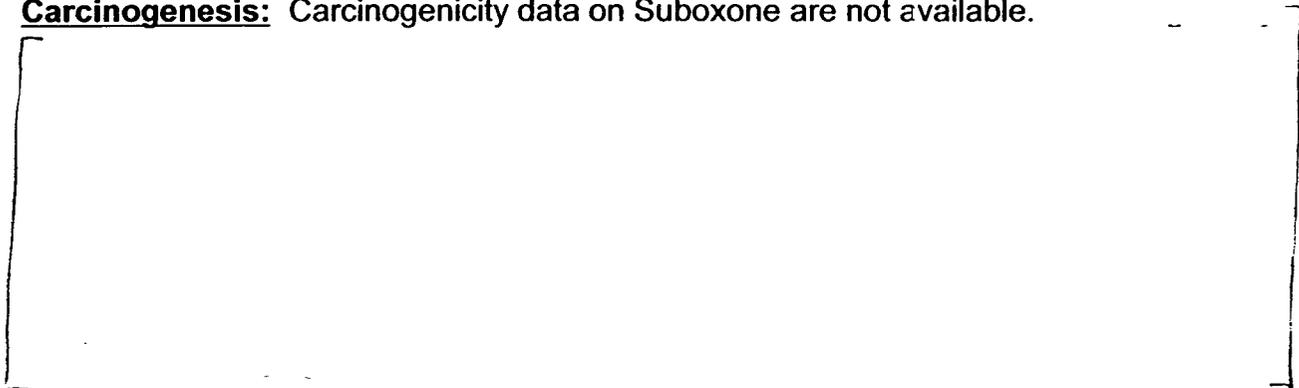
Despite the extensive number of studies employing several different routes of administration, no target organ or synergistic toxicities were readily apparent in rats or dogs. A sufficient number of preclinical toxicology studies have been conducted to support the safety of Suboxone as a treatment of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

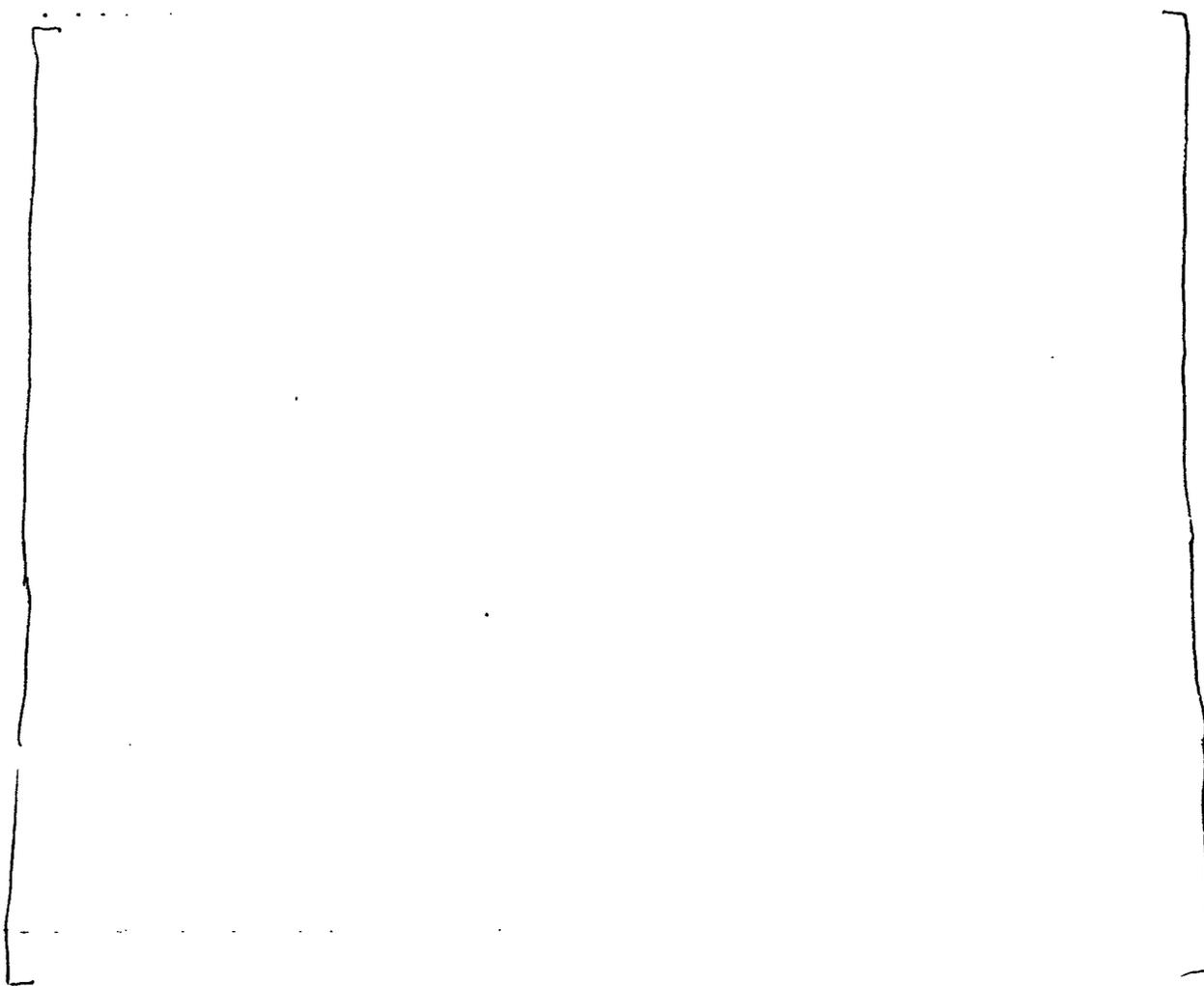
Impairment of Fertility:



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.

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