

PK parameters and rat brain levels:

TABLE 15: Mean plasma PK parameters and rat brain levels of unchanged [³H]-drug after oral, IV or IM [³H]-naloxone or [³H]-buprenorphine alone, or in combination with the other drug, unlabeled. (Sponsor's summary, Vol. 1.6)

Route and Parameter*	[³ H]-BUP alone		[³ H]-BUP+NAL		[³ H]-NAL alone		[³ H]-NAL+BUP	
	Rats	Dogs	Rats	Dogs	Rats	Dogs	Rats	Dogs
Dose, mg/kg	80	15	80+80	15+15	80	15	80+80	15+15
PO: C _{max} , ng/g	571	BLD	915	BLD	313	BLD	134	BLD
PO: T _{max} , min	90	BLD	30	BLD	15	BLD	15	BLD
PO: AUC _{0-8hr} **	188	BLD	243	BLD	48	8	26	6
PO: ng/g brain	1456 ³	ND	1564 ²	ND	8518 ²	ND	5853 ³	ND
Dose, mg/kg	4.5	1.5	4.5+3	1.5+1	3	1	3+4.5	1+1.5
IV: C _{max} , ng/g	1442	788	1306	739	761	349	574	357
IV: T _{max} , min.	5	5	5	5	5	5	5	5
IV: AUC _{0-8hr} **	1799	65	1653	67	726	18	690	20
IV: ng/g brain	4763 ⁰	ND	3626 ⁰	ND	2141 ⁰	ND	1736 ⁰	ND
IM: C _{max} , ng/g	892	103	839	129	865	258	833	258
IM: T _{max} , min.	5	15	5	15	5	15	5	15
IM: AUC _{0-8hr} **	2248	68	1453	64	908	26	760	26
IM: ng/g brain	1051 ²	ND	760 ¹	ND	1756 ¹	ND	1850 ¹	ND

*n = 3 for rats and 2 for dogs. **ng•hr/g of plasma.

⁰Measured at 5 min after dosing, the first sampling time in these studies. ¹Measured at 15 min after dosing. ²Measured at 30 min after dosing. ³Measured at 90 min after dosing. ND = Not determined. BLD = Below the limit of detection.

Elimination half-lives or total clearances were not calculated from the data in these studies. However, the plasma profiles (Tables 3, 6, 8, 11 and 13) indicate a longer elimination phase for buprenorphine (BUP) than for naloxone (NAL). Both rats and dogs excreted radioactivity from [³H]-BUP largely by the fecal route, whereas both species excreted radioactivity from [³H]-NAL largely into the urine. It is difficult to determine from this study whether one drug influenced the disposition of the other in a statistically significant manner, because of the low numbers of animals used per group, but there did not appear to be a major interaction. Naloxone may have caused an earlier T_{max} for oral BUP in rats by attenuating a delay of stomach emptying expected for BUP alone. Both BUP and NAL, although given orally at 10-15 times the doses used parenterally in dogs, displayed poor/oral bioavailability, such that the plasma levels in dogs were below the limits of detection at most time points. Peak brain levels of BUP in rats were much higher (4.5-4.8 times) after IV administration than after IM administration of the same dose. In contrast, peak brain levels of NAL were

comparable following either IV or IM administration. After 80 mg/kg, PO, peak brain levels of NAL were about 5 times greater than those of BUP. Consequently, at peak effect, the most advantageous route for the combination is IV for agonistic effects (high BUP:NAL ratio) and oral for antagonistic effects (low BUP:NAL ratio).

Metabolism: Some of the ADME studies with [³H]-buprenorphine and [³H]-naloxone in rats and dogs reviewed above also examined urine and/or feces for the metabolic profiles of these drugs. For the radiolabels in feces, this involved _____

TABLE 16: Metabolic patterns in the feces of rats and dogs following oral, IV or IM [³H]-buprenorphine alone, or in combination with unlabeled naloxone. (Vol. 1.18, tab 37386, p. 22; tab 37385, p. 22; tab 37387, p.25)

Radioactive Bands	% of t.l.c. plate radioactivity in two solvent systems							
	RAT				DOG			
	[³ H]-BUP alone		[³ H]-BUP+NAL		[³ H]-BUP alone		[³ H]-BUP+NAL	
Oral Route	Sys-1	Sys-2	Sys-1	Sys-2	Sys-1	Sys-2	Sys-1	Sys-2
Polar	15%	*	18%	*	11	*	13	*
nor-BUP	23%	23%	31%	29%	6	5	7	6
RX 1003-M	<1%	1%	<1	55%	<1	2	<1	82%
RX 2001-M	<1%	60%	51%		80%	82%	78%	
BUP	61%							
IV route	Sys-1	Sys-2	Sys-1	Sys-2	Sys-1	Sys-2	Sys-1	Sys-2
Polar	16%	10%	7%	5%	17%	11%	17%	9%
nor-BUP			47%	52%	7%	0%	6%	0%
BUP	79%	88%	40%	32%	70%	71%	69%	80%
IM Route	Sys-1	Sys-2	Sys-1	Sys-2	Sys-1	Sys-2	Sys-1	Sys-2
Polar	20%	*	13%	*	8%	*	16%	*
nor-BUP	43%	42%	39%	38%		4%		4%
RX 1003-M	<1%		<1%	2%	1%		1%	
RX 2001-M								
BUP	36%	40%	40%	48%	84%	86%	78%	79%

*Polar material in solvent system 2 (Sys-2) did not remain at the origin as a discreet spot, making quantification difficult.

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From Table 16, it appears that the major metabolic pathways of buprenorphine in the rat include N-dealkylation and conjugation (with glucuronide), whereas the major pathway in the dog is conjugation, as the dog excreted a much smaller fraction of the dose as norbuprenorphine than the rat. For naloxone, the only metabolite measured was naloxol, which appears to be a minor metabolite in both rats and dogs. The major metabolite in urine denoted as "Polar-1" may be different in the rat and dog, as the one in rat was definitely decreased by hydrolysis with glucuronidase treatment, but the one in dog was not appreciably diminished by hydrolysis (Table 18).

TABLE 17: Metabolic patterns in the feces of dogs following IM [³H]-naloxone alone, or in combination with unlabeled buprenorphine. (Vol. 1.18, tab 37387, p.27)

Radioactive Bands	% of t.l.c. plate radioactivity in two solvent systems			
	[³ H]-Naloxone alone		[³ H]-Naloxone+Buprenorphine	
	Sys-1	Sys-2	Sys-1	Sys-2
Polar-1	21%	51%	20%	31%
Polar-2			12%	
Polar-3	32%			
Naloxol	43%	2%	62%	2%
Naloxone		40%		61%

*Polar material in solvent system 2 (Sys-2) did not remain at the origin as a discreet spot, making quantification difficult. Did not study feces of rats after IM [³H]-naloxone.

TABLE 18: Metabolic patterns in the urine of rats and dogs following oral [³H]-naloxone alone, or in combination with unlabeled buprenorphine. (Vol. 1.18, tab 37386, p. 23)

Radioactive Bands	% of t.l.c. plate radioactivity per band in two solvent systems							
	RAT				DOG			
	[³ H]-NAL alone		[³ H]-NAL+BUP		[³ H]-NAL alone		[³ H]-NAL+BUP	
<i>Untreated</i>	Sys-1	Sys-2	Sys-1	Sys-2	Sys-1	Sys-2	Sys-1	Sys-2
Polar-1	69	99	93	97	69	98	51	85
Polar-2	24		3		6		6	
Polar-3	2		23		28			
Naloxol	<1	<1	<1	<1	<1	<1	13	<1
Naloxone	<1	<1	<1	<1	<1	<1		12
<i>Hydrolyzed</i>								
Polar-1	55	88	51	84	68	73	49	55
Polar-2	24		26		4		6	
Polar-3	5		6		5			
Naloxol		<1		1		<1		<1
Naloxone	13	12	14	15	24	23	41	41

TABLE 19: Metabolic patterns in the urine of rats and dogs following IV [³H]-naloxone alone, or in combination with unlabeled buprenorphine. (Vol. 1.18, tab 37385, p. 23)

Radioactive Bands	% of t.l.c. plate radioactivity per band in two solvent systems							
	RAT				DOG			
	[³ H]-NAL alone		[³ H]-NAL+BUP		[³ H]-NAL alone		[³ H]-NAL+BUP	
<i>Untreated</i>	Sys-1	Sys-2	Sys-1	Sys-2	Sys-1	Sys-2	Sys-1	Sys-2
Polar	96	53	93	60	94	88	96	78
Naloxol	0	24	0	26	ND	ND	0	4
Naloxone	2	15	4	6	0	3	3	5
<i>Hydrolyzed</i>								
Polar	66	35	77	41	38	26	81	43
Naloxol	3	22	0	29	ND	ND	3	6
Naloxone	28	23	18	14	55	57	5	26

TABLE 20: Metabolic patterns in the urine of rats and dogs following IM [³H]-naloxone alone, or in combination with unlabeled buprenorphine. (Vol. 1.18, tab 37387, p.26)

Radioactive Bands	% of t.l.c. plate radioactivity per band in two solvent systems							
	RAT				DOG			
	[³ H]-NAL alone		[³ H]-NAL+BUP		[³ H]-NAL alone		[³ H]-NAL+BUP	
<i>Untreated</i>	Sys-1	Sys-2	Sys-1	Sys-2	Sys-1	Sys-2	Sys-1	Sys-2
Polar-1	53%	86%	55%	86%	59%	95%	46%	90%
Polar-2	27%		26%		8%		17%	
Polar-3	5%		4%		26%		24%	
Naloxol		1%	1%		<1%		<1%	
Naloxone	10%	9%	11%	10%	4%	4%	7%	6%
<i>Hydrolyzed</i>	Sys-1	Sys-2	Sys-1	Sys-2	Sys-1	Sys-2	Sys-1	Sys-2
Polar-1	51%	84%	49%	83%	15%	61%	31%	59%
Polar-2	32%		28%		39%		30%	
Polar-3	4%		4%		7%		9%	
Naloxol		2%	2%		2%		3%	
Naloxone	11%	13%	16%	14%	34%	36%	27%	34%

Results: Apparently, neither solvent system effected a clear separation among the radioactive congeners of buprenorphine. In general after oral or IM administration, dogs excreted about 1½ to 2 times as much unchanged buprenorphine (+metabolite RX 2001-M) in feces as did rats, when expressed as a percentage of the total radioactivity, the difference being predominantly due to a much larger (~4 to 10-fold) excretion of radioactivity as nor-buprenorphine by rats than by dogs. Radioactivity from buprenorphine in urine was not assessed, as both species excreted most of it in the feces. In dogs, the addition of buprenorphine appeared to shift naloxone excretion in

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feces from about 40% to about 60% of total radioactivity as parent drug, with a concomitant decrease in the excretion of a metabolite designated as polar 3 after the IM route of administration (Table 17). The addition of buprenorphine did not appreciably affect the pattern of urinary excretion of radioactivity from [³H]-naloxone in the rat. Buprenorphine co-administration appeared to increase the amount of oral [³H]-naloxone that appeared in the urine as unchanged parent drug in dogs (Table 18). When expressed as a total % of excreted radioactivity in urine, the dog excreted about half as much [³H]-naloxone as the rat before hydrolysis, but more than twice as much parent drug as the rat after hydrolysis of the conjugate(s).

Summary of excretion studies: Other than the parent drugs, norbuprenorphine and two demethylated metabolites of buprenorphine, and naloxol, the other metabolites found in feces and urine were not identified and sometimes not adequately separated by t.l.c. However, the following table attempts to summarize the results of these studies:

TABLE 21: Dosing routes, doses, ratios and major excretion routes of radioactive buprenorphine and naloxone used in ADME studies in rats and dogs.

Species	Route of administration (n = 3 for rats and n = 2 for dogs)					
	Oral*		IV*		IM*	
Sample	Alone	+naloxone	Alone	+naloxone	Alone	+naloxone
[³ H]-BUP	Alone	+naloxone	Alone	+naloxone	Alone	+naloxone
Rat doses	80 mg/kg	80 + 80	4.5 mg/kg	4.5 + 3	4.5 mg/kg	4.5 + 3
Urine	9	12	10	14	14	6
Feces	64 (61)	55 (53)	74 (–)	62 (50)	56 (42)	56 (38)
Carcass	3	4	4	5	7	5
Dog doses	15 mg/kg	15 + 15	1.5 mg/kg	1.5 + 1	1.5 mg/kg	1.5 + 1
Urine	18	6	9	5	4	6
Feces	50 (81)	64 (80)	54 (70)	68 (74)	73 (83)	63 (78)
[³ H]-NAL	Alone	+BUP	Alone	+BUP	Alone	+BUP
Rat doses	80 mg/kg	80 + 80	3 mg/kg	3 + 4.5	3 mg/kg	3 + 4.5
Urine	35 (<1)	39 (<1)	45 (8)	43 (5)	37 (1)	45 (1)
Feces	13	12	7	6	8	6
Carcass	19	16	16	19	18	19
Dog doses	15 mg/kg	15 + 15	1 mg/kg	1 + 1.5	1 mg/kg	1 + 1.5
Urine	62 (<1)	50 (12)	48 (–)	65 (4)	54 (4)	43 (6)
Feces	25	22	26	20	23	26

*Numbers indicate the mean % of administered radioactivity found in the sample. The number in parentheses () indicates % of total radioactivity in the major excretion route (feces or urine) that is unchanged drug (buprenorphine or naloxone).

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TOXICOLOGY:

Acute Toxicity Studies of buprenorphine and naloxone alone, and in combination.
(For clinical signs, necropsy and causes of death, refer to overall toxicology summary)

Study Title: Naloxone hydrochloride and buprenorphine hydrochloride: Acute oral toxicity interaction study in the mouse. Experiment Number 349/8310

Study No.: 36585 Volume #: 1.11 Tab #: 36585

Conducting Laboratory: _____

Date of Study Initiation: October 14, 1983

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

Methods:

Dosing:

Species/strain: Mouse / _____

No./sex/group: 5/sex/dose (plus 2/sex/dose for range-finding studies)

Age: 4-6 weeks

Weight: 17-24 g males and 17-22 g females

Dosage groups:

Buprenorphine: 1000, 1710, 2924 and 5000 mg/kg

Naloxone: 1000, 1710, 2924 and 5000 mg/kg

Buprenorphine + Naloxone (3:2 ratio): 1000, 1710, 2924 and 5000 mg/kg (total)

Route, form, volume: Oral gavage, solution, 20 ml/kg

Drug lot no.: Buprenorphine, batch #17; naloxone, analysis #108 and #231

Formulation/vehicle: 2% aqueous methylcellulose

Observation period: 15 days

Clinical signs: Observed for mortality and overt signs of toxicity at 0.5, 1, 2, 3, 4 and 5 hours following dosing and at least once daily for 14 days.

Body weights: Recorded on days 0, 7 and 14.

Gross pathology: Mice dying during study and survivors euthanized on day 14 underwent a post mortem examination and any abnormalities were recorded.

Results:

TABLE 22: Acute oral toxicity of buprenorphine, naloxone and the 3:2 ratio in mice.

PARAMETER (mg/kg body weight)	BUPRENORPHINE ALONE	NALOXONE ALONE	BUPRENORPHINE PLUS NALOXONE
Max. non-lethal dose	<1000	<1000	1000
Minimum lethal dose	1000	1000	1710
LD ₅₀ (95% conf. lim.)	2025 (1547-2636)	1183 (849-1565)	2362 (1814-3088)

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Study Title: Naloxone hydrochloride and buprenorphine hydrochloride: Acute intravenous toxicity interaction study in the mouse. Experiment Number 310/8310

Study No.: 105293 Volume #: 1.11 Tab#: 36589

Conducting Laboratory: _____

Date of Study Initiation: October 13, 1983

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

Methods:

Dosing:

Species/strain: Mouse / LACA / _____

No./sex/group: 5/sex/dose (plus 2/sex/dose for range-finding studies)

Age: 4-6 weeks

Weight: 18-24 g males and 17-24 g females

Dosage groups:

Buprenorphine: 32, 40, 50, 63, 79 and 100 mg/kg

Naloxone: 79, 100, 126 and 159 mg/kg

Buprenorphine + Naloxone (2:3 ratio): 100, 126, 159, 200 and 252 mg/kg (total)

Route, form, volume: Intravenous, solution, 6.4-50.4 ml/kg

Drug lot no.: Buprenorphine, batch #17; naloxone, analysis #108

Formulation/vehicle: Water for injection

Observation period: 15 days

Clinical signs: Observed for mortality and overt signs of toxicity at 0.5, 1, 2, 3, 4 and 5 hours follow ; dosing and at least once daily for 14 days.

Body weights: Recorded on days 0, 7 and 14.

Gross pathology: Mice dying during study and survivors euthanized on day 14 underwent a post mortem examination and any abnormalities were recorded

Results:

TABLE 23: Acute IV toxicity of buprenorphine, naloxone and the 3:2 ratio in mice.

PARAMETER (mg/kg body weight)	BUPRENORPHINE ALONE	NALOXONE ALONE	BUPRENORPHINE PLUS NALOXONE
Max. non-lethal dose	<32	79	100
Minimum lethal dose	32	100	126
LD ₅₀ (95% conf. lim.)	50.1 (43.5-57.4)	106.6 (91.5-123.7)	180.2 (156.2-210.4)

Study Title: Naloxone hydrochloride and buprenorphine hydrochloride: Acute subcutaneous toxicity interaction study in the mouse. Experiment Number 311/8310

Study No.: 105293 Volume #: 1.11 Tab#: 36587

Conducting Laboratory: _____

Date of Study Initiation: October 13, 1983

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

Methods:

Dosing:

Species/strain: Mouse / _____

No./sex/group: 5/sex/dose (plus 2/sex/dose for range-finding studies)

Age: 4-6 weeks

Weight: 18-25 g males and 19-25 g females

Dosage groups:

Buprenorphine: 2500 mg/kg

Naloxone: 100, 271, 737 and 2000 mg/kg

Buprenorphine (1000 mg/kg) + Naloxone: 100, 271, 737 and 2000 mg/kg

Route, form, volume: Subcutaneous, solution, 1-50 ml/kg

Drug lot no.: Buprenorphine, batch #17; naloxone, analysis #108 and #231

Formulation/vehicle: 1% aqueous methylcellulose

Observation period: 15 days

Clinical signs: Observed for mortality and overt signs of toxicity at 0.5, 1, 2, 3, 4 and 5 hours following dosing and at least once daily for 14 days.

Body weights: Recorded on days 0, 7 and 14.

Gross pathology: Mice dying during study and survivors euthanized on day 14 underwent a post mortem examination and any abnormalities were recorded

Results:

TABLE 24: Acute SC toxicity of buprenorphine, naloxone and the combination in mice.

PARAMETER (mg/kg body weight)	BUPRENORPHINE ALONE	NALOXONE ALONE	BUPRENORPHINE PLUS NALOXONE
Max. non-lethal dose	2500	100	1000 + 100 (N)
Minimum lethal dose	>2500	271	1000 + 126 (N)
LD ₅₀ (95% conf. lim.)	N.D.	294 (214-401)	447 (325-614)

Study Title: Naloxone hydrochloride and buprenorphine hydrochloride: Acute oral toxicity interaction study in the rat. Experiment Number 348/8310

Study No.: 105296 Volume #: 1.11 Tab#: 105296

Conducting Laboratory: _____

Date of Study Initiation: October 14, 1983

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

Methods:

Dosing:

Species/strain: Rat / Sprague-Dawley _____

No./sex/group: 5/sex/dose (plus 2/sex/dose for range-finding studies)

Age: 4-6 weeks

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Weight: 104-147 g males and 100-155 g females

Dosage groups:

Buprenorphine: 5000 mg/kg

Naloxone: 500, 1077, 2321 and 5000 mg/kg

Buprenorphine (1000 mg/kg) + Naloxone: 500, 1077, 2321 and 5000 mg/kg

Route, form, volume: Oral, suspension, 10-25 ml/kg

Drug lot no.: Buprenorphine, batch #17; naloxone, analysis #231

Formulation/vehicle: 2% aqueous methylcellulose

Observation period: 15 days

Clinical signs: Observed for mortality and overt signs of toxicity at 0.5, 1, 2, 3, 4 and 5 hours following dosing and at least once daily for 14 days.

Body weights: Recorded on days 0, 7 and 14.

Gross pathology: Rats dying during study and survivors euthanized on day 14 underwent a post mortem examination and any abnormalities were recorded

Results:

TABLE 25: Acute oral toxicity of buprenorphine, naloxone and the combination of buprenorphine (1000 mg/kg) with various doses of naloxone in rats.

PARAMETER (mg/kg body weight)	BUPRENORPHINE ALONE	NALOXONE ALONE	BUPRENORPHINE PLUS NALOXONE
Max. non-lethal dose	5000	1077	1000 + 500 (NAL)
Minimum lethal dose	>5000	2321	1000 + 1017 (NAL)
LD ₅₀ (95% conf. lim.)	N.D.	3395 (2278-5919)	4732 (3097-9395)

Study Title: Naloxone hydrochloride and buprenorphine hydrochloride: Acute intravenous toxicity interaction study in the rat. Experiment Number 473/8312

Study No.: 36588 Volume #: 1.11 Tab#: 36588

Conducting Laboratory: _____

Date of Study Initiation: December 14, 1983

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

Methods:

Dosing:

Species/strain: Rat / Sprague-Dawley _____

No./sex/group: 5/sex/dose (plus 2/sex/dose for range-finding studies)

Age: 5-8 weeks

Weight: 115-223 g males and 110-198 g females

Dosage groups:

Buprenorphine: 79, 100, 126 and 159 mg/kg

Naloxone: 94, 119, 150, 189 and 238 mg/kg

Buprenorphine + Naloxone (45:55 ratio): 119, 150, 189 and 238 mg/kg

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Route, form, volume: Intravenous, solution, 4.7-31.8 ml/kg

Drug lot no.: Buprenorphine, batch #17; naloxone, analysis #231

Formulation/vehicle: water for injection, B.P.

Observation period: 15 days

Clinical signs: Observed for mortality and overt signs of toxicity at 0.5, 1, 2, 3, 4 and 5 hours following dosing and at least once daily for 14 days.

Body weights: Recorded on days 0, 7 and 14.

Gross pathology: Rats dying during study and survivors euthanized on day 14 underwent a post mortem examination and any abnormalities were recorded

Results:

TABLE 26: Acute IV toxicity of buprenorphine, naloxone and the 45:55 ratio in rats.

PARAMETER (mg/kg body weight)	BUPRENORPHINE ALONE	NALOXONE ALONE	BUPRENORPHINE PLUS NALOXONE
Max. non-lethal dose	<79	94	<119
Minimum lethal dose	79	119	119
LD ₅₀ (95% conf. lim.)	119 (101-204)	162 (139-189)	173 (148-204)

Study Title: Naloxone hydrochloride and buprenorphine hydrochloride: Acute subcutaneous toxicity interaction study in the rat. Experiment Number 312/8310

Study No.: 36586 Volume #: 1.11 Tab#: 36586

Conducting Laboratory: _____

Date of Study Initiation: October 13, 1983

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

Methods:

Dosing:

Species/strain: Rat / Sprague-Dawley _____

No./sex/group: 5/sex/dose (plus 2/sex/dose for range-finding studies)

Age: 4-6 weeks

Weight: 122-162 g males and 120-161 g females

Dosage groups:

Buprenorphine: 1500 mg/kg

Naloxone: 1000, 1710, 2924 and 5000 mg/kg

Buprenorphine (1000 mg/kg) + Naloxone: 1000, 1710, 2924 and 5000 mg/kg

Route, form, volume: Subcutaneous, solution, 5-30 ml/kg

Drug lot no.: Buprenorphine, batch #17; naloxone, analysis #108 and #231

Formulation/vehicle: 1% aqueous methylcellulose

Observation period: 15 days

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Clinical signs: Observed for mortality and overt signs of toxicity at 0.5, 1, 2, 3, 4 and 5 hours following dosing and at least once daily for 14 days.

Body weights: Recorded on days 0, 7 and 14.

Gross pathology: Rats dying during study and survivors euthanized on day 14 underwent a post mortem examination and any abnormalities were recorded.

Results:

TABLE 27: Acute SC toxicity of buprenorphine, naloxone and the combination of buprenorphine (1000 mg/kg) with various doses of naloxone in rats.

PARAMETER (mg/kg body weight)	BUPRENORPHINE ALONE	NALOXONE ALONE	BUPRENORPHINE PLUS NALOXONE
Max. non-lethal dose	1500	<1000	1000 + 1000 (N)
Minimum lethal dose	>1500	1000	1000 + 1710 (N)
LD ₅₀ (95% conf. lim.)	N.D.	1450 (1157-1793)	2550 (2054-3171)

Subacute Toxicity Studies

Study Title: Buprenorphine/Naloxone Preliminary Toxicity to Rats by Repeated Oral Administration for 4 Weeks.

Study No.: 105342 Volume #: 12 Tab#: 105342

Conducting Laboratory: _____

Date of Study Initiation: November 17, 1983

GLP Compliance: (X) Yes () No

QA Report: () Yes (X) No

Methods:

Dosing:

Species/strain: Rat / Sprague-Dawley _____

No./sex/group: 5/sex/dose

Age: ~5 weeks

Weight: 118-153 grams

Satellite group for health check: 5/sex were euthanized and necropsied before treatment of other groups began.

Satellite group for toxicokinetics: 4/sex at 150 mg/kg/day (2/sex 1 hr after the first dose and 2/sex 1 hr after day 28 dose).

Dosage groups: Buprenorphine + Naloxone (1:1): 0 (vehicle), 6, 30 and 150 mg/kg/day for 28 days.

Route, form, volume: Oral gavage, suspension, 5 ml/kg

Drug lot nos.: Buprenorphine, lot #17 (_____ pure); naloxone, lot #231 (_____ pure)

Formulation/vehicle: 1% aqueous methylcellulose

NDA 20-733 (Suboxone®)

Observation period: 4 weeks

Clinical signs: Observed for mortality or morbidity twice/day. Observed for signs of ill health, changes in behavior or reactions to treatment once/day on weekdays

Body weights: Measured at time of group assignment, initiation of treatment and weekly thereafter.

Food consumption: Measured weekly per cage, each housing 5 rats, unless death or separation for ill health or aggression intervened. Visual monitoring of water bottles was done daily.

Gross pathology: All surviving animals were euthanized by CO₂ asphyxiation on day 28 and necropsied.

Organs weighed: Adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes, thyroid and uterus.

Histopathology: Although samples from a number of tissues were preserved in buffered 10% formalin, including macroscopically abnormal tissue and adjacent normal tissue, the tissue samples were not processed further.

Results:

Clinical signs: One high dose female was euthanized in extremis on day 2, but its injuries were thought to be related to fighting. Localized hair loss (mostly on dorsum) at all doses, increased salivation shortly after dosing at mid and high doses in both sexes, and sedation during week 1 in high dose females were observed.

Body weights: Treatment-related decreases in body weight gain occurred in the male rats only.

TABLE 28: Body weight gains of rats given oral buprenorphine + naloxone for 28 days.

Dose	Body weight gain in males (g)		Body weight gain in females (g)	
	Mean ± S.D.	% of control	Mean ± S.D.	% of control
Control	213 ± 15.2		81 ± 6.3	
6 mg/kg/day	178 ± 7.5 *	84%	77 ± 6.1	95%
30 mg/kg/day	161 ± 15.1*	76%	83 ± 11.3	102%
150 mg/kg/day	160 ± 13.6*	75%	86 ± 8.2	106%

*P<0.001 compared with the control group.

Food Consumption: The treatment-related decreases in body weight gain that occurred only in the males were paralleled by decreases in food consumption by the males.

TABLE 29: Effect of oral buprenorphine + naloxone (1:1) on food consumption by rats.

Dose	Mean total food consumption (g/rat) in 28 days.			
	Males	% of control	Females	% of control
Control	759		506	
6 mg/kg/day	708	93%	495	98%
30 mg/kg/day	621	82%	477	94%
150 mg/kg/day	639	84%	528	104%

Gross Pathology: Health check group had one male with lung congestion (and alveolar hemorrhage) and one female with a nodule in the gastric mucosa of the antrum, but the group showed no sign of infectious disease. Males showed treatment-related decreases in liver weights at all doses and decreases in kidney weights at the middle and high doses, whereas females showed increases in kidney weights at the middle and high doses.

TABLE 30: Significant changes in organ weights in rats treated 4 weeks with a 1:1 oral combination of buprenorphine and naloxone (Vol. 1.12, tab 105342, p. 17-20).

ORGAN	SEX	ORGAN WEIGHTS, g (unadjusted mean ± S.D.)†			
		Control (vehicle)	6 mg/kg/day	30 mg/kg/day	150 mg/kg/d
Liver	Male	21.0 ± 3.46	18.1 ± 1.45*	15.2 ± 1.01**	15.7 ± 1.80**
	Female	11.3 ± 0.83	10.9 ± 1.24	9.9 ± 0.88	11.5 ± 1.13
Kidneys	Male	3.5 ± 0.47	3.4 ± 0.33	2.8 ± 0.18**	3.0 ± 0.13**
	Female	1.9 ± 0.16	2.0 ± 0.15	2.2 ± 0.23**	2.3 ± 0.35**

†Adjusted for final body weight as a covariate before statistical calculations,

*p<0.05 and **p<0.01, in comparison with control values (Williams' test).

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: 4-Week Toxicity of the Substance Mixture Buprenorphine-HCl, Lot No. 21 and Naloxone-HCl, Batch No. 231 (Ratio 1:1) — Called for Short 'BUP + NAL' — by Oral Administration to Sprague-Dawley Rats.

Study No.: 36935 Volume #: 15 Tab#: 36935

Conducting Laboratory: []

Date of Study Initiation: January 24, 1984

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

Methods:

Dosing:

Species/strain: Rat / Sprague-Dawley

No./sex/group: 25/sex/dose (main study)

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Age: 30 days for males; 32 days for females

Weight: 64-78 g

Satellite group for recovery: 5/sex from the control and high dose groups were retained for 4 weeks at the end of treatment.

Dosage groups: Buprenorphine + Naloxone (1:1): 0 (vehicle), 10, 80 and 640 mg/kg/day for 28 days.

Route, form, volume: Oral gavage, suspension, 5 ml/kg

Drug lot nos.: Buprenorphine, lot #17 (— , pure); naloxone, lot #231 (— , pure)

Formulation/vehicle: 0.8% aqueous hydroxypropyl-methylcellulose gel

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

Clinical signs: Observed for mortality or morbidity twice/day. Observed for external appearance and behavior once/day. Feces were monitored. Recovery group rats were monitored for the following withdrawal signs: wet-dog shakes, teeth-chattering, ptosis, writhing, vocalization at touch and diarrhea.

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

Food consumption: Determined weekly (rats housed individually). Monitoring of water bottles was done daily.

Ophthalmoscopy: Twenty-four hours after the last dosing at 4 weeks or in the recovery groups, the eyes were examined with a — ophthalmoscope (with slit lamp), if necessary, under dilation with 1% atropine sulfate for exact fundus-copy.

Hematology: Blood was drawn at week 4 in the first 10 rats/sex 24 hours after dosing (and fasting) under light ether anesthesia from the retrobulbar venous plexus, as well as in all surviving recovery rats at week 8 to measure hemoglobin, erythrocytes and leukocytes, differential blood count, hematocrit, thromboplastin time, 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, total cholesterol and plasma lactate dehydrogenase were measured.

Urinalysis: Urine was collected for a 16-hr period before [drug?] administration for analysis.

Gross pathology: All surviving animals were euthanized after 4 or 8 weeks and necropsied.

Organs weighed: Adrenals, brain, gonads, heart, kidneys, liver, lungs, pituitary, spleen, thymus and thyroid. Organs from prematurely deceased rats were weighed, but not included in the group means.

Histopathology: Samples from a number of tissues were preserved in buffered 10% formalin (see check list below), and those from the control and high dose groups were examined histologically after H & E staining. In addition, frozen sections of

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heart, liver and kidney were stained with Sudan III and those from kidney of the low and mid-dose females were examined microscopically.

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	
Gross lesions	
Gonads	X
Harderian gland	
Heart	
Ileum	X
Injection site	
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	
Parathyroid	
Peripheral nerve	X
Pharynx	
Pituitary	X
Prostate	X
Rectum	X
Salivary gland	X
Seminal vesicles	
Skeletal muscle	X
Skin	
Spinal cord	
Spleen	X
Stomach	X
Thymus	X
Thyroid	X
Tongue	

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Trachea	X
Urinary bladder	X
Uterus , including cervix	X
Vagina	

Results:

Clinical signs: One high-dose female was found dead on day 7 and one high-dose male was found dead on day 18. Necropsy failed to reveal the causes of these deaths, but lack of appetite and weight loss were noted in these rats prior to their deaths. The low dose was well tolerated, but some mid and high-dose rats showed hemochromodacryorrhea, abdominal distension and yellow-discolored feces during week 1. Sedation lasting about 30 minutes after dosing, followed by restlessness in most mid and high-dose rats and brief clonic convulsions in some rats, were noted from week 2 onwards. High dose rats also showed roughness of the coat. No withdrawal symptoms were reported during the recovery phase of the high-dose group, but they were sensitive to touch during the first two weeks of recovery.

Body weights: There was a treatment-related decrease in body weight gain, which only affected males and appeared to be unrelated to dose. This normalized during recovery.

TABLE 31: Body weight gains of rats given oral buprenorphine + naloxone for 28 days.

Dose oral buprenorphine	Body weight gain in males (g)		Body weight gain in females (g)	
	Mean ± S.D.	% of control	Mean ± S.D.	% of control
Control	153 ± 13 (30)		79 ± 15 (30)	
10 mg/kg/day	137 ± 18 (25)	90%	83 ± 12 (25)	105%
80 mg/kg/day	137 ± 19 (25)	90%	81 ± 11 (25)	103%
640 mg/kg/day	137 ± 15 (29)*	90%	86 ± 12 (29)	109%

*P<0.0001 compared with the control group (calculated from body weights by reviewer).

TABLE 32: Body weight gains in control and high dose rats during 4-week recovery after oral buprenorphine + naloxone (1:1) for 28 days.

Dose of oral buprenorphine	Body weight gain in males (g)		Body weight gain in females (g)	
	Mean ± S.D.	% of control	Mean ± S.D.	% of control
Control	80 ± 17 (5)		29 ± 13 (5)	
640 mg/kg/day	79 ± 11 (5)	99%	32 ± 9 (5)	110%

Food Consumption: The contract laboratory expressed food consumption data as grams per kg of body weight per day on a weekly basis. This method would tend to 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, all drug-treated groups except low dose females showed a slight, but significant, decrease in food consumption relative to body weight during the first week, but no decrease

occurred during week 2, when compared with the corresponding controls. During week 3, mid-dose females and both sexes at high dose showed significant decreases in food consumption, but no decreases occurred during week 4. During the 4-week recovery phase, there was a tendency for the high-dose males to consume more food per unit of body weight than the control males during 3 of the 4 weeks and for the high-dose females to exhibit increased consumption during all 4 weeks relative to the control females, but none of these differences were statistically significant with 5 rats per group.

Ophthalmoscopy: Sponsor indicated that the findings were "unremarkable."

Hematology: High-dose rats of both sexes had increased platelet counts. There was a dose-related increase in reticulocytes in both sexes. The two higher doses decreased thromboplastin time in males.

TABLE 33: Significant changes in hematological parameters after oral buprenorphine + naloxone (1:1) in rats for 28 days (n=10/group)

Parameter	Sex	Control	10 mg/kg	80 mg/kg	640 mg/kg
Platelets (x10 ³ /μl)	M	781 ± 73	N.S.	N.S.	894 ± 112*
	F	856 ± 91	N.S.	N.S.	961 ± 64**
Reticulocytes (as % of total erythrocytes)	M	8.3±0.95	8.8±1.23	9.8±1.40*	10.6±1.96†
	F	7.4±0.97	9.3±1.16***	9.8±1.32***	10.0±1.94†
Thromboplastin time (sec)	M	12.18±0.77	N.S.	11.23±0.63**	11.47±0.68*

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

Clinical chemistry: Although not mentioned by the sponsor, female rats had a treatment-related increase in glucose that would have been dose-related, except for one low value in each of the mid and high-dose groups, which gave these groups unequal variance relative to the control group. Serum sodium was increased slightly, but significantly, in both sexes at all three doses. Serum potassium was elevated slightly in males in a dose-related manner, but significant only at the high dose. Serum calcium tended to be elevated slightly in all treatment groups but was significant only for the mid-dose rats of both sexes. These changes in cations are still within the normal ranges. Serum creatinine was elevated in mid-dose males and females and in high-dose females.

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TABLE 34: Significant changes in blood chemistry parameters after oral buprenorphine + naloxone (1:1) in rats for 28 days (n=10/group)

Parameter	Sex	Control	10 mg/kg	80 mg/kg	640 mg/kg
Glucose, mmol/l	F	6.03±0.51	6.83±0.57**	6.71±0.92†	7.07±0.92†
Alkaline phosphatase	F	164.6±21.2	N.S.	N.S.	249.1±58.8†
Sodium, mmol/l	M	141.2±1.2	142.4±1.0*	144.3±0.8***	143.0±1.3**
	F	141.2±1.0	142.6±1.4*	143.2±1.6**	142.8±1.5*
Potassium, mmol/l	M	4.62±0.25	4.81±0.22	4.82±0.22	4.87±0.19*
Calcium, mmol/l	M	2.54±0.05	2.59±0.06	2.59±0.03*	2.59±0.06
	F	2.56±0.07	N.S.	2.65±0.07*	2.61±0.06
Creatinine, µmol/l	M	51.4±4.8	N.S.	56.8±3.6*	N.S.
	F	50.0±4.6	N.S.	56.4±5.6*	55.8±7.1*

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

Urinalysis: Unremarkable, except for an increase in volume by high-dose rats from week 2 onwards.

Gross Pathology: Unremarkable

Organ weights: Statistical evaluation of relative organ weights was not provided.

TABLE 35: Significant changes in organ weights after oral buprenorphine + naloxone (1:1) in rats for 28 days (n=10/group)

Parameter (grams)	Sex	Control	10 mg/kg	80 mg/kg	640 mg/kg
Body weight	M	273±14	256±21#	258±22#	252±14#
	F	192±13	195±14	193±13	199±14
Brain	M	1.87±0.08	N.S.	N.S.	1.80±0.10#
Liver	M	11.7±1.7	10.3±1.6#	11.1±1.5	12.8±2.3
	F	9.4±1.3	9.4±1.1	10.1±1.2	11.4±1.1#
Right kidney	M	1.08±0.10	0.99±0.09#	0.99±0.11#	0.98±0.09#
Left adrenal	M	0.031±0.009	N.S.	N.S.	0.038±0.009
Thyroid	F	0.011±0.003	N.S.	0.009±0.002#	N.S.

N.S.= not significant; *P<0.05, **p<0.01, ***p<0.001. † t-test not valid due to unequal variance. #Conducting laboratory did not report p-values, only t-values.

Histopathology: Unremarkable, except for a treatment-related increase in the incidence of microliths in the renal collecting ducts of females.

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Study Title: Buprenorphine/Naloxone Preliminary Toxicity to Rats by Repeated Intravenous Administration for Two Weeks.

Study No.: 105345 Volume #: 12 Tab#: 105345

Conducting Laboratory: _____

Date of Study Initiation: December 1, 1983

GLP Compliance: (X) Yes () No

QA Report: () Yes (X) No

Methods:

Dosing:

Species/strain: Rat / Sprague-Dawley _____

No./sex/group: 5/sex/dose

Age: Approximately 5 weeks.

Weight: 111-132 grams

Dosage groups: Buprenorphine + Naloxone (3:2): 0 (vehicle), 0.4, 2 and 10 mg/kg/day for 14 days.

Route, form, volume: Intravenous (tail vein), solution, 4 ml/kg

Drug lot nos.: Buprenorphine, lot #17 (_____ pure); naloxone, lot #231 (_____ pure)

Formulation/vehicle: Sterile saline, pH adjusted to 5.5.

Observation period: 2 weeks

Clinical signs: Observed for mortality or morbidity twice/day. Observed for signs of ill health, changes in behavior or reactions to treatment once/day on weekdays

Body weights: Measured at time of group assignment, initiation of treatment and weekly thereafter.

Food consumption: Measured weekly per cage, each housing 5 rats, unless death or separation for ill health or aggression intervened. Visual monitoring of water bottles was done daily.

Gross pathology: All surviving animals were euthanized by CO₂ asphyxiation on day 14 and necropsied.

Organs weighed: Adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes, thyroid and uterus.

Histopathology: Although samples from a number of tissues were preserved in buffered 10% formalin, including macroscopically abnormal tissue and adjacent normal tissue, the tissue samples were not processed further.

Results:

Clinical signs: One mid-dose female died on the second day of dosing immediately after receiving 2 mg/kg, but showed no postmortem abnormalities related to dosing. High-dose groups were killed on days 4 or 5 due to the severity of injection site reactions (swelling, dark discoloration and/or ulceration of the tail) that precluded further dosing. Similar injection difficulties were encountered in some mid-dose females during week 2.

Body weights:

TABLE 36: Body weight gains of rats receiving IV buprenorphine+naloxone for 14 days.

Dose	Body weight gain in males (g)		Body weight gain in females (g)	
	Mean ± S.D.	% of control	Mean ± S.D.	% of control
Control	108 ± 5.4		56 ± 6.5	
0.4 mg/kg/day	77 ± 4.3 ***	71%	46 ± 6.2*	82%
2.0 mg/kg/day	86 ± 6.8***	80%	45 ± 4.4*	80%
10 mg/kg/day	N/A	N/A	N/A	N/A

*P<0.05, compared with the corresponding control group.

***P<0.001, compared with the corresponding control group.

N/A: data not available due to premature euthanasia.

Food Consumption: No clear dose-related effects were apparent.

TABLE 37: Effect of IV buprenorphine + naloxone (3:2) on food consumption by rats.

Dose	Mean food consumption (g/rat/week)			
	Males	% of control	Females	% of control
Control	308		269	
0.4 mg/kg/day	325	106%	248	92%
2.0 mg/kg/day	304	99%	254	94%
10 mg/kg/day	N/A	N/A	N/A	N/A

N/A: data not available due to premature euthanasia.

Gross Pathology: The high-dose rats euthanized prematurely showed congestion, ulceration and edema of the tails, as well as enlargement or congestion of the lumbar and/or inguinal lymph nodes. Necropsy findings of rats in the lower dose groups were unremarkable.

Organ weights:

TABLE 38: Significant changes in organ weights after IV buprenorphine + naloxone (3:2) in rats for 14 days (n=5/group)

Parameter (grams)	Sex	Control	0.4 mg/kg	2 mg/kg	10 mg/kg
Body weight	M	230±10	202±9	211±11	N/A
	F	166±11	151±4	155±8	N/A
Liver	M	15.9±1.2	11.6±1.2*	12.0±0.8**	N/A
	F	9.8±1.8	8.3±1.3	8.9±1.4	N/A
Kidneys	F	2.0±0.05	1.7±0.05**	1.7±0.16**	N/A
Thyroid	F	0.011±0.002	0.014±0.002	0.015±0.002*	N/A

After adjustment for final body weight as a covariate, *p<0.05 and **p<0.01 (Williams' test). N/A: data not available due to premature euthanasia.

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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: 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 Intravenous and Subcutaneous Administration to Sprague-Dawley Rats.

Study No.: 36936 **Volume #:** 13 **Tab#:** 36936

Conducting Laboratory: []

Date of Study Initiation: January 31, 1984

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

Methods:

Dosing: Daily, 7 days/week for 4 weeks

Species/strain: Rat / Sprague-Dawley

No./sex/group: 25/sex/dose (main study)

Age: 28 days for males; 30 days for females

Weight (at receipt): males, 64-82 g; females, 64-81 g

Satellite group for recovery: 5/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 (IV + SC vehicle), 0.5 and 4.5 (IV) and 90 (SC) mg/kg/day

Route, form, volume: IV (two lower doses) or SC (high dose), solution, 10 ml/kg

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

Formulation/vehicle: 5% aqueous glucose

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

Clinical signs: Observed for mortality or morbidity twice/day. Observed for external appearance, local tolerance and behavior once/day. Feces were monitored daily.

Recovery group rats were monitored for the following withdrawal signs: wet-dog shakes, teeth-chattering, ptosis, writhing, vocalization upon touch and diarrhea.

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

Food consumption: Determined weekly (rats housed individually). Monitoring of water bottles was done daily.

Ophthalmoscopy: Twenty-four hours after the last dosing at 4 weeks or in the recovery groups, the eyes were examined with a ——— ophthalmoscope (with slit lamp), if necessary, under dilation with 1% atropine sulfate for exact funduscopy.

Hematology: Blood was drawn at week 4 in the first 10 rats/sex/group 24 hours after dosing (and fasting) under light ether anesthesia from the retrobulbar venous plexus, as well as in all surviving recovery rats at week 8, to measure hemoglobin,

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erythrocytes and leukocytes, differential blood count, hematocrit, thromboplastin time, 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, total cholesterol and plasma lactate dehydrogenase were measured.

Urinalysis: Urine was collected from the first 10 rats/sex/group after 4 test weeks for a 16-hr period for analysis of color, specific gravity, protein, glucose, bilirubin, hemoglobin, ketone bodies, pH, urobilinogen and identification of sediment.

Gross pathology: All surviving animals were euthanized after 4 or 8 weeks and necropsied.

Organs weighed: Adrenals, brain, gonads, heart, kidneys, liver, lungs, pituitary, spleen, thymus and thyroid. Organs from prematurely deceased rats were weighed, but not included in the group means.

Histopathology: Samples from a number of tissues were preserved in buffered 10% formalin (see check list below), and those from the control, medium (IV) and high (SC) dose groups, as well as tissues in the injection sites of the low dose group, were examined histologically after H & E staining. 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	
Gross lesions	
Gonads	X
Harderian gland	
Heart	
Ileum	X
Injection site	
Jejunum	X
Kidneys	X
Lachrymal gland	
Larynx	X
Liver	X
Lungs	X
Lymph nodes, cervical	
Lymph nodes, mandibular	

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Lymph nodes, submaxillary	
Lymph nodes, mesenteric	X
Mammary glands	X
Nasal cavity	
Pancreas	
Parathyroid	
Peripheral nerve	X
Pharynx	
Pituitary	X
Prostate	X
Rectum	X
Salivary gland	X
Seminal vesicles	
Skeletal muscle	X
Skin	
Spinal cord	
Spleen	X
Stomach	X
Thymus	X
Thyroid	X
Tongue	
Trachea	X
Urinary bladder	X
Uterus, including cervix	X
Vagina	

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Results:

Clinical signs: None at low IV dose. At high IV dose, inflammation of the tail affected all rats by week 3 and some progressed to necrosis. High-dose IV rats were sensitive to the touch during week 4. Skin lesions (wheals, open wounds, eschar formations) leading to sensitivity to the touch in the SC dosed rats also were observed.

Body weights: Sponsor reported no significant effects of the two IV doses on body weights relative to controls during the four weeks of treatment, but significant decreases in males receiving the SC dose, beginning at week 2 of treatment and continuing throughout treatment and recovery. These effects are also reflected in the body weight gain data, shown in the table below, except that the low IV dose also significantly affected body weight gain in males. A valid t-test on the higher IV dose could not be conducted because of unequal variance.

TABLE 39: Body weight gains of rats receiving buprenorphine+naloxone (3:2), 28 days.

Buprenorphine Dose (mg/kg/d)	Body weight gain in males (g)		Body weight gain in females (g)	
	Mean ± S.D.	% of control	Mean ± S.D.	% of control
Control, IV+SC	130 ± 12 (30)		67 ± 9 (30)	
0.5, IV	113 ± 13 (25)*	87%	68 ± 9 (25)	101%
4.5, IV	120 ± 20 (25)	92%	73 ± 11 (25)	109%
90, SC	100 ± 16 (30)*	77%	75 ± 9 (30)	112%

*P<0.0001 compared with the control group (calculated from body weights by reviewer).

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TABLE 40: Body weight gains during 4-week recovery after SC buprenorphine + naloxone (3:2) for 28 days.

Buprenorphine Dose (mg/kg/d)	Body weight gain in males (g)		Body weight gain in females (g)	
	Mean ± S.D.	% of control	Mean ± S.D.	% of control
Control, IV+SC	88 ± 25 (5)		40 ± 8 (5)	
90, SC	53 ± 26 (5)	60%	39 ± 5 (5)	98%

Food Consumption: Sponsor reported food consumption in terms of grams/kg of body weight per day and found no significant effects of treatment on this parameter. In the control rats, this parameter decreased over time from mean (± S.D.) starting male and female values of 131±12 and 134±9, respectively, to 85±5 and 91±13, respectively, at week 4 of treatment and to 74±14 and 88±10, respectively, at week 8 in the recovery animals.

Ophthalmoscopy: Unremarkable.

Hematology: The SC dose (90 mg/kg/day) decreased total protein and hemoglobin in both sexes and decreased erythrocytes (RBCs) and hematocrit in females, but increased leukocytes in females and platelets in males. Some of these effects may be secondary to severe injection site reactions. Reticulocytes were increased in both sexes by both the high IV dose (4.5 mg/kg) and the SC dose.

TABLE 41: Significant changes in hematological parameters after IV or SC buprenorphine + naloxone (3:2) in rats for 28 days (n=10/group)

Parameter	Sex	Control	0.5 mg/kg	4.5 mg/kg	90 mg/kg SC
Hemoglobin, mmol/l	M	8.1±0.3	N.S.	N.S.	7.5±0.4***
	F	8.2±0.3	N.S.	N.S.	7.2±0.2***
RBCs (x10 ⁶ /µl)	F	6.2±0.4	N.S.	N.S.	5.5±0.2***
Hematocrit (% v/v)	F	43.1±1.2	N.S.	N.S.	38.8±1.5***
Leukocytes (x10 ³ /µl)	F	9.6±1.9	N.S.	N.S.	14.0±2.9***
Platelets (x10 ³ /µl)	M	788±97	N.S.	N.S.	1047±212†
Reticulocytes (as % of total erythrocytes)	M	13.1±1.9	N.S.	19.5±2.2***	27.0±7.9†
	F	11.8±2.6	N.S.	19.1±3.8***	27.6±5.2†

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

Clinical chemistry: Intravenous administration had a tendency to increase blood glucose, which was significant for the lower dose (0.5 mg/kg) in both sexes. The high IV dose increased sodium in females and the SC dose increased urea and sodium in both sexes, as well as potassium in females. With the possible exception of urea, these changes are not of biological significance.

TABLE 42: Significant changes in blood chemistry parameters after IV or SC buprenorphine + naloxone (3:2) in rats for 28 days (n=10/group)

Parameter	Sex	Control	0.5 mg/kg	4.5 mg/kg	90 mg/kg SC
Glucose, mmol/l	M	5.74±0.60	6.65±0.60**	6.17±0.47	5.52±0.44
	F	5.98±0.62	6.78±0.89*	6.99±1.01	6.41±0.76
Alkaline phosphatase	F	178±39	N.S.	N.S.	329±188†
AST, U/l	M	40.1±3.2	N.S.	N.S.	56.3±8.2†
	F	32.5±3.0	N.S.	N.S.	54.1±8.1†
Sodium, mmol/l	M	144.8±1.1	N.S.	N.S.	147.0±1.2***
	F	144.2±1.0	N.S.	145.8±1.2**	145.5±1.3*
Potassium, mmol/l	F	3.81±0.18	N.S.	N.S.	4.44±0.27***
Calcium, mmol/l	M	2.61±0.06	N.S.	N.S.	2.53±0.07*
Urea, mmol/l	M	5.97±1.09	N.S.	N.S.	7.73±1.40**
	F	5.86±0.94	N.S.	N.S.	6.87±1.16*
Total protein, g/l	M	52.6±3.9	N.S.	N.S.	46.4±1.7†
	F	54.8±3.5	N.S.	N.S.	47.8±3.5***

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

Urinalysis: Unremarkable

Gross pathology: Lesions were observed at the injection sites.

Organ weights: Statistical evaluation of relative organ weights was not provided.

TABLE 43: Significant changes in organ weights after IV or SC buprenorphine + naloxone (3:2) in rats for 28 days (n=10/group)

Parameter (grams)	Sex	Control	0.5 mg/kg	4.5 mg/kg	90 mg/kg SC
Body weight	M	249±16	N.S.	N.S.	221±19***
Liver	M	10.6±1.3	9.5±1.3#	N.S.	N.S.
	F	8.8±1.0	N.S.	N.S.	10.6±1.0***
Spleen	M	0.60±0.08	N.S.	N.S.	0.81±0.18#
	F	0.51±0.08	N.S.	0.58±0.09#	0.86±0.09#
Kidney, left right left right	M	0.98±0.09	N.S.	N.S.	0.91±0.17#
		0.96±0.09	N.S.	N.S.	0.89±0.09#
	F	0.77±0.07	N.S.	N.S.	0.86±0.09#
		0.77±0.07	N.S.	N.S.	0.85±0.09#
Adrenal, left right	M	0.028±0.006	N.S.	N.S.	0.036±0.009#
		0.028±0.006	N.S.	N.S.	0.035±0.006#
Thymus	M	0.61±0.11	N.S.	0.52±0.11#	0.51±0.09#
Thyroid	F	0.008±0.002	0.01±.002#	N.S.	N.S.

N.S.= not significant; *P<0.05, **p<0.01, ***p<0.001. † t-test not valid due to unequal variance. #Conducting laboratory did not report p-values, only t-values.

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Histopathology: Rats dosed IV showed perivascular hemorrhage and/or inflammation at the injection sites. Rats dosed SC showed chronic splenitis, chronic peritonitis and peripancreatitis with adhesions, findings which are consistent with penetration of the injected material into the peritoneal cavity.

KEY STUDY FINDINGS

The 3:2 buprenorphine/naloxone combination selectively decreased body weight gain in male rats by both the IV and SC routes during a 4-week treatment period, an effect from which full recovery had not been achieved by 4 weeks following cessation of treatment. The SC dose (90 mg/kg/day as buprenorphine) decreased total protein and hemoglobin in both sexes and decreased erythrocytes and hematocrit in females, but increased leukocytes in females, platelets in males and sodium and urea in both sexes. Some of these effects may be secondary to severe injection site reactions.

Reticulocytes were increased in both sexes by both the high IV (4.5 mg/kg) and SC doses. Glucose was increased in rats receiving the low IV dose (0.5 mg/kg). Several organ weights were changed by the SC dose in a manner that was not uniform among the sexes, such as decreased kidney weights in males, but increased kidney weights in females. Gonad weights were decreased 10-13% by the SC dose in males.

Histological examination indicated injection site reactions, as well as penetration of the SC dosing into the peritoneal cavity with consequent organ inflammation.

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: 4-Week Toxicity of the Substance Mixture Buprenorphine-HCl, Lot No. 22 and Naloxone-HCl, Batch No. 231 (Ratio 3:2) — Called for Short 'BUP + NAL' — by Intramuscular Administration to Sprague-Dawley Rats.

Study No.: R06866 **Volume #:** 14 **Tab#:** R06866

Conducting Laboratory: []

Date of Study Initiation: August 21, 1984

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

Methods:

Dosing: Daily, 7 days/week for 4 weeks

Species/strain: Rat / Sprague-Dawley

No./sex/group: 25/sex/dose (main study)

Age: 28 days for males; 31 days for females

Weight: 73-84 g for males; 65-80 g for females

Satellite group for recovery: 5/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), 0.9, 9.0 and 90 mg/kg/day

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Route, form, volume: Intramuscular, solution (0.9 mg/kg) or suspension (9 and 90 mg/kg), 2.5 ml/kg

Drug lot nos.: Buprenorphine, lot #22; naloxone, lot #231 (—— pure)

Formulation/vehicle: 5% aqueous glucose

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

Clinical signs: Observed for external appearance, local tolerance and behavior once/day. Feces were monitored daily. Recovery group rats were monitored for the following withdrawal signs: wet-dog shakes, teeth-chattering, ptosis, writhing, vocalization upon touch and diarrhea.

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

Food consumption: Determined weekly (rats housed individually). Monitoring of water bottles was done daily.

Ophthalmoscopy: Twenty-four hours after the last dosing at 4 weeks or in the recovery groups, the eyes were examined with a —— ophthalmoscope (with slit lamp), if necessary, under dilation with 1% atropine sulfate for exact fundus-copy.

Hematology: Blood was drawn at week 4 in the first 10 rats/sex/group 24 hours after dosing (and fasting) under light ether anesthesia from the retrobulbar venous plexus, as well as in all surviving recovery rats at week 8, to measure hemoglobin, erythrocytes and leukocytes, differential blood count, hematocrit, thromboplastin time, 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, total cholesterol and plasma lactate dehydrogenase were measured.

Urinalysis: Urine was collected from the first 10 rats/sex/group after 4 test weeks for a 16-hr period for analysis of color, specific gravity, protein, glucose, bilirubin, hemoglobin, ketone bodies, pH, urobilinogen and identification of sediment.

Gross pathology: All surviving animals were euthanized after 4 or 8 weeks and necropsied.

Organs weighed: Adrenals, brain, gonads, heart, kidneys, liver, lungs, pituitary, spleen, thymus and thyroid (left only unless abnormal). Organs from prematurely deceased rats were weighed, but not included in the group means.

Histopathology: Samples from a number of tissues were preserved in buffered 10% formalin (see check list below), and those from the control, medium (IV) and high (SC) dose groups, as well as tissues in the injection sites of the low dose group, were examined histologically after H & E staining. In addition, frozen sections of heart, liver and kidney were stained with Sudan III. Injection sites from the 0.9 and 9 mg/kg/day groups were also examined microscopically.

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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	
Gross lesions	
Gonads	X
Harderian gland	
Heart	
Ileum	X
Injection site	
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	
Parathyroid	
Peripheral nerve	X
Pharynx	
Pituitary	X
Prostate	X
Rectum	X
Salivary gland	X
Seminal vesicles	
Skeletal muscle	X
Skin	
Spinal cord	
Spleen	X
Stomach	X
Thymus	X
Thyroid	X
Tongue	
Trachea	X
Urinary bladder	X
Uterus , including cervix	X
Vagina	

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Results:

Clinical signs: Two high-dose males were found dead on days 24-25 and a third high-dose male was found dead on the second day of recovery. Aside from injection site reactions in all three, the only other pathology reported was microscopic atrophy of the spleen, spermatogenic arrest and presence of giant cells in the testes of the third male. Sponsor considered cause of death in all three males to be possibly due to sudden cardiovascular collapse. However, the rat that died on the second day of recovery showed a body weight loss from a baseline of 129 grams to 101 grams at week 4. High-dose rats displayed sedation a few minutes after dosing during week 2, which lasted 2-3 hours and progressed to a longer duration (3-4 hours) during weeks 3 and 4.

Body weights:

TABLE 44: Body weight gains of rats receiving buprenorphine+naloxone (3:2), 28 days.

Buprenorphine Dose (mg/kg/d)	Body weight gain in males (g)		Body weight gain in females (g)	
	Mean ± S.D.	% of control	Mean ± S.D.	% of control
Control	141 ± 17 (30)		81 ± 14 (30)	
0.9	123 ± 13 (25)*	87%	71 ± 13 (25)*	88%
9.0	100 ± 11 (25) ¹	71%	70 ± 11 (25)*	86%
90	76 ± 19 (27)*	54%	61 ± 13 (29) ^{*2}	75%

*P<0.01 compared with the control group (calculated from body weights by reviewer).

¹t-test not valid due to unequal variance between test group and control.

²Does not include one female that lost 17 grams (14% of body weight) during treatment.

TABLE 45: Body weight gains during 4-week recovery after IM buprenorphine + naloxone (3:2) for 28 days.

Buprenorphine Dose	Body weight gain in males (g)		Body weight gain in females (g)	
	Mean ± S.D.	% of control	Mean ± S.D.	% of control
Control, vehicle	83 ± 26 (5)		37 ± 12 (5)	
90 mg/kg/day	100 ± 13 (4)	120%	66 ± 5 (5)†	178%

† t-test not valid due to unequal variance between test group and control.

Food Consumption: The testing laboratory reported food consumption in terms of grams/kg of body weight per day on a weekly basis. On this basis, food consumption was unaffected by treatment.

Ophthalmoscopy: Unremarkable.

Hematology: The NOAEL dose for hematological parameters was 0.9 mg/kg/day.

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TABLE 46: Significant changes in hematological parameters after IM buprenorphine + naloxone (3:2) in rats for 28 days (n=10/group)

Parameter	Sex	Control	0.9 mg/kg	9.0 mg/kg	90 mg/kg
Hemoglobin, mmol/l	M	8.9±0.3	N.S.	N.S.	8.2±0.3***
	F	8.9±0.5	N.S.	N.S.	7.8±0.4***
Hematocrit (% v/v)	M	46.2±1.6	N.S.	44.2±1.5**	44.3±3.2†
	F	44.4±1.9	N.S.	N.S.	41.4±2.2**
Leucocytes (x10 ³ /µl)	M	12.1±2.0	N.S.	N.S.	15.5±2.9**
	F	8.2±1.2	N.S.	N.S.	15.8±3.4†
Platelets (x10 ³ /µl)	F	858±178	N.S.	N.S.	1067±167*
Reticulocytes (as % of total erythrocytes)	M	12.3±3.6	N.S.	20.5±6.0**	42.6±17.4†
	F	11.1±2.8	N.S.	16.8±3.8**	35.9±12.3†

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

Clinical chemistry: The NOAEL dose for clinical chemistry parameters was <0.9 mg/kg/day, as glucose and sodium were elevated in both sexes by this dose. Although the elevation of sodium appeared to be dose-related and highly significant in the males at the two higher doses, the changes were very small and not of biological significance, as the sodium concentrations in samples from all of the animals were within the normal range.

TABLE 47: Significant changes in blood chemistry parameters after IM buprenorphine + naloxone (3:2) in rats for 28 days (n=10/group, except glucose)

Parameter	Sex	Control	0.9 mg/kg	9.0 mg/kg	90 mg/kg
Glucose, mmol/l (N = 7)	M	6.48±0.77	8.09±1.22*	N.S.	5.35±0.55**
	F	6.59±0.49	8.18±0.52***	N.S.	N.S.
AST, U/l	M	44.6±6.2	N.S.	59.5±13.1†	61.9±18.2†
	F	38.1±8.3	N.S.	N.S.	54.4±8.7***
Sodium, mmol/l	M	141.3±0.9	142.8±1.48*	144.0±0.9***	144.4±1.3***
	F	141.1±1.6	N.S.	143.1±1.7*	142.8±1.7*
Potassium, mmol/l	F	3.67±0.26	N.S.	4.10±0.44*	4.28±0.33***
Calcium, mmol/l	F	2.54±0.08	N.S.	2.68±0.10**	2.65±0.08**
Uric acid, µmol/l	M	66.7±20.8	N.S.	N.S.	100.7±20.3**

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

Urinalysis: Unremarkable

Gross Pathology: Unremarkable, except for a dose-related increase in the incidence of injection site intolerance, including lesions, indurations and swelling.

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Organ weights: Statistical evaluation of relative organ weights was not provided. Body weights of males decreased in a dose-related manner by 6.6%, 15.8% and 24.6%, with significant decreases in the weights of all organs measured at the high dose, except the adrenals which showed significant 16-19% increases in weight and the spleen which increased 21%. Most organ weights in males were also significantly decreased at the middle dose, except brain, heart, adrenals and spleen. Only the liver weight was significantly decreased (13.9%) at the low dose. Body weights of females decreased in a dose-related manner less markedly than in males by 4.8%, 6.0% and 10.7%, with significant decreases in the weights of liver at the low dose (12.4%) and the thymus at high dose (21.9%). As in the males, adrenal weights increased significantly at the high dose (14-16%), and spleen weights increased in a dose-related manner by 6, 33 and 48%, the latter two being statistically significant.

Histopathology: There was a dose-related increase in incidence and severity of intolerance at the injection site, including myositis with inflammatory lesions and necrotic changes, as well as neuritis and/or perineuritis of peripheral nerves. In the recovery group, evidence of healing at the injection sites was apparent.

KEY STUDY FINDINGS

In groups of rats injected IM daily for 4 weeks with 3:2 buprenorphine+naloxone mixtures containing 0.9, 9 or 90 mg/kg as buprenorphine, two high-dose males died during treatment (days 24 and 25) and another died during early recovery. Both sexes showed significant dose-related decreases in body weight gain at all doses. High dose rats showed significant decreases in hemoglobin and hematocrit, but significant increases in leucocytes and reticulocytes, the latter also being significantly elevated at mid dose. High-dose rats showed increased spleen and adrenal weights and decreased thymus weights. The NOAEL was <0.9 mg/kg/day and the LOAEL was 9.0 mg/kg/day.

Study Title: Buprenorphine/Naloxone Local Tolerance and Preliminary Toxicity to Rats by Repeated Subcutaneous Administration for Two Weeks.

Study No.: 105344 Volume #: 12 Tab#: 105344

Conducting Laboratory: _____

Date of Study Initiation: November 17, 1983

GLP Compliance: (X) Yes () No

QA Report: () Yes (X) No

Methods:

Dosing: Daily injections, 7 days/week for 2 weeks.

Species/strain: Rat / Sprague-Dawley (_____)

No./sex/group: 5/sex/dose

Age: Approximately 5 weeks.

Weight: 118-149 grams

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Satellite group for health check: 5/sex were euthanized and necropsied before treatment of other groups began.

Dosage groups: Buprenorphine + Naloxone (3:2): 0 (vehicle), 0.4, 2.0 and 10.0 mg/kg/day

Route, form, volume: Subcutaneous, suspension, 4 ml/kg

Drug lot nos.: Buprenorphine, lot #17 (——— pure); naloxone, lot #231 (——— pure)

Formulation/vehicle: Saline.

Observation period: 2 weeks

Clinical signs: Observed for mortality or morbidity twice/day. Observed for signs of ill health, changes in behavior or reactions to treatment once/day on weekdays.

Body weights: Measured at time of group assignment, initiation of treatment and weekly thereafter.

Food consumption: Measured weekly per cage, each housing 5 rats, unless death or separation for ill health or aggression intervened. Visual monitoring of water bottles was done daily.

Gross pathology: All surviving animals were euthanized by pentobarbital anesthesia and exsanguination on day 14 and necropsied.

Organs weighed: Adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes, thyroid and uterus.

Histopathology: Preservation and examination restricted to injection sites.

Results:

Clinical signs: The only signs of reaction to treatment were a localized hair loss that was observed at all doses and formation of small scabs and/or discoloration on the tails.

Body weights:

TABLE 48: Body weight gains of rats given SC buprenorphine+naloxone for 14 days.

Dose	Body weight gain in males (g)		Body weight gain in females (g)	
	Mean ± S.D.	% of control	Mean ± S.D.	% of control
Control	117 ± 12.3		56 ± 4.7	
0.4 mg/kg/day	83 ± 8.7***	71%	43 ± 5.3**	77%
2.0 mg/kg/day	72 ± 10.1***	62%	41 ± 5.1***	73%
10 mg/kg/day	88 ± 8.1***	75%	47 ± 7.1*	84%

*P<0.05, **p<0.01 and ***p<0.001, compared with the corresponding control group.

Food Consumption:

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TABLE 49: Effect of SC buprenorphine + naloxone (3:2) on food consumption by rats housed in groups of 5/cage.

Dose	Total mean food consumption (g/rat/2 weeks)			
	Males	% of control	Females	% of control
Control	342		256	
0.4 mg/kg/day	314	92%	248	97%
2.0 mg/kg/day	280	82%	244	95%
10 mg/kg/day	292	85%	245	96%

Gross Pathology: Health check group had one male with a nodule in the gastric antrum mucosa which, upon microscopic examination, consisted of an ectopic focus of non-glandular epithelium in the glandular region of the stomach; however, the group showed no sign of infectious disease. In the main study, high-dose males (3/5) showed enlargement of inguinal lymph nodes, and there was an increased incidence of opacity of the splenic capsule in 4/5 male and 1/5 female high-dose rats. Tail scabs and alopecia were also observed in some treated animals, but none of the controls.

Histopathology: Rats in the two higher dose groups showed a dose-related increase in the incidence of injection site necrosis and/or hemorrhage, with inflammatory cell infiltration and fibroblast proliferation, sometimes also with degeneration and inflammatory cell infiltration in the panniculus muscle.

KEY STUDY FINDINGS

In groups of rats injected SC daily for 2 weeks with 3:2 buprenorphine+naloxone in doses of 0.4, 2.0 or 10 mg/kg as buprenorphine, both sexes showed decreased body weight gains at all doses and the male groups showed decreased food consumption compared with controls. A dose-related injection site intolerance of moderate severity, including hemorrhage, necrosis, inflammatory cell infiltration and fibroblast infiltration, sometimes also involving the panniculus muscle, was documented for the two higher doses by gross and histopathological examination.

General Comments: The following is a dose range-finding study in the dog.

Study Title: Buprenorphine/Naloxone Preliminary Oral Toxicity Study in Beagle Dogs
(Repeated Daily Dosage for 4 Weeks)

Study No.: 36949 Volume #: 12 Tab#: 36949

Conducting Laboratory: _____

Date of Study Initiation: November 22, 1983

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

Methods: Dosing: Daily injections, 7 days/week for 4 weeks.

Species/strain: Dog / beagle (_____)

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No./sex/group: 1/sex/dose

Age: 24-25 weeks.

Weight: Males, 7.5 and 9.0 kg; females, 6.9 and 8.7 kg.

Dosage groups: Buprenorphine + Naloxone (1:1): 75 and 125 mg/kg/day.

Route, form, volume: Oral, gelatin capsule (separate capsules for each drug).

Drug lot nos.: Buprenorphine, lots #17 and #21; naloxone, lot #231 (pure)

Formulation/vehicle: Saline.

Observation period:

Clinical signs: Observed for signs of ill health, changes in behavior or reactions to treatment regularly throughout the day on weekdays and up to mid-day on holidays and weekends.

Body weights: Measured twice per week prior to feeding throughout the pre-dosing and dosing periods.

Food consumption: Amount consumed (out of 400 g/day offered) was measured daily.

Clinical chemistry: Blood samples were taken for PK analysis on day 1 and day 28 (3 survivors), but the results of these analyses were not included in this report.

Gross pathology: All surviving animals were euthanized by exsanguination under pentobarbital anesthesia and necropsied.

Organs weighed: Adrenals, brain, heart, kidneys, liver, lungs, pancreas, pituitary, spleen, testes or ovaries, thymus, thyroids and uterus or prostate.

Histopathology: Samples from a number of tissues were preserved in buffered 10% formalin (see check list below), together with any tissue showing macroscopic abnormality. However, they were not processed further.

Results:

Clinical signs: Abnormally quietness during the first two weeks. Occasional observations included salivation, vomiting, and unsteady gait associated with slight paresis of the hind limbs. The high-dose female convulsed repeatedly about 2 hours after dosing on day 18 and was euthanized.

Body weights:

TABLE 50: Summary of body weight changes in dogs receiving oral buprenorphine + naloxone (1:1) for 28 days.

Time Period	Body weights at 75 mg/kg/day		Body weights at 125 mg/kg/day	
	Male	Female	Male	Female
Initial weight, g	8200	8000	9800	9600
Low weight, g	7300 (1½ wks)	7400 (1½ wks)	9000 (at 2 wks)	9600 (1½ wks)
High weight, g	8500	8500	9800	9900
Last weight, g	8500	8500	9600	9900†(2½ wks)

†Last weighing before premature euthanasia on Day 18 due to repeated seizures.

Food Consumption:

TABLE 51: Effect of oral buprenorphine + naloxone (1:1) on food consumption by dogs.

Treatment week	% of Diet Offered (400 g/dog/day) Consumed per Treatment Week			
	75 mg/kg/day, PO		125 mg/kg/day, PO	
	Male	Female	Male	Female
-2	100%	100%	100%	100%
-1	100%	100%	100%	100%
1	36.8%	50.7%	61.1%	66.1%
2	65.7%	73.9%	55.0%	86.8%
3	65.4%	83.9%	75.7%	65.0%
4	70.7%	100%	86.1%	DEAD

Gross pathology: Unremarkable in the three surviving dogs. The euthanized high-dose female had a few scattered pale areas on the lungs, and the left diaphragmatic lobe was congested. A patchy subendocardial hemorrhage was present in the left ventricle of the heart. Discoloration of the stomach and jejunum, as well as minimal congestion of the duodenum, were also noted.

Organ weights: Unremarkable, except that the liver weight of the high-dose male exceeded the laboratory's historical range.

KEY STUDY FINDINGS

Individual dogs received oral capsules daily for 4 weeks containing 75 or 125 mg/kg each of buprenorphine and naloxone. Both sexes showed weight loss at both doses, which reached a trough at 1½-2 weeks and this was accompanied by decreased food consumption compared with pretreatment baseline data. The high-dose female was euthanized on day 18 due to repeated convulsions that began about 2 hr post-dosing. No target organ was identified in male dogs dosed with up to 125 mg/kg/day of each drug. Pathological findings in the high dose female that was euthanized involved lung, heart and gastrointestinal tract.

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. 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 1:1) — Called for Short 'BUP + NAL' — by Oral Administration to Beagle Dogs.

Study No.: 36937 Volume #: 16 Tab#: 36937

NDA 20-733 (Suboxone®)

Conducting Laboratory: []

Date of Study Initiation: January 26, 1984

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

Methods:

Dosing: Daily, 7 days/week for 4 weeks

Species/strain: Dog / beagle ()

No./sex/group: 4/sex/dose (main study)

Age: 9-10 months

Weight: 6.98-7.38 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 (1:1): 0 (cane sugar), 2.5, 15.0 and 90 mg/kg/day of each in one capsule.

Route, form, volume: Oral, by capsule (after feeding)

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

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. Feces were monitored. Recovery group dogs were monitored during the first week for the following withdrawal signs: Fright, baring of teeth, continuous yawning, chewing, swallowing, salivation; piloerection, shaking, tremor, muscle twitching, excitation, aggressiveness, vomiting, diarrhea, bizarre movement, stretching movement, dorsal position, abdominal position, lateral position with closed eyes; penile erection, and repeated vocalization (especially upon touch).

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

Food consumption: Estimated daily 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 and 2 hours 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 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

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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, total cholesterol and plasma lactate dehydrogenase were measured. Liver function was also measured with the bromsulphthalein test.

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 those from the control, medium (IV) and high (SC) dose groups, as well as tissues in the injection sites of the low dose group, were examined histologically after H & E staining. In addition, frozen sections of heart, liver and kidney were stained with Sudan III. Injection sites from the 0.9 and 9 mg/kg/day groups were also examined microscopically.

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 site	
Jejunum	X

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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
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	
Trachea	X
Urinary bladder	X
Uterus	X
Vagina	

**APPEARS THIS WAY
ON ORIGINAL**

Results:

Clinical signs: All four females receiving the middle dose vomited on a few occasions, as did most of the dogs (8/10) receiving the high dose. All high-dose dogs exhibited sedation during week 1.

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, only the control dogs showed a substantial gain in body weight during the 4-week treatment period (Table 52):

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TABLE 52: Body weight gains of dogs (male + female) receiving oral buprenorphine + naloxone (1:1) for 28 days (n=10/dose).

Buprenorphine Dose (mg/kg/d)	Body weight gain in dogs (kg)	
	Mean ± S.D.	% of Control
Control	0.59 ± 0.57 (10)	
2.5	-0.16 ± 0.32 (8)*	0%
15	0.04 ± 0.30 (8)†	7%
90	0.02 ± 0.42 (10)*	3%

*P<0.025 compared with the control group (calculated from body weights by reviewer).
†t-test not valid due to unequal variance between test group and control.

Food Consumption: During the first week food consumption was about 1/3 less in the two high-dose groups than in the controls, but normalized thereafter.

Ophthalmoscopy: Unremarkable

EKG: No significant differences between Week 4 and Day 1 in heart rate were noted, although means of low, mid and high dose groups increased by 13, 9 and 20 beats/min.

Hematology: Parameters were analyzed statistically by the sponsor by combining the sexes and no significant differences were found. Considering the increases in reticulocytes reported above for rats, however, this reviewer conducted separate t-tests on males (n=5/group) and females (n=5/group) for reticulocytes and found a significant increase (p<0.01) for females only in the high-dose group (6.4±1.7 %) compared with the female controls (2.8±1.3 %). There was no significant difference in reticulocytes between control (3.8±1.5 %) and high-dose males (3.2±0.8 %).

Clinical chemistry:

TABLE 53: Significant changes in blood chemistry parameters after oral buprenorphine + naloxone (1:1) in dogs for 28 days (n=4-5/group).

Parameter	Sex	Control	2.5 mg/kg/d	15 mg/kg/d	90 mg/kg/d
Glucose, mmol/l	F	5.34±0.22	5.96±0.36*	N.S.	5.95±0.26**
ALT, U/l	M	17.0±2.6	N.S.	N.S.	51.6±47.1†
	F	15.4±3.4	N.S.	N.S.	34.8±21.4†
Urea, mmol/l	M	2.78±0.59	N.S.	3.85±0.62*	4.30±1.13*
Cholesterol, mmol/l	M	2.25±0.49	N.S.	N.S.	3.62±0.46**
	F	2.27±0.44	N.S.	N.S.	3.36±0.23**

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

Urinalysis: Unremarkable

Gross pathology: Unremarkable

Histopathology: Unremarkable

KEY STUDY FINDINGS

Dogs received oral capsules daily for 4 weeks containing 1:1 buprenorphine HCl and naloxone HCl doses of 2.5, 15 and 90 mg/kg (each drug). The dogs failed to show significant body weight gain, being less than 10% of the controls' body weight gain at all doses. Food consumption was significantly decreased at the two higher doses during the first week. There were no behavioral effects at 2.5 mg/kg/day, the higher doses causing sedation and emesis. High-dose females showed >100% increase in reticulocytes. Glucose was significantly increased in the low and high dose females, urea was significantly increased in the mid and high dose males, and cholesterol was increased in a dose-related manner in both sexes, being significant in the high-dose groups. Alanine aminotransferase was increased sporadically in the high-dose groups. Except for effects on body weight gain, the NOAEL was 2.5 mg/kg/day and the LOAEL was 15 mg/kg/day. At 90 mg/kg, SC, increases in ALT, urea and reticulocytes were observed.

General Comments: The following is a dose range-finding study in the dog that was intended to involve daily drug administration for 4 weeks. The study had to be aborted, however, because of severe intravascular reaction at the injection site at all doses, which precluded prolonged administration.

Study Title: Buprenorphine/Naloxone Preliminary Intravenous Toxicity Study in Beagle Dog (Repeated Daily Dosage for up to 10 Days)

Study No.: 36942 Volume #: 12 Tab#: 36942

Conducting Laboratory: _____

Date of Study Initiation: November 24, 1983

GLP Compliance: Yes No

QA Report: Yes No

Methods:

Dosing:

Species/strain: Dog / beagle (_____)

No./sex/group: 1/sex/dose

Age: 16-27 weeks.

Weight: Males, 6.1-9.7 kg; females, 5.4-8.6 kg.

Dosage groups: Buprenorphine + Naloxone (3:2): Buprenorphine doses were 4, 8, 16 and 32 mg/kg in combination with naloxone doses of 2.67, 5.33, 10.67 and 21.33 mg/kg.

Route, form, volume: Intravenous, solution, 5.33 ml/kg.

Drug lot nos.: Buprenorphine, lots #17 and #21; naloxone, lot #231.

Formulation/vehicle: Water, pH 4 or 5% dextrose (two different vehicles were tried).

Observation period: 2 weeks

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Clinical signs: Observed for signs of ill health, changes in behavior or reactions to treatment regularly throughout the day on weekdays and up to mid-day on holidays and weekends.

Body weights: Measured twice per week prior to feeding throughout the pre-dosing and dosing periods.

Food consumption: Amount consumed (out of 400 g/day offered) was measured daily. Food was offered about 1 hour after dosing.

Gross pathology: Dogs were euthanized 17-28 days after the initiation of dosing.

Organs weighed: Adrenals, brain, heart, kidneys, liver, lungs, pancreas, pituitary, spleen, testes or ovaries, thymus, thyroids and uterus or prostate.

Histopathology: Samples from a number of tissues were preserved in buffered 10% formalin, together with any tissue showing macroscopic abnormality. However, they were not processed further.

Results:

Clinical signs: The high dose (32 mg/kg as buprenorphine) was given only once to only one dog (female), as immediate seizures precluded further testing of this dose level. The next highest dose (16 mg/kg) caused seizures within 2 minutes in the same dog after an 11-day drug holiday, and injection site reactions limited administration of this dose, as well as lower doses, to only a few days. The longest period of dosing possible was 10 days, which occurred in one female receiving 4 mg/kg of buprenorphine plus 2.67 mg/kg of naloxone, the lowest dose tested.

Body weights: Adverse effects were observed during treatment at all dose levels.

Food Consumption: Adverse effects were observed during treatment at all dose levels.

Gross pathology: Post mortem findings included thickening of the veins with dark material in the lumen at the injection site at all dose levels. The male dogs receiving 4 (n=1) or 16 (n=2) mg/kg buprenorphine doses (+ naloxone) had relative kidney weights that exceeded the historical control upper limit of 0.70% body weight, with values of 0.82, 0.74 and 0.77%. Two of the 8 dogs had roundworm infestation in the lumen of the jejunum.

KEY STUDY FINDINGS

This intended 4-week dose range-finding study with IV buprenorphine/naloxone (3:2) failed to achieve completion because severe injection site reaction at all doses tested (4-32 mg buprenorphine) prevented continued drug administration beyond 10 days, which was accomplished only with the lowest dose. All doses also adversely affected food consumption and body weights. Combination doses >8 mg/kg buprenorphine plus 5.33 mg/kg of naloxone, IV, produced seizures.

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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. 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 Intravenous and Subcutaneous Administration to Beagle Dogs.

Study No.: 36938 **Volume #:** 15 **Tab#:** 36938

Conducting Laboratory: []

Date of Study Initiation: February 26, 1984

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

Methods:

Dosing: Daily, 7 days/week for 4 weeks

Species/strain: Dog / beagle ()

No./sex/group: 4/sex/dose (main study)

Age: 9-10 months

Weight: 8.6-9.25 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, IV+SC), 0.15 and 1.5 mg/kg/day, IV, and 15.0 mg/kg/day, SC.

Route, form, volume: Solution, 0.5 ml/kg, IV, or 2.0 ml/kg, SC.

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

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. Feces were monitored. Recovery group dogs were monitored during the first week for the following withdrawal signs: Fright, baring of teeth, continuous yawning, chewing, swallowing, salivation; piloerection, shaking, tremor, muscle twitching, excitation, aggressiveness, vomiting, diarrhea, bizarre movement, stretching movement, dorsal position, abdominal position, lateral position with closed eyes; penile erection, and repeated vocalization (especially upon touch).

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

Food consumption: Estimated daily 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

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(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.

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

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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
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	

**APPEARS THIS WAY
ON ORIGINAL**

Results:

Clinical signs: The behavioral IV NOAEL dose was 0.15 mg/kg/day. The higher IV dose caused sedation (decreased motility) during week 1 and vomiting in 3 dogs after the first injection. One dog vomited after the SC dose. Swelling at the injection site occurred in 7/10 dogs receiving SC dosing.

Body weights: Sponsor reported significant differences in body weights between the control (combined male and female) group and dogs receiving 1.50 mg/kg/day, IV, at week 3 (88% of control) and at week 4 (86% of control), as well as the dogs receiving 15 mg/kg/day, SC, at week 2 (87% of control), at week 3 (84% of control)

and at week 4 (82% of control). When body weight gains are calculated for the individual treatments, all doses of buprenorphine+naloxone caused significant decrements in body weight gain during the 4-week treatment period (Table 54):

TABLE 54: Body weight gains of dogs (male + female) receiving IV or SC buprenorphine + naloxone (3:2) for 28 days.

Buprenorphine Dose (mg/kg/d)	Body weight gain in dogs (kg)	
	Mean ± S.D.	% of Control
Control, IV+SC	1.19 ± 0.52 (10)	
0.15, IV	0.28 ± 0.41 (8)*	24%
1.5, IV	0.05 ± 0.54 (8)*	4%
15, SC	0.00 ± 0.48 (10)*	0%

*P<0.001 compared with the control group (calculated from body weights by reviewer).

Food Consumption: Sponsor reported food consumption in terms of grams/kg of body weight per day and reported significant decreases between the control (combined male and female) group and dogs receiving 1.50 mg/kg/day, IV, at week 1 (55% of control), as well as the dogs receiving 15 mg/kg/day, SC, at week 1 (71% of control) and week 2 (82% of control). Significant decreases (p<0.05) also occurred in the IV low-dose group during week 3 (-10%) and week 4 (-13%).

Ophthalmoscopy: Unremarkable

EKG: Unremarkable

Hematology: Sponsor reported findings as unremarkable, but there was a significant elevation of reticulocytes (p=0.0139) with the 15 mg/kg/day SC dosing (5.6±1.1) compared with the controls (4.1±1.4). There also was a significant elevation of platelets (p<0.01) with the 15 mg/kg/day SC dosing (336±51 x10³/µl) compared with the controls (255±57 x10³/µl).

Clinical chemistry: A significant (p<0.05) 24% increase in α₂-globulin occurred at 1.5 mg/kg/day, IV, and a further increase (52%) at 15 mg/kg/day, SC. There also was a significant elevation of cholesterol (p<0.05) with the 15 mg/kg/day SC dosing (3.12±0.45 mmol/l serum) compared with the controls (2.69±0.40 mmol/l serum).

Urinalysis: Unremarkable

Gross pathology: Unremarkable, although 1-2 dogs/group, including a control dog, were described as having “whitish foci” in their kidneys.

Organ weights: Statistical evaluation of relative organ weights was not provided.