

1996. The FDA approved Suboxone® [sublingual tablets containing buprenorphine HCl and naloxone HCl dihydrate at 4:1 buprenorphine : naloxone ratio] on Dec. 07, 1999.

In this supplement, the sponsor has submitted a protocol for rat carcinogenicity study to fulfill their phase 4 commitment for Suboxone. Dose selection for the carcinogenicity study is based upon the results of a 13-week dietary toxicity study in rats, which is also included in this supplement.

Studies Reviewed within this Submission:

- (1) Suboxone: 28 Day palatability study in rats.
- (2) Suboxone: 13 Week dietary toxicity study in rats.
- (3) Suboxone: 2 Year oncogenicity study in the rat [protocol].

Studies Not Reviewed within this Submission: None

Note: Portions of this review were excerpted directly from the sponsor's submission.

- ** Statistically significant difference from the control group mean at the 1% level [Student's t-test, two sided]
- * Statistically significant difference from the control group mean at the 5% level [Student's t-test, two sided]

SUBOXONE: 28 DAY PALATABILITY STUDY IN RATS.

Purpose of the study: To investigate the palatability of Suboxone in diet.

Performing Laboratory: []

Study date: 14 January 1999-27 April 1999.

Report Number: — /P/6293

— Test Substance Reference Number: Y10150/002

— Study Number: KR1336

Sponsor: Reckitt & Colman, Danson Lane, Hull, UK.

Animals: Species: Rat
Strain: AlpK:ApfSD
Source: []

Sex/number: 50 males and 50 females

Specification: Approximately 5 weeks old and 120 – 145 g & 100 – 125 g body weight for males and females, respectively.

Test substance: Suboxone [buprenorphine hydrochloride and naloxone hydrochloride mixed as a 4:1 ratio of the bases.

Source: Reckitt & Colman / —

Color: White/Off white

Batch ref. #: Buprenorphine hydrochloride: V10291

Purity: Naloxone hydrochloride: V04434
 Buprenorphine hydrochloride: []
 Naloxone hydrochloride: []
 Control substance/vehicle: Rat's diet supplied by _____
 Diet preparation: Prepared by grinding the test substance with milled diet in a mortar.

Group	Dietary concentration of Suboxone (ppm)	Quantity (g) of Suboxone in 10 kg diet	Total quantity of diet per 10 kg batch (kg)	Color code
1	0	0	10.000	Blue
2	2000	22.125	9.978	Green
3	3000	33.188	9.967	Yellow
4	4000	44.251	9.956	Red

Dose levels and treatment groups: Animals received the dietary concentrations of Suboxone for 28 days as shown below.

Group	Suboxone [ppm]	Suboxone [~mg/kg/day]	Identities of rats	
			Males	Females
1	0	0	1-8	33-40
2	2000	200	9-16	41-48
3	3000	300	17-24	49-56
4	4000	400	25-32	57-64
4 [Satellite]	4000	400	65-82	83-100

Clinical observation, food consumption and body weights were recorded. Blood samples were collected at 1, 2, 4, 6, 12, or 24 hours after the dosing on day 29 from 3 animals per sex in the satellite group for toxicokinetic analysis. All animals were examined macroscopically. All data were evaluated using analysis of variance and covariance.

GLP/QA Statements: Yes

Date of Issue: 10 August 1999

Results:

Dosing preparation analysis: The mean achieved concentrations of homogenous Suboxone were within 7% of the target concentration.

Clinical observations: Increased activity and scabs on the tails, due to animals fighting, were observed in all treated animals. Due to fighting, animals were housed individually or scrunched up balls of tray lining papers were placed into each cage for animals to play.

Labored breathing, hind limb damage, swollen and/or torn ears, discharge from the eye, hair loss, piloerection and fur staining were also observed.

Mortality: Low dose group – 1 M on day 2
 Medium dose group – 2 F on day 2
 High dose group – 0

Body weights:

Intergroup comparison of body weight of males [g]

Day	Suboxone [ppm]			
	0	2000	3000	4000
1	197.1±6.6	198.1±7.5	173.4±24.8	174.8±24.7
4	217.8±6.5	206.1±8.4*	196.0±11.4**	194.8±10.8**
8	253.0±9.2	227.4±9.8**	218.5±13.4**	220.5±11.4**
15	307.5±11.4	276.3±11.4**	274.9±16.1**	271.3±9.5**
22	349.3±13.4	313.4±17.0**	317.9±16.1**	310.6±8.9**
29	380.8±15.1	340.9±23.3**	347.6±17.1**	343.0±14.6**

Intergroup comparison of body weight for females [g]

Day	Suboxone [ppm]			
	0	2000	3000	4000
1	159.3±7.6	161.5±6.1	158.5±5.7	160.9±8.1
4	168.0±8.2	170.9±6.6	167.8±10.1	169.6±11.8
8	183.3±8.1	183.4±8.9	178.7±13.0	185.3±9.5
15	206.9±9.3	208.0±8.2	205.2±7.3	211.6±13.2
22	219.6±9.5	225.8±9.6	222.2±10.6	223.9±12.1
29	231.3±9.4	236.8±10.1	233.5±10.5	237.8±11.6

Adjusted mean body weights for the males decreased ~10% below the control body weight. There was no apparent effect on the female body weight in all treated groups.

Food consumption:

Intergroup comparison of food consumption in males [g/rat/day]

Day	Suboxone [ppm]			
	0	2000	3000	4000
1	25.9±0.2	13.6±2.3	16.1±6.9	13.1±4.4
4	25.5±0.7	20.7±0.1**	19.0±1.4**	19.1±1.6**
8	28.5±0.0	24.8±0.6*	21.9±1.6**	22.5±1.1**
15	33.6±0.9	28.5±0.7**	28.3±0.4**	27.1±1.6**
22	35.3±0.4	32.1±0.2**	30.4±0.2**	29.6±0.5**
28	32.3±0.0	30.9±1.6	29.0±0.4	28.4±1.6

In all treated males, the food consumption was 10-32% reduced in comparison with the control group.

Intergroup comparison of food consumption in females [g/rat/day]

Day	Suboxone [ppm]			
	0	2000	3000	4000
1	23.3±1.8	14.9±0.2	12.3±3.2*	14.3±3.2

Day	Suboxone [ppm]			
	0	2000	3000	4000
4	21.1±1.6	19.9±1.4	21.0±0.0	21.5±2.8
8	19.5±0.0	19.3±2.1	20.8±4.0	18.4±1.9
15	20.1±0.5	21.0±1.1	20.3±3.3	21.3±3.5
22	22.9±1.9	22.0±0.7	20.7±1.9	22.3±2.1
29	19.2±0.2	19.5±0.4	19.5±0.2	18.8±0.7

In all females, food consumption was reduced during the first five days and thereafter; it was similar to that of the control group.

Toxicokinetics: Mean [n=3] minimum and maximum plasma concentrations of buprenorphine and naloxone are tabulated below. Blood sampling time [hours] is given in the parenthesis.

Plasma concentrations of Buprenorphine and Naloxone [page 24, vol. 3]

Dose [ppm]	Sex	Buprenorphine (ng/ml)		Naloxone (ng/ml)	
		Minimum	Maximum	Minimum	Maximum
4000	M				
4000	F				

Due to small number of samples (n=3) and a large variation in plasma concentrations at different times, it was not possible to calculate the area under the curve (AUC).

Post Mortem: Only gross pathology was done and no findings that could be attributed to treatment with Suboxone in the diet were found.

SUBOXONE: 13 WEEK DIETARY TOXICITY STUDY IN RATS.

Performing Laboratory: _____

Report Number: _____ PL1158/Regulatory Report

— Test Substance Reference Number: Y10150/002 (Y10142/002 plus Y10143/003)

— Study Number: PR1158

Sponsor: Reckitt & Colman Products Limited, Dansom Lane, Hull HU8 7DS, UK.

Sponsor Reference: 00600113

Animals: Species: Rat
Strain: Alpk:ApfSD
Source: _____

Sex/number: 152 males and 152 females

Specification: Approximately 5 weeks old and 187 – 245 g & 145 – 185 g body weight for males and females, respectively.

Test substance: Suboxone [buprenorphine hydrochloride and naloxone hydrochloride mixed at a 4:1 ratio of the bases.

Source: Reckitt & Colman / _____

Color: White/Off white

Batch ref. #: Buprenorphine hydrochloride: V10291
 Naloxone hydrochloride: V04434
 Purity: Buprenorphine hydrochloride: []
 Naloxone hydrochloride: []

Control substance/vehicle: Rat's diet supplied by _____
 Diet preparation: Prepared by grinding the test substance with milled diet in a mortar. Suboxone was stable in CTI diet for 28 days

Group	Nominal Suboxone concentration [ppm]	Number of batches	Mean Suboxone concentration [ppm]	% of nominal concentration	Concentration range [ppm]
1	control	4	ND		
2	100	5	102.5	102.5	
3	500	4	522	104.4	
4	1500	4	1488	99.2	
5	2000	5	2010	100.5	

Dose levels and treatment groups: Animals (16/sex/group) received the dietary concentrations of Suboxone for 90 days as shown below.

Group	Suboxone [ppm]	Suboxone [~mg/kg/day]	Identities of rats	
			Males	Females
1	0	0	1-16	81-96
2	100	10	17-32	97-112
3	500	50	33-48	113-128
4	1500	150	49-64	129-144
5	2000	200	65-80	145-160

Satellite groups contained 18/sex/group animals for toxicokinetics.

Group	Suboxone [ppm]	Suboxone [~mg/kg/day]	Identities of rats	
			Males	Females
2 (Satellite)	100	10	161-178	233-250
3 (Satellite)	500	50	179-196	251-268
4 (Satellite)	1500	150	197-214	269-286
5 (Satellite)	2000	200	215-232	287-304

Measurements and Observations:

General appearance, viability, clinical signs	Daily
Bodyweight	Weekly
Food consumption	Weekly
Ophthalmoscopy	Days 0 & 90
Functional Observational battery	Week 13.
Motor activity	Week 13
Hematology, serum chemistry	Termination
Pharmacokinetics	Week 13
Necropsy	Termination

Ophthalmoscopy: Hyaloid remnant & cornea

Functional Observational battery: landing foot splay, muscle weakness [fore- and hindlimb grip strength] and sensory perception [tail-flick test]

Hematology: Red blood cell count, hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, total white blood cell count, differential count and platelet count.
Prothrombin time and activated partial thromboplastin time with kaolin.

Blood clinical chemistry: Urea, creatinine, glucose, albumin, total protein, cholesterol, triglyceride, sodium, potassium, chloride, calcium, total bilirubin, phosphorus, gamma-glutamyl transferase activity, creatine kinase activity, alkaline phosphatase activity, aspartate aminotransferase activity, and alanine aminotransferase activity.

Organ weights: Adrenal glands, brain, epididymides, heart, kidneys, liver, lungs, ovaries, prostate gland, seminal vesicles, spleen, testes, thymus, and uterus

Macroscopic and microscopic examination:

Addendum 1
Histopathology Inventory for NDA # 20-733

Study	13/2	
	week	year
Species	Rat	Rat
Adrenals	X	x
Aorta	X	x
Bone Marrow smear	X	x
Bone (femur)	X	x
Brain	X	x
Cecum	X	x
Cervix	X	x
Colon	X	x
Duodenum	X	x
Epididymis	X	x
Esophagus	X	x
Eye	X	x
Fallopian tube		
Gall bladder		
Gross lesions		
Harderian gland	X	x
Heart	X	x
Hypophysis		
Ileum	X	x

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

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Data evaluation: Mixed procedure in SAS (1996)

GLP/QA Statements: Not submitted, unaudited report.

Results:

Diet preparation analysis: The mean achieved concentrations of Suboxone in the diet were within 9.4% of the target concentration.

Dose received:

Dosed concentration (ppm)	100	500	1500	2000
Dosed received by males (mg/kg/day)	7.5	36.6	112.0	151.8
Dosed received by females (mg/kg/day)	8.3	41.6	122.7	166.7

Mortality: None

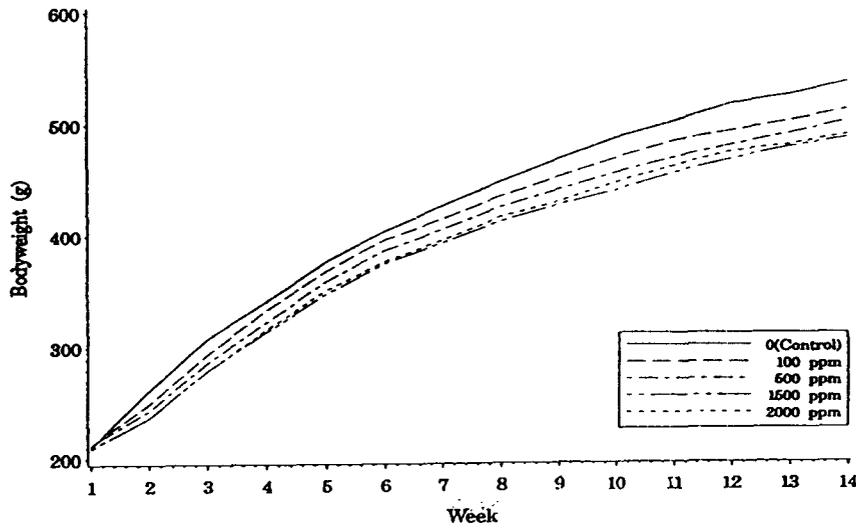
Clinical observations: [extracted from the submission, table 4, page 57, volume 2]

Dose (ppm)	0		100		500		1500		2000	
	M	F	M	F	M	F	M	F	M	F
Aggressive No. of animals				1	2	4	4	2	3	6
Anular constrictions: Tail No. of animals			4	1	4	1	4		3	
Fore limb damaged No. of animals	2		1		1	1		1	2	2
Hind limb damaged No. of animals									1	
Left ear torn No. of animals			2					1	1	2
Right ear torn No. of animals			3		2	1		1		1
Scabs No. of animals	1	1	9	1	4		9		7	
Stain around nose No. of animals		1		1		2		1		3
Tail damaged No. of animals	1	1	8		2	1	7		3	1

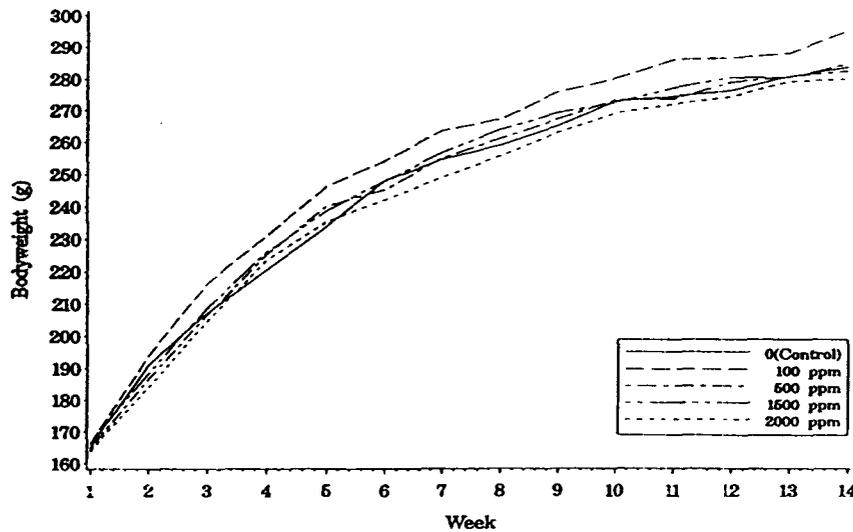
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Body weights:

GROUP MEAN BODYWEIGHT VERSUS TIME
MALES

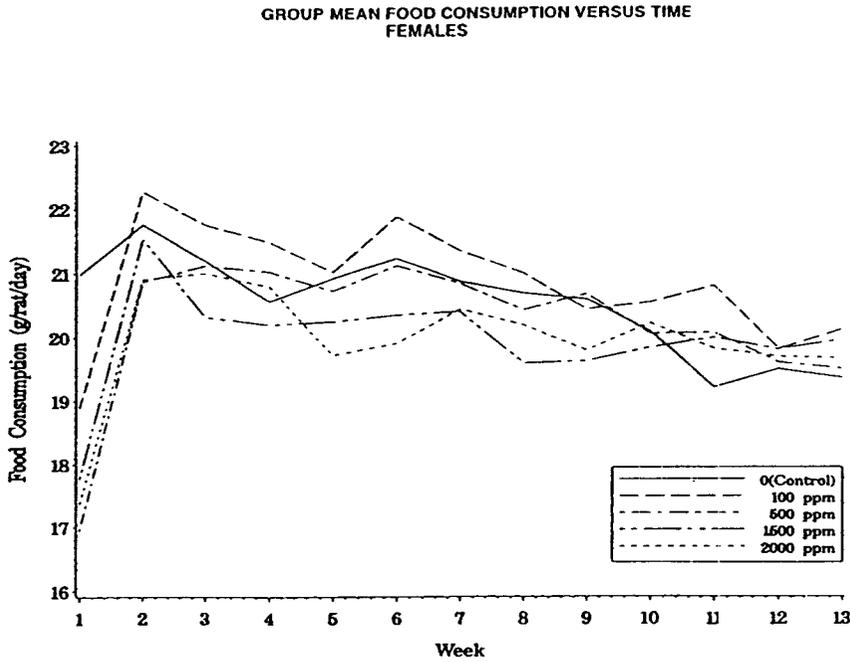
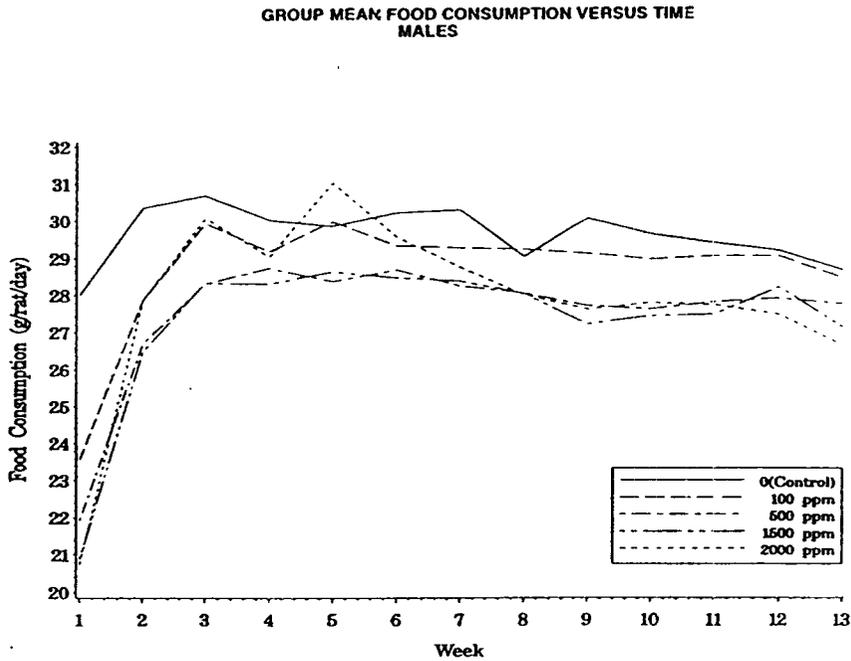


GROUP MEAN BODYWEIGHT VERSUS TIME
FEMALES



Adjusted mean male body weights were significantly lower in all groups treated with Suboxone as compared with the control group, although male adjusted body weight reduction was less than 10% irrespective of dieting concentrations of Suboxone. The body weight gains in all treated male groups were 13 to 22 % lower than the control. There was no effect on female body weights.

Food consumption and utilization:



Food consumption of all treated animals was significantly lower during the first week of treatment. Mean food consumption for the males administered Suboxone at 1500 and 2000 ppm was lower compared with the control group during the remaining treatment period. Group means female group consumption returned to level similar to that of the control group after the first week of dosing.

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Intergroup Comparison of Food Utilization:

Food utilization [g growth/100 g food]

Dose (ppm)	0		100		500		1500		2000	
	M	F	M	F	M	F	M	F	M	F
Weeks 1-4	19.8 ± 1.6	11.6 ± 1.9	20.0 ± 0.8	13.6* ± 1.4	19.8 ± 0.8	13.5* ± 0.8	19.0 ± 0.7	12.9 ± 0.5	18.7 ± 1.2	12.4 ± 0.9
Weeks 5-8	10.7 ± 1.1	5.5 ± 0.5	10 ± 0.4	4.9 ± 0.3	10.2 ± 1.5	4.6 ± 0.4	9.8 ± 1.2	5.4 ± 0.5	9.4* ± 1.1	4.9 ± 0.7
Weeks 9-13	6.6 ± 0.8	2.6 ± 0.8	5.9 ± 0.8	2.7 ± 0.6	6.2 ± 1.6	2.2 ± 0.6	6.1 ± 0.5	2.2 ± 0.6	6.1 ± 1.3	2.4 ± 0.7
Overall	12.0 ± 0.8	6.1 ± 0.2	11.4 ± 0.6	6.8 ± 0.7	11.5 ± 1.1	6.4 ± 0.5	11.1* ± 0.6	6.5 ± 0.3	10.9* ± 0.8	6.3 ± 0.2

Mean±S.D.

Ophthalmoscopy: No abnormalities detected.Functional observational battery:Functional observation One male in high dose group showed aggression, pupillary dilation, salivation and vocalization.Time to tail flick (sec) [table 10, page 76, volume 2]

Dose (ppm)	0	100	500	1500	2000
Males	7.4±4.0	9.5±5.7	10.5±5.6	9.4±5.4	10.4±6.6
Females	8.8±4.1	9.4±5.9	10.9±5.2	8.5±4.7	12.4*±5.5

High dose females showed significantly higher time to tail flick. Males of all groups and females of 100, 500 and 1500 ppm groups showed similar time to tail flick as in the control group.

Grip strength measurements: No effects on hindlimb or forelimb grip strength [g].Landing foot splay (mm) [table 13, page 79, vol. 2]

Dose (ppm)	0	100	500	1500	2000
Males	70.1±15.0	68.1±17.6	59.7±19.1	58.4±14.0	59.5±18.8
Females	76.3±19.8	67.0±18.6	68.5±15.2	64.9±10.7	60.9*±19.0

Group mean landing foot splay was lower for the females administered Suboxone at 2000 ppm than the control group.

Motor activity [from table 14, page 80, vol. 2]

Suboxone (ppm)	0	100	500	1500	2000
Overall males (1-50 minutes)	448±124	478±144	439±145	483±131	478±161
Overall females (1-50 minutes)	593±58	532±91	471*±122	471*±138	465*±158

A statistically significant lower motor activity count was observed for the females treated at 500, 1500 or 2000 ppm of Suboxone but there is no dose response. Motor activity in males was not effected by Suboxone administration.

Clinical pathology:Hematology [extracted from the submission, table 15, page 82, vol. 2]

Parameter	Sex	Dose (ppm)				
		0	100	500	1500	2000
Hemoglobin (g/dl)	M	14.6±0.5	14.8±0.4	15.1*±0.5	15.0±0.6	15.3**±0.5
	F	14.6±0.7	14.0**±0.5	14.4±0.7	14.5±0.4	14.5±0.5
Mean Cell Volume (fl)	M	50.8±1.7	53.2**±1.3	52.0*±1.3	52.1*±1.1	53.2**±1.0
	F	53.3±1.1	54.8*±1.3	54.6*±1.9	53.5±1.1	54.2±1.4
Mean Cell Hemoglobin (pg)	M	17.1±0.5	17.7**±0.4	17.5*±0.4	17.4±0.5	17.7**±0.4
	F	18.0±0.9	18.3±0.7	18.1±0.5	17.8±0.5	18.0±0.6
White Blood Cell Count (x10 ⁹ /l)	M	7.5±1.3	7.0±1.3	6.9±1.0	6.6±1.1	6.4*±1.7
Activated Partial Thromboplastin Time (s)	F	20.5±1.9	21.9±2.8	22.6*±1.6	22.9**±2.2	23.5**±2.2

Dose dependent increase in APTT was observed for the females. Although ↑group mean hemoglobin level for males at 2000 ppm, ↑mean cell volumes, ↑mean cell hemoglobin and ↓white blood cell count were statistically significant, these values were still within normal range.

Blood Clinical ChemistryBlood Clinical Chemistry [extracted from the submission, table 15, page 86, vol. 2]

Parameter	Sex	Dose (ppm)				
		0	100	500	1500	2000
Plasma triglycerides (mmol/l)	M	1.4±0.4	1.3±0.3	1.3±0.3	1.2±0.3	1.1±0.3*

Organ weights [extracted from the submission, table 23, page 96, vol. 2]

Organ	Sex	Parameter	Dose [ppm]				
			0	100	500	1500	2000
Adrenal gland	M	organ weight [mg]	65± 6	71± 7	74± 11*	78± 10**	80± 9**
		organ weight adjusted for body weight [%]	0.063	0.070*	0.074**	0.079**	0.081**
Liver	M	organ [g]	19.5± 1.5	18.0± 1.7*	17.3± 2.0**	17.2± 1.6**	17.0± 1.6**
		organ weight adjusted for body weight [%]	18.3	17.7	17.4*	17.9	17.6*
	F	organ weight [g]	10.2± 0.9	11.1± 1.2*	10.8± 1.2	11.6± 1.0**	11.2± 0.9**
		organ weight adjusted for body weight [%]	10.3	10.8	10.9*	11.6**	11.4**
Lung	M	organ weight [g]	1.9± 0.17	1.81± 0.17	1.74± 0.14**	1.70± 0.12**	1.71± 0.16**
Spleen	M	organ weight [g]	1.07± 0.12	1.01± 0.10	0.99± 0.09*	0.94± 0.1**	0.95± 0.1**
Seminal vesicles	M	organ weight [g]	2.31± 0.3	2.61± 0.29**	2.60± 0.32*	2.38± 0.32	2.50±0. 38
		organ weight adjusted for body weight [%]	2.27	2.60**	2.60**	2.40	2.52*
Testes	M	organ weight adjusted for bodyweight [%]	3.52	3.60	3.77*	3.76	3.77*

A dose-related increase in absolute adrenal gland weight [500-2000 ppm] and adrenal weight adjusted for body weight was observed in male animals. Group mean liver weight was higher for the females and lower for the males in comparison with control groups. The organ weight of adrenal gland [F], brain [M&F], epididymides, heart [M&F], kidneys [M&F], lungs [F], ovaries, prostate gland, spleen [F], thymus [M&F], and uterus with cervix were not effected by Suboxone in the diet.

Macroscopic findings: No treatment related abnormalities.

Microscopic findings:

Microscopic findings [extracted from the submission, table 24, page 111, vol. 2]

Organ	Sex	Observation	Dose [ppm]				
			0	100	500	1500	2000
Adrenal gland	M	Cortical vacuolation [total]	0	0	0	0	1/15
		minimal	0	0	0	0	1/15

Organ	Sex	Observation	Dose [ppm]				
			0	100	500	1500	2000
Harderian gland	M	Mononuclear cell infiltration [total]	3/16	1/16	6/16	5/16	6/16
		minimal	2	1	5	4	5
		slight	1	0	1	1	0
	F	Mononuclear cell infiltration [total]	1/16	4/15	5/15	6/16	9/16
		minimal	1	3	3	3	5
		slight	0	1	2	2	2
Heart	M	Degenerative cardiomyopathy [total]	1/16	-	-	-	3/16
		minimal	1				2
Liver	M	slight	0				1
		Mononuclear cell infiltration [total]	0/16	-	-	-	1/16
		minimal	0	-	-	-	1
Prostate gland		Hepatitis	2/16	-	-	-	0
		Epithelial hyperplasia	1/16	-	-	-	3/16
		minimal	0				1
Seminal vesicles	M	slight	1				1
		moderate	0				1
		Epithelial hyperplasia [total]	0/16	-	-	-	3/16
Thymus	F	minimal					1
		slight					1
		Congestion/hemorrhage [total]	0/16	3/16	1/16	1/16	2/16
		minimal	0	3	1	1	1
		slight	0	0	0	0	1

Treatment related increased incidence/severity of mononuclear cell infiltration in the Harderian gland was observed in all treated animals except 100 ppm male group. No treatment-related findings were observed in the adrenal glands, liver, lungs, seminal vesicles and thymus. There were no treatment-related abnormalities observed in other organs examined.

Overall Summary and Evaluation

Suboxone sublingual tablets contain buprenorphine HCl and naloxone HCl dihydrate at a ratio 4:1 buprenorphine : naloxone as free base. It is indicated for the treatment of opioid dependence. Suboxone is a partial agonist at the *mu*-opioid receptor and an antagonist at the *kappa*-opioid receptor. As a partial *mu*-opioid, buprenorphine behaves very much like classical *mu* agonist such as morphine up to doses of at least 16 mg. Naloxone is added to prevent diversion of the drug product.

Carcinogenicity of buprenorphine hydrochloride has been studied in Sprague-Dawley rats at dietary doses of 0.6, 5.6 and 56 mg/kg/day for 27 months. There were statistically significant increases in testicular interstitial (Leydig's) cell tumors based on the trend test adjusted for survival. Pair-wise comparison of the high dose against the control failed to show statistical significance. In an 86-week study in CD-1 mice, buprenorphine hydrochloride showed no evidence of tumorigenicity at dietary doses of up to 100 mg/kg/day. Carcinogenicity data on naloxone and Suboxone are not available.

Mutagenesis: Combinations (4:1) of buprenorphine hydrochloride and naloxone hydrochloride 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 assay in the rat [copied from the review by David Brase dated 10/26/99 on NDA 20-733 for Suboxone].

Suboxone: 28 Day palatability study in rats.

Male and female Alpk:APfSD rats (8/sex/group) were treated with 0, 2000, 3000 or 4000 ppm Suboxone in the diet for 28 consecutive days. These doses are approximately equivalent to daily intake of 0, 200, 300 or 400 mg/kg/day of Suboxone. A satellite group of 18 male and 18 female rats were treated with 4000 ppm Suboxone for 28 days for toxicokinetics. Chewing and biting of the tails and ears and discharge from the eyes were observed during the first few days of the study in all treated groups. Food consumption [up to 31%] and adjusted body weight [up to 14%] were reduced throughout the study in males only. There was no apparent effect on food consumption and body weight for the females. Gross pathology of the major organs showed no toxicological abnormalities related to treatment observed in male and female animals. Clinical pathology was not performed in this study.

Mean minimum and maximum plasma concentrations of buprenorphine and naloxone are tabulated below. Blood sampling time [hours] is given in the parenthesis.

Dose [ppm]	Sex	Buprenorphine (ng/ml)		Naloxone [ng/ml]	
		Minimum	Maximum	Minimum	Maximum
4000	M	75.9 (4)	160.0 (12)	4.32 (2)	13.5 (1)
4000	F	66.2 (24)	98.3 (12)	3.33 (6)	13.37 (24)

Due to small number of samples (n=3) and large variation in plasma concentrations at different times, the above-mentioned data was not suitable for calculation of the area under the curve (AUC).

Based on the results, 2000 ppm was selected as the high dose level for the 13 week dietary toxicity study in rats.

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Suboxone: 13 Week dietary toxicity study in rats.

A dose range finding toxicity study to support a future 2 year carcinogenicity study was carried out in male and female rats for 13 weeks. Animals received suboxone in the diet at concentrations of 0 (control), 100, 500, 1500 or 2000 ppm [\sim 3, 10, 50, 150 or 200 mg/kg/day]. Satellite groups were fed appropriate diet for 13 consecutive weeks for toxicokinetics. Blood samples were taken by cardiac puncture at selected times.

The food consumption was significantly lower [16% (L), 22% (ML), 25% (MH) and 26% (H)] in all treated groups during the first week of treatment as compared with control group. After first week of treatment, the food consumption of all male treated animals was less than 8% lower than the control animals. There was no significant difference in food consumption in female animals after one week of treatment.

Intergroup comparison of male body weights [g]

Week	Suboxone [ppm]				
	0	100	500	1500	2000
1	210.6±11.6	212.3±14.1	211.9±15.7	208.6±14.8	209.4±11.0
4	341.2±21.3	333.2±19.2	322.4±23.6	313.4±22.2	315.8±16.2
9	465.5±34.6	449.7±32.8	438.5±30.4	424.8±30.1	427.6±26.7
14	533.4±36.4	509.1±44.2	498.6±38.5	483.3±36.0	486.3±33.3

Intergroup comparison of female body weights [g]

Week	Suboxone [ppm]				
	0	100	500	1500	2000
1	165.1±8.5	166.1±10.3	164.4±10.5	166.7±11.5	163.8±10.0
4	220.5±12.8	231.0±14.8	225.2±16.0	226.1±11.8	223.5±13.6
9	264.8±15.0	275.4±14.9	266.9±15.2	268.9±12.9	262.6±16.4
14	283.2±15.1	294.7±16.2	281.9±17.9	284.3±11.0	279.4±17.2

The body weights of males in suboxone treated groups were lower than that of the control group throughout the study. Adjusted mean male body weights were significantly lower in all treated groups [less than 10%] as compared with control group. The body weight gains in males were 13 % [100 ppm], 18 % [500 ppm], 22 % [1500 ppm] and 20 % [2000 ppm] lower than the control group. The body weights of female animals were not effected by Suboxone treatment.

The time to tail flick was significantly higher for high dose females (2000 ppm) showing the expected pharmacological effect of Suboxone treatment. Ophthalmoscopy, functional observation battery measurements, clinical pathology, and organ weights were not affected. Although a statistically significant lower motor activity count was observed for the females treated at 500, 1500 or 2000 ppm of Suboxone, there was no effect of drug administration on motor activity in male rates.

Male hemoglobin concentration was higher and triglyceride level was lower in high dose group (2000 ppm). The increased female mean albumin and total protein were within the historical control ranges. Female activated partial thromboplastin time was higher in 500, 1500 and 2000 ppm treated animals. This increase in APTT values was dose-

related but still within the historical control range. Therefore, these results may not be related to treatment

Toxicokinetics

Mean Plasma concentration [ng/mL]

Substance	Sex	Suboxone Dose (ppm)			
		100	500	1500	2000
Buprenorphine	M	3.8 – 8.2	15.1 – 27.6	36.3 – 65.0	50.5 – 86.7
	F	3.2 – 13.6	15.9 – 27.2	42.9 – 75.0	41.1 – 86.2
Naloxone	M	<0.5	<0.5 – 0.6	1.1 – 3.3	1.8 – 3.5
	F	<0.5	<0.5 – 1.5	0.8 – 1.8	1.2 – 2.3

Mean Area under the Concentration Curve [AUC_{0-last}] values (h.ng/mL)

Substance	Sex	Suboxone Dose [ppm]			
		100	500	1500	2000
Buprenorphine	M	123	474	1238	1580
	F	134	455	1338	1424
Naloxone	M	N/A +	3.32	51.0	53.8
	F	N/A +	N/A +	31.7	37.8

Plasma concentration of both Buprenorphine and Naloxone increased with increasing dietary concentration of Suboxone. Plasma concentrations of Naloxone for group 2 and 3 were below the detection level. Area under the curve (AUC) for these groups (100 & 500 ppm) could not be calculated.

Human pharmacokinetics copied from the package insert for NDA 20-733.

Mean C_{max} and AUC of buprenorphine following single sublingual doses of Suboxone tablets in eight subjects are given below.

Pharmacokinetic Parameter	Suboxone 4 mg	Suboxone 8 mg	Suboxone 16 mg
C _{max} , ng/ml	1.84 (39)	3.0 (51)	5.95 (38)
AUC _{0-48h} , hour.ng/ml	12.52 (35)	20.22 (43)	34.89 (33)

Mean peak naloxone levels in humans ranged from 0.11-0.28 ng/ml in the dose range of 1-4 mg.

In rats and humans, buprenorphine undergoes both N-dealkylation to norbuprenorphine and glucuronidation. Norbuprenorphine is considered to be an inactive metabolite. The extents of plasma protein binding are 96% (human) and 86% (rat). Naloxone undergoes direct glucuronidation to naloxone 3-glucuronide as well as N-dealkylation and reduction of the 6-oxo group.

A treatment related increased adrenal absolute weight and adrenal weight adjusted for body weight was observed for male animals only. Other major organs did not show significant changes.

Increased incidence/severity of mononuclear cell infiltration of the Harderian gland was observed in males (500, 1500 and 2000 ppm) and all Suboxone treated females (100, 500, 1500 and 2000 ppm). The significance of this finding is not clear.

Dose selection for 2-years rat carcinogenicity study:

The 13 week Suboxone dietary toxicity study was conducted in rats at 0, 100, 500, 1500, and 2000 ppm [\sim 0, 10, 50, 150 and 200 mg/kg/day]. The study showed that males were more susceptible than females. The body weight gains in males were 13 % [100 ppm], 18 % [500 ppm], 22 % [1500 ppm] and 20 % [2000 ppm] lower than the control group. There was no effect on body weight gain in females. No mortality was observed.

Histopathological examination demonstrated an increased incidence/severity of mononuclear cell infiltration in the Harderian gland in all treated animals except low dose [100 ppm] males, but dose-related effects were seen only in the females. The significance of this finding is not clear. Minimal to moderate epithelial hyperplasia of both prostate gland (3/16 H vs 1/16 C) and seminal vesicles (3/16 H vs 0/16 C) were observed. Minimal to slight degenerative cardiomyopathy in males (3/16 H vs 1/16 C) and slight congestion/hemorrhage of thymus in females (2/16 H vs 0/16 C) were observed.

The AUC_{0-last} values of buprenorphine at 100, 500, 1500 and 2000 ppm of Suboxone were 123, 474, 1238 and 1580 hr.ng/mL in males and 134, 455, 1338 and 1424 hr.ng/mL in females. The AUC_{0-last} values of naloxone at 1500 and 2000 ppm of Suboxone were 51.0 and 53.8 hr.ng/mL in males and 31.7 and 37.8 hr.ng/mL in females. The AUC values of buprenorphine [1424 & 1580 hr.ng/mL] in male and female rats at 2000 ppm of Suboxone are approximately 43 fold higher than the human AUC [34.89 hr.ng/mL] following single administration of 16 mg of Suboxone. The human AUC of naloxone from Suboxone is not available. In humans mean peak naloxone levels ranged from 0.11 to 0.28 ng/ml in the dose range of 1-4 mg. In rats the mean plasma concentrations [ng/ml] of naloxone in 13 week toxicity study were 1.1-3.3 [m/1500 ppm], 0.8-1.8 [f/1500 ppm], 1.8-3.5 [m/2000 ppm] and 1.2-2.3 [f/2000 ppm].

In rats and humans, buprenorphine undergoes both N-dealkylation to norbuprenorphine and glucuronidation. Norbuprenorphine is considered to be an inactive metabolite. The extents of plasma protein binding are 96% (human) and 86% (rat). Naloxone undergoes direct glucuronidation to naloxone 3-glucuronide as well as N-dealkylation and reduction of the 6-oxo group.

Genotoxicity testing has been conducted with Suboxone. No genotoxicity was observed in the Ames test, the human lymphocytes chromosomal aberration assay and mouse micronucleus assay.

The sponsor proposes 1800 ppm as the high dose in the carcinogenicity study. This dose is acceptable for male rats based on the effect on body weight gain (20% ↓) and buprenorphine AUC exposure (45 fold ↑). In the females, dose-dependent effects on APTT (15% ↑), mononuclear cell infiltration of Harderian gland [1/16 (control) to 9/16 (HD)], motor activity (22% ↓) and buprenorphine AUC exposure (41 fold ↑) were

observed; although no effects on body weight gains and food consumption were observed. It is concluded that a high dose of 1800 ppm is also acceptable for females.

In summary, the dosing of both male and female rats at 100, 450 and 1800 ppm [~5, 22.5 and 90 mg/kg/day] for 2-year carcinogenicity study are acceptable. We recommend that animals be housed individually to manage the adverse effects of aggression seen in the 28-day palatability study and 90-day dietary toxicity study.

Recommendations: The proposed doses of 100, 450 and 1800 ppm [~5, 22.5 & 90 mg/kg/day] for two year carcinogenicity study in rats are acceptable.

[/S/]
M. Anwar Goheer, Ph.D.

[/S/] 3/21/2002
Lou Huey (Lucy) Jean, Ph.D.

Concur by Team Leader:

cc: IND 58,653 }
INDA - 20-733
HFD-170
/Goheer

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NDA 20-733 (Suboxone®)

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

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

NDA 20-733

Date of Submission: June 3, 1999
Type of Submission: New Drug Application / Original Submission
Received by reviewer: June 14, 1999
Date Completed: September 30, 1999 (Revised upon peer review, 10/26/99)

Reviewer: David A. Brase, Ph.D., Pharmacologist

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

Sponsor: Reckitt & Colman Pharmaceuticals, Inc.
1909 Huguenot Road, Richmond, VA 23235

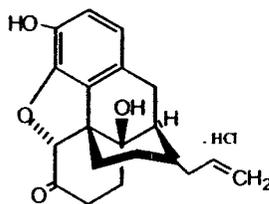
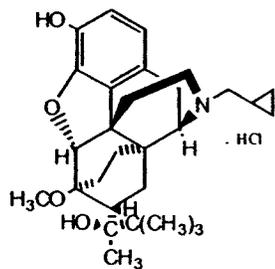
Drug: Suboxone® (sublingual tablets containing buprenorphine HCl and naloxone HCl dihydrate at a 4:1 buprenorphine:naloxone ratio)

Chemical names: [5 α ,7 α (S)]-17-(cyclopropylmethyl)- α -(1,1-dimethylethyl)-4,5-epoxy-18,19-dihydro-3-hydroxy-6-methoxy- α -methyl-6,14-ethenomorphinan-7-methanol hydrochloride (buprenorphine) and (5')-4,5-epoxy-3,14-dihydroxy-17-(2-propenyl)-morphinan-6-one hydrochloride dihydrate (naloxone)

CAS Registry Numbers: 53152-21-9 (buprenorphine HCl) and 51481-60-8 (naloxone HCl dihydrate)

Formula weights: 504.09 (buprenorphine HCl) and 399.87 (naloxone HCl·2H₂O)

Structures:



NDA 20-733 (Suboxone®)

Relevant IND/NDA/DMF: IND 45,220 / IND 58,653 / NDA 20-732 / DMF _____

Drug class: Buprenorphine: Opioid analgesic (partial *mu* agonist)
Naloxone: Opioid antagonist

Indication: Treatment of _____

Dose (MRHD): Buprenorphine HCl/naloxone HCl, 24/6 mg/day (0.40/0.10 mg/kg/day) for a 60-kg person.

Clinical formulation: Compressed buprenorphine/naloxone tablets, 2/0.5 and 8/2 mg, containing the following ingredients:

INGREDIENT	mg per 2.0/0.5-mg tablet	mg per 8/2-mg tablet
Buprenorphine HCl	[REDACTED]	[REDACTED]
Naloxone HCl		
Lactose		
Mannitol		

Lemon & lime flavor		
Povidone K30		
Citric acid, _____		
Magnesium stearate		
Acesulfame K		
Sodium citrate		

TABLET WEIGHT		

Route of administration: Sublingual

Proposed clinical use: Suboxone® is a 4:1 combination of buprenorphine hydrochloride and naloxone hydrochloride in a sublingual tablet formulation for the treatment of opioid addiction. The anticipated daily dose for this indication is in the range of 4 to 24 mg.

Previous clinical experience: An injectible formulation of buprenorphine hydrochloride (Buprenex®) is approved and has been marketed in the U.S. since 1982 for the treatment of moderate to severe pain at recommended doses of 0.3 mg, i.v. or 0.6 mg, i.m. A sublingual 0.2-mg formulation (Temgesic®, Buprex®) is marketed in 9 countries and a sublingual formulation containing 0.2 mg of buprenorphine and 0.18 mg of naloxone (Temgesic®-Nx) is marketed in New Zealand. Higher-dose buprenorphine sublingual tablets containing 0.4, 2 or 8 mg for treating opiate dependence have been marketed in France since 1996.

NDA 20-733 (Suboxone®)

Disclaimer: Some of the sponsor's material may be used in this review.

Introduction and drug history. The pharmacology of buprenorphine, a partial agonist of opioid *mu* receptors, has been reviewed in articles by Cowan, 1995; Rothman *et al.*, 1995; and Walter and Inturrisi, 1995, and non-clinical studies of buprenorphine conducted under NDA 20-732 (Subutex; Reckitt & Colman), except for carcinogenicity studies, have been reviewed previously by this reviewer (Pharm/tox review of NDA 20-732, December 5, 1997).

Studies reviewed within this submission (TABLE OF CONTENTS):	PAGE
Report 105341 in RC8782: Assessment of the analgesic profiles of buprenorphine and morphine given in combination with naloxone in the rat. (Volume 11)	15
Report R07012 in RC8782: Drug discrimination in rats trained to discriminate buprenorphine from saline (Volume 11)	16
Report 105306 in RC8782: Assessment of buprenorphine/naloxone mixtures for their ability to precipitate narcotic abstinence signs in rats previous infused with morphine. (Volume 11)	17
Report 37386: The effects of the oral co-administration of buprenorphine and naloxone on the metabolism of each compound in the rat and the dog. (Volume 18)	19
Report 37385: The effects of the intravenous co-administration of buprenorphine and naloxone on the metabolism of each compound in the rat and the dog. (Volume 18)	21
Report 37387: The effects of the intramuscular co-administration of buprenorphine and naloxone on the metabolism of each compound in the rat and the dog. (Volume 18)	24
Report 36585: Naloxone hydrochloride and buprenorphine hydrochloride: Acute oral toxicity interaction study in the mouse. (Volume 11)	33
Report 36589: Naloxone hydrochloride and buprenorphine hydrochloride: Acute intravenous toxicity interaction study in the mouse. (Volume 11)	34
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Report 105296: Naloxone hydrochloride and buprenorphine hydrochloride: Acute oral toxicity interaction study in the rat. (Volume 11)	35
Report 36588: Naloxone hydrochloride and buprenorphine hydrochloride: Acute intravenous toxicity interaction study in the rat. (Volume 11)	36
Report 36586: Naloxone hydrochloride and buprenorphine hydrochloride: Acute subcutaneous toxicity interaction study in the rat. (Volume 11)	37
Report 105342: Buprenorphine/Naloxone preliminary toxicity to rats by repeated oral administration for four weeks. (Volume 12)	38
Report 36935: 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 oral administration to Sprague-Dawley rats. (Volume 15)	40
Report 105345: Buprenorphine/Naloxone preliminary toxicity to rats by repeated intravenous administration for two weeks. (Volume 12)	46
Report 36936: 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. (Vol. 13)	48
Report R06866: 4-Week toxicity of the substance mixture buprenorphine-HCl, Lot 22 and naloxone-HCl, Batch No. 231 (Ratio 3:2) – called for short 'Bup + Nal' – by intramuscular administration to Sprague-Dawley rats. (Volume 14)	53
Report 105344: Buprenorphine/Naloxone local tolerance and preliminary toxicity to rats by repeated subcutaneous administration for two weeks. (Volume 12)	58
Report 36949: Buprenorphine/Naloxone Preliminary Oral Toxicity Study in Beagle Dogs (Repeated daily dosage for 4 weeks). (Volume 12)	60
Report 36937: 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. (Volume 16)	62
Report 36942: Buprenorphine/naloxone preliminary intravenous toxicity study in Beagle Dogs (Repeated daily dosage for up to 10 days). (Volume 12)	67

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Report 36938: 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. (Volume 15)	69
Report RC84198: 4-Week toxicity of the substance mixture Buprenorphine-HCl, Lot No. 21 and Naloxone-HCl, analysis No. 231 (Ratio 3:2) by intramuscular administration to beagle dogs. (Volume 16)	73
Report 38278: 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, embryo and fetus by oral administration. (Volume 17)	81
Report 38279: 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, embryo and fetus by intramuscular administration. (Volume 17)	84
Report 38282: 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, embryo and fetus by oral administration. (Volume 17)	87
Report 38283: 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 rabbit, embryo and fetus by intramuscular administration. (Volume 17)	89
Report RC980112: Study of buprenorphine / naloxone (4:1 mixture) in bacterial mutations assays using Salmonella typhimurium and Escherichia Coli. (— Study YV425; — Report — P/6017) (Volume 18)	94
Report 105983: Study to determine the ability of naloxone hydrochloride to induce mutation in five histidine-requiring strains of salmonella typhimurium. (Volume 18)	96
Report RC980113: Study of buprenorphine / naloxone (4:1 mixture) using an in vitro cytogenetic assay in human lymphocytes — Study SV0959; — Report — P/6052) (Volume 18)	99
Report 38746: Study to evaluate the chromosome damaging potential of naloxone hydrochloride by its effects on cultured human lymphocytes using an in-vitro cytogenetics assay. (Volume 18)	102

Report 980114: Study of buprenorphine / naloxone (4:1 mixture) using a rat bone marrow micronucleus test. (Study SR0958; Report P/6063)(Vol. 18) 104

Report 84204: 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. (Volume 11) 107

Report 84193: 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. (Vol. 11) 108

Studies not reviewed within this submission:

1. *The following studies were reviewed previously under NDA 20-732 [11/30/1997 (carcinogenicity) and 12/5/1997 (pharm/tox)]:*

Report RC80218: Acute Intravenous Toxicity Study in the Mouse, Buprenorphine and Identified Impurities. March 1980. (Volume 19)

Report RC79189: Primary Skin Irritation Study, U.S. Federal Register, 1973, Buprenorphine Hydrochloride, Batch No. PP1121/16. December 1979. (Volume 19)

Report RC79190: Primary Skin Irritation Study, U.S. Federal Register, 1973, Buprenorphine Injection, Batch No. 764. December 1979. (Volume 19)

Report RC79191: Eye Irritation Study, U.S. Federal Register, 1973, Buprenorphine Hydrochloride, Batch No. PP1121/16. December 1979. (Volume 19)

Report RC79192: Eye Irritation Study, U.S. Federal Register, 1973, Buprenorphine Injection, Batch No. 764. December 1979. (Volume 19)

Report RC80219: Acute Toxicity by Inhalation of Buprenorphine. February 1980. (Volume 19)

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Report RC80208: Buprenorphine Oral Toxicity Study in Beagle Dogs.
November 14, 1980. (Volume 22)

Report RC97020: Chronic (52-Week) Oral Toxicity Study of Buprenorphine in Dogs.
September 14, 1989. (Volume 22)

Report RC82211: Effect of Buprenorphine Hydrochloride on Pregnancy of the Rat (Oral Administration).
January 10, 1982. (Volume 24)

Report RC82212: Effect of Buprenorphine Hydrochloride on Pregnancy of the Rabbit.
December 21, 1982. (Volume 24)

Report CSR R07206: Effect of Buprenorphine Hydrochloride on Fertility and General Reproductive Performance of the Rat.
June 17, 1986. (Volume 24)

Report CSR R07067: Effect of Buprenorphine Hydrochloride on Peri- and Postnatal Development of the Rat.
April 17, 1986. (Volume 25)

Report RC84145: Mutagenicity Studies on Buprenorphine Hydrochloride.
January 1983. (Volume 25)

Report RC8532: Mutagenicity Studies of Buprenorphine Hydrochloride in In Vitro Bacterial Systems.
December 1984. (Volume 25)

Report RC8582: Study to Evaluate the Chromosome Damaging Potential of Buprenorphine Hydrochloride by its Effects on Cultured Chinese Hamster Ovary (CHO) Cells using an In Vitro Cytogenetics Assay.
October 11, 1985. (Volume 25)

Report RC8583: Study to Determine the Ability of Buprenorphine Hydrochloride to Induce Mutations in 6-Thioguanine Resistance in Mouse Lymphoma L5178Y Cells Using a Fluctuation Assay.
October 14, 1985. (Volume 25)

Report RC8202: Combined Chronic and Carcinogenicity Study in Rats with Buprenorphine (EU-4764).
July 7, 1981.
Volume 1. (Volume 26)

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Report RC8203: Combined Chronic and Carcinogenicity Study in Rats with
Buprenorphine (EU-4764). _____ July 7, 1981.
Volume 2. (Volume 27)

Report RC8204: Combined Chronic and Carcinogenicity Study in Rats with
Buprenorphine (EU-4764). _____ July 7, 1981.
Volume 3. (Volume 27)

Report RC8205: Combined Chronic and Carcinogenicity Study in Rats with
Buprenorphine (EU-4764). _____ July 7, 1981.
Volume 4. (Volume 28)

Report RC8206: Combined Chronic and Carcinogenicity Study in Rats with
Buprenorphine (EU-4764). _____ ; July 7, 1981.
Volume 5. (Volume 29)

Report RC8207: Combined Chronic and Carcinogenicity Study in Rats with
Buprenorphine (EU-4764). _____ ; July 7, 1981.
Volume 6. (Volume 30)

Report RC8208: Combined Chronic and Carcinogenicity Study in Rats with
Buprenorphine (EU-4764). _____ , July 7, 1981.
Volume 7. (Volume 31)

Report RC8777: Potential Tumorigenic Effects in Prolonged Dietary Administration to
Mice, Final Report (0-86 Weeks). _____
; August 26, 1987. Volume 1 (Volume 46)

Report RC8778: Potential Tumorigenic Effects in Prolonged Dietary Administration to
Mice, Final Report (0-86 Weeks). _____
August 26, 1987. Volume 2 (Volume 47)

Report RC8779: Potential Tumorigenic Effects in Prolonged Dietary Administration to
Mice, Final Report (0-86 Weeks). _____
August 26, 1987. Volume 3 (Volume 48)

Report RC97021: Potential Tumorigenic Effects in Prolonged Dietary Administration to
Mice, Final Report (0-99 Weeks). _____
August 21, 1989. Volume 1 (Volume 49)

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Report RC97022: Potential Tumorigenic Effects in Prolonged Dietary Administration to Mice, Final Report (0-99 Weeks).

August 21, 1989. Volume 2 (Volume 50)

Report RC97023: Potential Tumorigenic Effects in Prolonged Dietary Administration to Mice, Final Report (0-99 Weeks).

August 21, 1989. Volume 3 (Volume 51)

Report RC8915: Statistical Analysis of Selected Tumor Types from a Combined Chronic and Carcinogenicity Study in Rats with Buprenorphine.

June 10, 1986. (Volume 32)

Report RC 8910: Combined Chronic and Carcinogenicity Study in Rats with Buprenorphine (EU-4764), Pathology Report.

October 13, 1986. Volume 1. (Volume 32)

Report RC 8911: Combined Chronic and Carcinogenicity Study in Rats with Buprenorphine (EU-4764), Pathology Report.

October 13, 1986. Volume 2. (Volume 33)

Report RC 8912: Combined Chronic and Carcinogenicity Study in Rats with Buprenorphine (EU-4764), Pathology Report.

October 13, 1986. Volume 3. (Volume 34)

Report RC 8913: Combined Chronic and Carcinogenicity Study in Rats with Buprenorphine (EU-4764), Pathology Report.

October 13, 1986. Volume 4. (Volume 35)

Report RC 8914: Combined Chronic and Carcinogenicity Study in Rats with Buprenorphine (EU-4764). Addendum Pathology Report.

April 17, 1987. (Volume 36)

Report RC8916: Statistical Analysis of Selected Tumor Types from a Combined Chronic and Carcinogenicity Study in Rats with Buprenorphine.

July 14, 1988. (Volume 36)

Report RC97228: Combined Chronic and Carcinogenicity Study in Rats with Buprenorphine (EU-4764). Amended Pathology Report, February 26, 1988. Volume 1. (Volume 37)

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Report RC97229: Combined Chronic and Carcinogenicity Study in Rats with Buprenorphine (EU-4764) ; Amended Pathology Report, February 26, 1988. Volume 2. (Volume 38)

Report RC97230: Combined Chronic and Carcinogenicity Study in Rats with Buprenorphine (EU-4764) ; Amended Pathology Report, February 26, 1988. Volume 3. (Volume 39)

Report RC97231: Combined Chronic and Carcinogenicity Study in Rats with Buprenorphine (EU-4764) ; Amended Pathology Report, February 26, 1988. Volume 4. (Volume 40)

Report RC97232: Combined Chronic and Carcinogenicity Study in Rats with Buprenorphine (EU-4764) Amended Pathology Report, February 26, 1988. Volume 5. (Volume 41)

Report RC97226: Combined Chronic and Carcinogenicity Study in Rats with Buprenorphine (EU-4764). Special Report (6 July 1990) and Attachment 1, Volume 1: Pathology Peer Review Report. (Volume 42)

Report RC97227: Combined Chronic and Carcinogenicity Study in Rats with Buprenorphine (EU-4764). Special Report, Attachment 1, Volume 2: Pathology Peer Review Report, and Attachment 2: Pathology Review Committee Chairperson's Report. (Volume 43)

Report RC9733: Combined Chronic and Carcinogenicity Study in Rats with Buprenorphine (EU-4764) Special Report Attachment 3, Statistical Report. (Volume 44)

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

Report RC79126: Buprenorphine, Acute Intramuscular Toxicity in Rats. ; July 5, 1979. (Volume 19)

Report RC79127: Buprenorphine, Acute Intravenous Toxicity in Rats. ; May 14, 1979. (Volume 19)

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Report RC79128: Buprenorphine, Acute Intravenous Toxicity in Cross-Bred Dogs.
; April 2, 1979.
(Volume 19)

Report RC79129: Buprenorphine, Acute Intravenous Toxicity in Baboons.
June 11, 1979.
(Volume 19)

Report RC7668: Observations Upon the Acute Toxicity of Buprenorphine in Rodents by
R.L.F. Dawes. Reckitt & Colman Pharmaceutical Division, April 29, 1976. (Volume 19)

Report RC79125: Tolerance of Buprenorphine Injectable Solution following parenteral
administration in cross-bred dogs (with special reference to local reactions).
May
29, 1979. (Volume 19)

Report RC79100: Delayed Dermal Sensitization Study in the Guinea Pig.
November 1978. (Volume 19)

Report RC79124: In Vitro Haemolytic Properties of Buprenorphine Injection Solution.
May 29, 1979. (Volume 19)

Report RC8440: Evaluation of Haemolytic Activity of Buprenorphine in Human Blood.
March 1984. (Volume 19)

Report RC7203: Buprenorphine 30-Day Subcutaneous Toxicity Study in Rats. Reckitt
& Colman Products Ltd., Pharmaceutical Division, Hull, England; June 1972. (Volume
20)

Report RC79132: Buprenorphine, Four-Week Intravenous Toxicity in Beagle Dogs.
May 21, 1979.
(Volume 20)

Report RC79133: Buprenorphine, Four-Week Intravenous Toxicity in Baboons.
; May 21, 1979.
(Volume 20)

Report CSR 105817: Compound RX 6029-M, One-Month Oral Toxicity Study in Wistar
Rats.
September 1973.
(Volume 20)

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Report CSR 105816: Compound RX 6029-M, One-Month Oral Toxicity Study in Rhesus Monkeys. ; September 1973. (Volume 20)

Report RC77138: One-Month Sublingual Toxicity Study of Buprenorphine in Monkeys. , August 1977. (Volume 20)

Report CSR 105815: Compound RX 6029-M, Six-Month Intramuscular Toxicity Study in Wistar Rats. August 1974. (Volume 21)

Report CSR 105812: Compound RX 6029-M, Six-Month Intramuscular Toxicity Study in Olive Baboons. July 1974. (Volume 21)

Report CSR 103401: Compound RX 6029-M, Teratogenicity Studies in the Rabbit and Rat. February 1974. (Volume 23)

Report RC7205: Buprenorphine, Teratological Studies in Rats and Rabbits. Reckitt & Colman Products Ltd., Pharmaceutical Div., Hull, England; June 1972. (Volume 23)

Report RC79130: Buprenorphine, The Effect of Intravenous Administration on the Pregnancy of the Rat. July 5, 1979. (Volume 23)

Report RC79131: Buprenorphine, The Effect of Intravenous Administration on the Pregnancy of the Rabbit. July 2, 1979. (Volume 23)

Report CSR 103391: Buprenorphine, Fertility Study in the Rat. December 1975. (Volume 24)

Report CSR 103399: Buprenorphine, Peri- and Postnatal Toxicity Study in Rats. January 1976. (Volume 24)

Report RC7999: Mutagenicity Testing of Compound RX6029. November 1978. (Volume 25)

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PHARMACOLOGY:

Study Title: Assessment of the analgesic profile of buprenorphine and morphine given in combination with naloxone in the rat.

Study No.: 105341 Volume #: 1.11 Tab #: 8782 Pages: 1-13

Conducting Laboratory: Reckitt & Colman Pharmaceutical Division, Hull, England

Date of Study Initiation: September, 1983

GLP Compliance: Yes No

QA Report: Yes No

Methods: The antinociceptive effects of buprenorphine and morphine, alone, as well as in combination with naloxone, after SC injection, were tested with the tail-pressure to vocalization test in young rats at 15, 30 and 60 minutes after dosing. A cut-off of 400 mm Hg was employed to avoid damaging the tail.

Dosing:

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

No./sex/group: 10 males/group

Age: Not indicated

Weight: 30-40 grams

Dosage groups:

Buprenorphine alone: 0.003 to 3.0 mg/kg, SC

Morphine alone: 0.1 to 30 mg/kg, SC

Buprenorphine (0.03 mg/kg) + Naloxone (0.02, 0.04 or 0.08 mg/kg, SC)

Buprenorphine (0.3 mg/kg) + Naloxone (0.2, 0.4 or 0.8 mg/kg, SC)

Morphine (3.0 mg/kg) + Naloxone (0.02, 0.04 or 0.08 mg/kg, SC)

Route, form, volume: Subcutaneous, solution, 10 ml/kg

Drug lot no.: Buprenorphine, lot #13 (Reckitt & Colman); naloxone, batch #80-161

Formulation/vehicle: Saline

Results:

In the rat tail-pressure test, buprenorphine displayed a bell-shaped log dose-response relationship, with a maximum at 0.03 mg/kg, whereas morphine showed a

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sigmoidal log dose-response relationship, reaching maximal antinociception at 3 mg/kg. The low dose of naloxone (0.02 mg/kg) antagonized morphine, but not buprenorphine, at 15 or 30 minutes after dosing. Higher doses of naloxone antagonized buprenorphine at 15-30 minutes after dosing, but little or no antagonism remained by 60 minutes after dosing. Morphine was blocked more extensively by naloxone (>90%) than was buprenorphine (~70%) and displayed some residual antagonism by the two higher naloxone doses at 60 minutes after dosing.

Summary:

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 both maximum effect and duration. Buprenorphine (0.03 mg/kg) with naloxone (0.02 mg/kg) did not show less antinociceptive effect than buprenorphine (0.03 mg/kg) alone.

SAFETY PHARMACOLOGY:

Abuse Liability:

Study Title: Drug discrimination studies with buprenorphine-naloxone mixtures in rats.

Study No.: R07012 Volume #: 1.11 Tab #: 8782 Pages: 28-34

Conducting Laboratory: _____

Date of Study Initiation: September, 1985

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

Methods: Animals maintained at 85% of their free-feeding body weight were trained to discriminate with ≥80% accuracy between SC saline and buprenorphine in a 2-lever FR10 food reward drug discrimination paradigm, which was tested 30 min after dosing.

Dosing:

Species/strain: Rat / _____ strain

No./sex/group: 12 males Age: Not indicated

Weight: ~300 grams

Dosage groups:

Buprenorphine alone: Training (and testing doses), 0.03 mg/kg, SC

Naloxone alone: 0.02 mg/kg, SC

Buprenorphine (0.03 mg/kg) + Naloxone (0.002, 0.01 or 0.02 mg/kg, SC).

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

Drug lot no.: Buprenorphine, batch #17 (Reckitt & Colman); naloxone, lot 80-161

Formulation/vehicle: Saline

Results:

TABLE 1: Effect of naloxone on discrimination of buprenorphine by rats.

Dose of Drug, mg/kg		Buprenorphine-appropriate responding, %
Buprenorphine	Naloxone	
0.03	0	97%
0.03	0.002	93%
0.03	0.01	59%
0.03	0.02	23%
0 (saline)	0	2%
0	0.02	8%

Summary:

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 from 97% after buprenorphine alone (0.03 mg/kg, SC) to 93, 59 and 23%, respectively.

Study Title: Assessment buprenorphine/naloxone mixtures for their ability to precipitate narcotic abstinence signs in rats previously infused with morphine.

Study No.: 105306 Volume #: 1.11 Tab #: 8782 Pages: 14-27

Conducting Laboratory: Reckitt & Colman Pharmaceutical Division, Hull, England

Date of Study Initiation: October, 1983

GLP Compliance: () Yes (X) No

QA Report: (X) Yes () No

Methods: Animals were fitted with an IP cannula for continuous infusion of morphine at a rate of 100 mg/kg/24 hr for 48 hours. Thirty minutes post-infusion, saline, buprenorphine, naloxone or buprenorphine+naloxone were administered IV and the incidence of various withdrawal signs, including wet-dog shakes, escape attempts, writhes, teeth chattering, diarrhea, gland secretions, ptosis, penile erection, ear blanching, sensitivity (vocalization) to touch and stereotypy, were recorded during a 10-minute observation period. Weight loss was determined 3 hours after acute IV dosing.

Dosing:

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

No./sex/group: 10 males/group

Age: Not indicated

Weight: 140-160 grams

Dosage groups:

Buprenorphine alone: 0.003 to 3.0 mg/kg, SC

Morphine alone: 0.1 to 30 mg/kg, SC

Buprenorphine (0.03 mg/kg) + Naloxone (0.02, 0.04 or 0.08 mg/kg, SC)

Buprenorphine (0.3 mg/kg) + Naloxone (0.2, 0.4 or 0.8 mg/kg, SC)

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Morphine (3.0 mg/kg) + Naloxone (0.02, 0.04 or 0.08 mg/kg, SC)
 Route, form, volume: IP infusion, solution, 5.97 ml/24 hr;
 Drug lot no.: Buprenorphine, lot #13 (Reckitt & Colman); naloxone, batch #80-161
 _____, morphine sulfate, lot #11097 (_____
 Formulation/vehicle: Saline _____ lot #81210, _____)

Results:

TABLE 2: Precipitation by buprenorphine and naloxone, alone and in combination, of withdrawal signs in morphine-dependent rats.

Challenge drugs, mg/kg		Mean behavioral score out of 100	% Weight loss (mean ± SEM)	No. of signs in ≥50% of rats
Buprenorphine	Naloxone			
0 (saline)	0	6.7	2.7±0.4	1
0.03	0	11.7	3.9±0.4	2
0.3	0	14.2	3.4±0.2	2
0	0.02	40.8	4.1±0.4	5
0	0.2	63.3	6.7±0.4**	8
0.03	0.02	31.7	3.6±0.3	4
0.3	0.2	54.2	3.6±1.1	8

**P<0.01 (Dunnett's test).

The only group to show a significant weight loss during precipitated withdrawal was the high-dose naloxone only group, but it should be kept in mind that the saline-treated control rats were also physically dependent and likely were undergoing the beginning of abrupt withdrawal during the 3.5-hour period following the end of the morphine infusion. This is evidenced by the observations that half of the controls had a weight loss of >5 grams and 3/10 showed >2 wet-dog shakes. Similarly, dependent rats challenged with buprenorphine showed only two withdrawal signs with a frequency ≥50%: weight loss >5 grams (6/10 at both doses) and wet-dog shakes >2/10-min observation (7/10 at both doses). Naloxone alone showed a dose-dependent increase in number and incidence of the various withdrawal signs. The addition of buprenorphine to the naloxone in a 3:2 ratio had a tendency to attenuate slightly the effect of naloxone, but not significantly.

Summary:

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.

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PHARMACOKINETICS/TOXICOKINETICS:

Study Title: The effects of the oral co-administration of buprenorphine and naloxone on the metabolism of each compound in the rat and the dog.

Study No.: 37386 Volume #: 1.18 Tab #: 37386

Conducting Laboratory: Reckitt & Colman Pharmaceutical Division, Hull, England

Date of Study Initiation: June, 1984

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

Methods: Animals were fasted overnight prior to dosing and allowed food 2 hours post-dosing. Dogs were allowed a 4-week "washout" period between dosing. Test substances were administered by oral gavage, liquid in rats, capsule in dogs. At each time point studied, groups of 3 rats were exsanguinated by cardiac puncture into heparinized tubes under halothane (4%) anesthesia. Dog blood samples (5 ml) from the cephalic vein were also collected into heparinized tubes. Plasma was collected by centrifugation and then stored frozen before assay. Determination of unchanged drug in plasma involved

Dosing:

Species/strain: Rat / Sprague-Dawley ()
Dog / Beagle ()

No./sex/group: Rats, 3 males/group; dogs, 2 males/group. Age: Not indicated

Weight: Rats, 130-200 grams; dogs, 10.5-18.8 kg

Dosage groups (mg/kg):	<u>RATS</u>	<u>DOGS</u>
[³ H]-Buprenorphine alone:	80	15
[³ H]-Naloxone alone:	80	15
[³ H]-Buprenorphine + Naloxone:	80+80	15+15
Buprenorphine + [³ H]-Naloxone:	80+80	15+15

Route, form, volume: Oral, liquid in rats, 10 ml/kg; capsule in dogs.

Drug lot no., radiolabel and % purity: [15,16-³H]-Buprenorphine (Batch PMT 0540/066/1, specific activity 51.85 µCi/mg) was prepared by _____ and purified to _____ purity by Reckitt and Colman; [N-allyl-2,3-³H]-naloxone, lot #2079-082 was purchased from _____ and had _____ radiochemical purity in three test systems. Unlabeled buprenorphine was from lot #21. Unlabeled naloxone had certificate of analysis number CAJ 83/13.

Formulation/vehicle: Distilled water (adjusted to pH 4 with 0.01M HCl)

Results:

TABLE 3: Time course of unchanged [³H]-drug in plasma from rats after [³H]-naloxone or [³H]-buprenorphine alone, or in combination with the other drug, unlabeled.

Time after dosing	Mean concentration of unchanged [³ H]-drug, ng/g of rat plasma			
	[³ H]-BUP alone	[³ H]-BUP+NAL	[³ H]-NAL alone	[³ H]-NAL+BUP
5 min	300 ± 71	338 ± 60	162 ± 17	81 ± 18
15 min	313 ± 61	871 ± 163	313 ± 108	134 ± 18
30 min	437 ± 49	915 ± 243	166 ± 23	114 ± 17
60 min	559 ± 60	625 ± 123	59 ± 14	53 ± 30
90 min	571 ± 135	532 ± 41	132 ± 59	61 ± 21
2 hours	474 ± 100	454 ± 51	54 ± 13	47 ± 24
4 hours	133 ± 25	438 ± 221	41 ± 2	26 ± 3
6 hours	334 ± 7	253 ± 26	64 ± 26	27 ± 16
8 hours	288 ± 47	223 ± 50	31 ± 16	18 ± 18

TABLE 4: Time course of excretion of radioactivity by rats after oral [³H]-naloxone or [³H]-buprenorphine alone, or in combination with the other drug, unlabeled.

Sample (n=3)		Mean (± SEM) excretion of radioactivity, % of administered dose			
Type	Time	[³ H]-BUP alone	[³ H]-BUP+NAL	[³ H]-NAL alone	[³ H]-NAL+BUP
Urine	0-24 hr	1.98 ± 1.00	2.56 ± 1.59	27.91 ± 0.82	21.06 ± 1.24
	24-48h	3.30 ± 1.17	5.38 ± 3.50	4.15 ± 0.22	9.44 ± 0.92
	48-96h	3.69 ± 1.46	4.38 ± 1.62	3.33 ± 0.40	8.81 ± 0.21
	Total	8.97 ± 2.42	12.32 ± 6.70	35.39 ± 0.38	39.30 ± 0.45
Total labile [³ H]		1.06%	1.38%	7.48%	11.53%
Feces	0-24 hr	18.44 ± 2.15	2.61 ± 2.56	9.97 ± 1.33	4.99 ± 0.50
	24-48h	21.33 ± 5.84	27.75 ± 6.37	2.87 ± 1.36	4.04 ± 1.46
	48-96h	24.75 ± 2.78	24.75 ± 2.15	0.44 ± 0.11	3.26 ± 0.53
	Total	64.52 ± 4.72	55.11 ± 5.75	13.27 ± 0.70	12.29 ± 0.70
Carcass – 96 hr		2.63 ± 0.39	4.11 ± 0.69	18.57 ± 1.05	15.56 ± 2.37
Total labile [³ H]		2.43%	3.45%	17.46%	14.70%

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TABLE 5: Time course of excretion of radioactivity by dogs after oral [³H]-naloxone or [³H]-buprenorphine alone, or in combination with the other drug, unlabeled.

Sample (n=2)		Excretion of radioactivity by each dog, % of administered dose							
Type	Day	[³ H]-BUP alone	[³ H]-BUP+NAL	[³ H]-NAL alone	[³ H]-NAL+BUP				
Urine	1								
	2								
	3								
	4								
	5								
	6								
	7								
	Total								
Total labile [³ H]		1.19%	0.87%	0%	0.13%	0.25%	0.07%	0.72%	0.61%
Feces	1								
	2								
	3								
	4								
	5								
	6								
	7								
	Total								

NS=no sample

*Some urine lost.

† Urine sample contaminated with feces.

Study Title: The effects of the intravenous co-administration of buprenorphine and naloxone on the metabolism of each compound in the rat and the dog.

Study No.: 37385 Volume #: 1.18 Tab #: 37385

Conducting Laboratory: Reckitt & Colman Pharmaceutical Division, Hull, England

Date of Study Initiation: June, 1984

GLP Compliance: () Yes (X) No

QA Report: (X) Yes () No

Methods: Animals were fasted overnight prior to dosing and allowed food 2 hours post-dosing. Dogs were allowed a 4-week "washout" period between dosing. Test substances were administered intravenously by tail vein in rats or cephalic vein in dogs. At each time point studied, groups of 3 rats were exsanguinated by cardiac puncture into heparinized tubes under halothane (4%) anesthesia. Dog blood samples (5 ml) from the cephalic vein were also collected into heparinized tubes. Plasma was collected by centrifugation and then stored frozen before assay. Determination of unchanged drug in plasma involved extraction into ether from carbonate/bicarbonate - buffer at pH 9.8. Determination of metabolic profile involved e_____

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Dosing:

Species/strain: Rat / Sprague-Dawley _____

Dog / Beagle _____

No./sex/group: Rats, 3 males/group; dogs, 2 males/group

Age: Not indicated

Weight: Rats, 140-200 grams; dogs, 8.0-18.6 kg

Dosage groups (mg/kg): RATS DOGS

[³H]-Buprenorphine alone: 4.5 1.5

[³H]-Naloxone alone: 3.0 1.0

[³H]-Buprenorphine + Naloxone: 4.5+3.0 1.5+1.0

Buprenorphine + [³H]-Naloxone: 4.5+3.0 1.5+1.0

Route, form, volume: Intravenous, 2 ml/kg in rats; 0.2 ml/kg in dogs.

Drug lot no., radiolabel and % purity: [15,16-³H]-Buprenorphine (Batch PMT

0540/066/1, specific activity 51.85 µCi/mg) was prepared by _____

_____ and purified to _____ purity by Reckitt and Colman; [N-allyl-2,3-³H]-

naloxone, lot #2079-082 was purchased from _____

_____ and had _____ radiochemical purity in three test systems. Unlabeled

buprenorphine was from lot #21. Unlabeled naloxone had certificate of analysis

number CAJ 83/13.

Formulation/vehicle: 0.45% saline in rats; 5% sterile dextrose in dogs.

Results:

TABLE 6: Time course of unchanged [³H]-drug in rat plasma after IV [³H]-naloxone or [³H]-buprenorphine alone, or in combination with the other drug, unlabeled.

Time after IV dosing	Mean concentration of unchanged [³ H]-drug, ng/g of rat plasma			
	[³ H]-BUP alone	[³ H]-BUP+NAL	[³ H]-NAL alone	[³ H]-NAL+BUP
5 min	1442 ± 183	1306 ± 36	761 ± 22	574 ± 25
15 min	946 ± 104	994 ± 146	455 ± 22	456 ± 11
30 min	674 ± 31	731 ± 53	263 ± 19	255 ± 17
60 min	379 ± 22	344 ± 26	89 ± 5	92 ± 14
2 hours	136 ± 5	162 ± 6	24 ± 3	23 ± 3
4 hours	77 ± 9	47 ± 3	2 ± 1	10 ± 3
6 hours	55 ± 15	28 ± 9	1 ± 1	4 ± 0
8 hours	30 ± 3	23 ± 4	1 ± 1	5 ± 3

TABLE 7: Time course of excretion of radioactivity by rats after IV [³H]-naloxone or [³H]-buprenorphine alone, or in combination with the other drug, unlabeled.

Sample (n=3)		Mean (± SEM) excretion of radioactivity, % of administered dose			
Type	Time	[³ H]-BUP alone	[³ H]-BUP+NAL	[³ H]-NAL alone	[³ H]-NAL+BUP
Urine	0-24 hr	4.01 ± 0.81	6.51 ± 2.18	37.56 ± 0.43	34.68 ± 1.33
	24-48h	2.72 ± 0.60	3.97 ± 1.22	3.21 ± 0.66	3.70 ± 0.47
	48-96h	2.90 ± 1.78	3.41 ± 1.09	4.67 ± 0.68	4.42 ± 0.72
	Total	9.63 ± 1.87	13.89 ± 1.69	45.44 ± 1.76	42.80 ± 2.47
Total labile [³ H]		1.44%	1.80%	9.78%	9.44%
Feces	0-24 hr	32.34 ± 15.48	18.16 ± 11.26	6.87 ± 1.01	2.98 ± 1.86
	24-48h	14.59 ± 4.50	20.38 ± 2.09	0.25 ± 0.06	2.75 ± 0.32
	48-96h	26.92 ± 13.01	23.19 ± 9.74	0.20 ± 0.04	0.76 ± 0.45
	Total	73.86 ± 8.11	61.73 ± 2.62	7.32 ± 1.11	6.49 ± 1.27
Carcass – 96 hr		3.88 ± 0.16	5.13 ± 1.14	16.52 ± 1.55	18.91 ± 0.81
Total labile [³ H]		3.18%	4.35%	15.81%	18.48%

TABLE 8: Time course of unchanged [³H]-drug in plasma of dogs after IV [³H]-naloxone or [³H]-buprenorphine alone, or in combination with the other drug, unlabeled.

Time after IV dosing (n=2)	Mean concentration of unchanged [³ H]-drug, ng/g of dog plasma			
	[³ H]-BUP alone	[³ H]-BUP+NAL	[³ H]-NAL alone	[³ H]-NAL+BUP
5 min	788	739	349	357
15 min	539	496	237	263
30 min	436	323	140	181
60 min	184	161	69	94
2 hours	107	83	17	34
4 hours	39	39	5	7
6 hours	25	23	2	4
8 hours	21	18	2	3
24 hours	3	14	0.5	2
48 hours	2	2	1	1
72 hours	3	1	2	2
96 hours	4	0	1	0.5

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TABLE 9: Time course of excretion of radioactivity by dogs after IV [³H]-naloxone or [³H]-buprenorphine alone, or in combination with the other drug, unlabeled.

Sample (n=2)		Excretion of radioactivity by each dog, % of administered dose							
Type	Day	[³ H]-BUP alone	[³ H]-BUP+NAL	[³ H]-NAL alone	[³ H]-NAL+BUP				
Urine	1								
	2								
	3								
	4								
	5								
	6								
	7								
	Total								
Total labile [³ H]		0.45%	1.24%	0.46%	0.80%	0.41%	0.54%	2.51%	4.65%
Feces	1								
	2								
	3								
	4								
	5								
	6								
	7								
	Total								

NS=no sample.

Study Title: The effects of the intramuscular co-administration of buprenorphine and naloxone on the metabolism of each compound in the rat and the dog.

Study No.: 37387 Volume #: 1.18 Tab #: 37387

Conducting Laboratory: Reckitt & Colman Pharmaceutical Division, Hull, England

Date of Study Initiation: August, 1984

GLP Compliance: () Yes (X) No

QA Report: (X) Yes () No

Methods: Animals were fasted overnight prior to dosing and allowed food 2 hours post-dosing. Dogs were allowed a 4-week "washout" period between dosing. Test substances were administered intravenously by tail vein in rats or cephalic vein in dogs. At each time point studied, groups of 3 rats were exsanguinated by cardiac puncture into heparinized tubes under halothane (4%) anesthesia. After blood sampling, the injection site (whole left hind leg) was taken from each carcass for the determination of radioactivity. Dog blood samples (5 ml) from the cephalic vein were also collected into heparinized tubes. Plasma was collected by centrifugation and then stored frozen before assay. Determination of unchanged drug in plasma involved extraction into ether from carbonate/bicarbonate buffer at pH 9.8. Determination of metabolic profile

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involved extracting

Dosing:

Species/strain: Rat / Sprague-Dawley _____
 Dog / Beagle / _____

No./sex/group: Rats, 3 males/group; dogs, 2 males/group

Age: Not indicated

Weight: Rats, 130-200 grams; dogs, 9.0-16.0 kg

Dosage groups (mg/kg):	<u>RATS</u>	<u>DOGS</u>
[³ H]-Buprenorphine alone:	4.5	1.5
[³ H]-Naloxone alone:	3.0	1.0
[³ H]-Buprenorphine + Naloxone:	4.5+3.0	1.5+1.0
Buprenorphine + [³ H]-Naloxone:	4.5+3.0	1.5+1.0

Route, form, volume: Intramuscular, 1 ml/kg in rats; 0.2 ml/kg in dogs.

Drug lot no., radiolabel and % purity: [³H]-Buprenorphine (Batch PMT 0540/066/1, specific activity 51.85 µCi/mg) was prepared by Amersham International and purified to _____ purity by Reckitt and Colman; [N-allyl-2,3-³H]-naloxone, lot #2079-082 was purchased from _____

_____ and had _____ radiochemical purity in three test systems. Unlabeled buprenorphine was from lot #21. Unlabeled naloxone had certificate of analysis number CAJ 83/13.

Formulation/vehicle: 5% dextrose.

Results:

TABLE 10: Time course of the percentage of [³H]-drug remaining at injection site in rats after IM [³H]-naloxone or [³H]-buprenorphine alone, or in combination with the other drug, unlabeled (n=3/group).

Time after IM administration	% (±SEM) of administered [³ H]-drug remaining in rat hind thigh			
	[³ H]-BUP alone	[³ H]-BUP+NAL	[³ H]-NAL alone	[³ H]-NAL+BUP
5 min	63 ± 3	51 ± 2	53 ± 3	50 ± 8
15 min	58 ± 3	47 ± 1	18 ± 2	23 ± 3
30 min	40 ± 3	43 ± 3	6 ± 0.2	7 ± 2
60 min	37 ± 2	42 ± 11	3 ± 0.3	4 ± 0.6
2 hours	26 ± 2	26 ± 4	1 ± 0.2	3 ± 0.3
4 hours	23 ± 3	20 ± 2	1 ± 0.3	2 ± 0.2
6 hours	18 ± 6	18 ± 1	2 ± 0.1	2 ± 0.2
8 hours	23 ± 1	10 ± 2	2 ± 0.1	2 ± 0.1

TABLE 11: Time course of unchanged [³H]-drug in plasma of rats after IM [³H]-naloxone or [³H]-buprenorphine alone, or in combination with the other drug, unlabeled.

Time after IM administration	Mean concentration of unchanged [³ H]-drug, ng/g of rat plasma			
	[³ H]-BUP alone	[³ H]-BUP+NAL	[³ H]-NAL alone	[³ H]-NAL+BUP
5 min	892 ± 49	839 ± 23	865 ± 84	833 ± 29
15 min	647 ± 43	561 ± 80	594 ± 28	544 ± 60
30 min	772 ± 90	441 ± 89	329 ± 14	260 ± 25
60 min	576 ± 70	354 ± 65	127 ± 13	108 ± 8
2 hours	321 ± 21	206 ± 19	27 ± 5	22 ± 4
4 hours	93 ± 9	56 ± 12	4 ± 0	2 ± 1
6 hours	41 ± 10	37 ± 4	1	<1
8 hours	24 ± 3	36 ± 5	<1	<1

TABLE 12: Time course of excretion of radioactivity by rats after IM [³H]-naloxone or [³H]-buprenorphine alone, or in combination with the other drug, unlabeled.

Sample (n=3)		Mean (± SEM) excretion of radioactivity, % of administered dose			
Type	Time	[³ H]-BUP alone	[³ H]-BUP+NAL	[³ H]-NAL alone	[³ H]-NAL+BUP
Urine	0-24 hr	6.30 ± 2.51	3.07 ± 1.05	29.15 ± 6.00	35.87 ± 0.43
	24-48h	3.97 ± 1.14	1.42 ± 0.32	4.18 ± 0.06	4.35 ± 0.14
	48-96h	3.95 ± 1.53	1.20 ± 0.25	4.10 ± 0.18	4.43 ± 0.44
	Total	14.21 ± 3.68	5.69 ± 1.16	37.43 ± 5.97	44.66 ± 1.01
Total labile [³ H]		1.47%	1.42%	11.37%	9.48%
Feces	0-24 hr	22.42 ± 2.22	23.30 ± 6.36	6.87 ± 1.57	4.62 ± 0.70
	24-48h	21.62 ± 5.25	24.45 ± 5.37	0.98 ± 0.42	1.15 ± 0.44
	48-96h	12.41 ± 1.63	8.04 ± 1.64	0.12 ± 0.05	0.39 ± 0.16
	Total	56.45 ± 4.00	55.79 ± 2.01	7.97 ± 1.64	6.16 ± 0.70
Carcass – 96 hr		6.92 ± 0.18	4.70 ± 0.13	17.45 ± 0.97	18.81 ± 1.08
Total labile [³ H]		4.83%	3.46%	16.04%	17.85%

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TABLE 13: Time course of unchanged [³H]drug in plasma of dogs after IM [³H]naloxone or [³H]-buprenorphine alone, or in combination with the other drug, unlabeled.

Time after IM dosing (n=2)	Mean concentration of unchanged [³ H]-drug, ng/g of dog plasma			
	[³ H]-BUP alone	[³ H]-BUP+NAL	[³ H]-NAL alone	[³ H]-NAL+BUP
5 min	99	72	255	125
15 min	103	129	258	258
30 min	99	115	180	210
60 min	74	98	74	102
2 hours	51	79	19	30
4 hours	31	55	4	4
6 hours	49	50	1	2
8 hours	46	43	2	1
24 hours	13	8	2	1
48 hours	4	2	<1	1
72 hours	0.7	0.6	<1	0
96 hours	2.9	0.2	<1	0

TABLE 14: Time course of excretion of radioactivity by dogs after IM [³H]-naloxone or [³H]-buprenorphine alone, or in combination with the other drug, unlabeled.

Sample (n=2)		Excretion of radioactivity by each dog, % of administered dose							
Type	Day	[³ H]-BUP alone	[³ H]-BUP+NAL	[³ H]-NAL alone	[³ H]-NAL+BUP				
Urine	1								
	2								
	3								
	4								
	5								
	6								
	7								
	Total								
Total labile [³ H]		0.8%	0.8%	0.3%	0.2%	2.0%	4.9%	0.4%	1.3%
Feces	1								
	2								
	3								
	4								
	5								
	6								
	7								
	Total								

NS=no sample.

† Urine sample contaminated with feces.