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RESEARCH**

APPLICATION NUMBER:

20-732

20-733

PHARMACOLOGY REVIEW(S)

Division of Anesthetic, Critical Care and Addiction Drug Products
PHARMACOLOGY AND TOXICOLOGY REVIEW
Chemistry Consult #1

NDA No. 20-733

Date of Consult: NA

Reviewer: Timothy J. McGovern, Ph.D.

Review Completed: 07 OCT 2002

Information to be Conveyed to Sponsor: Yes (), No (✓)

Sponsor: Reckitt Benckiser Pharmaceuticals, Richmond, VA

Drug:

Trade Name: Suboxone

Generic: Buprenorphine/naloxone

Drug Class: opioid analgesic/opioid antagonist

Proposed Clinical Dose: The maximum recommended daily dose of Suboxone is 16 mg buprenorphine/4 mg naloxone per day. In a 50-kg adult, the maximum dose is equivalent to 0.32 mg/kg or 11.84 mg/m² buprenorphine and 0.08 mg/kg or 2.96 mg/m² naloxone.

Review:

A safety assessment of the proposed drug substance impurity specification for _____ was requested. The sponsor's proposed drug substance specification is as follows:

Drug substance impurity	Proposed specification
_____	_____

Safety Assessment of the Impurity in the Drug Substance:

The ICH guidance document Q3A, Impurities in New Drug Substances, recommends qualification of impurities exceeding the threshold limits of 0.15% or 1 mg TDI, whichever is lower, for a drug substance administered at < 2 g/day maximum daily dose. The sponsor performed a 3-month dietary general toxicity study (doses of 0, 100, 500, 1500 and 2000 ppm; 7.5-8.3, 36.6-41.6, 112-123, 152-167 mg/kg/day) in rats to provide dosing recommendations for a 2-year carcinogenicity study (reviewed in NDA 20-733 review dated March 21, 2000). The drug formulation for this study included the substance impurity in question at a level of _____ in naloxone batch V04434 and a concentration of _____ in the Suboxone formulation fed to the animals. Treatment-related effects include an increased incidence of aggressiveness at the 3 upper doses, dose-dependent increase in APTT in females at the three highest doses, a 20% decrease in triglycerides in high-dose males, a dose-dependent increase in adrenal gland weight in males with no correlating histological changes. At the NOAEL dose for this study (2000 ppm, 160 mg/kg), rats were administered the drug substance impurity at a dose of _____ µg/kg/day

(see calculations, page 4). These levels provide a 194-fold safety factor compared to the maximum recommended human dose. This safety margin is greater than the margin of 10 needed for qualification. Thus, the proposed level of _____ has been adequately qualified in terms of daily dosing of the drug product.

In a series of faxes dated October 7, 2002, the sponsor proposed a similar rationale for qualification although the calculated safety margin of 96.5-fold was based upon a maximum recommend human daily dose of 8 mg naloxone. Although the product label recommends a maximum dose of 4 mg/day, the sponsor's calculation does not affect the overall recommendation.

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Table 1:

Impurity	Proposed Limit % max $\mu\text{g/kg}$	Preclinical Dose % $\mu\text{g/kg}$	Species	Duration	Route	Safety Margin
[]	—	Rat	3 mos	diet	194

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/s/

Timothy McGovern
10/7/02 02:05:19 PM
PHARMACOLOGIST

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PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: 20-732/20-733

Review number: 3

Sequence number/date/type of submission: NDA 20-733/November 16, 2001/BC
NDA 20-732/December, 2001
NDA 20-733/June 28, 2002/000 BP

Information to sponsor: Yes (X) No ()

Sponsor and/or agent: Reckitt Benkiser Pharmaceuticals, Inc., Richmond, VA

Manufacturer for drug substance: Reckitt Benkiser Pharmaceuticals, Inc.

Reviewer name: Timothy J. McGovern, Ph.D.

Division name: Anesthetic, Critical Care and Addiction Drug Products

HFD #: 170

Review completion date: October 7, 2002

Drug:

Trade name: Subutex/Suboxone sublingual tablets

Generic name (list alphabetically): buprenorphine HCl, naloxone dihydrate HCl

Code name: NA

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

Naloxone: (5')-4,5-epoxy-3,14-dihydroxy-17-(2-propenyl)-morphinan-6-one hydrochloride dihydrate

CAS registry number: buprenorphine: 53152-21-9; Naloxone: 51481-60-8

Mole file number: NA

Molecular formula/molecular weight: buprenorphine: 504.09; Naloxone: 399.87

Structure: see previous NDA reviews

Relevant INDs/NDAs/DMFs: IND 45,220, IND 58,653

Drug class: buprenorphine: opioid analgesic (partial mu agonist); Naloxone: opioid antagonist

Indication: Treatment of 

Clinical Formulation: See previous NDA reviews

Route of administration: Sublingual

Proposed use: Suboxone is a 4:1 combination of buprenorphine HCl and naloxone HCl in a sublingual tablet formulation for the treatment of opioid addiction. Daily dosing up to a recommended maximum dose of 16 mg buprenorphine/4 mg naloxone. Subutex contains buprenorphine without the naloxone.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

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Executive Summary

I. Recommendations

- A. Recommendation on Approvability: These applications are approvable from a non-clinical perspective with the suggested label modifications (see section IX).
- B. Recommendation for Nonclinical Studies: The sponsor has recently completed a 2-year carcinogenicity study in rats with dietary administration of the buprenorphine/naloxone combination. The study should be reviewed upon submission and the results should be incorporated into the product label.
- C. Recommendations on Labeling: The sponsor's proposed labeling related to non-clinical information is acceptable with the proposed modifications in the "Carcinogenesis, mutagenesis, impairment of fertility" and "Pregnancy" section. See section IX for specific details.

II. Summary of Nonclinical Findings

- A. Brief Overview of Nonclinical Findings: See original NDA reviews for NDAs 20-732 and 20-733.
- B. Pharmacologic Activity: See original NDA reviews for NDAs 20-732 and 20-733.
- C. Nonclinical Safety Issues Relevant to Clinical Use: None at this time.

III. Administrative

- A. Reviewer signature: Timothy J. McGovern, Ph.D.
- B. Supervisor signature: Concurrence - Timothy J. McGovern, Ph.D.
- C. cc: list: NA

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PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

See original NDA reviews by Dr. David Brase for NDA 20-732 (January 12, 1998) and NDA 20-733 (October 26, 1999).

II. SAFETY PHARMACOLOGY:

See original NDA reviews by Dr. David Brase for NDA 20-732 (January 12, 1998) and NDA 20-733 (October 26, 1999).

III. PHARMACOKINETICS/TOXICOKINETICS:

See original NDA reviews by Dr. David Brase for NDA 20-732 (January 12, 1998) and NDA 20-733 (October 26, 1999).

IV. GENERAL TOXICOLOGY:

See original NDA reviews by Dr. David Brase for NDA 20-732 (January 12, 1998) and NDA 20-733 (October 26, 1999).

V. GENETIC TOXICOLOGY:

Genetic toxicology conclusions: Studies assessing the mutagenic potential of buprenorphine and the combination of buprenorphine and naloxone have been reviewed previously. Buprenorphine tested positively in the Green-Tweets (*E. coli*) survival test, a DNA synthesis inhibition (DSI) test with testicular tissue from mice, and an unscheduled DNA synthesis (UDS) test using testicular cells from mice. Negative results were found in yeast for recombinant, gene convertant, or forward mutations, in a *Bacillus subtilis* "rec" assay, in a clastogenicity assay in CHO cells, Chinese hamster bone marrow and spermatogonia cells and in the mouse lymphoma L5178Y assay. Equivocal results were found with the Ames assay. The 4:1 drug combination was negative in an in vitro bacterial mutation assay (Ames), an in vitro cytogenetic assay in human lymphocytes, and an in vivo IV rat micronucleus assay.

Labeling recommendations: The product label should reflect the results of the genetic toxicology studies summarized above.

VI. CARCINOGENICITY:

Carcinogenicity summary: Carcinogenicity studies have been completed for buprenorphine and the results have been reviewed previously and are described in the proposed labeling. The sponsor has a 2-year CA study in rats underway to assess the carcinogenic potential of the proposed drug combination. In an update dated June 28, 2002, the sponsor reports that the study is nearing completion of the in-life phase (week 102). The sponsor notes that mortalities in control males is approaching critical levels and proposes to monitor mortality status on a daily basis, but to continue the study to planned termination at week 104. If excessive mortality occurs during the final 2-3 weeks of exposure, it may be necessary to review this decision and terminate the male control groups before completion of week 104. The study will be reviewed once the study report is submitted and the results should then be included in the product label.

Carcinogenicity conclusions: Carcinogenicity studies in rats (27-month) and mice (86-week) demonstrated a statistically significant dose-related increase in testicular interstitial (Leydig's) cell tumors in rats, according to the trend test adjusted for survival. Pair-wise comparison of the high dose against control failed to show statistical significance. No significant findings were noted in the mouse study. Conclusions concerning the carcinogenic potential of the drug combination await receipt and review of the study report for a 2-year rat study that will be submitted as a Phase 4 commitment..

Recommendations for further analysis: None at this time

Labeling Recommendations: The results of carcinogenicity studies for buprenorphine HCL have been previously reviewed and the proposed labeling is acceptable. Information concerning the carcinogenic potential of the drug combination should be included in the product label once the 104-week rat study is submitted and reviewed.

Addendum/appendix listing: Not applicable

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VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

Study title: An oral (gavage) fertility and general reproduction toxicity study in rats with hydromorphone hydrochloride

Key study findings:

- Adverse clinical signs were observed at all doses
- Reduced body weight gain was noted at the two highest doses and reduced food consumption at the highest dose.
- No drug-related effects on mating or fertility parameters were observed.
- The NOAEL for paternal and maternal toxicity is
- The NOAEL for reproductive effects is.

Study no.: RR0857/REG/REPT

Volume #, and page #: 21, 1

Conducting laboratory and location: _____

Date of study initiation: February 2000

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, radiolabel, and % purity: buprenorphine/W08192, _____
naloxone/V04434/_____; Suboxone test substance reference: Y10150/003

Formulation/vehicle: mixed with rat diet

Methods:

Species/strain: Rat/Alpk:ApfSD (Wistar derived)

Doses employed: 0, 100, 500 or 2000 ppm suboxone (4:1 ratio of buprenorphine to naloxone; 10, 50, 200 mg/kg buprenorphine/day; 2.5, 12.5, 50 mg/kg naloxone/day).

Doses were selected based on results of a 13-week dietary toxicity study conducted with this strain of rat.

Route of administration: dietary

Study design: Male rats were administered the test article or vehicle beginning 28 days before mating (up to 14 days); Males given 100 or 500 ppm were sacrificed while those given 0 or 2000 ppm were given a test mating with stock females to clarify results obtained for pregnancy rate. Females were administered test article or vehicle once daily beginning 14 days before cohabitation and continuing through DG 7. Stock females were killed on day 13 of gestation.

Number/sex/group: 24/sex/group; 18/sex/group satellite animals

Parameters and endpoints evaluated:

Mortality: daily

Clinical signs: daily

Body weight: twice weekly pre-mating; males weighed weekly thereafter; females weighed days 1 and 7 of gestation.

Food consumption: monitored daily

Estrus cycling: examination of vaginal cytology/vaginal smears for 14 days prior to dosing initiation, for 14 days beginning with day after first administration and then until spermatozoa were observed in vaginal smear.

Gross necropsy:

Males: after cohabitation period; testes, epididymides, prostate with seminal vesicles weighed and retained.

Females: sacrificed on DG 13. Uterus and contents were assessed for number of corpora lutea in each ovary, live fetuses, early intra-uterine deaths, late intra-uterine deaths.

Female satellite animals killed day 7 of gestation and females, which failed to show positive evidence of mating were killed at end of 14 day mating period.

Male satellite animals were killed at end of study.

Results:

Achieved concentrations: Achieved buprenorphine concentrations ranged from -8 to +37% of the nominal concentrations. Achieved naloxone concentrations ranged from -3 to +39% of nominal concentration.

Buprenorphine: LD: — ppm; MD: — ppm; HD: — ppm
Naloxone: LD: — ppm; MD: — ppm; HD: — ppm

Suboxone dose received:

Males: 9.6, 48.3 and 189.4 mg/kg

Females: 9.1, 45.9, 181.9 mg/kg

Mortality: None

Clinical signs: After one-week treatment, some males in all drug dose groups and HD females showed aggressive behavior. Other observations included scabs on tail (from biting) and sores were seen in animals given Suboxone.

Observation	Males				Females			
	0	100	500	2000	0	100	500	2000
Aggressive	0	7	7	7	0	0	0	3
Coat stained	0	0	0	4	0	0	0	0
Dry sores one or more areas	0	2	0	4	0	1	1	1

Body weight:

Male: Mean body weight gain was reduced by 16, 16 and 18%, respectively, in the LD, MD and HD groups compared to control animals after 71 days of dosing. Body weights were significantly reduced by day 4 of dosing onward. After day 71, when HD males were housed with stock females for test mating, there was a marked loss of bodyweight in response to withdrawal from treatment (-17% day 78 and recovering to -9% by day 91).
Females: Body weight gain was reduced by 12% at the high dose following the 2-week pre-mating dosing period. The reduction was not statistically significant. No significant difference in body weight gain was noted through gestation day 7.

Food consumption: Food consumption was reduced in all male dose groups during the first 2 weeks of dosing (wk1 17-22%; wk 2 9-11%). In females, reduced consumption (14-19%) was observed at all doses during the first week of dosing but was comparable to control animals during the second week and through day 7 of gestation.

Toxicokinetics: Systemic exposure to both buprenorphine and naloxone increased with dose although the increase was sub-proportional from the MD to the HD. Exposure in females tended to be greater in males.

Dose (ppm)	Males			Females		
	100	500	2000	100	500	2000
Mean plasma concentration (ng/ml)						
Buprenorphine	5.1-7.8	23.5-56.7	69.4-137	5-9.5	21.2-61.6	57.7-149
Naloxone	<0.5-0.5	<0.5-1.4	0.7-4.4	<0.5	<0.5-3.2	1.2-3.1
AUC _{0-last} (ng.hr/ml)						
Buprenorphine	132	986	2425	150	759	2019
Naloxone	-	18.5	59.9	-	19.6	45.7

Terminal and necroscopic evaluations:

Males:

Gross: No definitive drug-related findings were observed.

Organ weight: A dose-dependent reduction in prostate weight was noted (10, 14, 26%, respectively) compared to control values. Of note, organ weights for LD and MD males were collected ~ 3 weeks before the control and HD males. Also, organ weights for males were collected ~ 3 weeks following withdrawal from treatment during which time the animals showed a marked loss of bodyweight.

Females:

Gross: No definitive drug-related findings were observed.

Mating and fertility:

Mean cycle length was longer in Suboxone treated females (4.27-5.21 days) vs 3.71 in control) although the response was not dose-related. Thus, the mean number of cycles was slightly lower in Suboxone treated groups versus controls (2.33-2.71 vs 2.96). As the expected cycle length for this strain of rat is 4-5 days, the increase duration is not though to be biologically significant. No significant effect on pre-coital interval was noted.

Pregnancy data: An increase in the number of non-pregnant dams was noted in the two highest dose groups. Also, 1 female in the 100 ppm group and 2 in the 500 ppm group had no live fetuses in utero, only intra-uterine deaths. Thus, there was a reduction in the number of females with live fetuses in utero on day 13 of gestation. The sponsor suggests that these findings may be due to loss of BW and food consumption in response to withdrawal from treatment, which occurred shortly after time of implantation such that all implants died without trace or in utero. However, body weight and food consumption changes in females occurred only early in the dosing period, were reversible and were of questionable significance.

Caesarean sectioning and litter parameters (corpora lutea, implantations, live fetuses, early and late resorptions, pre- and post-implantation loss) were comparable among the four dose groups.

Following the test mating of control and HD males with stock females, no effect on pre-coital interval was noted. Pre-implantation loss was increased in the females mated with males given 2000 ppm Suboxone compared with those mated with control males. Thus, the mean number of live fetuses was slightly reduced although not statistically significantly different from control group.

Dose (ppm)	Males				Females			
	0	100	500	2000	0	100	500	2000
Non-pregnant dams					2/24	3/24	6/24	7/24
Dams with totally resorbed fetuses					0	1	2	0
Dams with live fetuses in utero					22/24	20/24	16/24	17/24
Test mating:								
Non-pregnant dams					1/24			1/24
Dams with totally resorbed fetuses					0/24			1/24
Dams with live fetuses in utero					23/24			22/24
Mean # of live fetuses					12.8			11.3
Pre-implantation loss:								
Prop of implants affected					41/345			63/334
%					13.4			21.6

Summary of individual study findings: Administration of Suboxone resulted in adverse clinical signs at all doses, reduced body weight gain and food consumption in males at all doses. Transient decreases in food consumption were noted in females. No drug-related effects on mating parameters were observed. However, a dose-dependent reduction in pregnant females was observed. The NOAEL for paternal and maternal toxicity is less than 100 ppm mg/kg while the NOAEL for fertility effects is 100 ppm. The sponsor concluded that no fertility effects of Suboxone were noted at doses up to 2000 ppm as the reduction in the number of pregnant females was due to withdrawal symptoms and not the drug itself. However, the withdrawal symptoms described in females (reduced body weight gain and food consumption)

Reproductive and developmental toxicology summary: Reproductive toxicology studies performed with buprenorphine and with the combination were previously submitted and reviewed. In addition, the sponsor submitted a fertility study in rats with the drug combination that is reviewed above. Studies (fertility in rats, developmental studies in rats and rabbits, and pre- and post natal development in rats) demonstrated that buprenorphine alone had no effect on fertility at doses up to 80 mg/kg, and produced no teratogenic effects at doses up to 160 mg/kg in rats and 25 mg/kg in rabbits. However, drug-related findings included decreased fetal body weight, increased incidence of total litter losses postpartum, increased pup mortality, delayed occurrence of righting reflex and startle response, swelling of limbs and forepaws, and a non-significant increased incidence of skeletal anomalies. Studies with the drug combination demonstrated reduced female conception rates in a rat fertility study. No teratogenic effects were noted in rats and rabbits in drug combinations of 1:1 (oral, 250 mg/kg in rats, 40 mg/kg in rabbits) and 3:2 (intramuscular, 30:20 mg/kg in rats and rabbits). However, drug-related developmental effects included increased post-implantation losses and skeletal variations in rabbits.

Reproductive and developmental toxicology conclusions: Fertility effects were not observed with buprenorphine alone, although reduced female conception rates were noted with administration of the drug combination. No teratogenic effects were observed in rats or rabbits with either buprenorphine alone or with the drug combination. However, embryo-fetal toxicity and developmental effects were observed and included pre- and post-implantation losses, skeletal abnormalities in rats but not rabbits.

Labeling recommendations: The above noted findings should be included in the label with reference to dose and appropriate exposure comparisons. The Pregnancy Category should be a "C".

VIII. SPECIAL TOXICOLOGY STUDIES:

No studies have been performed.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions: NDAs 20-732 and 20-733 were considered to be approvable from a non-clinical viewpoint following the initial reviews of the original NDA submissions in 1998 and 1999, respectively. The sponsor is performing a study to evaluate the carcinogenic potential of Suboxone in rats. The in-life portion was completed in July 2002.

Buprenorphine hydrochloride exhibits a higher margin of safety than full agonists of opioid mu receptors. Buprenorphine has intrinsic subcutaneous and muscle irritating properties that appear to be concentration-related and are associated with secondary hematologic and serum chemistry changes. Significant adrenal changes were noted in dogs (increased weight, dilation of zona glomerulosa) and baboons (increased weight) although the physiologic significance is unknown. Chronic administration of buprenorphine also led to proliferation, fibrosis and hemosiderosis in the hepatobiliary system of dogs. Positive findings were noted in several genotoxicity assays and an increased incidence of testicular interstitial cell tumors was observed in rats. No effects on fertility and no teratogenic effects were noted, although some developmental effects were observed.

Studies with the combination of buprenorphine and naloxone included toxicity studies of up to 28 days duration with oral or parenteral administration. Drug related findings included deaths at high doses, decreased food consumption and body weight gain, increased reticulocytes and platelet counts. Negative findings were observed in the standard battery of genetic toxicology studies. A study to assess the carcinogenic potential of the drug combination has recently completed the in-life portion and will be reviewed once the study report is submitted. Reduced fertility was observed in rats although no teratogenic effects were noted in rats or rabbits. Findings included post-implantation losses, and increased skeletal variations.

General Toxicology Issues: A carcinogenicity study in rats has recently completed the in-life portion and will be submitted post-marketing.

Recommendations: This application is approvable from a non-clinical perspective with the inclusion of the proposed modifications to the product label described below.

Labeling with basis for findings: The following section describes the recommended edits to the sponsor's proposed label. Additions are highlighted by underlines and deletions are highlighted by cross-outs.

Carcinogenesis, Mutagenesis and Impairment of Fertility:

Carcinogenicity: Carcinogenicity data on SUBOXONE are not available. Carcinogenicity studies of buprenorphine ~~_____~~ were conducted in Sprague-Dawley rats and CD-1 mice. Buprenorphine ~~_____~~ was administered in the diet to rats at doses of 0.6, 5.5, and 56 mg/kg/day (estimated exposure was approximately 0.4, 3 and 35 times the recommended human daily sublingual dose of 16 mg on a mg/m² basis) for 27 months. Statistically significant dose-related increases in testicular interstitial (Leydig's) cell tumors occurred, according to the trend test adjusted for survival. Pair-wise comparison of the high dose against control failed to show statistical significance. ~~_____~~

~~_____~~ -In an 86-week study in CD-1 mice, buprenorphine ~~_____~~ was not carcinogenic at dietary doses ~~_____~~ up to 100 mg/kg/day; (estimated exposure was approximately ~~_____~~ 30 times the recommended human daily sublingual dose (of 16 mg) on a mg/m² basis).

Mutagenicity:

SUBOXONE: The 4:1 combination of buprenorphine ~~_____~~ and naloxone ~~_____~~ was not mutagenic in a bacterial mutation assay (Ames test) using four strains of *S. typhimurium* and two strains of *E. coli*. The combination was not clastogenic in an *in vitro* cytogenetic assay in human lymphocytes, or in an intravenous micronucleus test in the rat.

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

Impairment of Fertility:

SUBOXONE ~~_____~~ Dietary administration of SUBOXONE in the rat at dose levels ~~_____~~ of 500 ppm or greater (equivalent to approximately ~~_____~~ 47 mg/kg/day or greater; estimated exposure was ~~_____~~ approximately ~~_____~~ 28 times the recommended human daily sublingual dose of 16 mg on a mg/m² basis) produced a reduction in fertility demonstrated by reduced female conception rates. A dietary dose of 100 ppm (equivalent to approximately 10 mg/kg/day; estimated exposure was approximately 6 times the

recommended human daily sublingual dose of 16 mg on a mg/m^2 basis) had no adverse effect on fertility.

SUBUTEX: Reproduction studies of buprenorphine in rats demonstrated no evidence of impaired fertility at daily oral doses up to 80 $\text{mg}/\text{kg}/\text{day}$ (estimated exposure was approximately 50 times the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis) or up to 5 $\text{mg}/\text{kg}/\text{day}$ *im* or *sc* (estimated exposure was approximately 3 times the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis).

Pregnancy:

Pregnancy Category C:

Teratogenic effects:

SUBOXONE: Effects on embryo-fetal development were studied in Sprague-Dawley rats and Russian white rabbits following oral (1:1) and intramuscular (3:2) administration of mixtures of buprenorphine and naloxone. Following oral administration to rats and rabbits, no teratogenic effects were observed at doses up to 250 $\text{mg}/\text{kg}/\text{day}$ and 40 $\text{mg}/\text{kg}/\text{day}$, respectively (estimated exposure was approximately 150 times and 50 times, respectively, the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis). No definitive drug-related teratogenic effects were observed in rats and rabbits at intramuscular doses up to 30 $\text{mg}/\text{kg}/\text{day}$ (estimated exposure was approximately 20 times and 35 times, respectively, the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis). Acephalus was observed in one rabbit fetus from the low-dose group and omphalele was observed in two rabbit fetuses from the same litter in the mid-dose group; no findings were observed in fetuses from the high-dose group. Following oral administration to the rat, dose-related post-implantation losses, evidenced by increases in the numbers of early resorptions with consequent reductions in the numbers of fetuses, were observed at doses of 10 $\text{mg}/\text{kg}/\text{day}$ or greater (estimated exposure was approximately 6 times the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis). In the rabbit, increased post-implantation losses occurred at an oral dose of 40 $\text{mg}/\text{kg}/\text{day}$.

Following intramuscular administration in the rat and the rabbit, post-implantation losses, as evidenced by decreases in live fetuses and increases in resorptions, occurred at 30 $\text{mg}/\text{kg}/\text{day}$.

SUBUTEX: Buprenorphine was not teratogenic in rats or rabbits after *im* or *sc* doses up to 5 $\text{mg}/\text{kg}/\text{day}$ (estimated exposure was approximately 3 and 6 times, respectively, the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis), *iv* — doses up to 0.8 $\text{mg}/\text{kg}/\text{day}$ (estimated exposure was approximately 0.5 times and equal to, respectively, the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis), or oral doses up to 160 $\text{mg}/\text{kg}/\text{day}$ in rats (estimated exposure was approximately 95 times the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis) and 25 $\text{mg}/\text{kg}/\text{day}$ in rabbits (estimated exposure

was approximately ~~30~~ times the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis). Significant increases in skeletal abnormalities (e.g., extra thoracic vertebra or thoraco-lumbar ribs) were noted in rats after *sc* administration of 1 mg/kg/day and up (estimated exposure was approximately 0.6 times the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis), but were not observed at oral doses up to 160 mg/kg/day. Increases in skeletal abnormalities in rabbits after *im* administration of 5 mg/kg/day (estimated exposure was approximately 6 times the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis) or oral administration of 1 mg/kg/day or greater (estimated exposure was approximately equal to the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis) were not statistically significant

In rabbits, buprenorphine produced statistically significant pre-implantation losses ~~at oral doses of 1 mg/kg/day or greater~~ and post-implantation losses that were statistically significant ~~at iv doses of 0.2 mg/kg/day or greater~~ (estimated exposure was approximately 0.3 times the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis).

There are no adequate and well-controlled studies of SUBOXONE or SUBUTEX in pregnant women. SUBOXONE or SUBUTEX should only be used during pregnancy if the potential benefit justifies the potential risk to the fetus.

Non-teratogenic effects.

Dystocia was noted in pregnant rats treated *im* with buprenorphine 5 mg/kg/day (~~approximately 3 times the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis~~). Both fertility and peri- and postnatal development studies with buprenorphine in rats indicated increases in neonatal mortality after oral doses of 0.8 mg/kg/day and up (~~approximately 0.5 times the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis~~), after *im* doses of 0.5 mg/kg/day and up (approximately 0.3 times the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis), and after *sc* doses of 0.1 mg/kg/day and up (~~approximately 0.06 times the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis~~). Delays in the occurrence of righting reflex and startle response were noted in rat pups at an oral dose of 80 mg/kg/day (~~approximately 50 times the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis~~).

X. APPENDIX/ATTACHMENTS:

Other relevant materials (Studies not reviewed, appended consults, etc.): None.

Any compliance issues: None.

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/s/

Timothy McGovern
10/7/02 01:56:12 PM
PHARMACOLOGIST

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REVIEW AND EVALUATION OF TOXICOLOGY DATA
Division of Anesthetic, Critical Care, and Addiction Drug Products

Thomas Papoian, Ph.D.
Dec. 11, 2001

NDA # 20-732; #20-733
Submission # BZ (Supplement to NDAs)

SUBMISSION DATE: Nov. 20, 2001
CENTER RECEIPT DATE: Nov. 20, 2001
REVIEWER RECEIPT DATE: Nov. 27, 2001

SPONSOR: Reckitt Benckisser Pharmaceuticals
Richmond, VA

DRUGS: Subutex (buprenorphine; NDA #20-732)
Suboxone (buprenorphine/naloxone; NDA #20-733)

PROPOSED CLINICAL INDICATION: Treatment of —

ROUTE OF ADMINISTRATION: Sublingual

BACKGROUND:

On January 22, 2001, approvable letters were issued for Subutex and Suboxone asking the sponsor to provide safety qualification of each individual degradation product of buprenorphine and naloxone that occur at — or higher (Part 1c). This supplement to the NDA has addressed this issue by submitting toxicology studies consisting of a 28-day rat oral toxicity study and three genetic toxicity studies using — extracts of Suboxone (buprenorphine and naloxone at a 4:1 ratio) degraded under accelerated conditions (40° C/75% humidity for several months).

MATERIAL REVIEWED:

1. 28-day dietary toxicity study in rats (RC010107)
2. Bacterial mutation assay in *S. typhimurium* and *E. coli* (RC010110)
3. *In vitro* cytogenetic assay in human lymphocytes (RC010111)
4. Rat bone marrow micronucleus test (RC010112)

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REVIEW OF TOXICOLOGY STUDIES:

Preparation of Suboxone Extracts:

Extracts of Suboxone for all the toxicity studies performed below were prepared to test the safety of buprenorphine and naloxone when used at a 4:1 ratio. The main criteria used were that the extracts could be fed to rats without affecting palatability, and that they contained all the impurities in relatively the same proportions that would be found in Suboxone tablets after extended storage conditions.

Suboxone 8-mg tablets used in clinical trials (batch #06001/145) were degraded under accelerated conditions at 40° C at 75% humidity for several months (time not specified). Briefly,

A placebo extract was prepared using the same inactive ingredients. The extracts were analyzed by HPLC. The extracted material was enriched for buprenorphine and naloxone-containing impurities by 9-fold. The impurity analysis is shown in Table 1 (Sponsor's Table 2).

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1. 28-day dietary toxicity study in rats (Vol. #3 pp.1-345)

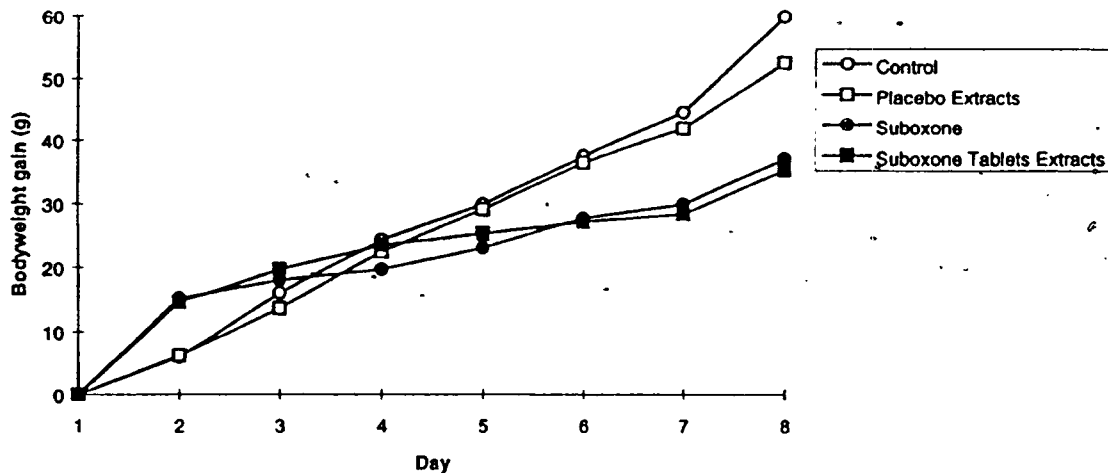
Testing Facility: _____
Study Number: KR1413
Study Date(s): Oct. 10, 2000 to Jan. 22, 2001
GLP Compliance: Yes
QA Report: Yes

Purpose: This study examined the toxicity of Suboxone tablet extracts in rats when given in the diet for 28 days. Results were compared to that seen with unextracted Suboxone.

Methods: Suboxone tablet extracts were prepared as described above. A preliminary palatability study in rats was performed. In that study (Study No. KR1412), male and female Alpk:AP₁SD rats (5/sex/group) were given either 0 (control diet), 0 (placebo extracts), Suboxone at 2000 ppm, or Suboxone tablet extracts at 2000 ppm in the diet for 7 days. Results showed peri-nasal staining in rats in both the Suboxone and Suboxone extract groups. Body weights in the Suboxone and Suboxone extract groups were decreased relative to normal controls, but there were no significant differences between the Suboxone and Suboxone extract groups (Figure 1A and 1B; Sponsor's Figure 1). No other significant toxicological effects were reported. It was concluded that since body weight decreases in the 2000 ppm Suboxone tablet extract group were similar to those seen with the 2000 ppm Suboxone group, diets containing 2000 ppm Suboxone tablet extracts were suitable for a 28 day rat oral toxicity study.

Figure 1A (Sponsor's Figure 1)

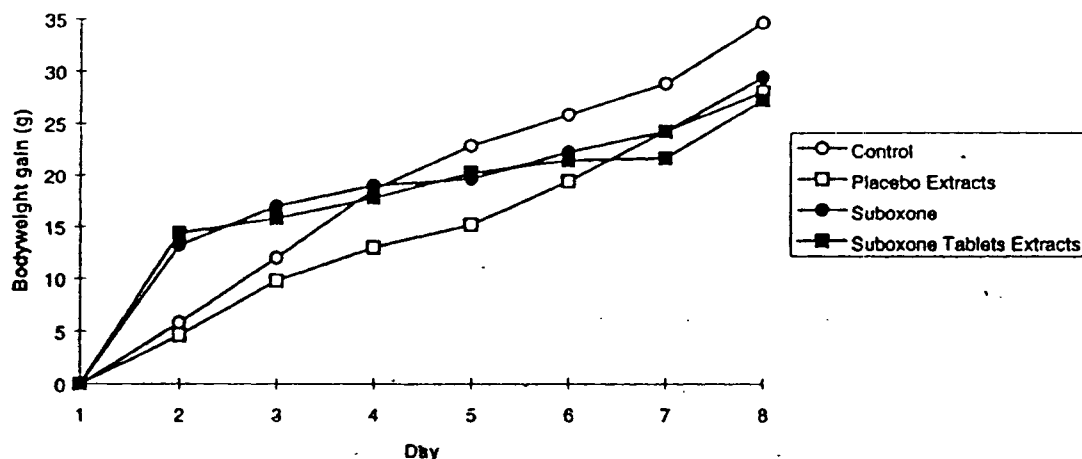
Group Mean Bodyweight Gain in Male Rats



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Figure 1B (Sponsor's Figure 1)

Group Mean Bodyweight Gain in Female Rats



Based on results of the preliminary palatability study, male and female Alpk:APSD rats (120-145 gms; 10/sex/group) were given either 0 (control diet), 0 (placebo extracts), Suboxone at 2000 ppm, or Suboxone tablet extracts at 2000 ppm in the diet for 28 days. Rats were observed twice daily for clinical signs, ophthalmoscopy was performed on the normal diet control and Suboxone tablet extract groups on Week 4, and body weights and food consumption were recorded weekly. On Day 29, blood was collected by cardiac puncture for hematology and clinical chemistry, and then rats were killed for *post mortem* examination. Weights of 14 organs were recorded, a gross exam conducted, and 47 tissues were collected. Tissues from the control diet and Suboxone tablet extract groups, as well as any abnormalities from other animals, were examined microscopically.

Results: Doses received were calculated and summarized in Table 2. As shown, there were no significant differences between the Suboxone and Suboxone tablet extract groups, or between males and females.

Table 2

Doses Received (mg/kg/day)

	Suboxone	Suboxone Tablet Extract
Males	198.3	197.4
Females	200.5	191.2

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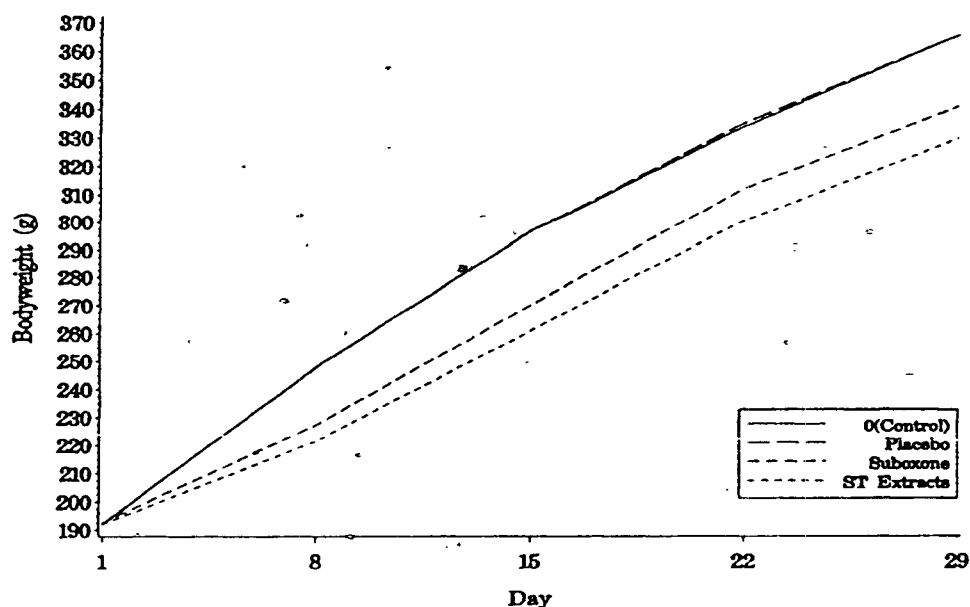
The only clinical signs noted in the drug-treated groups consisted of tail scabs and forelimb damage that was attributed to excess grooming due to decreased sensory perception, a known pharmacological effect of the opioid buprenorphine.

No ophthalmologic changes were noted in any of the drug-treated animals.

Body weight changes were similar to that seen in the preliminary palatability studies. Mean body weights on Day 29 were decreased in both the Suboxone (4-7%) and Suboxone tablet extract (8-10%) groups when compared to the placebo extract groups (Figures 2A and 2B; Sponsor's Figure 1).

Figure 2A (Sponsor's Figure 1)

Group Mean Body Weight (Males)

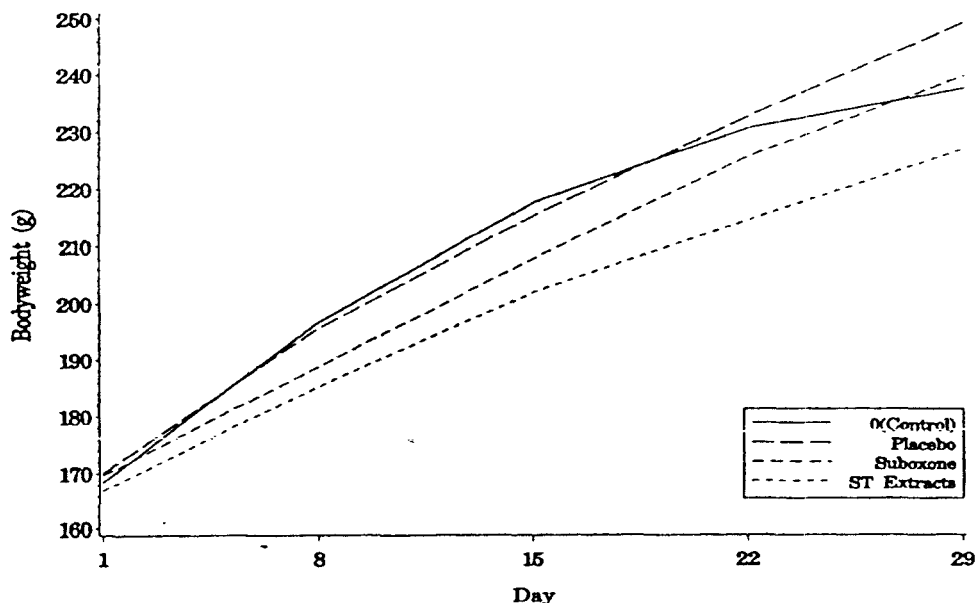


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Figure 2B (Sponsor's Figure 1)

Group Mean Body Weight (Females)



Food consumption was reduced 22% and 27% in female and male rats, respectively, during the first week of the study, but began approaching control intake by the fourth week.

Hematology results found small changes, including decreased activated partial thromoplastin time, in Suboxone tablet extract-treated females, but these decreases (9%) were relatively minor when compared to diet controls.

Clinical chemistry results found increased albumin (+7%) and total protein (+6%) in both Suboxone and Suboxone tablet extract-treated female animals. These changes have uncertain toxicological significance, but are easily monitorable, if found clinically.

No clear drug-related changes in mean organ weights were found.

Neither macroscopic nor microscopic examination of tissues revealed any drug-related changes. Minimal retinal degeneration was found in one eye of 2/10 male rats treated with Suboxone tablet extracts. This finding could be spontaneous in origin, and is difficult to attribute to drug treatment.

Conclusions: Rats treated with 2000 ppm Suboxone or Suboxone tablet extracts in their diets (approx. 200 mg/kg/day) had findings of minor drug-related effects. These included: tail scabs and forelimb damage that was attributed to excess grooming due to decreased sensory perception, a known pharmacological effect of the opioid buprenorphine; decreased body weights and food consumption; and minor hematological and clinical chemistry changes (slightly increased albumin and total protein). There were no microscopic findings that could be attributed to drug-treatment. Also, there did not appear to be any significant differences between

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2. Bacterial mutation assay in *S. typhimurium* and *E. coli*:

Testing Facility: _____

Study Number: — /YV5536

Study Date(s): Nov. 10, 2000 to Nov. 27, 2001

GLP Compliance: Yes

QA Report: Yes

Purpose: This test examined the ability of Suboxone tablet extracts and/or their metabolites to induce reverse mutations in the histidine locus in *Salmonella typhimurium* bacterial strains and at the tryptophan locus in *E. coli* with and without metabolic activation. This assay detects reverse mutations in the test strains that restore the functional capability of the bacteria to synthesize the essential amino acid histidine. The revertant or mutated bacteria are detected by their ability to grow in the absence of histidine required by the parent test strain.

Methods: *S. typhimurium* strains TA 1535, TA 1537, TA 98, and TA 100, and *E. coli* strains WP2P and WP2PuvrA were used. Suboxone tablet extracts were tested in a concentration range of 0.27 to 10.86 mg/plate (= 0.06 to 2.45 mg/plate Suboxone equivalent) [Note: According to ICH-S2A, bacterial mutagenicity studies should use concentrations of individual drug substances at up to 5 mg/plate. However, for qualification of impurities (ICH-Q3B), such studies can be conducted on the drug product or drug substance containing the degradation products to be controlled. Therefore, concentrations of up to 10 mg/plate for the Suboxone tablet extracts exceed the limit dose for this assay.]

Two incubation methods were used. The first method mixed bacteria, extract, \pm S9 with top agar, which were then plated onto agar plates (in triplicate), and cultures incubated at 37 C for 3 days. The second method premixed bacteria, extract, \pm S9, with preincubation at 37 C for 60 min to allow metabolites to form, then top agar was added, plated (in triplicate), and cultures incubated at 37 C for 3 days. Appropriate negative (DMSO vehicle and Suboxone Placebo — extracts) and positive controls were used. A positive response was defined as a reproducible 3X increase (strains TA 1535 and TA 1537) or a 2X increase (all other strains) in revertant colonies over control values in both experiments.

Results: With the exception of a 4.7X increase in revertants in strain TA 1535 at 5.4 mg/plate (-S9), there were no other increases in revertants that were greater than the 2-3X increase specified for a positive response (Table 4; Sponsor's Table I). The positive control cultures showed the expected increase in revertants.

Since this increase in TA 1535 did not occur in the second experiment and did not show a dose-related effect (the other concentrations were negative), the results were not reproducible, and therefore, not considered positive.

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Table 4 Sponsor's Table 1)

Suboxone Tablet Extracts in the Ames Test:
Results of Expt. 1 in TA 1535

COMPOUND Y10150/005 SUBOXONE TABLETS BATCH 06001/243 EX REKITT BENCKISER									
STUDY YV5536		TEST 00	SPONSOR	EXTERNAL SPONSOR			DATE TESTED 10/11/00		
DATE COUNTED 13/11/00									
COMMENTS									
COMPOUND PRECIPITATED ON 10.86/5.430mg DOSE PLATES:									
ALL 10.86mg, -S9 5.43mg PLATES SCORED BY EYE.									
BACKGROUND LAWN SPARSE ON -S9 10.86mg DOSE PLATES.									
STRAIN	DOSE LEVELS (MILLIGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	STATS SIGNIF.	NO REVERTANTS/PLATE			
						PLATE 1	PLATE 2	PLATE 3	
TA 1535 +S9	10.860	13.3	4.0	0.8		14	17	9	
	5.430	32.7	6.7	1.9	**	40	27	31	
	2.715	23.0	1.7	1.3	*	24	24	21	
	1.086	39.7	12.2	2.3	**	37	29	53	
	0.5430	35.7	10.8	2.1	**	48	31	28	
	0.2715	23.7	6.0	1.4		30	18	23	
TA 1535 -S9	10.860	6.7	3.8	0.5		5	4	11	
	5.430	66.0	20.0	4.7	**	56	89	53	
	2.715	17.3	15.7	1.2		12	5	35	
	1.086	32.0	17.4	2.3	*	40	44	12	
	0.5430	24.0	13.0	1.7		17	16	39	
	0.2715	12.7	4.0	0.9		15	8	15	

Conclusions: It was concluded that Suboxone tablet extracts at concentrations up to 10.86 mg/plate in either the presence or absence of metabolic activation (\pm S9) did not produce a positive response for mutagenicity in the Ames test when tested according to criteria outlined in ICH-Q3B.

3. In vitro cytogenetic assay in human lymphocytes:

Testing Facility: _____

Study Number: — /SV1062

Study Date(s): Oct. 31, 2000 to Feb. 7, 2001

GLP Compliance: Yes

QA Report: Yes

Purpose: This study examined the clastogenic potential of Suboxone tablet extracts using human peripheral blood lymphocytes.

Methods: Human blood was collected from 2-3 individuals and pooled. Lymphocyte cultures were prepared by the addition of 5% PHA and incubation at 37 C for 48 hours. Two separate experiments were performed. In Experiment 1, cells were exposed to Suboxone tablet extracts for 3 hours both with and without S9. In Experiment 2, cells were exposed for 3 hours with S9 and for 20 hours in the absence of S9. Concentrations of extracts tested are shown in the following table:

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Experiment 1		Experiment 2	
+S9-mix (3 hour treatment)	-S9-mix (3 hour treatment)	+S9-mix (3 hour treatment)	-S9-mix (20 hour treatment)
350µg/ml	260µg/ml	390µg/ml	90µg/ml
130µg/ml	130µg/ml	200µg/ml	40µg/ml
40µg/ml	40µg/ml	40µg/ml	10µg/ml

Control cultures were tested with Suboxone Placebo ————— Extracts as follows:

Experiment 1		Experiment 2	
+S9-mix (3 hour treatment)	-S9-mix (3 hour treatment)	+S9-mix (3 hour treatment)	-S9-mix (20 hour treatment)
290µg/ml	210µg/ml	320µg/ml	100µg/ml

Higher concentrations resulted in a reduced mitotic index (>50% cytotoxicity) sufficient to limit adequate cell counting (Tables 5A and 5B; Sponsor's Table 1 and 2). [Note: According to ICH-S2A, *in vitro* cytogenetic studies should use concentrations of individual drug substances at levels that produce an inhibition of the mitotic index by greater than 50%. However, for qualification of impurities (ICH-Q3B), such studies can be conducted on the drug product or drug substance containing the degradation products to be controlled that produce the same degree of cytotoxicity.]

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Table 5A (Sponsor's Table 1)

Mitotic Indices (without S9)

EXPERIMENT 1			EXPERIMENT 2		
Treatment	Mitotic Index %	Mean % Mitotic Index	Treatment	Mitotic Index %	Mean % Mitotic Index
Solvent Control (10µl/ml)	16.1 18.7	17.4	Solvent Control (10µl/ml)	13.0 11.3	12.2
Suboxone Tablet Extracts (µg/ml)			Suboxone Tablet Extracts (µg/ml)		
520	a a		260	a a	
480	a a		200	a a	
430	a a		130	2.1 7.6	4.9
390	a a		90	4.9 4.3	4.6
350	a a		40	15.1 13.2	14.2
260	8.5 7.9	8.2	20	13.5 10.8	12.2
200	14.5 13.9	14.2	10	12.8 15.0	13.9
130	13.8 16.7	15.3	5	b b	
90	b b				
40	19.3 18.6	19.0			
20	b b				
Suboxone Placebo Extracts(µg/ml)			Suboxone Placebo Extracts(µg/ml)		
210	19.9 20.9	20.4	100	18.9 19.1	19.0

a - Little or no mitosis observed

b - Mitotic index not required for selection of concentrations for chromosomal aberration analysis

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Table 5B (Sponsor's Table 2)

Mitotic Indices (with S9)

EXPERIMENT 1			EXPERIMENT 2		
Treatment	Mitotic Index %	Mean % Mitotic Index	Treatment	Mitotic Index %	Mean % Mitotic Index
Solvent Control (10µl/ml)	10.5 13.9	12.2	Solvent Control (10µl/ml)	15.3 14.9	15.1
Suboxone Tablet Extracts (µg/ml)			Suboxone Tablet Extracts (µg/ml)		
520	a a		390	8.7 6.6	7.7
480	a a		350	9.9 7.3	8.6
430	a a		260	b b	
390	a a		200	18.6 15.4	17.0
350	4.0 4.8	4.4	130	b b	
260	7.9 12.4	10.2	90	b b	
200	b c		40	13.9 16.5	15.2
130	8.8 9.9	9.4	20	b b	
90	b b				
40	9.9 10.7	10.3			
20	b b				
Suboxone Placebo —			Suboxone Placebo —		
Extracts(µg/ml)			Extracts(µg/ml)		
290	18.5 14.3	16.4	320	17.3 16.9	17.1

a - Little or no mitosis observed

b - Mitotic index not required for selection of concentrations for chromosomal aberration analysis

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Mitomycin C was used as the positive control. Two hours before the end of the indicated treatment times, duplicate cultures of cells were treated with colcemid to arrest cells in metaphase, and cells were fixed, stained with Giemsa stain, and examined microscopically for chromosomal aberrations. The mitotic index was determined by counting 1000 lymphocytes for the percentage of cells in metaphase. A positive response was defined as a statistically significant increase in the percentage of aberrant cells in at least one concentration and an increase that is substantially greater than lab historical solvent values.

Results: The percentage of cells with chromosomal aberrations, excluding gaps, are shown in Table 6A (no S9; Sponsor's Table 3) and Table 6B (plus S9; Sponsor's Table 4). As shown, cells treated with Suboxone tablet extracts at concentrations producing greater than 50% cytotoxicity, did not result in a significant increase in the percentage of cells with chromosomal aberrations when compared to either the solvent control or Placebo extracts. The positive control, mitomycin C, produced the expected increase in aberrations.

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Table 6A (Sponsor's Table 3)

Mean Chromosomal Aberrations and Mitotic Indices (No S9)

Treatment				Mean % Aberrant Cells Excluding Gaps	Mean % Mitotic Index
Experiment 1					
Solvent Control			10µl/ml	0.50	17.4
Suboxone Placebo	—	Extracts	210µg/ml	2.50	20.4
Mitomycin C			0.5µg/ml	40.00**	9.9Δ
Suboxone Tablet Extracts			260µg/ml	1.00	8.2
			130µg/ml	0.50	14.2
			40µg/ml	1.00	19.0
Experiment 2					
Solvent Control			10µl/ml	0.50	12.2
Suboxone Placebo	—	Extracts	100µg/ml	0.00	19.0
Mitomycin C			0.2µg/ml	36.00**	4.2Δ
Suboxone Tablet Extracts			90µg/ml	0.50	4.6
			40µg/ml	1.00	14.2
			10µg/ml	1.00	13.9

** Statistically significant increase in the percentage of aberrant cells at $p < 0.01$ using Fisher's Exact Test (one-sided).

Δ Positive control mitotic index and % aberrant cells are determined from a single culture.

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Table 6B (Sponsor's Table 4)

Mean Chromosomal Aberrations and Mitotic Indices (Plus S9)

Treatment		Mean % Aberrant Cells Excluding Gaps	Mean % Mitotic Index
Experiment 1			
Solvent Control	10µl/ml	0.50	12.2
Suboxone Placebo ——— Extracts	290µg/ml	1.50	16.3
Cyclophosphamide	50µg/ml	40.00**	7.9Δ
Suboxone Tablet Extracts	350µg/ml	1.00	4.4
	130µg/ml	1.00	9.4
	40µg/ml	1.00	10.3
Experiment 2			
Solvent Control	10µl/ml	0.50	15.1
Suboxone Placebo ——— Extracts	320µg/ml	1.00	17.1
Cyclophosphamide	50µg/ml	36.00**	11.1Δ
Suboxone Tablet Extracts	390µg/ml	1.00	7.7
	200µg/ml	1.00	17.0
	40µg/ml	1.00	15.2

** Statistically significant increase in the percentage of aberrant cells at $p < 0.01$ using Fisher's Exact Test (one-sided).

Δ Positive control mitotic index and % aberrant cells are determined from a single culture.

Conclusions: It was concluded that Suboxone tablet extracts, when tested at concentrations that produced greater than 50% cytotoxicity, did not produce evidence of clastogenicity in human peripheral lymphocytes either in the presence or absence of metabolic activation (\pm S9) when tested according to criteria outlined in ICH-Q3B.

4. Rat bone marrow micronucleus test:

Testing Facility: _____

Study Number: _____/SR1061

Study Date(s): Jan. 23, 2001 to Feb. 14, 2001

GLP Compliance: Yes

QA Report: Yes

Purpose: This *in vivo* study examined the clastogenic potential of Suboxone tablet extracts to induce micronuclei in bone marrow polychromatic erythrocytes of rats. Normally, when a bone marrow erythroblast matures into a polychromatic erythrocyte, the nucleus is completely extruded. However, if the animal is exposed to an agent that causes chromosomal damage (clastogenicity), a piece of chromosome (micronuclei) will remain in the erythrocyte cytoplasm after the nucleus is extruded. An increase in the frequency of micronucleated polychromatic erythrocytes is an index of drug-induced chromosomal damage or clastogenicity.

Methods: Male Alp:AP₁SD rats (5/group) were given a single oral dose of either:

- 10 ml/kg vehicle control (hydroxypropyl- β -cyclodextrin),
- 1600 mg/kg Suboxone Placebo extract,
- 20 mg/kg cyclophosphamide (positive control),
- 500-2000 mg/kg Suboxone tablet extract (2000 mg/kg was the OECD limit dose), or
- 460 mg/kg Suboxone.

[Note: According to OECD Guidelines, mammalian bone marrow chromosome aberration tests should use doses of drug substances up to the limit dose of 2000 mg/kg. However, for qualification of imputities (ICH-Q3B), such studies can be conducted on the drug product or drug substance containing the degradation products to be controlled.]

Animals were killed after 24 and 48 hours (Note: animals given cyclophosphamide and the two lower doses of Suboxone tablet extracts were sacrificed only after 24 hours), and bone marrow samples taken for preparation of smears. Slides were fixed, stained, and examined microscopically for polychromatic erythrocytes containing micronuclei. Evidence of cytotoxicity was assessed by counting the ratio of polychromatic to normochromatic erythrocytes. A positive response was defined as a statistically and biologically significant increase in micronucleated erythrocytes that is 3X greater than both historical and concurrent control incidences.

Results: Rats given Suboxone and Suboxone tablet extracts showed signs of altered behavior (agitation, excessive chewing, etc.) and other clinical signs consisting of diarrhea and wet urine stains.

An analysis of the percentage of micronucleated polychromatic erythrocytes did not reveal a significant increase in rats treated with either Suboxone or Suboxone tablet extracts (Table 7; Sponsor's Table 1). The positive control cyclophosphamide showed the expected increase. There was no evidence of bone marrow cytotoxicity that could account for the absence of findings (Table 8; Sponsor's Table 2).

Table 7 (Sponsor's Table 1)

Mean Incidence of Micronucleated Polychromatic Erythrocytes

Group	Treatment	Dose	Mean incidence of MPE/1000 PE \pm SD	
			24 hours	48 hours
11	Vehicle Control	10ml/kg	1.6 \pm 0.74	1.3 \pm 0.91
12	Suboxone Placebo Extracts	1600mg/kg	1.4 \pm 0.55	1.7 \pm 0.45
13	Cyclophosphamide	20mg/kg	29.7 \pm 10.80**	
14	Suboxone Tablet Extracts	2000mg/kg	2.6 \pm 0.65	0.9 \pm 0.96
15	Suboxone Tablet Extracts	1000mg/kg	1.2 \pm 0.91	
16	Suboxone Tablet Extracts	500mg/kg	2.2 \pm 1.15	
17	Suboxone	460.5mg/kg	1.4 \pm 0.82	0.7 \pm 0.45

PE = polychromatic erythrocytes.

MPE = micronucleated polychromatic erythrocytes.

SD = standard deviation.

** Statistically significant increase in micronucleated polychromatic erythrocytes at $p < 0.01$ in the Student's t-test (one-sided) on transformed data.

All means based on 5 counts of 2000 PE per animal.

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Table 8 (Sponsor's Table 2)

Mean Percentage of Polychromatic Erythrocytes

Group	Treatment	Dose	Mean % of Polychromatic Erythrocytes \pm SD	
			24 hours	48 hours
11	Vehicle Control	10ml/kg	39.1 \pm 8.5	45.0 \pm 4.9
12	Suboxone Placebo Extracts	1600mg/kg	45.1 \pm 8.7	40.2 \pm 9.8
13	Cyclophosphamide	20mg/kg	41.4 \pm 9.7	
14	Suboxone Tablet Extracts	2000mg/kg	40.7 \pm 6.5	46.2 \pm 3.7
15	Suboxone Tablet Extracts	1000mg/kg	41.7 \pm 8.0	
16	Suboxone Tablet Extracts	500mg/kg	42.4 \pm 7.5	
17	Suboxone	460.5mg/kg	35.5 \pm 7.6	41.4 \pm 6.1

SD = standard deviation.

All means based on 5 counts of 1000 erythrocytes per animal.

Conclusions: It was concluded that Suboxone tablet extracts when given orally to rats at the OECD limit dose did not produce evidence of clastogenicity (micronuclei) in bone marrow when tested according to criteria outlined in ICH-Q3B.

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OVERALL SUMMARY AND EVALUATION:

Approvable letters were issued for Subutex and Suboxone in Jan. 2001, asking the sponsor to provide safety qualification of each individual degradation product of buprenorphine and naloxone that occur at _____ or higher (Part 1c). In response, the Sponsor submitted a 28-day oral rat toxicity study and three genetic toxicity studies using ethanol extracts of Suboxone (buprenorphine and naloxone at a 4:1 ratio) degraded under accelerated conditions.

In the 28-day oral rat study, rats were fed either Suboxone or Suboxone tablet extracts in their diets at concentrations of 2000 ppm (= approx. 200 mg/kg/day). Clinical signs consisted of tail scabs and forelimb damage, an effect that was attributed to the pharmacological action of buprenorphine on reducing sensory perception. Body weights were decreased in both the Suboxone (4-7%) and Suboxone tablet extract (8-10%) groups when compared to the placebo extract groups. Food consumption was also reduced (22-27%) during the first week of the study, but began approaching control intake by the fourth week. Clinical chemistry results found increased albumin (+7%) and total protein (+6%) in both Suboxone and Suboxone tablet extract-treated female animals. These changes have uncertain toxicological significance, but are easily monitorable, if found clinically. There were no microscopic findings that could be attributed to drug-treatment. Also, there did not appear to be any significant differences between the Suboxone and Suboxone tablet extract-treated groups, indicating that a 9-fold concentration of degradants/impurities did not result in any additional toxicities from that found with Suboxone alone.

Based on the dietary doses achieved in this rat study, the individual impurities/degradants of Suboxone can be considered qualified up to the maximum limits proposed by the Sponsor (see Table 3). _____ can be considered qualified based or

Further qualification of Suboxone tablet extracts was performed for genetic toxicity potential in the standard battery. Results from the bacterial mutation assay (Ames test), *in vitro* cytogenetic assay in human lymphocytes, and the rat bone marrow micronucleus test were negative, indicating that Suboxone tablet extracts were not mutagenic or clastogenic when tested according to criteria outlined in ICH-Q3B.

Therefore, based on the qualification studies submitted, the proposed limits listed in Table 3 for the specified Suboxone impurities found in buprenorphine and naloxone at 4:1 are acceptable.


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NDA#s #20-732; 20-733

RECOMMENDATIONS:

Internal:

Part 1c ("Provide safety qualification of each individual degradation product of buprenorphine and naloxone that occur at  or higher") of the approvable letters dated January 22, 2001, for Subutex (NDA #20-732) and Suboxone (NDA #20-733) have been satisfactorily addressed. The proposed limits listed in Table 3 above for the specified Suboxone impurities found in buprenorphine and naloxone at 4:1 are acceptable.

Thomas Papoian, Ph.D.
Pharmacologist

Timothy McGovern, Ph.D.
Supervisory Pharmacologist

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NDAs #20-732; 20-733

REFERENCES:

Brewster D. *et al.*, Biliary excretion, metabolism and enterohepatic circulation of buprenorphine, *Xenobiotica* 1981; 11:189-196.

Ohtani M. *et al.*, Pharmacokinetic analysis of enterohepatic circulation of buprenorphine and its active metabolite, norbuprenorphine, in rats, *Drug Metabolism and Disposition* 1994; 22:2-7.

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this page is the manifestation of the electronic signature.**

/s/

Thomas Papoian
12/11/01 11:19:54 AM
PHARMACOLOGIST
Toxicology Review
Tim, Suggested changes were made. Tom

Timothy McGovern
12/11/01 01:28:08 PM
PHARMACOLOGIST
I concur.

**APPEARS THIS WAY
ON ORIGINAL**

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA

Keywords: Suboxone, buprenorphine, naloxone, rat carcinogenicity protocol, unaudited report

Reviewer: M. Anwar Goheer

Division: Division of Anesthetic, Critical Care and Addiction Drug Products.

HFD #: 170

Review Completed: March 20, 2000

NDA: 20-733

Submission: NDA Supplement Dated: Feb. 29, 2000

Received by HFD-170: March 1, 2000

Received by Reviewer: March 2, 2000

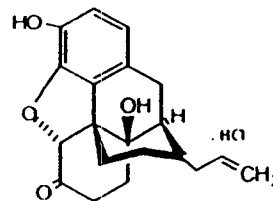
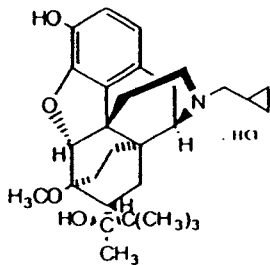
Information to Sponsor: Yes

Sponsor: Reckitt & Colman Pharmaceuticals Inc., 1901 Huguenot Road, Richmond, VA 23235.

Drug: Suboxone [sublingual tablets containing buprenorphine HCl and naloxone HCl dihydrate at a 4:1 buprenorphine : naloxone as free bases]

Formula weights: 504.09 [buprenorphine HCl] and 399.87 [naloxone HCl.2H₂O]

Structures:



CAS Registry Number: 53152-21-9 [buprenorphine HCl] and 51481-60-8 [naloxone HCl]

Related INDs/NDAs/DMFs: IND 45,220 / IND 58,653 / NDA 20-732 / DMF _____

Drug Class: Buprenorphine: Opioid analgesic [partial *mu* agonist]

Naloxone: Opioid antagonist

Indication: For the treatment of opioid dependence.

Route: Sublingual

Dose [MRHD]: Buprenorphine HCl/naloxone HCl, 24/6 mg/day
[0.4/0.1 mg/kg/day for a 60-kg person]

Introduction and Drug History: An injectable formulation of buprenorphine hydrochloride [Buprenex®] was approved by the FDA in 1982 for the treatment of moderate to severe pain at 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 naloxone [Temgesic®-NX] is marketed in New Zealand. Higher-dose buprenorphine sublingual tablets containing 0.4, 2.0, 8.0 mg for treating opiate dependence have been marketed in France since