CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-410**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**February 4, 2009**
PRODUCT:	**Suboxone (b) (4) (buprenorphine and naloxone)**
INTENDED CLINICAL POPULATION:	**Treatment of opioid dependence**
APPLICANT:	**Reckitt Benckiser Pharmaceuticals, Inc.**
DOCUMENTS REVIEWED:	**All nonclinical information in the above submission**
REVIEW DIVISION:	**Division of Analgesia, Anesthesia and Rheumatology Products (HFD-170)**
PHARM/TOX REVIEWER:	**Elizabeth A. Bolan, Ph.D.**
PHARM/TOX SUPERVISOR:	**R. Daniel Mellon, Ph.D.**
DIVISION DIRECTOR:	**Bob Rappaport, M.D.**
PROJECT MANAGER:	**Matthew Sullivan**

Date of review submission to Division File System (DFS): May 22, 2009

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

This NDA can be approved from a nonclinical pharmacology/toxicology perspective.

B. Recommendation for nonclinical studies

There are no recommendations for nonclinical studies.

C. Recommendations on labeling

The table below contains the draft labeling submitted by the Applicant, the proposed changes and the rationale for the proposed changes. For the final version of the label, please refer to the Action Letter. Note: The recommended changes from the proposed labeling are in red or strikeout font.

Applicant's proposed labeling	Reviewer's proposed changes	Rationale for changes
8 USE IN SPECIFIC POPULATIONS **8.1 Pregnancy** Pregnancy Category C.	**8 USE IN SPECIFIC POPULATIONS** **8.1 Pregnancy** Pregnancy Category C.	no changes to this section.
	(b) (4)	
Teratogenic effects: Effects on embryo-fetal development were studied in Sprague-Dawley rats and Russian white rabbits following oral (1:1) and intramuscular (IM) (3:2) administration of mixtures of buprenorphine and naloxone. Following oral administration to rats and rabbits, no teratogenic effects were observed at buprenorphine doses up to 250 mg/kg/day and 40 mg/kg/day, respectively (estimated exposure approximately 150 times and 50 times, respectively, the recommended human daily sublingual dose of 16 mg on a mg/m² basis). No definitive drug-related teratogenic effects were observed in rats and rabbits at IM doses up to 30 mg/kg/day (estimated	***Teratogenic effects:*** Effects on embryo-fetal development were studied in Sprague-Dawley rats and Russian white rabbits following oral (1:1) and intramuscular (IM) (3:2) administration of mixtures of buprenorphine and naloxone. Following oral administration to rats and rabbits, no teratogenic effects were observed at buprenorphine doses up to 250 mg/kg/day and 40 mg/kg/day, respectively (estimated exposure approximately 150 times and 50 times, respectively, the recommended human daily sublingual dose of 16 mg on a mg/m² basis). No definitive drug-related teratogenic effects were observed in rats and rabbits at IM doses up to 30 mg/kg/day (estimated	

exposure approximately 20 times and 35 times, respectively, the recommended human daily dose of 16 mg on a mg/m² basis). Acephalus was observed in one rabbit fetus from the low-dose group and omphacele 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 of buprenorphine to rats, 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 mg/kg/day or greater (estimated exposure approximately 6 times the recommended human daily sublingual dose of 16 mg on a mg/m² basis). In the rabbit, increased post implantation losses occurred at an oral dose of 40 mg/kg/day. Following IM administration in the rat and the rabbit, post-implantation losses, as evidenced by decreases in live fetuses and increases in resorptions, occurred at 30 mg/kg/day. 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 intravenous (IV) doses of 0.2 mg/kg/day or greater (estimated exposure approximately 0.3 times the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis). ***Non-teratogenic effects:*** Dystocia was noted in pregnant rats treated intramuscularly with buprenorphine 5 mg/kg/day (approximately 3 times the recommended human daily sublingual dose of 16 mg on a mg/m² basis). (b) (4) fertility, peri-, and post-natal 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/m² 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/m² basis), and after subcutaneous doses of 0.1	exposure approximately 20 times and 35 times, respectively, the recommended human daily dose of 16 mg on a mg/m² basis). Acephalus was observed in one rabbit fetus from the low-dose group and omphacele 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 of buprenorphine to rats, 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 mg/kg/day or greater (estimated exposure approximately 6 times the recommended human daily sublingual dose of 16 mg on a mg/m² basis). In the rabbit, increased post implantation losses occurred at an oral dose of 40 mg/kg/day. Following IM administration in the rat and the rabbit, post-implantation losses, as evidenced by decreases in live fetuses and increases in resorptions, occurred at 30 mg/kg/day. 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 intravenous (IV) doses of 0.2 mg/kg/day or greater (estimated exposure approximately 0.3 times the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis). ***Non-teratogenic effects:*** Dystocia was noted in pregnant rats treated intramuscularly with buprenorphine 5 mg/kg/day (approximately 3 times the recommended human daily sublingual dose of 16 mg on a mg/m² basis). (b) (4) fertility, peri-, and post-natal 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/m² 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/m² basis), and after subcutaneous 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/m² 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/m² basis).	mg/kg/day and up (approximately 0.06 times the recommended human daily sublingual dose of 16 mg on a mg/m² 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/m² basis).	
13 NONCLINICAL TOXICOLOGY **13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility** ***Carcinogenicity:*** Carcinogenicity data on SUBOXONE (b) (4) are not available. (b) (4)	**13 NONCLINICAL TOXICOLOGY** **13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility** ***Carcinogenicity:*** Carcinogenicity data on SUBOXONE (b) (4) are not available. A carcinogenicity study of buprenorphine/naloxone (4:1 ratio of the free bases) was performed in Alderley Park rats. Buprenorphine/naloxone was administered in the diet at doses of approximately 7, 31, and 123 mg/kg/day for 104 weeks (estimated exposure was approximately (b) (4) 4, 18 and 44 times the (b) (4) recommended human sublingual dose of (b) (4) 16/4 mg buprenorphine/naloxone based on buprenorphine AUC comparisons (b) (4) A statistically significant increase in Leydig cell adenomas was observed in all dose groups. (b) (4) No other drug-related ~~increases in~~ tumors were noted. 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/m^2 basis) for 27 months. As in the buprenorphine/naloxone carcinogenicity study in rat, statistically significant dose-	Alderley is misspelled AUC comparisons were added

(b) (4) 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/m^2 basis).	related increases in (b) (4) Leydig cell tumors occurred. (b) (4) 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/m^2 basis).	
Mutagenicity: 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.	***Mutagenicity:*** 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. 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 (*S. 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 [^{3}H]thymidine, and positive in unscheduled DNA synthesis (UDS) test using testicular cells from mice.	Buprenorphine mutagenicity data from the Suboxone/Subutex label were added in because they include additional positive findings
Impairment of Fertility: Dietary administration of buprenorphine in the rat at dose levels of 500 ppm or greater (equivalent to approximately 47 mg/kg/day or greater; estimated exposure approximately 28 times the recommended	***Impairment of Fertility:*** Dietary administration of buprenorphine in the rat at dose levels of 500 ppm or greater (equivalent to approximately 47 mg/kg/day or greater; estimated exposure approximately 28 times the recommended	

human daily sublingual dose of 16 mg on a mg/m^2 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 approximately 6 times the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis) had no adverse effect on fertility.	human daily sublingual dose of 16 mg on a mg/m^2 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 approximately 6 times the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis) had no adverse effect on fertility.	

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

The majority of the nonclinical data relied upon in NDA 22-410 for Suboxone (b) (4) is found in NDAs 20-732 (Subutex) and 20-733 (Suboxone).

The naloxone (NLX) drug substance contains (b) (4), an impurity with a structural alert for mutagenicity. As a post approval commitment for Suboxone (NDA 20-733), the Division requested adequate qualification of (b) (4) In studies submitted to this NDA, (b) (4) was not mutagenic in the Ames test but was found to be clastogenic in an *in vitro* cytogenetic assay in human lymphocytes. Because of the positive finding for clastogenicity, the levels of (b) (4) in the drug substance should be reduced to the currently acceptable threshold for known genotoxic impurities of NMT 1.5 mcg/day. The specification set by the Applicant for (b) (4) would result in levels NMT (b) (4) mcg/day when Suboxone (b) (4) is used as labeled, and are therefore acceptable.

The Applicant has conducted an *in vitro* study assessing the interaction of buprenorphine (BUP) and its metabolite norbuprenorphine (nor-BUP) with several cytochrome P450s in human liver and in cDNA expressed microsomes. At micromolar levels, BUP inhibited CYP2D6 and CYP3A and nor-BUP inhibited CYP2D6. However, plasma concentrations of BUP in the therapeutic range are unlikely to cause clinically significant inhibition of CYP2D6 or CYP3A in patients. The Applicant also demonstrated that BUP and nor-BUP do not bind to either central or peripheral benzodiazepine receptors. The current label for Subutex and Suboxone as well as reports in the literature (Ibrahim RB, et al., 2000b; Megarbane B, et al., 2006; Megarbane B, et al., 2005c) state that there is a pharmacodynamic interaction between BUP and benzodiazepines. Although the mechanism for this interaction remains unknown, in light of data submitted by the Applicant it is most likely not due to PK interactions or direct action of BUP or nor-BUP on central or peripheral benzodiazepine receptors.

A 2-year carcinogenicity study with Suboxone was conducted in the rat using doses yielding human exposure margins of 4, 18 and 44 times the human sublingual dose of 16/4 mg/mg BUP/NLX based on BUP AUC values. Treatment-related unilateral benign Leydig cell (testes) adenomas were observed at the high dose and bilateral benign Leydig cell (testes) adenomas were observed at all doses. These neoplasms are considered

treatment-related will be described in the product label. No other treatment-related neoplasms were observed in males and no treatment-related neoplasms were observed in females. This study confirms the findings of Leydig cell tumors that were seen in a prior carcinogenicity assessment in rats conducted with BUP alone for the Subutex NDA. The findings of Leydig cell tumors from the BUP study as well as negative findings from a mouse carcinogenicity study with BUP are described in the current Suboxone/Subutex label.

The results from the Suboxone carcinogenicity study as well as the BUP rat and mouse studies will be included in the Suboxone (b) (4) label. It is recommended that the Suboxone/Subutex label be updated to include results from the Suboxone carcinogenicity study.

B. Pharmacologic activity

Buprenorphine is a synthetic opioid agonist that is 10-20 times more potent than morphine with a very long duration of action. It acts as a partial mu opioid receptor agonist and a kappa opioid receptor antagonist. Naloxone is a nonspecific opioid receptor antagonist. At low doses BUP produces sufficient agonist effect to enable opioid-addicted individuals to discontinue the misuse of opioids without experiencing withdrawal symptoms. The NLX component of the formulation serves to attempt to prevent abuse of the product. Naloxone is rapidly metabolized via the oral and sublingual routes resulting in low bioavailability, however, with parenteral administration, as in an abuse situation, the NLX is bioavailable to block the effects of BUP.

C. Nonclinical safety issues relevant to clinical use

The Suboxone carcinogenicity assessment in rat submitted with this NDA confirms the findings of Leydig cell tumors that were seen in a carcinogenicity assessment in rats conducted with BUP for the Subutex NDA. The findings of Leydig cell tumors from the BUP study are described in the current Suboxone/Subutex label. The findings of Leydig cell tumors from the Suboxone carcinogenicity study as well as the BUP study will be included in the Suboxone (b) (4) label. The relevance of these findings to clinical use of Suboxone (b) (4) is unknown. No new clinical safety issues with Suboxone (b) (4) as compared to the currently marketed Suboxone/Subutex products have arisen.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-410
Review number: 1
Sequence number/date/type of submission: 000/February 4, 2009/original submission
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Reckitt Benckiser Pharmaceuticals, Inc. Richmond, VA
Manufacturer for drug substance: Buprenorphine HCl: Reckitt Benckiser Healthcare (UK) Limited, Hull UK; Naloxone HCl: (b) (4) and (b) (4)

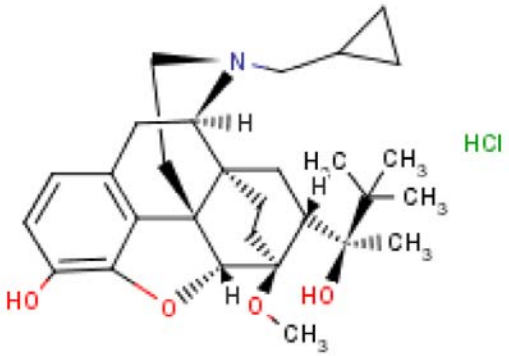
Reviewer name: Elizabeth A. Bolan, Ph.D.
Division name: Division of Anesthesia, Analgesia, and Rheumatology Products
HFD #: 170
Review completion date: May 14, 2009

Drug:

Trade name: Suboxone (b) (4)
Generic name: Buprenorphine HCl and Naloxone HCl
Code name: NA

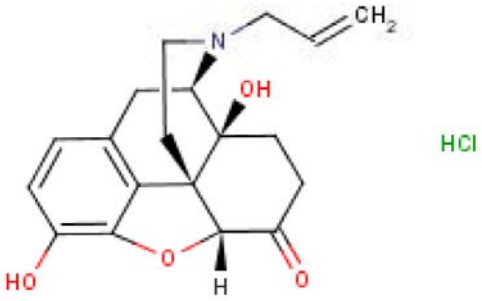
Buprenorphine hydrochloride

Chemical name: (2S)-2-[17-Cyclopropylmethyl-4,5α-epoxy-3-hydroxy-6-methoxy-6α,14-ethano-14α-morphinan-7α-yl]-3,3-dimethylbutan-2-ol hydrochloride
CAS registry number: 53152-21-9
Molecular formula/molecular weight: $C_{29}H_{41}NO_4$ HCl MW=504.1
Structure:



Naloxone hydrochloride

Chemical name: 4,5α-Epoxy-3,14-dihydroxy-17-(prop-2-enyl)morphinan-6-one hydrochloride
CAS registry number: 357-08-4
Molecular formula/molecular weight: $C_{19}H_{21}NO_4$ HCl $2H_2O$ MW=399.9
Structure:



Relevant INDs/NDAs/DMFs:

IND/NDA/MF	*drug/compound*	*Sponsor*	*Division*	*status*
IND 75,811	Suboxone (b) (4)	Reckitt Benckiser	DAARP	active
NDA 20-722	Subutex	Reckitt Benckiser	DAARP	approved 10/8/02
NDA 20-733	Suboxone	Reckitt Benckiser	DAARP	approved 10/8/02
MF 12412	buprenorphine	Reckitt Benckiser	NA	reviewed by CMC
MF (b) (4)	naloxone	(b) (4)	NA	reviewed by CMC
MF	naloxone		NA	reviewed by CMC

Drug class: Buprenorphine is a partial mu opioid receptor agonist and a kappa opioid receptor antagonist. Naloxone is a nonspecific opioid receptor antagonist.

Intended clinical population: Suboxone (b) (4) is indicated for treatment of opioid abuse.

Clinical formulation: The Suboxone (b) (4) drug product is a soluble sublingual film strip containing a fixed ratio of 4:1 buprenorphine: naloxone. The product will be available in 8/2 and 2/0.5 buprenorphine/naloxone strengths. The high strength (8/2) and low strength (2/0.5) strips utilize slightly different formulations as outlined in Table 1. All excipients can be found in approved drug products at equal or greater levels and therefore do not pose any unique toxicological concerns.

Excipients

Table 1. Quantitative Formula for Suboxone (b) (4) High and Low Strengths (reproduced from NDA)

	High Strength Suboxone (b) (4) (8 mg buprenorphine /2 mg naloxone) soluble film		Low Strength Suboxone (b) (4) (2 mg buprenorphine /0.5 mg naloxone) soluble film	
Component	**Composition % (w/w)**	**Quantity/strip (mg)**	**Composition % (w/w)**	**Quantity/strip (mg)**
Buprenorphine HCl	17.28	8.64	5.40	2.16
Naloxone HCl Dihydrate	4.88	2.44	1.53	0.61
Acesulfame Potassium	(b) (4)			
Citric Acid, (b) (4)				
(b) (4)				
Polyethylene Oxide (b) (4)				
Polyethylene Oxide (b) (4)				
Polyethylene Oxide (b) (4)				
Sodium Citrate, (b) (4)				
(b) (4)				
(b) (4) Lime Flavor (b) (4)				
FD&C Yellow No. 6				
White Ink				
Total	100.0	50.0	100.0	40.0

(b) (4)

Impurities in the drug substances

The qualification threshold according to the ICH Q3A(R2) guideline for impurities in the drug substances for a MDD of BUP or NLX of ≤ 2 g/day is 0.15% or 1 mg/day intake, whichever is lower. The identification threshold as per ICH Q3A(R2) guideline for impurities in the drug substances for a MDD of BUP or NLX of ≤ 2 g/day is 0.1% or 1

mg/day intake, whichever is lower. The Applicant has set the specifications for impurities in the buprenorphine drug substance obtained from Reckitt Benckiser (MF 12412) at NMT (b) (4) (Table 2) and no further identification or qualification will be necessary. The Applicant has set the specifications for impurities in the naloxone drug substances obtained from (b) (4) and (b) (4) below the thresholds for identification or qualification (unless otherwise noted, see Table 3) and no further qualification will be necessary. Specific impurities are discussed below. The specifications for the buprenorphine drug substance and two naloxone drug substances are acceptable from a pharmacology/toxicology perspective.

Table 2	***Specifications of buprenorphine hydrochloride drug substance impurities from Reckitt Benckiser***		
Impurity		***Specification limit***	***Acceptable?***
(b) (4)		NMT (b) (4)	YES
		NMT	YES
		NMT	YES
		NMT	YES
		NMT	YES

Table 3	***Specifications of naloxone hydrochloride drug substance impurities from*** (b) (4)			
Impurity		***Specification limit***		***Acceptable?***
		(b) (4)	(b) (4)	
(b) (4)		(b) (4) max	(b) (4) max	YES
		max	max	YES
		max	max	YES
		max	max	YES
		max	max	YES
		max	max	YES
		max	max	YES

(b) (4)

The naloxone drug substance from both suppliers contains (b) (4) , an impurity with a structural alert for mutagenicity. As a post approval commitment for Suboxone (NDA 20-733; see approval letter), the Division requested adequate qualification of (b) (4) by either demonstrating that it is a significant metabolite or by genotoxicity testing (one point mutation assay and one cytogenetic assay with the impurity tested up to the limit dose for each assay). The Division also stated that if (b) (4) is determined to be genotoxic, it must be limited via in-process controls or by drug substance acceptance criteria to (b) (4) For this NDA, the Applicant has submitted two genetic toxicology studies with (b) (4) was not mutagenic in the Ames test ((b) (4) YV62423) but was found to be clastogenic in an *in vitro* cytogenetic assay in human lymphocytes ((b) (4) SV1200). The current acceptable

threshold for known genotoxic impurities is NMT 1.5 mcg/day. The Applicant has set the specification of (b) (4) at (b) (4). At (b) (4) for a total daily dose of 8 mg of NLX, the total daily intake would be (b) (4) mcg of the impurity. The specification of (b) (4) for (b) (4) in the drug substance is acceptable.

(b) (4)

The Applicant has limited the naloxone impurity (b) (4) (also referred to as (b) (4)) to (b) (4) in the drug substances obtained from both (b) (4) and (b) (4). Although this level is above ICH Q3A(R2) guidelines, the Applicant has previously conducted a safety evaluation which qualifies the compound to a level of (b) (4). For the Suboxone NDA (NDA 20-733), the applicant had conducted a 3-month dietary general toxicology study with the impurity as well as a carcinogenicity assessment with Suboxone using a batch of naloxone containing the impurity (b) (4). These studies were reviewed by Dr. Timothy McGovern (NDA 20-733; Supplement review dated October 7, 2002). Dr. McGovern determined that the (b) (4) was qualified up to a level of (b) (4). The specification of (b) (4) for (b) (4) in the naloxone drug substances is considered acceptable.

Impurities in the drug product

The Suboxone (b) (4) drug product contains the same impurity/degradant profile as Suboxone SL tablets (NDA 20-733) (b) (4)

The qualification threshold according to the ICH Q3B(R2) guidelines for impurities/degradants in the drug product for a maximum daily dose (MDD) between 10 mg and 100 mg of BUP administered per day is 0.5% or 200 mcg TDI, whichever is lower. The Applicant has set the stability specifications for BUP-derived impurities/degradation products at levels which exceed this threshold: however, the four of the five impurities have been previously qualified for the Applicant's Suboxone NDA (NDA 20-733; Table 4). The Applicant conducted a 28-day dietary toxicology study as well as *in vitro* and *in vivo* genetic toxicology studies with ethanol extracts of Suboxone which had been degraded under accelerated conditions. These studies were reviewed and found to be acceptable by Dr. Thomas Papoian (NDA 20-733, Supplement review dated December 11, 2001). For the Suboxone (b) (4) product, the levels of the four impurities assayed in the submitted studies are below qualified levels (Table 4). The impurity (b) (4) is unique to the Suboxone (b) (4) product. The levels are below ICH Q3B(R2) guidelines for qualification for the high strength (8/2 mg/mg BUP/NLX) dose. However, the Applicant has set different impurity specifications for two of the impurities in the high strength (8/2 mg/mg BUP/NLX) and low strength (2/0.5 mg/mg BUP/NLX) dosages (Table 4). The specification for (b) (4) is set at (b) (4) for the high strength and (b) (4) for the low strength. The specification for the low strength exceeds ICH Q3B(R2) thresholds. The Applicant makes the argument that the low strength strips are unlikely to be utilized to achieve doses greater than the 8 mg

BUP dose of the high strength strips. I agree with this justification. The maximum daily dose for the low dose product would be < 10 mg so the limit of (b) (4) or (b) (4) mcg TDI, whichever is lower, would therefore not be exceeded by the specification of (b) (4). The specifications for the BUP-derived impurities in the drug product are considered acceptable.

The MDD of the NLX portion of the Suboxone (b) (4) product is < 10 mg/day, therefore the qualification threshold according to the ICH Q3B(R2) guidelines for impurities/degradants is 1.0% or 50 mcg TDI, whichever is lower. Several of the specifications for NLX-derived impurities/degradants exceed this threshold; however, those impurities have been previously qualified in the studies reviewed by Dr. Thomas Papioan mentioned above for the Applicant's Suboxone NDA (NDA 20-733). Three novel NLX-derived impurities occur in the drug product but specifications are set below (b) (4) and will not require qualification. The novel NLX-derived impurity, (b) (4) has a slightly higher specification in the low strength product but the higher specification does not exceed ICH thresholds for qualification. The specifications for the NLX-derived impurities in the drug product are considered acceptable.

Table 4	***Specifications of Suboxone (b) (4) drug product impurities/degradants***		
Source	***Impurity/degradant***	***Stability specification limit***	***Acceptable?***
buprenorphine	(b) (4)	NMT (b) (4)	YES
		NMT	YES
		NMT	YES
		NMT	YES
		NMT (b) (4)	YES
naloxone		NMT (b) (4)	YES
		NMT	YES
		NMT	YES
		NMT	YES
		NMT	YES
		NMT	YES
		NMT	YES
		NMT (b) (4)	YES
		NMT (b) (4)	YES
		NMT (b) (4)	YES

* impurity has been qualified (see Review by Dr. Thomas Papoian)
** impurity unique to Suboxone (b) (4) drug product
NOTE: Parentheses denote specifications for the low strength product when different from the high strength product

Route of administration: sublingual

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Data reliance: Except as specifically identified below, all data and information discussed below and necessary for approval of 22-410 are owned by Reckitt Benckiser or are data for which Reckitt Benckiser has obtained a written right of reference. Any information or data necessary for approval of 22-410 that Reckitt Benckiser does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Reckitt Benckiser does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 22-410.

Studies reviewed within this submission:

Study Title	***Study Number***
Pharmacology	
(b) (4) Screening Report: Buprenorphine and Norbuprenorphine Binding to Benzodiazapine Receptors	T/O 98-4005
Interaction of Buprenorphine and its Metabolite Norbuprenorphine with the Cytochrome P450s *in vitro*	RC010159
Toxicology	
Suboxone: Two-Year Dietary Oncogenicity Study in Rats	(b) (4) PR1175
Bacterial Mutation Assay in *S. typhimurium* and *E. coli* with (b) (4)	(b) (4) YV62423
(b) (4): *In vitro* Cytogenetic Assay in Human Lymphocytes	(b) (4) SV1200

Studies not reviewed within this submission:

(b) (4) The Applicant owns both Suboxone and Subutex. These studies have already been reviewed (refer to reviews by Dr. David Brase) and the reviews are in DFS.

Note: For NDA reviews, all section headings should be included.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary
Buprenorphine (BUP) was approved in injectable form (Buprenex®, NDA 18-401) for the treatment of moderate to severe pain in 1982. Buprenorphine as a single entity (Subutex; NDA 20-732) and the 4:1 fixed dose BUP/naloxone combination (Suboxone, NDA 20-733) were approved in 2002 as sublingual tablets for the treatment of opioid abuse. The current NDA for Suboxone (b) (4) (NDA 22-410) describes a reformulation of Suboxone SL tablet into a soluble sublingual film strip formulation.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: Buprenorphine is a synthetic opioid agonist that is 10-20 times more potent than morphine with a very long duration of action. It acts as a partial mu opioid receptor agonist and a kappa opioid receptor antagonist. Naloxone is a nonspecific opioid receptor antagonist.

Drug activity related to proposed indication: At low doses BUP produces sufficient agonist effect to enable opioid-addicted individuals to discontinue the misuse of opioids without experiencing withdrawal symptoms. The NLX component of the formulation serves to attempt to prevent abuse of the product. Naloxone is rapidly metabolized via the oral and sublingual routes resulting in low bioavailability, however, with parenteral administration, as in an abuse situation, the NLX is bioavailable to block the effects of BUP.

2.6.2.3 Secondary pharmacodynamics
For a detailed review of the secondary pharmacodynamics of BUP and NLX please refer to the Pharmacology/Toxicology reviews of Suboxone (NDA 20-733; September 30, 1999) and Subutex (NDA 20-732; January 12, 1998) by Dr. David Brase. The Applicant submitted one new secondary pharmacodynamics study in the current NDA. The study is discussed below.

Study title: (b) (4) Screening Report: Buprenorphine and Norbuprenorphine Binding to Benzodiazapine Receptors

Key study findings: Neither buprenorphine or its major metabolite norbuprenorphine show appreciable binding at central (GABA A) or peripheral benzodiazepine receptors in an *in vitro* assay.

Study no.: T/O 98-4005
Volume #, and page #: eCTD 000 4.2.1.1.1
Conducting laboratory and location: (b) (4)
Date of study initiation: December 7, 1998

Methods
Observations and times
The objective of this *in vitro* study was to assess the binding affinities of buprenorphine and the major metabolite, norbuprenorphine, at central (GABA A) and peripheral

benzodiazepine receptors. The test articles were assayed for receptor binding at concentrations between 10 pM and 100 μM. No appreciable binding was observed at either receptor for buprenorphine or norbuprenorphine up to the highest concentration tested.

(b) (4) has specified a set of criteria to interpret the results of their assays. The baseline range is between -20% to 20% inhibition. A compound would be considered to be a negative inhibitor if it shows inhibition greater than -20%. Compounds that show inhibition in the range of 20% to 49% are considered marginally active. An active compound is one that shows inhibition greater than 50% inhibition and displays a dose-dependent relationship.

Results, Discussions and Conclusions
The test articles were assayed for receptor binding at concentrations between 10 pM and 100 μM. No appreciable binding was observed at the central or peripheral benzodiazepine receptors for either buprenorphine or norbuprenorphine up to the highest concentration tested. The Applicant notes that these studies suggest that clinical CNS depressant effects reported following concomitant use of benzodiazepines and buprenorphine are not due to interactions at the receptor level.

2.6.2.4 Safety pharmacology
No new safety pharmacology studies were submitted by the Applicant. Please refer to the Pharmacology/Toxicology reviews of Suboxone (NDA 20-733; September 30, 1999) and Subutex (NDA 20-732; January 12, 1998) by Dr. David Brase for a discussion the safety pharmacology of BUP and NLX.

2.6.2.5 Pharmacodynamic drug interactions
No new pharmacodynamic interaction studies were submitted by the Applicant. Please refer to the Pharmacology/Toxicology reviews of Suboxone (NDA 20-733; September 30, 1999) and Subutex (NDA 20-732; January 12, 1998) by Dr. David Brase for a discussion of PD interactions between BUP and NLX, and BUP as well as the BUP/NLX combination with other drugs.

2.6.3 PHARMACOLOGY TABULATED SUMMARY
Not applicable

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary
No new PK/TK studies were submitted by the Applicant. Please refer to the Pharmacology/Toxicology reviews of Suboxone (NDA 20-733; September 30, 1999) and Subutex (NDA 20-732; January 12, 1998) by Dr. David Brase for a discussion the PK/TK of BUP and NLX.

2.6.4.2 Methods of Analysis
Not applicable

2.6.4.3 Absorption
See above

2.6.4.4 Distribution
See above

2.6.4.5 Metabolism
See above

2.6.4.6 Excretion
See above

2.6.4.7 Pharmacokinetic drug interactions
The human hepatic cytochrome P450s (CYPs) CYP1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1, and 3A4 are the major forms of CYPs in humans (Shimada T, et al., 1994). In human, the major metabolic pathway of BUP is via N-dealkylation to nor-BUP. The potential for BUP and nor-BUP to inhibit the major forms of CYP enzymes in order to identify potential drug-drug interactions was evaluated by the Applicant.

The Applicant has conducted an in vitro study assessing the interaction of BUP and its metabolite nor-BUP with several cytochrome P450s in human liver and in cDNA expressed microsomes (Study RC010159). The study was conducted in December of 1999 by Edward M. Sellers, M.D., Ph. D. who is a professor at Sunnybrook and Women's College Health Sciences Centre- Women's College Campus in Toronto, Canada. The study was not conducted under GLP and does not appear to have any quality control but otherwise appears to be adequate.

This study demonstrates that BUP and nor-BUP would not be predicted to interact with drugs metabolized by CYP1A2, 2A6, 2B6, 2C9, 2C19, or 2E1. Buprenorphine and nor-BUP were shown to be competitive inhibitors of CYP2D6 and BUP was shown to be a competitive inhibitor of CYP3A4. Micromolar concentrations of BUP (20 and 200 μM) and nor-BUP (200 μM only) inhibited CYP2D6 mediated O-demethylation of dextromethorphan. Although BUP inhibits CYP2D6, it is a poor substrate for the enzyme. The K_m for CYP2D6 for metabolism of BUP is in the millimolar range which is 1,000-fold above the steady state concentration of BUP when used at therapeutic doses. It is unlikely that therapeutic doses of BUP will interact with CYP2D6.

BUP is mainly metabolized by CYP3A4 to nor-BUP (Kobayashi K, et al., 1998). In this study BUP inhibited CYP3A4-mediated sulfoxidation of omeprazole.

The steady state trough plasma concentrations of BUP and nor-BUP in the range of 8-24 mg BUP/day are far below the K_i of the CYP2D6 and CYP3A4. It is concluded that at therapeutic doses of BUP inhibition of CYP2D6 or CYP3A4 is not likely to occur.

2.6.4.8 Other Pharmacokinetic Studies
No other PK studies were submitted with this application.

2.6.4.9 Discussion and Conclusions

According to Ibrahim et al., twenty deaths had been reported in patients who have ingested BUP in combination with benzodiazepines (Ibrahim RB, et al., 2000a). The current label for Subutex and Suboxone states that there may be an interaction between BUP and benzodiazepines and that there have been a number of post-marketing anecdotal reports of coma and death associated with intravenous misuse of BUP and benzodiazepines by addicts. Since BUP and many benzodiazepines are CYP3A substrates a pharmacokinetic interaction may be involved. The Applicant has provided *in vitro* data (Study RC010159) that demonstrate BUP is metabolized by CYP3A4. However, the steady state trough plasma concentrations of BUP in the therapeutic range are far below the K_i of CYP3A4. It is concluded that the inhibition of benzodiazepine metabolism via CYP3A4 inhibition is not likely to occur (refer to Clinical Pharmacology review by Dr. Sheetal Agarwal for further discussion). A report in the literature also describes the inhibition of CYP3A by BUP and notes that the IC_{50} for BUP is roughly 2000 times higher than the plasma concentration of BUP. According to the conclusion of this report, in the therapeutic range BUP is unlikely to cause clinically significant inhibition of CYP3A in patients (Ibrahim RB, et al., 2000).

The Applicant has conducted a study to assess binding affinities of BUP and its major metabolite nor-BUP to central and peripheral benzodiazepine receptors (Study T/O 98-4005). No appreciable binding was observed for either BUP or nor-BUP up to the highest concentration tested.

The current label for Subutex and Suboxone as well as reports in the literature (Ibrahim RB, et al., 2000;Megarbane B, et al., 2006;Megarbane B, et al., 2005) state that there is a pharmacodynamic interaction between BUP and benzodiazepines. Although the mechanism for this interaction remains unknown, in light of data submitted by the Applicant it is most likely not due to PK interactions or direct action of BUP or nor-BUP on central or peripheral benzodiazepine receptors.

2.6.4.10 Tables and figures to include comparative TK summary

Not applicable

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Not applicable

2.6.6 TOXICOLOGY

With the exception of a carcinogenicity assessment with Suboxone and genetic toxicology studies with a the NLX impurity (b) (4), no new toxicology studies were submitted by the Applicant. Please refer to the Pharmacology/Toxicology reviews of Suboxone (NDA 20-733; September 30, 1999) and Subutex (NDA 20-732; January 12, 1998) by Dr. David Brase for a discussion the toxicology data for BUP and NLX.

2.6.6.1 Overall toxicology summary

General toxicology: No new studies were conducted.

Genetic toxicology: The genotoxic potential of the structural alert-containing NLX impurity (b) (4) was evaluated in the *in vitro* Bacterial Reverse Mutation Assay (Ames Test) and an *in vitro* Chromosome Aberration Assay using human lymphocytes. (b) (4) was found to be negative in the Ames Test but was positive in the *in vitro* Chromosome Aberration Assay in both the presence and absence of metabolic activation. These findings suggest that (b) (4) is not mutagenic but is clastogenic and levels should be reduced to NMT (b) (4) mcg/day. The Applicant has set a specification for this impurity in the drug product of (b) (4) which yields levels below (b) (4) mcg/day.

Carcinogenicity: In a 2-year rat bioassay with Suboxone, unilateral benign Leydig cell (testes) adenomas reached statistical significance at the HD and bilateral benign Leydig cell (testes) adenomas reached statistical significance at all doses. The trend analysis reached statistical significance for both unilateral and bilateral Leydig cell adenomas. All doses for both unilateral and bilateral adenomas showed increased incidence over historical controls averaged over the past five years for unilateral and bilateral Leydig cell tumors combined. These neoplasms are considered treatment-related. No other treatment-related neoplasms were observed in males and no treatment-related neoplasms were observed in females.

Reproductive toxicology: No new studies were conducted.

Special toxicology: No new studies were conducted.

2.6.6.2 Single-dose toxicity

No new studies were conducted.

2.6.6.3 Repeat-dose toxicity

No new studies were conducted.

2.6.6.4 Genetic toxicology

No new genetic toxicology studies for BUP, NLX or the BUP/NLX combination were submitted with this NDA. Genetic toxicology data for Suboxone appears in the current version of the label. The studies have been previously reviewed by Dr. David Brase (see NDA 20-733 review dated September 30, 1999 and NDA 20-732 review dated January 12, 1998) by Dr. David Brase. (b) (4) is an impurity present in the NLX component of Suboxone which contains a structural alert for mutagenicity. As a Phase 4 commitment with NDA 20-733, the Applicant was asked to evaluate the potential for mutagenicity and clastogenicity of this compound. The Applicant conducted an Ames test and an *in vitro* Chromosome Aberration test with (b) (4) The studies are reviewed below.

Study title: (b) (4): Bacterial Mutation Assay in *S. typhimurium* and *E. coli*

Key findings: (b) (4) is not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, and TA 1537 and *E. coli* strains WP2P and WP2P *uvr*A in both the presence and absence of S9.

Study no.: (b) (4)/YV6423
Volume #, and page #: Module 4.2.3.7.6.1 (Study Report RC030408)
Conducting laboratory and location: (b) (4)
Date of study initiation: July 8, 2003
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: (b) (4) Batch reference # B4990P179; purity: >95%

Methods
The Applicant evaluated (b) (4) in a bacterial mutagenicity assay based on the method of Maron and Ames with modifications in accordance with procedures outlined in the OECD guideline 471 (Maron and Ames, 1983). Six concentrations of test article as well as water vehicle and positive controls were plated in triplicate with overnight cultures of *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 (Ames, et al., 1975) and *Escherichia coli* strains WP2P and WP2 *uvr*A (Venitt S and Crofton-Sleigh C, 1979) on selective minimal agar in the presence and absence of S9 prepared from phenobarbital/β-napthoflavone-induced rat liver using the plate incorporation method. The positive controls utilized were appropriate for each tester strain and metabolic activation condition. Five-hundred µL of S9 or sham mix, 100 µL of tester strain and 100 µL of vehicle, test article dilution or positive control were added to melted selective top agar, vortexed and overlaid onto the surface of 25 mL Vogel-Bonner minimal medium (Vogel and Bonner, 1956). After solidification of the overlay, plates were inverted and incubated for approximately 72 hours at 37°C. Following examination for contamination, revertant colonies for a given tester strain and activation condition were counted by an automated colony counter (Cardinal® automated counter linked to the Ames Study Manager system, Perceptive Instruments).

The Applicant states that test data from individual experiments are considered valid if the concurrent solvent control data are acceptable and the positive control data show acceptable increases. The Applicant does not state what would be considered "acceptable".

The reviewer's criteria for a valid assay are as follows. The mean of each positive control must exhibit at least a 3-fold increase in the number of revertants as compared to vehicle. A minimum of three non-toxic dose levels is also required to evaluate the assay data. Toxicity is described as a ≥50% reduction in the mean number of revertants per plate as compared to vehicle accompanied by an abrupt dose-dependent drop in the

revertant count and/or a moderate reduction in the background lawn. The study satisfies these criteria and will be considered valid.

According to the Applicant's criteria, a positive response would be indicated by a statistically significant dose-related increase in the mean revertants observed and a ≥2-fold increase in mean number of revertant colonies at one or more concentrations.

Strains/species/cell line: *S. typhimurium* histidine auxotrophs utilized included: TA98, TA100, TA1535 and TA1537. *E. coli* tryptophan auxotroph utilized: WP2 (pKM101) and WP2 *uvr*A.

Doses used in definitive study: The doses used for TA98, TA1535 and TA1537, WP2 and WP2 *uvr*A in the presence of S9 are: 20, 50, 100, 200, 500 and 1000 mcg/plate. The doses used for TA100 in the presence of S9 are: 10, 20, 50, 100, 200, and 500 mcg/plate. The doses used for TA98, TA100, TA1535 and TA1537 in the absence of S9 are: 10, 20, 50, 100, 200, and 500 mcg/plate. The doses used for WP2 and WP2 *uvr*A in the absence of S9 are: 20, 50, 100, 200, 500 and 1000 mcg/plate. All doses are given in free base equivalents. Water was used as the vehicle for all conditions.

Basis of dose selection: The initial assay tested concentrations over a range of 100 mcg to 5000 mcg (free base equivalent) per plate of the test article in water vehicle ±S9 using the plate incorporation method. Due to the observed toxicity, the test article was re-tested over a range of 10 to 1000 mcg ±S9 with the plate incorporation method. The test article was also tested over a range of 10 to 500 mcg +S9 with the pre-incubation method. Toxicity, as evidenced by bacterial lawn clearing, was observed at concentrations down to 500 mcg/plate in at least one experiment with each strain and at ≥1000 mcg/plate in all cases. No precipitate was observed at any concentration.

Negative controls: A negative control was not used in this study.

Positive controls: The positive controls utilized for the respective strains are indicated in Table 1.

Incubation and sampling times: Plates were incubated for 72 hours at 37°C.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The study is valid. Three separate studies were conducted with each strain. Suitable numbers of replicate plates and appropriate counting methods were utilized. The positive controls demonstrated clear increases in tester strain revertants while the vehicle control was within historical range for the tester strains for this vehicle.

Study outcome: It is concluded that under conditions of the assays conducted, (b) (4) is not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, and TA 1537 and *E. coli* strains WP2P and WP2P *uvr*A in both the presence and absence of S9. The results of the confirmative assay are summarized in Table 2 (+S9) and Table 3 (-S9) below (tables

reproduced from NDA). The data in the table indicate negative mutagenic responses in the presence and absence of exogenous metabolic activation with S9.

Table 1. Positive controls utilized in the various strains		
Strain	***S9***	***Positive Control***
all Salmonella strains and WP2P	+	2-Aminoanthracene
WP2P *uvr*A	+	Benzo (a) pyrene
TA98	-	Daunomycin HCl
WP2P *uvr*A	-	N-Ethyl-N'-nitro-N-nitrosoguanidine
WP2P	-	Mitomycin C
TA1537	-	Acridine mutagen ICR 191
TA1535 and TA100	-	Sodium azide

Table 2. Data from the definitive study: Plate incorporation method in the presence of S9.

Study Name: YV6423 (b) (4)
Experiment: YV6423 Phase 2

Study Code: YV6423
Date Plated: 25/07/2003
Counted: 28/07/2003

With S9-mix

Strain	Compound	Dose level per plate: Actual test substance (free base equivalent)	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	(b) (4)	650µg (500µg)	*39.7*	*11.8*	*0.3*	26 S, 47 S, 46 S
		260µg (200µg)	*104.7*	*11.5*	*0.9*	93, 116, 105
		130µg (100µg)	*130.7*	*7.6*	*1.1*	136, 134, 122
		65µg (50µg)	*134.0*	*23.1*	*1.1*	136, 156, 110
		26µg (20µg)	*104.0*	*7.2*	*0.9*	110, 96, 106
		13µg (10µg)	*123.3*	*16.5*	*1.0*	140, 107, 123
	Water		*121.6*	*14.7*		110, 111, 129, 114, 144
TA1535	(b) (4)	1299µg (1000µg)	*0.0*	*0.0*	*0.0*	0 A, 0 A, 0 A
		650µg (500µg)	*0.0*	*0.0*	*0.0*	0 S, 0 S, 0 S
		260µg (200µg)	*28.7*	*3.1*	*1.6*	26, 28, 32
		130µg (100µg)	*24.0*	*7.9*	*1.4*	21, 33, 18
		65µg (50µg)	*19.3*	*2.3*	*1.1*	18, 22, 18
		26µg (20µg)	*25.3*	*2.3*	*1.5*	28, 24, 24
	Water		*17.4*	*3.9*		21, 18, 12, 21, 15
TA1537	(b) (4)	1299µg (1000µg)	*0.0*	*0.0*	*0.0*	0 A, 0 A, 0 A
		650µg (500µg)	*1.3*	*0.6*	*0.1*	1 S, 2 S, 1 S
		260µg (200µg)	*7.0*	*1.7*	*0.5*	6, 9, 6
		130µg (100µg)	*9.3*	*3.5*	*0.7*	6, 9, 13
		65µg (50µg)	*4.3*	*2.5*	*0.3*	4, 2, 7
		26µg (20µg)	*11.7*	*4.6*	*0.9*	9, 9, 17
	Water		*12.8*	*3.2*		11, 15, 12, 17, 9
TA98	(b) (4)	1299µg (1000µg)	*0.0*	*0.0*	*0.0*	0 A, 0 A, 0 A
		650µg (500µg)	*1.0*	*1.0*	*0.0*	1 A, 2 A, 0 A
		260µg (200µg)	*18.7*	*4.9*	*0.6*	21, 13, 22
		130µg (100µg)	*30.0*	*6.9*	*1.0*	22, 34, 34
		65µg (50µg)	*28.3*	*9.5*	*1.0*	28, 19, 38
		26µg (20µg)	*18.7*	*8.3*	*0.6*	12, 16, 28
	Water		*28.8*	*3.7*		34, 24, 27, 29, 30
WP2 (pKM101)	(b) (4)	1299µg (1000µg)	*11.3*	*5.5*	*0.1*	11 S, 17 S, 6 S
		650µg (500µg)	*47.7*	*9.3*	*0.5*	40, 58, 45
		260µg (200µg)	*79.0*	*6.2*	*0.9*	86, 77, 74
		130µg (100µg)	*94.3*	*12.0*	*1.1*	82, 106, 95
		65µg (50µg)	*107.7*	*2.5*	*1.2*	105, 108, 110
		26µg (20µg)	*66.0*	*3.0*	*0.7*	66, 63, 69
	Water		*88.4*	*12.4*		71, 88, 85, 105, 93

Key to Plate Postfix Codes

S	Sparse/Incomplete lawn
A	Lawn absent

Study Name: YV6423 (b) (4)
Experiment: YV6423 Phase 2

Study Code: YV6423
Date Plated: 25/07/2003
Counted: 28/07/2003

With S9-mix

Strain	Compound	Dose level per plate: Actual test substance (free base equivalent)	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 uvrA (pKM101)	(b) (4)	1299µg (1000µg)	*60.0*	*11.4*	*0.3*	52 S, 73 S, 55 S
		650µg (500µg)	*122.7*	*10.0*	*0.7*	122, 113, 133
		260µg (200µg)	*117.7*	*5.1*	*0.6*	119, 122, 112
		130µg (100µg)	*166.3*	*9.2*	*0.9*	161, 161, 177
		65µg (50µg)	*203.0*	*12.3*	*1.1*	198, 217, 194
		26µg (20µg)	*172.7*	*23.6*	*0.9*	146, 191, 181
	Water		*187.8*	*49.2*		160, 150, 168, 189, 272
TA100	**2AA**	1µg/plate	*447.7*	*40.5*	*3.7*	401, 474, 468
TA1535	**2AA**	2µg/plate	*101.0*	*13.2*	*5.8*	116, 91, 96
TA1537	**2AA**	2µg/plate	*121.0*	*8.0*	*9.5*	129, 121, 113
TA98	**2AA**	1µg/plate	*844.0*	*123.8*	*29.3*	759, 986, 787
WP2 (pKM101)	**2AA**	20µg/plate	*257.7*	*40.0*	*2.9*	298, 257, 218
WP2 uvrA (pKM101)	**BP**	5µg/plate	*897.3*	*154.0*	*4.8*	742, 900, 1050

Key to Positive Controls

2AA 2-Aminoanthracene
BP Benzo[a]pyrene

Key to Plate Postfix Codes

S Sparse/Incomplete lawn

Table 3. Data from the definitive study: Plate incorporation method in the absence of S9.

Study Name: YV6423: (b) (4)
Experiment: YV6423 Phase 2

Study Code: YV6423
Date Plated: 25/07/2003
Counted: 28/07/2003

Without S9-mix

Strain	Compound	Dose level per plate: Actual test substance (free base equivalent)	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	(b) (4)	650µg (500µg)	*11.3*	*1.5*	*0.1*	11 A, 13 A, 10 A
		260µg (200µg)	*86.2*	*9.6*	*0.7*	76, 95, 88
		130µg (100µg)	*112.7*	*3.8*	*0.9*	117, 111, 110
		65µg (50µg)	*113.0*	*8.9*	*0.9*	123, 106, 110
		26µg (20µg)	*103.3*	*7.0*	*0.8*	101, 93, 107
		13µg (10µg)	*92.0*	*9.2*	*0.8*	90, 102, 84
	Water		*121.2*	*17.9*		99, 118, 122, 149, 118
TA1535	(b) (4)	650µg (500µg)	*0.0*	*0.0*	*0.0*	0 A, 0 A, 0 A
		260µg (200µg)	*5.0*	*1.0*	*0.3*	4 S, 6 S, 5 S
		130µg (100µg)	*15.7*	*5.5*	*0.9*	16, 21, 10
		65µg (50µg)	*21.7*	*5.0*	*1.2*	21, 27, 17
		26µg (20µg)	*23.7*	*6.0*	*1.3*	23, 30, 18
		13µg (10µg)	*3.0*	*1.7*	*0.2*	2, 2, 5
	Water		*18.0*	*4.1*		23, 21, 18, 13, 15
TA1537	(b) (4)	650µg (500µg)	*0.0*	*0.0*	*0.0*	0 A, 0 A, 0 A
		260µg (200µg)	*4.7*	*1.2*	*0.6*	4 S, 6 S, 4 S
		130µg (100µg)	*5.7*	*0.6*	*0.7*	6, 5, 6
		65µg (50µg)	*8.3*	*2.1*	*1.1*	6, 9, 10
		26µg (20µg)	*9.7*	*5.0*	*1.2*	9, 5, 15
		13µg (10µg)	*10.7*	*6.5*	*1.4*	4, 11, 17
	Water		*7.8*	*2.7*		6, 10, 10, 4, 9
TA98	(b) (4)	650µg (500µg)	*5.3*	*4.2*	*0.3*	4 A, 2 A, 10 A
		260µg (200µg)	*7.0*	*2.0*	*0.4*	7 S, 9 S, 5 S
		130µg (100µg)	*12.3*	*3.2*	*0.7*	11, 10, 16
		65µg (50µg)	*10.0*	*4.4*	*0.5*	5, 12, 13
		26µg (20µg)	*10.7*	*4.0*	*0.6*	13, 6, 13
		13µg (10µg)	*17.7*	*1.5*	*0.9*	18, 16, 19
	Water		*18.8*	*4.1*		24, 18, 15, 22, 15
WP2 (pKM101)	(b) (4)	1299µg (1000µg)	*0.3*	*0.6*	*0.0*	0 A, 0 A, 1 A
		650µg (500µg)	*22.3*	*24.8*	*0.3*	49 S, 0 S, 18 S
		260µg (200µg)	*82.3*	*9.6*	*1.1*	91, 84, 72
		130µg (100µg)	*68.7*	*7.6*	*0.9*	60, 74, 72
		65µg (50µg)	*74.3*	*4.9*	*1.0*	71, 72, 80
		26µg (20µg)	*74.0*	*7.8*	*1.0*	78, 65, 79
	Water		*72.8*	*4.6*		65, 73, 75, 74, 77

Key to Plate Postfix Codes

A	Lawn absent
S	Sparse/Incomplete lawn

Study Name: YV6423: (b) (4)
Experiment: YV6423 Phase 2

Study Code: YV6423
Date Plated: 25/07/2003
Counted: 28/07/2003

Without S9-mix

Strain	Compound	Dose level per plate: Actual test substance (free base equivalent)	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 uvrA (pKM101)	(b) (4)	1299µg (1000µg)	*0.0*	*0.0*	*0.0*	0 A, 0 A, 0 A
		650µg (500µg)	*2.3*	*2.5*	*0.0*	5 S, 0 S, 2 S
		260µg (200µg)	*121.7*	*21.4*	*0.8*	113, 106, 146
		130µg (100µg)	*132.7*	*11.6*	*0.9*	125, 127, 146
		65µg (50µg)	*147.0*	*5.3*	*1.0*	151, 141, 149
		26µg (20µg)	*138.0*	*22.3*	*0.9*	158, 114, 142
	Water		*151.6*	*14.3*		132, 144, 153, 160, 169
TA100	**NaZ**	2µg/plate	*650.0*	*36.6*	*5.4*	615, 647, 688
TA1535	**NaZ**	2µg/plate	*519.7*	*17.5*	*28.9*	537, 502, 520
TA1537	**ICR**	2µg/plate	*161.3*	*27.5*	*20.7*	163, 133, 188
TA98	**DR**	1µg/plate	*378.3*	*50.5*	*20.1*	342, 436, 357
WP2 (pKM101)	**MMC**	1µg/plate	*253.7*	*34.5*	*3.5*	252, 289, 220
WP2 uvrA (pKM101)	**ENNG**	1µg/plate	*538.3*	*133.4*	*3.6*	397, 556, 662

Key to Positive Controls

NaZ	Sodium Azide
ICR	Acridine Mutagen ICR191
DR	Daunomycin Hydrochloride
MMC	Mitomycin C
ENNG	N-Ethyl-N'-nitro-N-nitrosoguanidine

Key to Plate Postfix Codes

A	Lawn absent
S	Sparse/Incomplete lawn

Study title: (b) (4): *In vitro* Cytogenetic Assay in Human Lymphocytes

Key findings: (b) (4) is clastogenic in human peripheral blood lymphocytes in the presence of S9 metabolic activation with 3 hr incubation and in the absence of metabolic activation with 20 hr incubation.

Study no.: (b) (4)/SV1200
Volume #, and page #: Module 4.2.3.7.6.1 (Study Report RC030407)
Conducting laboratory and location: (b) (4)
Date of study initiation: June 16, 2003
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: (b) (4), Batch reference # B4990P179; purity: >95%

Methods
Strains/species/cell line: Human peripheral blood lymphocytes

Doses used in definitive studies:

Assay 1: ±S9 3 hr: water vehicle; (b) (4): 1, 5, and 10 mcg/mL
Assay 2: +S9 3 hr: water vehicle; (b) (4): 2.5, 10, and 20 mcg/mL; -S9 20 hr: water vehicle; (b) (4): 1, 5, and 10 mcg/mL

Basis of dose selection: The Applicant conducted two separate assays with a wide range of (b) (4) concentrations. The highest concentrations selected for chromosomal aberration analysis were limited by cytotoxicity and reductions in mitotic activity. The following assay parameters and doses were used in the two assays:

Assay 1: ±S9 3 hr: water vehicle; (b) (4) 0.5, 1, 2.5, 5, 10, 20, 30, 40, 50 mcg/mL
Assay 2: +S9 3 hr/-S9 20 hr: water vehicle; (b) (4) 0.5, 1, 2.5, 5, 10, 20, 30 mcg/mL

The procedures and assay design complied with the recommendations of the OECD guideline 473 (1997), EEC Annex V BIO (2000), ICH guidelines (1995 and 1997) and the UKEMS Recommended Procedures for Basic Mutagenicity Tests (Scott, et al., 1990).

Mitotic index was determined by examining 1000 lymphocytes per culture and calculating the percentage of cells in metaphase. One hundred cells in metaphase were analyzed from each culture for the incidence of structural chromosomal damage.

Negative controls: No negative control was used in this study.

Positive controls: Mitomycin C (MMC) was used as the positive control at 0.5 mcg/mL for the 3 hour groups and at 0.2 mcg/mL for the 20 hour non-activated groups.

Incubation and sampling times: In Assay 1, cultures were treated for a period of 3 hours both in the presence and absence of S9. In Assay 2, cultures were treated for 3 hours in the presence of S9 and 20 hours in the absence of S9. All cultures were harvested 68 hours after culture initiation.

The percentages of aberrant metaphases and the number of aberrations per cell were calculated for each treatment scored, including and excluding cells with only gap-type aberrations. The Fisher Exact Probability Test (one-sided) was used to evaluate statistically the percentage of metaphases showing aberrations (excluding cells with only gap-type aberrations). Data from each treatment group, in the presence and absence of S9, was compared with the respective control group value.

The study was considered negative if any of the following criteria were met:

- No statistically significant increase in the percentage of aberrant cells (at any concentration) above concurrent solvent control values were observed.
- A statistically significant increase in the percentage of aberrant cells above concurrent solvent control values, which falls within the laboratory historical solvent control range.

The study was considered positive if any of the following criteria were met:

- An increase in the percentage of aberrant cells, at least at one concentration, which is substantially greater than the laboratory historical solvent control values.

- A statistically significant increase in the percentage of aberrant cells which is above concurrent solvent values and which is above the historical solvent control range upper value but below that described in the first bullet may require further evaluation.

Results

Study validity: This study is valid. It utilizes appropriate replicates and cell counting/viability methodology. The vehicles and positive controls for the S9-activated and non-activated groups with 3 hr incubation and non-activated 20 hr incubation are within the range of the historical data set. The positive controls are significantly higher than vehicle controls for all groups.

Study outcome: It is concluded that under conditions of the assays conducted (b) (4) is clastogenic in both the presence and absence of S9 activation.

The Applicant conducted two assays using concentration ranges of (b) (4) between 0.5-50 mcg/mL (Assay 1) and 0.5-30 mcg/mL (Assay 2). The highest concentrations selected for chromosomal aberration analysis were limited by cytotoxicity and reductions in mitotic activity. Significant reductions in mean mitotic activity, compared to the control values, were observed in cultures from both Assay 1 (10 mcg/mL +S9: 41%; 10 mcg/mL -S9: 53%) and Assay 2 (20 mcg/mL +S9: 46%; 10 mcg/mL -S9: 38%) treated with the highest concentrations of (b) (4) selected for chromosomal aberration analysis. Cultures treated with higher concentrations of (b) (4) were considered not to be suitable for chromosomal aberration analysis due to severe cytotoxic effects. Treatment of the culture medium with (b) (4) had no significant effect on osmolality or pH at all doses tested.

Dose related, but not statistically significant, increases in the percentage of aberrant cells treated with (b) (4) in both the absence and presence of S9 (3 hour incubations) were observed in Assay 1 (Tables 1 and 2). In Assay 2 in the presence of S9 (3 hr incubation), a statistically significant increase in the percentage of aberrant cells was seen in the high dose (b) (4) group (20 mcg/mL: 5.5%) as compared to control (control: 1.0%; Table 2). In Assay 2 in the absence of S9 (20 hour incubation), statistically significant increases above control values (control: 0.5%) in the percentage of aberrant cells treated with (b) (4) were seen at the high dose (10 mcg/mL: 9.0%) and the mid dose (5 mcg/mL: 3.5%; Table 1).

Table 1. Mean chromosomal aberrations and mitotic indices in the absence of S9 metabolic activation

Treatment			Mean % Aberrant Cells Excluding Gaps	Mean % Mitotic Index
Assay 1				
Solvent Control	10µl/ml		1.00	9.0
Mitomycin C	0.5µg/ml		40.00**	5.9Δ
(b) (4)				
	10µg/ml	(7.7µg/ml)	3.50	4.2
	5µg/ml	(3.9µg/ml)	1.50	6.9
	1µg/ml	(0.8µg/ml)	1.50	7.9
Assay 2				
Solvent Control	10µl/ml		0.50	17.4
Mitomycin C	0.2µg/ml		60.00**	7.3Δ
(b) (4)				
	10µg/ml	(7.7µg/ml)	9.00**	10.8
	5µg/ml	(3.9µg/ml)	3.50*	12.7
	1µg/ml	(0.8µg/ml)	1.50	17.1

* Statistically significant increase in the percentage of aberrant cells at $p<0.05$ using Fisher's Exact Test (one-sided).

** 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.

Concentrations expressed as free base are shown in parentheses

Table 2. Mean chromosomal aberrations and mitotic indices in the presence of S9 metabolic activation

Treatment			Mean % Aberrant Cells Excluding Gaps	Mean % Mitotic Index
Assay 1				
Solvent Control	10µl/ml		1.50	8.7
Cyclophosphamide	50µg/ml		44.00**	4.4Δ
(b) (4)				
	10µg/ml	(7.7µg/ml)	4.50	5.1
	5µg/ml	(3.9µg/ml)	3.00	7.9
	1µg/ml	(0.8µg/ml)	2.00	9.6
Assay 2				
Solvent Control	10µl/ml		1.00	16.1
Cyclophosphamide	50µg/ml		44.00**	7.4Δ
(b) (4)				
	20µg/ml	(15.4µg/ml)	5.50*	8.7
	10µg/ml	(7.7µg/ml)	2.00	10.1
	2.5µg/ml	(1.9µg/ml)	2.00	13.9

* Statistically significant increase in the percentage of aberrant cells at $p<0.05$ using Fisher's Exact Test (one-sided).

** 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.

Concentrations expressed as free base are shown in parentheses

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2.6.6.5 Carcinogenicity

The Suboxone/Subutex label contains information describing carcinogenicity studies with BUP in rat and mouse. No carcinogenicity studies are described for NLX or the

BUP/NLX combination. The current study is a carcinogenicity assessment in rat with Suboxone, the 4:1 combination of BUP/NLX.

Carcinogenicity studies with BUP alone have been conducted in rat and mouse and appear in the Suboxone/Subutex label. Buprenorphine was administered in the diet to rats at doses of 0.6, 5.5, and 56 mg/kg/day for 27 months. Estimated exposure was approximately 0.4, 3 and 35 times the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis. Statistically significant dose-related increases in testicular Leydig cell tumors were seen, according to the trend test adjusted for survival. Pairwise comparison of the high dose with control did not show statistical significance. In an 86-week study in mice, BUP was not carcinogenic at dietary doses up to 100 mg/kg/day with estimated exposure approximately 30 times the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis. No carcinogenicity studies with NLX have been conducted.

Study title: Suboxone: Two-Year Dietary Oncogenicity Study in Rats

Key study findings:

- Unilateral benign Leydig cell (testes) adenomas reached statistical significance at the HD. Statistical significance was also observed in the trend test. These neoplasms are considered treatment-related.

- Bilateral benign Leydig cell (testes) adenomas reached statistical significance at all doses. Statistical significance was also observed in the trend test. These neoplasms are considered treatment-related.

- All doses for both unilateral and bilateral adenomas (analyzed individually) showed increased incidence over historical controls averaged over the past five years for unilateral and bilateral Leydig cell tumors combined.

- No treatment-related neoplasms were observed in females.

- Aggression was observed in all rats in the treated groups.

- Male group mean body weights in all treated groups were lower than controls throughout the study. The maximal decreases in males as compared to control were 10%, 14%, and 14% for the LD, MD, and HD, respectively.

- Female group mean body weights were similar to controls until about halfway through the study at which point all treated groups showed lower weights as compared to controls. The maximal decreases in females as compared to control were 9%, 10%, and 13% for the LD, MD and HD groups, respectively.

- Group mean food consumption in males and females was lower than controls in all treated groups at the beginning of the study. From approximately study week

40 until the completion of the study, food consumption in both sexes in all treated groups was increased as compared to controls.

Adequacy of the carcinogenicity study and appropriateness of the test model:
The rodent model used in this study is appropriate for assessment of the carcinogenic potential of Suboxone. However, the strain of rat used in this study was the Alderley Park strain. This strain is not commonly used in carcinogenicity assessments and does not have an extensive historical control database. The Applicant states that the Alderley Park strain of rat was used because of the substantial background data available for this strain in their laboratory ((b) (4)).
Although the ECAC recommended that the animals be individually housed due to aggressive behavior seen in the 28 day and 13 week studies (see meeting minutes from March 20, 2000), the study was conducted with rats in the main study housed 4 per cage. Aggressive behavior was observed in the study but it does not appear that it compromised the study in any way (i.e., it did not lead to life threatening injuries). Group housing is also not the optimal situation for a study with dietary administration.

Animal survival was sufficient for an adequate assessment of tumorigenic potential.

Although not conducted under optimal conditions, the study is considered adequate to assess the carcinogenic potential of Suboxone.

Mutagenicity/genotoxicity:
***Suboxone* (combination of 4:1 buprenorphine: naloxone):** The following studies appear in the label of the approved sublingual tablet product:
Negative: Ames test
Negative: *in vitro* chromosomal aberration assay in human lymphocytes
Negative: *in vivo* micronucleus assay in rat bone marrow

Numerous genetic toxicology studies with buprenorphine and naloxone tested individually have been conducted with some positive findings for both compounds.

Buprenorphine:
Positive: Ames test (positive in TA1538 ±S9 and TA98 ±S9)
Negative: *in vitro* chromosomal aberration assay in human lymphocytes
Negative: *in vivo* mouse micronucleus assay
Positive: Unscheduled DNA synthesis assay
Negative: *in vitro* mouse lymphoma assay (L5178Y cells)

Naloxone:
Positive: Ames test (positive in TA1535 +S9 and TA100 +S9)
Negative: *in vitro* mouse lymphoma assay (L5178Y cells)
Positive: *in vitro* chromosomal aberration assay in human lymphocytes

Evaluation of tumor findings: A variety of tumors were observed in this study. Several tumors including pituitary gland adenomas in males and females, adnexal tumors of the

skin in males, thymoma in females, and pilomatricoma of the subcutaneous tissue in males showed decreases in the MD and HD treated groups as compared to controls.

In pairwise comparisons, unilateral benign Leydig cell (testes) adenomas reached statistical significance at the HD and bilateral benign Leydig cell (testes) adenomas reached statistical significance all doses. The trend analysis reached statistical significance for both unilateral and bilateral Leydig cell adenomas. All doses for both unilateral and bilateral adenomas showed increased incidence over historical controls averaged over the past five years for unilateral and bilateral Leydig cell tumors combined. These neoplasms are considered treatment-related.

The incidence of uterine adenocarcinoma was increased in all female treated groups. The trend analysis was statistically significant; however, none of the pairwise comparisons reached statistical significance. All treated groups and one of the control groups showed increased incidence above historical controls for uterine adenocarcinoma averaged over the past five years, however, a wide degree of variability was observed in the historical controls. The incidence of uterine adenoma in the present study may reflect a trend of increases in background levels in the strain of rat used in the study. The increases in uterine adenocarcinoma in the treated groups were not accompanied by increases in uterine adenoma or endometrial hyperplasia. The increase in incidence of uterine adenocarcinoma is not considered treatment-related.

There was a small increase in the incidence of large granular lymphocyte (LGL) leukemia in males at all doses and females in the MD and HD. Although none of the pairwise comparisons or trend analyses reached statistical significance, all treated male groups and the MD and HD females fell outside the historical control range. The slight increases in LGL lymphoma observed in males and females are considered spurious and unrelated to the test article.

Various neoplasms or pre-neoplastic lesions were observed in the treated groups with incidences similar to controls and/or similar to levels observed in the historical controls. None of these tumors were considered to be treatment-related.

Study no.: Applicant reference number: 00600114; (b) (4) study number: PR1175
Volume #, and page #: eCTD 4.2.3.4.1
Conducting laboratory and location: (b) (4)

Date of study initiation: June 16, 2000
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Buprenorphine HCL: batch # X04033; 99.7%
Naloxone HCl dihydrate: batch # 82650008; 98.8%
CAC concurrence: YES; Minutes from the ECAC meeting discussing the study report results (May 12, 2009) can be found in Appendix 2.

Methods

Doses: Doses of 100, 450 and 1800 ppm of Suboxone in the diet were administered to the rats in the main study. Suboxone is the combination of a 4:1 ratio of buprenorphine (BUP) to naloxone (NLX). The low, mid and high dietary doses of Suboxone given in ppm are equivalent to 5, 22.5, and 90 mg/kg/day of the sum of the BUP and NLX free bases. Individually, the low, mid and high doses of Suboxone correspond to 4, 18, and 72 mg/kg/day BUP and 1, 4.5, and 18 mg/kg/day NLX (Table 1). The control groups did not have study drug added to their diet. The maximum concentration of Suboxone was well below 5% of the total diet.

Table 1. Dose comparison of the Suboxone components

dose group	*dietary concentration of Suboxone*, ppm*	*Suboxone concentration, mg/kg/day*	*Buprenorphine concentration, mg/kg/day*	*Naloxone concentration, mg/kg/day*
low	100	5	4	1
med	450	22.5	18	4.5
high	1800	90	72	18

*expressed as the sum of BUP and NLX free bases

Basis of dose selection (MTD, MFD, AUC etc.): A 13-week dose range finding study with dietary administration using doses up to 2000 ppm was conducted with Suboxone. The review states that no MTD could be established for females in the 13-week dose range finding study. Males showed decreases in body weight gain of 13% (100 ppm), 18% (500 ppm), 22% (1500 ppm), and 20% (2000 ppm) which were attributed to decreased food consumption. The dose selection for both males and females for this carcinogenicity assessment was based on AUC comparisons with the clinical dose of a single administration of 16 mg of Suboxone. The AUC values at 2000 ppm (1580 and 1424 hr.ng/mL in M and F, respectively) of buprenorphine (BUP) are approximately 43-fold higher than human AUC (34.89 hr.ng/mL). The AUC values for naloxone (NLX) from the 13-week rat study are not available due to low oral bioavailability but the mean plasma concentrations of NLX at 2000 ppm are approximately 10-fold higher that the maximum concentration of NLX detected in humans.

The 13-week dose range finding study and 2-year carcinogenicity assessment protocol were reviewed by Dr. Anwar Goheer. The proposed doses used in this study received ECAC concurrence (March 28, 2000). The ECAC meeting minutes are included as Appendix 1.

Species/strain: Rat/Alpk:AP$_f$SD (Alderley Park)
Number/sex/group (main study): 52/sex/group
Route, formulation, volume: dietary administration
Frequency of dosing: food was available *ad libitum* for 104 weeks
Satellite groups used for toxicokinetics or special groups: TK groups: 18/sex each low, med and high dose groups. Note that TK was not evaluated in the control groups.
Age: The rats were approximately 5 weeks at initiation of dosing.

Animal housing: The main study rats were housed 4 per cage; satellite rats (TK) were housed 3 per cage.
Restriction paradigm for dietary restriction studies: N/A
Drug stability/homogeneity: Samples from all dietary levels (including controls) were taken prior to the start of the study and at approximately three-monthly intervals throughout the study and analyzed quantitatively for Suboxone. Drug uniformity and stability were confirmed in study # (b) (4)/WC0304. The results were considered satisfactory with percent deviations from the overall mean being within 4.9%.
Dual controls employed: Two identical control groups were used in this study.
Interim sacrifices: none
Deviations from original study protocol: No deviations in the study protocol were described by the applicant.

Histopathology Inventory

Study Number			
Species	Alpk:APfSD rat		
Adrenal	X*	Ovary	X*
Aorta	X	Oviduct	X
Bone (femur)	X	Pancreas	X
Brain	X*	Parathyroid	X
Cecum	X	Pharynx	X
Cervix	X	Pituitary	X
Colon	X	Prostate	X
Duodenum	X	Rectum	X
Epididymis	X*	Salivary gland	X
ex-orbital lacrimal gland	X	Seminal vesicles	X
Eye	X	Skin	X
Esophagus	X	Spinal cord	X
Gross lesions	X	Spleen	X*
Harderian gland	X	Sternum	X
Heart	X*	Stomach	X
Ileum	X	Testes	X*
Jejunum	X	Thymus	X
Kidney	X*	Thyroid	X
Larynx	X	Tongue	X
Liver	X*	Tumors, suspected tumors and associated tissues	X
Lung	X	Trachea	X
Lymph nodes, cervical	X	Urinary bladder	X
Lymph nodes mediastinal	X	Uterus	X*
Mammary Gland (inguinal, F only)	X	Vagina	X
Nerve	X	Zymbal gland	X
Nasal epithelium	X	Voluntary muscle	X

*organ weighed

Observation times (stated in the Results section)

Results

Mortality: Cage-side observations were made twice daily. Any rats euthanized *in extremis* were examined *post mortem*. Any rats found dead were examined as soon as possible after death. No statistically significant differences in survival were seen in the individual group comparisons. Across doses, males showed a statistically significant trend for increased survival ($p<0.05$; Figure 1). Female survival in the MD and HD groups was significantly lower than controls ($p<0.05$, $p<0.01$, respectively). Across doses, there was a statistically significant trend for decreased survival in females ($p<0.01$; Figure 2).

Figure 1. Kaplan-Meier Survival Curve for Males (reproduced from NDA submission)

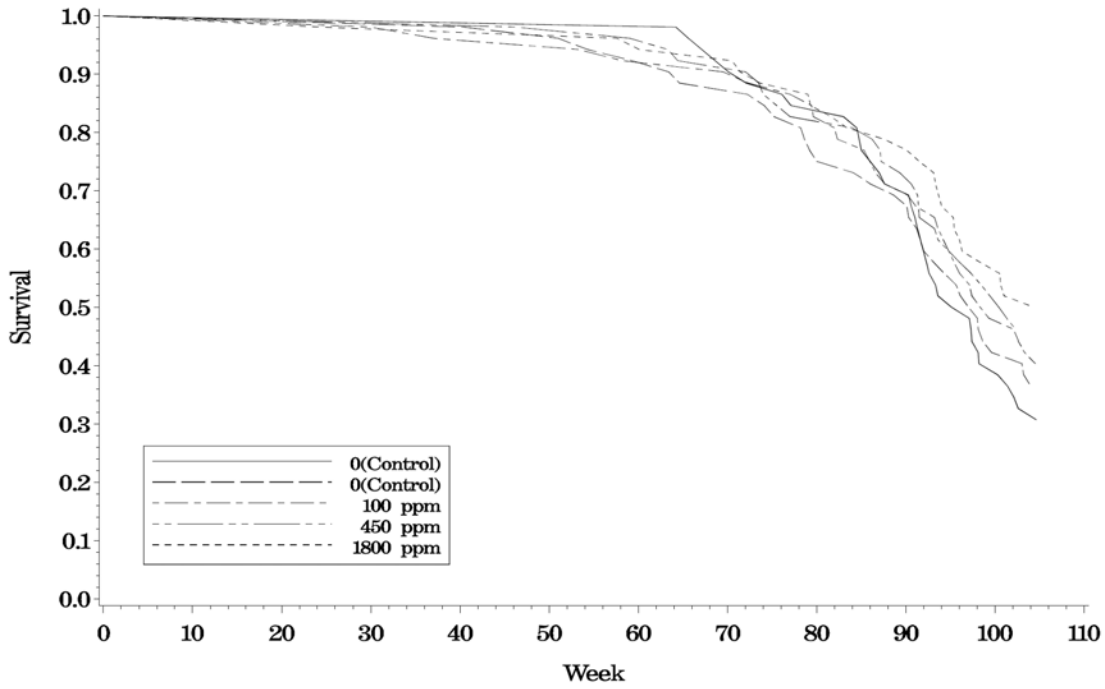
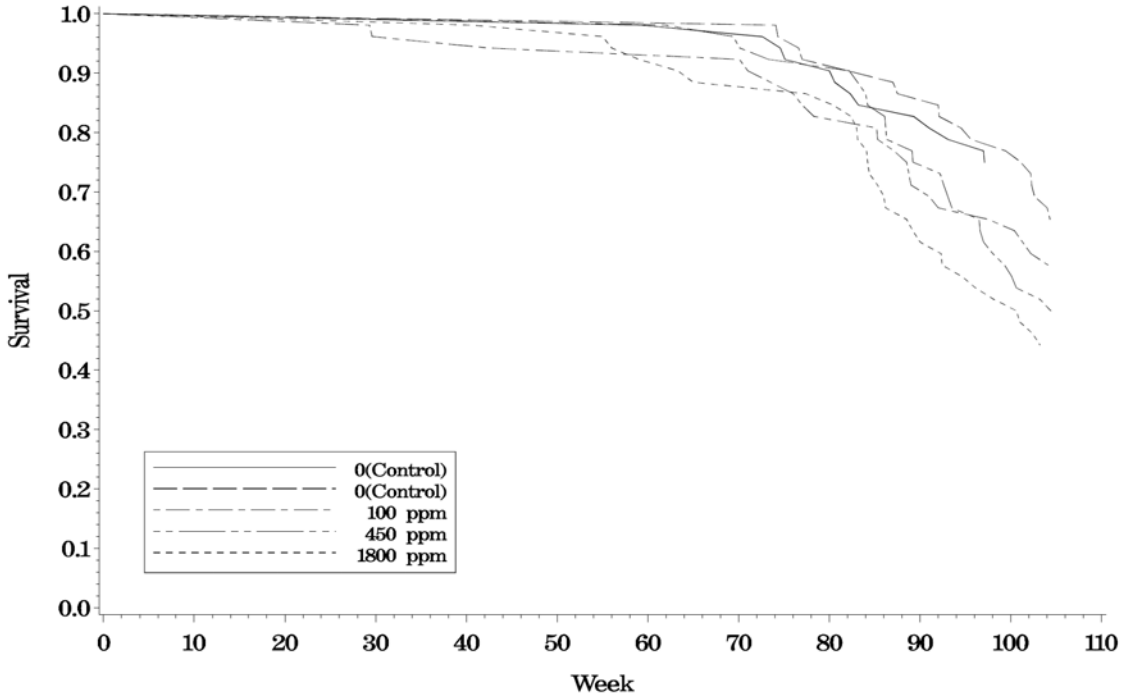


Figure 2. Kaplan-Meier Survival Curve for Females (reproduced from NDA submission)



Clinical signs: Prior to the start of the study all rats were examined to ensure that they were normal. Cage-side observations included recording of any changes in clinical condition or behavior and were made twice daily. A detailed examination was performed at least weekly. The rats in the Suboxone groups were more aggressive and showed observations including torn ears, scabs, tail damage and vocalization. These behaviors are considered due to the pharmacologic action of the drug. They are consistent with behaviors observed in the 28-day and 13-week studies. Age-related signs (*i.e.,* urine staining of coat and ears, pallor, piloerection) were observed in all groups were not considered drug-related.

Body weights: Body weights were recorded immediately before feeding of the experimental diets every other week for weeks 2-15 of the study, week 17 and then every 4 weeks until termination. All rats were weighed immediately prior to termination. The adjusted group mean male body weights in all treated groups were lower than controls. The maximal decreases in males as compared to control were 10%, 14%, and 14% for the LD, MD, and HD, respectively (Figures 3 and 4). The adjusted group mean female body weights were similar to control until about halfway through the study. Subsequently, the lower body weights in the treated groups as compared to controls reached statistical significance. Maximal decreases in females as compared to control of

9%, 10%, and 13% in the LD, MD and HD groups, respectively, were observed (Figures 5 and 6).

The description of the analysis represented graphically in Figures 4, 6, 8 and 10 is reproduced verbatim from the NDA:

> The differences from pooled control based on the analysis of bodyweight and food consumption are also presented graphically. The centre of each bar represents the mean percentage difference between pooled control and treated group least squares means, and the top and bottom of each bar represent the upper and lower 95% confidence limits for this difference. A statistically significant difference between the treated group and the pooled control group is present when the bar does not cross the zero difference line. For ease of reference, lines have been added to the plots to show differences of ± 10%.

Figure 3. Group Mean Body Weights in Main Study Males (reproduced from NDA submission)

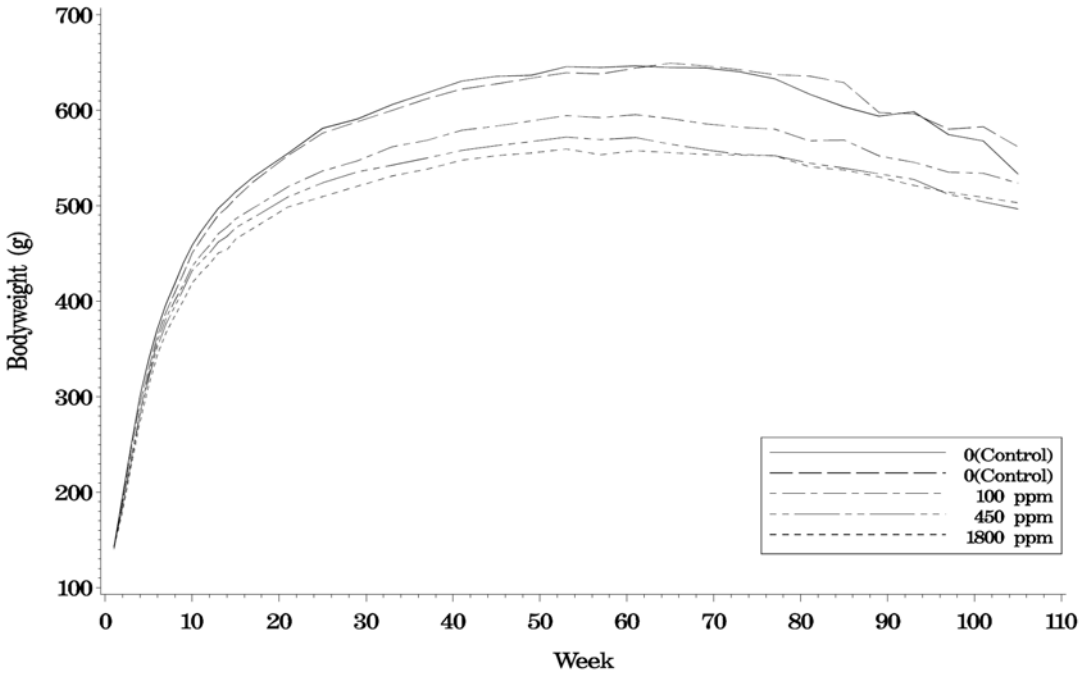


Figure 4. Analysis of Bodyweight Adjusted for Initial Weight in Males (reproduced from NDA submission)

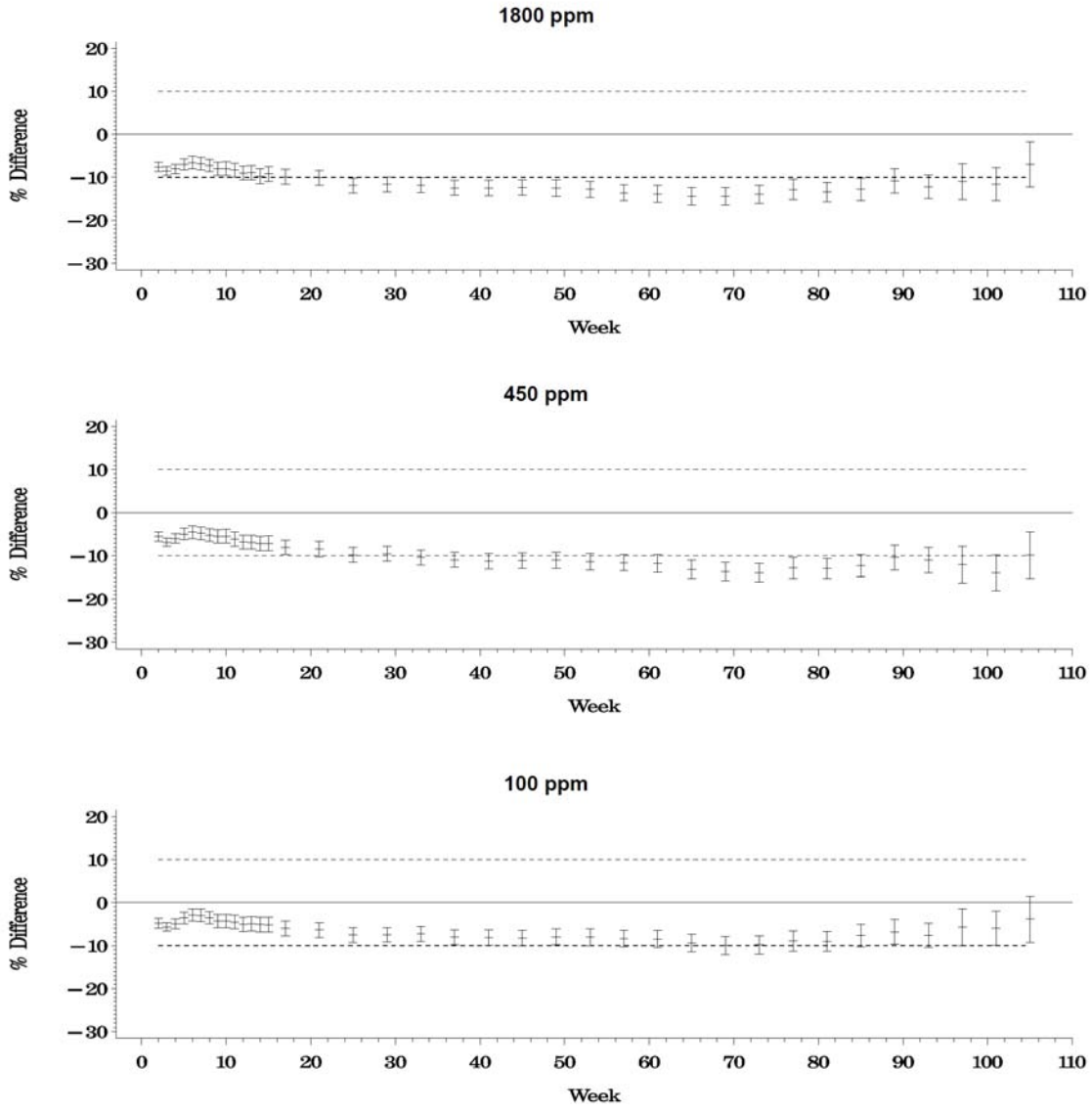


Figure 5. Group Mean Body Weights in Main Study Females (reproduced from NDA submission)

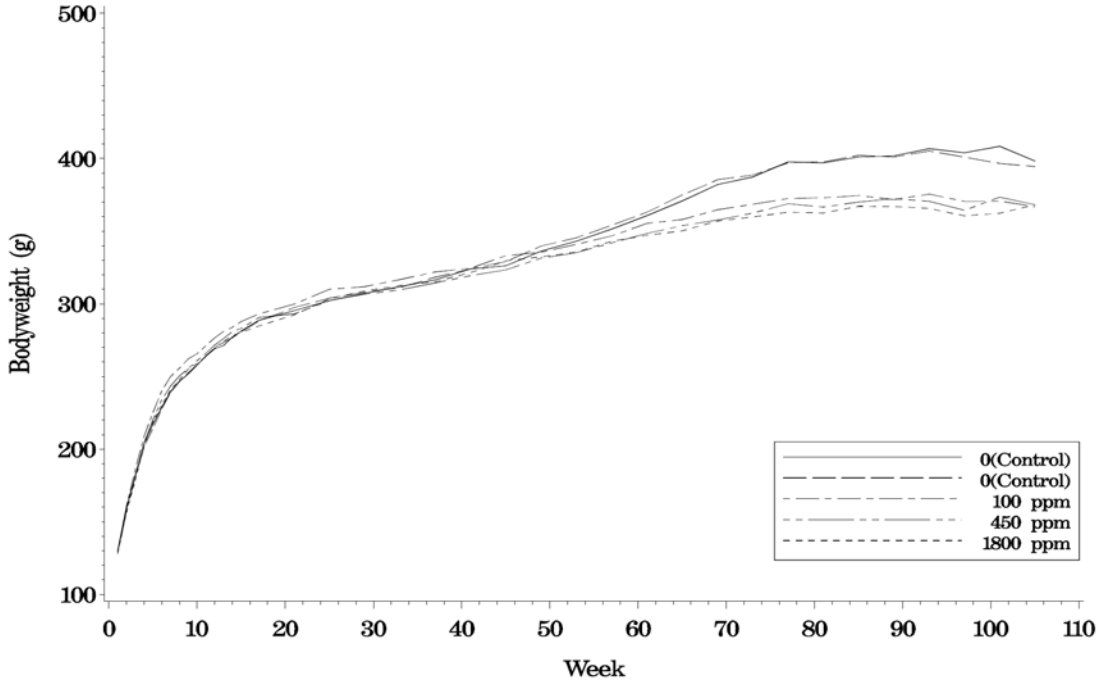
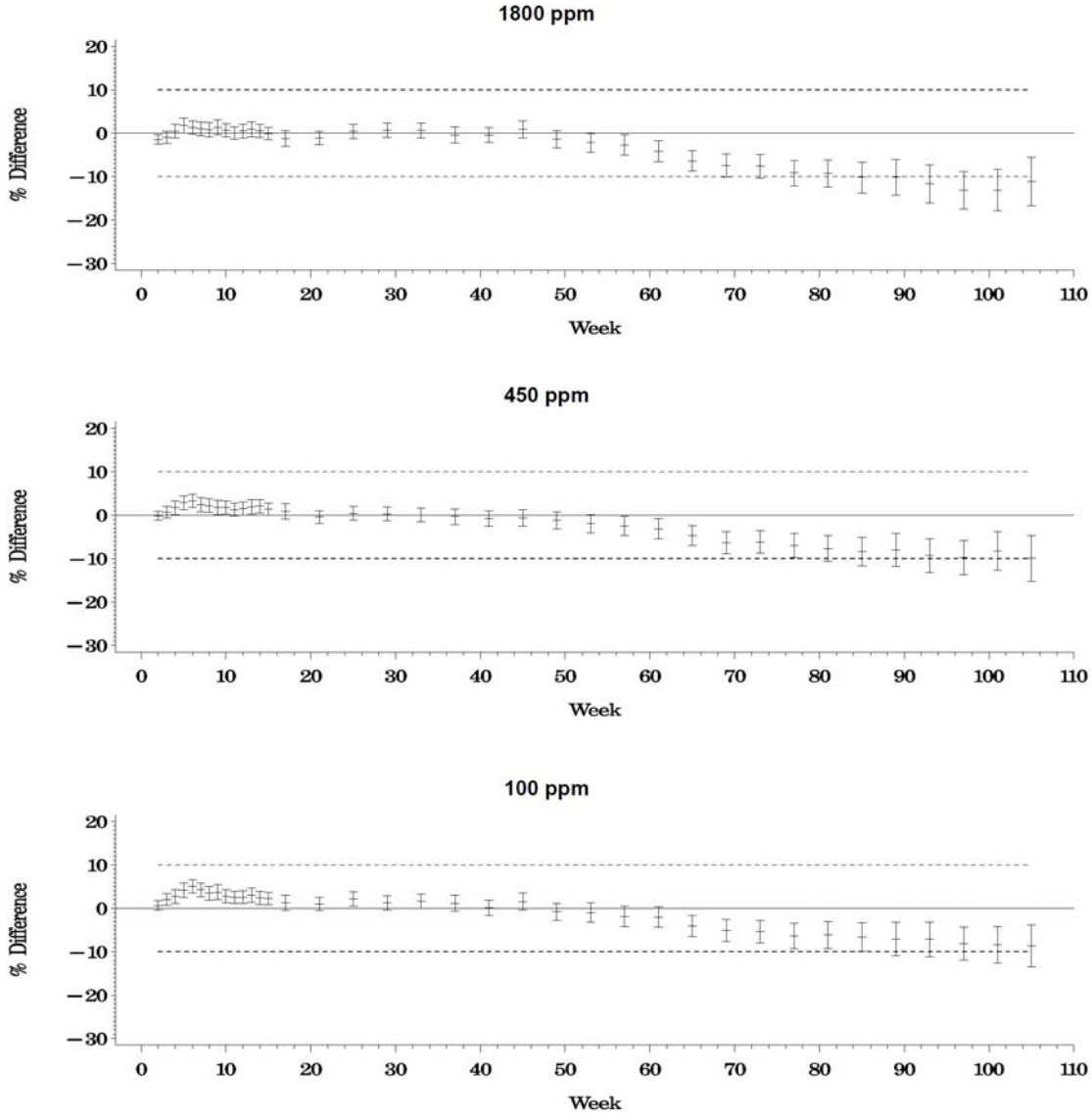


Figure 6. Analysis of Bodyweight Adjusted for Initial Weight in Females (reproduced from NDA submission)



Food consumption: Food consumption for each cage was recorded weekly for the first 14 weeks of the study, week 16 and thereafter every fourth week. Food consumption was calculated, at respective intervals, as a mean value (g food/rat/day) for each cage. Group mean food consumption in males was significantly lower in all treated groups for the first sixteen weeks of the study as compared to control groups (Figures 7 and 8). From approximately study week 40 until the completion of the study, food consumption in all treated groups showed statistically significant increases as compared to controls although these increases were not always dose dependent. Group mean food consumption in

females was significantly lower at the HD for the first two weeks of the study and at the MD for the first week of the study as compared to control groups (Figures 9 and 10). From approximately study week 24 until the completion of the study, food consumption in all treated groups showed increases as compared to controls. For the most part, these increases were statistically significant but the increases were not always dose dependent.

Figure 7. Group Mean Food Consumption in Main Study Males (reproduced from NDA submission)

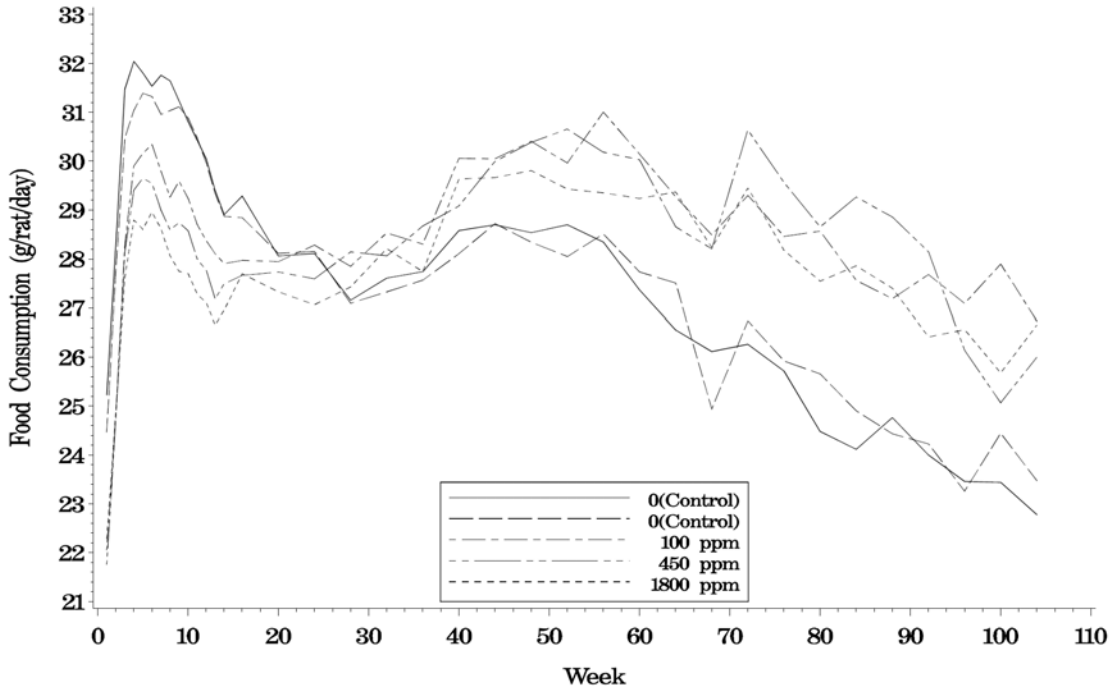


Figure 8. Analysis of Food Consumption in Males (reproduced from NDA submission)

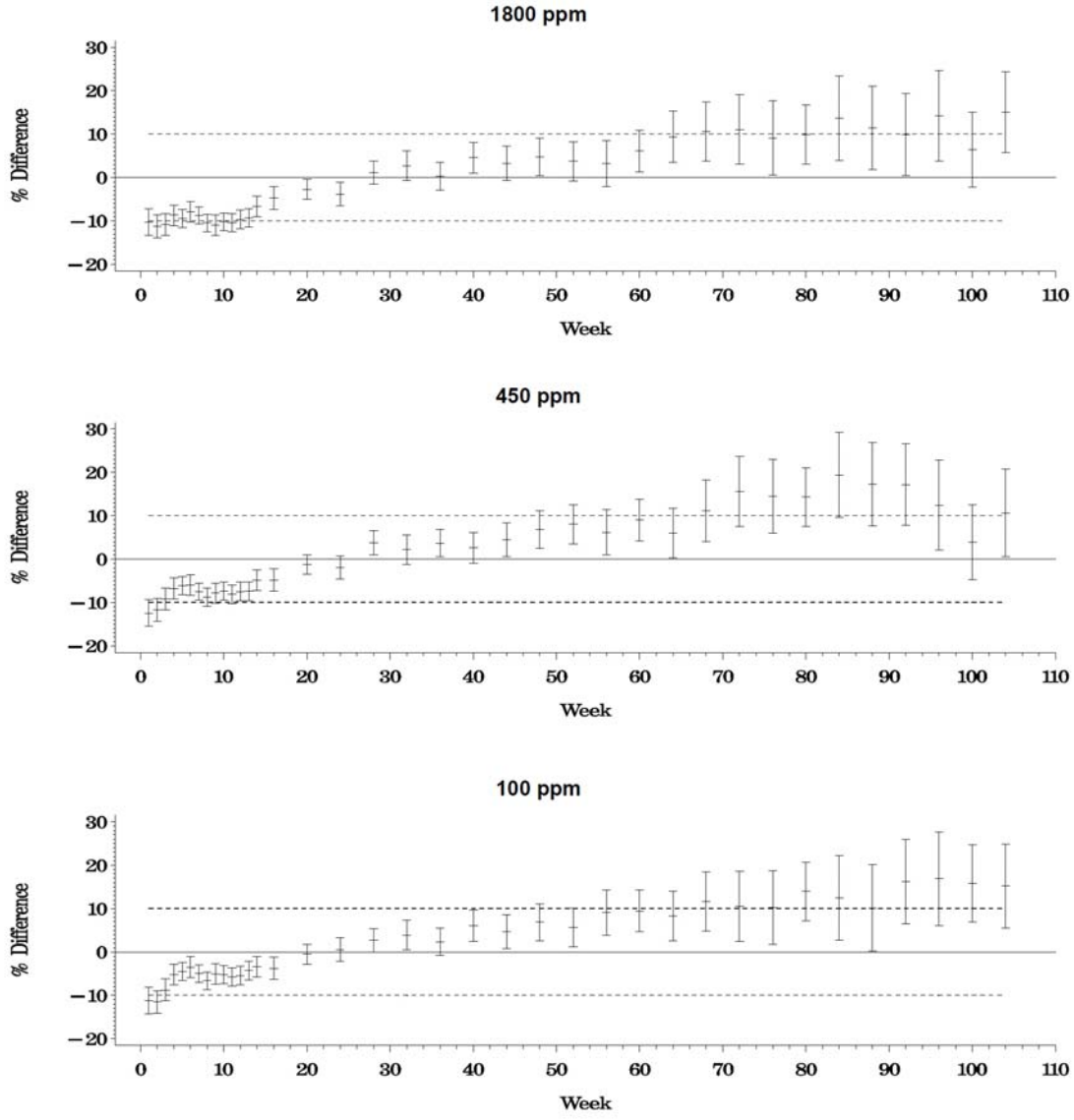


Figure 9. Group Mean Food Consumption in Main Study Females (reproduced from NDA submission)

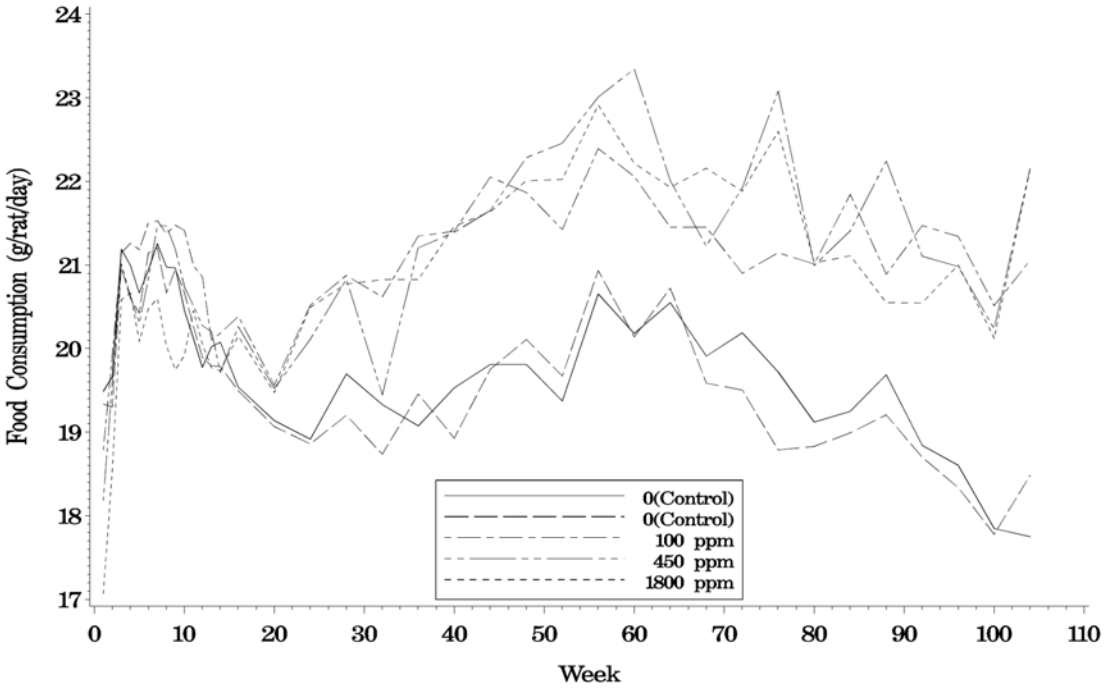
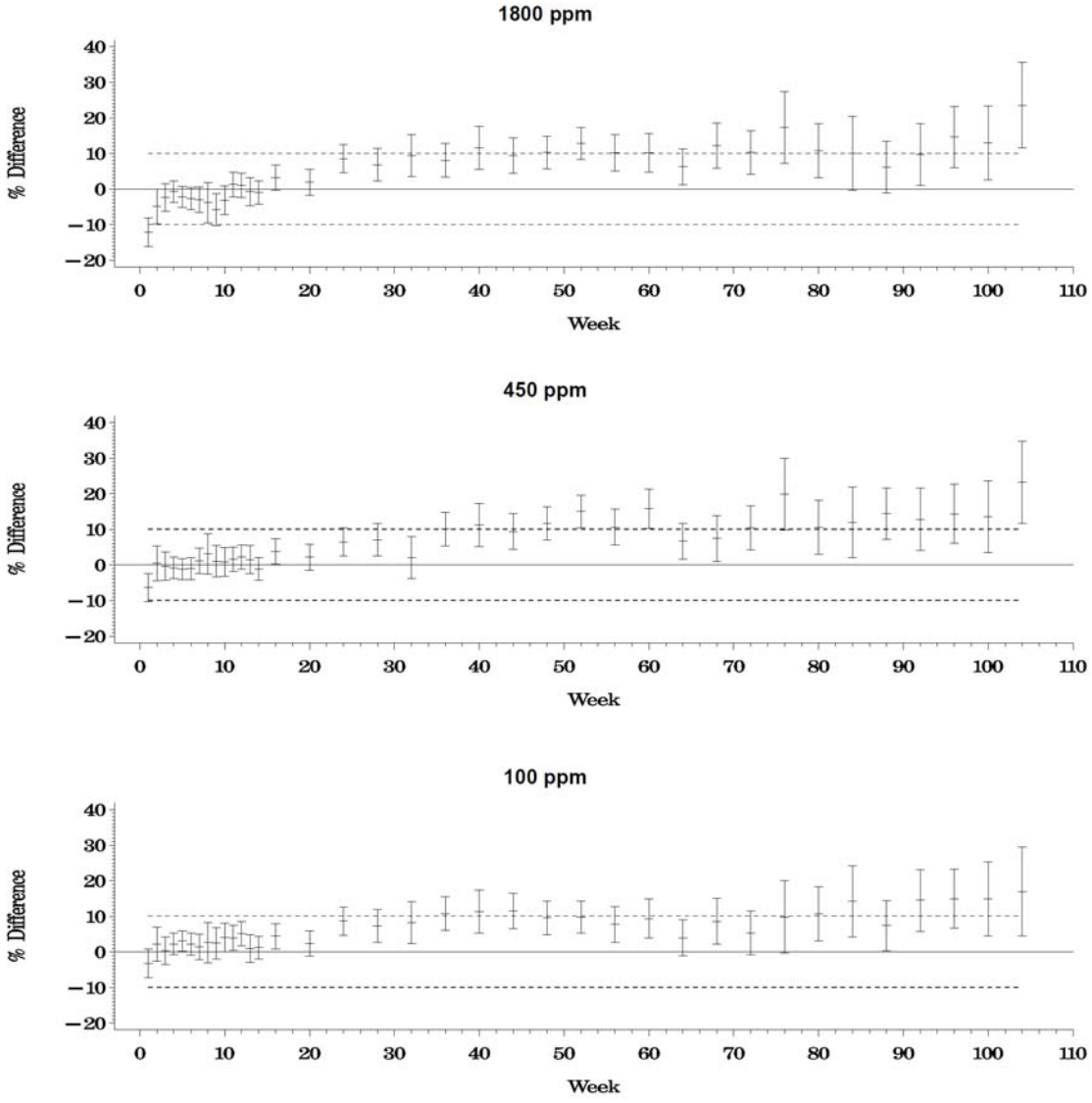


Figure 10. Analysis of Food Consumption in Females (reproduced from NDA submission)



Gross pathology:

Organ Weights: The following organs were weighed at the scheduled necropsy of all Main Study animals: adrenal gland, epididymis, heart, kidney, liver, ovary, spleen, testes and uterus. The applicant notes that several organs had been excluded from statistical analysis due to reasons such as the organ/tissue being enlarged (with or without masses), or reduced at macroscopic examination *post mortem*.

Several organ weights were significantly increased in the male treated groups.

Both absolute organ weight and organ weight adjusted for body weight were higher for treated males in epididymides (HD) and testes (LD, MD). Adrenal weights, both adjusted and absolute, were increased at all doses in males but the increases were not dose dependent. Liver (LD, HD) weights adjusted for body weight were also increased as compared to controls. No dose responses or pathological changes (with the exception of the testes) were observed so the increased weights will not be considered drug-related.

In treated females, heart (HD), and brain (LD, HD) absolute and adjusted weights were significantly increased as compared to controls. Liver (MD, HD) weights adjusted for body weight were increased as compared to controls. No dose responses or pathological changes were observed so the increased weights will not be considered drug-related.

Macroscopic findings: In treated males, an increased incidence of enlarged and discolored testes was observed at all doses as compared to controls. These changes corresponded to Leydig cell adenomas in the majority of rats. An increased incidence of enlarged seminal vesicles, which corresponded microscopically to luminal dilation in most rats was also seen at all doses. These findings are considered treatment-related.

In both males and females, a decreased incidence of pituitary masses at the HD was seen and shown to correspond microscopically to adenoma of the pars distalis.

No other drug-related changes in organ weights were observed.

Histopathology: Peer review: yes ( ), no (X) No signed pathology report was provided by the applicant. See the Histopathology Inventory table in the Methods section for a listing of organs. All tissues from any main study rat found dead or killed intercurrently, all animals in both control groups and the HD group, all gross lesions, tumors, suspected tumors and associated tissues were submitted for histology. Representative portions were embedded in paraffin and sectioned at 5 microns. All sections were stained with hematoxylin and eosin. The following tissues from the LD and MD groups were submitted for histology:
Males: testes, pituitary gland, liver, spleen, adrenal glands, eye, lachrymal gland, seminal vesicles and voluntary muscle
Females: adrenal glands, uterus, pituitary gland, mammary gland, liver, spleen and eye.

The incidence of each tumor type was analyzed by comparing each treated group and the pooled control group using Fisher's Exact Test. A trend test was also performed using the Cochran-Armitage Test.

Non-neoplastic: Several tissues in males showed dose-related increases in non-neoplastic lesions (Table 2). Unilateral and bilateral Leydig cell hyperplasia was increased in all treated groups. Dose-related increases in vascular ectasia of the adrenal gland were also observed. Increases in luminal dilatation and inflammatory cell infiltrates in the seminal vesicles and harderian metaplasia and mononuclear cell infiltrates in the lacrimal glands were also observed. With the exception of the Leydig cell lesions, none of the non-neoplastic lesions showed progression to neoplastic lesions.

Several tissues in females showed decreases in non-neoplastic lesions (secretory activity and hyperplasia of the mammary gland). A few tissues showed increases in various non-neoplastic lesions in the treated groups as compared to controls but changes were not dose-dependent and not outside historical controls and are not considered test article-related.

Table 2. Selected non-neoplastic lesions in Males						
organ	***microscopic finding***	***Control 1***	***Control 2***	***100 ppm***	***450 ppm***	***1800 ppm***
	n	***52***	***52***	***52***	***52***	***52***
Testes	***unilateral hyperplasia***	5 (10%)	4 (8%)	9 (17%)	9 (17%)	9 (17%)
	bilateral hyperplasia	2 (4%)	2 (4%)	22 (42%)	20 (38%)	27 (52%)
Adrenal gland	***vascular ectasia***	6 (12%)	6 (12%)	28 (54%)	28 (54%)	35 (67%)
Seminal vesicle	***luminal dilatation***	0 (0%)	2 (4%)	8 (15%)	7 (13%)	9 17%)
	inflam. cell infiltrates	4 (8%)	4 (8%)	11 (21%)	12 (23%)	12 (23%)
Lacrimal gland	***harderian metaplasia***	29 (56%)	26 (50%)	41 (79%)	43 (83%)	44 (85%)
	mononuclear cell infiltration	15 (29%)	19 (37%)	32 (62%)	30 (58%)	32 (62%)

Neoplastic: A variety of tumors were observed in this study. Several tumors including pituitary gland adenomas in males and females, adnexal tumors of the skin in males, thymoma in females, and pilomatricoma of the subcutaneous tissue in males showed decreases in the MD and HD treated groups as compared to controls. In some cases, these differences reached statistical significance. The decreases in selected tumor incidence in the treated groups as compared to controls were attributed to the decreased weights of the treated groups throughout most of the study and are not considered directly test article-related.

Several other tumors occurred at a low incidence at various doses in the treated groups. The occurrence of these tumors was not dose related, did not reach statistical significance and were within historical control values. None of these tumors were considered to be treatment-related.

The incidence of uterine adenocarcinoma was increased in all female treated groups (Table 3). Although none of the pairwise comparisons reached statistical significance, the trend analysis was significant. A few benign uterine adenomas were observed in both

the control and treated groups. McConnell et al. recommends combining adenomas and carcinomas of the uterus (McConnell EE, et al., 1986). Incidence of uterine adenocarcinoma alone and combined with benign uterine adenomas is presented in Table 3. All treated groups and one of the control groups showed increased incidence above historical controls for uterine adenocarcinoma averaged over the past five years. However, the variability was high over the previous five year span 1994-1998 with an average incidence of 5.2% and a range between 0% to 11.5% for the six studies conducted within that five year window. The two previous spans of five year data, 1984-1988 (average: 3%; range: 0-5.8%) and 1989-1993 (average: 1.5%; range: 0-3.8%) showed smaller incidences. The incidence of uterine adenocarcinoma in the present study may reflect a trend of increases in background levels in the strain of rat used in the study. The increases in uterine adenocarcinoma in the treated groups were not accompanied by increases in uterine adenoma or endometrial hyperplasia. The increase in incidence of uterine adenocarcinoma is not considered treatment-related.

There was a small increase in the incidence of large granular lymphocyte (LGL) leukemia in males at all doses and females in the MD and HD (Table 3). Although none of the pairwise comparisons or trend analyses reached statistical significance, the incidence in males for all doses (LD: 17%; MD: 15%; HD: 12%) and one control group (C2: 10%) fell outside of the historical control range over the last five years (7%). The incidence in females at the MD (23%) and HD (21%) was just outside of the historical control range over the last five years (20.1%). The slight increases in LGL lymphoma observed in males and females are considered spurious and unrelated to the test article.

Table 3. Selected neoplastic lesions in males and females (not including Leydig cell adenomas)

Organ	***Tumor type***	***Control 1***	***Control 2***	***100 ppm***	***450 ppm***	***1800 ppm***	***Trend analysis***
	n	***52***	***52***	***52***	***52***	***52***	-
Uterus	***adenocarcinoma (malignant)***	8 (15%)	4 (8%)	11 (21%)	10 (19%)	12 (23%)	***p= 0.036***
	adenoma (benign)	0 (0%)	1 (2%)	1 (2%)	2 (4%)	1 (2%)	ND
	combined	8 (15%)	5 (10%)	12 (23%)	12 (23%)	13 (25%)	ND
Lympho-reticular system	***large granular lymphocyte (LGL) leukemia (malignant)***	M: 2 (4%)	M: 5 (10%)	M: 9 (17%)	M: 8 (15%)	M: 6 (12%)	M: ND
		F: 4 (8%)	F: 10 (19%)	F: 6 (12%)	F: 12 (23%)	F: 11 (21%)	F: p= 0.79

Trend Analysis: Cochran-Armitage test
Historical Control uterine adenocarcinoma: 5%
Historical Control LGL leukemia: M: 7%; F: 20%
ND= not done

Unilateral benign Leydig cell adenomas reached statistical significance at the HD (p= 0.008; Table 4). Bilateral benign Leydig cell adenomas reached statistical significance all doses (p< 0.001; Table 4). A trend analysis (p=0.01; p<0.001) was statistically

significant for unilateral and bilateral Leydig cell adenomas, respectively (Table 4). All doses for both unilateral and bilateral adenomas showed increased incidence over historical controls averaged over the past five years for unilateral and bilateral Leydig cell tumors combined (7%). However, both control groups for combined unilateral and bilateral tumors were above the historical control as well, but the percentages were only slightly higher (Table 4). These neoplasms are considered treatment-related and will be discussed in the label for Suboxone (b) (4) and Suboxone (b) (4) tablets.

Table 4. Leydig cell adenomas (benign) in males						
	Control 1	***Control 2***	***100 ppm***	***450 ppm***	***1800 ppm***	***Trend analysis***
n	***52***	***52***	***52***	***52***	***52***	-
unilateral	6 (12%)	3 (6%)	10 (19%)	7 (13%)	***14* (27%)***	***p= 0.01***
bilateral	0 (0%)	1 (2%)	***11** (21%)***	***18** (35%)***	***19** (37%)***	***p< 0.001***
combined	6 (12%)	4 (8%)	21 (40%)	25 (48%)	33 (63%)	ND

*p=0.008; **p<0.001 (Fisher's Exact Test)
Trend analysis: Cochran-Armitage test
Historical Control combined unilateral and bilateral: 7.2%
ND= not done

Toxicokinetics: Three rats/sex/dose/time point were bled for toxicokinetic analysis after approximately six months of dietary administration of Suboxone. Toxicokinetic parameters of BUP and NLX are detailed in Table 5. The last time point included in $AUC_{0\text{-last}}$ calculations was 22 hr.

Buprenorphine: The plasma concentrations of BUP for male and female mice were fairly consistent throughout the 22 hour period. Exposure to BUP was similar for male and female rats at all doses. Buprenorphine showed a T_{max} between 6-9 h. The $T_{1/2}$ was not calculated in this study but Megarbane *et al.* calculate the elimination $T_{1/2}$ of BUP in rat to be 7.7 +/-2.5 h (Megarbane B, et al., 2005). The mean $AUC_{0\text{-last}}$ values for BUP increased with dose in a non-linear manner (Table 5).

Naloxone: Plasma NLX concentrations for male and female rats administered Suboxone at the LD and males at the MD were below the lower limit of quantitation of the assay. Data were available for female animals in the MD and both males and females at the HD to allow for the calculation of toxicokinetic parameters. At the HD, the plasma concentration data demonstrated exposure to NLX and appeared to remain fairly consistent throughout the 22 hour period. The mean $AUC_{0\text{-last}}$ values obtained for the HD for both males and females were similar. Naloxone showed a T_{max} between 6-9 h (Table 5).

Exposure comparisons between rat and human at the C_{max} and AUC for the recommended daily dose of Suboxone (16/4 mg/mg BUP/NLX) are provided in Table 6. The human C_{max} and $AUC_{0\text{-}inf}$ values are for males and females combined and were taken from the applicant's proposed labeling. The AUC comparison in Table 6 is between rat $AUC_{0\text{-}last}$ and human $AUC_{0\text{-}inf}$. The last value used to calculate the AUC value in the rat study was 22 h. The appropriateness of the direct comparison between rat $AUC_{0\text{-}22}$ and human $AUC_{0\text{-}inf}$ was discussed with the Clinical Pharmacology team. Dr. Sheetal Agarwal and Dr. Suresh Doddapaneni (Clinical Pharmacology) explained that $AUC_{0\text{-}24}$ at steady state is equal to $AUC_{0\text{-}inf}$ after single dosing, therefore the comparison between rat and human values would be appropriate (W.A.Ritschel, 1986). No gender differences in exposure were observed so averaged male and female AUC values for human ($AUC_{0\text{-}inf}$) and rat ($AUC_{0\text{-}22}$) will be used for the exposure comparison in the label (Table 6). Exposure comparisons based on body surface area in mg/m^2 are included in Table 6 for comparison.

Table 5. Toxicokinetic parameters of buprenorphine and naloxone following approximately 6 months of dietary administration of Suboxone (reproduced from NDA submission)

Compound	Sex	Group	T_{max} (h)	C_{max} (ng/mL)	$AUC_{0\text{-}last}$ (hr.ng/mL)
Buprenorphine	M	6	6	6.2	113
		7	6	30.7	500
		8	9	82.8	1380
Buprenorphine	F	6	9	6.2	114
		7	6	56.6	581
		8	9	82.8	1310
Naloxone	M	6	n/c	<lloq	n/c
		7	n/c	<lloq	n/c
		8	6	3.9	46.5
Naloxone	F	6	n/c	<lloq	n/c
		7	6	2.0	8.8
		8	9	2.0	33.4

<lloq = Less than lower limit of quantitation (0.5ng/mL)

n/c = Not calculated

Group 6 = 100 ppm Suboxone

Group 7 = 450 ppm Suboxone

Group 8 = 1800 ppm Suboxone

Table 6. Exposure Comparison Between Rat and Human (16/4 mg/mg BUP/NLX dose)					
	Dose, ppm	***Dose, mg/kg/day****	***Rat/human C_{max}, (ng/mL)***	***Rat AUC_{0-22}/ human AUC_{0-inf}, (h.ng/mL)***	***exposure ratio based on mg/m^2***
Male	***100***	6.3	2	4	3
	450	28.8	9	16	14
	1800	115.4	26	47	56
Female	***100***	7.2	2	4	4
	450	32.9	17	19	16
	1800	130.2	25	43	63
Combined	***100***	6.8	2	**4**	3
	450	30.9	13	**18**	15
	1800	122.8	25	**45**	60

Human combined male and female C_{max}= 3.4 ng/mL
Human combined male and female AUC_{0-inf}= 30.5 h.ng/mL
*mg/kg/day is calculated by averaging the weekly value of nominal dietary concentration*food consumption/body weight for each cage
Note: Bolded values will be included in the label

Summary and Evaluation:
The high dose of Suboxone used in this study was 1800 ppm (equivalent to 90 mg/kg/day of Suboxone: 72 mg/kg/day BUP and 18 mg/kg/day NLX). The doses (100, 450 and 1800 ppm) used in this study were recommended by the ECAC (see Appendix 1).

Aggression was observed in all rats in the treated groups. The aggression was attributed the pharmacodynamic effects of BUP and did not compromise the study. Toxicity observed in this study was minor, however, dose-related decreases in body weights in males throughout the study (up to 14%) and females in the last half of the study (up to 13%) were observed. Group mean food consumption in males and females was lower than controls in all treated groups at the beginning of the study. From approximately study week 40 until the completion of the study, food consumption in both sexes in all treated groups was increased as compared to controls. No other findings of drug-related toxicity were observed in the study.

Daily treatment with Suboxone at doses up to 1800 ppm (~122 mg/kg) did not result in an increase of neoplastic lesions in females. In males, increases in unilateral and bilateral testicular Leydig cell adenomas were observed. Incidence of unilateral Leydig cell adenomas reached statistical significance at the HD as compared to controls and bilateral Leydig cell adenomas reached statistical significance at all doses. The trend analysis reached a level of statistical significance for both unilateral and bilateral Leydig cell

adenomas. Both unilateral and bilateral Leydig cell adenomas (separately) at all doses were well outside of the historical control range for combined Leydig cell adenomas averaged over the past five years. These tumors are considered treatment-related and will be included in the label.

Uterine adenocarcinomas were observed in females and LGL leukemia was observed in both males and females. A trend test for uterine adenoma was significant; however, no significant pairwise comparisons were seen. The incidence of uterine adenomas was outside the historical control values provided by the applicant, however, a trend toward increased background levels in the strain of rat used in the study may account for the observed increases. None of the pairwise comparisons or the trend test reached statistical significance for LGL leukemia but the incidence in males for the LD and MD fell just out side the historical control range. The incidence of LGL leukemia was increased for treated females at the MD and HD as compared to concurrent controls, but was within historical control values. The slight increases in LGL lymphoma observed in males and females are considered spurious and unrelated to the test article.

Various other neoplasms or pre-neoplastic lesions were observed in the treated groups but all were similar to levels observed in controls and/or similar to levels observed in the historical controls. Selected neoplasms (pituitary gland adenomas in males and females, adnexal tumors of the skin in males, thymoma in females, and pilomatricoma of the subcutaneous tissue in males) were *decreased* in treated groups as compared to control rats. The decreased incidence of these tumors was attributed to the lower body weights of the treated rats as compared to control rats throughout most of the study and was not considered to be directly test article-related.

2.6.6.6 Reproductive and developmental toxicology
No new reproductive and developmental toxicology studies were submitted by the Applicant. Please refer to the Pharmacology/Toxicology reviews of Suboxone (NDA 20-733; September 30, 1999) and Subutex (NDA 20-732; January 12, 1998) by Dr. David Brase for a discussion of reproductive and developmental toxicology with BUP and NLX.

2.6.6.7 Local tolerance
No new studies were conducted.

2.6.6.8 Special toxicology studies
No new studies were conducted.

2.6.6.10 Tables and Figures
Not applicable

2.6.7 TOXICOLOGY TABULATED SUMMARY
Not applicable

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

The majority of the nonclinical data to support approval of NDA 22-410 for Suboxone (b) (4) are found in NDAs 20-732 (Subutex) and 20-733 (Suboxone).

All excipients in the Suboxone (b) (4) formulation can be found in approved drug products at equal or greater levels and therefore do not pose any unique toxicological concerns. All impurities in the drug substances and drug product are below ICH thresholds or have been adequately qualified. The naloxone drug substance contains (b) (4) , an impurity with a structural alert for mutagenicity. The Applicant conducted two genetic toxicology assays with (b) (4) (b) (4) was not mutagenic in the Ames test but was found to be clastogenic in an *in vitro* cytogenetic assay in human lymphocytes. Because of the positive finding for clastogenicity, the levels of (b) (4) in the drug substance should be reduced to the currently acceptable threshold for known genotoxic impurities of NMT 1.5 mcg/day. The specification set by the Applicant for (b) (4) would result in levels NMT (b) (4) mcg/day when Suboxone (b) (4) is used as labeled.

No drug-drug interactions with Suboxone are predicted to occur. The Applicant conducted an *in vitro* study assessing the interaction of BUP and its metabolite nor-BUP with several cytochrome P450s in human liver and in cDNA expressed microsomes. Although BUP and nor-BUP were shown to be competitive inhibitors of CYP2D6 and BUP was shown to be a competitive inhibitor of CYP3A4, plasma concentrations in the therapeutic range are unlikely to cause clinically significant inhibition of CYP2D6 or CYP3A in patients. The Applicant also demonstrated that BUP and nor-BUP do not bind to either central or peripheral benzodiazepine receptors.

The Applicant conducted a 2-year carcinogenicity assessment in the rat with Suboxone. Treatment-related Leydig cell adenomas were observed at all doses tested. The results of this study confirm the findings of Leydig cell tumors that were seen in a carcinogenicity assessment in rats conducted with BUP alone for the Subutex NDA. The findings of Leydig cell tumors from the BUP study are described in the current Suboxone/Subutex label. The findings of Leydig cell tumors from the Suboxone carcinogenicity study as well as the BUP study will be included in the Suboxone (b) (4) label. The relevance of these findings to clinical use of Suboxone (b) (4) is unknown. No new clinical safety issues with Suboxone (b) (4) as compared to the currently marketed Suboxone/Subutex products have arisen.

Unresolved toxicology issues (if any): There are no unresolved toxicology issues. There are no recommendations for nonclinical studies.

Recommendations: This NDA can be approved from a nonclinical pharmacology/toxicology perspective.

Suggested labeling:

The table below contains the draft labeling submitted by the Applicant, the proposed changes and the rationale for the proposed changes. For the final version of the label, please refer to the Action Letter. Note: The recommended changes from the proposed labeling are in red or strikeout font.

Applicant's proposed labeling	Reviewer's proposed changes	Rationale for changes
8 USE IN SPECIFIC POPULATIONS **8.1 Pregnancy** Pregnancy Category C. (b) (4)	**8 USE IN SPECIFIC POPULATIONS** **8.1 Pregnancy** Pregnancy Category C. (b) (4)	no changes to this section.
Teratogenic effects: Effects on embryo-fetal development were studied in Sprague-Dawley rats and Russian white rabbits following oral (1:1) and intramuscular (IM) (3:2) administration of mixtures of buprenorphine and naloxone. Following oral administration to rats and rabbits, no teratogenic effects were observed at buprenorphine doses up to 250 mg/kg/day and 40 mg/kg/day, respectively (estimated exposure approximately 150 times and 50 times, respectively, the recommended human daily sublingual dose of 16 mg on a mg/m² basis). No definitive drug-related teratogenic effects were observed in rats and rabbits at IM doses up to 30 mg/kg/day (estimated exposure approximately 20 times and 35 times, respectively, the recommended human daily dose of 16 mg on a mg/m² basis). Acephalus was observed in one rabbit fetus from the low-dose group and omphacele 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 of buprenorphine to rats, 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 mg/kg/day or greater	***Teratogenic effects:*** Effects on embryo-fetal development were studied in Sprague-Dawley rats and Russian white rabbits following oral (1:1) and intramuscular (IM) (3:2) administration of mixtures of buprenorphine and naloxone. Following oral administration to rats and rabbits, no teratogenic effects were observed at buprenorphine doses up to 250 mg/kg/day and 40 mg/kg/day, respectively (estimated exposure approximately 150 times and 50 times, respectively, the recommended human daily sublingual dose of 16 mg on a mg/m² basis). No definitive drug-related teratogenic effects were observed in rats and rabbits at IM doses up to 30 mg/kg/day (estimated exposure approximately 20 times and 35 times, respectively, the recommended human daily dose of 16 mg on a mg/m² basis). Acephalus was observed in one rabbit fetus from the low-dose group and omphacele 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 of buprenorphine to rats, 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 mg/kg/day or greater	

(estimated exposure approximately 6 times the recommended human daily sublingual dose of 16 mg on a mg/m² basis). In the rabbit, increased post implantation losses occurred at an oral dose of 40 mg/kg/day. Following IM administration in the rat and the rabbit, post-implantation losses, as evidenced by decreases in live fetuses and increases in resorptions, occurred at 30 mg/kg/day. 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 intravenous (IV) doses of 0.2 mg/kg/day or greater (estimated exposure approximately 0.3 times the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis). ***Non-teratogenic effects:*** Dystocia was noted in pregnant rats treated intramuscularly with buprenorphine 5 mg/kg/day (approximately 3 times the recommended human daily sublingual dose of 16 mg on a mg/m² basis). (b) (4) fertility, peri-, and post-natal 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/m² 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/m² basis), and after subcutaneous 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/m² 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/m² basis).	(estimated exposure approximately 6 times the recommended human daily sublingual dose of 16 mg on a mg/m² basis). In the rabbit, increased post implantation losses occurred at an oral dose of 40 mg/kg/day. Following IM administration in the rat and the rabbit, post-implantation losses, as evidenced by decreases in live fetuses and increases in resorptions, occurred at 30 mg/kg/day. 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 intravenous (IV) doses of 0.2 mg/kg/day or greater (estimated exposure approximately 0.3 times the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis). ***Non-teratogenic effects:*** Dystocia was noted in pregnant rats treated intramuscularly with buprenorphine 5 mg/kg/day (approximately 3 times the recommended human daily sublingual dose of 16 mg on a mg/m² basis). (b) (4) fertility, peri-, and post-natal 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/m² 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/m² basis), and after subcutaneous 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/m² 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/m² basis).	
13 NONCLINICAL TOXICOLOGY **13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility** ***Carcinogenicity:***	**13 NONCLINICAL TOXICOLOGY** **13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility** ***Carcinogenicity:***	

Carcinogenicity data on SUBOXONE (b) (4) are not available. (b) (4)	Carcinogenicity data on SUBOXONE (b) (4) are not available. A carcinogenicity study of buprenorphine/naloxone (4:1 ratio of the free bases) was performed in Alderley Park rats. Buprenorphine/naloxone was administered in the diet at doses of approximately 7, 31, and 123 mg/kg/day for 104 weeks (estimated exposure was approximately (b) (4) 4, 18 and 44 times the (b) (4) recommended human sublingual dose of (b) (4) 16/4 mg buprenorphine/naloxone based on buprenorphine AUC comparisons (b) (4) A statistically significant increase in Leydig cell adenomas was observed in all dose groups. (b) (4) other drug-related tumors were noted.	Alderley is misspelled AUC comparisons were added
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/m^2 basis) for 27 months. (b) (4) 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/m^2 basis). ***Mutagenicity:*** The 4:1 combination of buprenorphine and naloxone was not mutagenic in a	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/m^2 basis) for 27 months. As in the buprenorphine/naloxone carcinogenicity study in rat, statistically significant dose-related increases in (b) (4) Leydig cell tumors occurred. (b) (4) 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/m^2 basis). ***Mutagenicity:*** 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.	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. 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 (*S. 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 [^{3}H]thymidine, and positive in unscheduled DNA synthesis (UDS) test using testicular cells from mice.	Buprenorphine mutagenicity data from the Suboxone/Subutex label were added in because they include additional positive findings
Impairment of Fertility: Dietary administration of buprenorphine in the rat at dose levels of 500 ppm or greater (equivalent to approximately 47 mg/kg/day or greater; estimated exposure approximately 28 times the recommended human daily sublingual dose of 16 mg on a mg/m^2 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 approximately 6 times the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis) had no adverse effect on fertility.	***Impairment of Fertility:*** Dietary administration of buprenorphine in the rat at dose levels of 500 ppm or greater (equivalent to approximately 47 mg/kg/day or greater; estimated exposure approximately 28 times the recommended human daily sublingual dose of 16 mg on a mg/m^2 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 approximately 6 times the recommended human daily sublingual dose of 16 mg on a mg/m^2 basis) had no adverse effect on fertility.	

APPENDIX/ATTACHMENTS

Appendix 1: ECAC Meeting Minutes from March 28, 2000 for protocol review

I. Executive CAC

March 28, 2000

Committee: Joseph F. Contrera, Ph.D., HFD-901, Acting Chair
Nakissa Sadrieh, Ph.D., HFD-160, Alternate Member
Glenna Fitzgerald, Ph.D., HFD-120, Alternate Member
Lucy Jean, Ph.D., HFD-170, Team Leader
Anwar Goheer, Ph.D., HFD-170, Presenting Reviewer

Author of Draft: Anwar Goheer

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA # **20-733**
Drug Name: **Suboxone (buprenorphine HCl and naloxone HCl dihydrate at a 4:1 buprenorphine : naloxone ratio as bases)**
Sponsor: **Reckitt & Colman Pharmaceuticals, Inc., Richmond, VA 23235. Telephone (804) 379-1090, Fax (804) 379-1215.**

Background:

Suboxone, sublingual tablets, is indicated for the treatment of opioid dependence. Naloxone is added to prevent abuse and 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 was a statistically significant increase in testicular interstitial (Leydig's) cell tumors based on the trend test adjusted for survival. Pairwise 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. Suboxone is not mutagenic in Ames, human lymphocyte and rat micronucleus assays.

A 13-week dose range finding toxicity study to support a future 2-year carcinogenicity study was carried out in male and female rats. Animals received suboxone in the diet at concentrations of 0 (control), 100, 500, 1500 or 2000 ppm (approximately 10, 50, 150 or 200 mg/kg/day). Satellite groups were fed appropriate diet for 13 consecutive weeks for toxicokinetics.

The study showed that males were more susceptible to effects on weight and appetite 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. These decreases in body weight gain were accompanied by the reduction of food consumption. In females, there was no effect on body weight gain or food consumption. No mortality in either sex was observed.

The MTD could not be used because there was no target organ toxicity, no effects on body weight gain or food consumption in females, the decreases of body weight gain in males were related to the decrease of food consumption, and there were no drug-related clinical signs that can limit the dosing.

The AUC values of buprenorphine in female and male rats at 2000 ppm of Suboxone (1424 & 1580 hr.ng/ml, respectively) are approximately 43-fold higher than the human AUC (34.89 hr ng/mL) following single administration of 16 mg of Suboxone, a recommended human daily dose. The human AUC of naloxone from Suboxone is not available due to low oral bioavailability, consistent with the intent for naloxone not to contribute any activity following sublingual use, yet prevent illicit parenteral use of Suboxone. In rats, the mean plasma concentrations of naloxone at 2000 ppm are approximately 10-fold higher than the maximum concentration of naloxone detected in humans

Executive CAC Recommendations and Conclusions:

(1) The Committee concurred with the sponsor's proposed doses (100, 450 and1800 ppm i.e. approximately 5, 22.5 and 90 mg/kg/day) for the two-year carcinogenicity study in rats based on AUC values of buprenorphine in female and male rats at 2000 ppm of Suboxone (1424 & 1580 hr.ng/ml, respectively) that are approximately 43-fold higher than the human AUC (34.89 hr.ng/mL) following single administration of 16 mg of Suboxone, a recommended human daily dose.

(2) If the sponsor plans histological evaluation of tissues from only control and high dose treatment groups, they will also need to conduct histopathologic examination of other dose groups under any of the following circumstances:

(a) For any macroscopic findings in the low and mid dose groups for a given tissue, they will need to look at that tissue for all of the dose groups.
(b) For an increase in the incidence of tumors (rare or common) in the high dose group for a tissue, even if not statistically significant, they will also need to look at the next lower dose group.
(c) For an increase in tumors in an organ for a tumor type that should be analyzed across tissue sites as well as by tissue site (e.g., hemangiosarcoma, lymphoma etc.; see McConnell et al, JNCI 76:283, 1986) they should look at all relevant tissues for that dose level and the next lower dose level.
(d) For an excessive decrease in body weight or survival in the examined dose group, they should examine lower dose groups.

(3) The division recommends that animals be housed individually to manage the adverse effects of aggression (fighting behavior) seen in the 28-day palatability study and 90-day dietary toxicity study.

Joseph F. Contrera, Ph.D.
Acting Chair, Executive CAC.

cc: \
/Division File, HFD-170
/Lucy Jean, HFD-170
/Anwar Goheer, HFD-170
/ASeifried, HFD-024

Appendix 2: ECAC Meeting Minutes from May 12, 2009 for study report

Executive CAC
Date of Meeting: May 12, 2009

Committee: David Jacobson Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Karen Davis-Bruno, Ph.D., DMEP, Alternate Member
Dan Mellon, Ph.D., DAARP, Supervisor
Elizabeth A. Bolan, Ph.D., DAARP, Presenting Reviewer

Author of Minutes: Elizabeth Bolan, Ph.D., DAARP

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA # : 22-410
Drug Name: Suboxone (b) (4) (buprenorphine/naloxone SL film strip)
Sponsor: Reckitt Benckiser

Background
Suboxone (b) (4) is a 4:1 fixed combination of buprenorphine and naloxone in a sublingual soluble film strip formulation indicated for the treatment of opioid abuse. Buprenorphine is a partial mu opioid receptor agonist and a kappa opioid receptor antagonist. Naloxone is a nonspecific opioid receptor antagonist.

At low doses buprenorphine produces sufficient agonist effect to enable opioid-addicted individuals to discontinue the misuse of opioids without experiencing withdrawal symptoms. The presence of naloxone in the formulation serves as a means to try to prevent abuse of the product. Naloxone is rapidly metabolized via the oral and sublingual routes resulting in low bioavailability, however, with parenteral administration, as in an abuse situation, the naloxone is bioavailable to block the effects of the buprenorphine.

Study findings
The dose selection for both males and females for this carcinogenicity assessment was based on AUC comparisons from a 13-week dose range finding study with the clinical dose of a single administration of 16/4 mg of Suboxone (BUP/NLX). The proposed doses used in this study received ECAC concurrence (March 28, 2000).

Suboxone (4:1 BUP/NLX) was administered to Alderley Park rats at doses of 100, 450, and 1800 ppm (5, 22.5, and 90 mg/kg/day) for 2 years via dietary administration. Two identical control groups were utilized. Toxicokinetic analysis was conducted after approximately six months administration of Suboxone. The systemic levels of NLX were

extremely low in both rat and human. Exposure to BUP was similar in male and female rats. The exposure margins for the sexes combined based on BUP AUC comparisons with a human dose of 16/4 mg/mg BUP/NLX are 4x at the LD, 18x at the MD and 44x at the HD.

In pairwise comparisons, unilateral benign Leydig cell (testes) adenomas reached statistical significance at the HD (p= 0.008) and bilateral benign Leydig cell (testes) adenomas reached statistical significance all doses (all p<0.001; Table 1). The trend analysis reached statistical significance for both unilateral (p=0.01) and bilateral (p<0.001) Leydig cell adenomas. All doses for both unilateral and bilateral adenomas showed increased incidence over historical controls averaged over the past five years for unilateral and bilateral Leydig cell tumors combined. These neoplasms are considered treatment-related. Various other neoplasms were observed in the treated groups with incidences similar to concurrent controls and/or similar to levels observed in the historical controls. None of these tumors were considered to be treatment-related. No other treatment-related neoplasms were observed in males and no treatment-related neoplasms were observed in females.

Table 1 Leydig cell adenomas (benign) in males

	Control 1	***Control 2***	***100 ppm***	***450 ppm***	***1800 ppm***	***Trend analysis***
n	***52***	***52***	***52***	***52***	***52***	-
unilateral	6 (10%)	3 (6%)	10 (19%)	7 (13%)	***14* (27%)***	***p= 0.01***
bilateral	0 (0%)	1 (2%)	***11** (21%)***	***18** (35%)***	***19** (37%)***	***p< 0.001***
combined	6 (12%)	4 (8%)	21 (40%)	25 (48%)	33 (63%)	ND

*p=0.008; **p<0.001 (Fisher's Exact Test)
Trend analysis: Cochran-Armitage test
Historical Control combined unilateral and bilateral: 7.2%
ND= not done

Executive CAC Recommendations and Conclusions:

- The committee agreed that the study was adequate, noting prior Exec CAC protocol concurrence.
- The committee found that the carcinogenicity study was positive for Leydig cell adenomas in male rats
- The committee agreed that no other treatment-related neoplasms were observed in male rats and no treatment related neoplasms were observed in female rats.

David Jacobson Kram, Ph.D.
Chair, Executive CAC

cc:\
/Division File, DAARP, NDA 22-410
/Team leader, DMellon, DAARP
/Reviewer, EBolan, DAARP
/PM, MSullivan, DAARP
/DJacobson-Kram, OND IO
/AJacobs, OND IO
/ASeifried, OND IO

REFERENCES

Ames BN, McCann J and Yamasaki E (1975) Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat Res 31:347-364.

Ibrahim RB, Wilson JG, Thorsby ME and Edwards DJ (2000) Effect of buprenorphine on CYP3A activity in rat and human liver microsomes. Life Sci 66:1293-1298.

Kobayashi K, Yamamoto T, Chiba K, Tani M, Shimada N, Ishizaki T and Kuroiwa Y (1998) Human buprenorphine N-dealkylation is catalyzed by cytochrome P450 3A4. Drug Metab Dispos 26:818-821.
Maron DM and Ames BN (1983) Revised methods for the Salmonella mutagenicity test. Mutat Res 113:173-215.

McConnell EE, Solleveld HA, Swenberg JA and Boorman GA (1986) Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. J Natl Cancer Inst 76:283-289.

Megarbane B, Hreiche R, Pirnay S, Marie N and Baud FJ (2006) Does high-dose buprenorphine cause respiratory depression?: possible mechanisms and therapeutic consequences. Toxicol Rev 25:79-85.

Megarbane B, Pirnay S, Borron SW, Trout H, Monier C, Risede P, Boschi G and Baud FJ (2005) Flunitrazepam does not alter cerebral distribution of buprenorphine in the rat. Toxicol Lett 157:211-219.

Scott D, Danford N, Dean B and Kirkland D (1990) Metaphase Chromosome Aberration Assays In Vitro, in Basic Mutagenicity Tests: UKEMS Recommended Procedures (Kirkland D ed) pp 62-86, Cambridge University Press, Cambridge.

Shimada T, Yamazaki H, Mimura M, Inui Y and Guengerich FP (1994) Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. J Pharmacol Exp Ther 270:414-423.

Venitt S and Crofton-Sleigh C (1979) Bacterial mutagenicity tests of phenazine methosulphate and three tetrazolium salts. Mutat Res 68:107-116.

Vogel H and Bonner D (1956) Acetylornithinase of Escherichia coli: partial purification and some properties. J Biol Chem 218:97-106.

Ritschel WA (1986) Multiple Dose Pharmacokinetics, in Handbook of Basic Pharmacokinetics pp 302-331, Drug Intellegence Publications, Inc., Hamilton, IL.

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/s/

Elizabeth Bolan
5/22/2009 03:42:47 PM
PHARMACOLOGIST

R. Daniel Mellon
5/22/2009 03:50:22 PM
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I concur.