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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 NDA REVIEW AND EVALUATION

Application number:	202245
Supporting document/s:	SDN-1 (Original Application; electronic)
Applicant's letter date:	September 27, 2010
CDER stamp date:	September 27, 2010
Product:	Codeine Sulfate Oral Solution (30 mg/5 mL)
Indication:	Mild to moderately severe pain
Applicant:	Roxane Laboratories
Review Division:	Division of Anesthesia, Analgesia, and Addiction Products
Reviewer:	Marcus S. Delatte, Ph.D.
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1 Executive Summary

1.1 Recommendations

1.1.1 Approvability

NDA 202245 may be approved from a nonclinical pharmacology and toxicology perspective.

1.1.2 Additional Non Clinical Recommendations

None.

1.1.3 Labeling

The Reviewer did not identify any issues in the proposed label, which is identical to Roxane's codeine tablet drug product label. No changes are recommended.

1.2 Brief Discussion of Nonclinical Findings

Brief overview of nonclinical findings

The Agency has previously approved a single entity codeine tablet from Roxanne Laboratories, Inc. (i.e., codeine sulfate 15, 30, and 60 mg) for the treatment of mild to moderate pain. Prior to the approval of this product, the Sponsor agreed to **postmarketing requirements** to qualify the drug substance impurity (b) (4) ((b) (4) since its proposed specification of NMT (b) (4) exceeded the ICH Q3A(R2) qualification threshold. (b) (4) has been reported to be a known impurity of codeine; however, the Sponsor did not provided adequate safety qualification for this impurity at the time of the application review period. Findings from the postmarketing studies were reviewed and discussed below. Briefly, the Sponsor provided data from rats in two separate repeat-dose toxicology studies; as well as data from four genetic toxicology studies. In the two-week repeat-dose toxicology study, a NOAEL estimated in rats at (b) (4) (b) (4) based on the observation of clinical signs that included protruded and constricted eyes, and hypoactivity across sexes at 100 mg/kg group; as well as decreased body weight in males at the same dose. In the thirteen-week repeat-dose toxicology study, a NOAEL was estimated at (b) (4) in males and a LOAEL was estimated at (b) (4) in females. The NOAEL in males was based on the observation of tremors and the reduction of body weight at ≥ 30 mg/kg, as well as the observation of mortalities (n=2) at 60 mg/kg. The LOAEL estimated in females was based on the observation of tremors

at all of the doses tested; as well as mortality at 100 mg/kg (n=1). In the genetic toxicology studies, two in vitro assays and two in vivo assays were employed. Findings in the in vitro studies demonstrated that (b) (4) was not mutagenic in the Ames assay; whereas it was clastogenic in the chromosome aberrations assay. In the chromosome aberration assay, (b) (4) induced chromosomal aberrations in lymphocytes in the presence of metabolic activation (three hour treatment). Given these findings, in vivo genetic toxicology tests were conducted in rats as part of an evaluation of the toxicological significance of the positive in vitro findings. In the in vivo studies, (b) (4) was deemed negative in the induction of micronuclei in bone marrow and the induction of DNA damage in the liver, stomach, jejunum, and blood in rats; which suggested that the positive findings in the in vitro chromosome aberration assay were not toxicologically significant. See below for further details on the methods and results from these studies evaluating (b) (4) as well as findings from published studies evaluating the pharmacological and toxicological effects of codeine in various animal species.

Pharmacologic activity

The Sponsor did not conduct formal pharmacological studies in support of the submitted NDA application. The application is partly supported by published nonclinical studies demonstrating the therapeutic potential of codeine. Codeine has analgesic and antitussive properties that have been demonstrated in experimental animal species (for further information see Adcock JJ et al., 1988; Chau TT and Harris LS, 1980; Erichsen, H. K. et al., 2005; Meert TF and Vermeirsch HA, 2005). Studies have examined the receptor pharmacology of codeine, in regard to these properties, as well as its binding affinity to mu- and delta-opioid receptors (Kotzer CJ et al., 2000; for review see, Trescot AM et al., 2008).

The pharmacology of codeine has been thoroughly studied using various animal models to demonstrate its efficacy as an analgesic and antitussive agent. Previous studies have demonstrated that codeine produces anti-nociceptive effects in mice, rats, and guinea pigs using a variety of tests (Adcock JJ et al., 1988; Chau TT and Harris LS, 1980; Meert TF and Vermeirsch HA, 2005). For example, the anti-nociceptive effects of codeine in rats have been demonstrated in studies using the formalin, tail withdrawal, Von Frey, as well as other experimental tests (Erichsen, H. K. et al., 2005; Meert TF and Vermeirsch HA, 2005). Studies have demonstrated that codeine produces antitussive effects in cats, guinea pigs and mice using variety of approaches to stimulate cough reflex (Chau TT and Harris LS, 1980; Kotzer CJ et al., 2000; Saha K et al., 1997). The antitussive effects of codeine have been demonstrated to be dose-related and reversed by opioid-receptor antagonists such as naloxone (Karlsson JA et al., 1990; Kotzer CJ et al., 2000).

Nonclinical safety issues relevant to clinical use

There are no new nonclinical safety issues relevant to this drug product. Nonclinical studies have previously demonstrated that codeine in experimental animals alters cardiovascular, respiratory, and gastrointestinal function, which is consistent with clinical

findings (Adcock JJ et al., 1988; Chau TT and Harris LS, 1980; Liguori A et al., 1996; Meert TF and Vermeirsch HA, 2005). In general, the magnitude of these effects produced by codeine is lower than those produced by the prototypical opioid analgesic morphine. For example, gastrointestinal studies have demonstrated that the peristalsis ratio in rats was reduced to approximately 40% and 20%, respectively, by codeine and morphine at 40 mg/kg (Meert TF and Vermeirsch HA, 2005). These safety issues are typical of opioid agonists and are monitorable in clinical settings.

2 Drug Information

2.1 Drug

Table 1. Drug Information

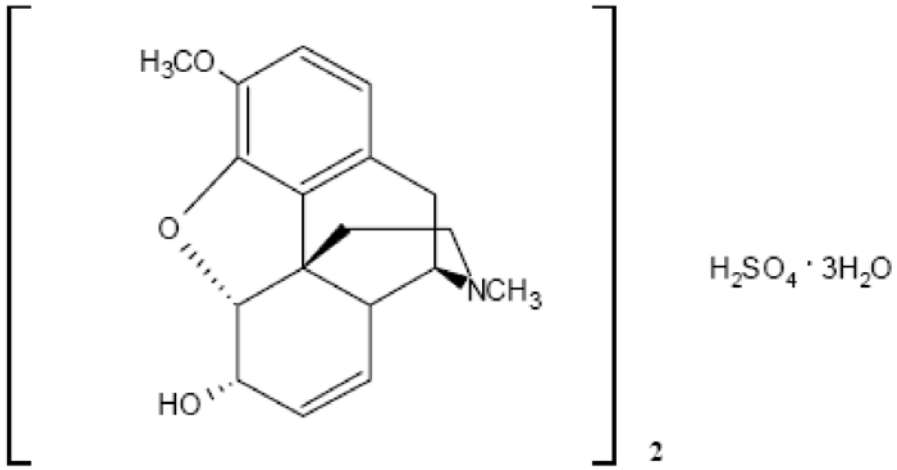
Generic Name	**Codeine Sulfate (Oral Solution)**
Pharmacological Class	Opioid agonist
Structure	 $H_2SO_4 \cdot 3H_2O$
CAS Registry#	6854-40-6
Molecular Weight	750.85
Molecular Formula	$C_{18}H_{21}NO_3 \cdot H_2SO_4 \cdot 3H_2O$
Chemical Name	Morphinan-6-ol, 7, 8-didehydro-4,5-epoxy-3-methoxy-17-methyl-,(5alpha, 6alpha)-, sulfate (2:1) (salt), trihydrate

Table 2. Relevant NDA Applications

NDA#	Product Name	Route	Formulation	Codeine Strength	Approval Date
22-402	Codeine Sulfate	Oral	Tablet	15, 30, and 60 mg	16 Jul 2009

Table 3. Relevant Master Files (MFs)

MF#	Subject of MF	Holder
(b) (4)	Type II for Codeine Sulfate	(b) (4)
	Type III (b) (4)	
	Type III (b) (4)	
	Type III (b) (4)	
	Type III (b) (4)	
	Type III (b) (4)	
	Type III (b) (4)	
	Type III (b) (4)	
	XBF-709818 Orange	

Table 4. Relevant IND applications

IND #	Sponsorship	Product Name	Indication	Division	Status	Submit Date
75,764	Roxane Laboratories, Inc.	Codeine Sulfate Tablets	(b) (4) of mild to moderate pain	DAAP	PreIND	17 July 2006

2.3 Clinical Formulation

2.3.1 Drug Formulation

The drug product is formulated as an oral solution. See **Table 5** for the ingredients used. The Sponsor noted that none of the excipients in the formulation are from human or animal origin.

As noted in the table below, there are no novel excipients in this drug product formulation. Although the maximum daily dose for an opioid is not clearly identified, the Division has concluded that a reasonable maximum theoretical daily dose (MTDD) is 360 mg of codeine via this formulation. Although the daily exposure to many of these excipients is greater than the Maximum Potency Listed in the Inactive Ingredient Database (IID), the Division has been able to identify adequate coverage for the MTDD via cross reference with the Drug Product Reference File.

Table 5. Drug Product Ingredients

Ingredients	Purpose	Amount (mg/mL) per administration	Maximum exposure (based on 360 mg amount in 60 mL volume)
Codeine Sulfate, USP	Active Ingredient	6 mg	360 mg
Sorbitol Solution, USP (b) (4)	(b) (4)	200 mg	12,000 mg
Glycerin, USP		250 mg	15,000 mg
Ascorbic Acid, USP		2 mg	120 mg
Citric Acid (b) (4) USP (b) (4)		2 mg	120 mg
Disodium Edetate, USP		0.75 mg	45 mg
Sucralose, NF (b) (4)		1.5 mg	90 mg
Sodium Benzoate, NF (b) (4)		1 mg	60 mg
FD&C Yellow No. 6 (b) (4)	Coloring	0.01 mg	0.6 mg
FD&C Red No. 40	Coloring	0.01 mg	0.6 mg
Orange Flavor, XBF-709818	Flavor	2.9 mg	174 mg

Table 6. Components of Orange Flavor, XBF-709818

(b) (4)

(b) (4)

Extractable/Leachable Studies

The container closure system has been previously used in numerous FDA-approved drug products. Nonetheless, additional extractable/leachable data were submitted. These studies (**report/study no. 1674-011**) were conducted to determine an extractables/leachables profile for the PET bottles (500 mL; Item NC 1006471) and caps (Item 10005814) for the Codeine Sulfate (30 mg/5 mL) drug product. For extraction studies, the Sponsor reportedly extracted the packaging components by (b) (4) [redacted] Separately, leachable studies were conducted to determine if materials with peaks that correspond to those detected in the extractable study were detected. Leachable studies were conducted using drug product stored for three months under accelerated conditions (40°C/75% RH SIDE). Based on results from these studies, the Sponsor reported that several peaks were detected in the (b) (4) [redacted] in the extractable studies; but that none of them were detected in the leachables study. There were no leachable materials detected in samples of the drug product (lot G1928-80B) at levels (b) (4) [redacted] versus the caffeine reference employed. There are no nonclinical safety concerns with the proposed drug product container closure system.

2.3.2 Comments on Novel Excipients

There were no novel excipients identified in the drug product.

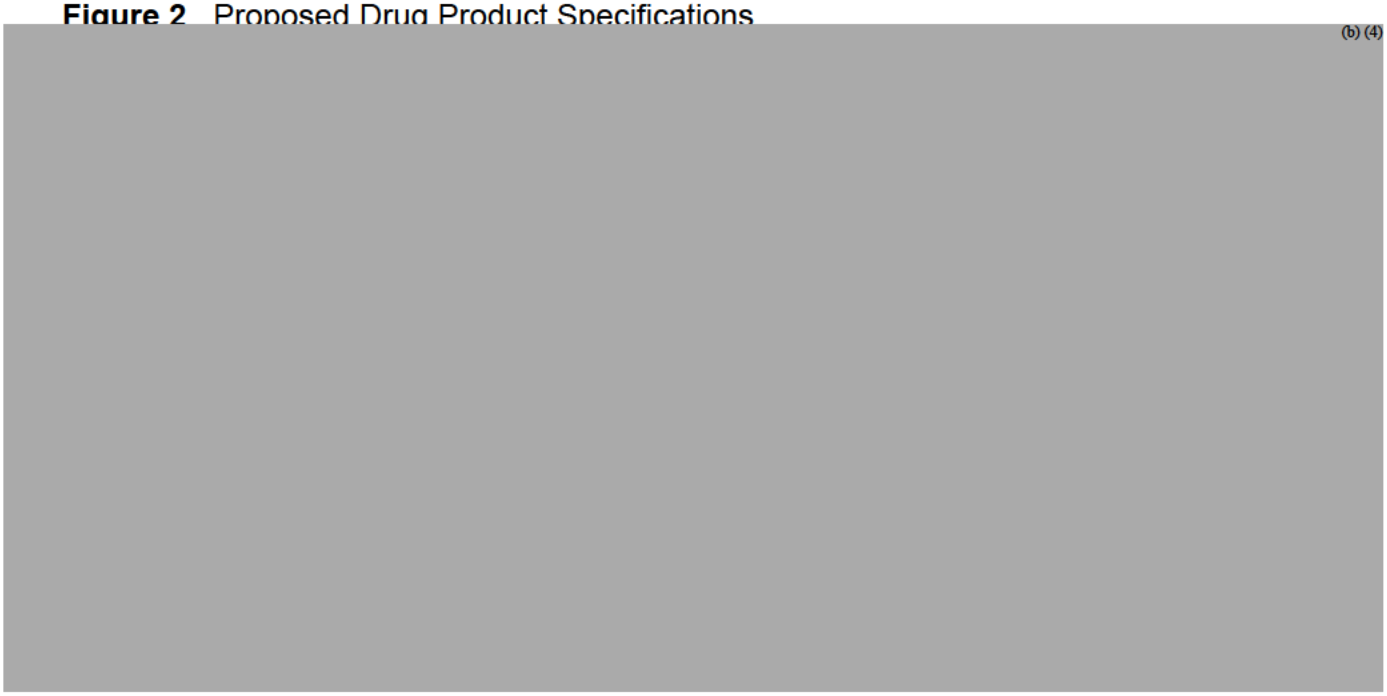
2.3.3 Comments on Impurities/Degradants of Concern

There were no impurities/degradants of concern identified by the Reviewer. Those reported were at or below that found in the Sponsor's codeine sulfate tablet product that is approved by the Agency (see NDA 22-402 pharmacology/toxicology review).

Figure 1. Proposed Drug Substance Specifications

As per ICHQ3A(R2), for a drug with a maximum daily dose (MDD) of ≤ 2 grams per day, the drug substance impurity qualification threshold is NTM (b) (4) (b) (4) For this drug product, a reasonable MDD is deemed 360 mg/day; therefore, (b) (4) With the exception of (b) (4) ((b) (4) all specifications are in accordance with ICHQ3A(R2). Adequate safety justification for the (b) (4) specification was provided in this NDA. The proposed specification of NMT (b) (4) is acceptable.

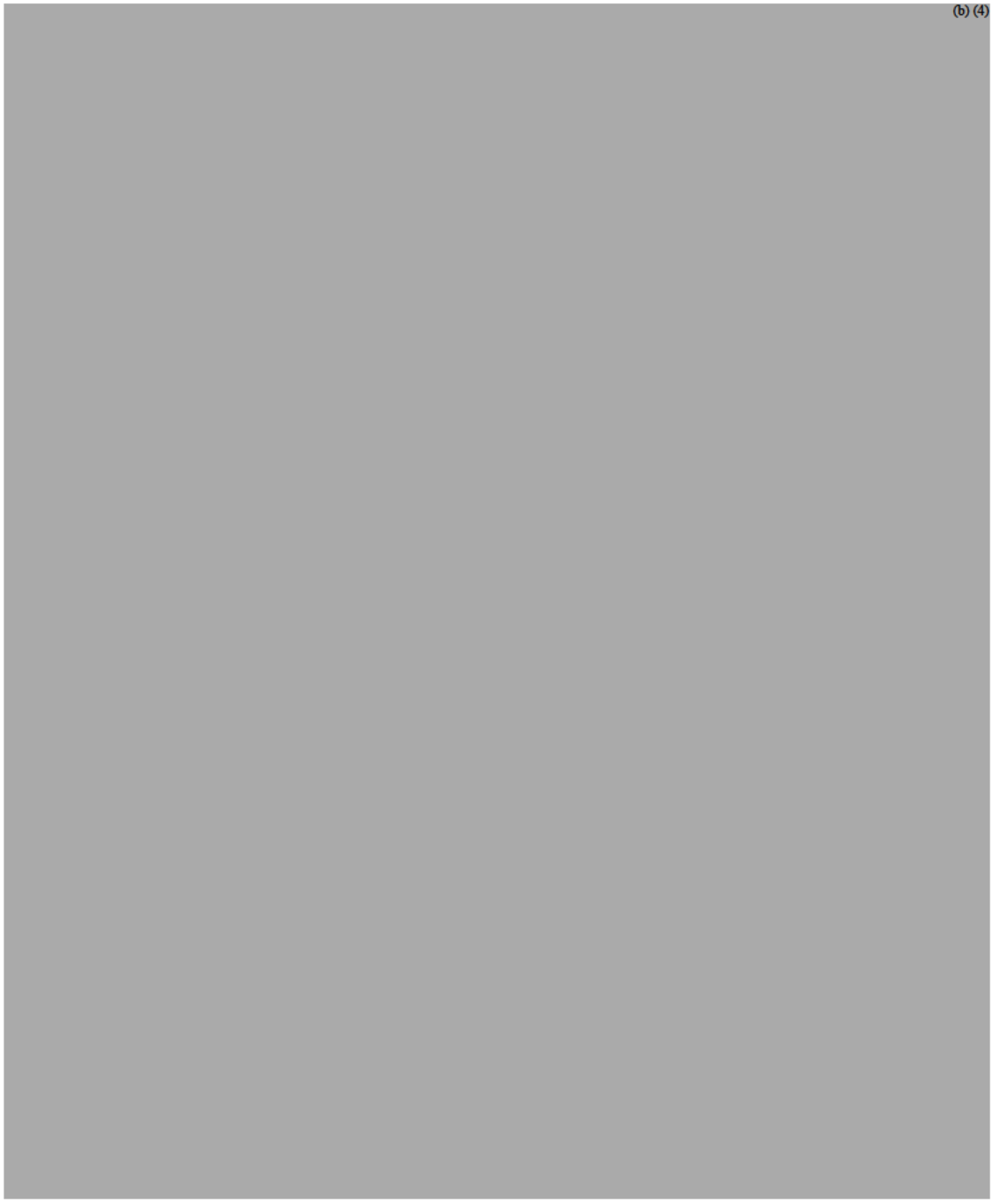
Figure 2 Proposed Drug Product Specifications



As per ICHQ3B(R2), the impurity qualification threshold for a drug with a maximum daily dose > 100 mg – 2 g is 0.2% or 3 mg/day (whichever is lower). For a drug with an MDD of 360 mg, (b) (4) (b) (4) contains and (b) (4) (b) (4) which is a structural alert for mutagenicity. However, adequate safety justification was provided for this impurity as part of NDA 22402. The proposed specifications are acceptable.

Figure 3. Impurities And Degradation Products Identified In The Drug Product

IMPURITIES AND DEGRADATION PRODUCTS



2.4 Proposed Clinical Population and Dosing Regimen

Codeine oral solution is intended for adult patients only at this time. Like any opioid, the dosing regimen will be individually tailored to take into account the patient's previous

opioid analgesic experience. As per the proposed product labeling, “The usual adult dosage is 15 mg to 60 mg (2.5 mL to 10 mL) repeated up to every four hours as needed for pain. The maximum 24 hour dose is 360 mg.”

2.5 Regulatory Background

Based on a search of DARRTS, there were no record of any formal meetings between the Agency and the Sponsor to discuss this application. For information on relevant NDAs, INDs and DMFs please see **Tables 2-4**.

3 Studies Submitted

3.1 Studies Reviewed

The Sponsor referenced the studies listed in **Table 7**. Note that the repeat-dose toxicology and genetic toxicology studies noted were originally submitted by the Sponsor for review under NDA 22402, as part of post-marketing requirements agreed to with the Agency (prior to the marking approval of codeine sulfate tablets).

Table 7. Studies Reviewed

Study Title	Study#	Electronic submission
Extractables/Leachables		
Results for the Extractables/Leachables Study for PET Bottles and Caps for Codeine Sulfate Oral Solution, 30 mg/5 mL	1674-011	3.2.P.2
Repeat-dose Toxicology		
2-Week Non-GLP Toxicity Oral Gavage Study in Rats with (b) (4)	88894F-1 (Proposal) 8218703 (b) (4)	4.2.3.2.1
13-week Toxicity and Toxicokinetics Oral Gavage Study in Rats with (b) (4)	8218694 (b) (4)	4.2.3.2.1
Genetic Toxicology		
Chromosomal Abberations in Cultured Human Peripheral Blood Lymphocytes (in vitro)	8218266	4.2.3.3.1
Bacterial Reverse Mutation Assay with a Confirmatory Assay (in vitro)	8218265	4.2.3.3.1
In Vivo Rat Bone Marrow Micronucleus Assay with Liver, Stomach, Jejunum, and Blood Comet Assay	8222569	4.2.3.3.1

4 Pharmacology

4.1 Primary Pharmacology

Codeine. Codeine is a mu-opioid agonist that has been demonstrated in experimental animals to produce a variety of physiological and behavioral effects (Karlsson JA et al., 1990; Liguori A et al., 1996; Meert TF and Vermeirsch HA, 2005). For example, codeine administered to rats produced antinociception, increased constipation, and decreased gastrointestinal motility and respiration across a range of doses (Meert TF and Vermeirsch HA, 2005). Note that codeine is an opioid receptor agonist and its effects at this site are blocked by naltrexone.

4.2 Secondary Pharmacology

Secondary pharmacodynamics: The secondary pharmacodynamic effects of codeine in experimental animal species include decreased locomotor behavior, respiration and GI

motility; as well as increased abuse liability (Carney JM et al., 1976; Liguori A et al., 1996; Meert TF and Vermeirsch HA, 2005; Teiger DG, 1974).

4.3 Safety Pharmacology

Published studies have demonstrated in experimental animals that codeine decreases respiration, GI motility, and heart rate when administered by various routes (Adcock JJ et al., 1988; Chau TT and Harris LS, 1980; Liguori A et al., 1996; Meert TF and Vermeirsch HA, 2005). These effects are consistent with those produced by other opioid agonists such as morphine. Note that the magnitude of various effects produced by codeine in experimental animals have been demonstrated to be lower than that of opioid agonists such as morphine. For example, codeine subcutaneously administered to rats decreases respiration and GI motility, but the magnitude of this effect is lower than that produced by morphine (Meert TF and Vermeirsch HA, 2005).

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Note that the studies identified by the Reviewer evaluated the pharmacokinetic profile of codeine administered to experimental animals via various routes. See below for a brief summary of studies that have evaluated the pharmacokinetic profile of codeine.

Absorption. The absorption of codeine has been evaluated in species that include monkeys, dogs, and rats following its administration via various routes (Findlay JW et al., 1979; KuKanich, B., 2010; Shah J and Mason WD, 1990; WOODS LA et al., 1956). In particular, for findings on plasma levels of orally administered codeine in rats and in dogs see **Figures 4 and 5** (excerpts from Findlay JW et al., 1979; Shah J and Mason WD, 1990). In oral studies, codeine serum levels in these animal species peaked ≤ 1 hour following administration; and its absorption was low, based on bioavailability values that ranged from 4-8% in these animals (Findlay JW et al., 1979; KuKanich, B., 2010; Shah J and Mason WD, 1990).

Figure 4. Pharmacokinetic Findings In Rats Administered Codeine

TABLE 1

Pharmacokinetic parameters following intravenous (3 mg/kg) and oral (5 mg/kg) doses of codeine phosphate

All values are mean ± SD. $N = 6$.

Parameter	Intravenous	Oral
Codeine		
C_{max} (ng/ml)		101.3 ± 42.4
T_{max} (min)		6.4 ± 4.5
β (min^{-1})	0.021 ± 0.004	0.019 ± 0.005
$t_{½}$ (min)	34.1 ± 5.7	39.6 ± 10.7
$AUC_{0\text{-last}}$ (ng*min/ml)	29732 ± 6050	3936 ± 1762
$AUC_{0-\infty}$ (ng*min/ml)	30279 ± 6238	4074 ± 1673
CL_s (liter/kg/hr)	6.2 ± 1.5	6.3 ± 1.5
Vd_{area} (liter/kg)	5.1 ± 1.7	
MRT (min)	34.1 ± 6.9	58.3 ± 12.2
MAT (min)		22.4 ± 13.9
F (%)		8.3 ± 3.2
Morphine		
C_{max} (ng/ml)	24.8 ± 7.3	68.7 ± 32.6
T_{max} (min)	4.2 ± 4.1	6.5 ± 4.9
$AUC_{0\text{-last}}$ (ng*min/ml)	1387 ± 560	3656 ± 1839
$AUC_{morphine}/AUC_{codeine}$	0.05 ± 0.02	0.90 ± 0.19
MRT (min)	91.3 ± 36.7	83.5 ± 42.9

Figure 5. Pharmacokinetic Data In Dogs Orally Administered Codeine

Bioavailability and O-demethylation of codeine and hydrocodone in the dog

Dog No.	Alkaloid Administered	Free Parent Drug AUC[a]		Absolute % Bio-avail-ability[b]	Total Codeine AUC[a, c]	Metabolite[d] AUC[a]	
		po	iv			Free	Total[c]
1	Codeine	42	706	6	5464	6	13
	Hydrocodone	223	511	44		118	408
2	Codeine	46	631	7	4284	10	17
	Hydrocodone	211	615	34		107	748

[a] Area under the plasma concentration-time curve from 0–12 hr (ng·ml^{-1}·hr).

[b] % ratio of AUC (free drug po)/AUC (free drug iv).

[c] Data derived from β-glucuronidase-treated samples.

[d] Morphine or hydromorphone, in po studies.

Distribution. The Reviewer identified limited findings on the distribution of codeine in experimental animals (Hartvig, P. et al., 1984; KuKanich, B., 2010). In monkeys, intravenous codeine was reportedly taken up in the brain to a larger extent than morphine, a difference that may be due to the former being more lipophilic than the later (Hartvig, P. et al., 1984). Separately, intravenous codeine in dogs reportedly had a large volume of distribution (i.e., 3.17 L/kg). Note that these data are limited and do not provide any insight into the distribution of codeine to organs other than the brain.

Metabolism. The metabolic profile of codeine has been evaluated in species that include humans, dogs, rabbits, guinea pigs and rats (Cone EJ et al., 1979; Findlay, J. W. et al., 1977; WOODS LA et al., 1956). Cone et al. has published urinalysis findings demonstrating that the metabolite norcodeine is detected in all of these species; however, the metabolite hydrocodone is only detected in man, dog and guinea pig following codeine administration (Cone EJ et al., 1979). In humans, codeine is metabolized primarily by the liver (24-89%), with some metabolism also occurring in the brain and intestines. Approximately 50-70% of orally administered codeine is converted to codeine-6-glucuronide by UGT2B7. Codeine-6-glucuronide has similar affinity for the mu opioid receptor as morphine; however, it does not enter the brain to the same extent. Approximately 10-15% of codeine is N-demethylated to norcodeine by CYP 3A4. Norcodeine also has activity at the mu-opioid receptor comparable to codeine itself. Approximately 0-15% of codeine is O-demethylated to morphine by CYP2D6.

Excretion. The excretion of codeine has been studied in humans and various animal species (Oguri K et al., 1990; WOODS LA et al., 1956). Codeine is predominately excreted via renal elimination.

5.2 Toxicokinetics

Toxicokinetic studies were conducted in F344 rats (male/female) administered codeine (0, 400, 800, 1600 ppm) orally in NIH-07 feed available *ad libitum* over a two-year period (Yuan J et al., 1994). In blood samples taken, codeine plasma levels decreased between 1 week and 16 months of treatment and then increased at 24 months when measured. The plasma levels of codeine increased in a dose-related manner at the time points studied. Similarly, the bioavailability of codeine increased in a dose-related manner. Yuan et al. (1994) reported that based on the results from day 7 the bioavailability of codeine from the diet was estimated to be 10, 24, and 25% for the 400, 800, and 1,600 ppm dose groups, respectively. See **Figure 6** for findings (excerpt from Yuan J et al., 1994).

Figure 6. Codeine and Morphine Exposure Data From 2-Year Rat Feeding Study

Estimated AUC values for codeine in rat dosed feed codeine studies[a]

Sex	Dose in Feed	Day 7		Day 21		Day 90	
		Codeine[b]	Total Codeine[c]	Codeine	Total Codeine	Codeine	Total Codeine
	ppm	*ng·hr/ml*	*ng·hr/ml*	*ng·hr/ml*	*ng·hr/ml*	*ng·hr/ml*	*ng·hr/ml*
Male	400	350 ± 30	358 ± 33	143 ± 21	175 ± 37	224 ± 26	289 ± 26
	800	1,566 ± 117	1,567 ± 120	635 ± 49	632 ± 46	1,237 ± 118	1,492 ± 144
	1600	4,894 ± 336	4,958 ± 387	2,668 ± 236	2,689 ± 234	1,681 ± 136	3,042 ± 150
Female	400	572 ± 41	581 ± 35	368 ± 24	395 ± 26	394 ± 29	639 ± 48
	800	1,633 ± 90	1,676 ± 104	1,319 ± 77	1,380 ± 84	985 ± 65	1,547 ± 101
	1600	4,820 ± 292	4,870 ± 270	3,631 ± 353	3,727 ± 380	3,350 ± 391	3,876 ± 395

[a] AUC values are expressed as mean ± SD for the 12-hr dark cycle period.
[b] Unconjugated codeine.
[c] Unconjugated and conjugated codeine.

Estimated AUC values for morphine in rat dosed feed codeine studies[a]

Sex	Dose in Feed	Day 7		Day 21		Day 90	
		Morphine[b]	Total Morphine[c]	Morphine	Total Morphine	Morphine	Total Morphine
	ppm	*ng·hr/ml*	*ng·hr/ml*	*mg·hr/ml*	*ng·hr/ml*	*ng·hr/ml*	*ng·hr/ml*
Male	400	427 ± 17	3,752 ± 296	472 ± 15	3,422 ± 122	368 ± 18	3,206 ± 368
	800	1,018 ± 32	13,728 ± 1,427	676 ± 22	3,944 ± 180	764 ± 30	7,050 ± 491
	1600	2,069 ± 155	24,970 ± 1,357	1,429 ± 135	15,692 ± 1,883	1,449 ± 35	13,102 ± 1,195
Female	400	382 ± 8	3,638 ± 143	394 ± 16	2,900 ± 87	275 ± 25	5,084 ± 514
	800	867 ± 76	18,236 ± 1,014	840 ± 36	3,778 ± 238	609 ± 27	12,002 ± 470
	1600	1,447 ± 97	22,506 ± 1,325	1,508 ± 105	13,944 ± 1,362	1,079 ± 89	24,254 ± 1,647

[a] AUC values are expressed as mean ± SD for the 12-hr dark cycle period.
[b] Unconjugated morphine.
[c] Unconjugated and conjugated morphine.

6 General Toxicology

6.1 Single-Dose Toxicity

Table 8. See For LD_{50} Values For Oral Codeine

Organism	LD_{50} value (mg/kg)	Source
Mouse	250	Medicinal Chemistry: A series of monographs. Vol. 5, Pg. 318, 1965
Rat	427	Journal of Medicinal Chemistry. Vol. 16, Pg. 782, 1973.

Source: ChemIDPlus Lite database.

6.2 Repeat-Dose Toxicity

The toxicity of codeine in experimental animal species has been evaluated in repeat-dose toxicology studies. These studies have been conducted in rats and mice treated for up to 13 weeks (Dunnick JK and Elwell MR, 1989; National Toxicology Program, 1996). See below for a summary of findings from these studies.

A 13-week repeat-dose toxicity study was conducted to evaluate the potential toxicities of codeine (0, 390, 781, 1562, 3125, and 6250 ppm) in male/female F344/N rats and $B6C3F_1$ mice (Dunnick JK and Elwell MR, 1989). In these studies, both species (10 animals/sex/dose level) were administered codeine mixed in NIH-07 feed daily for 13 weeks. Mortalities were reported in 2 male mice, which were exposed to 3125 ppm. During the initial 3 weeks of drug exposure in rats and mice, the 1562, 3125, and 6500 ppm treatments of codeine decreased feed consumption. In subsequent weeks, feed consumption returned to control levels until the conclusion of the study. In regard to body weight, the effects of codeine varied across species and genders. Findings in rats demonstrated that codeine decreased body weight in males at every exposure amount, and in females at 1562, 3125, 6250 ppm. In mice, codeine decreased body weight only at 6250 ppm in males. Treatment with codeine did not produce histopathological lesions or clinical signs at the amounts studied.

The effects of codeine in the daily diet of F344/N rats was evaluated in a 14-day toxicity study (National Toxicology Program, 1996). The daily diets of rats (5/sex/group) contained 0, 1562, 3125, 6250, 12500 or 25000 ppm of codeine. Mortalities were reported in the 6,250 ppm (1 female), 12500 ppm (1 male and 3 females), and 25000 ppm (all animals) groups. The final body weight was decreased in a dose-related manner in treated animals. At treatment day 1, there was a substantial decrease in food consumption in codeine-treated animals compared to controls. There were no apparent relationships at day 1 between the amount of codeine exposure and alterations in food consumption. At day 14, food consumption was increased in

codeine-treated animals when compared to day 1; and was slightly lower than control. In regard to organ weight, the absolute and relative weights for the thymus in both genders and the testis in males from the 12500 ppm group were significantly lower when compared to control. Reportedly, there were no toxicologically significant gross lesions observed in codeine-treated rats at necropsy. However, nonneoplastic lesions were observed in both genders, generally at ≥12500 ppm. These lesions included lymphoid depletion of the thymus, and hyperplasia and hyperkeratosis in the forestomach mucosa. Testicular degeneration was reported in males across the same dose range.

In $B6C3F_1$ mice, the effects of codeine mixed in the daily diet of animals were evaluated in a 14-day toxicity study (National Toxicology Program, 1996). The daily diets of mice (5/sex/group) contained 0, 781, 1562, 3125, 6250, or 12500 ppm of codeine. No mortalities were reported. Mean body weight was significantly increased in females from the 3125 ppm group and decreased in both genders from the 12500 ppm group when compared to control. In males, absolute and relative weights were significantly decreased for the right kidney in the 12500 ppm group; as well as for the liver in the 3125, 6250, and 12500 ppm groups when compared to control. These weights were also significantly decreased for the liver in females from the 12500 ppm group. No gross or histopathology findings were reported in codeine-treated groups.

The effects of codeine in the daily diet of F344/N rats were evaluated in a 13-week toxicity study (National Toxicology Program, 1996). The daily diets of rats (10/sex/group) contained 0, 390, 781, 1562, 3125 or 6250 ppm of codeine. A single mortality was reported in codeine-exposed rats (male from 390 ppm group; week 2) during treatment. In week 1 there was a decrease in food intake with increasing concentrations of codeine; however, by the conclusion of the study food intake was comparable to control levels. Significant decreases in the final mean body weights and mean body weight gains were reported in males from each treatment group and females in the 1562, 3125 and 6250 ppm groups when compared to control. Mild dose-dependent lymphopenia was reported in females fed ≥1562 ppm codeine and in males fed 6250 ppm codeine. Minimal to mild macrocytosis was reported in all treated males and in females treated with ≥781 ppm codeine. There were no toxicologically significant effects observed in vaginal cytology parameters or sperm morphology. In regard to absolute and relative organ weights in males from treated groups, weights for the liver were significantly decreased, whereas those for the adrenal gland were significantly increased when compared to controls In females, these weights for the adrenal gland were increased in the 3125 and 6250 ppm groups. In males from the 3125 and 6250 ppm groups, the relative weight for the thymus was significantly lower than control. There were no gross or histopathological lesions reported.

In $B6C3F_1$ mice, the effects of codeine mixed in the daily diet of animals were evaluated in a 13-week toxicity study (National Toxicology Program, 1996). The daily diets of mice (10/sex/group) contained 0, 390, 781, 1562, 3125 or 6250 ppm of codeine. Two mortalities reportedly occurred during week 7 in male animals exposed to 3125 ppm. In the surviving animals, the food consumption and mean body weights of treated animals

were comparable to those of controls. No biologically significant alterations in hematology, urinalysis, clinical chemistry, and vaginal cytology or sperm morphology parameters were reported in treated animals. Significant decreases in the absolute and relative kidney weights in males from the 3125 and 6250 ppm groups were observed. No gross or histopathology lesions were observed in codeine-treated animals.

Studies on [redacted] (drug substance impurity) that were submitted for review (see also **NDA 22402, postmarketing requirement studies**)

Study title: 2-Week Non-GLP Toxicity Oral Gavage Study in Rats with [redacted]

Study no.:	Proposal 88894F-1
Study report location:	[redacted] 8218703 [redacted]
	[redacted] (1 year after report finalization)
Conducting laboratory and location:	[redacted]
Date of study initiation:	December 10, 2009
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	[redacted] ([redacted] P10764, and [redacted]

Key Study Findings

Oral [redacted] was administered for up to two weeks in rats at either 0, 10, 30, 60 or 100 mg/kg with the following key findings:

1) the observation of clinical signs that included protruded and constricted eyes, and hypoactivity across sexes; and swollen paws (both), face, and nose in males at 100 mg/kg;
2) the reduction of body weight in males at 100 mg/kg;
3) the determination that there were no toxicologically significant test article effects in clinical chemistry, hematology, urinalysis, gross pathology and histopathology findings evaluated; and
4) the estimation of the NOAEL in rats at [redacted] based on the observation of hypoactivity and protruding eyes across sexes, as well as swollen paws, face and nose, and decreased body weight in males at 100 mg/kg.

Methods

Doses:	See **Figure 7** (Excerpt from Final Protocol, page 7)
Frequency of dosing:	Daily. Note that animals from each group were dosed for at least fourteen days; except those from Group 5 (60 mg/kg), which were dosed for nine days. See **Figure 7** for more details.
Route of administration:	Oral (PO) via gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	The test article was formulated as a oral solution that was suspended in 0.5% methyl cellulose in reverse osmosis/deionized water
Species/Strain:	Crl:CD (SD) rats
Number/Sex/Group:	See **Figure 7**
Age:	6-8 weeks
Weight:	100 to 300 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Yes. Note that the Reviewer concurred with the Sponsor that the deviation reported affected neither the overall interpretation of the study findings nor compromised the integrity of the study.

Figure 7. Study Design for 2-Week Non-GLP Toxicology Study

Group[a]	No. of Animals Male	No. of Animals Female	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)
1 (Control)	5	5	0	0
2 (Low)	5	5	10	1
3 (Mid)	5	5	30	3
4 (High)	5	5	100	10
5 (Mid High)[b]	4	4	60	6

a Group 1 will receive vehicle control article only.

b Group will be dosed for nine days (add to study on 22 Dec 2009 which will be designated as Day 1 for Group 5). Detailed observations, body weights, and food consumption (full feeders) will be collected on Day 1, then again on Days 3 (24 Dec 2009) and 9 (30 Dec 2009). Protocol designated intervals for cageside observations, eye exams, and clinical pathology collections will be applicable to this group as well; scheduled terminal sacrifice will be on (b) (4)

Observations and Results

Toxicokinetics

Note that the Sponsor did not evaluate toxicokinetic endpoints in treated animals.

General daily observations of the animals were conducted twice daily (a.m. and p.m.). Animals were reportedly observed for mortality, abnormalities, and signs of pain or distress. Note that cage-side observations were conducted once daily approximately two hours after animals were dosed. Also, detailed observations were conducted and body weights and food consumption were measured at least once prior to the initiation of treatment, as well as during this phase. The observations conducted during the treatment phase occurred on the eighth and fourteenth day of dosing (except for Group five, which was the third and ninth day) and on the day of necropsy.

Mortality

There were no test article-related deaths observed in the treated groups prior to the scheduled sacrifice.

Clinical Signs

Test article-related clinical signs in rats were observed at 100 mg/kg. Clinical signs such as hypoactivity, constricted pupils, and protruding eyes were observed across both sexes. In males, hypoactivity and constricted pupils were observed in 5/5 animals, generally on day 1 only; whereas protruding eyes (i.e., exophthalmos) were observed in 1/5 animals from this group on day 2. In females, hypoactivity (day 1) and protruding eyes were observed in ≥ 4/5 animals (see **Table 9**); whereas constricted pupil-eyes were observed in 1/5 animals from this group on day 1. In regard to protruding eyes, this effect reportedly occurs in animals treated with opioids such as morphine and under conditions such as Graves' Disease (SZENTAGOTHAI, J. and SCHAB, R., 1956; Wallenstein, M. C., 1983). In morphine treated animals, exophthalmos appears to be mediated by the activation of the sympathetic system (Wallenstein, M. C., 1983). Protruding eyes can cause eye lids to fail to close during sleep, which may result in corneal dryness and damage. Note that there were no test article related ophthalmic findings reported in treated animals. Clinical signs such as swollen paws (both), nose, and face were observed in males only. In males, these signs were observed in ≤ 3/5 animals from the 100 mg/kg group. Of the clinical signs observed, hypoactivity and protruding eyes across sexes; and swollen paws, nose and face in males were deemed adverse.

Table 9. Incidence of Clinical Signs in 2-Week Non-GLP Toxicology Study

Sign	Dose Group (mg/kg/day)									
	0		10		30		60		100	
	M	F	M	F	M	F	M	F	M	F
Swollen, All Paws	0/5	0/5	0/5	0/5	0/5	0/5	0/4	0/4	**3/5**	0/5
Swollen, Nose	0/5	0/5	0/5	0/5	0/5	0/5	0/4	0/4	**2/5**	0/5
Swollen, Face	0/5	0/5	0/5	0/5	0/5	0/5	0/4	0/4	**2/5**	0/5
Hypoactive	0/5	0/5	0/5	0/5	0/5	0/5	0/4	0/4	**5/5**	**5/5**
Constricted Pupil-Eyes	0/5	0/5	0/5	0/5	0/5	0/5	0/4	0/4	**5/5**	**1/5**
Protruding Eyes	0/5	0/5	0/5	0/5	0/5	**1/5**	0/4	0/4	**1/5**	**4/5**

Body Weights

(b) (4) only produced a toxicologically significant decrease in the mean body weight of males from the 100 mg/kg group on treatment day 14. The mean body weight of males from the 100 mg/kg group was decreased to 88% of control. There were no toxicologically significant alterations in the averaged body weights of treated females.

Food Consumption

Based on those data submitted, there were no apparent toxicologically significant alterations in food consumption in treated animals, given that the statistically significant alterations observed did not appear to be associated with reductions in the averaged body weight of animals when compared to control. Note that statistically significant decreases in the mean food consumption were observed in males from the 30 and 100 mg/kg groups. Given that there were no statistically significant decreases at 60 mg/kg, the alterations observed at 30 mg/kg were attributed to biological variation amongst animals from that group. Separately, those effects at 100 mg/kg were observed across a time period in which the mean body weights of animals were not significantly reduced, which suggested that those changes were not toxicologically significant. The Reviewer acknowledges the reduction in feed consumption at 100 mg/kg and that it may be test article-related; however, this effect is likely not toxicologically significant.

Ophthalmoscopy

Ophthalmic examinations were conducted once prior to treatment and within three days before the end of this phase. The eyes of animals were dilated with a mydriatic agent prior to examinations that involved the use of indirect ophthalmoscope. Note that there were no test article-related alterations in the endpoints evaluated under the ophthalmoscopy examination in treated animals.

ECG

Electrocardiograms were not recorded in the study animals.

Clinical Pathology

Clinical pathology was evaluated by collecting blood and urine samples from treated animals on the day of scheduled sacrifice and using standard endpoints to evaluate potential test article-related alterations (see **Table 10**). Animals were fasted overnight prior to the collection of blood samples. Blood samples were collected from the jugular vein, unless an alternate site was needed, to evaluate clinical chemistry, hematology, and coagulation endpoints. The anticoagulants used were potassium EDTA for hematology tests and sodium citrate for coagulation tests. In clinical chemistry tests, an anticoagulant was not used. Urine samples were collected and stored during the overnight period prior to blood collection.

Table 10. Clinical Pathology Parameters for 2-Week Non-GLP Toxicology Study

Hematology	
Red blood cell (erythrocyte) count	Platelet count
Hemoglobin	White blood cell (leukocyte) count
Hematocrit	Differential blood cell count
Mean corpuscular volume	Blood smear
Mean corpuscular hemoglobin	Reticulocyte count
Mean corpuscular hemoglobin concentration	

Coagulation	
Prothrombin time	Activated partial thromboplastin time

Clinical Chemistry	
Glucose	Alkaline phosphatase
Urea nitrogen	Gamma glutamyltransferase
Creatinine	Aspartate aminotransferase
Total protein	Calcium
Albumin	Inorganic phosphorus
Globulin	Sodium
Albumin/globulin ratio	Potassium
Cholesterol	Chloride

Clinical Chemistry	
Total bilirubin	Triglycerides
Alanine aminotransferase	

Urinalysis	
Appearance (clarity and color)	pH
Bilirubin	Protein
Blood	Specific gravity
Glucose	Urobilinogen
Ketones	Volume
Microscopic examination of sediment	

Hematology

There were no test article-related alterations in the hematology endpoints measured in treated animals.

Clinical Chemistry

There were no test article-related alterations in the clinical chemistry endpoints measured in treated animals.

Urinalysis

There were no test article-related alterations in the urinalysis endpoints measured in treated animals.

For macroscopic and microscopic examinations, animals were anesthetized with sodium pentobarbital and exsanguinated. Note that animals were anesthetized with CO_2/O_2 and exsanguinated (blood collected via vena cava) if their weight was less than 160 grams. Macroscopic examinations involved the evaluation of external features of the carcass; external body orifices; abdominal, thoracic, and cranial cavities; and organs. A variety of organs were weighed to evaluate potential test article related alterations (see **Table 11**; paired organs weighed together). These data were presented as overall weight; as well as organ-to-body weight and organ-to-brain weight ratios, which were reported as percentages. Microscopic examinations involved the evaluation of various tissues collected from animals. Collected tissues were preserved in 10% neutral-buffered formalin, unless otherwise indicated (see **Table 12** for list). Note that tissues from the epididymis, heart, kidneys, lesions, liver, lungs, spleen and testis from all animals (when present) in the control and high dose groups (i.e., 100 mg/kg) were evaluated. These tissues were embedded in paraffin and stained with hematoxylin and eosin following collection. The histopathology battery was deemed adequate by the Reviewer.

Table 11. Organs weighed for 2-Week Non-GLP Toxicology Study

Organs		
Adrenal*	Lung	Spleen
Brain	Ovary*	Testis*
Epididymis*	Pituitary gland	Thymus
Heart	Prostate	Thyroid* with parathyroid
Kidney*	Salivary gland (mandibular)	Uterus
Liver	Seminal vesicle*	

*Paired

Table 12. Histopathology Summary Table for 2-Week Non-GLP Toxicology Study

Tissue	
Adrenal*	Optic nerve*
Aorta	Ovary*
Brain	Pancreas
Cecum	Pituitary gland
Cervix	Prostate
Colon	Rectum
Duodenum	Salivary gland (mandibular*)
Epididymis*	Sciatic nerve
Eye*	Seminal vesicle
Femur with bone marrow (articular surface of the distal end)	Skin/subcutis
Harderian gland†	Spinal cord (cervical, thoracic, and lumbar)
Heart	Spleen
Ileum	Sternum with bone marrow
Jejunum	Stomach
Kidney*	Testis*
Lesions	Thymus
Liver	Thyroid (lobes*) with parathyroid
Lung with large bronchi	Tongue
Lymph node (mandibular)	Trachea
Lymph node (mesenteric)	Urinary bladder
Mammary gland	Uterus
Muscle, biceps femoris	Vagina

*Paired

†Preserved in modified Davidson's fixative

Gross Pathology

There were no macroscopic findings that were deemed test article-related in the treated animals.

Organ Weights

There were no test article-related alterations in the organ weights from treated animals.

Histopathology

There were no microscopic findings that were deemed test article-related in the treated animals. Note that animal #B58946 was observed with focal, unilateral and minimal testicular atrophy/degeneration. The Reviewing Pathologist interpreted this finding as being consistent with a spontaneous change (Greaves, 2007a). Given the low incidence and severity of this finding in the male animal, the Reviewer concurred with the Pathologist's interpretation.

Dosing Solution Analysis

Note that the Sponsor did not conduct a dosing solution analysis.

Study title: 13-Week Toxicity and Toxicokinetics Oral Gavage Study in Rats with (b) (4)

Study no.:	(b) (4)
Study report location:	(b) (4)
	(b) (4) (1 year after report finalization)
Conducting laboratory and location:	(b) (4) (b) (4)
Date of study initiation:	16 March 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) ((b) (4) P10764, and (b) (4)

Key Study Findings

Oral (b) (4) was administered daily for up to 13 weeks to male rats at either 0, 10, 30, or 60 mg/kg; and to female rats at either 0, 30, 60, or 100 mg/kg with the following key findings:

1) the observation of clinical signs that included hyperactivity, vocalizations, excessive grooming, and tremors;
2) the reduction of body in males at 30 and 60 mg/kg;
3) the observation of mortality in males at 60 mg/kg and in a females at 100 mg/kg;
4) the determination that there were no toxicologically significant test article effects in clinical chemistry, hematology, urinalysis, gross pathology and histopathology findings evaluated; and
5) the estimation of a NOAEL in males at (b) (4) person) based on the observation of tremors, the reduction of weight, and observation of mortality at ≥ 30 mg/kg; and a LOAEL in females at (b) (4) based on the observation of tremors at all of the doses tested, as well as mortality at 100 mg/kg.

Methods

Doses:	See **Figure 8** (excerpt from Sponsor's final protocol, page 374)
Frequency of dosing:	Daily
Route of administration:	Oral (PO) via gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	The test article was formulated as a oral solution that was suspended in 0.5% methyl cellulose in reverse osmosis/deionized water
Species/Strain:	Crl:CD (SD) rats
Number/Sex/Group:	See **Figure 8**
Age:	6-8 weeks
Weight:	189 to 246 g for males and 164 to 201 g for females
Satellite groups:	Yes, note that a toxicokinetics group was employed
Unique study design:	None
Deviation from study protocol:	Yes. Note that the Reviewer concurred with the Sponsor that the deviation reported affected neither the overall interpretation of the study findings nor compromised the integrity of the study.

Figure 8. Study Design for 13-Week (b) (4) Toxicology Study

Group[a]	No. of Animals		Dose Level		Dose Concentration	
	Male	Female	Male (mg/kg/day)	Female (mg/kg/day)	Male (mg/mL)	Female (mg/mL)
Main Study Animals						
1 (Control)[a]	10	10	0	0	0	0
2 (Low)	10	10	10	30	1	3
3 (Mid)	10	10	30	60	3	6
4 (High)	10	10	60	100	6	10
Toxicokinetic Animals[b]						
5 (Control)[a]	3	3	0	0	0	0
6 (Low)	9	9	10	30	1	3
7 (Mid)	9	9	30	60	3	6
8 (High)	9	9	60	100	6	10

a Group 1 and 5 will receive vehicle control article only.
b Toxicokinetic animals will be used for blood collection only.

Observations and Results

Toxicokinetics

Toxicokinetic endpoints were measured in a satellite group in which blood samples (0.5 mL) were obtained via a jugular vein. Animals were not fasted and samples were collected during day 1 (week 1) and week 13 at the times noted in **Table 13**. The

samples were analyzed to measure endpoints that included C_{max}, AUC_{0-24}, AUC_{0-t}, $AUC_{0-\infty}$, and $t_{1/2}$. Note that given the comparability of the values for the various AUC endpoints within each treatment group, the Reviewer only discussed the AUC_{0-t} value, which represents the area under the concentration-time curve from hour 0 to the last measurable concentration, as estimated by the linear trapezoidal rule.

Table 13. Toxicokinetic Time Points for 13-Week (b) (4) Toxicology Study

Group	Set	Time points
5	3 animals/sex	1 hour
6-8	1st 3 animals/sex	Predose, 1 and 24 hours
6-8	2nd 3 animals/sex	0.5 and 2.0 hours
6-8	3rd 3 animals/sex	4 and 8 hours

Findings in rats administered oral (b) (4) demonstrated that its averaged maximum observed concentration (T_{max}) and elimination half-life, respectively, ranged from 0.5-1 and 0.8-5.1 hours at the doses levels tested. In treated rats, C_{max} and AUC_{0-t} increased in a dose-related manner at the time points studied (see **Table 14**). For example, at weeks 1 and 13 in male rats administered 60 mg/kg/day (i.e., the high dose), the averaged values for C_{max} was 33.8 and 1367 ng/mL and AUC_{0-t} was 217.0 and 2401.0 ng·hr/mL. At weeks 1 and 13 in females administered 100 mg/kg (i.e., the high dose), the averaged values for C_{max} were 193.0 and 3243.0 ng/mL and AUC_{0-t} was 1418.0 and 10,622.0 ng·hr/mL. These and other data demonstrated that the plasma levels of the test article were much higher on week 13 when compared to week 1, which suggests that (b) (4) accumulated in the plasma of treated animals. Although ratios in **Table 16** demonstrated that (b) (4) accumulation was greater in males when compared to females, note that test article exposure (peak and overall systemic exposure) was less in males when compared to females (see **Table 14**). The levels shown in **Table 14** demonstrated that exposure was greater in females when compared to males at weeks 1 and 13. Differences in exposure to (b) (4) were also demonstrated when C_{max} and AUC_{0-t} were normalized based on the dose tested (see **Table 15**). For instance, C_{max} was less than dose-proportional in males from the 10 mg/kg group at week 13; and greater than dose-proportional in females from the 30 mg/kg group. AUC_{0-t} was less than dose-proportional in males from the 10 mg/kg group at weeks 1 and 13; and greater than dose-proportional in females from the 60 mg/kg group at week 1, as well as those from the 100 mg/kg group at week 13. At the other doses tested, C_{max} and AUC_{0-t} values were deemed dose-proportional. Together, these data provide evidence that suggest that the kinetics of (b) (4) is different across genders at the doses tested.

Table 14. Pharmacokinetics (C_{max} and AUC_{0-t}) for 13-Week [redacted] (b) (4) Toxicology Study

Dose	C_{max} (ng/mL)				AUC_{0-t} (ng·hr/mL)			
	Week 1		Week 13		Week 1		Week 13	
	M	F	M	F	M	F	M	F
10	5.7		67.4		5.6		86.9	
30	25.7	210.0	573.0	1093.0	58.6	359.0	949	2126
60	33.8	164.0	1367.0	2633.0	217.0	1291.0	2401	5067
100		193.0		3243.0		1418.0		10622

Table 15. Dose Proportion Values for 13-Week [redacted] (b) (4) Toxicology Study

Dose	C_{max} (ng/mL)				AUC_{0-t} (ng·hr/mL)			
	Week 1		Week 13		Week 1		Week 13	
	M	F	M	F	M	F	M	F
10	0.6		**6.7**		**0.6**		**8.7**	
30	0.9	**7.0**	19.1	36.4	2.0	12.0	31.6	70.9
60	0.6	2.7	22.8	43.9	3.6	**21.5**	40.0	84.5
100		1.9		32.4		14.2		**106.2**

Table 16. Drug Accumulation Ratios for 13-Week [redacted] (b) (4) Toxicology Study 13/week 1

Dose	C_{max} (ng/mL)		AUC_{0-t} (ng·hr/mL)	
	M	F	M	F
10	11.9		15.5	
30	22.3	5.2	16.2	5.9
60	40.4	16.1	11.1	3.9
100		16.8		7.5

Mortality

Mortalities were observed in males (n = 2) at 60 mg/kg and a female at 100 mg/kg. These rats were found dead during the study at various time points. See **Table 17** for more information. Note that no explanation was provided to explain the deaths of these animals. The Reviewer deemed these deaths test article-related, given that the effect only occurred at the highest dose tested in each sex and the lack of findings to suggest that these mortalities may be related to the dosing procedure or some other issue.

Table 17. Mortality for 13-Week [redacted] (b) (4) Toxicology Study

Gender	Animal #	Study Group	Treatment dose (mg/kg)	Day of Death
Female	B62064	Main	**100**	73

Gender	Animal #	Study Group	Treatment dose (mg/kg)	Day of Death
Male	B62027	Toxicokinetic	**60**	78
	B62033	Toxicokinetic	**60**	91

Clinical Signs

Animals from the main study and toxicokinetic study were observed for clinical signs twice daily. Animals were observed for signs related to their general appearance, behavior, bodily discharges and excretions, skin and pelage condition. A variety of clinical signs deemed adverse by the Pharmacology Toxicology Reviewer are noted in **Table 18** (findings in Main study animals only). The signs noted include hyperactivity, vocalizations, excessive grooming, and tremors. In general, the frequency of these signs, across treatment days, increased in a dose-related manner in both sexes from the main study and toxicokinetic study. In particular, tremors were observed in several treated animals (see **Table 19**). Head tremors were observed in three females from the main study (B62056, 60 mg/kg; B62073 and B62071, 100 mg/kg) and a male from the toxicokinetic study (B62026, 60 mg/kg). Limb tremors were also observed in two females (B62056, 60 mg/kg; and B62065, 100 mg/kg) from the main study. Note that the days and frequency in which the tremors were observed varied across subjects (see **Table 19** for days observed). Together, these data suggest that orally administered (b) (4) in rats may be proconvulsive at ≥ 30 mg/kg, especially when considering the observation of signs such as vocalizations and tremors (limb and head). However, in the absence of convulsions at the doses tested, the only clinical sign observed and deemed adverse was tremors. This finding is noteworthy, especially since convulsions were observed across sexes at ≥ 200 mg/kg in rats employed in in vivo genetic toxicology studies discussed below (**study #8222569**)

Table 18. Incidence of Clinical Signs for 13-Week [redacted] (b) (4) Toxicology Study

Sign	Dose Group (mg/kg/day; n=10/sex/group)									
	0		10		30		60		100	
	M	F	M	F	M	F	M	F	M	F
Hyperactive	0/10	0/10	1/10		**6/10**	**8/10**	**9/10**	**10/10**		**10/10**
Vocalization	0/10	2/10	3/10		**6/10**	**6/10**	**10/10**	**7/10**		**6/10**
Excessive Grooming	0/10	0/10	2/10		**7/10**	**8/10**	**9/10**	**10/10**		**10/10**
Head Tremors	0/10	0/10	0/10		0/10	0/10	0/10	**1/10**		**2/10**
Limb Tremors, front legs	0/10		0/10		0/10	0/10	0/10	**1/10**		**1/10**

Table 19. Tremor Observations for 13-Week [redacted] (b) (4) Toxicology Study

Gender	Animal #	Study Group	Treatment dose (mg/kg)	Tremor site	Days observed
Female	B62073	Main	100	Head	7
	B62071	Main	100	Head	74, 88
	B62056	Main	60	Head	38, 45, 55, 59, 60, 63-64, 76, 78, 79, 83, 85-88
				Limbs, front legs	59, 63
	B62065	Main	100	Limbs	88
Males	B62026	Toxicokinetics	60	Head	75-76, 86, 88-89

Body Weights

Body weights were recorded at least once during the predose phase, prior to day 1, and once weekly during the treatment phase. (b) (4) produced dose-related decreases in body weight in males only (see **Table 20**). Body weight in males was decreased to 86-88% of control in the 30 mg/kg group at weeks 4-14 and to 81-88% of control in the 60 mg/kg group. Note that no toxicologically significant alterations in body weight were noted in females tested.

Table 20. Body Weights (Percentage of Control) for 13-Week (b) (4) Toxicology Study

Week	Dose (mg/kg)							
	10		30		60		100	
	M	F	M	F	M	F	M	F
1	100%		101%	101%	101%	100%		102%
2	98%		94%	98%	92%	95%		96%
3	94%		90%	97%	**88%**	93%		96%
4	93%		**88%**	95%	**86%**	93%		93%
5	92%		**88%**	95%	**86%**	93%		93%
6	92%		**88%**	95%	**85%**	92%		92%
7	91%		**88%**	95%	**84%**	92%		93%
8	90%		**88%**	95%	**84%**	93%		94%
9	90%		**87%**	96%	**83%**	93%		94%
10	91%		**86%**	96%	**82%**	93%		95%
11	90%		**86%**	96%	**82%**	93%		92%
12	91%		**86%**	95%	**81%**	92%		93%
13	92%		**87%**	95%	**82%**	90%		91%
14	91%		**86%**	95%	**83%**	91%		92%

Food Consumption

Food consumption was measured (g/animal/period) weekly in the main study animals. There were no biologically significant alterations in the amount of food consumed by (b) (4) treated animals. Note that food consumption in (b) (4) treated animals ranged from 87-105% of control.

Ophthalmoscopy

Indirect ophthalmoscopic examinations were performed prior to the initiation of treatment and during week 13 of the study. Based on findings from these examinations there were no test article-related ophthalmic toxicities. Note that Corneal dystrophy was

reported in the right eye of a male in the 30 mg/kg and 60 mg/kg group, as well as a female in the control group. Corneal dystrophy reportedly occurs spontaneously in commonly used strains of laboratory rats and mice (e.g., Fischer 344, Sprague Dawley and Wistar rats; and CD-1 mice); and may be caused by the accumulation of extraneous materials in the cornea (for further information see Greaves, 2007b).

ECG

Electrocardiograms were not recorded in the study animals.

Clinical Pathology

Clinical pathology data were obtained via hematology, coagulation, clinical chemistry, and urinary endpoints measured in fasted animals at the scheduled necropsy. Blood (via a jugular vein) and urine samples were collected and stored at week 13 prior to necropsy. See **Table 21** for the endpoints evaluated in treated animals.

Table 21. Clinical Pathology Endpoints Evaluated for 13-Week (b) (4) Toxicology Study

Hematology	
Red blood cell (erythrocyte) count	Platelet count
Hemoglobin	White blood cell (leukocyte) count
Hematocrit	Differential blood cell count
Mean corpuscular volume	Blood smear
Mean corpuscular hemoglobin	Reticulocyte count
Mean corpuscular hemoglobin concentration	

Coagulation	
Prothrombin time	Activated partial thromboplastin time

Clinical Chemistry	
Glucose	Alkaline phosphatase
Urea nitrogen	Gamma glutamyltransferase
Creatinine	Aspartate aminotransferase
Total protein	Calcium
Albumin	Inorganic phosphorus
Globulin	Sodium
Albumin/globulin ratio	Potassium
Cholesterol	Chloride
Total bilirubin	Triglycerides
Alanine aminotransferase	

Urinalysis	
Appearance (clarity and color)	pH

Urinalysis	
Bilirubin	Protein
Blood	Specific gravity
Glucose	Urobilinogen
Ketones	Volume
Microscopic examination of sediment	

Hematology

There were no test article related alterations to the hematology endpoints measured.

Clinical Chemistry

There were no test article related alterations to the clinical chemistry endpoints measured.

Urinalysis

There were no toxicologically significant alterations in the urinalysis endpoints measured. Note that the averaged urine volume in males from the 60 mg/kg group was increased to 184% of control; however, given the lack of histopathological findings in the kidney, this finding was not deemed toxicologically significant (see **Table 22**).

Table 22. The Averaged Urine Volume (Percentage of Control)

Dose	Urine Volume (mL)	
	M	F
10	132%	
30	112%	92%
60	184%	127%
100		135%

Gross Pathology

There were no test article-related macroscopic findings observed in (b) (4) treated animals. Note that two females in the 100 mg/kg group reportedly had a distended uterus. Given the lack of histopathology findings in the uterus and the possibility that this organ was distended because of other factors such as cyclical endometrial changes in estrous, this finding was not deemed toxicologically significant by the Reviewer.

Organ Weights

There were no alterations in organ weight that were deemed test article related by the Reviewer. See **Table 23** for the organs weighed at necropsy (excerpt from Sponsor's Final Protocol, page 382).

Table 23. Organs Weighed at Necropsy for 13-Week [redacted] (b) (4) Toxicology Study

Organs		
Adrenal*	Lung*	Spleen
Brain	Ovary*	Testis*
Epididymis*	Pituitary gland	Thymus
Heart	Prostate	Thyroid* with parathyroid
Kidney*	Salivary gland (mandibular)	Uterus
Liver	Seminal vesicle*	

*Paired

Histopathology

The histopathology battery was deemed adequate by the Reviewer. See **Table 24** for a list of the tissues collected and preserved in 10% neutral-buffered formalin, unless otherwise noted (excerpt from the Sponsor's Final Protocol, page 383). The tissues collected from main study animals were embedded in paraffin and prepared with hematoxylin and eosin stain. Note that toxicokinetic animals that died at unscheduled intervals were subjected to a specialized necropsy of the oral, cervical, and thoracic cavities. No tissues from these animals were preserved. In regard to the other animals from the toxicokinetic study, they were sacrificed and their remains discarded after the final blood sample collection.

There were no test article-related toxicities noted in the tissues prepared from main study animals.

Table 24. Histpathology Summary Table for 13-Week (b) (4) Toxicology Study

Tissue	
Adrenal*	Optic nerve*
Aorta	Ovary*
Brain	Pancreas
Cecum	Pituitary gland
Cervix	Prostate
Colon	Rectum
Duodenum	Salivary gland (mandibular*)
Epididymis*	Sciatic nerve
Eye*	Seminal vesicle
Femur with bone marrow (articular surface of the distal end)	Skin/subcutis
Harderian gland†	Spinal cord (cervical, thoracic, and lumbar)
Heart	Spleen
Ileum	Sternum with bone marrow
Jejunum	Stomach
Kidney*	Testis*
Lesions	Thymus
Liver	Thyroid (lobes*) with parathyroid
Lung with large bronchi	Tongue
Lymph node (mandibular)	Trachea
Lymph node (mesenteric)	Urinary bladder
Mammary gland	Uterus
Muscle, biceps femoris	Vagina

***Paired**

†Preserved in modified Davidson's fixative

Dosing Solution Analysis

According to the dose analysis report, the concentration verification results for day 1 and week 6 of the dosing phase met acceptance criteria; however, the initial results for week 13 did not. Findings from two sets of daily dosing aliquots reportedly suggested that the 1-, 3-, and 10- mg/mL formulations were prepared within target; unlike the 6-mg/mL formulation. To address this issue, the 6-mg/mL formulation was remixed and yielded an acceptable mean result. The Sponsor collected two sets of blood samples (days 86 and 91), from which the plasma was analyzed and the results demonstrated that animals were dosed with the appropriate dose formulation; findings that support the use of those data submitted by the Sponsor.

7 Genetic Toxicology

Codeine. In vitro tests that included the *Salmonella*/mammalian-liver homogenate test and *E.coli* mammalian-liver homogenate test, as well as in vivo assays that included the intrasanguineous host-mediated test, sex-linked recessive lethal test in *Drosophilia melanogaster*, and the micronucleus test were used to evaluate the potential mutagenic effects of codeine phosphate (King MT et al., 1979). Codeine did not produce mutagenicity in the *Salmonella typhimurium* (18 μg/plate) and *Escherichia coli* (30 mM) tests. Similarly, codeine did not produce mutagenicity in the intrasanguineous host-mediated test (0.18 mmoles/kg), sex-linked recessive lethal test in *Drosophilia melanogaster* (5 mM) and micronucleus (0.25 mmoles/kg – ip; 0.5 mmoles/kg – po) tests.

The National Toxicology Program (NTP) evaluated codeine phosphate (100 to 10000 μg/plate) for its potential to induce genetic mutations in strains of *Salmonella typhimurium* (TA97, TA98, TA100, or TA1535; with or without liver S9 enzymes), as well as mutations in sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells (National Toxicology Program, 1996). No mutagenesis was produced in the Salmonella strains exposed to the codeine concentrations tested. In cultured Chinese hamster ovary cells (with and without S9 enzymes), codeine increased the frequency of sister chromatid exchanges in a concentration-related manner. However, the increase occurred at concentrations that produced marked cell cycle delay, which is indicative of a high level of cytotoxicity. These findings are difficult to interpret given the delays in cell cycling, which may prolong DNA exposure to various factors (e.g., 5-bromodeoxyuridine) known to increase levels of sister chromatid exchanges. There were no reports of increases in the frequency of chromosomal aberrations in the cultured Chinese hamster ovary cells exposed to codeine.

Potential mutagenic effects of codeine phosphate were evaluated using in vivo (micronucleus and sperm abnormality assays) and in vitro (Ames *Salmonella* assay) tests (Bruce WR and Heddle JA, 1979). In the in vivo tests, hybrid mice (genotype C57BL/6 X C3H/He; F1 generation) at 11-14 weeks of age served as subjects (8/dose group). Males served as subjects in the sperm assay, whereas their female litter mates served as subjects in the micronucleus assay. The highest dose was selected based on the LD_{50} values determined in separate studies. Three lower doses were selected, which were 1/2, 1/4 and 1/8 of the highest dose tested in animals. Note that the authors presented data in tabular and graphical form; however, the actually doses were not mentioned. Codeine or vehicle was administered intraperitoneally for five consecutive days. In the micronucleus assay, the mice were sacrificed on the 5th day of treatment, approximately 4 hr after the final treatment. Bone marrow cells were prepared from the femur, and a total of 1000 reticulocytes were scored per group evaluated. In the sperm abnormality assay, the mice were sacrificed 35 days following the final treatment. Sperm cell suspensions were prepared from the cauda epididymus, and 1000 sperms were scored per treatment group. Positive findings were reported when the treated

group values exceeded those of the control by 1%, which was reportedly twice the control value for both assays. In the Ames assay several concentrations of codeine (0.05, 0.5, 5, 50, and 500 mcg/plate; with and without S9 metabolic activation) were evaluated for potential mutagenicity in several *Salmonella typhimurium* strains that included TA1537, TA1535, TA100, and TA98. These strains, without S9, were irradiated at 10, 100, 1000, 10000, and 100000 rads. The criterion for a positive response was considered a 50% increase above the spontaneous frequency obtained at the same time. In these studies, codeine phosphate was not mutagenic in the micronucleus and sperm abnormality assays, as well as the Ames Salmonella assay.

As many of the studies described above are not part of the standard genetic toxicology battery or were not deemed adequate by current standards to be included in drug product labeling, only the studies from the standard battery conducted by NTP were described in the referenced label for NDA 22402 as follows:

> Mutagenesis: Codeine was not mutatgenic in the *in vitro* bacterial reverse mutation assay or clastogenic in the *in vitro* Chinese hamster ovary cell chromosome aberration assay.

Given that the drug substance impurity (b) (4) was detected at levels higher greater than allowed based on ICH standards, it was evaluated as part of a postmarketing commitment for NDA 22402 in the studies reviewed below. The methods and results from the four genetic toxicology studies conducted are discussed below.

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Bacterial Reverse Mutation Assay with a Confirmation Assay

Study no.:	8218265
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 27, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) ARS, P10764, and (b) (4)

Key Study Findings

(b) (4) was deemed negative in the Bacterial Reverse Mutation Assay under the conditions employed.

Methods

Strains:	See **Figure 9**
Concentrations in definitive study:	313, 625, 1250, 2500, and 5000 mcg/plate with and without S9
Basis of concentration selection:	Concentration selection was based on the following guidelines: –the cytotoxicity of (b) (4) to the test system was determined in order to select the doses tested in the definitive assay (minimum of three non-toxic concentrations of (b) (4) employed); –the highest dose was expected to produce a decrease in revertant frequency and/or thinning of the bacterial background lawn; and –the maximum dose evaluated was the highest able to be administered or scored (with at least four additional lower doses), if limited by solubility, and did not exceed 5000 mcg/plate
Negative control:	Dimethylsulfoxide (DMSO)
Positive control:	See **Figure 10**
Formulation/Vehicle:	(b) (4) was provided to conducting laboratory as a white, crystalline powder and dissolved in DMSO
Incubation & sampling time:	The Sponsor incubated inverted plates for 52 ± 4 hours. Note that tester strain culture titers were ≥ 10^9 cells/mL

Figure 9. Tester Strains Employed in Ames Assay

Tester Strain	*his*/*trp* Mutation	Additional Mutations		Plasmid
		Repair	LPS	
TA98	*his*D3052	*uvr*B	*rfa*	pKM101
TA100	*his*G46	*uvr*B	*rfa*	pKM101
TA1535	*his*G46	*uvr*B	*rfa*	–
TA1537	*his*C3076	*uvr*B	*rfa*	–
WP2*uvr*A	*trp*	*uvr*A	–	–

Figure 10. Positive Controls Employed in the Ames Assay

Tester Strain(s)	S9	Positive Control	Dose (μg/plate)	CAS No.	Lot No.
TA98	–	2-nitrofluorene	1.0	607-57-8	01508BE
TA100, TA1535	–	sodium azide	2.0	26628-22-8	017K0136
TA1537	–	ICR-191	2.0	17070-45-0	116K1026
WP2*uvr*A	–	4-nitroquinoline-N-oxide	1.0	56-57-5	117K1485
TA98	+	benzo[a]pyrene	2.5	50-32-8	087K0733
TA100, TA1535, TA1537	+	2-aminoanthracene	2.5	613-13-8	12317CE
WP2*uvr*A	+	2-aminoanthracene	25.0	613-13-8	12317CE

Study Validity

In order for the initial toxicity-mutation and confirmatory mutagenicity assays to be considered valid the following criteria was met:

- tester strain integrity was demonstrated;
- tester strains cultures (all) exhibited characteristic numbers of spontaneous revertants per plate in the vehicle controls, based on historical data and published reports;
- tester strain culture titers reached a target optical density ($OD_{650(1:4)}$) of 0.4 to 0.6, which reportedly was representative of cultures in the late exponential or early stationary phase with ≥ 10^9 cells/mL;
- positive control values exhibited at least a 3-fold increase over the respective mean, negative control value for each tester strain; and
- dose levels included a minimum of three non-toxic doses to evaluate the assay data and did not exceed 5000 mcg/plate.

Given these criteria, the Reviewer concurred with the Sponsor that the study was valid.

Results

Revertant frequencies at the doses of (b) (4) up to 5000 mcg/plate reportedly approximated or were less than those observed in vehicle control cultures in the initial and confirmatory mutagenicity assay (see results below). All positive and negative controls produced the expected effects. Based on these results, the criteria for a valid study were met and (b) (4) was deemed negative in the Bacterial Reverse Mutation Assay under the conditions employed.

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Figure 11. Initial Mutagenicity Assay Results With S9

Study No.: 8218265
Trial No.: 8218265-B1 Date Plated: 11/20/2009
Plating Method: Plate incorporation assay Date Counted: 11/25/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	(b) (4)	5000	*21.7*	*1.2*	*1.2*	23 N, 21 N, 21 N
		2500	*20.0*	*4.6*	*1.1*	21 N, 15 N, 24 N
		1250	*21.7*	*3.5*	*1.2*	25 N, 22 N, 18 N
		625	*19.7*	*1.2*	*1.1*	19 MN, 21 N, 19 N
		313	*19.7*	*11.0*	*1.1*	16 N, 32 N, 11 N
	Dimethyl Sulfoxide		*17.7*	*3.1*		15 N, 17 N, 21 N
TA100	(b) (4)	5000	*74.0*	*11.5*	*0.9*	63 MN, 73 MN, 86 N
		2500	*78.7*	*5.5*	*1.0*	76 MN, 85 MN, 75 MN
		1250	*78.7*	*3.1*	*1.0*	78 N, 76 N, 82 N
		625	*78.3*	*6.0*	*1.0*	72 N, 79 N, 84 N
		313	*75.3*	*5.7*	*0.9*	69 MN, 80 N, 77 MN
	Dimethyl Sulfoxide		*80.7*	*4.6*		78 N, 86 N, 78 N
TA1535	(b) (4)	5000	*10.7*	*4.0*	*0.7*	10 N, 7 N, 15 N
		2500	*10.3*	*2.9*	*0.7*	12 N, 12 N, 7 N
		1250	*9.0*	*4.6*	*0.6*	5 N, 8 MN, 14 MN
		625	*12.0*	*4.0*	*0.8*	12 N, 16 N, 8 N
		313	*14.7*	*3.5*	*1.0*	11 N, 15 N, 18 MN
	Dimethyl Sulfoxide		*14.3*	*7.1*		22 N, 8 MN, 13 MN
TA1537	(b) (4)	5000	*7.7*	*3.8*	*1.0*	5 MN, 6 N, 12 N
		2500	*6.7*	*1.5*	*0.9*	7 N, 5 N, 8 MN
		1250	*6.7*	*2.3*	*0.9*	8 MN, 8 N, 4 MN
		625	*5.0*	*2.6*	*0.7*	6 MN, 7 N, 2 MN
		313	*8.0*	*2.6*	*1.1*	6 N, 7 N, 11 N
	Dimethyl Sulfoxide		*7.3*	*1.2*		8 N, 8 N, 6 N
WP2*uvrA*	(b) (4)	5000	*8.3*	*2.3*	*0.4*	11 N, 7 N, 7 N
		2500	*10.7*	*1.2*	*0.6*	10 MN, 12 N, 10 N
		1250	*15.3*	*0.6*	*0.8*	16 MN, 15 MN, 15 N
		625	*16.3*	*6.1*	*0.9*	15 N, 11 N, 23 N
		313	*17.0*	*3.5*	*0.9*	13 N, 19 N, 19 N
	Dimethyl Sulfoxide		*19.0*	*8.2*		12 N, 17 N, 28 MN

Key to Plate Postfix Codes

M	Plate counted manually
N	Normal background bacterial lawn

Study No.: 8218265
Trial No.: 8218265-B1 Date Plated: 11/20/2009
Plating Method: Plate incorporation assay Date Counted: 11/25/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	BP	2.5	*325.0*	*49.2*	*18.4*	270 N, 365 N, 340 N
TA100	2AA	2.5	*1362.7*	*254.4*	*16.9*	1069 N, 1502 N, 1517 N
TA1535	2AA	2.5	*208.7*	*6.7*	*14.6*	207 N, 216 N, 203 N
TA1537	2AA	2.5	*117.0*	*63.2*	*16.0*	79 N, 82 N, 190 N
WP2*uvrA*	2AA	25.0	*635.0*	*36.3*	*33.4*	594 N, 648 N, 663 N

Key to Positive Controls

BP	Benzo(a)pyrene
2AA	2-aminoanthracene

Key to Plate Postfix Codes

M	Plate counted manually
N	Normal background bacterial lawn

Figure 12. Initial Mutagenicity Assay Results Without S9

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Study No.: 8218265
Trial No.: 8218265-B1 Date Plated: 11/20/2009
Plating Method: Plate incorporation assay Date Counted: 11/25/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	(b) (4)	5000	*17.0*	*4.0*	*1.5*	21 MN, 13 MN, 17 N
		2500	*13.7*	*3.1*	*1.2*	17 N, 11 MN, 13 N
		1250	*14.7*	*3.2*	*1.3*	11 N, 17 N, 16 N
		625	*14.7*	*2.5*	*1.3*	17 N, 12 N, 15 N
		313	*16.7*	*0.6*	*1.5*	17 N, 17 MN, 16 MN
	Dimethyl Sulfoxide		*11.3*	*1.2*		10 N, 12 N, 12 N
TA100	(b) (4)	5000	*62.3*	*0.6*	*0.8*	63 N, 62 N, 62 MN
		2500	*74.3*	*4.0*	*0.9*	78 N, 75 N, 70 MN
		1250	*76.3*	*2.5*	*1.0*	74 MN, 76 N, 79 MN
		625	*76.7*	*9.7*	*1.0*	66 MN, 85 N, 79 N
		313	*82.7*	*6.4*	*1.0*	90 N, 78 N, 80 MN
	Dimethyl Sulfoxide		*79.7*	*5.0*		79 MN, 75 N, 85 N
TA1535	(b) (4)	5000	*13.0*	*2.0*	*1.3*	15 N, 13 N, 11 N
		2500	*14.0*	*2.6*	*1.4*	11 N, 16 N, 15 N
		1250	*13.0*	*4.6*	*1.3*	17 MN, 8 N, 14 MN
		625	*11.0*	*1.0*	*1.1*	11 N, 10 N, 12 N
		313	*11.7*	*1.5*	*1.1*	13 N, 10 MN, 12 N
	Dimethyl Sulfoxide		*10.3*	*2.5*		13 N, 8 N, 10 N
TA1537	(b) (4)	5000	*2.7*	*0.6*	*0.7*	2 MN, 3 MN, 3 MN
		2500	*5.0*	*2.6*	*1.4*	4 N, 3 MN, 8 MN
		1250	*5.0*	*3.0*	*1.4*	2 MN, 5 N, 8 N
		625	*7.3*	*0.6*	*2.0*	8 N, 7 N, 7 N
		313	*6.0*	*1.7*	*1.6*	7 MN, 7 N, 4 N
	Dimethyl Sulfoxide		*3.7*	*1.5*		2 N, 4 N, 5 N
WP2*uvrA*	(b) (4)	5000	*13.0*	*3.5*	*0.5*	17 MN, 11 N, 11 MN
		2500	*14.0*	*1.7*	*0.5*	16 N, 13 N, 13 N
		1250	*14.0*	*3.6*	*0.5*	10 N, 15 MN, 17 MN
		625	*14.7*	*2.3*	*0.6*	12 N, 16 MN, 16 N
		313	*14.7*	*3.5*	*0.6*	18 N, 11 N, 15 MN
	Dimethyl Sulfoxide		*26.0*	*1.7*		24 N, 27 N, 27 MN

Key to Plate Postfix Codes

N	Normal background bacterial lawn
M	Plate counted manually

Study No.: 8218265
Trial No.: 8218265-B1 Date Plated: 11/20/2009
Plating Method: Plate incorporation assay Date Counted: 11/25/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	2NF	1.0	*226.7*	*6.4*	*20.0*	234 N, 223 N, 223 N
TA100	SA	2.0	*1251.7*	*42.6*	*15.7*	1286 N, 1204 N, 1265 N
TA1535	SA	2.0	*964.7*	*101.6*	*93.4*	1082 N, 907 N, 905 N
TA1537	ICR	2.0	*252.7*	*43.5*	*68.9*	296 N, 253 N, 209 N
WP2*uvrA*	4NQO	1.0	*106.0*	*28.1*	*4.1*	133 N, 108 MN, 77 MN

Key to Positive Controls

2NF	2-nitrofluorene
SA	sodium azide
ICR	ICR-191
4NQO	4-nitroquinoline-N-oxide

Key to Plate Postfix Codes

N	Normal background bacterial lawn
M	Plate counted manually

Figure 13. Confirmatory Mutagenicity Assay Results with S9

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Study No.: 8218265
Trial No.: 8218265-C1
Plating Method: Plate incorporation assay

Date Plated: 12/3/2009
Date Counted: 12/9/2009 to 12/10/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	(b) (4)	5000	16.0	5.0	0.9	16 N, 11 MN, 21 N
		2500	21.0	6.6	1.2	22 N, 14 MN, 27 N
		1250	16.0	3.0	0.9	13 N, 19 N, 16 N
		625	19.0	6.6	1.1	26 N, 18 N, 13 MN
		313	21.7	1.2	1.2	23 N, 21 N, 21 N
	Dimethyl Sulfoxide		18.0	1.7		16 N, 19 N, 19 N
TA100	(b) (4)	5000	82.0	3.6	0.9	86 N, 79 MN, 81 N
		2500	82.0	14.5	0.9	83 N, 96 N, 67 MN
		1250	84.7	11.0	0.9	72 MN, 90 N, 92 N
		625	80.0	9.5	0.9	79 N, 90 N, 71 MN
		313	90.3	5.9	1.0	86 N, 97 N, 88 N
	Dimethyl Sulfoxide		94.0	3.6		95 N, 97 N, 90 N
TA1535	(b) (4)	5000	10.0	1.7	0.7	11 N, 8 MN, 11 N
		2500	12.0	3.0	0.9	15 N, 9 N, 12 N
		1250	9.7	5.5	0.7	6 MN, 7 N, 16 MN
		625	10.7	2.3	0.8	12 N, 8 MN, 12 N
		313	11.7	2.5	0.8	9 N, 14 N, 12 N
	Dimethyl Sulfoxide		14.0	2.0		12 N, 14 N, 16 N
TA1537	(b) (4)	5000	7.0	2.0	0.7	5 N, 7 N, 9 N
		2500	7.7	1.5	0.8	9 N, 8 N, 6 N
		1250	5.7	2.9	0.6	4 N, 4 N, 9 MN
		625	5.0	1.0	0.5	4 N, 6 N, 5 N
		313	4.3	0.6	0.4	4 MN, 5 MN, 4 N
	Dimethyl Sulfoxide		9.7	2.3		11 N, 7 N, 11 MN
WP2*uvrA*	(b) (4)	5000	16.0	3.0	0.7	16 N, 19 N, 13 N
		2500	16.3	4.6	0.7	19 N, 11 N, 19 N
		1250	15.7	3.1	0.7	13 N, 19 N, 15 N
		625	14.3	4.9	0.6	12 N, 20 N, 11 MN
		313	16.7	4.2	0.7	18 N, 20 N, 12 N
	Dimethyl Sulfoxide		24.0	5.6		30 N, 19 N, 23 N

Key to Plate Postfix Codes

N	Normal background bacterial lawn
M	Plate counted manually

Study No.: 8218265
Trial No.: 8218265-C1
Plating Method: Plate incorporation assay

Date Plated: 12/3/2009
Date Counted: 12/9/2009 to 12/10/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	BP	2.5	351.0	23.6	19.5	376 N, 329 N, 348 N
TA100	2AA	2.5	1837.3	93.5	19.5	1853 N, 1922 N, 1737 N
TA1535	2AA	2.5	174.7	9.5	12.5	182 N, 164 N, 178 N
TA1537	2AA	2.5	115.3	32.0	11.9	150 N, 109 N, 87 MN
WP2*uvrA*	2AA	25.0	344.0	37.7	14.3	349 N, 304 N, 379 N

Key to Positive Controls

BP	Benzo(a)pyrene
2AA	2-aminoanthracene

Key to Plate Postfix Codes

N	Normal background bacterial lawn
M	Plate counted manually

Figure 14. Confirmatory Mutagenicity Assay Results Without S9

Best Available Copy

Study No.: 8218265
Trial No.: 8218265-C1
Plating Method: Plate incorporation assay

Date Plated: 12/3/2009
Date Counted: 12/9/2009 to 12/10/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	(b) (4)	5000	*14.0*	*3.5*	*1.1*	12 N, 18 MN, 12 N
		2500	*10.3*	*1.5*	*0.8*	12 N, 10 MN, 9 N
		1250	*11.0*	*2.6*	*0.8*	13 MN, 12 N, 8 MN
		625	*10.0*	*3.0*	*0.8*	10 MN, 13 MN, 7 MN
		313	*8.7*	*2.1*	*0.6*	11 N, 7 N, 8 N
	Dimethyl Sulfoxide		*13.3*	*3.5*		10 MN, 13 N, 17 MN
TA100	(b) (4)	5000	*80.0*	*12.5*	*1.0*	79 N, 68 MN, 93 N
		2500	*73.3*	*6.7*	*0.9*	70 N, 69 N, 81 N
		1250	*71.0*	*9.5*	*0.9*	70 N, 62 N, 81 N
		625	*76.7*	*8.1*	*1.0*	86 N, 72 MN, 72 MN
		313	*76.7*	*2.5*	*1.0*	79 N, 74 N, 77 N
	Dimethyl Sulfoxide		*80.0*	*1.7*		79 N, 79 N, 82 N
TA1535	(b) (4)	5000	*19.0*	*4.6*	*1.5*	20 N, 23 N, 14 MN
		2500	*12.0*	*7.0*	*0.9*	5 N, 19 N, 12 N
		1250	*13.0*	*1.0*	*1.0*	13 N, 14 N, 12 N
		625	*12.0*	*1.0*	*0.9*	13 N, 12 N, 11 N
		313	*14.3*	*8.6*	*1.1*	22 N, 16 N, 5 N
	Dimethyl Sulfoxide		*13.0*	*6.2*		11 N, 20 N, 8 N
TA1537	(b) (4)	5000	*4.3*	*2.5*	*0.8*	4 N, 2 N, 7 N
		2500	*7.3*	*4.2*	*1.3*	6 N, 4 MN, 12 N
		1250	*4.0*	*1.7*	*0.7*	2 MN, 5 N, 5 N
		625	*7.7*	*1.5*	*1.4*	8 N, 6 N, 9 N
		313	*5.3*	*0.6*	*0.9*	5 N, 6 N, 5 N
	Dimethyl Sulfoxide		*5.7*	*1.5*		6 N, 7 N, 4 N
WP2*uvr*A	(b) (4)	5000	*12.7*	*4.2*	*1.1*	16 N, 14 N, 8 N
		2500	*12.0*	*1.0*	*1.0*	13 N, 12 MN, 11 N
		1250	*15.0*	*2.6*	*1.3*	14 N, 18 N, 13 N
		625	*16.7*	*2.3*	*1.4*	18 N, 18 N, 14 N
		313	*17.0*	*5.3*	*1.4*	11 MN, 19 N, 21 N
	Dimethyl Sulfoxide		*12.0*	*3.6*		13 N, 8 N, 15 N

Key to Plate Postfix Codes

N	Normal background bacterial lawn
M	Plate counted manually

Study No.: 8218265
Trial No.: 8218265-C1
Plating Method: Plate incorporation assay

Date Plated: 12/3/2009
Date Counted: 12/9/2009 to 12/10/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	**2NF**	1.0	*161.7*	*18.7*	*12.1*	183 N, 154 N, 148 N
TA100	**SA**	2.0	*1167.7*	*140.1*	*14.6*	1021 N, 1182 N, 1300 N
TA1535	**SA**	2.0	*839.7*	*55.7*	*64.6*	898 N, 787 N, 834 N
TA1537	**ICR**	2.0	*248.3*	*20.6*	*43.8*	272 N, 239 N, 234 N
WP2*uvr*A	**4NQO**	1.0	*295.7*	*10.2*	*24.6*	300 N, 303 N, 284 N

Key to Positive Controls

2NF	2-nitrofluorene
SA	sodium azide
ICR	ICR-191
4NQO	4-nitroquinoline-N-oxide

Key to Plate Postfix Codes

N	Normal background bacterial lawn
M	Plate counted manually

7.2 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

Study title: Chromosomal Aberrations in cultured human peripheral blood lymphocytes

Study no.:	
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 21, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) ((b) (4) ARS P10764, and (b) (4)

Key Study Findings

(b) (4) **induced** chromosomal aberrations in cultured lymphocytes in the presence of metabolic activation (three hour treatment); whereas it did not in the absence of metabolic activation. Under both conditions, (b) (4) did not induce polyploidy or endoreduplication.

Methods

Cell line:	Human lymphocytes
Concentrations in definitive study:	20.3, 29.1, 41.5, 59.3, 84.7, 121.0, 173.0, 247.0, 353.0, 504.0, 720.0, 1030.0, 1470.0, 2100.0, and 3000.0 mcg/mL with and without metabolic activation
Basis of concentration selection:	The concentrations employed were selected based on the reduction of cell growth (i.e., cell growth inhibition) relative to the solvent control. Note that the highest concentration selected induced ≥ 50% cell growth inhibition relative to control; with a sufficient number of scorable metaphase cells, as determined based on the mitotic index. As part of the analysis, at least two lower concentrations were evaluated in addition to the high concentration selected.
Negative control:	Dimethylsulfoxide (DMSO)
Positive control:	Mitomycin C (MMC; 0.75, 1.00, 1.50 mcg/mL; without S9) Cyclophosphamide (CP; 20.00, 25.00, 40.00 mcg/mL; with S9)
Formulation/Vehicle:	The test article was provided as a white crystalline powder and dissolved in DMSO
Incubation & sampling time:	The cultures were incubated for three hours and harvested twenty two hours after the initiation of treatment

Study Validity

The criteria for a valid test were reportedly based on the following:

- the frequency of cells with structural chromosome aberrations in the solvent control contained approximately 5% cells with aberrations;
- the positive control results were significantly higher ($p \leq 0.01$) when compared to the solvent control;
- the assay included the highest applicable dose (10 mM or 5 mg/mL, whichever was lower) or a dose exceeding the solubility limit in culture medium if there was no significant reduction (approximately ≥ 50%) in mitotic index and the aberration results were negative; and
- the assay employed at least three analyzable dose levels.

Given these criteria, the Reviewer concurred with the Sponsor that the study was valid.

Results

Solubility and dose determination. Based on solubility testing, DMSO was chosen as the vehicle, based on the lack of precipitation at the final concentration of 1620 mcg/mL. At the mentioned concentration, the media formed a transparent, light red solution, and pH was 7.5 (pH of the culture medium was 7.0). Note that the highest concentration tested in the assay was 3000 mcg/mL, which was ~ 10 mM of (b) (4) (molecular weight 313.4) after dosing in culture medium. The Sponsor reported that precipitate was observed at 2100 mcg/mL and 3000 mcg/mL in the presence and absence of metabolic activation in the initial chromosomal aberration assay. In the absence of metabolic activation, precipitate was observed during treatment and wash at both concentrations. In the presence of metabolic activation, precipitation was observed during wash only at 2100 mcg/mL and during treatment and wash at 3000 mcg/mL.

Dose Analysis. According to the final report, the dose formulations tested (high and low dose) for each mutagenicity assay were solutions and stable for 24 hours at room temperature (all values were within ± 1.7% of the initial concentration). Note that the test article was not detected in vehicle control formulations.

Initial Chromosomal Aberration Assay. A statistically significant increase in cells with chromosomal aberrations was observed at 2100 mcg/mL (3 hour treatment) in the presence of metabolic activation only. Note that this concentration produced ≥ 50% reduction in the mitotic index in the absence and presence of metabolic activation. At the highest concentration tested (3000 mcg/mL), the slides prepared for both cultures were observed to have dead cells and sparse cells, respectively, following treatment in the absence and presence of metabolic activation. (b) (4) was considered negative for inducing chromosomal aberrations in the absence of metabolic activation and there were no significant increases in cells with polyploidy or endoreduplication in the cultures analyzed.

Confirmatory Chromosomal Aberrations Assay. Given that (b) (4) was demonstrated to induce chromosomal aberrations in the presence of metabolic activation after a (three hour treatment) the confirmatory assay was not conducted.

Conclusions. (b) (4) was demonstrated to be positive for inducing chromosomal aberrations (i.e., clastogenic) in cultured lymphocytes with metabolic activation; whereas it was negative without metabolic activation after a three hour treatment period. Note that (b) (4) did not induce polyploidy or endoreduplication in the presence or absence of metabolic activation. Also, the positive controls employed induced chromosomal aberrations, as expected.

Figure 15. Chromosomal Aberrations in Human Lymphocyte Cultures

Without Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest

Study No.: 8218266 Trial No.: B1 Date: 11/23/09 Test Article: (b) (4)

					# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judge-Ment (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations							Judge ment (+/-)[d]
												simple breaks				Totals[c]		
											gaps		chte	chre	mab	-g	+g	
Controls																		
Vehicle:	DMSO	20.0	µL/mL	A	100		100	0	0			1				1	1	
				B	100		100	1	0							0	0	
				Total	200		200					1				1	1	
				Average	%	0		**0.5**	**0.0**			**0.5**				**0.5**	**0.5**	
Positive:	MMC	1.00	µg/mL	A	50		100	0	0		1	14	7			19	19	
				B	50		100	0	0			10	4		1	15	15	
				Total	100		200				1	24	11		1	34	34	
				Average	%	--		**0.0**	**0.0**	-	**1.0**	**24.0**	**11.0**		**1.0**	**34.0**	**34.0**	+
Test Article		720	µg/mL	A	100		100	0	0							0	0	
				B	100		100	0	0		1					0	1	
				Total	200		200				1					0	1	
				Average	%	0		**0.0**	**0.0**	-	**0.5**					**0.0**	**0.5**	-
		1470	µg/mL	A	100		100	0	0		3	1				1	4	
				B	100		100	0	0			1				1	1	
				Total	200		200				3	2				2	5	
				Average	%	21		**0.0**	**0.0**	-	**1.5**	**1.0**				**1.0**	**2.5**	-
		2100	µg/mL	A	100		100	0	0			5	4			7	7	
				B	100		100	0	0							0	0	
				Total	200		200					5	4			7	7	
				Average	%	57		**0.0**	**0.0**	-		**2.5**	**2.0**			**3.5**	**3.5**	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. DMSO = dimethylsulfoxide MMC = Mitomycin C

With Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest

Study No.: 8218266 Trial No.: B1 Date: 11/23/09 Test Article: (b) (4)

					# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judge-Ment (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations							Judge-ment (+/-)[d]
												simple breaks	chte	chre	mab	Totals[c] -g	Totals[c] +g	
Controls																		
Vehicle:	DMSO	20.0	µL/mL	A	100		100	0	0			3				3	3	
				B	100		100	0	0		1					0	1	
				Total	**200**		**200**				1	3				3	4	
				Average	%	**0**		**0.0**	**0.0**		**0.5**	**1.5**				**1.5**	**2.0**	
Positive:	CP	25.0	µg/mL	A	50		100	0	0		1	18	3			20	21	
				B	50		100	0	0		5	15	5			17	20	
				Total	**100**		**200**				6	33	8			37	41	
				Average	%	--		**0.0**	**0.0**	-	**6.0**	**33.0**	**8.0**			**37.0**	**41.0**	+
Test Article		720	µg/mL	A	100		100	1	0			3				3	3	
				B	100		100	0	0			3				3	3	
				Total	**200**		**200**					6				6	6	
				Average	%	**0**		**0.5**	**0.0**	-		**3.0**				**3.0**	**3.0**	-
		1470	µg/mL	A	100		100	1	0			4	1			5	5	
				B	100		100	1	0		3	1			1	2	5	
				Total	**200**		**200**				3	5	1		1	7	10	
				Average	%	**23**		**1.0**	**0.0**	-	**1.5**	**2.5**	**0.5**		**0.5**	**3.5**	**5.0**	-
		2100	µg/mL	A	100		100	0	0		1	11				11	12	
				B	100		100	0	0		3	4	1		1	6	7	
				Total	**200**		**200**				4	15	1		1	17	19	
				Average	%	**50**		**0.0**	**0.0**	-	**2.0**	**7.5**	**0.5**		**0.5**	**8.5**	**9.5**	+

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. DMSO = dimethylsulfoxide CP = Cyclophosphamide

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: In Vivo Rat Bone Marrow Micronucleus Assay with Liver, Stomach, Jejunum, and Blood Comet Assay

Study no.:	8222569
Study report location:	(b) (4)
	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	28 January 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) ARS, #s L11937 and P10764, and (b) (4)

Key Study Findings

(b) (4) did not produce a statistically significant increase in the averaged % micronucleated polychromatic erythrocytes and ratio of PCE:NCE; and the averaged values for the tail intensity and tail moment endpoints measured in tissue samples obtained from test article treated animals when compared to control. Note that (b) (4) produced mortalities at the highest dose tested in each sex (male, 400 mg/kg; female, 200 mg/kg), as well as produced clinical signs across the doses tested. The clinical signs observed were generally produced in a dose-related manner in the treated animals. Across sexes, the clinical signs included convulsions; yellow haircoat-perineal area; protruding eyes; hypoactivity; and swollen nose. In females, clear oral discharge; red hair coat-nose; recumbent-sternal; lacrimation; rough hair coats; hunched posture; irregular respiration; cold to touch; and hyperactivity were also observed. Together, these data demonstrated that (b) (4) was tested at an appropriately toxic dose and was deemed negative in the induction of micronuclei in bone marrow and the induction of DNA damage in the liver, stomach, jejunum, and blood in rats administered toxicologically relevant doses.

Methods

Doses in definitive study:	See **Figure 16**
Frequency of dosing:	Daily for three consecutive days
Route of administration:	Oral via gavage
Dose volume:	See **Figure 16**

Formulation/Vehicle:	Test article was provided as a white crystalline powder that was dissolved in a vehicle consisting of Methylcellulose USP and Reverse Osmosis/Deionized Water
Species/Strain:	HSD: Sprague Dawley (SD) rats
Number/Sex/Group:	See **Figure 16**
Satellite groups:	None
Basis of dose selection:	A dose-finding study was conducted in SD rats in order to define the highest appropriate dose level for the definitive study, based on the observation of the observation of toxic signs and/or mortality in treated animals.
Negative control:	See **Figure 16**
Positive control:	See **Figure 16**

Figure 16. Study Design for Definitive Micronucleus/Comet assay

Target Dose Level (mg/kg)	Stock Concentration (mg/mL)	Dosing Volume (mL/kg)	Route of Administration	Animals/Harvest		Replacement Animals[a]
				Male	Female	Female
Positive Controls, CP/EMS: 60/200	6/20	10	Oral Gavage	5	5	--
Vehicle Control, 0	0	20	Oral Gavage	5	5	--
50	2.5	20	Oral Gavage	--	5	--
100	5	20	Oral Gavage	5	5	--
200	10	20	Oral Gavage	5	5	3
400	20	20	Oral Gavage	5	--	--

Vehicle Control = 0.5% methyl cellulose in reverse osmosis/deionized (RO/DI) water
Positive Controls: CP = Cyclophosphamide, EMS = Ethyl methanesulfonate
a Animals were dosed as potential replacements for the original high-dose groups.

Study Validity

The assay acceptance criteria:

1. Acceptable Controls
   - the vehicle control group mean was within the historical control range and usually was less than 0.4% micronucleated PCEs; and
   - the positive control group mean was elevated in a statistically significant manner relative to that of the vehicle control group and the positive control response was consistent with the historical positive control data

2. Acceptable High Dose
   - the high dose reached the limit dose or produced some observable toxicity (e.g., reduce PCE:NCE ratio, induce toxic signs, and/or produced mortality in test article treated animals); unless there were solubility constraints, in which case the high dose was the solubility limit or higher doses if a well dispersed suspension was obtained that did not settle out rapidly

Assay Evaluation Criteria

1. Micronucleus Assay:
   - The criteria for a positive result was based on the observation of a statistically significant positive response in micronucleated PCEs for at least one dose, as well as a statistically significant dose-related response for the micronucleus assay; otherwise the result was deemed negative.
2. Comet Assay:
   - The criteria for a positive result was based on the observation of a statistically significant positive response in the parameters of Tail moment or Tail Intensity for at least one dose and/or a statistically significant dose-response; otherwise, the result was deemed negative.

The Sponsor noted that biological relevance could also be considered as an additional determinant of a positive response.

Results

Mortality. Mortalities were reported in males at 400 mg/kg/day (n=3/5) and in females at 200 mg/kg (n=6/8; see **Figure 17**). Note that three males and five females from these dose groups were found dead prior to the scheduled sacrifice. On day 2, the male animals were found dead either immediately after treatment (n=1; #3848) or one hour after treatment (n=2; #s 3850 and 3861). The five females were found dead one hour after treatment on day 2. A single female from the 200 mg/kg/day group was euthanized prior to the scheduled sacrifice during the morning of day 2 (prior to treatment).

Figure 17. Mortality Summary for In Vivo Micronucleus/Comet Assay

Target Dose Level (mg/kg/day)	Sex	Number Dosed	Number Surviving to Scheduled Sacrifice	Number Died	Number Euthanized*
100	M	5	5	0	0
200	M	5	5	0	0
400	M	5	2	3	0
50	F	5	5	0	0
100	F	5	5	0	0
200	F	8	2	5	1

* Number euthanized refers to animals humanely euthanized prior to scheduled sacrifice due to excessive toxicity in animal.

Clinical Observations. A variety of clinical signs were observed in treated animals. These signs varied in regard to the incidence in which they were reported and the

treatment days on which they were observed. Note that the severity of the clinical signs reported below was not discussed in the final report reviewed.

Those clinical signs that were observed in both sexes included convulsions; yellow haircoat-perineal area; protruding eyes; hypoactivity; and swollen nose. Convulsions were observed one hour following treatment in a female from the 200 mg/kg group (#3918) on day 1 and immediately after treatment in a male animal (#3848) from the 400 mg/kg group on day 2. The female animal was found dead one hour after treatment on day 2. The male animal reportedly was found dead immediately after treatment; and was observed with yellow haircoat–perineal area, wet haircoat–perineal area, and red ocular discharge. Animal #3918 was found dead one hour after treatment on day 2. Yellow hair coat–perineal area was also reported immediately after treatment in a female (#3912) from the 200 mg/kg group on day 2. Those signs observed across sexes that were observed on multiple treatment days are noted in **Table 25**. For example, a swollen nose was reported in a female (#3919) from the 200 mg/kg group prior to termination on day 3 and in males from each dose group one hour following treatment on various days. In males, swollen noses were observed in animals from the 100 mg/kg group on days 2 and 3 (n ≤ 2/5); the 200 mg/kg group on day 1, 2, and 3 (n ≤ 3/5); and the 400 mg/kg group on days 1 (n = 5/5), 2 (n = 1/4), and 3 (n = 1/2). Protruding eyes were observed in both sexes in all of the treatment groups. In females, this sign was observed in animals from the 50 mg/kg group on days 1 and 2 (n = 5/5); the 100 mg/kg group on days 1, 2, and 3 (n = 5/5); and the 200 mg/kg group on days 1 (n = 8/8), 2 (n = 2/8), and 3 (n = 2/8). In males, this sign was observed in animals from the 100 mg/kg group on days 1 (n = 5/5), 2 (n = 1/5), and 3 (n = 5/5); the 200 mg/kg group on days 1 (n = 5/5), 2 (n = 2/5), and 3 (n = 5/5); and the 400 mg/kg group on days 1 (n = 5/5), 2 (n = 2/4), and 3 (n = 2/2). Hypoactivity was observed in both sexes at ≥ 200 mg/kg. In males, hypoactivity was observed in animals from the 200 mg/kg group (n = 5/5) on day 3; as well as the 400 mg/kg group on days 1 (n = 2/5) and 3 (n = 2/2). In females, hypoactivity was observed in animals from the 200 mg/kg group only on days 1 (n = 8/8) and 2 (n = 2/7).

Clinical signs observed in females only included clear oral discharge; red hair coat-nose; recumbent-sternal; lacrimation; rough hair coats; hunched posture; irregular respiration; cold to touch; hyperactivity. Clear oral discharge was observed one hour following treatment in 2/8 females from the 200 mg/kg group on day 1 (#s 3918 and 3924) and day 2 (#s 3919 and 3925). Red hair coat – nose was reported in the 100 mg/kg group on day 2 in the morning prior to treatment in 2/5 females; and in the 200 mg/kg group on day 2 in immediately post dose in 6/8 females and on day 3 immediately post dose and one hour post dose in 1/8 females. Recumbent-sternal was reported in a female (#3916) from the 200 mg/kg group in the morning prior to treatment on day 2. Lacrimation was observed on day 2 in a female during the morning prior to treatment (#3916) and immediately after treatment (#3912). Rough haircoats and hunched posture were observed in ≥ 6/8 females from the 200 mg/kg group on day 2 during the morning prior to treatment and immediately after treatment. Irregular respiration and cold to touch was reported in a female (#3916) from the 200 mg/kg group during the morning prior to treatment on day 2. On day 2, hyperactivity was

reported in 3/8 females immediately following treatment. Female #3929 was noted as thin immediately after treatment on day 2.

Table 25. Clinical Signs Observed in Both Sexes on Multiple Treatment Days

Clinical Sign	Treatment Groups																							
	50 mg/kg/day						100 mg/kg/day						200 mg/kg/day						400 mg/kg/day					
	D1		D2		D3		D1		D2		D3		D1		D2		D3		D1		D2		D3	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Protruding Eyes		5/5		5/5		0/5	5/5	5/5	1/5	5/5	5/5	5/5	5/5	8/8	2/5	2/8	5/5	2/8	5/5		2/4		2/2	
Swollen nose		0/5		0/5		0/5	0/5	0/5	1/5	0/5	2/5	0/5	2/5	0/5	3/5	0/5	1/5	0/5	5/5		1/4		1/2	
Hypoactive		0/5		0/5		0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	8/8	0/5	2/7	5/5	0/2	2/5		0/4		2/2	

Micronucleus assay. (b) (4) in treated animals did not produce a statistically significant increase in the averaged % micronucleated polychromatic erythrocytes and ratio of PCE:NCE when compared to control (see **Figure 18**). Note that the positive control CP did produce a statistically significant increase in the averaged % micronucleated polychromatic erythrocytes and decrease in the averaged ratio of PCE:NCE when compared to control.

Figure 18. Summary of in Vivo Micronucleus Data

Study No.: 8222569
Test Article: (b) (4)
Initiation of Dosing - Males: 23 February 2010
Initiation of Dosing - Females: 02 March 2010 Species/Strain: Rat/Sprague-Dawley

Treatment	Dose	Harvest Time[b]	% Micronucleated PCEs Mean ± SD Male	% Micronucleated PCEs Mean ± SD Female	Ratio PCE:NCE Mean ± SD Male	Ratio PCE:NCE Mean ± SD Female
Controls						
Vehicle	VC 20 mL/kg/day	3	0.05 ± 0.04	0.04 ± 0.04	0.77 ± 0.10	0.58 ± 0.08
Positive	60 mg/kg[a]	24[c]	1.40 ± 0.48*	0.94 ± 0.41*	0.64 ± 0.04**	0.65 ± 0.06
Test Article	100 mg/kg/day	3	0.03 ± 0.04	--	0.78 ± 0.11	--
	200 mg/kg/day	3	0.09 ± 0.07	--	0.81 ± 0.11	--
	400 mg/kg/day	3	0.05 ± 0.00	--	0.72 ± 0.06	--
	50 mg/kg/day	3	--	0.05 ± 0.04	--	0.61 ± 0.10
	100 mg/kg/day	3	--	0.06 ± 0.09	--	0.65 ± 0.12
	200 mg/kg/day	3	--	0.10 ± 0.07	--	0.54 ± 0.02

* Significantly greater than the corresponding vehicle control, $p \leq 0.01$.
** Significantly less than the corresponding vehicle control, $p \leq 0.05$.
VC = 0.5% methyl cellulose in reverse osmosis/deionized water
PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte
[a] Positive control animals were dosed once with cyclophosphamide at 60 mg/kg ~24 hours prior to bone marrow collection and once with ethyl methanesulfonate at 200 mg/kg ~3 hours prior to organ/tissue collection.
[b] Animals were dosed with test article once daily for 3 days. The first two doses were ~24 hrs apart. The third dose was started ~21 hr after second dose. Bone marrow harvest occurred ~3 hours after the last dose administration.
[c] Bone marrow was harvested ~24 hours after the cyclophosphamide administration.

Comet assay. (b) (4) did not produce statistically significant increases in averaged values for the tail intensity and tail moment endpoints measured in the tissue samples collected when compared to control (see **Figures 19** and **20**). Note that the positive control produced a statistically significant increase in the averaged values for these endpoints when compared to control, as expected.

Figure 19. Comet Assay Summary Tables for Males

Study No.: 8222569
Test Article: (b) (4)
Initiation of Dosing: 23 February 2010
Species/Strain: Rat/Sprague-Dawley
Tissue: Liver

Treatment Group	Dose Level (mg/kg/day)	Total no. cells scored	Tail Intensity Mean	Tail Intensity SEM	Tail Moment Mean	Tail Moment SEM	Mean % clouds	Mean % Diffused cells
Positive	60/200[a]	500	28.96	± 4.08*	6.34	± 1.51*	0.00	0.20
Vehicle	20 mL/kg/day	500	0.34	± 0.14	0.06	± 0.03	0.00	0.20
(b) (4)								

* Significantly greater than the corresponding vehicle control, $p \leq 0.01$.
Vehicle = 0.5% methyl cellulose in reverse osmosis/deionized water
SEM = Standard error of the mean
[a] Positive control animals were dosed once with cyclophosphamide at 60 mg/kg ~24 hours prior to bone marrow collection and once with ethyl methanesulfonate at 200 mg/kg ~3 hours prior to organ/tissue collection.

Study No.: 8222569
Test Article (b) (4)
Initiation of Dosing: 23 February 2010
Species/Strain: Rat/Sprague-Dawley
Tissue: Blood

Treatment Group	Dose Level (mg/kg/day)	Total no. cells scored	Tail Intensity Mean	Tail Intensity SEM	Tail Moment Mean	Tail Moment SEM	Mean % clouds	Mean % Diffused cells
Positive	60/200[a]	500	4.40	± 1.24*	0.63	± 0.19*	0.00	0.20
Vehicle	20 mL/kg/day	500	0.15	± 0.04	0.03	± 0.01	0.00	0.00
(b) (4)								

* Significantly greater than the corresponding vehicle control, $p \leq 0.01$.
Vehicle = 0.5% methyl cellulose in reverse osmosis/deionized water
SEM = Standard error of the mean
[a] Positive control animals were dosed once with cyclophosphamide at 60 mg/kg ~24 hours prior to bone marrow collection and once with ethyl methanesulfonate at 200 mg/kg ~3 hours prior to organ/tissue collection.

Study No.: 8222569
Test Article: (b) (4)
Initiation of Dosing: 23 February 2010
Species/Strain: Rat/Sprague-Dawley
Tissue: Stomach

Treatment Group	Dose Level (mg/kg/day)	Total no. cells scored	Tail Intensity Mean	Tail Intensity SEM	Tail Moment Mean	Tail Moment SEM	Mean % clouds	Mean % Diffused cells
Positive	60/200[a]	500	9.20	± 3.26	1.68	± 0.73	7.00	0.40
Vehicle	20 mL/kg/day	500	1.95	± 0.42	0.32	± 0.06	6.20	1.00
(b) (4)								

Vehicle = 0.5% methyl cellulose in reverse osmosis/deionized water
SEM = Standard error of the mean
[a] Positive control animals were dosed once with cyclophosphamide at 60 mg/kg ~24 hours prior to bone marrow collection and once with ethyl methanesulfonate at 200 mg/kg ~3 hours prior to organ/tissue collection.

Study No.: 8222569
Test Article: (b) (4)
Initiation of Dosing: 23 February 2010
Species/Strain: Rat/Sprague-Dawley
Tissue: Jejunum

Treatment Group	Dose Level (mg/kg/day)	Total no. cells scored	Tail Intensity Mean	Tail Intensity SEM	Tail Moment Mean	Tail Moment SEM	Mean % clouds	Mean % Diffused cells
Positive	60/200[a]	500	12.32	± 1.93*	2.31	± 0.56*	8.20	0.20
Vehicle	20 mL/kg/day	500	1.39	± 0.26	0.23	± 0.04	4.40	0.20
(b) (4)								

* Significantly greater than the corresponding vehicle control, $p \leq 0.01$.
Vehicle = 0.5% methyl cellulose in reverse osmosis/deionized water
SEM = Standard error of the mean
[a] Positive control animals were dosed once with cyclophosphamide at 60 mg/kg ~24 hours prior to bone marrow collection and once with ethyl methanesulfonate at 200 mg/kg ~3 hours prior to organ/tissue collection.

Figure 20. Comet Assay Summary Tables for Females

(b) (4)

Study No.: 8222569
Test Article: (b) (4)
Initiation of Dosing: 02 March 2010
Species/Strain: Rat/Sprague-Dawley
Tissue: Blood

Treatment Group	Dose Level (mg/kg/day)	Total no. cells scored	Tail Intensity Mean	Tail Intensity SEM	Tail Moment Mean	Tail Moment SEM	Mean % clouds	Mean % Diffused cells
Positive	60/200[a]	500	19.93	± 2.12*	3.60	± 0.48*	0.00	0.00
Vehicle	20 mL/kg/day	500	0.19	± 0.06	0.03	± 0.01	0.00	0.00
(b) (4)								

* Significantly greater than the corresponding vehicle control, $p \leq 0.01$.
Vehicle = 0.5% methyl cellulose in reverse osmosis/deionized water
SEM = Standard error of the mean
[a] Positive control animals were dosed once with cyclophosphamide at 60 mg/kg ~24 hours prior to bone marrow collection and once with ethyl methanesulfonate at 200 mg/kg ~3 hours prior to organ/tissue collection.

Study No.: 8222569
Test Article: (b) (4)
Initiation of Dosing: 02 March 2010
Species/Strain: Rat/Sprague-Dawley
Tissue: Stomach

Treatment Group	Dose Level (mg/kg/day)	Total no. cells scored	Tail Intensity Mean	Tail Intensity SEM	Tail Moment Mean	Tail Moment SEM	Mean % clouds	Mean % Diffused cells
Positive	60/200[a]	500	19.47	± 6.24*	4.56	± 1.92	6.60	0.40
Vehicle	20 mL/kg/day	500	2.50	± 0.38	0.38	± 0.06	5.40	1.40
(b) (4)								

* Significantly greater than the corresponding vehicle control, $p \leq 0.01$.
Vehicle = 0.5% methyl cellulose in reverse osmosis/deionized water
SEM = Standard error of the mean
[a] Positive control animals were dosed once with cyclophosphamide at 60 mg/kg ~24 hours prior to bone marrow collection and once with ethyl methanesulfonate at 200 mg/kg ~3 hours prior to organ/tissue collection.

Study No.: 8222569
Test Article: (b) (4)
Initiation of Dosing: 02 March 2010
Species/Strain: Rat/Sprague-Dawley
Tissue: Jejunum

Treatment Group	Dose Level (mg/kg/day)	Total no. cells scored	Tail Intensity Mean	Tail Intensity SEM	Tail Moment Mean	Tail Moment SEM	Mean % clouds	Mean % Diffused cells
Positive	60/200[a]	500	25.82	± 5.16*	6.11	± 1.53*	9.70	0.20
Vehicle	20 mL/kg/day	500	2.39	± 0.40	0.34	± 0.06	5.20	0.20
(b) (4)								

* Significantly greater than the corresponding vehicle control, $p \leq 0.01$.
Vehicle = 0.5% methyl cellulose in reverse osmosis/deionized water
SEM = Standard error of the mean
[a] Positive control animals were dosed once with cyclophosphamide at 60 mg/kg ~24 hours prior to bone marrow collection and once with ethyl methanesulfonate at 200 mg/kg ~3 hours prior to organ/tissue collection.

8 Carcinogenicity

Codeine. The carcinogenic potential of oral codeine in rats and mice has been evaluated (National Toxicology Program, 1996). In these studies, codeine was presented in the daily diet of the animals for up to 24 months. The findings are briefly summarized below.

The NTP evaluated the effects of codeine fed in a daily diet to F344/N rats in a 24 month study (National Toxicology Program, 1996). The daily diets of rats (60/sex/group) consisted of 0, 400, 800, or 1600 ppm of codeine. There were no apparent drug-related differences in the survival rates of the animals tested. Food consumption in codeine-treated rats was not significantly altered; however, mean body weight was decreased in the 1600 ppm group when measured during weeks 53-105. In regard to absolute and relative organ weights in males at the 15 month interim necropsy, there was a significant increase in the weight of the adrenal gland in the 800 and 1600 ppm groups and a decrease in the weight of the liver in all treated groups. Histological findings reported in males from these groups demonstrated that there was a larger amount of adrenal cortex relative to adrenal medulla; vascular dilation in the medulla and inner cortex was slightly increased; and the zona reticularis was slightly thickened and the cells of the inner portion of the zona fasicularis were decreased when compared to control at 15 months. At the 24 month necropsy, data in the 1600 ppm group demonstrated that there was a significant increase in the incidence of clitoral gland ectasia in females and preputial gland hyperplasia in males when compared to control. There were no reports of significant (i.e., dose-related) toxicological findings in codeine-treated animals.

In the same study, the NTP evaluated the effects of codeine fed in a daily diet to $B6C3F_1$ mice in a 24 month study (National Toxicology Program, 1996). The daily diets of mice (60/sex/group) consisted of 0, 750, 1500, or 3000 ppm of codeine. There were no apparent drug-related differences in the survival rates of the animals tested. Food consumption in codeine-treated mice was not significantly altered; however, mean body weight was slightly decreased in the 3000 ppm group, during weeks 14-52 and 53-104, when compared to control. The weights of collected organs were reportedly comparable across groups and there were no significant toxicological effects in gross pathology studies in codeine-treated mice. Histological findings were limited to thyroid gland follicular cell hyperplasia, which significantly increased at 15 months in males from the 3000 ppm group and at 24 months in both genders from all codeine treatment groups. By 24 months, this hyperplasia was described as focal in distribution; minimal to mild in severity; and involved up to 3 adjacent follicles, in which the number of epithelial cells was increased. In females, the incidence of this hyperplasia increased in a dose-related manner.

The FDA-approved labeling for Roxane's codeine tablet (NDA 22402) summarizes the findings of the NTP studies, as follows:

> Carcinogenesis: Two year carcinogenicity studies have been conducted in F344/N rats and B6C3F1 mice. There was no evidence of carcinogenicity in male and female rats, respectively, at dietary doses up to 70 and 80 mg/kg/day of codeine (approximately 2 times the maximum recommended daily dose of 360 mg/day for adults on a mg/m^2 basis) for two years. Similiarly there was no evidence of carcinogenicity activity in male and female mice at dietary doses up to 400 mg/kg/day of codeine (approximately 5 times the maximum recommended daily dose of 360 mg/day for adults on a mg/m^2 basis) for two years.

9 Reproductive and Developmental Toxicology

Codeine. Reproductive toxicity studies evaluating codeine administered using various dosing schedules and routes of administration have been conducted in animal species that include rabbits, hamsters, rats and mice. These studies evaluated the effects of codeine on embryo-fetal development and pre- and postnatal development in experimental animals. See below for brief summaries of findings from these studies.

Fertility. There were no fertility studies identified in the literature by either the Sponsor or the Reviewer. As such the FDA-approved labeling for Roxane's codeine tablet (NDA 22402) contains the following:

> Impairment of fertility: No animal studies were conducted to evaluate the effect of codeine on male or female fertility.

Embryo-fetal development. Published embryofetal development studies for codeine have been reported using the hamster, rat, mouse, and rabbit models. The results of these studies served as the basis of the labeling for Roxane's codeine tablet NDA (22402).

In a single-treatment study, the toxicity of codeine (0, 73, 96, 240 or 360 mg/kg; subcutaneous route) was evaluated in timed pregnant Lakeview outbred golden hamsters at gestation day 8 (Geber WF and Schramm LC, 1975). At 240 and 360 mg/kg, codeine produced dose-related increases in the percentage of maternal deaths when compared to control (10 and 20%, respectively). In codeine dose groups, there was a slight decrease in the mean number of fetuses/litter when compared to control. Codeine increased the number of malformed fetuses, an effect that wasn't dose-related. Across codeine dose groups, all of the malformed fetuses exhibited cranioschisis. A dose of 73 mg/kg represents a human equivalent dose of 592 mg/60 kg person based on body surface area. As the dose tested was far greater than the typical dose for an opioid naïve individual, the clinical significance of this finding is not clear. Further the study is difficult to interpret as the maternal toxicity at non-lethal doses was not described. It should be noted that GLP studies conducted by the National Toxicology Program tested lower doses of codeine and were able to identify a NOAEL for fetal effects (see below). Although the interpretation of the results of this study is challenging due to evidence of maternal toxicity, these results were deemed adequate to be

included in the referenced product labeling based on their relevance to toxicological findings reported at lower doses in other studies (National Toxicology Program, 1987b; Zellers JE and Gautieri RF, 1977).

The potential reproductive toxicity of oral codeine was evaluated in rabbits (5, 12.5 and 30 mg/kg) and rats (10, 35 and 120 mg/kg) during organogenesis (Lehmann H, 1976). Codeine in the rabbit did not produce any apparent teratogenic and embryotoxic effects at the doses tested. In the rat, no apparent teratogenic effects were produced up to 120 mg/kg; however, embryotoxic effects were observed at this dose. This toxicity was observed as an increase in resorptions in one of several groups of rats treated at this dose, which was reportedly in the toxic range for dams. This increase was observed in dams sacrificed at the 11th day of pregnancy, unlike those sacrificed at the 21st day.

The effects of 100 mg/kg codeine (SC) administered in CF-1 albino mice either on a single day during gestation from Day 7-12 or on Days 8 and 9 were studied to evaluate its potential embryo-fetal toxicities (Zellers JE and Gautieri RF, 1977). Codeine increased the starting and terminal weights of dams when administered at Gestation Day (GD) 10. In dams treated on days other than GD 11 or 12, the mean weight (g) of harvested fetuses was significantly decreased when compared to control. All treatment groups, except those administered codeine on GD 8 and 9 had increased skeletal abnormalities almost 15-fold higher than control. In the litters present, codeine reportedly increased delayed ossification of the supraoccipital bone, xiphoid, and paws in various treatment groups. Delayed ossification of the supraoccipital bone and paws reported in at least one litter present in the control and codeine treated groups. In regard to the supraoccipital bone, there was a significant increase in the number of litters from dams administered codeine on GD 9 or 10, and GD 8 and 9 when compared to control. Delayed ossification of the xiphoid was reported in fetuses harvested from dams treated with codeine on either GD 9, 10, 12, or GD 8 and 9. There were a significant number of litters present with delayed ossification of the paws in fetuses from dams for every treatment group, except those treated with codeine on GD 12 when compared to control. Sternebrae defects reportedly occurred in significantly more litters of fetuses from dams administered codeine, except GD 8 and GD 12, when compared to control. It appears as though the mouse data in the previously approved referenced drug product labeling is from this publication.

The National Toxicology Program conducted two definitive reproductive toxicology studies, which were also later summarized in a single journal article (see, Williams J et al., 1991). Codeine (0, 10, 50, or 150 mg/kg; bid; oral) was administered in Lakeview outbred golden (LVG) hamsters on gestational days 5-13 to evaluated its potential teratogenic effects (National Toxicology Program, 1987b). In the pregnant LVG hamsters (12-18/group), maternal toxicity was clearly evident at the high dose, presenting as significantly reduced body weight gain compared to controls. In the litters studied, mean fetal body weights were significantly reduced in the 50 and 150 mg/kg treatment groups. The highest dose of codeine (150 mg/kg) increased the incidence of resorptions per litter. Dead fetuses (n=3) were reportedly observed only in dams treated with 150 mg/kg. The number of live fetuses was comparable across groups,

and litters from dams treated at each dose had comparable amounts of fetuses from both genders. Body weights of fetuses from dams treated with 50 and 150 mg/kg were substantially decreased compared to control. At the highest dose tested (150 mg/kg; bid), there was a non-statistically significant increase in fetuses demonstrating the external malformation meningoencephalocele and there was an increase in the number of variations such as supernumerary rib (in the Lumbar I full; n=7) and incomplete ossification (cartilage present; n=9) were observed. The authors report a NOAEL for developmental toxicity in hamsters as 20 mg/kg/day (100 mg/m^2, based on body surface area). This dose is approximately 0.5 times the maximum recommended human dose of 360 mg/day for adults based on body surface area.

The study in Swiss CD-1 mice administered codeine (0, 75, 150 or 300 mg/kg; bid; oral) during gestational days 6-15 evaluated its potential teratogenic effects (National Toxicology Program, 1987a). The 150 and 300 mg/kg dose significantly reduced maternal weight gain. The high dose also resulted in mortality in 19% of the dams, and increased the percentage of resorptions per litter by > 2-fold the level observed in controls. At the 150 and 300 mg/kg doses of codeine, fetal body weight was significantly decreased. Codeine exposure during gestation reportedly did not increase the incidence of major fetal malformations up to the 300 mg/kg dose. The authors report a NOAEL for developmental toxicity in mice as 150 mg/kg/day(450 mg/m^2, based on body surface area). This dose is approximately 2 times the maximum recommended human dose of 360 mg/day for adults based on body surface area.

Pre- and postnatal development studies. In repeat dose-studies the toxicity of codeine phosphate was evaluated in female Sprague Dawley rats subcutaneously administered 30 mg/kg starting 3 days after sperm were observed in their vaginal smears and continuing 25 days after neonates were delivered (Ching M and Tang L, 1986). Dams were treated during gestation and lactation periods. In codeine-treated dams, the mortality rate of neonates (3/41) was 7.3% at birth compared to 0% in controls; and their body weights (male and female) were slightly decreased compared to control. No neonate deaths were reported 1 week after delivery. In neonates, hypothalamic growth hormone releasing activity at 25 days of age in both genders and vaginal opening at 39 days of age in females were comparable to control.

Based on these data, the FDA-approved labeling for Roxane's codeine tablet (NDA 22402) contains the following:

> **8 USE IN SPECIFIC POPULATIONS**
> **8.1 Pregnancy**
> Teratogenic Effects, Pregnancy Category C
> There are no adequate and well-controlled studies in pregnant women. Codeine should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.
>
> Codeine has been shown to have embryolethal and fetotoxic effects (reduced fetal body weights and delayed or incomplete ossification) in the hamster, rat and mouse models at approximately 2-4 times the maximum recommended human

dose of 360 mg/day based on a body surface area comparison. Maternally toxic doses that were approximately 7 times the maximum recommended human dose of 360 mg/day, were associated with evidence of resorptions and incomplete ossification, including meningioencephalocele and cranioschisis. In contrast, codeine did not demonstrate evidence of embrytoxicity or fetotoxicity in the rabbit model at doses up to 2 times the maximum recommended human dose of 360 mg/day based on a body surface area comparison. *[see Nonclinical Toxicology (13.3)]*

13.3 Reproduction and Developmental Toxicology
Studies on the reproductive and developmental effects of codeine have been reported in the published literature in hamsters, rats, mice and rabbits.

A study in hamsters administered 150 mg/kg bid of codeine (PO; approximately 7 times the maximum recommended daily dose of 360 mg/day for adults on a mg/m^2 basis) reported the development of cranial malformations (i.e., meningoencephalocele) in several fetuses examined; as well as the observation of increases in the percentage of resorptions per litter examined. Doses of 50 and 150 mg/kg, bid resulted in fetotoxicity as demonstrated by decreased fetal body weight. In an earlier study in hamsters, doses of 73-360 mg/kg level (PO; approximately 2-8 times the maximum recommended daily dose of 360 mg/day for adults on a mg/m^2 basis), reportedly produced cranioschisis in all of the fetuses examined.

In studies in rats, doses at the 120 mg/kg level (PO; approximately 3 times the maximum recommended daily dose of 360 mg/day for adults on a mg/m^2 basis), in the toxic range for the adult animal, were associated with an increase in embryo resorption at the time of implantation.

In pregnant mice, a single 100 mg/kg dose (SC; approximately 1.4 times the recommended daily dose of 360 mg/day for adults on a mg/mg^2 basis) reportedly resulted in delayed ossification in the offspring.

No teratogenic effects were observed in rabbits administered up to 30 mg/kg (approximately 2 times the maximum recommended daily dose of 360 mg/day for adults on a mg/m^2 basis) of codeine during organogenesis.

10 Special Toxicology Studies

There were no special toxicology studies submitted.

11 Integrated Summary and Safety Evaluation

Codeine as a drug substance was first approved by the FDA in 1950 in drug combination products. This NDA would be the second single entity codeine product approved by the Agency. Given the long history of use and the current marketed status of the referenced oral tablet drug product (NDA 22402), nonclinical toxicology studies

for codeine were not required for this NDA application. Codeine has been demonstrated to produce analgesic and adverse effects in humans that correspond to effects observed in animal species. In animal species, codeine has not been demonstrated to be mutagenic, but does produce reproductive toxicities. For example, reproductive organ toxicity (i.e., testicular degeneration) has been reportedly produced in male rats administered ≥ 12500 ppm (i.e., ≥ 650 mg/kg) of daily codeine (at least 20 times the maximum recommended daily dose for adults on a mg/m^2 basis) for 14 days. Mortality was observed at these doses, unlike lower doses (i.e., ≥ 450 mg/kg) which did not produce testicular degeneration or, as an added measure, did not induce sperm morphology alterations in male rats and mice across longer exposure periods. In pregnant animals, embryotoxicity and teratogenic effects of codeine have been demonstrated in hamsters, rats and mice. Such findings support the Pregnancy Category C label given to codeine.

In addition to the published studies submitted to support the safety of oral codeine, the Sponsor provided original studies evaluating (b) (4) ((b) (4) an impurity identified in the drug substance. In the studies reviewed, oral (b) (4) in rats produced a variety of adverse effects. For example, (b) (4) administered for up to two weeks produced toxicities that included hypoactivity and protruding eyes across sexes, as well as swollen paws, face and nose in males at 100 mg/kg. (b) (4) administered up to thirteen weeks produced toxicities that included mortality and tremors across sexes; as well as decreased body weight in males at ≥ 30 mg/kg. Based on these findings safety margins were established, which demonstrated that the NOAEL/LOAEL values estimated in rats were (b) (4) (b) (4) estimated in the maximum dose approved for codeine (see **Table 26**) given the proposed specification of NMT (b) (4) Therefore, there is an adequate safety margin for this impurity specification. Given these findings and the extensive clinical history of codeine, this NDA may be approved from the pharmacology/toxicology perspective.

Table 26. Safety Margins Estimated for (b) (4)

Repeat-Dose Study Duration	Gender	NOAEL (mg/kg)	LOAEL (mg/kg)	mg/m^2	Safety Margin[1] (based on mg/m^2)
2-week (non-GLP)	Male/Female	(b) (4)		(b) (4)	(b) (4)
13-week (GLP)	Male	(b) (4)		(b) (4)	(b) (4)
	Female		(b) (4)	(b) (4)	(b) (4)

[1]Safety Margins are based on the amount of (b) (4) proposed at NMT of the maximum amount of daily codeine (360 mg/day) in a 70 kg individual and expressed as mg/m^2. **The maximum dose of (b) (4) expected in humans is** (b) (4)

12 Appendix/Attachments

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/s/

MARCUS S DELATTE
06/09/2011

RICHARD D MELLON
06/09/2011
I concur with Dr. Delatte's recommendation that NDA 202245 may be approved from the nonclinical pharmacology toxicology perspective.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 202-245 Applicant: Roxane Laboratories Stamp Date: 27 Sep 2010

Drug Name: Codeine Sulfate NDA/BLA Type: 505 (b)(2)

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		Note that according to the Sponsor's cover letter (27 Sep 2010), the Agency agreed with the proposed approach of referencing codeine sulfate tablets (i.e., NDA 22-402) in support of the current 505 (b) (2)

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
				submission.
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		The Sponsor previously agreed to qualify the drug substance impurity (b) (4) as a post-marketing commitment for NDA 22-402. Note that findings from several genetic toxicology studies evaluating this impurity have been provided; and that data from a 90-day repeat-dose toxicology study will be submitted by 31 Dec 2010. (b) (4) in the DP requires further justification in terms of general toxicology
11	Has the applicant addressed any abuse potential issues in the submission?	X		
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable.

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes

Potential review issues to be forwarded to the Applicant for the 74-day letter.

1. Your drug product stability specification for (b) (4) of NMT (b) (4) exceeds the safety qualification threshold of NMT 0.2%. Although there are adequate genetic toxicology data available to support the safety of this specification, you have not submitted adequate justification regarding the general toxicity of this impurity. Either (b) (4) the specification to NMT (b) (4) or conduct a repeat-dose toxicology study of at least 90-days duration to support the proposed specification.

2. Your have not provided adequate safety justification for the novel excipient, Orange Flavor, XBF-709818. You must provide the quantitative formulation, including CAS numbers, for all components of the flavor and provide justification for the safety of up to (b) (4) of this flavoring agent in your drug product. We refer you to the following guidance document: Guidance for Industry: Nonclinical Studies for Safety Evaluation of Pharmaceutical Excipients (May 2005) which is available on the CDER web page at the following

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

Marcus S. Delatte, Ph.D.	8 November 2010
Reviewing Pharmacologist	Date
Team Leader/Supervisor	Date

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/s/

MARCUS S DELATTE
11/18/2010

RICHARD D MELLON
11/18/2010