CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

206627Orig1s000

PHARMACOLOGY REVIEW(S)

FDA Center for Drug Evaluation and Research
Division of Anesthesia, Analgesia, and Addiction Products
10903 New Hampshire Avenue, Silver Spring, MD 20993

MEMORANDUM TO FILE

Date: October 30, 2014

To: **NDA 206627**

From: R. Daniel Mellon, PhD
Supervisory Pharmacologist, Division of Anesthesia, Analgesia, and Addiction Products DAAAP

Subject: **Secondary review and PMRs based on DMF deficiencies**

Background: Purdue Pharma submitted NDA 206627 seeking approval of Hysingla ER (hydrocodone bitartrate) extended-release tablets, indicated for the management of pain severe enough to require daily, around-the-clock, long-term opioid treatment and for which alternative treatment options are inadequate. The drug product formulation contains polyethylene oxide (PEO), which is intended to impart abuse-deterrent properties to the drug product formulation. There is no maximum daily dose for single entity full agonist opioid drug products due to the development of tolerance necessitating an increase in dose in order to maintain efficacy. As such, the risk assessment for these drug product formulations is based on a maximum theoretical daily doses (MTDD) that is either based on actual clinical use data or extrapolations of potency across the class when actual use data for the compound does not exist. As there is limited clinical experience with single entity hydrocodone drug products, the Division has determined that the MTDD for hydrocodone controlled-release drug products intended for chronic use is 3 grams per day. If a person consumed 3 grams of hydrocodone per day via the largest dosage form of Hysingla, they would consume (b) (4) of PEO. (b) (4) t. Therefore the safety assessment of this drug product formulation was based, in part, on the data supporting the safety of the PEO. Purdue did not conduct toxicology studies for PEO or the drug product formulation. They referenced (b) (4) Drug Master File for PEO (DMF (b) (4)) to support the safety of the PEO.

Elizabeth Bolan, PhD completed the primary review of the NDA and recommended approval. However, she notes that there were deficiencies in the Drug Master File; but these deficiencies may not be deemed approval issues given the long history of use of (b) (4) PEO in (b) (4) FDA-approved drug products that reference DMF (b) (4) . With the assistance of Dr. Bolan, I reviewed the toxicology studies in the Drug Master File to determine if there were adequate data to justify the safety of the PEO in Hysingla. During the course of the review of the DMF, multiple deficiencies

were identified in the DMF and numerous communications with (b) (4) occurred in an attempt to resolve those deficiencies prior to the NDA action date. The pharmacology toxicology review of the DMF was finalized on October 28, 2014. There were a total of 6 deficiencies identified and these will be communicated to (b) (4). Several of these deficiencies request that emailed commitments from (b) (4) to update the DMF be formalized. However, several other deficiencies will require additional studies including analysis of the PEO for low molecular weight impurities and possible reproductive and developmental toxicology studies, since these studies are not currently in the DMF and there was evidence for absorption of radiolabeled material in the distribution studies. The reader is referred to the review of the DMF dated October 28, 2014 for additional details.

Recommendation: As noted with my signature of Dr. Bolan's review, I concur with Dr. Bolan's recommendation that the NDA 206627 may be approved from a pharmacology toxicology perspective; however, given the deficiencies noted in the DMF, I recommend that the Agency institute the following Post-Marketing Requirement to Purdue, in order to assure that the deficiencies are adequately addressed. We encourage (b) (4) and Purdue to work together to address these deficiencies, given the daily dose of PEO that could be consumed via the Hysingla drug product.

PMR 1 Analyze the (b) (4) PEO products employed in Hysingla for low molecular weight impurities. Identify and quantitate the impurities. Submit a toxicological risk assessment for the exposure to the impurities taking into consideration the maximum theoretical daily dose of Hysingla.

PMR 2 Conduct an embryo-fetal development study in the rat model to assess the potential impact of PEO on development. The study must be designed to adequately qualify the safety of the low molecular weight PEO components (impurities/degradants) in the PEO used to manufacture Hysingla when the product is consumed up to the MTDD of Hysingla.

PMR 3 Conduct an embryo-fetal development study in the rabbit model to assess the potential impact of PEO on development. The study must be designed to adequately qualify the safety of the low molecular weight PEO components (impurities/degradants) in the PEO used to manufacture Hysingla when the product is consumed up to the MTDD of Hysingla.

PMR 4 Conduct a pre- and post-natal development study in the rat model to assess the potential impact of PEO on development. The study must be designed to adequately qualify the safety of the low molecular weight PEO components (impurities/degradants) in the PEO used to manufacture Hysingla when the product is consumed up to the MTDD of Hysingla.

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/s/

RICHARD D MELLON
10/30/2014

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:	206627
Supporting document/s:	SDN 1
Applicant's letter date:	4/26/2014
CDER stamp date:	4/28/2014
Product:	Hydrocodone bitartrate q24h Film-coated tablets
Indication:	Management of pain severe enough to require daily, around-the-clock, long-term opioid treatment for which alternative options are inadequate
Applicant:	Purdue Pharma LP
Review Division:	Division of Anesthesia, Analgesia, and Addiction Products
Reviewer:	Elizabeth A. Bolan, PhD & Huiqing Hao, PhD
Supervisor/Team Leader:	R. Daniel Mellon, PhD
Division Director:	Sharon Hertz, MD
Project Manager:	Lisa Basham

Template Version: September 1, 2010

Disclaimer

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1 Executive Summary

1.1 Introduction

Purdue has submitted NDA 206627 for Hysingla ER, an extended-release hydrocodone bitartrate (HC) product which contains excipients that are intended to confer abuse-deterrent properties. Hysingla ER is formulated in strengths of 20, 30, 40, 60, 80, 100, and 120 mg HC per tablet and is intended for q24h dosing. The indication for this product is management of pain severe enough to require daily, around-the-clock, long-term opioid treatment for which alternative options are inadequate. This NDA is a 505(b)(2) application and is relying on the Agency's findings of safety and the description of the pharmacology of HC in the label of Vicoprofen (NDA 20716).

The Applicant has submitted studies to assess the safety pharmacology, general toxicology, genetic toxicology, developmental and reproductive toxicology and the carcinogenic potential of HC.

1.2 Brief Discussion of Nonclinical Findings

All impurities in the drug substance and drug product are controlled at acceptable levels. Hysingla ER contains excipients that are intended to confer abuse-deterrent properties and resist alcohol-induced dose dumping. (b) (4) With the exception of the PEO, the levels of the excipients in this product, when calculated for the maximum theoretical daily dose of HC, are considered acceptable and do not require qualification. (b) (4) . To support the safety of the levels of the PEO in this product, the Applicant is referencing MF (b) (4) ((b) (4)). Master File (b) (4) has been found to be inadequate because of the lack of adequate characterization of low molecular weight entities in the polymer. These low molecular weight entities could include (b) (4) and specifications for these impurities in the excipient master file may be required. However, because of the longstanding history of use of PEO in many products which reference MF (b) (4) , this deficiency will not be an approval issue for NDA 206627. The levels of PEO in Hysingla ER when used at the MTDD of hydrocodone are considered acceptable from a pharmacology/toxicology perspective.

The neurobehavioral and respiratory safety pharmacology studies showed results consistent with the known effects of opioids. The cardiac safety pharmacology assessment showed the potential for effects of HC on the heart. Hydrocodone did not show meaningful inhibition of the hERG potassium channel at concentrations >300-fold higher than human exposure at an oral dose of 120 mg. However, the in vitro Purkinje fiber assay showed HC-dependent increases in action potential duration and an in vivo single-dose study in conscious, freely

moving telemetered dogs showed increases in RR and QRS interval durations as well as increases in QT and QTc intervals with HC. The findings in dog were seen at C_{max} exposures 0.7-fold the human C_{max} exposure of a 120 mg HC dose. No effects on the heart were observed in the toxicology studies with chronic administration of HC to rat and dog. To address the potential for cardiotoxicity in humans, the Applicant has conducted a clinical study to evaluate the effect of HC on the QT/QTc interval.

The highest available strength for this product will be 120 mg HC and the product is labeled to be used q24h, therefore, the systemic levels at the human dose of 120 mg/day at steady state will be used as the exposure comparison with the toxicology studies described below.

The results of the general toxicology studies in rat and dog were typical of an opioid agonist and no clinically-relevant toxicities unique to HC were demonstrated. No target organs were identified in either species. No adverse findings were identified in the dog study; therefore, the NOAEL in dog was greater than the highest dose tested. The highest dose tested yielded exposure margins in male and female dogs of 0.9-fold and 0.8-fold, respectively, the human systemic exposure of a 120 mg HC dose (based on AUC comparisons). No adverse findings were identified in the rat study; therefore, the NOAEL in rat is greater than the highest dose tested. The highest dose tested yielded exposure margins in male and female rats of 0.2-fold and 0.1-fold, respectively, the human systemic exposure of a 120 mg HC dose (AUC).

The standard ICH battery of genetic toxicology studies was conducted with HC and the weight-of-evidence suggests that HC does not have mutagenic or clastogenic potential. Additionally, two-year carcinogenic assessments were conducted in rat and mouse and no HC-related neoplasms were observed. Exposure margins for the highest dose tested in male and female rats were 0.2-fold and 0.1-fold, respectively, the systemic levels at the human dose of 120 mg/day (AUC). Exposure margins for the highest dose tested in male and female mice were 3.5-fold and 3.1-fold, respectively, the systemic levels at the human dose of 120 mg/day (AUC).

A full reproductive and developmental toxicology battery was conducted with HC. No embryotoxicity, teratogenicity or effects on fertility were observed in rats at exposures 0.1-fold the human HC dose of 120 mg/day based on exposure (AUC) comparisons. However, the embryofetal development studies in rat and rabbit showed reduced pup survival rates and reduced fetal/pup body weights. A pre- and post-natal development study in rat showed decreases in pup viability, pup survival indices, litter size, and pup body weight. These effects in rat and rabbit were seen at exposures approximately 0.1 and 0.3-fold, respectively, the human HC dose of 120 mg/day based on exposure comparisons (AUC). The observed toxicities in the reproductive and developmental toxicology battery are consistent with a Pregnancy Category C designation and the findings will be described in the label.

The pharmacologic effects of opioids limit the dosing in nonclinical species and typically multiples of human clinical exposures are not achieved. Although the mouse carcinogenicity study provided exposure margins of ~3-fold at the NOAEL, all of the rat and dog exposures of HC in the studies conducted are below the systemic exposure in humans at the dose of 120 mg.

1.3 Recommendations

1.3.1 Approvability

Pharmacology Toxicology recommends approval for NDA 206627.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

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

Applicant's proposed labeling	**Reviewer's proposed changes**	**Rationale for changes**
(b) (4)		The format has been changed to comply with the Pregnancy and Lactation Labeling Rule. To comply with this rule, the *Teratogenic and Nonteratogenic Effects* headings have been replaced with an *Animal Data* section. The Maternal Health

(b) (4)	Team will add the appropriate language for the human lactation data. Exposure comparisons were based on systemic levels at the human dose of 120 mg/day. The dose of 120 mg is the highest dosage form of this product which is labeled to be dosed once per day. (b) (4) .

(b) (4)	(b) (4) were removed.
	(b) (4) The Maternal Health Team will add the appropriate language for the human lactation data.
	The Established Pharmacologic Class for HC was added.

(b) (4)	
	Exposure comparisons were based on systemic levels at the human dose of 120 mg/day (from Study # HYD1002). The dose of 120 mg is the highest dosage

(b) (4)	form of this product which is labeled to be dosed once per day. (b) (4) were removed. The data from the genetic toxicology battery conducted by the Applicant were added. Positive findings were placed first.

(b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number: 34195-34-1 (hydrate)
143-71-5 (anhydrous)

Generic Name: Hydrocodone bitartrate

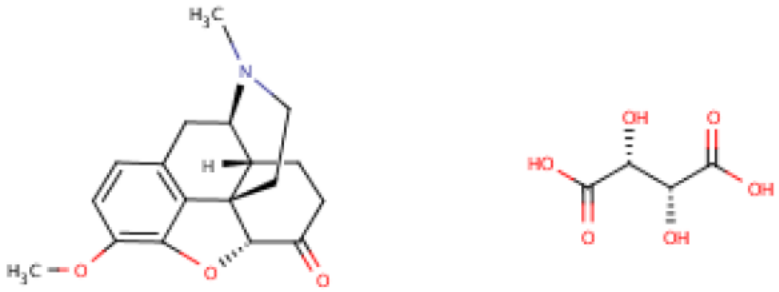
Code Names: HYD, HydroContin

Chemical Name: 4,5-Alpha-Epoxy-3-Methoxy-17-Methyl-Morphinan-6-one Tartrate (1:1) Hydrate (2:5)

Molecular Formula/Molecular Weight: $C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2.5H_2O$ / 494.90 (hydrate)

Structure:

Figure 1. Structure of Hydrocodone Bitartrate



Pharmacologic Class: Opioid Agonist (EPC)

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA#	Drug Name	Div	Strength (route)	Marketing Status	AP Date	Indication	Company
20716	Vicoprofen	DAAAP	Oral	Approved	9/23/1997	Moderate to Severe Pain	Abbvie Inc.

IND#	Drug	Status	Division	Indication	Stamp Date	Sponsor
59175	Hydrocodone bitartrate CR Tablets	Active	DAAAP	Management of Pain	2/13/2009	Purdue Pharma LP

DMF#	Subject of DMF	Holder	Submit Date	Reviewer's Comment
(b) (4)	Hydrocodone bitartrate, USP as manufactured in (b) (4)	(b) (4)		

(b) (4)

Of the above MFs, the MF for the drug substance and the (b) (4) (PEO) excipient were evaluated for potential toxicology data.

2.3 Drug Formulation

The drug product is formulated as an oral tablet for once-a-day delivery in seven strengths (20, 30, 40, 60, 80, 100, and 120 mg). The formulation is intended to provide abuse-deterrent properties and be resistant to alcohol-induced dose dumping.

The composition of the drug product formulation is depicted in the table below:

Table 1. Composition of the Hydrocodone Bitartrate q24h Film Coated Tablets

Component	Tablet Strength (mg)							Function	Reference to Standard
	20	30	40	60	80	100	120		
(b) (4)									

2.4 Comments on Novel Excipients

As with any single-entity opioid drug product approved for chronic use, there is no maximum daily dose listed in the labeling due to the development of tolerance. The development of tolerance necessitates increased doses with time in order to obtain the same desired effect. To establish the safety of the product for opioid-tolerant

individuals, the Division has employed a “maximum theoretical daily dose” (MTDD) based on clinical use data. As there are limited clinical use data on single-entity HC drug products, the Division elected to employ a rough potency comparison to morphine in order to estimate the MTDD and has established 3 grams per day as the MTDD for single-entity HC drug products. The table below summarizes the MTDD of the excipients in this drug product and assumes that if these levels were to be reached, it would be via use of the highest dosage strength.

The quantitative composition of the 120 mg tablet and the amount of each inactive ingredient at the MTDD of HC is presented in Table 2. The microcrystalline cellulose, hydroxypropyl cellulose and magnesium stearate can all be found in previously approved chronic use products at higher levels and do not present any unique toxicologic concerns in this product.

Polyvinyl alcohol

Polyvinyl alcohol is considered GRAS (GRAS Notice #GRN 00014; April 28, 2004) for use in aqueous film coating formulations applied to dietary supplement products (i.e., tablets or capsules), where the coating formulation is up to four percent (by weight) of the tablet or capsule, and PVA is up to 45 percent (by weight) of the coating formulation. The GRAS notice states that the maximum daily intake of PVA from its intended use in aqueous film coatings applied to pharmaceutical products is 360 mg/day. Additionally, the Joint Food and Agriculture Organization/World Health Organization's (FAO/WHO) Expert Committee on Food Additives (JECFA) assigned an acceptable daily intake to PVA of 50 mg/kg body weight/day (61st JECFA 2003). Fifty mg/kg for a 60 kg human equals 3 g which is greater than the (b) (4) of PVA when this product is used at the MTDD of HC. Therefore, the amount of PVA in the Hysingla ER product is considered acceptable.

Titanium dioxide

An acceptable daily intake of "not limited" was established at the 13th JECFA (1969). Additionally, titanium dioxide may be used for coloring foods if the quantity does not exceed 1% by weight of the food. Titanium dioxide may also be used for coloring ingested and externally applied drugs in amounts consistent with good manufacturing practice (21 CFR 73.1575). The percentage of titanium dioxide by weight of the 120 mg tablet of Hysingla ER is (b) (4)%. The level of titanium dioxide in the Hysingla ER product is considered acceptable.

Polyethylene Oxide
To support the safety of the levels of the PEO in this product, the Applicant is referencing MF (b) (4) ((b) (4) Master File (b) (4) has been found to be inadequate because of the lack of adequate characterization of low molecular weight entities in the polymer. These low molecular weight entities could include (b) (4) and specifications may be required. However, because of the longstanding history of use of PEO in (b) (4) products which reference MF (b) (4), this deficiency will not be an approval issue for NDA 206627. The levels of PEO in Hysingla ER when used at the MTDD of hydrocodone are considered acceptable from a pharmacology/toxicology perspective. Refer to the review of MF (b) (4) for details.

Table 2. Acceptability of Levels of Inactive Ingredients in the 120 mg Tablet at the MTDD of Hydrocodone

Inactive Ingredient	***Total dosage via single 120 mg tablet, mg***	***Total Dose at MTDD (25 pills, mg)***	***Rationale***
(b) (4)			

IIG: FDA Inactive Ingredients Guide

Table 3. Composition of (b) (4) (b) (4) and (b) (4) (b) (4) film coatings (as provided in the NDA)

Component	***Total dosage via single 120 mg***	***Total Dose at MTDD (25 pills),***	***Rationale***

(b) (4)

2.5 Comments on Impurities/Degradants of Concern

Drug Substance Impurities.

Although the drug substance to be used in the marketed drug product will only be obtained from (b) (4), the drug substance used in the toxicology studies was obtained from multiple manufacturers, including (b) (4) as summarized in the table below, reproduced from the submission. The qualification threshold according to the ICH Q3A(R2) guideline for impurities in the drug substance for a maximum daily dose of drug substance > 2 g/day is 0.05%. For this product, the clinical team determined that the MTDD of HC is 3 g. The Applicant is obtaining the HC drug substance for the marketed drug product from (b) (4) (MF (b) (4)). (b) (4) contains a structural alert for mutagenicity and the Applicant has adequately qualified (b) (4) for genotoxic potential. (b) (4) was found to be negative for genotoxic potential (see reviews in Genetic Toxicology section) and can therefore be regulated as a typical DS impurity according to ICH Q3A(R2) thresholds for qualification. All drug substance impurity specifications meet the ICH Q3A(R2) guideline specification of 0.05% and are considered acceptable (Table 5).

Table 4. Batch Analysis Summary for Hydrocodone Bitartrate Toxicology Lots

(b) (4)

Table 5. Drug Substance Specifications: (b) (4) MF (b) (4)

Test	*Proposed Specification*	*ICH Qualification Thresholds or Limits*	*Comments*
Residual Solvents			
(b) (4)			
Chromatographic Purity			
(b) (4)			

(b) (4)			
Total Impurities	NMT (b) (4)		Defer to CMC as this is a quality issue

The structure of (b) (4) is depicted below. This compound does not contain a structural alert for mutagenicity and can be control as per ICH Q3A(R2).

Figure 2. Structure of (b) (4) DS Impurity

(b) (4)

The Applicant indicated that safety qualification studies are being completed to support a drug substance specification for this impurity that exceeds the qualification threshold. According to the submission the studies will be completed in either the 3rd or 4th quarter of 2014 and submitted to the NDA. However, the specification of NMT (b) (4) is currently acceptable.

Drug Product Degradants.

The qualification threshold according to the ICH Q3B(R2) guidance for impurities/degradants in the drug product for a MDD of drug substance > 2 g is 0.15%. For this product, DAAAP has determined that the MTDD of HC is 3 g. During the review cycle, the specification of (b) (4)% for (b) (4) originally submitted in the NDA was tightened to 0.15%. The 0.15% specification for (b) (4) is acceptable. Justification in support of the originally proposed (b) (4) specification was provided by the Applicant and is reviewed below. The justification was determined to not support a specification in excess of the ICH Q3B(R2) threshold for qualification. This was communicated to the Applicant and a specification of 0.15% was ultimately submitted.

Table 6. Drug Product Degradant Specifications

Degradant	***Proposed Specification***	***ICH Qualification Thresholds or Limits***	***Comments***
(b) (4)	NMT 0.15%	The ICH Q3B(R2) qualification threshold is NMT 0.15%.	The specification is acceptable.
Each Individual Unknown	NMT 0.1%	The ICH Q3B(R2) identification threshold is NMT 0.1%.	The specification is acceptable.
Total Degradation Products	NMT (b) (4)%		Defer to CMC as this is a quality issue

Figure 3. Structure of (b) (4)

(b) (4)

When the NDA was submitted, the drug product specification for (b) (4) was set at (b) (4)% and exceeded the ICH Q3B(R2) threshold for qualification of 0.15%. The Applicant provided a rationale to justify their proposed specification. During the review cycle, the justification was determined to be inadequate to qualify the levels of (b) (4) at the (b) (4)% specification in the drug product. The CMC reviewer also had concerns with the (b) (4)% specification based on stability data that showed that the actual levels of (b) (4) detected were below the limit of quantitation (LOQ= (b) (4) %). Information Requests from both

Pharmacology Toxicology and CMC were sent to the Applicant during the review cycle. The information requests are below.

Information request from Pharmacology Toxicology sent to the Applicant on July 10, 2014:

> Provide data to demonstrate that (b) (4) is a significant metabolite present in either a toxicology species or in humans. The ICH Q3B(R2) Guidance document states that a drug product degradant is deemed adequately qualified if it is also a significant metabolite. Although the guidance does not define the term "significant," in general, if a metabolite is formed at levels that are equal to or greater than the levels that would occur via administration of your drug product, the overall toxicity assessment can be considered to have been established by the existing animal toxicology data or human experience. However, you have not provided adequate data to support the conclusion that the levels of (b) (4) formed in humans provides adequate coverage for the total daily dose of (b) (4) that could occur via use of your drug product with the proposed (b) (4)% specification. In the absence of adequate data to support your conclusion that (b) (4) is a significant metabolite, tighten the drug product specification to NMT 0.15%.

Excerpt from the information request from CMC sent to the Applicant on June 13, 2014:

> The release specification for (b) (4) in the drug product is too high and is not supported by the batch release data which demonstrate levels of (b) (4) to be at or below the limit of quantitation. Tighten the specification for (b) (4) to be consistent with the batch data and further tighten the specification for the total impurities which includes the current high specification for (b) (4).

The Applicant responded the queries from the Division on July 17, 2014. They agreed to tighten the (b) (4) specification to (b) (4)% and re-submitted the same justification for the (b) (4) specification. The (b) (4)% specification for (b) (4) still exceeds the ICH Q3B(R2) threshold for qualification.

The Division had a teleconference with the Applicant on July 22, 2014 to discuss the proposed specification of (b) (4)% for (b) (4). The Division communicated to the Applicant that the drug product specification for (b) (4) was still in excess of the ICH Q3B(R2) threshold for qualification and that their justification did not support a specification in excess of the ICH Q3B(R2) threshold for qualification. The Applicant subsequently submitted an updated specification of 0.15% for (b) (4). This specification meets ICH Q3B(R2) and is considered acceptable.

The Applicant's justification for the (b) (4)% specification of (b) (4) is discussed below.

A DEREK assessment of (b) (4) was submitted as justification for the (b) (4)% specification of (b) (4). No structural alerts for genotoxicity were identified in this report. The data show

that (b) (4) does not contain a structural alert for mutagenicity but it does not provide qualification for safety of levels of a degradant in excess if ICH Q3B(R2).

Receptor binding data (Study # HYD-N-058) was provided by the Applicant that shows that (b) (4) has lower affinity for mu, delta, and kappa opioid receptors than HC. They argue that "(b) (4) would be expected to have less intrinsic pharmacotoxicity or on-target toxicity than hydrocodone". While (b) (4) may have lower affinity at the mu, delta, and kappa opioid receptors than HC, the data do not provide support for a specification in excess of the ICH Q3B(R2) threshold for qualification.

The Applicant states that (b) (4) was found to "spontaneously revert" to HC when incubated with human liver S9 fractions. The Applicant conducted an in vitro study (Study # HYD-P-055) to assess the stability of HC and (b) (4) in human plasma and human liver S9 fractions in the presence and absence of NADPH and NADH cofactors. No conversion of HC to (b) (4) was observed in plasma or human S9 liver fractions +/- cofactors. Minimal HC levels were observed when (b) (4) was incubated with human plasma and S9 in the absence of cofactors. Significant levels of HC were observed when (b) (4) was incubated with S9 plus cofactors. Although this study shows that (b) (4) can be converted to HC under certain circumstances, it does not provide adequate evidence that (b) (4) is a metabolite of HC. The data actually suggest that HC is not metabolized to (b) (4) under the conditions of the assays conducted.

(b) (4) No extrapolation to the production of (b) (4) in human can be concluded.

The Applicant measured HC and (b) (4) in human urine samples collected from six subjects after a single administration of 60 mg HC (Study # HCZ, HYD1008). Five samples of urine over 24 h were pooled and analyzed. The batch analysis of the drug product (lot # CB-2011-08) used in the study showed that the amount of (b) (4) at release was below the limit of quantitation (LOQ= (b) (4) %), although since (b) (4) is a degradant of HC, the levels of (b) (4) at the time of the study cannot be accurately assessed. The concentrations of (b) (4) in urine pooled over 24 h ranged from 24.2–65.6 ng/mL across the six patients. Plasma levels of (b) (4) were not measured. The occurrence of the (b) (4) in the urine could be qualitative evidence of the metabolism of HC to (b) (4) although it should be noted than none of the other assessments of the metabolism of

HC conducted by the Applicant identified (b) (4) as a metabolite. The source of the low levels of (b) (4) detected in urine could also be as a result of the degradation of HC over 24 h in the urine. If (b) (4) is a human metabolite of HC, it is a very minor metabolite.

Data have not been provided to demonstrate that (b) (4) is a significant metabolite present in either a toxicology species or in humans. No quantitative data have been provided to support the conclusion that the levels of (b) (4) formed in humans provide adequate coverage for the total daily dose of (b) (4) that could occur via use of the drug product with the proposed (b) (4) % specification. However, the Applicant has submitted an updated specification of 0.15% (b) (4) in the drug product. This specification now meets the ICH Q3B(R2) threshold for qualification and is considered acceptable.

2.6 Proposed Clinical Population and Dosing Regimen

This extended-release HC product is planned to be marketed as 20, 30, 40, 60, 80, 100, and 120 mg tablets intended for q24h dosing in adults. The indication is management of moderate-to-severe chronic pain when a continuous around-the-clock opioid analgesic is needed for an extended period of time. The formulation is intended to provide abuse-deterrent properties and be resistant to alcohol-induced dose dumping.

2.7 Regulatory Background

The Applicant is submitting NDA 206627 via the 505(b)(2) regulatory pathway and is relying on the Agency's previous findings of safety and efficacy for Vicoprofen (NDA 20716). IND 59175 was originally opened on October 27, 1999 by Purdue Pharma and inactivated on May 12, 2004. The IND was reactivated by Purdue with the current formulation on February 13, 2009.

Hydrocodone bitartrate has been approved by FDA as immediate-release formulations in combination with various non-opioid drugs (e.g., acetaminophen, aspirin, and ibuprofen). Hydrocodone bitartrate has also recently been approved by FDA in the first single-entity extended-release formulation. The formulation of the product in NDA 206627 has purported abuse-deterrent properties, which the currently marketed HC ER product does not possess. Because of the abuse-deterrent properties of this product, NDA 206627 was granted a priority review.

3 Studies Submitted

3.1 Studies Reviewed

The studies in the table below are located in the EDR in eCTD format.

Study Title	***Study #***
Pharmacology and Pharmacokinetics	
Evaluation of the Effects of HYDROCODONE Bitartrate on Cloned hERG Channels Expressed in HEK293 Cells	HYD-N-014

Evaluation of the Potential Effects of HYDROCODONE Bitartrate on Cardiac Action Potential Parameters Measured in Canine Isolated Purkinje Fibers	HYD-N-015
Potential Cardiovascular Effects of Hydrocodone Bitartrate in the Beagle Dog	HYD-N-016
Neurobehavioral Evaluation of Orally Administered Hydrocodone Bitartrate in Rats	HYD-N-017
A Pharmacological Assessment of the Effect of Hydrocodone Bitartrate and HYDROCODONE Bitartrate Plus Naltrexone Hydrochloride on the Respiratory Function of the Albino Rat	NDSE-729-GLP
An *in Vitro* Stability of Hydrocodone and (b) (4) in Human Plasma and Human Liver S9 Fractions	HYD-P-055
In Vitro Opioid Receptor Binding Properties Of Hydrocodone And (b) (4) (Non-GLP)	HYD-N-058
Determination of Concentration of Hydrocodone and (b) (4) in Human Urine	HYD-P-059
Toxicology	
A 9-Month Oral (Capsule) Toxicity Study in Dogs with Hydrocodone Bitartrate	NDSE-572-GLP
A 2-Year Oral (Gavage) Carcinogenicity Study in Rats with Hydrocodone Bitartrate	NDSE-559-GLP
A 2-Year Oral (Gavage) Carcinogenicity Study in Mice with Hydrocodone Bitartrate	NDSE-558-GLP
An Oral Gavage Fertility Study of Hydrocodone Bitartrate in the Rat	901670 HYD-N-011
An Oral Teratology Study of Hydrocodone Bitartrate in the Rat	901671 HYD-N-008
An Oral Teratology Study of Hydrocodone Bitartrate in the Rabbit	901672 HYD-N-009
An Oral Pre and Postnatal Study of Hydrocodone Bitartrate in the Rat	901673 HYD-N-001
Salmonella-Escherichia Coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay Using Hydrocodone Bitartrate	DSE-362-GKP
L5178Y TK+/- Mouse Lymphoma Forward Mutation Assay with a Confirmatory Assay Using Hydrocodone Bitartrate	DSE-367-GLP
L5178Y TK+/- Mouse Lymphoma Forward Mutation Assay With and Without Human S9 Using Hydrocodone Bitartrate	NDSE-682-GLP
In Vivo Mouse Micronucleus Assay with Toxicokinetics Using Hydrocodone Bitartrate	DSE-366-GLP
In Vitro Mammalian Chromosome Aberration Test with (b) (4)	HYD-N-031
Bacterial Reverse Mutation Assay with (b) (4)	HYD-N-024
In Vivo Micronucleus and Comet Assay in Rats with (b) (4)	HYD-N-048

3.2 Studies Not Reviewed

The studies below were evaluated for relevance to the application but not formally reviewed because they were not deemed necessary for approval. The studies are located in the EDR in eCTD format.

Study Title	Study #
(b) (4)	

3.3 Previous Reviews Referenced

No previous reviews have been referenced.

4 Pharmacology

4.1 Primary Pharmacology

Hydrocodone is a semi-synthetic opioid agonist. The primary intended pharmacodynamic activity of HC is analgesia. The analgesic effect is mediated through mu opioid receptors. Hydrocodone is relatively selective for mu receptors though it can bind to other opioid receptor subtypes at higher doses.

4.2 Secondary Pharmacology

Hydrocodone, like other opioids, can cause respiratory depression, drowsiness, change in mood, decreased gastrointestinal motility, nausea, vomiting, and miosis. Additionally, effects on various other body systems, including but not limited to, the neuroendocrine system, the immune system, and the autonomic nerve system have been noted.

4.3 Safety Pharmacology

Although extensive clinical experience exists with HC, the Applicant has conducted a battery of safety pharmacology studies to characterize the effects of HC on the central nervous system, the respiratory system, and the cardiovascular system.

The acute effects of HC on the central nervous system were assessed with a neurobehavioral assessment in the rat (Study # HYD-N-017). Single oral doses of 20, 60, and 200 mg/kg were administered and a Functional Observational Battery and locomotor evaluation were conducted. Salivation was observed in one mid-dose rat and a few high-dose rats and resolved by 24 h in all cases. The finding was not considered adverse. Decreases in locomotor activity (basic and fine movement, rearing, and total distance) were observed at the high dose. Decreased locomotor activity is a typical pharmacologic effect of opioids in the rat and other species and was not considered adverse in this study. Swelling of one or more limbs was observed at the mid and high doses but was not considered adverse. Treatment-related swelling of limbs/paws was also observed in the rat carcinogenicity bioassay. No mortality was observed in this study and the NOAEL was considered the highest dose tested.

Acute use of opioids is typically associated with decreased salivation in humans. In animals and humans, opioid withdrawal is associated with increased salivation. The observed salivation in this study may be explained by the effect of HC on either the sympathetic or parasympathetic nervous systems, as stimulation of either can result in increased salivation. Kappa agonists and mixed mu/kappa agonists have also been associated with salivation in rats (Bowen, et al., 2003).

Swelling of the paws was noted in this study as well as the rat carcinogenicity assessment. Opioid-induced edema is a pharmacologic effect observed in both nonclinical species and humans. The mechanism is unclear but it has been hypothesized that opioid-mediated histamine release results in vesodilation leading to edema (Gardner-Nix, 2002).

A well-known effect of opioids, including HC, is respiratory depression. To assess the effect of HC on the respiratory system, the Applicant conducted a single-dose study in rat with oral doses of 125, 250, 500, 600, and 1200 mg/kg HC (Study # NSDE-729-GLP). Only respiratory rate and tidal volume were assessed in this study. Hydrocodone was shown to dose-dependently decrease respiratory rate in all treated groups and the effect was naloxone reversible. Tidal volume was unaffected by HC treatment. Multiple deaths in the higher doses of HC were observed and attributed to HC-mediated respiratory depression.

The Applicant conducted several studies to assess the effect of HC on the cardiovascular system. The effect of HC on hERG channel current in stably transfected mammalian cells was evaluated at concentrations of 0.5, 2, 10, and 100 mcM (Study # HYD-N-014). At the highest concentration tested, HC inhibited the hERG current by 33%. The human C_{max} of HC at dose of 120 mg (135 ng/mL, Study # HYD1002) is 367-fold higher than the concentration of 100 mcM that produced a 33% inhibition in this assay. In this assay, HC does not produce appreciable inhibition of the hERG channel at concentrations much higher than what would be seen with physiologic levels.

An evaluation of the effects of HC on cardiac action potential parameters in isolated Purkinje fiber cells was conducted using HC concentrations of 0.5, 10, and 100 mcM (Study # HYD-N-015). Action potentials were recorded at stimulation rates of 0.5, 1.0, and 2.0 Hz. At 10 and 100 mcM, HC produced concentration-dependent increases in APD_{50} and APD_{90} measurements with APD_{90} changes being of a higher magnitude than APD_{50}. The largest increase in APD_{90} was a 17% increase from baseline was observed at the lowest stimulation rate (0.5 Hz). Hydrocodone had no effect on other parameters such as rate of depolarization (V_{max}), overshoot, and resting membrane potential.

A single-dose study in conscious, freely moving telemetered dogs was conducted to characterize the effect of HC at doses of 1, 3, and 10 mg/kg on cardiovascular function (Study # HYS-N-016). The high dose in this study was chosen as the dose at which toxicity was expected based on results from a previous dose-range finding study. HC was associated with decreases in body temperature at all doses with a maximal decrease of approximately 2 degrees in the high dose group. Small, dose-dependent decreases in heart rates were observed with HC at the mid and high doses. Increases in RR and QRS interval durations and an increase in QT and QTc intervals were observed at 10 mg/kg. At doses up to 10 mg/kg HC did not produce changes in systolic, diastolic, or mean arterial blood pressure, PR interval duration, qualitative ECG evaluations, or troponin levels. The C_{max} in dogs for a single oral dose of 10 mg/kg in this study (97.1 ng/mL) is 0.7-fold the C_{max} in humans after a dose of 120 mg/day (135 ng/mL, Study # HYD1002). The Applicant notes that the results from this study prompted the Thorough-QT evaluation in humans.

The abuse liability of HC is well-known and is discussed in detail in the review by the Controlled Substances Staff.

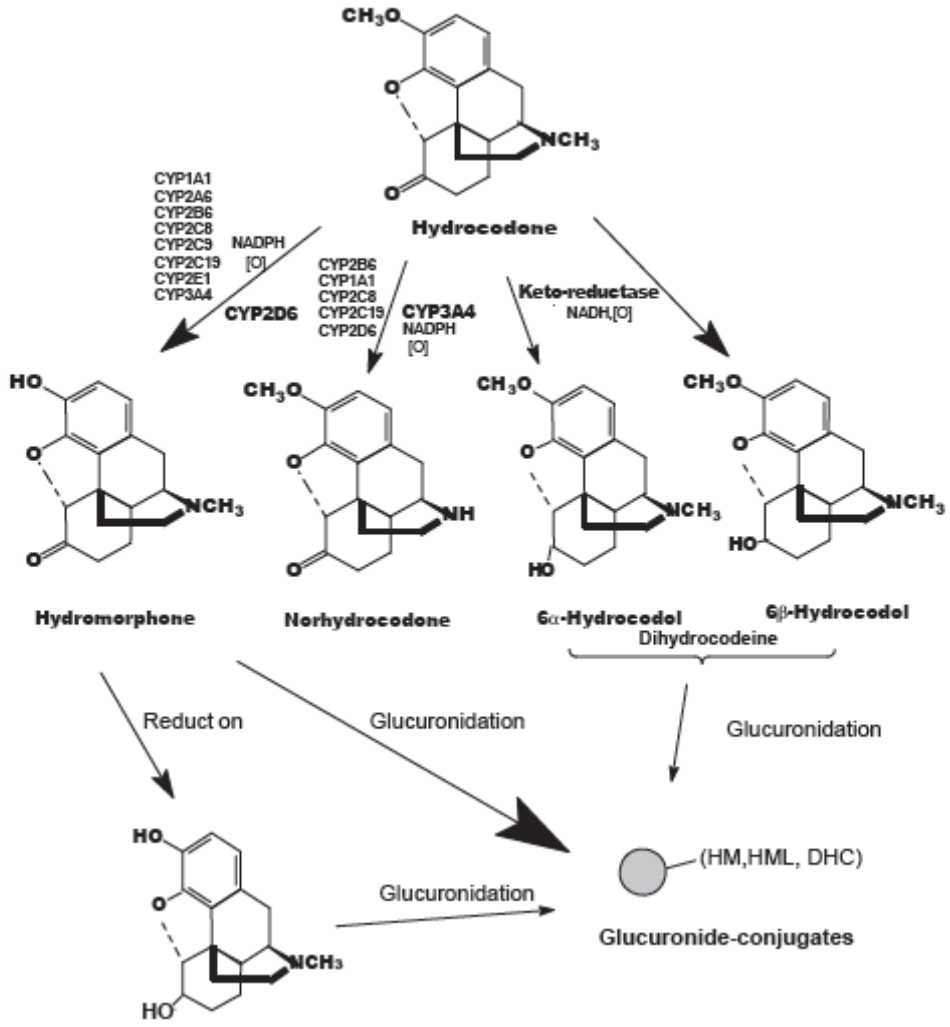
5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

In humans, HC undergoes complex hepatic metabolism with the major metabolic pathways resulting in the O- and N-demethylated metabolites. Hydrocodone is O-demethylated by CYP2D6 to hydromorphone and N-demethylated by CYP3A4 to norHC. Six-keto reduction to the corresponding 6-alpha- and 6-beta-hydroxy active metabolites also occurs (Clinical Pharmacology Online). The percent of the parent HC at which each metabolite is produced is not well-defined in the literature. Individual

differences in CYP enzymes and other non-CYP metabolic pathways may play a role in this inability to identify clear levels of metabolites (Hutchinson, et al., 2004). Comparison of recalculated published clearance data for HC with those predicted in Hutchinson, et al. 2004, indicate that about 40% of the clearance of HC is via non-CYP pathways (Hutchinson, et al., 2004).

Figure 4. Metabolism of Hydrocodone



5.2 Toxicokinetics

Toxicokinetics are discussed in reviews of the individual toxicology studies.

6 General Toxicology

6.1 Single-Dose Toxicity

No single-dose toxicology studies were conducted.

6.2 Repeat-Dose Toxicity (Rat)

Study title: A 2-Year Oral (Gavage) Carcinogenicity Study in Rats with Hydrocodone Bitartrate

Reviewer's Note: An extensive clinical history exists with HC. In lieu of a dedicated repeat-dose toxicology study in rodent with HC, the Division agreed to accept the rat carcinogenicity study if interim lab values were measured and endpoints were analyzed in terms of a NOAEL.

Study no.:	NDSE-559-GLP ((b) (4) 6770-142)
Study report location:	EDR 4.2.3.4.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 20, 2002
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Hydrocodone Bitartrate, Lots B13205 (99.2%), E01157 (98.6%), B13205 (98.7%)

Key Study Findings

- Significant body weight decreases (>20% at the HD) in males and females as well as decreases in food consumption were observed in all treated groups.
- Treatment-related increases in survival were observed and attributed to the decreased body weights.
- Hyperactivity, vocalizations, and sensitivity to touch were observed throughout the study and are attributed to the pharmacologic effects of HC. The findings are not considered adverse.
- Non-dose dependent increases in convulsions were also observed in both sexes.
- Treated rats (all doses) were generally more aggressive and had swollen hindpaws (males $\geq$12 mg/kg) sores and scabs (referred to as "pododermatitis", males and females $\geq$4 mg/kg).
- Increases in sternum bone marrow hypercellularity were observed in males at all doses. All females, including control, showed a higher incidence of bone marrow

hypercellularity than males. This finding is attributed to inflammation and is not considered adverse.

- In males, significant decreases in triglycerides (all doses, 26 and 105 weeks) and cholesterol (HD, 105 weeks) were observed.

- Treatment-related decreases in tumors in the anterior pituitary and mammary gland were observed.

- No NOEL can be established for this study. The NOAEL is >25 mg/kg in both males and females because none of the findings observed were particularly adverse.

Methods

Doses:	4, 12, and 25 mg/kg
Frequency of dosing:	Daily
Route of administration:	10 mL/kg
Dose volume:	Gavage
Formulation/Vehicle:	Distilled Water
Species/Strain:	Rat; Crl:CD(SD)IGS BR
Number/Sex/Group:	70/sex/group
Age:	At least 6 weeks
Weight:	Not given
Satellite groups:	9/sex/group for control TK, 12/sex/group for treated TK
Unique study design:	This study was designed as a 2-year carcinogenicity bioassay. The maximum dose was determined using a maximum tolerated dose based on body weight decrements in a 13-week dose range-finding study. Interim assessments at 26 weeks were conducted for clinical chemistry, hematology, and urinalysis parameters.
Deviation from study protocol:	None that affected the integrity of the study

Observations and Results

Mortality

Cage-side observations were made twice daily. Any rats euthanized *in extremis* were examined *post mortem*. Any rats found dead were examined as soon as possible after death. At the conclusion of the study, mortality among the controls and low dose males and females was similar throughout the study. In both males and females, the mid and high dose groups had a lower rate of mortality as compared to controls (Table 7). The increased survival in these groups is attributed to the lower body weights observed in the treated groups throughout the study.

Table 7. Survival at the Conclusion of the Study

Group	***Male Surviving/total***	***Female Surviving/total***
Control 1	28/50 (56%)	17/50 (34%)
Control 2	22/50 (44%)	17/50 (34%)
4 mg/kg	31/50 (62%)	19/50 (38%)
12 mg/kg	36/50 (72%)	27/50 (54%)
25 mg/kg	41/50 (82%)	41/50 (82%)

Clinical Signs

Cage-side observations included recording of any changes in clinical condition or behavior and were made twice daily. A detailed examination was performed at least weekly. The rats in the drug-treated groups were more aggressive and showed observations including swollen hindpaws (males ≥12 mg/kg) sores and scabs (males and females ≥4 mg/kg). Hyperactivity, vocalizations, and sensitivity to touch were also observed more commonly in all groups of treated males. These behaviors are considered to be due to the pharmacologic action of HC and are consistent with behaviors observed in the 13-week study. Convulsions were also observed in all groups. Incidences of convulsions in the mid-dose male and female groups exceeded levels in control animals (Table 8). The incidence of convulsions appears to be treatment-related but the observation was not dose-dependent. High-dose opioids have been shown to inhibit GABAergic systems leading to decreased seizure thresholds. Labeling for opioids notes that use of an opioid can induce or potentiate seizures in some clinical settings. Age-related signs were observed in all groups were not considered drug-related.

Table 8. Incidence of Convulsions

	Male					***Female***				
Clinical sign	***C1***	***C2***	***mg/kg***			***C1***	***C2***	***mg/kg***		
			4	***12***	***25***			***4***	***12***	***25***
n	70	70	70	70	70	70	70	70	70	70
Convulsions	2 (3%)	3 (4%)	4 (6%)	7 (10%)	5 (7%)	1 (1%)	0 (0%)	2 (3%)	5 (7%)	2 (3%)

Body Weights

Body weights were recorded prior to treatment, weekly through Week 14 and every two weeks thereafter. All rats were weighed immediately prior to termination. All treated groups had net gains in body weight, but at terminal sacrifice, both male and female body weights were dose-dependently lower than controls. Body weights are presented in the figures and tables below. Mean body weights of the two control groups were similar for both males and females and were combined. The reduced body weights throughout the study likely contributed to the increased survival and decreased incidence of certain neoplasms in the treated groups.

Table 9. Body Weights at the Conclusion of the Study

	Group, mg/kg	***Body weight difference: Treated - control at last time point, g***	***Percent change from control at last time point, %***
Male	*4*	-3	-5
	12	-116	-16
	25	-164	-23
Female	*4*	-30	-6
	12	-60	-13
	25	-100	-21

Figure 5. Body Weights in Males

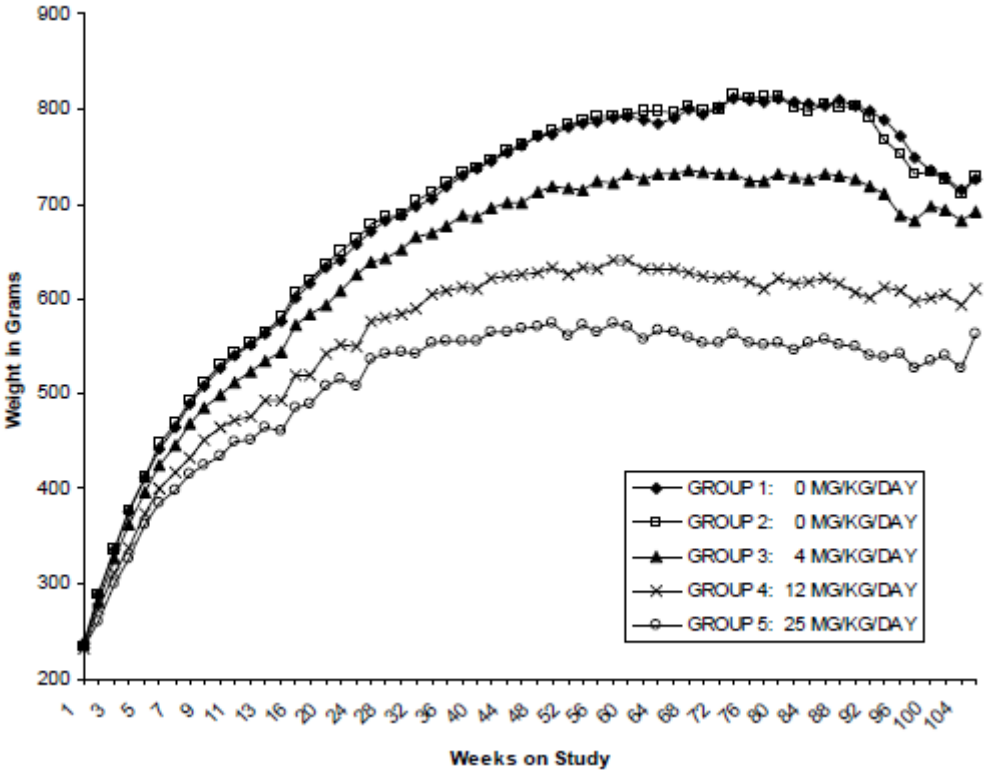
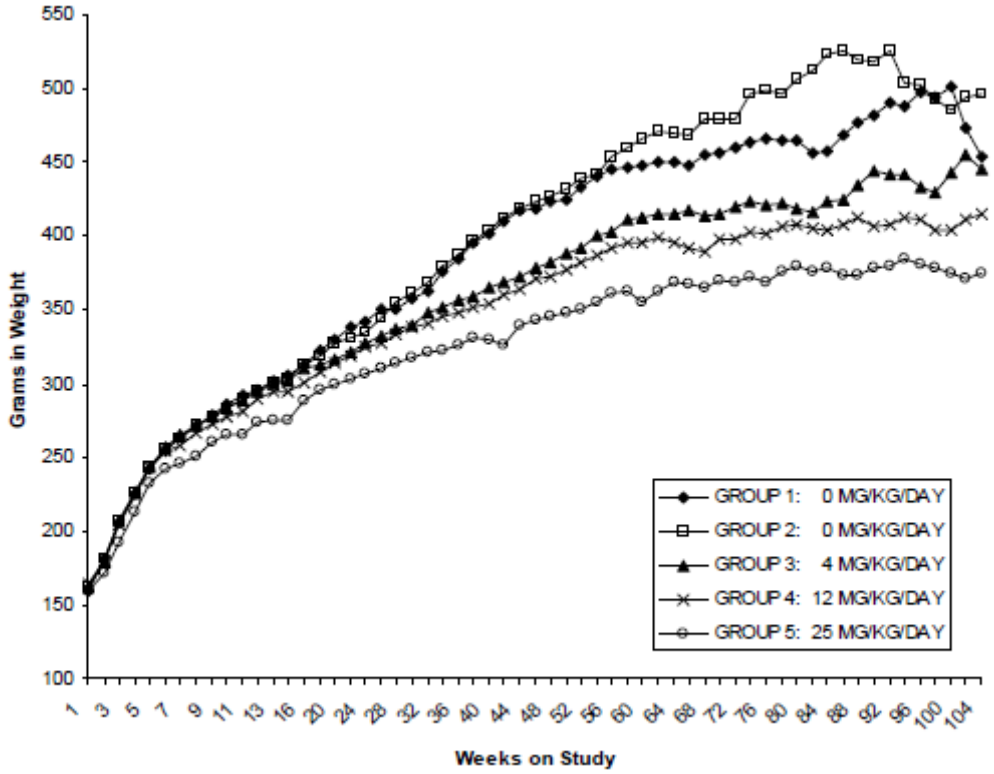


Figure 6. Body Weights in Females



Food Consumption

Food consumption was recorded prior to treatment, weekly through Week 14 and every two weeks thereafter. In most weeks during the study, males showed dose-dependently lower food consumption than control groups. To a lesser degree than males, treated females also showed lower food consumption than control groups. Decreases in food consumption correlated with decreased body weights in both males and females. Decreased food consumption and body weights are expected pharmacologic effects of opioid agonists and at no point in the study were either considered to be adverse.

Ophthalmoscopy

Ophthalmic examination was conducted at Weeks 26 and 105. No treatment-related findings were observed.

Hematology

Samples for hematology were collected during Weeks 26 and 105. The parameters in the table below were measured.

Erythrocyte cell count

Leukocyte cell count
Differential blood cell count
Blood cell morphology

No test article-related hematology changes were observed.

Clinical Chemistry

Samples for clinical chemistry were collected during Weeks 26 and 105. The parameters in the table below were measured.

Alkaline Phosphatase	Globulin
Total Bilirubin	Albumin/Globulin Ratio
Aspartate Aminotransferase	Cholesterol
Alanine Aminotransferase	Triglycerides
Glucose	Sodium
Urea Nitrogen	Potassium
Creatinine	Chloride
Total Protein	Calcium
Albumin	Phosphorus

Significant decreases in triglycerides were observed in males at all doses at both the 26- and 105-week time points. A small but significant decrease in cholesterol was observed at the high dose in males at the 105-week time point. Decreases in calcium were observed in females at 26 weeks only. Significance was reached at the mid and high doses. No other test article-related changes in clinical chemistry parameters were observed in males or females. The observed changes did not correlate with any organ specific changes and will be considered treatment-related.

Table 10. Selected Clinical Chemistry Results

	Parameter	***Week***	***C1***	***C2***	***4 mg/kg***	***12 mg/kg***	***25 mg/kg***
Male	Triglycerides (mg/dL)	26	133+/- 56	214+/- 113	102*+/- 37	83*+/- 24	59*+/-18
		105	151+/-98	150+/-127	81*+/-28	64*+/-20	50*+/-16
	Total Cholesterol (mg/dL)	105	116+/-48	135+/-60	113+/-34	101+/-33	76*+/-13
Female	Total Cholesterol (mg/dL)	26	107+/-28	101+/-9	97+/-18	78*+/-18	76*+/-11
	Calcium (mg/dL)	26	11.4+/-0.5	11.7+/-0.4	11.3+/-0.3	11.1*+/-0.5	11.1*+/-0.4

*P≤0.05

Urinalysis

Samples for urinalysis were collected during Weeks 26 and 105. The parameters in the table below were measured.

Bilirubin
Blood
Glucose
Appearance/color
Ketones
Protein
Microscopic analysis of sediment
pH
Specific gravity
Urobilinogen

No test article-related urinalysis changes were observed.

Gross Pathology

Test article-related macroscopic findings included a decrease in enlarged pituitaries and a concomitant decrease in the number of brains reported to be indented as a result of the pituitary tumors. Decreased body weights have been associated with decreased incidences of certain types of tumors in rats including tumors of the pituitary (Haseman, et al., 1997).

Dose-dependent increases in the number of sores were observed in both males and females. These sores mostly occurred on the hindpaws and were referred to as pododermatitis by the pathologist. No further analysis was conducted to determine whether the cause of the pododermatitis was bacterial or fungal. Chronic use of opioids can lead to immunosuppression which could explain the increased incidence and severity of the observed dermatitis, if the dermatitis is due to overgrowth of endogenous skin flora. Alternatively, some opioids, including HC, have been reported to lead to histamine release which could result in increased scratching and dermatitis.

Organ Weights

Organs were not weighed.

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Histopathology Inventory			
Study Number	NDSE-559-GLP ((b) (4) Study # 6770-142)		
Species	Rat		
Organ	***assessed***	***Organ***	***assessed***
Adrenal	X	Nasopharynx	X
Aorta	X	Ovary	X
Brain	X	Oviduct	X
Cecum	X	Pancreas	X
Cervix	X	Preputial gland	X
Clitoral gland	X	Pituitary	X
Colon	X	Prostate	X
Duodenum	X	Rectum	X
Epididymis	X	Salivary gland	X
Eye	X	Seminal vesicles	X
Esophagus	X	Skin	X
Femur	X	Spinal cord	X
Gross lesions	X	Spleen	X
Harderian gland	X	Sternum	X
Heart	X	Stomach	X
Ileum	X	Testes	X
Jejunum	X	Thymus	X
Kidney	X	Thyroid with parathyroid	X
Lacrimal gland	X	Tongue	X
Larynx	X	Trachea	X
Liver	X	Urinary bladder	X
Lung	X	Ureters	X
Lymph nodes, mesenteric	X	Vagina	X
Mammary gland	X	Voluntary muscle	X
Nerve, sciatic	X		

Histological Findings
Decreased incidences in tumors in the anterior pituitary and mammary gland were observed (Table 11). Decreased body weights in long-term studies have been associated with decreased incidences of mammary and pituitary tumors in rats and mice (Haseman, et al., 1997).

An increase above control animals in sternum bone marrow hypercellularity was observed in males at all doses (Table 12). All females, including controls, had higher levels of bone marrow hypercellularity than those observed in males. No treatment-related changes were observed in the females. Increased bone marrow cellularity can be considered a normal response to inflammation. In this study, the inflammation was most likely caused by the pododermatitis. However, pododermatitis was also observed in treated females. Increases in hypercellularity in females may not have been

observed because of the higher background levels in the control group. No hematologic changes or related neoplasms were observed in the study in either males or females. The increase in bone marrow hypercellularity in males will be considered treatment-related.

Table 11. Selected Neoplastic Lesions

Tissue	***Tumor type***	***Incidence (observed/examined)***							
		Males, mg/kg				***Females, mg/kg***			
		0	***4***	***12***	***25***	***0***	***4***	***12***	***25***
Pituitary gland (pars distalis)	adenoma	67/140 48%	20/69 29%	19/69 28%	18/70 26%	113/139 81%	52/68 76%	50/70 71%	37/70 53%
	carcinoma	0/140 0%	0/69 0%	0/69 0%	0/70 0%	6/139 4%	3/68 4%	2/70 3%	0/70 0%
	combined	**67/140 48%**	**20/69 29%**	**19/69 28%**	**18/70 26%**	**119/139 86%**	**55/68 81%**	**52/70 74%**	**37/70 53%**
Mammary gland	fibroadenoma	0/124 0%	1/64 2%	0/67 0%	1/63 2%	51/140 36%	34/70 49%	30/69 43%	18/70 26%
	adenoma	0/124 0%	0/64 0%	1/67 1%	0/63 0%	10/140 7%	6/70 9%	5/69 7%	3/70 4%
	carcinoma	0/124 0%	0/64 0%	1/67 1%	0/63 0%	36/140 26%	18/70 26%	20/69 29%	9/70 13%
	combined	**0/124 0%**	**1/64 2%**	**2/67 3%**	**1/63 2%**	**97/140 69%**	**58/70 83%**	**55/69 80%**	**30/70 43%**

Table 12. Selected Histopathologic Observations

Tissue: finding	***Incidence (observed/examined)***							
	Males, mg/kg				***Females, mg/kg***			
	0	***4***	***12***	***25***	***0***	***4***	***12***	***25***
Marrow, sternum: hypercellular	7/135 5%	7/70 10%	14/70 20%	15/70 21%	35/140 25%	15/70 21%	19/69 28%	20/70 29%

Toxicokinetics

Nine rats/sex/group and 12 rats/sex/group were bled for control and treated toxicokinetics, respectively. Toxicokinetic parameters of HC are detailed in the table below.

Exposure to HC was similar for male and female rats at all doses. Hydrocodone showed a T_{max} between 0.5-1 h. The $t_{1/2}$ values in this study ranged from 3.1-8.1 h on Day 1 and 1.3-2.5 h during Weeks 26 and 52. For the mid and high dose groups, mean C_{max} and AUC_{0-24} values were approximately 2-fold higher at Weeks 26 and 52 as

compared to Day 1, indicating an accumulation of HC after multiple dosing. The increases in C_{max} and AUC_{0-24} values for males and females were less than dose proportional on Day 1 but greater than dose proportional during Weeks 26 and 52. Systemic levels of a 120 mg human dose at steady state (Study # HYD1002) are compared to systemic exposure of rats (Table 14). In the clinical Study # HYD1002, 120 mg HC was dosed q24h for 5 days. Steady state was reached by Day 5 and AUC_{tau}= 1938 +/-729. In the clinical Study # HYD1002, the dosing interval was 24h therefore the AUC_{0-24} animal data can be compared to the AUC_{tau} human data (Table 14). All rat exposures of HC in this study are below the systemic exposure at the human dose of 120 mg. The pharmacologic effects of opioids limit the dosing and typically multiples of human clinical exposures are not achieved in rat studies.

Table 13. Toxicokinetic Parameters of Hydrocodone in Rat Plasma

Interval	Dose Group	Hydrocodone Bitartrate Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	DN C_{max} (ng/mL)/ (mg/kg/day)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} (ng•hr/mL)/ (mg/kg/day)	$AUC_{0-\infty}$ (ng•hr/mL)	$t_{1/2}$ (hr)	AUC_{0-24} AR
Day 1	7	4	M	3.53	0.883	1.00	7.42	9.21	2.30	NC	NC	NA
			F	4.18	1.04	0.500	8.27	11.6	2.89	NC	NC	NA
	8	12	M	5.24	0.437	1.00	20.9	20.9	1.74	21.2	4.22	NA
			F	9.10	0.758	0.500	21.5	38.3	3.19	29.9	3.12	NA
	9	25	M	7.49	0.300	0.500	50.1	50.1	2.00	58.5	8.13	NA
			F	13.0	0.518	0.500	58.7	58.7	2.35	63.5	7.04	NA
Week 26	7	4	M	6.24	1.56	1.00	11.3	13.8	3.46	NA	NC	1.50
			F	6.11	1.53	0.500	9.06	10.8	2.71	NA	1.27	0.938
	8	12	M	25.4	2.12	0.500	48.8	59.8	4.98	NA	1.34	2.87
			F	21.8	1.82	0.500	33.6	42.6	3.55	NA	1.47	1.11
	9	25	M	91.5	3.66	1.00	186	239	9.55	NA	NC	4.77
			F	79.4	3.18	0.500	128	143	5.71	NA	NC	2.43
Week 52	7	4	M	6.37	1.59	1.00	12.6	15.0	3.75	NA	NC	1.63
			F	5.68	1.42	0.500	10.5	13.1	3.28	NA	1.35	1.13
	8	12	M	36.0	3.00	0.500	80.0	80.0	6.67	NA	2.49	3.84
			F	34.6	2.88	0.500	55.0	71.5	5.96	NA	1.61	1.87
	9	25	M	239	9.56	0.500	255	328	13.1	NA	NC	6.55
			F	107	4.28	1.00	173	193	7.71	NA	NC	3.28

Table 14. Exposure Comparison Between Rat and Human

	Dose, mg/kg	***Rat/human* AUC_{0-24}, (ng.h/mL)***
Male	***4***	0.008
	12	0.041
	25	0.169
Female	***4***	0.007
	12	0.037
	25	0.100

*120 mg/day at steady state; AUC_{tau}= 1938 ng.h/mL, Study # HYD1002.

Dosing Solution Analysis

The homogeneity of samples and the sample concentrations were both within an acceptable range of the respective target concentrations for HC.

6.2 Repeat-Dose Toxicity (Dog)

Study title: A 9-Month Oral (Capsule) Toxicity Study in Dogs with Hydrocodone Bitartrate

The following study was reviewed by Dr. Huiqing Hao.

Study no.:	NDSE-572-GLP
Study report location:	EDR 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	07/31/2001
GLP compliance:	Yes, a signed GLP compliance statement was included in the study report
QA statement:	Yes
Drug, lot #, and % purity:	Hydrocodone Bitartrate, U.S.P., Lot No. B13869, purity 100.5%

Key Study Findings

Dogs were given hydrocodone bitartrate orally at 0.2, 0.6, 2.0, and 6.0 mg/kg for 9 months. The main toxicities were reduced food consumption and transient reductions in body weight gain or body weight loss at the 6.0 mg/kg. These findings were not considered adverse. The NOAEL in this study is greater than the highest dose tested. The highest dose tested yielded exposure margins in male and female dogs of 0.9-fold and 0.8-fold, respectively, the human systemic exposure of a 120 mg HC dose (based on AUC comparisons; data from Study # HYD1002).

Methods

Doses:	0.2, 0.6, 2.0, and 6.0 mg/kg (free base)
Frequency of dosing:	Once a day
Route of administration:	Oral (capsule)
Dose volume:	NA
Formulation/Vehicle:	Empty capsules were filled with active drug substance only; control animals were given empty capsules
Species/Strain:	Dog, beagle
Number/Sex/Group:	4/sex/dose
Age:	9 months
Weight:	Males, 9.0-14.0 kg; females, 7.2-10.6 kg
Satellite groups:	2/sex for recovery study (control and high dose)
Unique study design:	All treatment groups were initially treated with 0.2 mg/kg/day for two weeks and then given the target doses of 0.2, 0.6, 2.0 or 6.0 mg/kg/day for additional 9 months
Deviation from study protocol:	No significant deviations that affect the result interpretation.

Observations and Results

Mortality

None

Clinical Signs

Dose-related signs of toxicity were observed at 2.0 and 6.0 mg/kg/day including salivation prior to and following dosing, and decreased activity following dosing. Post-dose rapid breathing was noted in the 2.0 and 6.0 mg/kg/day males and in the 6.0 mg/kg/day females. During the 28-day recovery period, there were no remarkable signs of toxicity for animals in 6.0 mg/kg/day group.

Body Weights

Body weights were not affected during the first two weeks when all treatment groups were given 0.2 mg/kg. During the first week (Days 14-21) with the increased doses, treatment groups showed dose-related decrease of body weight gain or body weight loss, most significantly in the high dose group (6 mg/kg) where body weight loss of 9.2% in males (control gained 2.7%) and 5.6% in females (control gained 3.8%) occurred. This body weight loss correlated with reduced food consumption in the 6.0 mg/kg group during Days 14-21. It took about 3-5 weeks for the 6.0 mg/kg/day animals to regain the body weight they lost over the first week following dosage escalation. By the end of treatment of 9 months, no significant treatment effect on body weight was observed.

Table 15. Body Weights in the 9-Month Dog Study (kg)

Dose, mg/kg		0	0.2	0.6	2	6
Day 0	M	10.82	10.70	10.81	10.75	11.10
	F	9.24	8.62	8.32	8.83	8.70
Day 14	M	10.92	10.93	10.80	10.87	11.05
	F	9.22	8.74	8.32	8.73	8.72
Day 21	M	11.22	10.61	10.89	10.93	10.03
	F	9.57	9.11	8.66	8.88	8.23
% change Day 21 from Day 14	M	+2.7	-2.9	+0.8	+0.5	-9.2
	F	+3.8	+4.2	+0.4	+1.7	-5.6
Day 280 (end of treatment)	M	11.76	11.69	12.13	11.28	11.79
	F	10.53	9.49	8.93	9.35	9.39
Day 313 (recovery)	M	12.75				10.90
	F	9.87				9.76

Food Consumption

Food consumption was lower in the high dose group, most significantly (1/2-1/3 of control levels) during the first week with 6.0 mg/kg, with a non-statistically significant trend throughout the treatment period. Mean food consumption in the 0.6 and 2.0 mg/kg females was also generally lower than control during the treatment period. During the recovery phase, no treatment effects on food consumption were observed.

Ophthalmoscopy

No treatment related findings

ECG (Lead II in Days -13, -7, 99, 198 and 282 of treatment phase, Day 309 of recovery phase)

No treatment related findings

Hematology (weekly)

No meaningful findings were observed. On Day 281 (but not any other time points) females had increased APTT (11.4, 12.2, 41.4, 35.8, and 35.6 seconds for the control, 0.2, 0.6, 2.0, and 6.0 mg/kg, respectively), but no similar findings were seen in males. The increased APTT was not associated with any abnormal histopathologic findings. The toxicological significance is not clear.

Clinical Chemistry

No treatment related findings

Urinalysis

No treatment related findings

Gross Pathology

No remarkable findings

Organ Weights

No treatment related findings

Histopathology

Adequate Battery: Yes. All tissues and organs from the control and high dose animals and gross lesions from all animals were examined.

Peer Review: Yes

Histological Findings: There were no treatment related findings.

Special Evaluation

None

Toxicokinetics

Systemic exposures to HC were linear (dose proportional). No major difference in the exposure was found between Study Days 104, 194, and 283, suggesting that steady-state was reached after 104 days of daily dosing. No gender related difference in the metrics of HC was detected. The following table (excerpted from the report) presents the details.

Table 16. Toxicokinetics in Dog (AUC)

Day	Gender	Hydrocodone Doses (mg/kg/day)			
		0.2	0.6	2.0	6.0
14	Male	21.6	64.5	285	902
	Female	9.15	51.6	186	758
104	Male	30.4	125	466	1509
	Female	28.2	60.8	256	1077
283	Male	46.1	170	528	1746
	Female	30.3	75.1	372	1529

Dosing Solution Analysis

Hydrocodone capsules contained 95.1-101.0% of labeled dose.

7 Genetic Toxicology

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

Study title: Salmonella-Escherichia Coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay Using Hydrocodone Bitartrate

Study no.:	20442-0-409OECD (Purdue DSE-362-GLP)
Study report location:	EDR 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 18, 1999
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Hydrocodone bitartrate, B1067-980302, 99.8%

Key Study Findings

- **Hydrocodone bitartrate is not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 and *E. coli* strain WP2*uvr*A in either the presence or absence of S9.**

Methods

Strains:	*Salmonella typhimurium*: TA98, TA100, TA1535, TA1537 and *Escherichia coli*: WP2 uvrA
Concentrations in definitive study:	100, 333, 1000, 3330, and 5000 mcg +/- S9
Basis of concentration selection:	Initial study used 6.7 – 5000 mcg
Negative control:	Distilled water
Positive control:	See table below
Formulation/Vehicle:	Distilled water
Incubation & sampling time:	52 h at 37 degrees

Table 17. Ames Assay Positive Controls

Tester Strain	S9 Mix	Positive Control	Conc. per plate
TA98	+	benzo[a]pyrene	2.5 µg
TA98	–	2-nitrofluorene	1.0 µg
TA100	+	2-aminoanthracene	2.5 µg
TA100	–	sodium azide	2.0 µg
TA1535	+	2-aminoanthracene	2.5 µg
TA1535	–	sodium azide	2.0 µg
TA1537	+	2-aminoanthracene	2.5 µg
TA1537	–	ICR-191	2.0 µg
WP2*uvr*A	+	2-aminoanthracene	25.0 µg
WP2*uvr*A	–	4-nitroquinoline-N-oxide	1.0 µg

Study Validity

The study is valid. All strains were shown to contain the appropriate genetic markers. Suitable numbers of replicate plates and appropriate counting methods were utilized. The positive controls demonstrated clear increases in tester strain revertants while the vehicle control was within historical range for the tester strains for this vehicle.

Results

It is concluded that under conditions of the assays conducted, HC is not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 and *E. coli* strain WP2*uvr*A in either the presence or absence of S9. The results of the confirmative assay are summarized in the table below. No reduction in bacterial lawn was observed at any concentration. For all strains, at least three concentrations of test article were able to be evaluated. All of the strains at all of the concentrations tested showed negative mutagenic responses in the presence and absence of exogenous metabolic activation with S9.

Table 18. Hydrocodone Ames Confirmatory Assay Summary of Results

		Mean Revertants Per Plate With Standard Deviation										
	Dose/Plate	TA98		TA100		TA1535		TA1537		WP2*uvr*A		Background Lawn[c]
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Microsomes: Rat Liver												
Vehicle Control		15	5	118	11	10	1	5	3	14	3	1
Test Article	100 µg	16	5	125	10	13	5	6	2	20	5	1
	333 µg	25	3	107	4	14	2	5	3	17	4	1
	1000 µg	15	3	121	11	10	2	10	6	15	3	1
	3330 µg	18	4	103	4	9	1	9	4	9	1	1
	5000 µg	24	7	102	8	12	3	5	1	12	3	1
Positive Control[a]		383	10	761	33	116	1	131	23	328	57	1
Microsomes: None												
Vehicle Control		14	3	99	10	11	1	5	3	12	3	1
Test Article	100 µg	8	3	90	9	12	2	6	2	19	4	1
	333 µg	11	1	87	15	9	2	4	2	17	6	1
	1000 µg	8	1	95	15	11	2	6	1	13	5	1
	3330 µg	15	1	106	20	13	2	3	1	16	3	1
	5000 µg	16	2	99	6	8	2	3	2	10	3	1
Positive Control[b]		169	11	647	42	493	62	402	46	400	51	1

[a] TA98 benzo[a]pyrene 2.5 µg/plate
TA100 2-aminoanthracene 2.5 µg/plate
TA1535 2-aminoanthracene 2.5 µg/plate
TA1537 2-aminoanthracene 2.5 µg/plate
WP2*uvr*A 2-aminoanthracene 25.0 µg/plate

[b] TA98 2-nitrofluorene 1.0 µg/plate
TA100 sodium azide 2.0 µg/plate
TA1535 sodium azide 2.0 µg/plate
TA1537 ICR-191 2.0 µg/plate
WP2*uvr*A 4-nitroquinoline-N-oxide 1.0 µg/plate

[c]Background Lawn Evaluation Codes:
1 = Normal
2 = Slightly reduced
3 = Moderately reduced
4 = Extremely reduced
5 = Absent
6 = Obscured by precipitate
sp = Slight precipitate
mp = Moderate precipitate (requires hand count)
hp = Heavy precipitate (requires hand count)

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

Study title: Bacterial Reverse Mutation Assay

Study no.: AD22NH.503.[(b) (4)] (Purdue HYD-N-024)
Study report location: EDR 4.2.3.3.1
Conducting laboratory and location: [(b) (4)]
Date of study initiation: April 11, 2011
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: [(b) (4)] RT0323-23, 97.2%

Key Study Findings

(b) (4) is not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 and *E. coli* strain WP2*uvr*A in either the presence or absence of S9.

Methods

Strains:	*Salmonella typhimurium*: TA98, TA100, TA1535, TA1537 and *Escherichia coli*: WP2 uvrA
Concentrations in definitive study:	15, 50, 150, 500, 1500, and 5000 mcg +/- S9
Basis of concentration selection:	Initial study used 1.5 – 5000 mcg
Negative control:	DMSO
Positive control:	See table below
Formulation/Vehicle:	DMSO
Incubation & sampling time:	48-72 h at 37 degrees

Table 19. Ames assay: Positive Controls

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
TA98, TA1535 and TA1537	Rat	2-aminoanthracene (b) (4) Lot No. 03403ED Exp. Date 22-Jan-2012 CAS No. 613-13-8 Purity 99.8%	1.0
TA100			2.0
WP2 *uvr*A			15
TA98	None	2-nitrofluorene (b) (4) Lot No. S43858 Exp. Date 31-Jan-2014 CAS No. 607-57-8 Purity 97.9%	1.0
TA100, TA1535		sodium azide (b) (4) Lot No. A23U048 Exp. Date 04-Dec-2012 CAS No. 26628-22-8 Purity 100.0%	1.0
TA1537		9-aminoacridine (b) (4) Lot No. 106F06682 Exp. Date 28-Oct-2011 CAS No. 90-45-9 Purity >97%	75
WP2 *uvr*A		methyl methanesulfonate (b) (4) Lot No. 76296KJ Exp. Date 09-Jun-2012 CAS No. 66-27-3 Purity 99.8%	1,000

Study Validity

The study is valid. All strains were shown to contain the appropriate genetic markers. Suitable numbers of replicate plates and appropriate counting methods were utilized. The positive controls demonstrated clear increases in tester strain revertants while the vehicle control was within historical range for the tester strains for this vehicle.

Results

It is concluded that under conditions of the assays conducted, (b) (4) is not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 and *E. coli* strain WP2*uvr*A in either the presence or absence of S9. The results of the confirmative assay are summarized in the tables below. Reduction in bacterial lawn was observed at 1500 mcg per plate for TA1537 in the absence of metabolic activation and 5000 mcg/plate for TA1537 in the presence of metabolic activation all other strains in both the presence and absence of metabolic activation. For all strains, at least three concentrations of test article were able to be evaluated. All of the strains at the concentrations tested showed negative mutagenic responses in the presence and absence of exogenous metabolic activation with S9.

Table 20. (b) (4) Ames Confirmatory Assay Results Without S9 Activation

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	(b) (4)	5000 µg	0	0	0.0	0^M 4, 0^M 4, 0^M 4
		1500 µg	1	0	0.1	1^A, 1^A, 1^A
		500 µg	3	1	0.2	3^A, 3^A, 4^A
		150 µg	8	2	0.4	6^A, 10^A, 9^A
		50 µg	12	5	0.7	17^A, 8^A, 10^A
		15 µg	14	1	0.8	15^A, 13^A, 13^A
	DMSO	100 µL	18	7		13^A, 14^A, 26^A
TA100	(b) (4)	5000 µg	0	0	0.0	0^M 4, 0^M 4, 0^M 4
		1500 µg	35	11	0.4	41^A, 23^A, 42^A
		500 µg	92	9	1.1	94^A, 82^A, 99^A
		150 µg	85	12	1.0	75^A, 98^A, 83^A
		50 µg	99	12	1.2	92^A, 92^A, 113^A
		15 µg	91	18	1.1	111^A, 87^A, 75^A
	DMSO	100 µL	81	9		73^A, 91^A, 80^A
TA1535	(b) (4)	5000 µg	0	0	0.0	0^M 4, 0^M 4, 0^M 4
		1500 µg	21	4	1.6	23^A, 24^A, 17^A
		500 µg	27	8	2.1	29^A, 19^A, 34^A
		150 µg	14	4	1.1	18^A, 11^A, 13^A
		50 µg	11	2	0.8	13^A, 11^A, 10^A
		15 µg	14	5	1.1	13^A, 19^A, 10^A
	DMSO	100 µL	13	5		11^A, 10^A, 19^A
TA1537	(b) (4)	5000 µg	0	0	0.0	0^M 4, 0^M 4, 0^M 4
		1500 µg	0	1	0.0	1^M 3, 0^M 3, 0^M 3
		500 µg	1	2	0.3	1^A, 3^A, 0^A
		150 µg	4	4	1.0	4^A, 8^A, 1^A
		50 µg	3	1	0.8	3^A, 4^A, 3^A
		15 µg	3	2	0.8	1^A, 4^A, 3^A
	DMSO	100 µL	4	2		3^A, 6^A, 3^A

WP2*uvr*A	(b) (4)	5000 µg	*0*	*0*	*0.0*	0^{M} 4, 0^{M} 4, 0^{M} 4
		1500 µg	*5*	*4*	*0.3*	4^{A}, 1^{A}, 9^{A}
		500 µg	*12*	*4*	*0.8*	10^{A}, 17^{A}, 9^{A}
		150 µg	*15*	*5*	*0.9*	20^{A}, 11^{A}, 15^{A}
		50 µg	*18*	*8*	*1.1*	11^{A}, 26^{A}, 17^{A}
		15 µg	*19*	*9*	*1.2*	14^{A}, 29^{A}, 13^{A}
	DMSO	100 µL	*16*	*7*		23^{A}, 17^{A}, 9^{A}
TA98	**2NF**	1.0 µg	*261*	*22*	*14.5*	260^{A}, 284^{A}, 240^{A}
TA100	**SA**	1.0 µg	*452*	*87*	*5.6*	363^{A}, 537^{A}, 455^{A}
TA1535	**SA**	1.0 µg	*404*	*8*	*31.1*	413^{A}, 400^{A}, 399^{A}
TA1537	**9AAD**	75 µg	*584*	*95*	*146.0*	645^{A}, 633^{A}, 474^{A}
WP2*uvr*A	**MMS**	1000 µg	*333*	*4*	*20.8*	333^{A}, 330^{A}, 337^{A}

Key to Positive Controls	
2NF	2-nitrofluorene
SA	sodium azide
9AAD	9-Aminoacridine
MMS	methyl methanesulfonate

Key to Automatic & Manual Count Flags

^{M}: Manual count ^{A}: Automatic count

Key to Plate Postfix Codes	
4	Extremely reduced background
3	Moderately reduced background

Table 21. (b) (4) Ames Confirmatory Assay Results With S9 Activation

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	(b) (4)	5000 µg	*0*	*0*	*0.0*	0^{M} 4, 0^{M} 4, 0^{M} 4
		1500 µg	*4*	*4*	*0.3*	8^{A}, 3^{A}, 0^{A}
		500 µg	*10*	*4*	*0.8*	6^{A}, 10^{A}, 14^{A}
		150 µg	*16*	*7*	*1.3*	24^{A}, 10^{A}, 15^{A}
		50 µg	*14*	*1*	*1.2*	13^{A}, 15^{A}, 15^{A}
		15 µg	*17*	*6*	*1.4*	22^{A}, 19^{A}, 11^{A}
	DMSO	100 µL	*12*	*2*		13^{A}, 13^{A}, 9^{A}
TA100	(b) (4)	5000 µg	*0*	*0*	*0.0*	0^{M} 4, 0^{M} 4, 0^{M} 4
		1500 µg	*34*	*7*	*0.3*	31^{A}, 29^{A}, 42^{A}
		500 µg	*91*	*13*	*0.8*	106^{A}, 82^{A}, 84^{A}
		150 µg	*112*	*6*	*0.9*	119^{A}, 108^{A}, 110^{A}
		50 µg	*109*	*7*	*0.9*	117^{A}, 106^{A}, 103^{A}
		15 µg	*118*	*1*	*1.0*	117^{A}, 117^{A}, 119^{A}
	DMSO	100 µL	*120*	*20*		125^{A}, 98^{A}, 136^{A}
TA1535	(b) (4)	5000 µg	*0*	*0*	*0.0*	0^{M} 4, 0^{M} 4, 0^{M} 4
		1500 µg	*5*	*3*	*0.5*	3^{A}, 3^{A}, 8^{A}
		500 µg	*7*	*3*	*0.7*	5^{A}, 10^{A}, 6^{A}
		150 µg	*14*	*3*	*1.4*	11^{A}, 17^{A}, 15^{A}
		50 µg	*10*	*3*	*1.0*	9^{A}, 14^{A}, 8^{A}
		15 µg	*9*	*2*	*0.9*	11^{A}, 9^{A}, 8^{A}
	DMSO	100 µL	*10*	*3*		13^{A}, 8^{A}, 8^{A}
TA1537	(b) (4)	5000 µg	*0*	*0*	*0.0*	0^{M} 4, 0^{M} 4, 0^{M} 4
		1500 µg	*2*	*2*	*0.4*	0^{A}, 4^{A}, 1^{A}
		500 µg	*5*	*1*	*1.0*	6^{A}, 5^{A}, 4^{A}
		150 µg	*5*	*5*	*1.0*	10^{A}, 4^{A}, 1^{A}
		50 µg	*7*	*3*	*1.4*	10^{A}, 5^{A}, 6^{A}
		15 µg	*4*	*1*	*0.8*	5^{A}, 3^{A}, 5^{A}
	DMSO	100 µL	*5*	*3*		3^{A}, 9^{A}, 3^{A}

WP2*uvrA*	(b) (4)	5000 µg	*0*	*0*	*0.0*	0^{M} 4, 0^{M} 4, 0^{M} 4
		1500 µg	*7*	*3*	*0.3*	5^{A}, 5^{A}, 11^{A}
		500 µg	*22*	*5*	*0.9*	27^{A}, 17^{A}, 23^{A}
		150 µg	*15*	*2*	*0.6*	13^{A}, 15^{A}, 17^{A}
		50 µg	*22*	*10*	*0.9*	15^{A}, 17^{A}, 34^{A}
		15 µg	*18*	*3*	*0.7*	19^{A}, 15^{A}, 20^{A}
	DMSO	100 µL	*25*	*7*		33^{A}, 23^{A}, 20^{A}
TA98	2AA	1.0 µg	*155*	*16*	*12.9*	170^{A}, 138^{A}, 156^{A}
TA100	2AA	2.0 µg	*631*	*44*	*5.3*	597^{A}, 616^{A}, 681^{A}
TA1535	2AA	1.0 µg	*102*	*24*	*10.2*	129^{A}, 92^{A}, 85^{A}
TA1537	2AA	1.0 µg	*59*	*9*	*11.8*	50^{A}, 68^{A}, 60^{A}
WP2*uvrA*	2AA	15 µg	*179*	*42*	*7.2*	139^{A}, 176^{A}, 223^{A}

Key to Positive Controls		Key to Plate Postfix Codes	
2AA	2-aminoanthracene	4	Extremely reduced background

Key to Automatic & Manual Count Flags

^{M}: Manual count ^{A}: Automatic count

7.2 *In Vitro* Assays in Mammalian Cells

Study title: L5178Y TK+/- Mouse Lymphoma Forward Mutation Assay with a Confirmatory Assay Using Hydrocodone Bitartrate

Study no.:	DSE-367-GLP (b) (4) 20442-0-431)
Study report location:	EDR 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 23, 1999
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Hydrocodone bitartrate, Lot B1067-980302, 99.8%

Key Study Findings

- Under the conditions of this assay, hydrocodone bitartrate is considered negative in the absence of metabolic activation and positive in the presence of rat S9 for inducing forward mutations at the TK locus of L5178 mouse lymphoma cells.

Methods

Cell line:	L5178Y cells
Concentrations in definitive study:	-S9: 9.85-750 mcg/mL, +S9: 9.85-625 mcg/mL
Basis of concentration selection:	Dose range finding study using 9.85-4980 mcg/mL HC
Negative control:	Water
Positive control:	Methyl methanesulfonate (-S9) and

	Methylcholanthrene (+S9)
Formulation/Vehicle:	Water
Incubation & sampling time:	-S9: 4 h and 24 h, +S9: 4 h

Study Validity

The study was deemed valid for the following reasons: 1) vehicle control cultures exhibited a mean cloning efficiency of 50% or greater; 2) vehicle control cultures gave a mean mutant frequency less than 150 x 10^{-6} and at least 12 x 10^{-6}; 3) positive controls exhibited appropriate responses; 4) concentrations were appropriate in that either a positive mutant frequency response or an 80% reduction in RTG or a persistent precipitate was achieved and 5) the ability to recover small colonies was demonstrated by sizing of the positive control.

Results

Two trials and a confirmatory assay in the absence of metabolic activation were performed. One trial and a confirmatory assay in the presence of metabolic activation were performed. The in vitro metabolic activating system used in this assay was comprised of rat liver enzymes (S9 fraction) and the appropriate cofactors. Sizing analysis was performed on all treatments. The maximum concentration used in each trial was determined by the solubility and cytotoxicity of HC. In the absence of S9 at both 4 h and 24 h incubation, HC did not induce an increase in mutation frequency (Tables 22-24). In the presence of rat S9 with 4 h incubation, the three highest concentrations induced mutant frequencies higher than the background and were considered positive (Table 25). A confirmatory trial showed similar results with the four highest concentrations inducing mutant frequencies higher than the background (Table 26). Responses deemed positive were outside the historical control range of 27.6 x 10^{-6} - 150.3 x 10^{-6} for metabolically activated conditions. Under the conditions of this assay, HC bitartrate is considered negative in the absence of metabolic activation and positive in the presence of rat S9 for inducing forward mutations at the TK locus of L5178 mouse lymphoma cells.

Table 22. Trial 1: Summary of Results -S9, 4 h Incubation

Mutation Assay Without Activation – Initial 4-Hour Treatment Assay

Test Article: Hydrocodone Bitartrate
Vehicle: Water
Selective Agent: TFT 3.0 µg/mL
Test Date: 5/18/99
Treatment Period: 4 Hours

Test Condition	Daily Cell Counts (Cell/ML, 10E5 Units)		RSG[a]		Total Mutant Colonies[h]	Total Viable Colonies[h]	Cloning Efficiency[b]		Relative Growth(%)[c]	Mutant Frequency (10E-6 Units)[d]
	Day 1	Day 2								
Nonactivation Controls[e]				AVG VC[g]				AVG VC[g]		
VEHICLE CONTROL	12.3	13.2	18.0		255	378	62.9		87.9	135.1
VEHICLE CONTROL	13.0	12.9	18.6		242	405	67.6		97.5	119.2
VEHICLE CONTROL	14.5	13.8	22.2	19.6	239	401	66.8	65.8	115.0	119.4
MMS 6.5 µg/mL	6.4	17.7	12.6		221	218	36.4		35.5	202.0
MMS 13 µg/mL	9.0	12.3	12.3		253	165	27.5		26.2	306.8[f]
TEST COMPOUND			Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
9.85 µg/mL	15.0	10.3	87.4		223	328	83.2		72.7	135.7
19.7 µg/mL	15.5	12.1	106.1		219	342	86.6		92.0	128.3
39.3 µg/mL	16.9	14.1	134.8		194	285	72.2		97.4	135.9
78.5 µg/mL	16.0	12.6	114.1		188	443	112.3		128.1	84.9
157 µg/mL	13.0	14.3	105.2		196	364	92.3		97.1	107.6
313 µg/mL	11.8	16.3	108.8		208	395	100.2		109.0	105.4
625 µg/mL	11.1	15.7	98.6		197	405	102.7		101.3	97.3
1250 µg/mL	5.4	9.5	29.0		164	372	94.3		27.4	88.0

[a]RSG =Relative Suspension Growth= [Day 1 Cell Concentration divided by Cell Concentration Seeded on Day 0 (which would be 3 x 10E5/mL)] multiplied by [Day 2 Cell Concentration divided by Cell Concentration seeded on Day 1 (3 x 10E5/mL if subcultured; Day 1 Cell Concentration if not subcultured)]
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded (600) * 100
[c]Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4.
Decimal is moved to express the frequency in units of 10E-6.
[e]Vehicle Control = Water
Positive Control: MMS = Methyl Methanesulfonate
[f]Mutagenic. Exceeds Minimum Criterion of 249.2 X 10E-6, which is twice the average mutant frequency of the concurrent vehicle controls.
[g]AVG VC=average of the vehicle controls.
[h]Colony counts increased by 11.3751% to compensate for area of dish not scanned.

Table 23. Trial 2: Summary of Results -S9, 4 h Incubation

Mutation Assay Without Activation – Second Trial With A 4-Hour Treatment

Test Article: Hydrocodone Bitartrate
Vehicle: Water
Selective Agent: TFT 3.0 µg/mL
Test Date: 6/8/99

Test Condition	Daily Cell Counts (Cell/ML, 10E5 Units)		RSG[a]		Total Mutant Colonies[h]	Total Viable Colonies[h]	Cloning Efficiency[b]		Relative Growth(%)[c]	Mutant Frequency (10E-6 Units)[d]
	Day 1	Day 2								
Nonactivation Controls[e]				AVG VC[g]				AVG VC[g]		
VEHICLE CONTROL	16.5	12.7	23.3		97	456	76.0		100.7	42.6
VEHICLE CONTROL	17.9	12.0	23.9		96	472	78.7		106.9	40.6
VEHICLE CONTROL	14.5	16.8	27.1	24.7	63	350	58.4	71.0	89.9	36.1
MMS 6.5 µg/mL	10.8	14.0	16.8		240	344	57.3		54.8	139.7[f]
MMS 13 µg/mL	7.9	13.6	11.9		323	265	44.2		30.0	243.6[f]
TEST COMPOUND			Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
62.5 µg/mL	11.3	13.8	70.0		75	443	103.9		72.8	34.0
125 µg/mL	14.2	15.4	98.2		86	357	83.7		82.2	48.3
250 µg/mL	13.7	12.7	78.1		85	397	93.2		72.8	42.9
500 µg/mL	13.8	13.6	84.3		70	392	91.9		77.5	35.7
750 µg/mL	12.4	12.6	70.2		85	350	82.2		57.7	48.6
1000 µg/mL	10.7	14.4	69.2		77	397	93.2		64.5	39.0
1250 µg/mL	5.5	15.9	39.3		69	358	84.0		33.0	38.4
1500 µg/mL	4.4	11.4	22.5		73	425	99.8		22.5	34.4

[a]RSG =Relative Suspension Growth= [Day 1 Cell Concentration divided by Cell Concentration Seeded on Day 0 (which would be 3 x 10E5/mL)] multiplied by [Day 2 Cell Concentration divided by Cell Concentration seeded on Day 1 (3 x 10E5/mL if subcultured; Day 1 Cell Concentration if not subcultured)]
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded (600) * 100

[c]Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100

[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4.

Decimal is moved to express the frequency in units of 10E-6.

[e]Vehicle Control = Water

Positive Control: MMS = Methyl Methanesulfonate

[f]Mutagenic. Exceeds Minimum Criterion of 79.6 X 10E-6, which is twice the average mutant frequency of the concurrent vehicle controls.
[g]AVG VC=average of the vehicle controls.
[h]Colony counts increased by 9.099% to compensate for area of dish not scanned.

Table 24. Confirmatory Trial: Summary of Results -S9, 24 h Incubation

Mutation Assay Without Activation – Confirmatory Trial

Test Article: Hydrocodone Bitartrate
Vehicle: Water
Selective Agent: TFT 3.0 µg/mL
Test Date: 6/8/99
Treatment Period: 24 Hours

Test Condition	Daily Cell Counts (Cell/ML, 10E5 Units)			RSG[a]		Total Mutant Colonies[h]	Total Viable Colonies[h]	Cloning Efficiency[b]		Relative Growth (%)[c]	Mutant Frequency (10E-6 Units)[d]
	Day 1	Day 2	Day 3								
Nonactivation Controls[e]					AVG VC[g]				AVG VC[g]		
VEHICLE CONTROL	14.1	10.2	9.2	49.0		89	477	79.5		94.3	37.5
VEHICLE CONTROL	13.1	12.5	9.8	59.4		87	449	74.9		107.7	38.8
VEHICLE CONTROL	13.6	14.0	7.9	55.7	54.7	84	432	72.0	75.5	97.1	38.9
MMS 6.5 µg/mL	10.1	11.6	7.7	33.4		712	329	54.9		44.4	432.5[f]
MMS 6.5 µg/mL	14.6	8.4	7.3	33.2		651	281	46.9		37.7	462.8[f]
TEST COMPOUND				Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
31.3 µg/mL	14.6	10.9	12.4	133.6		116	525	115.9		154.8	44.1
62.5 µg/mL	12.9	10.6	10.7	99.0		75	370	81.7		80.9	40.7
125 µg/mL	12.3	9.3	7.5	58.1		95	502	110.8		64.4	37.8
250 µg/mL	14.3	5.8	8.4	47.2		121	613	135.4		63.9	39.5
375 µg/mL	11.5	8.4	8.8	57.5		79	394	87.0		50.0	39.9
500 µg/mL	8.3	6.7	13.5	50.8		99	485	107.2		54.5	40.9
625 µg/mL	5.6	9.0	10.5	35.8		137	467	103.1		36.9	58.9
750 µg/mL	2.4+	7.0	11.5	13.1		98	345	76.1		10.0	57.0

[a]RSG =Relative Suspension Growth= [Day 1 Cell Concentration divided by Cell Concentration Seeded on Day 0 (which would be 3 x 10E5/mL)] multiplied by [Day 2 Cell Concentration divided by Cell Concentration seeded on Day 1 (3 x 10E5/mL if subcultured; Day 1 Cell Concentration if not subcultured) multiplied by [Day 3 Cell Concentration divided by Cell Concentration seeded on Day 2 (3 x 10E5/mL if subcultured; Day 2 Cell Concentration if not subcultured)]
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded (600) * 100
[c]Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4.
Decimal is moved to express the frequency in units of 10E-6.
[e]Vehicle Control = Water
Positive Control: MMS = Methyl Methanesulfonate
[f]Mutagenic. Exceeds Minimum Criterion of 76.8 X 10E-6, which is twice the average mutant frequency of the concurrent vehicle controls.
[g]AVG VC=average of the vehicle controls.
[h]Colony counts increased by 9.099% to compensate for area of dish not scanned.
+ Not subcultured

Table 25. Trial 1: Summary of Results +S9, 4 h Incubation

Test Article: Hydrocodone Bitartrate
Vehicle: Water
Selective Agent: TFT 3.0 µg/mL
Test Date: 5/18/99
Treatment Period: 4 hours

Test Condition	Daily Cell Counts (Cell/ML, 10E5 Units)		RSG[a]		Total Mutant Colonies[h]	Total Viable Colonies[h]	Cloning Efficiency[b]		Relative Growth (%)[c]	Mutant Frequency (10E-6 Units)[d]
	Day 1	Day 2								
S9-Activation Controls S9 Batch No: 0800				AVG VC[g]				AVG VC[g]		
VEHICLE CONTROL	13.5	13.1	19.7		168	381	63.5		81.8	88.3
VEHICLE CONTROL	13.5	13.2	19.8		166	404	67.4		87.5	82.1
VEHICLE CONTROL	16.3	12.6	22.8	20.8	210	537	89.5	73.4	133.9	78.4
MCA 2 µg/mL	8.0	15.5	13.8		347	262	43.6		39.4	265.5[f]
MCA 4 µg/mL	7.9	15.3	13.4		352	312	52.0		45.8	225.7[f]
TEST COMPOUND			Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
9.85 µg/mL	14.5	15.4	119.5		185	391	88.7		106.0	94.6
19.7 µg/mL	13.5	17.6	127.2		146	399	90.5		115.1	73.2
39.3 µg/mL	14.0	14.5	108.7		195	378	85.7		93.1	103.2
78.5 µg/mL	11.3	13.5	81.7		275	385	87.4		71.4	142.8
157 µg/mL	9.1	14.4	70.1		270	316	71.8		50.3	170.4[f]
313 µg/mL	4.6	13.1	32.3		273	212	48.0		15.5	257.9[f]
625 µg/mL	1.3+	5.1	8.2		159	139	31.6		2.6	228.8[f]

[a]RSG =Relative Suspension Growth= [Day 1 Cell Concentration divided by Cell Concentration Seeded on Day 0 (which would be 3 x 10E5/mL)] multiplied by [Day 2 Cell Concentration divided by Cell Concentration seeded on Day 1 (3 x 10E5/mL if subcultured; Day 1 Cell Concentration if not subcultured)]
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded * 100
[c]Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4.
Decimal is moved to express the frequency in units of 10E-6.
[e]Vehicle Control = Water
Positive Control: MCA = Methylcholanthrene
[f]Mutagenic. Exceeds Minimum Criterion of 165.9 X 10E-6, which is twice the average mutant frequency of the concurrent vehicle controls.
[g]AVG VC=average of the vehicle controls.
[h]Colony counts increased by 11.3751% to compensate for area of dish not scanned.
+Not Subcultured.

Table 26. Confirmatory Trial: Summary of Results +S9, 4 h Incubation

Mutation Assay With Activation – Confirmatory Trial

A. Test Article: Hydrocodone Bitartrate
C. Vehicle: Water
D. Selective Agent: TFT 3.0 µg/mL
E. Test Date: 6/8/99
F. Treatment Period: 4 hours

Test Condition	Daily Cell Counts (Cell/ML, 10E5 Units)		RSG[a]		Total Mutant Colonies[h]	Total Viable Colonies[h]	Cloning Efficiency[b]		Relative Growth (%)[c]	Mutant Frequency (10E-6 Units)[d]
	Day 1	Day 2								
S9-Activation Controls S9 Batch No: 0800				AVG VC				AVG VC		
VEHICLE CONTROL	9.6	16.1	17.2		127	506	84.4		101.9	50.0
VEHICLE CONTROL	9.8	16.6	18.1		144	491	81.8		104.0	58.7
VEHICLE CONTROL	10.3	16.1	18.4	17.9	163	433	72.2	79.5	93.6	75.1
MCA 2 µg/mL	6.5	19.7	14.2		453	313	52.2		52.2	289.2[f]
MCA 4 µg/mL	9.5	13.8	14.6		505	332	55.3		56.6	304.6[f]
TEST COMPOUND			Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
15.7 µg/mL	10.2	14.4	91.2		206	472	99.1		90.4	87.3
31.3 µg/mL	12.0	14.3	106.6		168	441	92.4		98.5	76.2
62.5 µg/mL	7.3	19.6	88.9		293	400	84.0		74.6	146.6[f]
125 µg/mL	7.7	14.9	71.3		338	281	59.0		42.1	240.3[f]
250 µg/mL	3.9+	15.9	29.6		547	248	51.9		15.4	441.4[f]
375 µg/mL	3.4+	13.0	24.2		485	206	43.2		10.5	470.9[f]

[a]RSG =Relative Suspension Growth= [Day 1 Cell Concentration divided by Cell Concentration Seeded on Day 0 (which would be 3 x 10E5/mL)] multiplied by [Day 2 Cell Concentration divided by Cell Concentration seeded on Day 1 (3 x 10E5/mL if subcultured; Day 1 Cell Concentration if not subcultured)]
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded * 100
[c]Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4.
Decimal is moved to express the frequency in units of 10E-6.
[e]Vehicle Control = Water
Positive Control: MCA = Methylcholanthrene

[f]Mutagenic. Exceeds Minimum Criterion of 122.5 X 10E-6, which is twice the average mutant frequency of the concurrent vehicle controls.
[g] AVG VC=Average vehicle control values
[h]Colony Counts increased by 9.099% to compensate for area of dish not scanned
[i]Not Subcultured

7.2 *In Vitro* Assays in Mammalian Cells

Study title: L5178Y TK+/- Mouse Lymphoma Forward Mutation Assay With and Without Human S9 Using Hydrocodone Bitartrate

Study no.:	NDSE-682-GLP ((b) (4)
Study report location:	EDR 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 8, 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Hydrocodone bitartrate, Lot B13205 99.2% and E01157 100.4%

Key Study Findings

- Under the conditions of this assay, hydrocodone bitartrate is considered negative in both the presence and absence of human S9 for inducing forward mutations at the TK locus of L5178 mouse lymphoma cells.

Methods

Cell line:	L5178Y cells
Concentrations in definitive study:	-S9: 9.85-1100 mcg/mL +S9: 9.85-1600 mcg/mL
Basis of concentration selection:	Dose range finding study using 3.93-2000 mcg/mL HC
Negative control:	Water
Positive control:	Methyl methanesulfonate (-S9) and Aflatoxin B1 (+S9)
Formulation/Vehicle:	Water
Incubation & sampling time:	-S9: 4 h and 24 h, +S9: 4 h

Study Validity

The study was deemed valid for the following reasons: 1) vehicle control cultures exhibited a mean cloning efficiency of 50% or greater; 2) vehicle control cultures gave a mean mutant frequency less than 150 x 10^{-6} and at least 12 x 10^{-6}; 3) positive controls exhibited appropriate responses; 4) concentrations were appropriate in that either a positive mutant frequency response or an 80% reduction in RTG or a persistent precipitate was achieved and 5) the ability to recover small colonies was demonstrated by sizing of the positive control.

Results

Two trials and a confirmatory assay in the absence of metabolic activation were performed. One trial and a confirmatory assay in the presence of metabolic activation were performed. The in vitro metabolic activating system used in this assay was comprised of human liver enzymes (S9 fraction) and the appropriate cofactors. Sizing

analysis was performed on all treatments. The maximum concentration used in each trial was determined by the solubility and cytotoxicity of HC. In the absence of S9 at both 4 h and 24 h incubations, HC did not induce an increase in mutation frequency (Tables 27-29). In the presence of S9 with 4 h incubation in the initial trial, two low concentrations induced mutant frequencies slightly higher than the background levels (Table 30). The initial assay used concentrations of 9.85, 19.7, 750, 1000, 1250, 1400, 1500 and 1600 mcg/mL. The conditions from 39.3 to 625 mcg/mL were terminated because a sufficient number of higher concentrations were available for analysis. The concentrations of 19.7 and 750 mcg/mL induced mutant frequencies (94.7×10^{-6} and 91.5×10^{-6}, respectively) slightly higher than the minimum criterion for a positive response of 87.5×10^{-6}. A confirmatory trial using concentrations of 10.0-1600 mg/mL showed no induction of mutations (Table 31). The increased frequencies were not dose dependent and were within historical control ranges for activated conditions. The confirmatory assay did not replicate the finding observed in the initial trial. Under the conditions of this assay, HC bitartrate is considered negative in both the presence and absence of human S9 metabolic activation for inducing forward mutations at the TK locus of L5178 mouse lymphoma cells.

Table 27. Initial Trial: Summary of Results -S9, 4 h Incubation

A. TEST ARTICLE: Hydrocodone Bitartrate
B. ASSAY NO.: 24595-0-431H
C. VEHICLE: Water
D. SELECTIVE AGENT: TFT 3.0 µg/mL
E. TREATMENT DATE: 08/19/2003
F. CELLS ANALYZED: 3 x 10^6
G. TREATMENT PERIOD: ~4 hours
H. EXPRESSION PERIOD: 2 days

Test Condition	Daily Cell Density/mL (x 10^5)		Cumulative RSG[a]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]		Relative Growth (%)[c]	Mutant Frequency (x 10^{-6})[d]
	Day 1	Day 2								
Nonactivation Controls[e]				AVG VC				AVG VC		
Vehicle Control	15.5	12.0	20.7		134	340	56.7		97.0	78.8
Vehicle Control	17.0	10.8	20.4		142	371	61.8		104.4	76.5
Vehicle Control	17.2	11.8	22.6	21.2	93	314	52.4	57.0	97.7	59.0
MMS 13 µg/mL	7.9	9.8	8.6		273	128	21.3		15.1	427.4[f]
MMS 13 µg/mL	9.2	8.7	8.9		345	183	30.5		22.5	376.2[f]
Test Article (µg/mL)			Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
9.85	16.0	10.3	86.3		155	427	124.8		107.8	72.6
19.7	16.1	11.7	98.7		166	349	102.1		100.8	95.0
39.3	15.2	12.3	98.0		145	348	101.8		99.7	83.4
157	15.1	12.1	95.7		155	363	106.3		101.7	85.3
500	13.0	12.3	83.8		146	520	152.2		127.5	56.2
625	10.5	10.6	58.3		157	528	154.5		90.1	59.5
750	9.2	11.2	54.0		119	447	130.9		70.6	53.2

[a] RSG = (Day 1 Count/3) x (Day 2 Count)/3 (or Day 1 Count if not subcultured)
[b] Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100
[c] Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100
[d] Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10^{-4})
Decimal is moved to express the frequency in units of 10^{-6}
[e] Vehicle Control = Water
Positive Control: MMS = Methyl methanesulfonate
[f] Mutagenic. Exceeds Minimum Criterion of 142.9 x 10^{-6}
[g] Not subcultured

Table 28. Repeat Trial: Summary of Results -S9, 4 h Incubation

A. TEST ARTICLE: Hydrocodone Bitartrate
B. ASSAY NO.: 24595-0-431H
C. VEHICLE: Water
D. SELECTIVE AGENT: TFT 3.0 µg/mL
E. TREATMENT DATE: 09/09/2003
F. CELLS ANALYZED: 3 x 10^6
G. TREATMENT PERIOD: ~4 hours
H. EXPRESSION PERIOD: 2 days

Test Condition	Daily Cell Density/mL (x 10^5)		Cumulative RSG[a]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]		Relative Growth (%)[c]	Mutant Frequency (x 10^{-6})[d]
	Day 1	Day 2		AVG VC				AVG VC		
Nonactivation Controls[e]										
Vehicle Control	10.8	12.5	15.0		204	717	119.5		102.5	56.9
Vehicle Control	13.2	12.0	17.6		194	597	99.5		100.1	65.1
Vehicle Control	14.6	11.2	18.2	16.9	167	547	91.1	103.3	94.6	61.1
MMS 13 µg/mL	10.1	9.4	10.5		550	470	78.4		47.3	233.9[f]
MMS 13 µg/mL	10.3	11.0	12.6		538	334	55.6		40.1	322.2[f]
Test Article (µg/mL)			Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
500	11.3	9.4	69.7		277	873	140.8		98.2	63.5
600	13.2	8.1	70.2		238	895	144.3		101.3	53.2
700	8.8	10.3	59.5		190	696	112.3		66.8	54.5
800	8.4	9.1	50.2		204	707	114.0		57.2	57.7
900	6.1	9.1	36.4		196	643	103.6		37.8	61.1
1000	3.0[g]	10.6	20.9		217	736	118.8		24.8	59.0
1100	2.5[g]	9.8	19.3		177	731	117.9		22.8	48.4

[a] RSG = (Day 1 Count/3) x (Day 2 Count)/3 (or Day 1 Count if not subcultured)
[b] Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100
[c] Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100
[d] Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10^{-4})
Decimal is moved to express the frequency in units of 10^{-6}
[e] Vehicle Control = Water
Positive Control: MMS = Methyl methanesulfonate
[f] Mutagenic. Exceeds Minimum Criterion of 122.1 x 10^{-6}
[g] Not subcultured

Table 29. Confirmatory Trial: Summary of Results -S9, 24 h Incubation

A. TEST ARTICLE: Hydrocodone Bitartrate
B. ASSAY NO.: 24595-0-431H
C. VEHICLE: Water
D. SELECTIVE AGENT: TFT 3.0 µg/mL
E. TREATMENT DATE: 09/09/2003
F. CELLS ANALYZED: 3 x 10^6
G. TREATMENT PERIOD: ~24 hours
H. EXPRESSION PERIOD: 2 days

Test Condition	Daily Cell Density/mL (x 10^{-5}) Day 1	Day 2	Day 3	Cumulative RSG[a]	AVG VC	Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]	AVG VC	Relative Growth (%)[c]	Mutant Frequency (x 10^{-6})[d]
Nonactivation Controls[e]											
Vehicle Control	12.8	10.2	11.7	56.6		149	795	132.6		125.3	37.6
Vehicle Control	12.7	8.9	9.7	40.6		166	675	112.6		76.4	49.1
Vehicle Control	13.0	9.4	12.1	54.8	50.6	119	656	109.3	118.1	100.0	36.3
MMS 6.5 µg/mL	12.0	5.7	6.1	15.5		542	317	52.9		13.7	341.6[f]
MMS 6.5 µg/mL	12.4	5.2	6.3	15.0		707	388	64.7		16.3	364.0[f]
Test Article (µg/mL)				Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
30.0	12.1	9.8	10.4	90.2		221	729	102.8		92.7	60.8
50.0	9.8	8.8	11.8	74.4		172	739	104.2		77.5	46.7
100	11.4	6.6	11.0	60.5		185	687	97.0		58.7	54.0
200	9.2	7.3	11.0	54.0		199	611	86.2		46.6	65.0
250	9.7	9.2	12.3	80.3		153	592	83.6		67.1	51.6
300	7.2	7.2	9.7	36.8		192	840	118.5		43.6	45.7
350	7.7	6.7	11.6	43.8		156	742	104.7		45.8	42.1
400	5.4	6.7	9.2	24.3		218	861	121.4		29.6	50.7

[a] RSG = [Treatment termination (Day 1) cell density/3] x [Day 2 cell density/3 or Day 1 density if not split back] x [Day 3 cell density/3 or Day 2 density if not split back]
[b] Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100
[c] Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100
[d] Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10^{-4})
Decimal is moved to express the frequency in units of 10^{-6}
[e] Vehicle Control = Water
Positive Control: MMS = Methyl methanesulfonate
[f] Mutagenic. Exceeds Minimum Criterion of 82.0 x 10^{-6}

Table 30. Initial Trial: Summary of Results +S9 Activation, 4 h Incubation

A. TEST ARTICLE: Hydrocodone Bitartrate
B. ASSAY NO.: 24595-0-431H
C. VEHICLE: Water
D. SELECTIVE AGENT: TFT 3.0 µg/mL

E. TREATMENT DATE: 08/19/2003
F. CELLS ANALYZED: 3 x 10^6
G. TREATMENT PERIOD: ~4 hours
H. EXPRESSION PERIOD: 2 days

Test Condition	Daily Cell Density/mL (x 10^5)		Cumulative RSG[a]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]		Relative Growth (%)[c]	Mutant Frequency (x 10^{-6})[d]
	Day 1	Day 2								
Activation Controls[e]				AVG VC				AVG VC		
Vehicle Control	15.1	13.1	22.0		123	652	108.7		114.3	37.8
Vehicle Control	14.3	13.8	21.9		134	581	96.9		101.6	46.2
Vehicle Control	12.2	13.5	18.3	20.7	130	581	96.9	100.9	84.8	44.7
AFB1 0.5 µg/mL	8.0	11.4	10.1		500	383	63.8		30.9	261.0[f]
AFB1 1.0 µg/mL	6.4	6.1	4.3		454	259	43.1		8.9	351.1[f]
AFB1 1.5 µg/mL	5.5	4.8	2.9		321	95	15.8		2.2	675.9[f]
Test Article (µg/mL)			Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
9.85	10.0	12.0	64.3		273	656	108.4		69.7	83.2
19.7	11.5	12.4	76.4		196	415	68.5		52.4	94.7[f]
750	10.2	14.7	80.3		165	360	59.5		47.8	91.5[f]
1000	7.8	12.5	52.2		129	381	62.9		32.9	67.6
1250	7.1	12.4	47.2		153	357	59.0		27.8	85.6
1400	3.8[g]	11.4	18.3		159	635	104.9		19.2	50.2
1500	2.7[g]	11.5	18.5		148	690	113.9		21.1	43.0
1600	0.9[g]	8.8	14.1		97	538	88.9		12.6	36.1

[a] RSG = (Day 1 Count/3) x (Day 2 Count)/3 (or Day 1 Count if not subcultured)
[b] Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100
[c] Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100
[d] Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10^{-4})
Decimal is moved to express the frequency in units of 10^{-6}
[e] Vehicle Control = Water
Positive Control: AFB1 = Aflatoxin B1
[f] Mutagenic. Exceeds Minimum Criterion of 85.7 x 10^{-6}
[g] Not subcultured

Table 31. Confirmatory Trial: Summary of Results +S9, 4 h Incubation

A. TEST ARTICLE: Hydrocodone Bitartrate
B. ASSAY NO.: 24595-0-431H
C. VEHICLE: Water
D. SELECTIVE AGENT: TFT 3.0 µg/mL
E. TREATMENT DATE: 09/09/2003
F. CELLS ANALYZED: 3 x 10^6
G. TREATMENT PERIOD: ~4 hours
H. EXPRESSION PERIOD: 2 days

Test Condition	Daily Cell Density/mL (x 10^5)		Cumulative RSG[a]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]		Relative Growth (%)[c]	Mutant Frequency (x 10^{-6})[d]
	Day 1	Day 2		AVG VC				AVG VC		
Activation Controls[e]										
Vehicle Control	14.9	9.3	15.4		196	873	145.5		117.1	45.0
Vehicle Control	12.4	9.8	13.5		202	807[h]	134.4		94.9	50.0
Vehicle Control	13.0	10.1	14.6	14.5	166	695	115.8	131.9	88.4	47.7
AFB1 0.5 ug/mL	9.3	8.8	9.1		525	628	104.7		49.8	167.0[f]
AFB1 1.0 ug/mL	8.9	6.9	6.8		482	298	49.6		17.7	323.8[f]
AFB1 1.5 ug/mL	5.5	6.3	3.8		454	219	36.5		7.4	413.9[f]
Test Article (µg/mL)			Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
10.0	14.5	8.0	88.9		213	867	109.6		97.4	49.1
100	14.6	8.5	95.1		213	730	92.2		87.7	58.3
200	13.0	11.6	115.6		218	593	75.0		86.7	73.5
400	13.2	9.0	91.1		223	636	80.4		73.2	70.0
800	6.1	11.6	54.2		205	679	85.7		46.5	60.5
1200	4.2	7.9	25.4		190	717	90.6		23.0	53.0
1400	3.5[g]	9.7	22.3		215	571	72.1		16.1	75.3
1500	3.9[g]	7.1	16.3		261	650	82.2		13.4	80.2

[a] RSG = (Day 1 Count/3) x (Day 2 Count)/3 (or Day 1 Count if not subcultured)
[b] Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100
[c] Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100
[d] Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10^{-4})
Decimal is moved to express the frequency in units of 10^{-6}
[e] Vehicle Control = Water
Positive Control: AFB1 = Aflatoxin B1
[f] Mutagenic. Exceeds Minimum Criterion of 95.2 x 10^{-6}
[g] Not subcultured
[h] One plate contaminated; value determined by weight proportion

7.2 *In Vitro* Assays in Mammalian Cells

Study title: In Vitro Mammalian Chromosome Aberration Test with (b) (4)

Study no.:	HYD-N-031 ((b) (4) AD22NH.341. (b) (4)
Study report location:	EDR 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 20, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) Lot RT0323-23, 99.5%

Key Study Findings

- It is concluded that (b) (4) produces structural aberrations in the in vitro chromosomal aberration assay in HPBLs in the absence of metabolic activation under the conditions of this assay. (b) (4) did not produce structural aberrations in the presence of metabolic activation and did not produce numerical aberrations in either the presence or absence of metabolic activation.

Methods

Cell line:	Human peripheral blood lymphocytes
Concentrations in definitive study:	5, 7.5, 10 mcg/L
Basis of concentration selection:	A preliminary toxicity test using concentrations up to 715 mcg/mL was conducted.
Negative control:	DMSO
Positive control:	-S9: Mitomycin C; +S9: Cyclophosphamide
Formulation/Vehicle:	DMSO was used as the vehicle.
Incubation & sampling time:	5, 7.5, and 10 mcg/mL for both +/- S9

Study Validity

The study appears to be valid for the following reasons: 1) The appropriate positive controls were employed according to FDA/CFSAN Redbook guidelines and produced expected results. 2) The appropriate number of cells was evaluated and two replicates of each test concentrations were tested which is in accordance with the current practice. 3) Metaphase cells with 46 centromeres were examined under oil immersion. Whenever possible, a minimum of 200 metaphase spreads (100 per duplicate treatment condition) were examined and scored for chromatid-type and chromosome-type aberrations. The counting method was in compliance with the currently accepted procedure and therefore considered valid. 4) According to the protocol a test article was considered to induce a positive response when the percentage of cells with aberrations (minus gaps) was increased in a concentration-responsive manner with one or more concentrations being statistically elevated relative to the solvent control group. A reproducibly statistically significant increase at the high concentration only or one

other concentration only with no concentration-response was considered positive. The criteria for the evaluation of the positive results were considered valid. 5) The conditions of the assays were appropriate given the use of the limit dose for 4 h incubations and toxicity measured in the 20 h incubation (FDA/CFSAN Redbook guidelines). The dose selection based upon mitotic index was acceptable.

Results

Increases in structural aberrations were observed with (b) (4) under non-activated conditions at both 4 and 20 h time points (Table 32). For the 4 h incubation, the highest concentration tested produced a significant increase over vehicle for structural aberrations (Veh=1.5%, 10 mcg/mL= 9.5%). For the 20 h incubation, increases in structural aberrations were observed with (b) (4) at the mid and high concentrations (Veh= 0.0%, 7.5 mcg/mL= 9.0%, 10 mcg/mL=16%). No structural aberrations were seen with (b) (4) under non-activated conditions. No increases in numerical aberrations were seen for either the activated or non-activated assay. The positive controls yielded appropriate increases in structural aberrations under both activated and non-activated conditions. It is concluded that (b) (4) produces structural aberrations in the in vitro chromosomal aberration assay in HPBLs in the absence of metabolic activation. (b) (4) did not produce structural aberrations in the presence of metabolic activation and did not produce numerical aberrations in either the presence or absence of metabolic activation.

Table 32. Summary of Cytogenetic Analysis Results with (b) (4)

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations	
				Numerical	Structural			Numerical (%)	Structural (%)
DMSO	-S9	4	15.6	200	200	0.015	±0.122	0.0	1.5
(b) (4)									
5	-S9	4	12.2	200	200	0.000	±0.000	0.0	0.0
7.5	-S9	4	11.3	200	200	0.040	±0.262	0.0	3.0
10	-S9	4	10.9	200	150	0.173	±0.702	0.0	9.5**
MMC, 0.6	-S9	4	7.3	200	100	0.340	±0.655	0.0	25.0**
DMSO	+S9	4	10.4	200	200	0.000	±0.000	0.0	0.0
(b) (4)									
5	+S9	4	9.8	200	200	0.010	±0.100	0.5	1.0
7.5	+S9	4	9.1	200	200	0.000	±0.000	0.0	0.0
10	+S9	4	9.9	200	200	0.005	±0.071	0.0	0.5
CP, 5	+S9	4	4.4	200	100	0.280	±0.552	0.0	23.0**
DMSO	-S9	20	12.1	200	200	0.000	±0.000	0.0	0.0
(b) (4)									
5	-S9	20	12.0	200	200	0.000	±0.000	0.0	0.0
7.5	-S9	20	8.4	200	150	0.080	±0.296	0.0	9.0**
10	-S9	20	5.8	200	100	0.290	±1.104	0.0	16.0**
MMC, 0.3	-S9	20	6.3	200	100	0.190	±0.419	0.0	18.0**

Treatment: Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, p≤0.05; **, p≤0.01; using the Fisher's Exact test.

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

Study title: In Vivo Mouse Micronucleus Assay with Toxicokinetics Using Hydrocodone Bitartrate

Study no:	DSE-266-GLP ((b) (4) 20442-455OECD)
Study report location:	EDR 4.2.3.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 3, 1999
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Hydrocodone bitartrate, Lot 0598031, 100.5%

Key Study Findings

- **Hydrocodone bitartrate was found to be negative in the in vivo micronucleus assay with bone marrow**.

Methods

Doses in definitive study:	Male: 125, 250, and 500 mg/kg Female: 50, 100, and 200 mg/kg
Frequency of dosing:	Single dose
Route of administration:	Oral gavage
Dose volume:	20 mL/kg
Formulation/Vehicle:	Water
Species/Strain:	Mouse, Crl:CD-1 (ICR)BR
Number/Sex/Group:	6/sex/group
Satellite groups:	None
Basis of dose selection:	Dose range-finding study
Negative control:	Water
Positive control:	Cyclophosphamide

Study Validity

The study was deemed valid for the following reasons:

- Previous pharmacokinetic assessments demonstrated systemic exposure.
- Dosing appeared to be adequate based upon the results of the dose-ranging study.
- Preparation and administration of the test substance was acceptable.
- The species and number of animals/sex/group were acceptable.
- Tissue sampling and analysis was acceptable.
- Positive controls exhibited appropriate responses.

Results

The Applicant stated that since differing morbidity was observed between the sexes in the dose range-finding study, both male and female mice were used in the MN assay. Mice treated with HC bitartrate showed a group mean %PCE that was similar to controls in both males and females indicating the lack of bone marrow toxicity. Group mean frequencies of MN PCE were similar to controls. Micronucleus data are summarized in the table below. Based on the results of this study, HC bitartrate did not induce any increase in micronucleated polychromatic erythrocytes in the bone marrow in either males or females. Hydrocodone bitartrate can be considered negative the in vivo micronucleus assay in bone marrow.

Table 33. Summary of Results in Micronucleus Assay with Hydrocodone Bitartrate

Study 20442-0-455OECD
Micronucleus Summary Data

TEST ARTICLE: Hydrocodone Bitartrate

ASSAY: 20442

TREATMENT	DOSE	HARVEST TIME	% MICRONUCLEATED PCEs MEAN OF 2000 PER. ANIMAL ± S.E.		RATIO PCE:NCE MEAN ± S.E	
			MALES	FEMALES	MALES	FEMALES
CONTROLS						
VEHICLE	Water	24 hr	0.03 ± 0.01	0.01 ± 0.01	0.73 ± 0.12	0.70 ± 0.15
		48 hr	0.10 ± 0.05	0.05 ± 0.04	1.24 ± 0.14	1.87 ± 0.30
POSITIVE	CP 80.0 mg/kg	24 hr	3.51 ± 0.32*	3.28 ± 0.35*	1.41 ± 0.18**	0.38 ± 0.06
TEST ARTICLE	50mg/kg	24 hr		0.07 ± 0.03		0.61 ± 0.06
	125mg/kg	24 hr	0.07 ± 0.02		0.87 ± 0.09	
	100mg/kg	24 hr		0.03 ± 0.02		0.60 ± 0.13
	250mg/kg	24 hr	0.07 ± 0.03		0.76 ± 0.10	
	200mg/kg	24 hr		0.03 ± 0.01		0.43 ± 0.08
	500mg/kg	24 hr	0.03 ± 0.01		0.60 ± 0.06	
	200mg/kg	48 hr		0.07 ± 0.06		3.00 ± 0.53
	500mg/kg	48 hr	0.06 ± 0.03		0.84 ± 0.22	

*Significantly greater than the corresponding vehicle control, $p < 0.01$.
**Significantly greater than the corresponding vehicle control, $p < 0.05$.
CP = Cyclophosphamide
PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte

7.3 *In Vivo* Clastogenicity Assay in Rodent (Combined Micronucleus/Comet Assay)

Study title: *In Vivo* Micronucleus and Comet Assay in Rats with (b) (4)

Study no:	HYD-N-048 (AD22NH.433ICH.(b) (4)
Study report location:	EDR 4.2.3.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 24, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) Lot RT0473-38-1, 99.7%

Key Study Findings

- (b) (4) **was found to be negative in the comet assay using liver and negative in the in vivo micronucleus assay using bone marrow**.

Methods

Doses in definitive study:	40, 75, and 150 mg/day
Frequency of dosing:	Daily for three days
Route of administration:	Oral gavage
Dose volume:	20 mL/kg
Formulation/Vehicle:	1% Methylcellulose in water
Species/Strain:	Rat, Hsd:Sd
Number/Sex/Group:	5/group
Satellite groups:	None
Basis of dose selection:	Dose range-finding study
Negative control:	1% Methylcellulose in water
Positive control:	Ethyl methanesulfonate

Study Validity

The micronucleus assay was deemed valid for the following reasons: 1) previous pharmacokinetic assessments demonstrated systemic exposure, 2) dosing appeared to be adequate based upon the results of the dose-ranging study, 3) preparation and administration of the test substance was acceptable, 4) the species and number of animals/sex/group were acceptable, 5) tissue sampling and analysis was acceptable, 6) positive controls exhibited appropriate responses, and 7) the proportion of immature erythrocytes among total erythrocytes was not less than 20% of the control value.

The comet study was deemed valid for the following reasons: 1) At least 5 animals per group could be evaluated. 2) The DNA damage (mean of % tail DNA) in the negative control group was within the historical negative control range for liver cells data generated by the testing facility using other vehicles.

Results

Micronucleus Assay

Mice treated with (b) (4) showed a group mean %PCE that was similar to controls indicating the lack of bone marrow toxicity. Group mean frequencies of MN PCE were similar to controls. Micronucleus data are summarized in the table below.

Table 34. Summary of Results for Bone Marrow Micronucleus Assay with (b) (4)

Treatment (10 mL/kg/day)	Sex	Time (hr)	Number of Animals	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Number of MPCE/1000 PCE (Mean +/- SD)	Number of MPCE/PCE Scored
1% Methylcellulose in deionized water (400 cps)							
	F	24	5	0.511 ± 0.02	---	0.5 ± 0.35	5 / 10000
(b) (4)							
40 mg/kg/day	F	24	5	0.462 ± 0.05	-10	0.6 ± 0.42	6 / 10000
75 mg/kg/day	F	24	5	0.471 ± 0.04	-8	0.4 ± 0.42	4 / 10000
150 mg/kg/day	F	24	5	0.454 ± 0.03	-11	0.4 ± 0.42	4 / 10000
EMS							
200 mg/kg/day	F	24	5	0.446 ± 0.03	-13	10.4 ± 1.08	*104 / 10000

*Statistical signficant increase compared to vehicle control $p \leq 0.05$ (Kastenbaum-Bowman Tables)

Comet Assay

No dose-related increase in percent of clouds or percent of diffused cells in the liver following treatment with (b) (4) indicating that the drug treatment did not cause excessive DNA damage that could have interfered with the comet analysis. Comet analysis of liver provided tail intensities and tail moment values that were similar to the control group indicating the absence of DNA damage. Comet assay data are summarized in the table below.

Table 35. Summary of Results for the Comet Assay with (b) (4)

Treatment (10 mL/kg/treatment)	Number of Animals	Mean % of Clouds	Tail DNA (%)[A] Mean ± S.D.
Vehicle Control:			
1% methylcellulose in deionized water (400 cps)	5	2.8	2.06 ± 0.53
Test Article: (b) (4)			
40 mg/kg/day	5	2.8	2.47 ± 0.69
75 mg/kg/day	5	3.4	1.73 ± 0.29
150 mg/kg/day	5	2.6	2.16 ± 0.38
Positive Control:			
EMS 200 mg/kg/day[B]	5	6.0	38.65 ± 6.25*

[A] Mean of 5 animals means
[B] Ethyl methanesulfonate (EMS), positive control for Comet assay, orally administered at 24 hours and at 3 to 4 hours prior to organ collection.

*$p < 0.05$ (pair t-test); Statistically significant increase relative to the vehicle control
S.D. = Standard Deviation

Conclusions

(b) (4) did not induce DNA damage in the liver of male mice following oral gavage administration at doses of 40, 75, and 150 mg/day. At these same doses, (b) (4) did not induce any increase in micronucleated polychromatic erythrocytes in the bone marrow. (b) (4) can be considered negative in the comet assay (in liver) and the in vivo micronucleus assay (in bone marrow).

7.4 Other Genetic Toxicity Studies

8 Carcinogenicity

8.1 Two-Year Rat Bioassay

Study title: A 2-Year Oral (Gavage) Carcinogenicity Study in Rats with Hydrocodone Bitartrate

Study no.:	NDSE-559-GLP ((b) (4) 6770-142)
Study report location:	EDR 4.2.3.4.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 20, 2002
GLP compliance:	Yes
QA statement:	Yes
Drug, lot # (% purity):	Hydrocodone Bitartrate, Lots B13205 (99.2%), E01157 (98.6%), B13205 (98.7%)
CAC concurrence:	Yes

Key Study Findings

- No test HC-related neoplasms were observed in either sex in this study.
- Survival in both males and females at the low dose was comparable to controls, in the mid- and high-dose groups survival was significantly higher than controls.
- Significant test article-related decreases in body weights at the end of the study were observed at the mid and high dose groups in both sexes. The increases in survival are attributed to the lower body weights at the mid and high dose groups.

Adequacy of Carcinogenicity Study

The study was conducted with doses chosen based on a 13-week dose range-finding study. The SPA was presented to the ECAC and doses for the carcinogenicity study were recommended. This study employed an appropriate rat strain, an adequate number of animals per sex for each dose and concurrent control groups, and the clinically relevant route of administration. Animals were individually housed, provided appropriate food and care, and were administered test article or vehicle control for 104 weeks. The study is considered adequate.

Appropriateness of Test Models

The rat is an appropriate model for a 2-year bioassay and the strain, Crl:CD(SD)IGS BR, is acceptable.

Evaluation of Tumor Findings

No HC-mediated increases in neoplastic lesions were observed in this study. Several treatment-related decreases in tumor incidences were seen. Decreased incidences in tumors in the anterior pituitary and mammary gland were observed. Decreased body weights in long-term studies have been associated with decreased incidences of mammary and pituitary tumors in rats and mice (Haseman, et al., 1997). Statistical analysis of the data was conducted by Dr. Min Min of the Division of Biostatistics VI. Tumors were combined for statistical analysis according to McConnell, et al, 1986 (McConnell EE, et al., 1986). According to Dr. Min's review, no tumor types were considered to have a statistically significant positive dose-response relationship or a statistically significant pair-wise comparison for either males or females.

Methods

Doses:	4, 12, and 25 mg/kg
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Gavage
Formulation/Vehicle:	Distilled water
Basis of dose selection:	The ECAC recommended the high dose based on the determination of an MTD (body weight gain decrements) in a 13-week study in Sprague-Dawley rats (see ECAC meeting minutes dated June 14, 2002).
Species/Strain:	Rat; Crl:CD(SD)IGS BR
Number/Sex/Group:	70/sex/group (Table 1)
Age:	At least 6 weeks
Animal housing:	Individually housed in suspended cages
Paradigm for dietary restriction:	None
Dual control employed:	Yes
Interim sacrifice:	No
Satellite groups:	9/sex/group for control TK, 12/sex/group for treated TK (Table 36)
Deviation from study protocol:	None that affected the integrity of the study

Table 36. Study Design

Group	No. of Animals[a]		Dose Level (mg/kg/day)[b]	Concentration (mg/mL)
	Male	Female		
Main Study				
1 (Control)	70	70	0	0
2 (Control)	70	70	0	0
3 (Low)	70	70	4	0.4
4 (Mid)	70	70	12	1.2
5 (High)	70	70	25	2.5
Toxicokinetic Study[b]				
6 (Control)	9	9	0	0
7 (Low)	12	12	4	0.4
8 (Mid)	12	12	12	1.2
9 (High)	12	12	25	2.5
Sentinels				
10 (Untreated)	25	25	0	0

a Twenty-five animals/sex (in addition to main and toxicokinetic animals) were assigned as sentinel animals. Five rats/sex were bled prestudy and at Weeks 26 and 52 and four rats/sex were bled (due to survival) at Weeks 78 and 104 for viral screening purposes.

b Toxicokinetic animals were bled on Day 1 and during Weeks 26 and 52 at the following time points: predose and 0.5, 1, 2, 6, and 24 hours postdose.

Observations and Results

Mortality

Cage-side observations were made twice daily. Any rats euthanized *in extremis* were examined *post mortem*. Any rats found dead were examined as soon as possible after death. At the conclusion of the study, mortality among the controls and low dose males and females was similar throughout the study. In both males and females, the mid- and high-dose males had a lower rate of mortality as compared to controls (Table 37, Figures 7 and 8). The increased survival in these groups is attributed to the lower body weights observed in the treated groups throughout the study.

Table 37. Survival at the Conclusion of the Study

Group	***Male Surviving/total***	***Female Surviving/total***
Control 1	28/50 (56%)	17/50 (34%)
Control 2	22/50 (44%)	17/50 (34%)
4 mg/kg	31/50 (62%)	19/50 (38%)
12 mg/kg	36/50 (72%)	27/50 (54%)
25 mg/kg	41/50 (82%)	41/50 (82%)

Figure 7. Survival in Males

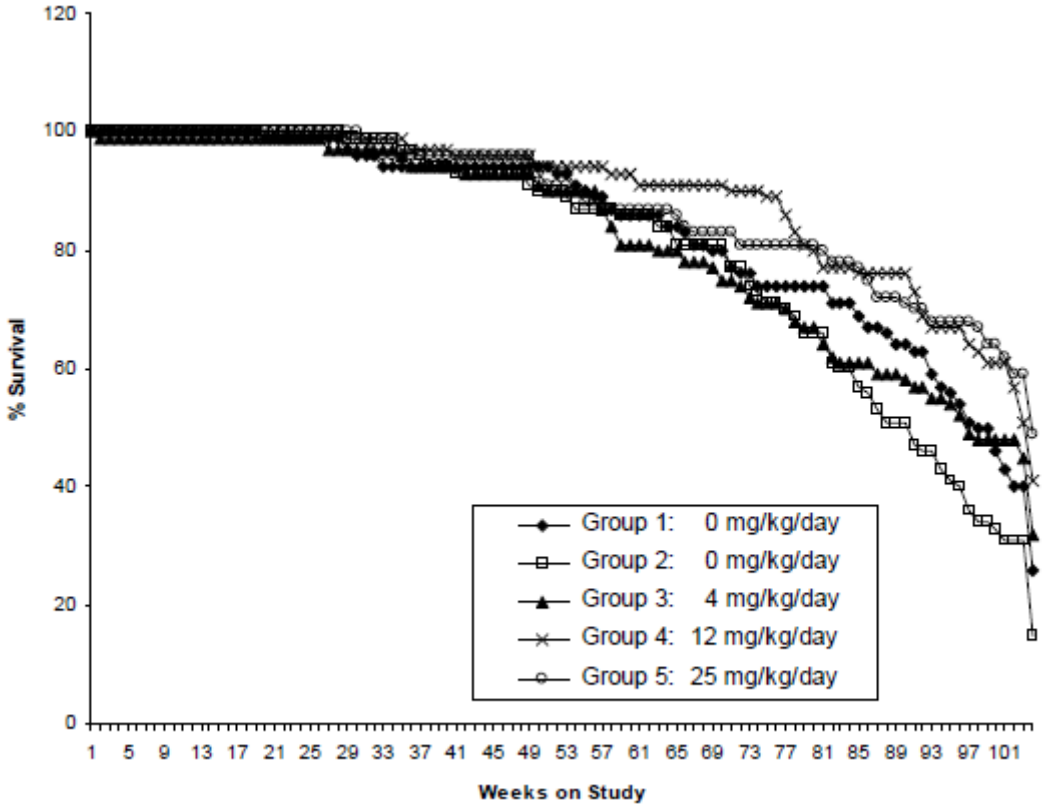
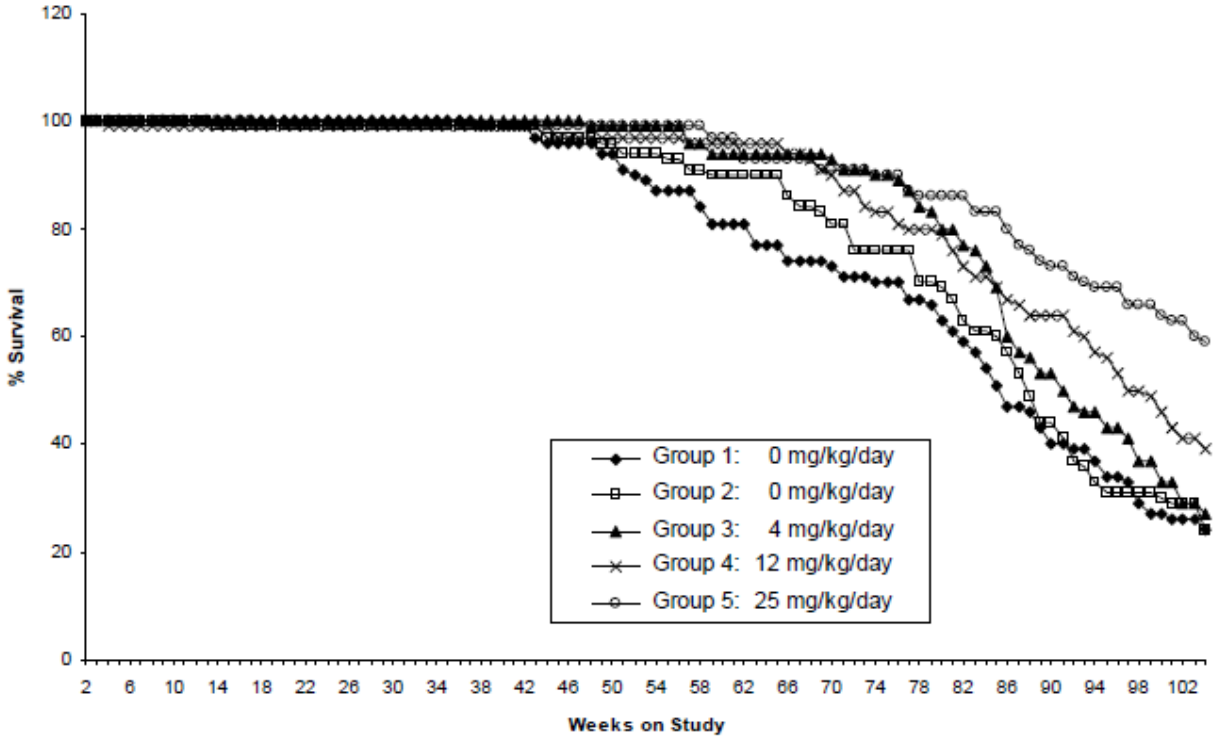


Figure 8. Survival in Females



Clinical Signs

Cage-side observations included recording of any changes in clinical condition or behavior and were made twice daily. A detailed examination was performed at least weekly. The rats in the drug-treated groups were more aggressive and showed observations including sores and scabs (on paws, limbs, mouth, tail). Hyperactivity, vocalizations, and sensitivity to touch were also observed more commonly in the treated groups. These behaviors are considered to be due to the pharmacologic action of HC and are consistent with behaviors observed in the 13-week study. Convulsions were also observed in the mid-dose males and females and exceeded levels in control animals. Age-related signs were observed in all groups were not considered drug-related.

Body Weights

Body weights were recorded prior to treatment, weekly through Week 14 and every two weeks thereafter. All rats were weighed immediately prior to termination. All treated groups had net gains in body weight, but at terminal sacrifice, both male and female body weights were dose-dependently lower than controls. Body weights are presented in the figures and tables below. Mean body weights of the two control groups were similar for both males and females and were combined. The reduced body weights throughout the study likely contributed to the increased survival and decreased incidence of certain neoplasms in the treated groups.

Table 38. Body Weights at the Conclusion of the Study

	Group, mg/kg	***Body weight difference: Treated - control at last time point, g***	***Percent change from control at last time point, %***
Male	*4*	-3	-5
	12	-116	-16
	25	-164	-23
Female	*4*	-30	-6
	12	-60	-13
	25	-100	-21

Figure 9. Body Weights in Males

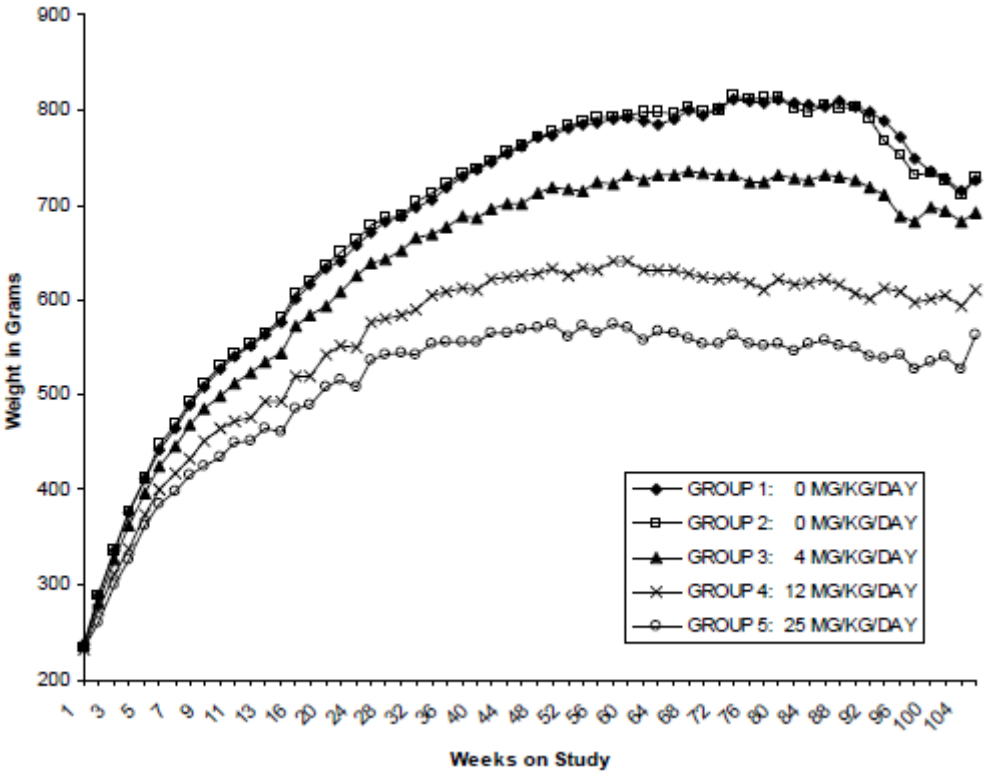
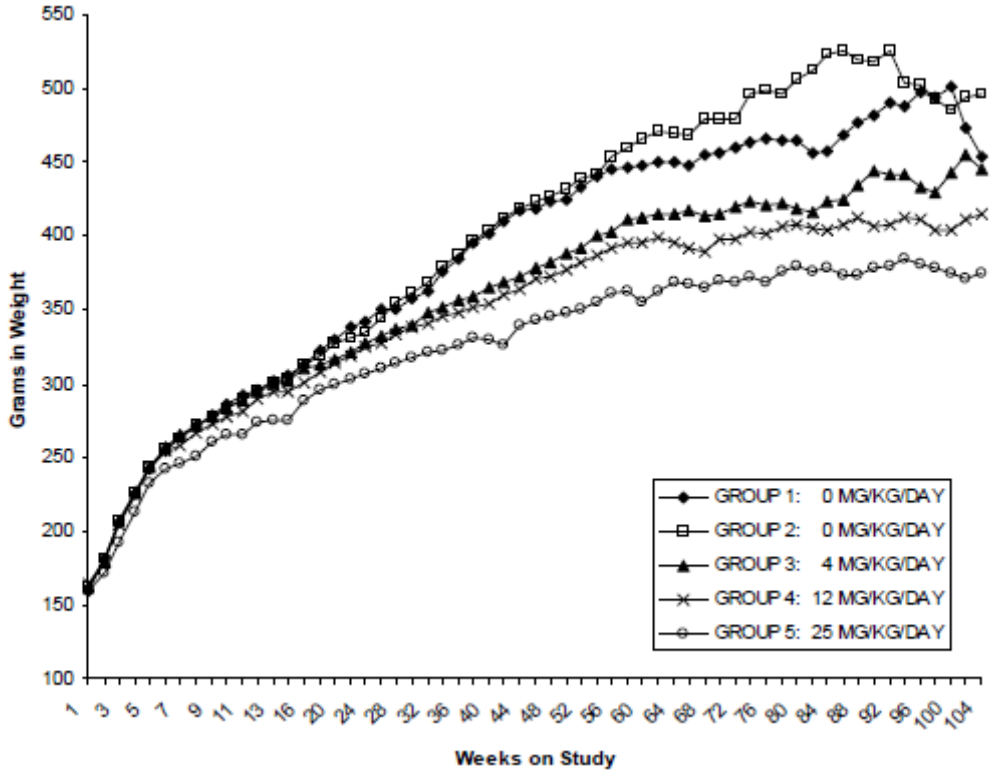


Figure 10. Body Weights in Females



Food Consumption

Food consumption was recorded prior to treatment, weekly through Week 14 and every two weeks thereafter. In most weeks during the study, males showed dose-dependently lower food consumption than control groups. To a lesser degree than males, treated females also showed lower food consumption than control groups. Decreases in food consumption correlated with decreased body weights in both males and females. Decreased food consumption and body weights are expected pharmacologic effects of opioid agonists and at no point in the study were either considered to be adverse.

Hematology

Samples for hematology were collected during Weeks 26 and 105. The parameters in the table below were measured.

Erythrocyte cell count
Leukocyte cell count
Differential blood cell count
Blood cell morphology

No test article-related hematology changes were observed.

Clinical Chemistry

Samples for clinical chemistry were collected during Weeks 26 and 105. The parameters in the table below were measured.

Alkaline Phosphatase	Globulin
Total Bilirubin	Albumin/Globulin Ratio
Aspartate Aminotransferase	Cholesterol
Alanine Aminotransferase	Triglycerides
Glucose	Sodium
Urea Nitrogen	Potassium
Creatinine	Chloride
Total Protein	Calcium
Albumin	Phosphorus

Significant decreases in triglycerides were observed in males at all doses at both the 26- and 105-week time points. A small but significant decrease in cholesterol was observed at the high dose in males at the 105-week time point. Decreases in calcium were observed in females at 26 weeks only. Significance was reached at the mid and high doses. No other test article-related changes in clinical chemistry parameters were observed in males or females. The observed changes did not correlate with any organ specific changes and, although probably treatment-related, these findings do not impact the carcinogenicity endpoints in this study.

Table 39. Selected Clinical Chemistry Results

	Parameter	***Week***	***C1***	***C2***	***4 mg/kg***	***12 mg/kg***	***25 mg/kg***
Male	Triglycerides (mg/dL)	26	133+/- 56	214+/- 113	102*+/- 37	83*+/- 24	59*+/-18
		105	151+/-98	150+/-127	81*+/-28	64*+/-20	50*+/-16
	Total Cholesterol (mg/dL)	105	116+/-48	135+/-60	113+/-34	101+/-33	76*+/-13
Female	Total Cholesterol (mg/dL)	26	107+/-28	101+/-9	97+/-18	78*+/-18	76*+/-11
	Calcium (mg/dL)	26	11.4+/-0.5	11.7+/-0.4	11.3+/-0.3	11.1*+/-0.5	11.1*+/-0.4

*P≤0.05

Urinalysis

Samples for urinalysis were collected during Weeks 26 and 105. The parameters in the table below were measured.

Bilirubin

Blood
Glucose
Appearance/color
Ketones
Protein
Microscopic analysis of sediment
pH
Specific gravity
Urobilinogen

No test article-related urinalysis changes were observed.

Gross Pathology

Test article-related macroscopic findings included a decrease in enlarged pituitaries and a concomitant decrease in the number of brains reported to be indented as a result of the pituitary tumors. Decreased body weights have been associated with decreased incidences of certain types of tumors in rats including tumors of the pituitary (Haseman, et al., 1997). Dose-dependent increases in the number of sores were observed in both males and females. These sores mostly occurred on the hindpaws and were referred to as pododermatitis by the pathologist. No further analysis was conducted to determine whether the cause of the pododermatitis was bacterial or fungal. Chronic use of opioids can lead to immunosuppression which could be an explanation for the increased incidence and severity of the observed dermatitis, if the dermatitis is due to overgrowth of endogenous skin flora. Alternatively, some opioids, including HC, have been reported to lead to histamine release in a non-opioid receptor mediated manner. This could result in increased scratching and dermatitis. Although clearly treatment-related, the sores were not associated with any type of neoplastic or proliferative lesion and do not impact the assessment of any potential carcinogenic effect of HC.

Histopathology

Peer Review: Yes

Histopathology Inventory			
Study Number	NDSE-559-GLP ((b) (4) Study 6770-142)		
Species	Rat		
Organ	***assessed***	***Organ***	***assessed***
Adrenal	X	Nasopharynx	X
Aorta	X	Ovary	X
Brain	X	Oviduct	X
Cecum	X	Pancreas	X
Cervix	X	Preputial gland	X
Clitoral gland	X	Pituitary	X
Colon	X	Prostate	X
Duodenum	X	Rectum	X
Epididymis	X	Salivary gland	X
Eye	X	Seminal vesicles	X
Esophagus	X	Skin	X
Femur	X	Spinal cord	X
Gross lesions	X	Spleen	X
Harderian gland	X	Sternum	X
Heart	X	Stomach	X
Ileum	X	Testes	X
Jejunum	X	Thymus	X
Kidney	X	Thyroid with parathyroid	X
Lacrimal gland	X	Tongue	X
Larynx	X	Trachea	X
Liver	X	Urinary bladder	X
Lung	X	Ureters	X
Lymph nodes, mesenteric	X	Vagina	X
Mammary gland	X	Voluntary muscle	X
Nerve, sciatic	X		

Neoplastic

No HC-mediated increases in neoplastic lesions were observed in this study. Several treatment-related decreases in tumor incidences were seen. Decreased incidences in tumors in the anterior pituitary and mammary gland were observed (Table 40). Decreased body weights in long-term studies have been associated with decreased incidences of mammary and pituitary tumors in rats and mice (Haseman, et al., 1997). Various neoplasms or pre-neoplastic lesions were observed in the treated groups with incidences similar to controls and/or similar to levels observed in the historical controls. None of these tumors were considered to be treatment-related.

Table 40. Selected Neoplastic Lesions

Tissue	***Tumor type***	***Incidence (observed/examined)***							
		Males, mg/kg				***Females, mg/kg***			
		0	***4***	***12***	***25***	***0***	***4***	***12***	***25***
Pituitary gland (pars distalis)	adenoma	67/140 48%	20/69 29%	19/69 28%	18/70 26%	113/139 81%	52/68 76%	50/70 71%	37/70 53%
	carcinoma	0/140 0%	0/69 0%	0/69 0%	0/70 0%	6/139 4%	3/68 4%	2/70 3%	0/70 0%
	combined	**67/140 48%**	**20/69 29%**	**19/69 28%**	**18/70 26%**	**119/139 86%**	**55/68 81%**	**52/70 74%**	**37/70 53%**
Mammary gland	fibroadenoma	0/124 0%	1/64 2%	0/67 0%	1/63 2%	51/140 36%	34/70 49%	30/69 43%	18/70 26%
	adenoma	0/124 0%	0/64 0%	1/67 1%	0/63 0%	10/140 7%	6/70 9%	5/69 7%	3/70 4%
	carcinoma	0/124 0%	0/64 0%	1/67 1%	0/63 0%	36/140 26%	18/70 26%	20/69 29%	9/70 13%
	combined	**0/124 0%**	**1/64 2%**	**2/67 3%**	**1/63 2%**	**97/140 69%**	**58/70 83%**	**55/69 80%**	**30/70 43%**

Non Neoplastic

An increase above control animals in sternum bone marrow hypercellularity was observed in males at all doses. All females, including controls, had higher levels of bone marrow hypercellularity than those observed in males. No treatment-related changes were observed in the females. Incidence data are presented in the table below. Increased bone marrow cellularity can be considered a normal response to inflammation. In this study, the inflammation was most likely caused by the pododermatitis. However, pododermatitis was also observed in treated females. Increases in hypercellularity in females may not have been observed because of the higher background levels in the control group. No hematologic changes or related neoplasms were observed in the study in either males or females. The increase in hypercellularity in males will be considered treatment-related but does not impact the assessment of the carcinogenic potential of HC in this study.

Table 41. Selected Non Neoplastic Lesions

Tissue: finding	***Incidence (observed/examined)***							
	Males, mg/kg				***Females, mg/kg***			
	0	***4***	***12***	***25***	***0***	***4***	***12***	***25***
Marrow, sternum: hypercellular	7/135 5%	7/70 10%	14/70 20%	15/70 21%	35/140 25%	15/70 21%	19/69 28%	20/70 29%

Toxicokinetics

Nine rats/sex/group and 12 rats/sex/group were bled for control and treated toxicokinetics, respectively. Toxicokinetic parameters of HC are detailed in the table below.

Exposure to HC was similar for male and female rats at all doses. Hydrocodone showed a T_{max} between 0.5-1 h. The $t_{1/2}$ values in this study ranged from 3.1-8.1 h on Day 1 and 1.3-2.5 h during Weeks 26 and 52. For the mid- and high-dose groups, mean C_{max} and AUC_{0-24} values were approximately 2-fold higher at Weeks 26 and 52 as compared to Day 1, indicating an accumulation of HC after multiple dosing. The increases in C_{max} and AUC_{0-24} values for males and females were less than dose proportional on Day 1 but greater than dose proportional during Weeks 26 and 52. Systemic levels of a 120 mg human dose at steady state (Study # HYD1002) are compared to systemic exposure of rats (Table 43). In the clinical Study # HYD1002, 120 mg HC was dosed q24h for 5 days. Steady state was reached by Day 5 and AUC_{tau}= 1938 +/-729. In the clinical Study # HYD1002, the dosing interval was 24h therefore the AUC_{0-24} animal data can be compared to the AUC_{tau} human data (Table 8). All rat exposures of HC in this study are below the systemic exposure at the human dose of 120 mg. The pharmacologic effects of opioids limit the dosing and typically multiples of human clinical exposures are not achieved in rat studies.

Table 42. Toxicokinetic Parameters of Hydrocodone in Rat Plasma

Interval	Dose Group	Hydrocodone Bitartrate Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	DN C_{max} (ng/mL)/ (mg/kg/day)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} (ng•hr/mL)/ (mg/kg/day)	$AUC_{0-\infty}$ (ng•hr/mL)	$t_{1/2}$ (hr)	AUC_{0-24} AR
Day 1	7	4	M	3.53	0.883	1.00	7.42	9.21	2.30	NC	NC	NA
			F	4.18	1.04	0.500	8.27	11.6	2.89	NC	NC	NA
	8	12	M	5.24	0.437	1.00	20.9	20.9	1.74	21.2	4.22	NA
			F	9.10	0.758	0.500	21.5	38.3	3.19	29.9	3.12	NA
	9	25	M	7.49	0.300	0.500	50.1	50.1	2.00	58.5	8.13	NA
			F	13.0	0.518	0.500	58.7	58.7	2.35	63.5	7.04	NA
Week 26	7	4	M	6.24	1.56	1.00	11.3	13.8	3.46	NA	NC	1.50
			F	6.11	1.53	0.500	9.06	10.8	2.71	NA	1.27	0.938
	8	12	M	25.4	2.12	0.500	48.8	59.8	4.98	NA	1.34	2.87
			F	21.8	1.82	0.500	33.6	42.6	3.55	NA	1.47	1.11
	9	25	M	91.5	3.66	1.00	186	239	9.55	NA	NC	4.77
			F	79.4	3.18	0.500	128	143	5.71	NA	NC	2.43
Week 52	7	4	M	6.37	1.59	1.00	12.6	15.0	3.75	NA	NC	1.63
			F	5.68	1.42	0.500	10.5	13.1	3.28	NA	1.35	1.13
	8	12	M	36.0	3.00	0.500	80.0	80.0	6.67	NA	2.49	3.84
			F	34.6	2.88	0.500	55.0	71.5	5.96	NA	1.61	1.87
	9	25	M	239	9.56	0.500	255	328	13.1	NA	NC	6.55
			F	107	4.28	1.00	173	193	7.71	NA	NC	3.28

Table 43. Exposure Comparison Between Rat and Human

	Dose, mg/kg	***Rat/human* AUC_{0-24}, (ng.h/mL)***
Male	***4***	0.008
	12	0.041
	25	0.169
Female	***4***	0.007
	12	0.037
	25	0.100

*120 mg/day at steady state; AUC_{tau}= 1938 ng.h/mL, Study # HYD1002.

Dosing Solution Analysis

The homogeneity of samples and the sample concentrations were both within an acceptable range of the respective target concentrations for HC.

8.1 Two-Year Mouse Bioassay

Study title: A 2-Year Oral (Gavage) Carcinogenicity Study in Mice with Hydrocodone Bitartrate

Study no.:	NDSE-558-GLP ((b) (4) Study # 6770-151)
Study report location:	EDR 4.2.3.4.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 10, 2002
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Hydrocodone bitartrate, Lot B13205, 99.2% and Lot E01157, 98.6%
CAC concurrence:	Yes

Key Study Findings

- No hydrocodone-related neoplasms were observed in either sex in this study.
- Survival in males at the high dose was significantly lower than controls. All other groups were similar to control groups.

Adequacy of Carcinogenicity Study

The study was conducted with doses chosen based on a 13-week dose range-finding study. The SPA was presented to the ECAC and doses for the carcinogenicity study

were recommended. This study employed an appropriate mouse strain, an adequate number of animals per sex for each dose and concurrent control groups, and the clinically relevant route of administration. Animals were housed appropriately, provided appropriate food and care, and were administered test article or vehicle control for 104 weeks. The study is considered adequate.

Appropriateness of Test Models

The mouse is an appropriate model for a 2-year bioassay and the strain, Crl:CD(SD)IGS BR, is acceptable.

Evaluation of Tumor Findings

No HC-mediated increases in neoplastic lesions were observed in this study. Various neoplasms or pre-neoplastic lesions were observed in the treated groups with incidences similar to controls and/or similar to levels observed in the historical controls. None of these tumors were considered to be treatment-related. Statistical analysis of the data was conducted by Dr. Min Min of the Division of Biostatistics VI. Tumors were combined for statistical analysis according to McConnell, et al, 1986 (McConnell EE, et al., 1986). According to Dr. Min's review, no tumor types were considered to have a statistically significant positive dose-response relationship or a statistically significant pair-wise comparison for either males or females.

Methods

Doses:	M: 20, 60, 200 mg/kg; F: 10, 30, 100 mg/kg
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Distilled water
Basis of dose selection:	The ECAC recommended the high dose based on the determination of an MTD (mortality) in a 13-week study in the mouse (see ECAC meeting minutes dated June 14, 2002).
Species/Strain:	Mouse, Crl:CD-1 (ICR) BR
Number/Sex/Group:	See table below
Age:	6 weeks
Animal housing:	Individually housed in suspended cages
Paradigm for dietary restriction:	No
Dual control employed:	Yes
Interim sacrifice:	No
Satellite groups:	Yes, TK groups, see table below
Deviation from study protocol:	None that affected the integrity of the study.

Table 44. Study Design

Group	No. of Animals		Dose Level (mg/kg/day)		Concentration (mg/mL)	
	Male	Female	Male	Female	Male	Female
Main Study						
1 (Control 1)	60	60	0	0	0	0
2 (Control 2)	70	70	0	0	0	0
3 (Low)	70	70	20	10	2	1
4 (Mid)	70	70	60	30	6	3
5 (High)	70	70	200	100	20	10
TK Study[a]						
6 (Control)	12	12	0	0	0	0
7 (Low)	36	36	20	10	2	1
8 (Mid)	36	36	60	30	6	3
9 (High)	36	36	200	100	20	10
10 (Sentinel)[b]	25	25	0	0	0	0

a Toxicokinetic animals in Groups 7-9 were bled on Day 1 and during Week 26 at the following time points: predose, 0.5, 1, 2, 6, and 24 hours postdose. Toxicokinetic mice in Group 6 were bled on Day 1 and Week 26 at 0.5 hour postdose.

b Five (5) mice/sex were bled for viral screening purposes prestudy, and at Weeks 26, 52, 78, and 104, except for during Week 78 when 4 males and 3 females were bled and Week 104 when two/sex were bled.

Observations and Results

Mortality

Mice were checked twice daily for moribundity and mortality. Survival at the termination of the study is detailed in the table below. Males at the high dose had decreased survival as compared to controls. Due to high mortality in the male high dose group, as per advice from the Division (Tcon July 9, 2004), dosing was suspended during Week 94 for the remainder of the study. Surviving animals in this group were sacrificed at the conclusion of the study.

Table 45. Survival at the Conclusion of the Study

Group	***Male Surviving/total***	***Female Surviving/total***
Control 1	26/60 (43%)	23/60 (38%)
Control 2	27/70 (39%)	28/70 (40%)
Low dose	30/70 (43%)	24/70 (34%)
Mid dose	30/70 (43%)	28/70 (40%)
High dose	15/70 (21%)	27/70 (39%)

Figure 11. Survival in Males

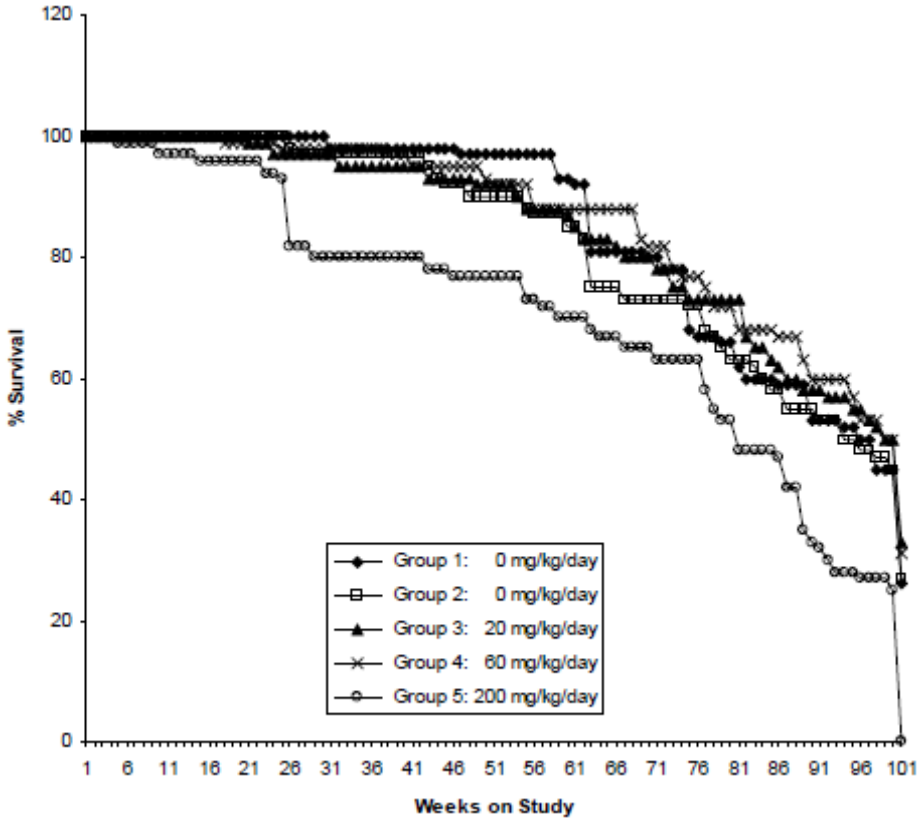
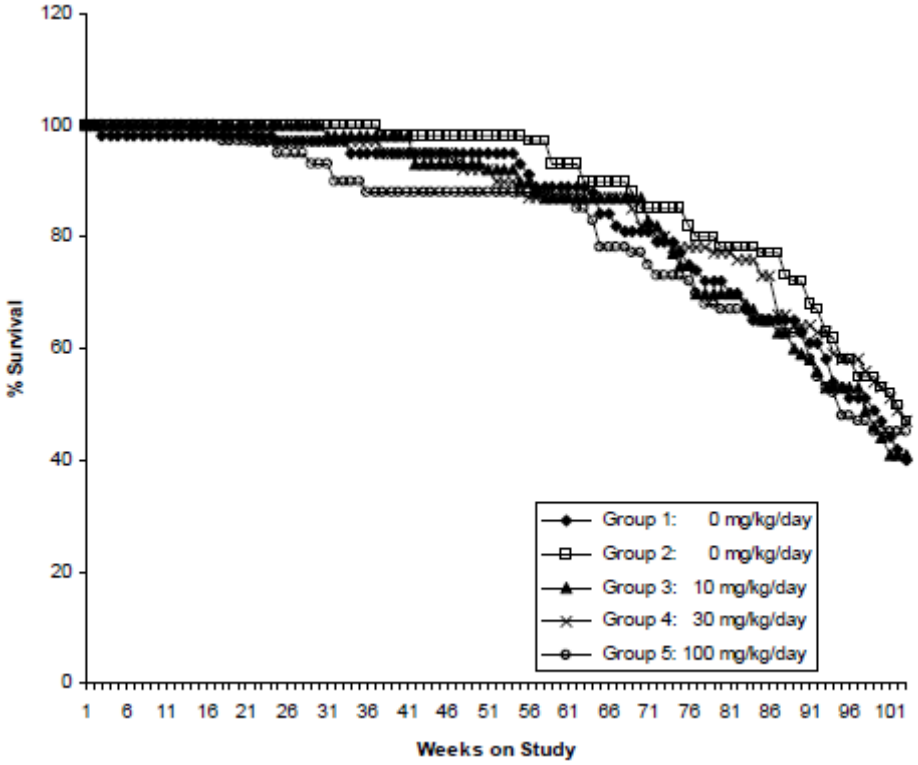


Figure 12. Survival in Females



Clinical Signs

Detailed clinical observations were conducted once weekly. Beginning at Week 52, all main study mice were examined weekly for the presence of palpable masses. Several clinical signs were noted in the treated males including rough hair coat, swollen perineal area, sore/scab in perineal area and yellow hair coat in perineal area. In males and females, hunched posture was observed at higher incidences in the treated groups. No treatment-related palpable masses were observed.

Food Consumption

Food consumption was recorded one week prior to treatment, weekly through Week 14 and every two weeks thereafter. For the first half of the study, male and female treated groups consistently showed lower food consumption than controls. Later in the study the differences between treated groups and controls were reduced. At no point in the study were the reductions in food consumption considered aversive.

Body Weights

Body weights were recorded prior to treatment, weekly through Week 14 and every two weeks thereafter. All rats were weighed immediately prior to termination. All treated groups had net gains in body weight, but at terminal sacrifice, both male and female body weights were dose-dependently lower than controls. Body weights are presented

in the table and figures below. Mean body weights of the two control groups were similar for both males and females and were combined.

Table 46. Body Weights at the Conclusion of the Study

	Group, mg/kg	***Body weight difference: Treated - control at last time point, g***	***Percent change from control at last time point, %***
Male	*20*	-4	-9
	60	-6	-14
	200	-7	-18
Female	*10*	-2	-6
	30	-4	-13
	100	-6	-16

Figure 13. Body Weights in Males

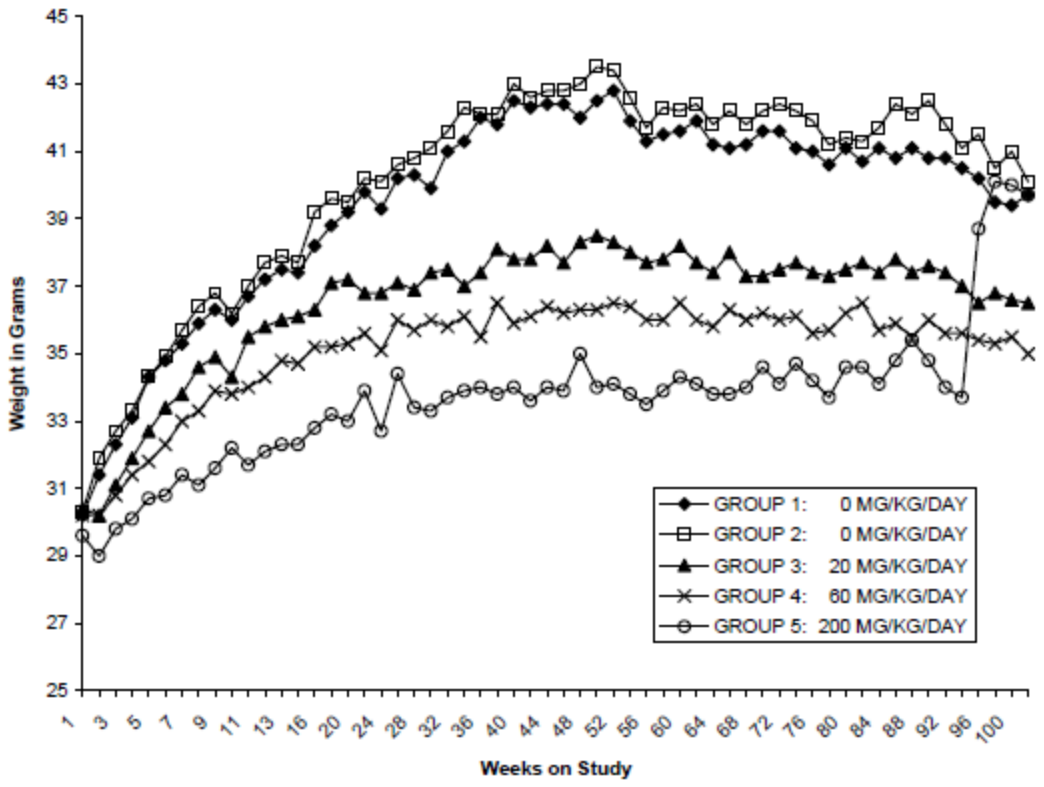
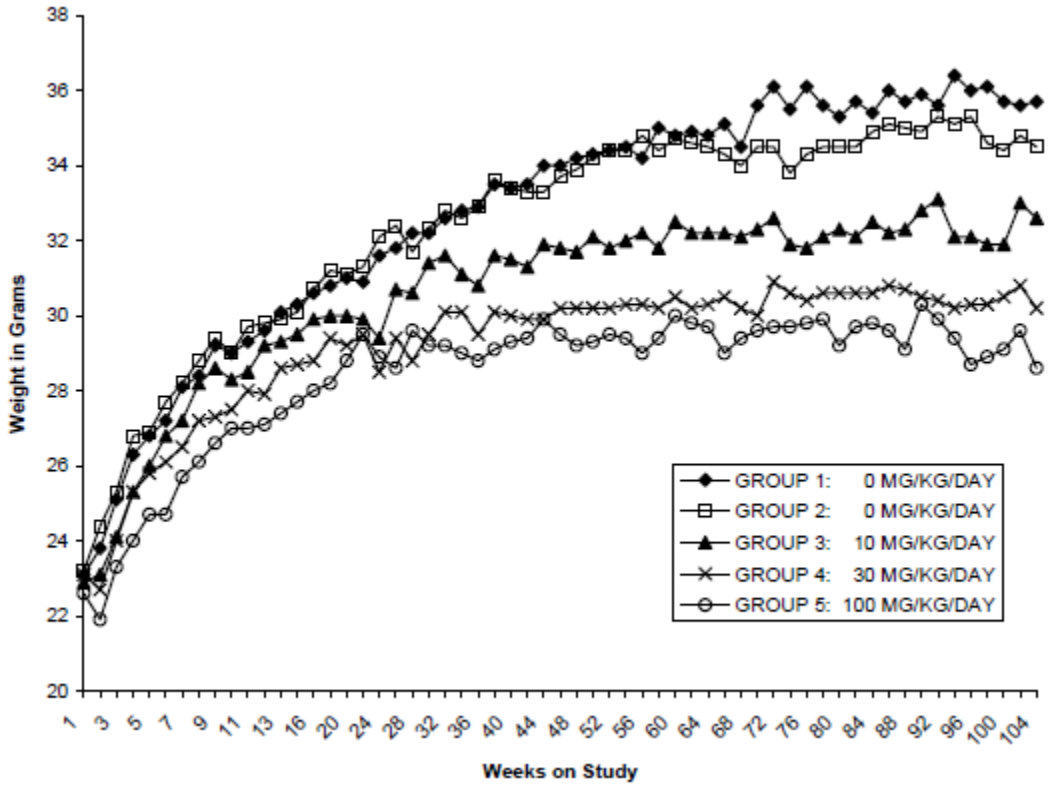


Figure 14. Body Weights in Females



Ophthalmoscopy

Ophthalmic observations were conducted on all animals prior to treatment and on main study animals during Week 26 and prior to terminal euthanasia. No treatment-related ophthalmic findings were observed.

Hematology

Samples for hematology were collected during Weeks 26 and 105. The parameters in the table below were measured.

Erythrocyte cell count
Leukocyte cell count
Differential blood cell count
Blood cell morphology

No test article-related hematology changes were observed.

Clinical Chemistry

Samples for clinical chemistry were collected during Weeks 26 and 105. The parameters in the table below were measured.

Alkaline Phosphatase	Globulin
Total Bilirubin	Albumin/Globulin Ratio
Aspartate Aminotransferase	Cholesterol
Alanine Aminotransferase	Triglycerides
Glucose	Sodium
Urea Nitrogen	Potassium
Creatinine	Chloride
Total Protein	Calcium
Albumin	Phosphorus

No test article-related changes in clinical chemistry parameters were observed

Gross Pathology

An increased incidence of distended urinary bladders were observed at necropsy of the unscheduled deaths in high dose males as compared to the unscheduled deaths in control males (C1: 10/34; C2: 12/33; HD: 22/45). This finding is attributed to the known pharmacologic activity of HC of inhibition of the voiding reflex. No histopathologic findings suggesting obstruction were observed. The distended bladder appeared to contribute to the increased mortality observed in high dose males. No other treatment-related gross pathology findings were observed.

Histopathology

Peer Review: Yes

Histopathology Inventory			
Study Number	NDSE-558-GLP ((b) (4) Study # 6770-151)		
Species	Mouse		
Organ	***assessed***	***Organ***	***assessed***
Adrenal	X	Nasal turbinates with paranasal sinus	X
Aorta	X	Nasopharynx	X
Brain	X	Ovary with oviduct	X
Cecum	X	Pancreas	X
Cervix	X	Preputial gland	X
Clitoral gland	X	Pituitary	X
Colon	X	Prostate	X
Duodenum	X	Rectum	X
Epididymis	X	Salivary gland	X
Eye	X	Seminal vesicles	X
Esophagus	X	Skeletal muscle (thigh)	X
Femur	X	Skin	X
Gallbladder	X	Spinal cord	X
Gross lesions	X	Spleen	X
Harderian gland	X	Sternum	X
Heart	X	Stomach	X
Ileum	X	Testes	X
Jejunum	X	Thymus	X
Kidney	X	Thyroid with parathyroid	X
Lacrimal gland	X	Tongue	X
Larynx	X	Trachea	X
Liver	X	Urinary bladder	X
Lung	X	Ureters	X
Lymph nodes, mesenteric	X	Vagina	X
Mammary gland	X	Voluntary muscle	X
Nerve, sciatic	X	Zymbal gland	X

Neoplastic

No HC-mediated increases in neoplastic lesions were observed in this study. The most commonly observed neoplasms were hematopoietic neoplasms, pulmonary neoplasms (alveolar adenomas and carcinomas), hepatocellular neoplasms, and hemangiosarcomas. All lesions were observed in the treated groups with incidences similar to controls and/or similar to levels observed in the historical controls. None of these tumors were considered to be treatment-related.

Non Neoplastic
Necrotizing dermatitis was observed at a higher incidence in treated males and females. Clinical signs in this study also included sore/scab in perineal area. Treatment-related increases of skin-related neoplasms including squamous cell carcinoma and basal cell adenoma were not observed. No other treatment-related non neoplastic findings were observed.

Toxicokinetics

Six mice/sex/group and three mice/sex/group were bled for control and treated toxicokinetics, respectively. Toxicokinetic parameters of HC are detailed in the table below.

Exposure to HC was similar in male and female mice at all doses. Hydrocodone showed a T_{max} between 0.5-1 h for both males and females. The $t_{1/2}$ values in this study ranged from 1.1 to 2.7 and were similar between males and females. Generally, mean C_{max} and AUC_{0-24} values were higher at Weeks 26 as compared to Day 1, indicating an accumulation of HC after multiple dosing. The increases in C_{max} and AUC_{0-24} values with dose for males and females were roughly dose proportional during Week 26 but greater than dose proportional on Day 1.

Systemic levels of a 120 mg human dose at steady state (Study # HYD1002) are compared to the systemic exposure in mice for this study (Table 48). In the clinical Study # HYD1002, 120 mg HC was dosed q24h for 5 days. Steady state was reached by Day 5 and AUC_{tau}= 1938 +/-729. In the clinical Study # HYD1002, the dosing interval was 24h, therefore the AUC_{0-24} animal data can be compared to the AUC_{tau} human data (Table 13). The high doses in male and female mice provide 3.5 and 3.1 fold exposure margins, respectively, above the clinical dose of 120 mg/day.

Table 47. Toxicokinetic Parameters of Hydrocodone in Mouse Plasma

Interval	Dose Group	Hydrocodone Bitartrate Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	DN C_{max} (ng/mL)/(mg/kg/day)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} (ng•hr/mL)/(mg/kg/day)	$AUC_{0-\infty}$ (ng•hr/mL)	$t_{1/2}$ (hr)	AUC_{0-24} AR
Day 1	7	20	M	125	6.24	0.500	286	370	18.5	305	1.45	NA
		10	F	57.8	5.78	1.00	82.9	113	11.3	NC	NC	NA
	8	60	M	352	5.87	0.500	914	1199	20.0	1001	1.89	NA
		30	F	241	8.05	0.500	370	441	14.7	386	1.38	NA
	9	200	M	2246	11.2	0.500	11684	11684	58.4	11695	2.73	NA
		100	F	1608	16.1	0.500	3407	3407	34.1	3408	2.30	NA
Week 26	7	20	M	323	16.1	0.500	590	667	33.4	NA	1.06	1.80
		10	F	250	25.0	0.500	364	433	43.3	NA	1.30	3.82
	8	60	M	918	15.3	0.500	2959	2959	49.3	NA	2.06	2.47
		30	F	891	29.7	0.500	1347	1530	51.0	NA	1.20	3.47
	9	200	M	1553	7.77	1.00	6795	6795	34.0	NA	2.19	0.582
		100	F	1926	19.3	0.500	6001	6001	60.0	NA	1.95	1.76

Table 48. Exposure Comparisons Between Mouse and Human

	Dose, mg/kg	*Mouse/human AUC_{0-24}, (ng.h/mL)*
Male	***20***	0.3
	60	1.5
	200	3.5
Female	***10***	0.2
	30	0.7
	100	3.1

*120 mg/day at steady state; AUC_{tau}= 1938 ng.h/mL, Study # HYD1002

Dosing Solution Analysis

The homogeneity of samples and the sample concentrations were both within an acceptable range of the respective target concentrations for HC.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

The reproductive and developmental toxicology studies were reviewed by Dr. Huiqing Hao.

Study title: An Oral Gavage Fertility Study of Hydrocodone Bitartrate in the Rat

Study no.:	HYD-N-011 (901670)
Study report location:	EDR 4.2.3.5.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	11/24/2008
GLP compliance:	Yes, a signed GLP compliance statement was included in the report
QA statement:	Yes
Drug, lot #, and % purity:	Hydrocodone bitartrate, Lot 07DW257-4, 100%

Key Study Findings

Treatment related effects in rats given HC bitartrate orally at doses of 3, 12.5, and 25 mg/kg were decreased body weight gain and reduced food consumption, most significantly at the early days of treatment. Male reproductive system (sperm motility, spermatozoa count, sperm morphology, and testicular histopathology) was not affected. Organ weight increase in the prostate (18%) and left seminal vesicle (20%) were observed without associated histopathology changes. Fertility parameters (day of mating, mating and fertility indices, and conception rates) were not affected. There were no treatment-related increases in pre- and post-implantation loss. The total number of corpora lutea, implantation sites and the number of live embryos were decreased at the MD and HD (9-14%, more in MD than HD). However, these changes were reported to be within normal range.

In conclusion, treatment with HC orally at doses up to 25 mg/kg did not induce significant findings in rat fertility in males or females. The dose of 25 mg/kg ($AUC_{0\text{-last}}$ of 118 ng.h/mL in males and 149 ng.h/mL in females) was considered to be the NOAEL in this study.

Methods

Doses:	0, 3, 12.5, and 25 mg/kg
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral
Formulation/Vehicle:	Deionized water
Species/Strain:	Rat, Sprague-Dawley CD (Crl:CD[SD])
Number/Sex/Group:	22/sex
Satellite groups:	9/sex for TK study
Study design:	Males were dosed for 28 days prior to placement for mating, during mating and until necropsy; Females were dosed for 14 days prior to mating, during mating and up to Day 7 of gestation inclusively and sacrificed at Day 14 of gestation.
Deviation from study protocol:	No significant deviation from protocol that affects result interpretation

Observations and Results

Mortality

There was no treatment-related mortality.

Clinical Signs

Treatment-related behavior changes were observed primarily during the pre-mating treatment period, including but not limited to the following: excessive licking and grooming, self-biting of paws, chewing action, limbs and cage, excessive scratching and alternating periods of decreased and increased activity. At 25 mg/kg, abnormal gait and

head shaking were also noted in a few occasions. All of these signs were seen within 2 ± 0.5 hours of treatment and were no longer apparently by the end of each treatment day.

Body Weight

For males, decreased body weight or body weight losses were dose-related and most significantly during the first week of treatment. Over time, the effect became less significant. Nevertheless, at the end of treatment of 55 days, dose-related lower body gain was still apparent (% body weight gain from Day 0 was 34%, 30%, 19%, and 14% for control, LD, MD, and HD, respectively).

For females, dose-related lower body weight gain was observed in the premating period of 14 days (% body weight gain from Day 0 was 11%, 10%, 7%, and 5% for control, LD, MD, and HD, respectively). Lower body weight gain was also seen in pregnant females when treatment was remained (for the control, LD, MD, and HD groups, body weight gain was 13%, 13%, 9%, and 10% during the first week of gestation with maintained treatment and 24%, 27%, 26%, and 25% during Days 0 to 14 of gestation).

Food Consumption

Consistent with the body weight changes, decreased food consumption in a dose dependent manner was noted throughout the pre-mating treatment period (Study Days 0-27 overall 18% less than control for HD males, Study Days 0-14 overall 13% less than control for female MD and HD), with the most significant decreases appearing between Study Days 0 and 7. During gestation, overall food consumption was 10% less than control in Gestation Days 0-10 for the MD and HD, but higher than control in Gestation Days 10-13 when animals were no longer given the drug for all treatment groups (LD, 6.5%; MD, 8.6%; HD, 8.6%).

Toxicokinetics

Blood concentrations of hydrocodone (HC) and norHC (NHD, metabolite) rapidly declined at the LD, but relatively slowly declined at MD and HD. Therefore, systemic exposures (AUC) increased with increasing doses in a more than dose proportional manner. Systemic exposures to hydromorphone (HM, metabolite) and hydromorphone 3β-glucuronide (HM3G, metabolite) were in a dose proportional and less than dose proportional manner, respectively. There was a general trend of decrease in metabolite-parent ratios with increase in dose level, suggesting a possibility of saturation in biotransformation of HC to HM, HM3G, and NHC with increase in dose. The exposure data are presented below.

Note, notes for the parameters reported were provided as the following. The same was provided for other studies reviewed in this document.

T_{max}	Time of maximum observed concentration	T or t = time
T_{last}	Time of last observed concentration	T or t = time
C_{max}	Maximum observed concentration	C or c = concentration
C_{max}/Dose	The C_{max} divided by the dose administered.	
$AUC_{(0\text{-}tlast)}$	Area Under the Curve from the dosing time to the final observation	$AUC\Big\|_{t_1}^{t_2} = (t_2 - t_1)\left(\frac{C_1 + C_2}{2}\right)$
$AUC_{(0\text{-}tlast)}$/Dose	The AUC(0-tlast) divided by the dose administered.	
K_{el}	Terminal elimination phase rate constant	-1 (slope of ln specified conc.)
R^2	Coefficient of determination for the terminal elimination phase regression model[1]	$\frac{s_c^{\,2} - s_{error}^{\,2}}{s_c^{\,2}}$, where s^2 = variance
T ½	Terminal half-life	$\frac{\ln 2}{K_{el}}$
$AUC_{(0\text{-}\infty)}$	Area Under the Curve from the dosing time extrapolated to infinity	$AUC_{(0\text{-}tlast)} + \frac{C_n}{K_{el}}$
$AUC\%_{(tlast\text{-}\infty)}$	Percentage of $AUC_{(0\text{-}\infty)}$ extrapolated from the final observation to infinity	$\frac{\frac{C_n}{K_{el}}}{AUC_{(0-\infty)}} * 100$

[1] $C = \beta \cdot T + \alpha + \varepsilon$

Where β = slope, α = intercept, and ε = error

Table 49. Hydrocodone Exposure in Rat Fertility Study

Day 7 of Gestation (Females)

Group No.	Dose Level (mg/kg/day)	C_{max} (ng/mL)	$AUC_{(0\text{-}tlast)}$ (ng•h/mL)	$AUC_{(0\text{-}\infty)}$ (ng•h/mL)	AUC% (tlast-∞)	C_{max}/ Dose	$AUC_{(0\text{-}tlast)}$/ Dose
2	3	6.47	9.80	10.9	10.2	2.16	3.27
3	12.5	30.3	41.5	42.1	1.45	2.42	3.32
4	25	46.1	145	149	2.22	1.84	5.81

Day 14 (Males)

Group No.	Dose Level (mg/kg/day)	C_{max} (ng/mL)	$AUC_{(0\text{-}tlast)}$ (ng•h/mL)	$AUC_{(0\text{-}\infty)}$ (ng•h/mL)	AUC% (tlast-∞)	C_{max}/ Dose	$AUC_{(0\text{-}tlast)}$/ Dose
2	3	3.25	4.09	5.08	19.4	1.08	1.36
3	12.5	17.4	38.2	43.1	11.4	1.39	3.05
4	25	30.6	118	118	0.186	1.22	4.72

Table 50. Norhydrocodone Exposure in Rat Fertility Study

Day 7 of Gestation (Females)

Group No.	Dose Level (mg/kg/day)	C_{max} (ng/mL)	$AUC_{(0\text{-}tlast)}$ (ng•h/mL)	$AUC_{(0\text{-}\infty)}$ (ng•h/mL)	AUC% (tlast-∞)	C_{max}/ Dose	$AUC_{(0\text{-}tlast)}$/ Dose
2	3	7.61	10.8	a	12.9	2.54	3.60
3	12.5	20.9	46.9	47.5	1.45	1.67	3.75
4	25	40.2	150	155	3.10	1.61	6.00

Day 14 (Males)

Group No.	Dose Level (mg/kg/day)	C_{max} (ng/mL)	$AUC_{(0\text{-}tlast)}$ (ng•h/mL)	$AUC_{(0\text{-}\infty)}$ (ng•h/mL)	AUC% (tlast-∞)	C_{max}/ Dose	$AUC_{(0\text{-}tlast)}$/ Dose
2	3	10.5	22.3	23.0	2.96	3.49	7.43
3	12.5	36.8	153	153	0.103	2.95	12.2
4	25	83.1	346	347	0.295	3.32	13.9

a Values are not reported because the $AUC_{(0\text{-}\infty)}$ was extrapolated by more than 20% or R^2 was <0.800.

Table 51. Hydromorphone Exposure in Rat Fertility Study

Day 7 of Gestation (Females)

Group No.	Dose Level (mg/kg/day)	C_{max} (ng/mL)	$AUC_{(0\text{-}tlast)}$ (ng•h/mL)	$AUC_{(0\text{-}\infty)}$ (ng•h/mL)	AUC% (tlast-∞)	C_{max}/ Dose	$AUC_{(0\text{-}tlast)}$/ Dose
2	3	5.06	21.0	21.2	1.02	1.69	6.99
3	12.5	17.8	102	105	2.91	1.43	8.18
4	25	47.9	187	213	12.3	1.92	7.47

Day 14 (Males)

Group No.	Dose Level (mg/kg/day)	C_{max} (ng/mL)	$AUC_{(0\text{-}tlast)}$ (ng•h/mL)	$AUC_{(0\text{-}\infty)}$ (ng•h/mL)	AUC% (tlast-∞)	C_{max}/ Dose	$AUC_{(0\text{-}tlast)}$/ Dose
2	3	4.01	15.6	16.9	7.71	1.34	5.18
3	12.5	14.0	76.4	84.3	9.44	1.12	6.11
4	25	35.0	163	186	12.2	1.40	6.52

Table 52. Hydromorphone-3β–Glucuronide Exposure in Rat Fertility Study

Day 7 of Gestation (Females)

Group No.	Dose Level (mg/kg/day)	C_{max} (ng/mL)	$AUC_{(0\text{-}tlast)}$ (ng•h/mL)	$AUC_{(0\text{-}\infty)}$ (ng•h/mL)	AUC% (tlast-∞)	C_{max}/ Dose	$AUC_{(0\text{-}tlast)}$/ Dose
2	3	134	809	842	3.92	44.5	270
3	12.5	387	2291	2451	6.51	30.9	183
4	25	811	5598	a	25.6	32.4	224

Day 14 (Males)

Group No.	Dose Level (mg/kg/day)	C_{max} (ng/mL)	$AUC_{(0\text{-}tlast)}$ (ng•h/mL)	$AUC_{(0\text{-}\infty)}$ (ng•h/mL)	AUC% (tlast-∞)	C_{max}/ Dose	$AUC_{(0\text{-}tlast)}$/ Dose
2	3	94.8	598	741	19.3	31.6	199
3	12.5	245	2035	a	20.8	19.6	163
4	25	425	3302	a	40.3	17.0	132

a Values are not reported because the $AUC_{(0\text{-}\infty)}$ was extrapolated by more than 20% or R^2 was <0.800.

Dosing Solution Analysis

Study sample analyzed were within the acceptance criteria of ±10% of their nominal concentrations except for Week 7, Group 2 and 3 (mean results of 130 and 129%, respectively). The high results were determined to be due to an error in the preparation of the formulation. Dosing solutions were therefore re-formulated and analyzed, and results (mean results: 97.3, 90.7% for Group 2 and 3, respectively) were within acceptance criteria.

Necropsy

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.) were not affected by the treatment.

Higher lengths of estrous cycle in two females with prolonged periods of diestrus were observed and were interpreted as a result of biological variation.

The mean day of mating, mating and fertility indices and the conception rates were unaffected by treatment.

The total number of corpora lutea, implantation sites, and the number of live embryos were decreased at the MD and HD (see table below). The decreases were in a range of 9-14% and slightly more severe in the MD than the HD. The Applicant reported that these levels of changes are within normal range although no normal range data were provided. There were no differences noted for the numbers of dead embryos, early resorptions, or the pre/post-implantation loss compared to controls (see table below).

Table 53. Group Mean Uterine Findings

Group 1 - Vehicle Control
Group 2 - Hydrocodone 3 mg/kg/day
Group 3 - Hydrocodone 12.5 mg/kg/day
Group 4 - Hydrocodone 25 mg/kg/day

Group	Summary Information	Total Number of Corpora Lutea	Total Number of Implantation Sites	Number of Live Embryos	Number of Dead Embryos
1	Mean	19.3	18.0	16.8	0.0
	SD	2.0	2.1	2.9	0.0
	N	21	21	21	21
2	Mean	19.2	17.1	16.4	0.0
	SD	1.7	2.3	2.0	0.0
	N	21	21	21	21
3	Mean	17.1 b	15.5 c	14.6 b	0.0
	SD	2.3	2.2	2.7	0.0
	N	21	21	21	21
4	Mean	17.3 c	16.4 c	15.3 b	0.1
	SD	1.2	1.1	1.4	0.3
	N	22	22	22	22

Significantly different from control group (group 1) value: a - P ≤ 0.05 b - P ≤ 0.01 c - P ≤ 0.001 (Wilcoxon)

Table 54. Group Mean Uterine Finding, Continued

Group 1 - Vehicle Control
Group 2 - Hydrocodone 3 mg/kg/day
Group 3 - Hydrocodone 12.5 mg/kg/day
Group 4 - Hydrocodone 25 mg/kg/day

Group	Summary Information	Number of Early Resorptions	Sum of Early Resorptions and Dead Embryos	Preimplantation Loss %	Post Implantation Loss %
1	Mean	1.2	1.2	6.86	6.95
	SD	1.9	1.9	6.85	11.12
	N	21	21	21	21
2	Mean	0.8	0.8	10.93	4.09
	SD	1.2	1.2	8.94	6.23
	N	21	21	21	21
3	Mean	0.9	0.9	9.50	6.14
	SD	1.0	1.0	8.39	6.75
	N	21	21	21	21
4	Mean	1.0	1.0	5.35	6.40
	SD	0.9	1.0	5.19	5.74
	N	22	22	22	22

Significantly different from control group (group 1) value: a - P ≤ 0.05 b - P ≤ 0.01 c - P ≤ 0.001 (Wilcoxon)

Gross pathology: No remarkable findings

Organ weight: Decreased prostate (up to 18%) and left seminal vesicle weight (up to 20%), and increased testicular weight (right side up to 3.8%) were observed (see table

below) without associated histopathological findings. Toxicological significance of these findings is not clear.

Table 55. Selected Organ Weights (g)

Organ	Dose (mg/kg)			
	0	3	12.5	25
Prostate	1.48	1.25	1.22	1.21
Seminal vesicle, left	0.75	0.62	0.67	0.60
Seminal vesicle, right	0.65	0.65	0.64	0.61
Testicle, left	1.81	1.83	1.83	1.83
Testicle, right	1.81	1.83	1.85	1.88

Male reproductive assessment: Sperm motility, spermatozoa count, and sperm morphology, as well as testicular histopathology were not affected by the treatment.

9.2 Embryonic Fetal Development (Rat)

The following study was reviewed by Dr. Huiqing Hao.

Study title: An Oral Teratology Study of Hydrocodone Bitartrate in the Rat

Study no.:	HYD-N-008 (901671)
Study report location:	EDR 4 2 3 5 3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	10/27/2008
GLP compliance:	Yes, a signed GLP compliance statement was included in the report
QA statement:	Yes
Drug, lot #, and % purity:	Hydrocodone bitartrate, Lot 07DW257-4, 100.6%

Key Study Findings

Administration of HC bitartrate from Days 6 to 17 of gestation at 3, 10, and 30 mg/kg in the rat resulted in maternal toxicity as evidenced by reduced food consumption and lower body weight gain or body weight loss. Fetal toxicity was evidenced by lower fetal weights and some delays in ossification at 30 mg/kg. No embryolethality or teratogenicity was observed at any dose. The NOAEL for maternal toxicity was 3 mg/kg ($AUC_{0\text{-last}}$ of 11.1 ng.h/mL) and the NOAEL for embryo-fetal development was 10 mg/kg ($AUC_{0\text{-last}}$ of 28.3 ng.h/mL) based on a decrease in fetal weights and delays in ossification at the HD.

Methods

Doses:	0, 3, 10, 30 mg/kg
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Deionized water
Species/Strain:	Rat, Sprague Dawley CD
Number/Sex/Group:	22/dose
Satellite groups:	12/dose (2 for control) for TK study
Study design:	Gravid rats were given the test article orally on Days 6-17 of gestation and sacrificed on Day 21.
Deviation from study protocol:	No deviations impacting the outcome of the study occurred

Observations and Results

F_0 Generation

Mortality

One TK animal was euthanized in extremis on Gestation Day 11 due to a deep skin laceration in the neck region as a result of self-biting. No other mortality occurred in this study.

Clinical Signs

Excessive licking and grooming; self-biting of paws, limbs or cage; excessive scratching or alternating periods of decreased and increased activity were observed, within 2 ± 0.5 hours of treatment. Upon cessation of treatment, after Gestation Day 17, the condition of the animals returned to normal.

Body Weight

A dose-related reduction in body weight gain was seen at the MD and HD over the entire treatment period. The overall mean body weight gain (Gestation Days 6-18) at the MD and HD were 24% and 49% lower than control animals, respectively.

Food Consumption

A dose dependent decrease in food consumption was noted at the MD and HD treatment groups from first day of dosing to sacrifice (last 4 days were free of treatment) with the worst decrease at 24% less than control.

Toxicokinetics

The maximum concentration of HC and its metabolites were generally observed between 0.5-2 hours post dose. The elimination from the systemic circulation was rapid

for the parent compound (Day 17, $t_{1/2}$ of 1.29-2.51 hours) and the metabolite norHC (Day 17, $t_{1/2}$ of 1.32-2.31 hours) but slow for the metabolites hydromorphone (Day 17, $t_{1/2}$ of 5.38-5.88 hours) and hydromorphone 3β-glucuronide (Day 17, $t_{1/2}$ of 5.24-8.58 hours).

Systemic exposure to the parent compound was dose proportional in general, although the AUCs of hydromorphone and norhydromorphone at high dose appeared more than dose proportional compared to the MD.

Table 56. Toxicokinetic Parameters in the Rat Fertility Study

Dose (mg/kg)		3	10	30
	Hydrocodone bitartrate			
C_{max}, ng/mL	Day 6	3.45	8.38	16.9
	Day 17	5.69	12.2	44.3
AUC_{0-last} ($AUC_{0-\infty}$), ng.h/mL	Day 6	9.03 (10.3)	24.2 (24.9)	73.7 (77.3)
	Day 17	11.1 (11.6)	28.3 (30.2)	164 (164)
	Norhydrocodone			
C_{max}, ng/mL	Day 6	2.3	6.7	13.2
	Day 17	3.7	11.6	45.6
AUC_{0-last} ($AUC_{0-\infty}$), ng.h/mL	Day 6	6.17 (7.56)	23.2 (23.7)	75.4 (79.0)
	Day 17	6.98 (7.35)	28.4 (30.1)	213 (213)
	Hydromorphone			
C_{max}, ng/mL	Day 6	3.57	11.3	18.0
	Day 17	5.45	14.9	41.0
AUC_{0-last} ($AUC_{0-\infty}$), ng.h/mL	Day 6	23.0 (23.2)	70.3 (NO)	160 (NO)
	Day 17	9.51 (NO)	54.3 (56.9)	165 (172)
	Hydromorphone 3β-glucuronide			
C_{max}, ng/mL	Day 6	84.2	236	349
	Day 17	117	446	825
AUC_{0-last} ($AUC_{0-\infty}$), ng.h/mL	Day 6	494 (551)	1639 (NO)	3631 (NO)
	Day 17	555 (574)	1756 (2025)	4062 (4297)

NO=not obtained

Dosing Solution Analysis

Measured concentrations of HC bitartrate in the dose formulations deviated from nominal concentrations by a maximum of 3%. No test article was detected in the control sample analyzed.

Necropsy

There were no macroscopic findings considered to be related to HC bitartrate administration.

The pregnancy rate was 100% in each group.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

The number of corpora lutea, total number of implantation sites, the sex ratio, number of live and dead fetuses, resorptions (early, middle, late, total), and pre and post implantation loss were unaffected by treatment with HC bitartrate.

Offspring (Malformations, Variations, etc.)

Fetal weights (male, female and combined) were lower than control at the HD of 30 mg/kg. Fetal weights were unaffected at MD and LD.

There were no treatment-related findings of major malformations, external or visceral anomalies, or minor visceral anomalies.

The percentage of fetuses with thoracic centrum variants was increased at 30 mg/kg as compared to control (67.8% versus 42.94%). This finding likely represents a slight transitory delay in the rate of ossification that is related to the lower fetal weights at this dose. Incidence of sternebral variants were not affected by treatment.

	Affected Fetuses/Litters (Mean %)			
Dose (mg/kg)	**0**	**3**	**10**	**30**
Thoracic centrum variants (unossified/incomplete/semi-bipartate/bipartite)	42.94	27.52	44.05	67.82*

* p<0.01 (Wilcoxon)

9.2 Embryonic Fetal Development (Rabbit)

The following study was reviewed by Dr. Huiqing Hao.

Study title: An Oral Teratology Study of Hydrocodone Bitartrate in the Rabbit

Study no.:	HYD-N-009 (901672)
Study report location:	EDR 4.2.3.5.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	11/10/2008
GLP compliance:	Yes, a signed GLP compliance statement was included in the report
QA statement:	Yes
Drug, lot #, and % purity:	Hydrocodone bitartrate, Lot 07DW257-4, 100.6%

Key Study Findings

Treatment of female rabbits from Days 7 to 19 of gestation with HC bitartrate at 3, 10, and 30 mg/kg resulted in lower food intake and related clinical signs at 10 and 30 mg/kg and lower body weights in the 30 mg/kg dose level, which was considered a maternally toxic effect. The LOAEL for maternal toxicity was 10 mg/kg (reduced food consumption without effects on body weights). Fetotoxicity was evidenced by decreased fetal weights at 30 mg/kg. The lower fetal weights were likely due to maternal effects of lower food intake. There was no embryolethality or teratogenicity noted during the course of this study. The NOAEL for embryo-fetal development was 10 mg/kg ($AUC_{0\text{-last}}$ of 130 ng.h/mL) and was based on decreased fetal weights.

Methods

Doses:	0, 3, 10 and 30 mg/kg
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Deionized water
Species/Strain:	Rabbit, New Zealand White
Number/Sex/Group:	22 females
Satellite groups:	4/dose (2 for control) were used for TK study
Study design:	Pregnant rabbits were given test article on Days 7-19 of gestation, inclusive, and euthanized on Day 29 of gestation
Deviation from study protocol:	No significant deviations occurred impacting on the study results.

Observations and Results

Mortality

One high dose animal was sacrificed due to moribundity at Gestation Day 13. Macroscopic findings in the stomach and duodenum including dark discoloration, dark raised or depressed areas on the fundus, swelling of the mucosa in the pyloric region, thickening of the wall of the fundus and pyloric region and in the duodenum, and multiple dark areas noted on the mucosa. Pale and gelatinous material was also found in both the stomach and colon, suggesting mucoid enteropathy.

Clinical Signs

Reduced food intake at the MD and HD and related decreased activity and thin and backbone prominent were observed.

Body Weight

Lower body weight gains or body weight losses were noted from Days 7-16 of gestation at 30 mg/kg. Although these animals started to gain weight during the post dosing

period, the discrepancy persisted until the end of the study, the resultant terminal body weights of 30 mg/kg group were approximately 5% lower than control.

Food Consumption

During the treatment (Gestation Days 7-19), the MD and HD groups exhibited lower food intake (MD, -23%; HD, -49%). During the post dosing period, however, these groups were noted to be eating more than control from Gestation Day 23 until termination on Gestation Day 29.

Toxicokinetics

The absorption of hydrocodone (HC) and its conversion to the metabolites hydromorphone (HM), hydromorphone 3β-glucuronide (HM3G) and norhydromorphone (NHC) was rapid (C_{max} was ranged 0.5-2 hours for hydrocodone and its metabolites). The exposure of the gravid rabbits to the metabolites hydromorphone and norhydromorphone was lower than parent compound, while the exposure of HM3G was much higher.

Table 57. Toxicokinetics in the Embryofetal Development Rabbit Study

Dose (mg/kg)		**3**	**10**	**30**
	Hydrocodone bitartrate (HC)			
C_{max} (ng/mL)	Day 7	5.54	25.6	122
	Day 19	16.6	61.9	239
AUC_{0-last} ($AUC_{0-\infty}$) (ng.h/mL)	Day 7	9.3 (10.5)	57.2 (57.9)	294 (302)
	Day 19	23.4 (24.2)	130 (131)	606 (612)
	Norhydrocodone (NHC)			
C_{max} (ng/mL)	Day 7	1.69	5.49	18.2
	Day 19	2.98	14.0	40.7
AUC_{0-last} ($AUC_{0-\infty}$) (ng.h/mL)	Day 7	3.41 (3.82)	18.4 (20.3)	72.9 (77.0)
	Day 19	4.62 (5.22)	39.0 (39.7)	157 (158)
	Hydromorphone (HM)			
C_{max} (ng/mL)	Day 7	2.97	6.24	14.3
	Day 19	3.01	6.62	10.2
AUC_{0-last} ($AUC_{0-\infty}$) (ng.h/mL)	Day 7	9.08 (9.60)	29.2 (30.4)	75.8 (79.8)
	Day 19	7.32 (7.95)	25.0 (26.0)	61.0 (62.6)
	Hydromorphone 3β-glucuronide (HM3G)			
C_{max} (ng/mL)	Day 7	3322	6270	10531
	Day 19	3175	5901	9281
AUC_{0-last} ($AUC_{0-\infty}$) (ng.h/mL)	Day 7	12530 (12553)	38076 (38220)	93294 (97216)
	Day 19	9398 (9437)	27552 (27682)	69261 (69607)

Dosing Solution Analysis

The dosing solution concentrations were within 10% of their nominal concentrations. No test article was detected in the control samples.

Necropsy

There were no remarkable macroscopic findings in F_0 animals.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

The number of corpora lutea, total number of implantation sites, numbers of live and dead fetuses, resorptions, and pre- and post-implantation losses were unaffected by treatment with HC bitartrate. Although the sex ratio was noted to be slightly higher for males in the HD group at 57.51%, this value was within the historical range (37.4%-59.7%) and not considered to be of toxicologic significance. One animal at the LD group aborted on Day 25 of gestation, however, in the absence of any additional instances of abortion, this was thought to be a result of biological variation and not test article related.

Offspring (Malformations, Variations, etc.)

Fetal weights (male, female, and combined) were lower than control at 30 mg/kg. No similar changes were observed at lower doses (3 mg/kg and 10 mg/kg).

There were no treatment related findings as major malformations, external or visceral anomalies, or minor skeletal anomalies. There was no test article related effect on the 13th rib or sternebral variants.

9.3 Prenatal and Postnatal Development

The following study was reviewed by Dr. Huiqing Hao.

Study title: An Oral Pre and Postnatal Study of Hydrocodone Bitartrate in the Rat

Study no.:	HYD-N-001 (901673)
Study report location:	EDR 4 2 3 5 3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	8/30/2008
GLP compliance:	Yes, a signed GLP compliance statement was included in the report
QA statement:	Yes
Drug, lot #, and % purity:	Hydrocodone bitartrate, Lot 07DW257-4, 100.6%

Key Study Findings

Administration of HC bitartrate from Day 6 of gestation to Day 21, 22, or 23 post-partum, inclusive at 0, 3, 10, and 30 mg/kg in the rat, resulted in maternal toxicity (F_0 generation) at 10 and 30 mg/kg, evidenced by lower body weight and food consumption.

For the offspring of dams given 30 mg/kg, lower viability and reduced pre-weaning body weights were noted. The lower body weights continued for males and females throughout the post-weaning period. Effects of lower body weights were associated with transitory retardation of hair growth and to a slight retardation of preputial separation. There were no effects on behavior or reproductive performance.

The NOAEL is 3 mg/kg ($AUC_{0\text{-last}}$ of 16.3 ng.h/mL) for the F_0 generation and 10 mg/kg ($AUC_{0\text{-last}}$ of 54.4 ng.h/mL) for the F_1 generation.

Methods

Doses:	0, 3, 10, 30 mg/kg
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Deionized water
Species/Strain:	Rat, Sprague Dawley CD
Number/Sex/Group:	24/dose
Satellite groups:	13/dose (5 for control) for TK study
Study design:	Gravid rats were given the test article orally on Days 6 of gestation to Day 21, 22, or 23 post-partum, inclusive (depending on their day of necropsy).
Deviation from study protocol:	No deviations impacting the outcome of the study occurred.

Observations and Results
F_0 Dams

Survival:

During the gestation phase, one pregnant female at HD was sacrificed moribund on Gestation Day 22. Necropsy examination revealed pale foci on the liver and pale discoloration of the pancreas and small thymus, but the cause of the deteriorating condition was not determined. During lactation phase, additional four females at HD (Nos. 4501, 4506, 4515, and 4521) were sacrificed due to complete loss (death) of their litter, on Days of 0-7 post-partum. The cause of litter loss was related to scabs on the nipples and failed lactation in Animal No. 4506. The causes of other litter loss were unidentified although it was noted that one dead fetus was found in the uterine horn of Animal No. 4501, and the carcass of Animal No. 4515 was pale and had an enlarged spleen.

In the TK subset, premature mortality occurred in two HD females (one was found dead on Gestation Day 18 and one was sacrificed on Day 11 post-partum due to litter loss), and one MD female (on Day 0 post-partum due to complete loss of this litter). The

animal found dead was associated with Elizabethan collar related neck swelling and the two animals that had complete loss of litters were associated with finding of scabs on their nipples (possible results from self-mutilation) and inability to feed their pups. The Elizabethian collars were placed on animals in the HD group when signs of self-mutilation were observed.

Clinical signs:

Mainly within 2 ± 0.5 hours of treatment, animals at 10 and 30 mg/kg showed the following: pale eyes, self-biting on paws and tail, excessive grooming and excessive licking of the cage, fur loss, signs of thinness, weakness, and alternating periods of decreased activity and increased respiratory rate.

Body weight:

Decreased body weight gains were noted at 10 and 30 mg/kg throughout the gestation period and until Day 4 post-partum (after Day 4 post-partum, body weight gain was similar or higher than control). Of note, body weight loss was observed in all groups during Days 17-21 post-partum. The cause of such a change was not provided in the report.

Table 58. Dam Body Weight (g)

	control	LD	MD	HD
GD 0	227.6	229.7	226.3	229.6
GD 20	404.2	403.0	378.3	349.5
LD 0	322.1	317.3	298.5	281.7
LD 4	335.9	330.4	300.6	280.2
LD17	368.2	360.2	343.7	323.6
LD 21	347.5	345.2	341.9	321.7

GD: Gestation Day; LD: Lactation Day

Food consumption

Food consumption was measured during Gestation Days 6 to 18. The MD and HD groups showed reduced food consumption (up to 27%), which was more significantly in earlier days of treatment.

Uterine content

Pregnancy rate was at least 95.8% in each group. The gestation index (percentage of pregnant rats with live litters), number of malformed pups, number of implantation scars and sex ratio were unaffected by the treatment.

Necropsy observation

Skin scabs (abdominal, digit or tail regions) were seen in four dams at the HD.

Toxicokinetics

Plasma concentrations of test article and its metabolites were measured on Day 10 post-partum. Hydrocodone was rapidly absorbed (T_{max} of 0.5-1 hour) following the oral dosing. Hydrocodone, NHC, and HM displayed similar TK profiles, characterized by $t_{1/2}$ of 0.74-1.92 hour, and dose proportional exposures. The exposure of NHC was equivalent to that of parent compound, and exposure of HM was 50-70% of that of parent compound. The metabolite HM3G presented dissimilar TK profiles, with longer $t_{1/2}$ (3.3-6.97 hours), higher exposure than that of parent compound (5-42 fold of parent exposure), but in a less than dose proportional manner with dose increase.

Plasma concentrations of the test article HC and the metabolites NHC and HM were below the LLOQ in most samples collected from pups. HM3G was the only metabolite quantified in pups originating from HC treated dams, and plasma concentrations of HM3G were one to two orders of magnitude higher in dams than in pups. There were no sex-related differences in HM3G plasma concentrations among pups.

Measurement of HC in milk and plasma from the same animals indicated that HC concentrations in milk were 1.3-5 fold greater than in plasma.

The two tables below present TK data details and milk and plasma concentration comparison.

Table 59. Concentrations Ratio of Hydrocodone in Milk and Plasma

Occasions	Sample Description	Group 2: 3 mg/kg/day - Concentration (ng/mL) Animal 2525	Animal 2530	Animal 2533	Animal 2534	Mean ± SD
Day 10 2 h	Milk	5.02	2.98	4.41	6.81	4.80 ± 1.59
	Plasma	2.37	1.53	1.30	1.64	1.71 ± 0.461
	Ratio [a]	2.12	1.95	3.40	4.15	2.91 ± 1.05

Occasions	Sample Description	Group 3: 10 mg/kg/day - Concentration (ng/mL) Animal 3530	Animal 3536	Animal 3537	Mean ± SD
Day 10 2 h	Milk	1.37	34.2	8.16	14.6 ± 17.4
	Plasma	1.08	7.37	1.65	3.37 ± 3.47
	Ratio [a]	1.26	4.65	4.93	3.62 ± 2.04

Occasions	Sample Description	Group 4: 30 mg/kg/day - Concentration (ng/mL) Animal 4525	Animal 4528	Animal 4529	Mean ± SD
Day 10 2 h	Milk	10.8	13.5	22.4	15.6 ± 6.04
	Plasma	3.67	8.51	7.96	6.72 ± 2.65
	Ratio [a]	2.95	1.58	2.81	2.45 ± 0.753

a = Calculated as milk ÷ plasma.

Table 60. Toxicokinetic Parameters

Group No.	Dose Level (mg/kg/day)	Analyte Name	T_{max} (h)	T_{last} (h)	C_{max} (ng/mL)	$AUC_{0\text{-}tlast}$ (ng•h/mL)	K_{el} (1/h)	R^2	T½ (h)	$AUC_{(0\text{-}\infty)}$ (ng•h/mL)	AUC% (T_{last}-∞)	C_{max}/ Dose	$AUC_{(0\text{-}tlast)}$/ Dose
2	3	Hydrocodone (HC)	0.50	6.00	10.4	16.3	0.836	0.988	0.829	16.4	0.770	3.45	5.44
		Norhydrocodone (NHC)	0.50	6.00	10.8	19.7	0.762	0.999	0.910	20.0	1.10	3.60	6.58
		Hydromorphone (HM)	0.50	6.00	5.53	11.1	0.360	0.808	1.92	13.0	15.0	1.84	3.69
		Hydromorphone-3β-Glucuronide (HM3G)	0.50	24.0	199	693	0.210	0.999	3.30	696	0.405	66.4	231
3	10	Hydrocodone (HC)	1.00	6.00	26.8	54.4	0.937	0.998	0.740	54.7	0.446	2.68	5.44
		Norhydrocodone (NHC)	1.00	6.00	34.8	60.1	0.823	0.988	0.842	60.7	0.968	3.48	6.01
		Hydromorphone (HM)	1.00	6.00	16.6	35.0	a	0.796	a	a	15.2	1.66	3.50
		Hydromorphone-3β-Glucuronide (HM3G)	1.00	24.0	444	1925	0.157	0.994	4.43	1961	1.84	44.4	193
4	30	Hydrocodone (HC)	0.50	6.00	158	199	a	0.725	a	a	12.5	5.27	6.63
		Norhydrocodone (NHC)	0.50	6.00	110	182	0.397	0.837	1.75	208	12.2	3.66	6.08
		Hydromorphone (HM)	0.50	24.0	69.4	141	0.135	0.999	5.13	144	2.21	2.31	4.70
		Hydromorphone-3β-Glucuronide (HM3G)	0.50	24.0	830	3519	0.0994	0.990	6.97	3772	6.71	27.7	117

a = Values are not reported because the $AUC_{(0\text{-}\infty)}$ was extrapolated by more than 20% or R^2 was <0.800.

Dosing Solution Analysis

All dosing formulation samples had mean concentrations within the acceptance criteria of ± 10%, with individual values within ± 15%.

F_1 Generation Survival

The following parameters were obtained

$$\text{Viability index (\%)} = \frac{\text{No. of live pups on Day 4 } \textit{post partum} \text{ (precull)}}{\text{No. of live pups on Day 0 } \textit{post partum}} \times 100$$

$$\text{Survival index (\%)} = \frac{\text{No. of live pups on Day 7 or 14 } \textit{post partum}}{\text{No. of live pups on Day 4 } \textit{post partum} \text{ (post cull)}} \times 100$$

$$\text{Lactation index (\%)} = \frac{\text{No. of live pups on Day 21 } \textit{post partum}}{\text{No. of live pups on Day 4 } \textit{post partum} \text{ (post cull)}} \times 100$$

Note, on Day 4 post-partum the litter were culled to 8 pups using a randomization procedure. Culled pups were euthanized and discarded.

Treatment related effects were seen at HD including lower values of viability indices (HD, 80.96%; control, 98.36%), and survival indices (Day 7 post-partum: HD, 95.39%, control, 100%; Day 14 post-partum: HD, 94.74%, control, 100%). The lower viability index mainly resulted from three litters (No. 4501, 4515, and 4521) in which all pups died before Day 4 post-partum and the lower survival indices were caused by one litter (No. 4506), in which all pups were dead after Day 7 post-partum. These values are also below historical control data. The lactation index was not affected by treatment. The litter size was slightly lower for HD (Day 0: HD, 11.6; control, 12.6; Day 4 post-partum, HD, 10.0; control, 12.4).

There was no mortality for F1 generation adults.

Clinical Signs

No treatment related findings in F_1 pups or F_1 generation adults.

Body Weight

Treatment related lower pup body weights (up to 22% lower) were observed at 30 mg/kg, from Day 4 post-partum to Day 21 post-partum (last time point examined). The extent of the differences typically decreased as the pups were growing older (10-12% lower than control at Day 21). Nevertheless, adult F_1 from dams at 30 mg/kg remained lower than control body weights throughout the study (approximately 10% lower in males throughout Day 105 post-partum, and 12% lower in females prior to mating at Day 77 post-partum).

Food consumption

Not reported

Physical Development

The average time of development for hair growth was delayed at 30 mg/kg compared to control (9.94 days versus 8.90 days) which might be related to the lower body weight of these animals. Development of eye opening and pinna unfolding was not affected by treatment. The average time of development for vaginal opening and preputial separation was unaffected.

Neurological assessment

The average time of development for the righting reflex and negative geotaxis response were not affected. F_1 generation adults showed no treatment-related effects on motor activity (Day 35 and 60 post-partum), startle habituation (Day 55 post-partum), or performance in water maze tests (Days 60-70 post-partum).

Terminal Procedure

One male pup from a HD dam (Litter 4501) was euthanized on Day 0 post-partum and revealed total cleft palate. One female pup from a HD dam (litter 4506) was also euthanized on Day 0 post-partum, confirmed finding of anal atresia and microcaudia noted in clinical examination. Additionally, two preterminal deaths occurred in main study pups (Nos. 2508/4 and 3510/6) during the pre-weaning period. Animal No. 2508/4 was found dead on Day 18 post-partum and gross pathology revealed a blood clot at the surface of the brain which was considered to be the cause of death. Animal No. 3510/6 was moribund sacrificed on Day 17 part partum without cause of death identified in gross pathology examination. No treatment-related external or visceral findings were observed in terminal sacrificed F_1 pups. The number of stillborn pups was not affected by treatment. F_1 generation adults showed no macroscopic findings.

Reproduction

The mating index, fertility index, conception rate and mean day to mating were unaffected by treatment. The pregnancy rate was 78.3 and 83.3% at 3 and 30 mg/kg, respectively and 100% at 10 mg/kg and in the control group. The lower pregnancy rate at 3 and 30 mg/kg were considered the result of normal biological variability, due to lack

of dose-relationship. Other maternal performance parameters including gestation index, length of gestation, number of live, dead and malformed pups, number of implantation scars and sex ratio and the live birth index were not affected.

F_2 Generation Survival

The viability and litter size were unaffected.

Body Weight

Pup weight was not affected

External and Visceral Evaluation

There were no external or visceral findings attributable to the treatment to the F_0 generation females.

Male/Female Ratio

Not provided

10 Special Toxicology Studies

N Special toxicology studies were conducted.

11 Integrated Summary and Safety Evaluation

The Applicant has assessed the safety pharmacology, acute and repeat-dose toxicity, genetic toxicology, reproductive and developmental toxicology, and carcinogenic potential of HC bitartrate.

This formulation of extended-release HC bitartrate uses a PEO-based formulation to confer abuse-deterrent properties and provide resistance to alcohol-induced dose dumping. All excipients, with the exception of the PEO, can be found either in previously approved products or have acceptable daily intakes at higher levels and do not require further justification for the levels when the product is consumed at the MTDD of HC. To support the safety of the (b) (4) levels of the PEO in this product, the Applicant is referencing MF (b) (4). Master File (b) (4) has been found to be inadequate because of the lack of adequate characterization of low molecular weight entities in the polymer. These low molecular weight entities could potentially be absorbed systemically and might include (b) (4). Because of the longstanding use of PEO in (b) (4) products referencing MF (b) (4), this deficiency will not be an approval issue

for NDA 206627 and the levels of PEO in this product are considered acceptable from a pharmacology/toxicology perspective.

The Applicant is referencing MF (b) (4) ((b) (4)) for the HC drug substance (DS). The specifications for all DS impurities meet ICH Q3A(R2) qualification thresholds. The DS impurity (b) (4) contains a structural alert for mutagenicity. The Applicant conducted an Ames assay, an in vitro chromosome aberration (CA) assay, and a combined comet/micronucleus assay with (b) (4) was negative for mutagenicity in the Ames assay, but positive for clastogenicity in the absence of S9 in the in vitro CA assay. An in vivo combined comet/micronucleus assay was conducted and (b) (4) was found to be negative in both the presence and absence of metabolic activation. The weight of evidence suggests that (b) (4) is not mutagenic or clastogenic and can therefore be regulated as per ICH Q3A(R2) as a typical non-genotoxic impurity. The specifications for the drug substance are acceptable.

The drug product degradant specifications for Hysingla ER meet ICH Q3B(R2) qualification thresholds and are considered acceptable.

The Applicant conducted an Ames assay, two in vitro mouse lymphoma assays and an in vivo micronucleus assay with HC bitartrate. Hydrocodone tested negative in the in Ames assay. However, HC yielded a positive result in the presence of rat S9 in the in vitro mouse lymphoma assay. The Applicant repeated the mouse lymphoma assay using human S9 and HC was negative in both the presence of metabolic activation (human S9) and absence of metabolic activation. Hydrocodone was negative for clastogenicity in the in vivo mouse micronucleus assay in both the presence and absence of metabolic activation. The weight-of-evidence suggests that HC does not have mutagenic or clastogenic potential.

The pharmacology of HC is well-characterized and extensive clinical experience with HC combination products exists. The Applicant conducted a battery of safety pharmacology studies to assess the effects of HC of the CNS, respiratory system, and cardiovascular system. The pharmacologic effects of HC on the central nervous system and respiratory system have been fairly well-characterized in the published literature and the results from the studies conducted by the Applicant were consistent with the known effects of HC. In the neurobehavioral assessment in rat, HC showed increased salivation, decreased locomotor activity, and swelling of limbs. A HC-mediated decrease in respiratory rate was observed in the assessment of respiratory function in rat. This is consistent with the well-known effects of opioids on the pontine and medullary centers in the brain.

A battery of studies to assess the effects of HC on cardiac function was also conducted by the Applicant. Hydrocodone did not inhibit the hERG potassium currents in cells expressing the channel. However, concentration-dependent increases in action potential duration were observed with HC treatment in isolated Purkinje fiber cells. A study in conscious, freely moving telemetered dogs demonstrated HC-mediated decreases in body temperature and small decreases in heart rate. Increases in RR and

QRS interval durations as well as an increase in QT and QTc intervals were also observed in the study at the highest dose tested. The C_{max} exposure in dogs at the dose where the increases in QT and QTc intervals were seen was 0.7-fold the human C_{max} exposure of HC with a 120 mg/day human dose. To address the potential for HC-mediated effects on QT interval in humans, the Applicant conducted a clinical thorough-QT study.

The table below outlines exposure margins for the toxicology studies with HC.

Decreases in food consumption and concomitant decreases in body weights were the main observations in the rat and dog toxicology studies with chronic oral HC administration. No other toxicities were observed in the 9-month dog study and no target organs were identified. No adverse findings were noted, therefore, the NOAEL was greater than 6 mg/kg, the highest dose tested in the dog (0.9-fold and 0.8-fold in males and females, respectively, the human systemic exposure of a 120 mg HC dose (based on AUC comparisons; data from Study # HYD1002). Because extensive clinical experience with HC exists, an agreement was made with the Applicant that in lieu of a dedicated repeat-dose toxicology study with HC in rat, the Division would accept the rat carcinogenicity study if interim lab values were measured and endpoints were analyzed in terms of a NOAEL. Although several treatment-related findings were noted in the rat with chronic treatment of HC, none of them were considered adverse. Significant body weight decreases in both male and female rats as well as decreased food consumption were observed in this study. Dose-dependent increases in survival at 105 weeks were observed and attributed to the decreased body weights. Hyperactivity, vocalizations, and sensitivity to touch were observed throughout the study and are attributed to the pharmacologic effects of HC. They were not considered adverse. Clinical chemistry and hematologic values were measured at 26 and 105 weeks. In males, small but significant decreases in triglycerides (at all doses, 26 and 105 weeks) and cholesterol (high dose, 105 weeks) were observed. Treatment-related decreases in tumors in the anterior pituitary and mammary gland were observed in this study. This decrease is attributed to the reduced weights in the treated groups throughout the study (Haseman, et al., 1997). An increased incidence of swollen hindpaws (males) and sores and scabs (males and females; referred to as pododermatitis by the pathologist) was also noted. These findings are commonly seen with opioids in rats but are not considered clinically relevant. Histopathology was conducted at 105 weeks in this study. Dose-dependent increases in sternum bone marrow hypercellularity were observed in males. All groups of females (including control) showed higher levels of hypercellularity than males. Bone marrow hypercellularity is typically associated with inflammation and in this study it is attributed the inflammation from the pododermatitis. Although increases in bone marrow hypercellularity were not treatment-related in females, all females showed high levels. This could be attributed to age or other underlying inflammation. No HC-related changes in hematologic parameters or other relevant hyperplasia/neoplasia was observed in this two-year study. No NOEL was established for this study but the NOAEL is >25 mg/kg in both males and females because none of the findings observed were particularly adverse. No target organs were identified in this study.

A full battery of developmental and reproductive toxicology studies has been conducted with HC. The fertility and early embryofetal development study in rats with oral doses of 3, 12.5, and 25 mg/kg HC showed decreased food consumption and reduced body weight gain, especially at the early days of treatment. Male reproductive system (sperm motility, spermatozoa count, sperm morphology, and testicular histopathology), and fertility parameters (day of mating, mating and fertility indices, and conception rates) were not affected. There were treatment-related increases in pre- and post-implantation loss, but the values were within historical control ranges. The dose of 25 mg/kg was considered to be the NOAEL in this study.

Embryofetal development studies with oral HC were conducted in rats and rabbits. The toxicity findings were similar in the two species: reduced maternal food consumption and lower body weight gain or body weight loss, lower fetal weights (with some delays in ossification in rats). No embryolethality or teratogenicity was noted in either of these studies. The rat maternal NOAEL was 3 mg/kg and fetal NOAEL was 10 mg/kg; the rabbit maternal LOAEL and fetal NOAEL were both 10 mg/kg.

A pre- and post-natal development study in rats with oral doses of 3, 10, and 30 mg/kg was conducted. Maternal toxicities of lower body weight and reduced food consumption were observed at 10 and 30 mg/kg and effects on the offspring including lower viability, reduced pre-weaning body weights were seen at 30 mg/kg. The lower offspring body weight continued throughout the post-weaning period. The lower body weights were associated with transitory retardation of hair growth and to a slight retardation of preputial separation. There were no effects on behavior or reproductive performance for the offspring. The NOAEL was 3 mg/kg for the F_0 generation and 10 mg/kg for the F_1 generation.

A Pregnancy Category C is recommended for this product and the relevant results will be described in the label. The highest available strength for this product will be 120 mg HC and the product is labeled to be used q24h, therefore, the systemic levels at the human dose of 120 mg/day will be used as the exposure comparison with the nonclinical studies.

Two-year rat and mouse carcinogenicity studies were conducted with HC. The Executive CAC provided concurrence on dose selection and study results for both the mouse and rat carcinogenicity studies (minutes dated 6/14/02 and 6/26/14, respectively). No HC-related tumors were observed in either study. In mice, HC bitartrate was administered orally by gavage at doses of 0, 20, 60, 200 mg/kg in males and 0, 10, 30, 100 mg/kg in females. This study is considered valid and no treatment-related increases in any tumor type were observed. In rats, HC bitartrate was administered orally by gavage at doses of 0, 4, 12, and 25 mg/kg in both males and females. This study is considered valid and no treatment-related increases in any tumor type were observed.

All rat and dog exposures of HC in the studies conducted are below the systemic exposure in humans at the dose of 120 mg. The pharmacologic effects of opioids limit

the dosing and typically multiples of human clinical exposures are not achieved in rat or dog studies.

Table 61. Summary of Exposure Margins at NOAEL

Study	Species	NOAEL (mg/kg) M/F	Exposure Margin at NOAEL Based on AUC*	Finding that defined NOAEL
Chronic Toxicology	Rat	>25 (M&F)	M; 0.2; F: 0.1	NA
Chronic Toxicology	Dog	>6 (M&F)	M: 0.9; F: 0.8	NA
Fertility	Rat	25	0.06 (M&F)	↓food intake and body weight
Embryofetal development	Rat	10	0.014 (F)	↓fetal weight delayed ossification
Embryofetal development	Rabbit	10	0.07 (F)	↓fetal weight
Pre- and Post-natal	Rat	10	0.03 (F)	↓pup body weight ↓pup survival rate
Carcinogenicity	Rat	>25 (M&F)	M; 0.2; F: 0.1	NA
Carcinogenicity	Mouse	>200 (M) >100 (F)	M: 3.5; F: 3.1	NA

*Human dose of 120 mg/day at steady state; AUC_{tau}= 1938 ng.h/mL, Study # HYD1002 (dose interval tau was 24 hours).

12 Appendix/Attachments

Reference List

Bowen CA, Negus SS, Zong R, Neumeyer JL, Bidlack JM and Mello NK (2003) Effects of mixed-action kappa/mu opioids on cocaine self-administration and cocaine discrimination by rhesus monkeys. *Neuropsychopharmacology* **28**:1125-1139.

Gardner-Nix J (2002) Opioids causing peripheral edema. *J Pain Symptom Manage* **23**:453-455.

Haseman JK, Young E, Eustis SL and Hailey JR (1997) Body weight-tumor incidence correlations in long-term rodent carcinogenicity studies. *Toxicol Pathol* **25**:256-263.

Hutchinson MR, Menelaou A, Foster DJ, Coller JK and Somogyi AA (2004) CYP2D6 and CYP3A4 involvement in the primary oxidative metabolism of hydrocodone by human liver microsomes. *Br J Clin Pharmacol* **57**:287-297.

(b) (4)

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

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/s/

ELIZABETH BOLAN
10/10/2014

RICHARD D MELLON
10/10/2014
I concur with Dr. Bolan's recommendation that, from a nonclinical pharmacology toxicology perspective, NDA 206627 may be approved.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 206627 **Applicant: Purdue Pharma** **Stamp Date: 4/28/2014**

Drug Name: Hydrocodone bitartrate CR **NDA/BLA Type: 505(b)(2) referencing Vicoprofen NDA 20716**

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

	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		

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
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m^2 or comparative serum/plasma levels) and in accordance with 201.57?	X		The proposed labeling is based on the MTDD of hydrocodone. It will be adjusted to a more typical daily dose. The human multiple is based on body surface area comparisons.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)		X	Sponsor claims data support conclusion that (b) (4) is a minor metabolite in humans. DS qualification studies for (b) (4) (b) (4) are unde but have not been submitted with this application. The Sponsor intends to submit the studies in Quarter 3-4 of 2014, Within 6 months of the submission. Until submitted and reviewed by the Agency, Purdue plans to only select DS batches that meet the ICH specification. This will be acceptable for filing.
11	Has the applicant addressed any abuse potential issues in the submission?	X		From a nonclinical perspective, the application contains adequate data. We defer to CSS for a more comprehensive examination of these data.
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_____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

None at this time.

R. Daniel Mellon, PhD	5/20/2014
Reviewing Pharmacologist	Date
R. Daniel Mellon, PhD	5/20/2014
Team Leader/Supervisor	Date

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

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/s/

RICHARD D MELLON
06/04/2014