

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**202880Orig1s000**

**PHARMACOLOGY REVIEW(S)**

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

**PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION**

Application number: 202880  
Supporting document/s: EDR 0000 received 5/1/12  
Applicant's letter date: May 1, 2012  
CDER stamp date: May 1, 2012  
Product: Zohydro ER (hydrocodone bitartrate)  
Indication: Treatment of chronic, moderate-to-severe pain  
requiring continuous around-the-clock opioid  
therapy for an extended period of time  
Applicant: Zogenix, Inc.  
Review Division: Division of Anesthesia, Analgesia, and Addiction  
Products (DAAAP)  
Reviewer: Elizabeth A. Bolan, Ph.D.  
Supervisor/Team Leader: R. Daniel Mellon, Ph.D.  
Division Director: Bob Rappaport, M.D.  
Project Manager: Dominic Chiapperino, Ph.D.

*Template Version: September 1, 2010*

**Disclaimer**

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 202880 are owned by Zogenix or are data for which Zogenix has obtained a written right of reference. Any information or data necessary for approval of NDA 202880 that Zogenix does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 202880.

## TABLE OF CONTENTS

<b>1</b>	<b>EXECUTIVE SUMMARY .....</b>	<b>7</b>
1.1	INTRODUCTION .....	7
1.2	BRIEF DISCUSSION OF NONCLINICAL FINDINGS .....	7
1.3	RECOMMENDATIONS .....	8
<b>2</b>	<b>DRUG INFORMATION .....</b>	<b>14</b>
2.1	DRUG .....	14
2.2	RELEVANT INDS, NDAs, BLAs AND DMFs .....	15
2.3	DRUG FORMULATION .....	15
2.4	COMMENTS ON NOVEL EXCIPIENTS .....	16
2.5	COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN .....	16
2.6	PROPOSED CLINICAL POPULATION AND DOSING REGIMEN .....	18
2.7	REGULATORY BACKGROUND .....	18
<b>3</b>	<b>STUDIES SUBMITTED.....</b>	<b>18</b>
3.1	STUDIES REVIEWED.....	18
3.2	STUDIES NOT REVIEWED .....	20
3.3	PREVIOUS REVIEWS REFERENCED.....	20
<b>4</b>	<b>PHARMACOLOGY .....</b>	<b>20</b>
4.1	PRIMARY PHARMACOLOGY .....	20
4.2	SECONDARY PHARMACOLOGY .....	21
4.3	SAFETY PHARMACOLOGY .....	21
<b>5</b>	<b>PHARMACOKINETICS/ADME/TOXICOKINETICS .....</b>	<b>21</b>
5.1	PK/ADME.....	21
5.2	TOXICOKINETICS .....	21
<b>6</b>	<b>GENERAL TOXICOLOGY.....</b>	<b>22</b>
6.1	SINGLE-DOSE AND ACUTE TOXICITY .....	22
6.2	REPEAT-DOSE TOXICITY .....	22
<b>7</b>	<b>GENETIC TOXICOLOGY .....</b>	<b>56</b>
7.1	<i>IN VITRO</i> REVERSE MUTATION ASSAY IN BACTERIAL CELLS (AMES).....	56
7.2	<i>IN VITRO</i> ASSAYS IN MAMMALIAN CELLS.....	58
7.3	<i>IN VIVO</i> CLASTOGENICITY ASSAY IN RODENT (MICRONUCLEUS ASSAY).....	61
7.4	OTHER GENETIC TOXICITY STUDIES.....	64
<b>8</b>	<b>CARCINOGENICITY .....</b>	<b>69</b>
<b>9</b>	<b>REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY .....</b>	<b>69</b>
9.1	FERTILITY AND EARLY EMBRYONIC DEVELOPMENT .....	69
9.2	EMBRYONIC FETAL DEVELOPMENT .....	73
9.3	PRENATAL AND POSTNATAL DEVELOPMENT .....	83

<b>10</b>	<b>SPECIAL TOXICOLOGY STUDIES.....</b>	<b>87</b>
	REPEAT-DOSE TOXICITY.....	87
<b>11</b>	<b>INTEGRATED SUMMARY AND SAFETY EVALUATION.....</b>	<b>92</b>
<b>12</b>	<b>APPENDIX/ATTACHMENTS.....</b>	<b>94</b>

## Table of Tables

Table 1. Proposed Label for Zohydro ER .....	9
Table 2. HC-ER formulation .....	16
Table 3. Acceptance criteria specifications for the hydrocodone bitartrate drug substances from (b) (4) and (b) (4) .....	17
Table 4. Drug product acceptance criteria specifications for release and stability .....	18
Table 5. Studies reviewed .....	18
Table 6. Studies reviewed previously by Dr. Violetta Klimek .....	19
Table 7. Studies not reviewed .....	20
Table 8. Summary of mortality (mouse 90-day study) .....	25
Table 9. Mean body weights, males: percent change from control (mouse 90-day study) .....	26
Table 10. Mean body weights, females: percent change from control (mouse 90-day study) .....	26
Table 11. Mean body weights in grams, males (mouse 90-day study) .....	27
Table 12. Mean body weights in grams, females (mouse 90-day study) .....	27
Table 13. Body weight gain (mouse 90-day study) .....	29
Table 14. Absolute and relative organ weight changes (mouse 90-day study) .....	33
Table 15. Toxicokinetic parameters (mouse 90-day study) .....	36
Table 16. Exposure ratios (plasma levels) between mouse and humans (mouse 90-day study) .....	37
Table 17. Mean body weights, males: percent change from control (rat 90-day study) .....	39
Table 18. Mean body weights, females: percent change from control (rat 90-day study) .....	39
Table 19. Mean body weights in grams, males (rat 90-day study) .....	40
Table 20. Mean body weights in grams, females (rat 90-day study) .....	40
Table 21. Body weight gain (rat 90-day study) .....	41
Table 22. Significant changes in mean clinical pathology values (rat 90-day study) .....	44
Table 23. Significant changes in mean organ weight values (rat 90-day study) .....	45
Table 24. Summary of toxicokinetic parameters (rat 90-day study) .....	47
Table 25. Exposure ratios (plasma levels) between rat and human (rat 90-day study) .....	48
Table 26. Mean glucose levels (mg/dL +/-SD) in females (28-day dog study) .....	52
Table 27. Histopathologic findings (28-day dog study) .....	54
Table 28. Summary of toxicokinetics (reproduced from NDA) .....	56
Table 29. Positive controls (reproduced from NDA) .....	57
Table 30. Summary of Ames assay results (reproduced from NDA) .....	58
Table 31. Study design for in vitro chromosome aberration test .....	58
Table 32. Summary of chromosomal aberration assay results (reproduced from NDA) .....	60
Table 33. Summary of bone marrow MN assay results (reproduced from NDA) .....	63
Table 34. Positive controls for the Ames assay (reproduced from NDA) .....	65
Table 35. Summary of Ames assay results .....	66
Table 36. Summary of Comet data for (b) (4) .....	68
Table 37. Summary of micronucleus data for (b) (4) .....	68
Table 38. Fertility index and selected estrous cycle parameters .....	72

Table 39. Selected fertility parameters ..... 72  
Table 40. Pregnancy rates in different dose groups ..... 74  
Table 41. Toxicokinetic parameters for hydrocodone in pregnant rats ..... 76  
Table 42. Summary of toxicokinetics ..... 80  
Table 43. Mean live fetus weight ..... 80  
Table 44. Gross, soft tissue and skeletal variations and malformations ..... 82  
Table 45. Body weight gain ( (b) (4) rat study) ..... 89  
Table 46. Summary of toxicokinetics ( (b) (4) rat study) ..... 90

### Table of Figures

Figure 1. Mean body weights in males (mouse 90-day study) .....	28
Figure 2. Mean body weights in females (mouse 90-day study) .....	29
Figure 3. Mean food consumption, males (mouse 90-day study).....	30
Figure 4. Mean food consumption, females (mouse 90-day study).....	30
Figure 5. Mean body weights in males (rat 90-day study).....	41
Figure 6. Mean body weights in females (rat 90-day study).....	42
Figure 7. Mean body weights in males (28-day dog study) .....	50
Figure 8. Mean body weights in females (28-day dog study) .....	51
Figure 9. Maternal body weights during gestation (rat) .....	75
Figure 10. Mean maternal body weights (rabbit).....	79
Figure 11. Mean maternal body weights: gestation and lactation (rat) .....	85
Figure 12. Mean body weights in F1 males.....	86
Figure 13. Mean body weights in F1 females.....	86
Figure 14. Mean body weights in males ( (b) (4) rat study).....	88
Figure 15. Mean body weights in females ( (b) (4) rat study).....	89
Figure 16. Structure of (b) (4).....	91
Figure 17. Structure of (b) (4).....	91

## **1 Executive Summary**

### **1.1 Introduction**

Zogenix has submitted NDA 202880 for Zohydro ER (hydrocodone bitartrate, HC, extended release) for the indication of management of moderate to severe pain in patients requiring continuous around-the-clock opioid therapy for an extended period of time. The product will be available in strengths of 10, 15, 20, 30, 40, and 50 mg HC per capsule and is intended for b.i.d. dosing. 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).

Although HC-containing combination products have been approved and marketed for decades, the doses of HC have been limited by the toxicity of the non-opioid component of the combination (i.e. acetaminophen, ibuprofen, or chlorpheniramine). This product would be the first approved single-entity HC product and due to tolerance to HC, could be used at very high doses.

The Applicant has assessed the acute and repeat-dose toxicology, genetic toxicology, and developmental and reproductive toxicology of HC. The results of the general toxicology studies were typical of an opioid agonist and no toxicities unique to HC were demonstrated. Several adverse findings on fertility and embryonic and peri- and post-natal development with seen with HC. A pregnancy category C is being recommended and the findings will be described in the label. One of the genetic toxicology studies with HC showed a positive result. A fourth-tier study is being required as a PMR in order to fully characterize the clastogenic potential of HC. The genetic toxicology results will be included in the label. Carcinogenicity studies in the mouse and rat are required for this NDA and are currently being conducted by the Applicant. An agreement with FDA was made to conduct the studies as a PMR as long as the studies were underway by NDA filing. The levels of excipients in this formulation when the product is used at the maximum theoretical daily dose of HC are acceptable and do not pose any toxicologic concerns. All impurities/degradants in the drug substance and drug product are controlled at acceptable levels.

### **1.2 Brief Discussion of Nonclinical Findings**

The Zohydro ER formulation uses SODAS (Spheroidal Oral Drug Absorption System) drug delivery technology to confer controlled-release properties. The excipients, when calculated for the maximum theoretical daily dose of HC, can all be found in previously approved products and do not present any unique toxicologic concerns. All impurities/degradants in the drug substance and drug product are controlled at acceptable levels. Hydrocodone-related toxicities in acute and repeat-dose general toxicology studies were consistent with the known toxicities of other opioid agonists.

The standard ICH battery of genetic toxicology studies was conducted for HC. Hydrocodone tested negative in the in vitro bacterial reverse mutation assay, the in vivo mouse micronucleus assay, and the in vitro chromosome aberration assay in the

absence of metabolic activation. In contrast, HC tested positive for clastogenic activity in the in vitro chromosome aberration assay in the presence of metabolic activation. HC is considered to have clastogenic potential and a fourth test will be required to be conducted post-marketing. Carcinogenicity assessments in mice and rats with HC are currently being conducted by the Applicant and will be submitted to the NDA as a post-marketing requirement (PMR). At the time of this review, the results of the two carcinogenicity assessments are not available.

A full battery of developmental and reproductive toxicology studies has been conducted with HC. Decreases in female fertility were observed at all doses tested in the fertility study. No NOAEL was established for effects on female fertility, the lowest dose tested was two-times the human dose of 100 mg/day on a mg/m<sup>2</sup> basis. However, the changes in fertility observed in the rat may be related to known opioid-mediated effects on prolactin, which is essential for estrous cycling in the rat. The clinical relevance of the fertility finding is not known. No effects of HC on male fertility parameters were observed (NOAEL is ten-times the human dose of 100 mg/day on a mg/m<sup>2</sup> basis), however, decreased weights of male reproductive organs were observed at all doses. No effects of HC were seen in a rat embryofetal development study at any dose tested, although HC-mediated decreases in fertility limited the dosing in the study (NOAEL is approximately two-times the human dose of 100 mg/day on a mg/m<sup>2</sup> basis). In the rabbit embryofetal development study, fetal body weights were significantly decreased in all treated groups. Increases in the number of fetal malformations, including umbilical hernia and various irregularly shaped bones (ulna, femur, tibia, fibula) were observed in the highest dose group. Decreases in the number of ossified hyoid bodies and xiphoid bones, considered a developmental variation, were also observed in the highest dose group. The NOAEL for teratogenicity in the rabbit study is ten-times the human dose of 100 mg/day on a mg/m<sup>2</sup> basis. In the peri- and post-natal study, HC-mediated decreases on pup body weights, viability and lactation indices were observed (NOAEL is 0.5-times the human dose of 100 mg/day on a mg/m<sup>2</sup> basis). A pregnancy category C is recommended for this product and the relevant results will be described in the label.

### **1.3 Recommendations**

#### **1.3.1 Approvability**

The recommendation from pharmacology/toxicology is that NDA 202880 be approved with post-marketing requirements to conduct an additional fourth tier genetic toxicology study and complete the two ongoing carcinogenicity studies (mouse and rat) with hydrocodone bitartrate.

#### **1.3.2 Additional Non Clinical Recommendations**

A post-marketing requirement from pharmacology/toxicology will be needed in order to further characterize the potential clastogenicity of hydrocodone bitartrate. The mouse and rat carcinogenicity studies that are currently being conducted by the Applicant will be considered post-marketing requirements. These studies are deemed acceptable as PMRs since hydrocodone has been approved in combination drug products for many

years and, to date, there are no signals of adverse effects related to genotoxic or carcinogenic potential.

### 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.

Table 1. Proposed Label for Zohydro ER

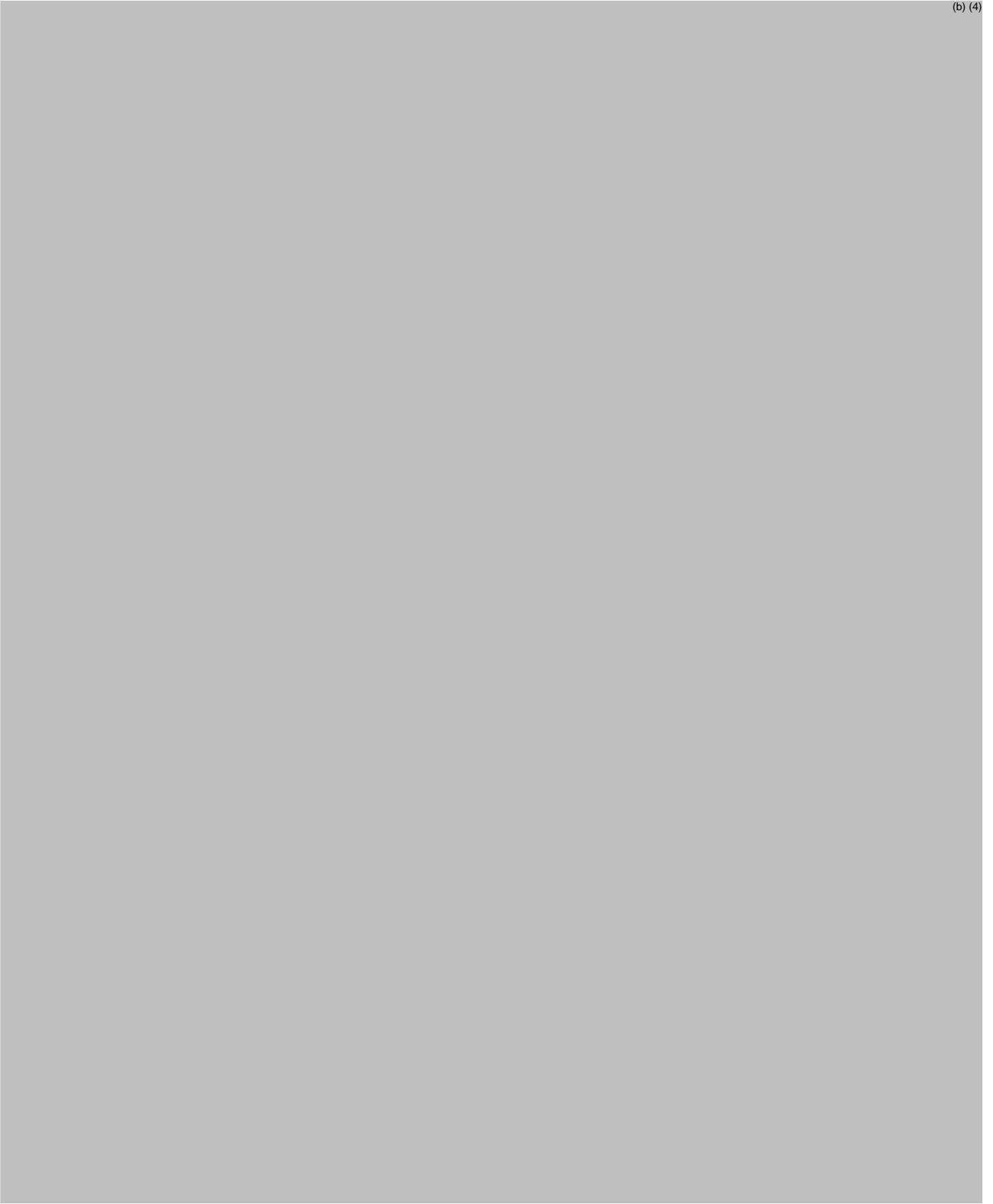


(b) (4)





(b) (4)





## 2 Drug Information

### 2.1 Drug

CAS Registry Number  
34195-34-1

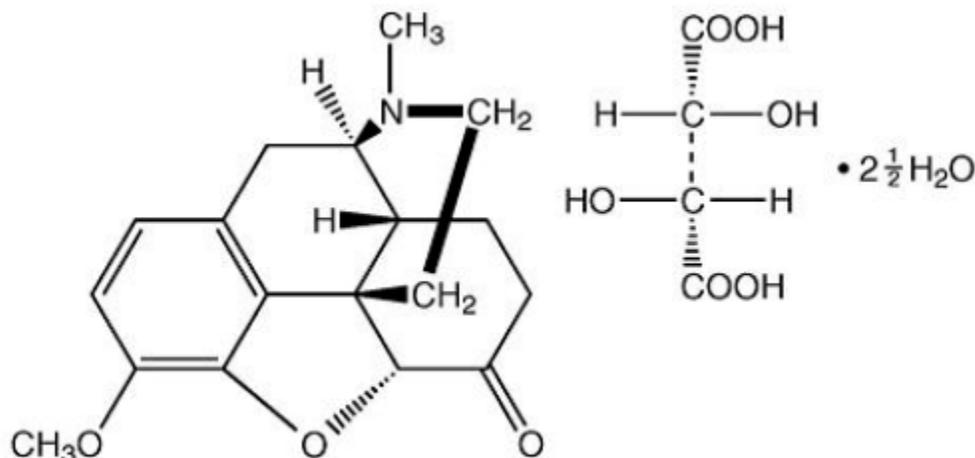
Generic Name  
Hydrocodone bitartrate

Code Name  
ELN154088

Chemical Name  
Morphinan-6-one, 4,5-epoxy-3-methoxy-17-methyl-, (5 $\alpha$ )-, [R(R\*,R\*)]-2,3-dihydroxybutanedioate (1:1), hydrate (2:5) 4,5 $\alpha$ -Epoxy-3-methoxy-17-methyl-morphinan-6-one tartrate (1:1), hydrate (2:5)

Molecular Formula/Molecular Weight  
C<sub>18</sub>H<sub>21</sub>NO<sub>3</sub> • C<sub>4</sub>H<sub>6</sub>O<sub>6</sub> • 2 1/2 H<sub>2</sub>O MW= 494.50

## Structure or Biochemical Description



## Pharmacologic Class

Opioid agonist (FDA Established Pharmacologic Class)

## 2.2 Relevant INDs, NDAs, BLAs and DMFs

<i>IND/NDA/MF</i>	<i>Drug/compound</i>	<i>Sponsor</i>	<i>Division/Office</i>	<i>status</i>
IND 65111	Hydrocodone bitartrate ER	Zogenix	DAAAP	active
NDA 20716	Vicoprofen (referenced drug)	AbbVie	DAAAP	approved
MF (b) (4)	Hydrocodone bitartrate	(b) (4)	ONDQA	not adequate*
MF (b) (4)	Hydrocodone bitartrate	(b) (4)	ONDQA	adequate

\* ONDQA has deemed MF (b) (4) not adequate. This has been communicated to the DMF holder and Applicant. As per Dr Yong Hu (ONDQA), the Applicant has committed to not using the (b) (4) drug substance for the manufacture of the drug product until the problems with the MF are rectified.

## 2.3 Drug Formulation

Hydrocodone Bitartrate Extended Release (HC-ER) Capsules are an extended-release capsule formulation which uses Alkermes SODAS (Spheroidal Oral Drug Absorption System) drug delivery technology. This technology is based on coating sugar spheres with the drug substance and excipients to form immediate-release (IR) beads. These IR beads are then coated with rate-controlling polymers to confer sustained-release characteristics. The target in vitro dissolution rate for HC-ER is achieved by combining IR and SR beads in a defined ratio followed by encapsulation in hard gelatin capsules. Several approved drugs (Verelan, Avinza, Ritalin LA and Focalin XR) utilize SODAS technology. It should be noted that the excipient formulations of these approved products are similar to the formulation used in HC-ER, but not identical. The capsules

will be available in strengths of 10, 15, 20, 30, 40, and 50 mg HC per capsule and are intended to be dosed b.i.d.

#### Determination of the maximum daily dose of hydrocodone

The maximal dosing information for hydrocodone is relevant to this review in that the ICH specifications for impurity levels for the drug substances and the drug product as well as the acceptable levels of total amount of inactive ingredients are based on the total daily dose of the drug substance. All of the FDA-approved HC-containing products available to date are combination products. In these combinations, the drug combined with the HC is responsible for toxicities which effectively limit the dose of the combination product. No data exists to assess how a single-entity HC product will be used. Using potency ratios with other opioids, DAAAP has determined that the maximum theoretical daily dose (MTDD) of HC for this product in an opioid-tolerant population could be as high as 3 g.

## 2.4 Comments on Novel Excipients

The total daily intake of each excipient in the HC-ER formulation, when calculated at the MTDD of 3 g of HC, is considered acceptable from the pharmacology/toxicology perspective. All excipients in the HC-ER formulation are either considered GRAS without limits by the oral route or can be found at higher levels in products previously approved by FDA. The excipients, their amounts and the acceptability of the amounts are presented in Table 2.

Table 2. HC-ER formulation

<b>Excipient</b>	<b>amt per 50 mg capsule, mg</b>	<b>Total daily intake for 60 capsules, mg</b>	<b>Acceptability, Rationale</b>
Sugar spheres	(b) (4)	(b) (4)	Yes, GRAS with no limits by the oral route
Hypromellose (b) (4)			Yes, IIG
Ammonio Methacrylate Copolymer (b) (4)			Yes, IIG
(b) (4)			
Silicon Dioxide (b) (4)			Yes: GRAS with no limits by the oral route
Talc		Yes, IIG	

IIG: FDA Inactive Ingredients Guide

## 2.5 Comments on Impurities/Degradants of Concern

### Impurities in the drug substance

The qualification threshold according to the ICH Q3A(R2) guideline for impurities in the drug substance for a MDD of drug substance > 2 g/day is 0.05%. For HC-ER, the

clinical team determined that the MTDD of hydrocodone is 3 g. The Applicant is obtaining the HC drug substance from two suppliers, (b) (4) (MF (b) (4) and (b) (4) (MF (b) (4). With the exception of (b) (4) in the (b) (4) sourced drug substance, all drug substance impurity specifications meet the ICH Q3A(R2) guideline specification of 0.05% (Table 3). (b) (4) contains a structural alert for mutagenicity and (b) (4) has adequately qualified (b) (4) for genotoxic potential. It was found to be negative for genotoxic potential and can therefore be regulated as a typical DS impurity according to ICH Q3A(R2) thresholds for qualification. The comment below was sent to the Applicant in the 74-day letter.

As the proposed specifications may be able to be (b) (4) based on manufacturing capability this potential issue is not deemed a filing issue.

The specification for (b) (4) in the hydrocodone drug substance obtained from (b) (4) exceeds ICH Q3A(R2) qualification thresholds. Although adequate genetic toxicology data exist, there are no repeat-dose toxicity data to support your proposed specification. You must either (b) (4) the specification to meet ICH Q3A(R2) thresholds or provide adequate justification for the proposed level in the form of a repeat-dose toxicology study of 90-days duration in a single species.

As per the ONDQA chemist, Dr Yong Hu, MF (b) (4) has been deemed not adequate due to some impurity specification issues. The Applicant has committed to not using the (b) (4) sourced HC for the manufacture of the drug product until the MF is deemed adequate.

Table 3. Acceptance criteria specifications for the hydrocodone bitartrate drug substances from (b) (4) and (b) (4)

<b>Impurity</b>	<b>DS Source</b>	<b>Specification</b>	<b>Acceptable?</b>
(b) (4)	(b) (4)	(b) (4)	Yes
(b) (4)	(b) (4)	(b) (4)	Yes
(b) (4)	(b) (4)	(b) (4)	Yes
(b) (4)	(b) (4)	(b) (4)	Yes
(b) (4)	(b) (4)	(b) (4)	Yes
(b) (4)	(b) (4)	(b) (4)	No

\*structural alert for mutagenicity but see above

**Impurities in the drug product**

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 HC-ER, DAAAP has determined that the MTDD of hydrocodone is 3 g. The Applicant has conducted a DEREK evaluation on the two DP impurities, (b) (4) (also called (b) (4) and (b) (4). The DEREK analysis did not identify any potential mutagenic/genotoxic activity for either

compound. The specification of (b) (4) is set at (b) (4) % and exceeds ICH Q3B(R2) specifications for qualification. The Applicant has conducted the necessary genetic and repeat-dose toxicology studies and (b) (4) can be considered qualified at the proposed specification of (b) (4) %. Release specifications for all drug product impurities/degradants in the HC-ER drug product are considered acceptable (Table 4).

Table 4. Drug product acceptance criteria specifications for release and stability

<b>Impurity</b>	<b>Specification</b>	<b>Acceptable?</b>
(b) (4)	(b) (4)	Yes, qualified
(b) (4)	(b) (4)	Yes

## 2.6 Proposed Clinical Population and Dosing Regimen

Hydrocodone bitartrate extended-release capsules (HC-ER) are planned to be marketed in 10, 15, 20, 30, 40 and 50 mg capsules intended for b.i.d. dosing in adults. The indication for HC-ER is management of moderate-to-severe chronic pain in when a continuous around-the-clock opioid analgesic is needed for an extended period of time.

## 2.7 Regulatory Background

The Applicant is submitting NDA 202880 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 65111 was opened on June 26, 2002 by Elan Pharmaceuticals. On January 31, 2008 Elan transferred sponsorship to Zogenix. Type B and PNDA meetings were held with Zogenix on June 4, 2008 and November 17, 2011, respectively.

## 3 Studies Submitted

### 3.1 Studies Reviewed

Table 5. Studies reviewed

<b>Study Title</b>	<b>Study Report #</b>	<b>EDR Location</b>
A 90-Day Oral Gavage Toxicity Study with Hydrocodone in CD-1 Mice	022615	4.2.3.2.1
A 3-Month Oral (Gavage) Toxicity Study of ELN154088 in Fischer 344 Rats	3344.262	4.2.3.2.1
A 28-Day Oral Toxicity Study of ELN 154008 (HC ER) Capsules in Beagle Dogs	3344.266	4.2.3.2.1
Bacterial Reverse Mutation Assay with Hydrocodone Bitartrate	214-029-01	4.2.3.3.1
In Vitro Mammalian Chromosome Aberration Test with Hydrocodone Bitartrate	214-030-01	4.2.3.3.1

Mammalian Erythrocyte Micronucleus Test with Hydrocodone Bitartrate	214-031-01	4.2.3.3.1
Oral (Gavage) Fertility and General Reproduction Toxicity Study of Hydrocodone Bitartrate in Rats	20007043	4.2.3.5.1
Oral (Gavage) Developmental Toxicity Study of Hydrocodone Bitartrate in Rats	20007040	4.2.3.5.1
Oral (Stomach Tube) Developmental Toxicity Study of Hydrocodone Bitartrate in Rabbits	20007042	4.2.3.5.1
Oral (Gavage) Developmental and Perinatal/Postnatal Reproduction Toxicity Study of Hydrocodone Bitartrate in Rats, Including a Postnatal Behavioral/Functional Evaluation	20007044	4.2.3.5.1
Reverse mutation in four histidine-requiring strains of <i>Salmonella typhimurium</i> and one tryptophan-requiring strain of <i>Escherichia coli</i> (b) (4)	8243018	4.2.3.7.6.1
Combined Comet assay in the liver and a bone marrow micronucleus test in treated mice (b) (4)	8243025	4.2.3.7.6.1
13-Week Oral Gavage Toxicity and Toxicokinetic Study with Hydrocodone and (b) (4) in Rats with a 2-Week Recovery Phase (b) (4)	1000159	4.2.3.6.1
Derek Evaluation of the Toxicities of (b) (4) and (b) (4)	NA	4.2.3.7.7.1

Table 6. Studies reviewed previously by Dr. Violetta Klimek

<b>Study Title</b>	<b>Study Report #</b>	<b>EDR Location</b>
Acute Oral Toxicity Study of Hydrocodone Bitartrate in Sprague-Dawley Rats With a 14-Day Observation Period	214-021-01	4.2.3.1.1
Acute Oral Toxicity Study of Hydrocodone Bitartrate in Beagle Dogs With a 14-Day Observation Period	214-022-01	4.2.3.1.1
A 28-Day Oral Toxicity Study of Hydrocodone Bitartrate in Sprague-Dawley Rats	214-023-01	4.2.3.2.1
28-Day Oral Toxicity Study of Hydrocodone Bitartrate in Beagle Dogs	214-024-01	4.2.3.2.1

**Studies Not Reviewed**

Table 7. Studies not reviewed

<i>Study Title</i>	<i>Study Report #</i>	<i>EDR Location</i>
(b) (4)		

**3.3 Previous Reviews Referenced**

The studies in Table 6 were previously reviewed by Dr Violetta Klimek (June 8, 2002). The reviews are reproduced verbatim in Appendix 1.

**4 Pharmacology****4.1 Primary Pharmacology**

Portions of the pharmacology summary of HC (denoted by indented text) are reproduced from the review by Dr. Violetta Klimek (June 8, 2002)

Hydrocodone is a semi-synthetic narcotic analgesic and antitussive with multiple actions qualitatively similar to those of codeine. The precise mechanism of action of hydrocodone and other opioids is not known, although it is believed to relate to the existence of opiate receptors in the CNS. Three classical opioid receptor types,  $\mu$  (mu),  $\delta$  (delta), and  $\kappa$  (kappa) have been identified. Hydrocodone binds to all three of them with the highest degree of selectivity for the  $\mu$  type. The analgesic, euphorant, respiratory depressant and physiologic dependence of hydrocodone (like other compounds of this class) are primarily results of its action at  $\mu$  type of opioid receptor. It has been shown that hydrocodone binding affinity for  $\mu$  type of opioid receptor was 10-fold greater than its affinity for  $\delta$  and  $\kappa$  receptors. Opioid receptors are coupled, via pertussis toxin-sensitive GTP-binding proteins, to inhibition of adenylyl cyclase activity, activation of receptor-operated  $K^+$  currents, and suppression of voltage-gated  $Ca^{2+}$  currents. The hyperpolarization of the membrane potential by  $K^+$

current-activation and the limiting of  $\text{Ca}^{2+}$  entry by suppression of  $\text{Ca}^{2+}$  currents are tenable but unproven mechanisms for explaining blockade by opioids of neurotransmitter release and pain transmission in varying neuronal pathways (Gutstein and Akil H, 2001).

In addition, recent animal and human studies have demonstrated that both endogenous and exogenous opioids can produce opioid-mediated analgesia at sites outside the CNS. Animal models of pain have indicated the presence of peripheral opioid receptors (Ko, et al., 1998). Expression of opioid receptor mRNA and/or protein has been demonstrated in human epidermis (Bigliardi, et al., 1998) and vascular epithelium (Cadet, et al., 2000).

#### **4.2 Secondary Pharmacology**

In addition to analgesia and cough suppression, opioids can induce respiratory depression, drowsiness, change in mood, decreased gastrointestinal motility, nausea, vomiting, and miosis. Opioids also have effects on various other body systems including the neuroendocrine system, biliary tract, genito-urinary tract, the immune system, autonomic nerve system and body temperature.

#### **4.3 Safety Pharmacology**

The CNS and GI effects of HC are well-known and extensive clinical experience exists with HC. No safety pharmacology studies were conducted for NDA 202880.

### **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 CYP3A to norhydrocodone. 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 hydrocodone is via non-CYP pathways (Hutchinson, et al., 2004).

#### **5.2 Toxicokinetics**

Toxicokinetic data are discussed in the reviews of the toxicology studies.

## 6 General Toxicology

### 6.1 Single-Dose and Acute Toxicity

Acute oral toxicity studies with HC were conducted in rat and dog. The reviews of these studies by Dr. Violetta Klimek can be found in Appendix 1. The summary of the findings below (denoted by indented text) is reproduced verbatim from her review dated June 8, 2002.

Acute oral administration of hydrocodone bitartrate at doses up to 30 mg/kg/day to Sprague Dawley rats within 24 hrs period showed no adverse effects on any of the parameters evaluated during 15 days post-dose other than minor, not dose related though incidental occurrences. Acute oral administration of hydrocodone bitartrate (3, 10, and 30 mg/kg/day) to Beagle Dogs within 24 hrs period showed no test article-related adverse effects on mortality, body weights, organ weights, or gross pathology through 15 days postdose. Clinical signs noted in dogs at the highest-dose (30 mg/kg/day) included diarrhea, red discharge, emesis and languid behavior. All signs had resolved at study Day-3. Food consumption was decreased in a dose-dependent fashion, significantly at 30 mg/kg/day, beginning on study Day-1, and returned to the control levels in all test article-treated groups by study Day-4. A NOAEL was not identified in the acute studies as tissue histopathology was not performed.

### Repeat-Dose Toxicity

Ninety-day oral toxicity studies in mouse and rat were conducted with HC and a 28-day oral toxicity study in dog was conducted using the drug product. The reviews are below. Hydrocodone-related toxicities in the repeat-dose general toxicology studies were consistent with the known toxicities of other opioid agonists.

Twenty eight-day toxicity studies with HC were conducted in rat and dog. The reviews of these studies by Dr Violetta Klimek can be found in Appendix 1. The summary of the findings below (denoted by indented text) is reproduced verbatim from her review dated June 8, 2002.

In the rat repeated dose toxicity studies, no test article-related deaths occurred during the study. Clinical signs were incidental and occurred across test groups. Signs included dyspnea, hyperactivity, languid behavior, nasal discharge, pale looks, rough hair-coat, rales and thin appearance. At termination, mean total body weight gain was significantly reduced in females at 30 mg/kg/day by approx. 34% that was correlated with lower food consumption by approx. 21% in this group of female rats.

Increases in hemoglobin (+6) and mean corpuscular volume by approx. 4% were noted in males at 10 and 30 mg/kg/day, respectively. Elevated chloride (+2%) in females at 10 and 30 mg/kg/day was also assessed. The urinalysis revealed decreased urine volume (-16%) in females at 10 mg/kg/day and increased mean urine specific gravity in males at 30 mg/kg/day. Organ weights

obtained at necropsy demonstrated lower kidney weights in males at 10 and 30 mg/kg/day by 10% and 9%, respectively as well as increased weight of thymus (39%) and decreased weight of thyroid (-33%) in males at 10 mg/kg/day. The mean weight of the liver was decreased by approx. 13% in the female rats at 30 mg/kg/day. These organ weight changes were not associated with any histological or clinical pathology observations that could be related to kidney or liver function. A NOAEL was identified at 10 mg/kg/day in females and 30 mg/kg in males based on body weight changes.

The repeated-dose (oral) toxicity of hydrocodone bitartrate in dogs, revealed clinical signs of diarrhea, red discharge in the feces, and few or no feces, as well as emesis, salivation, thin appearance and languid behavior at 30 mg/kg/day (all resolved by study Day-19). Transient weight gain reduction and reduced food consumption (during the first week of treatment) were observed in males and females of all groups but one (male –control). Overall body weight was reduced in males by 11%, 12%, 19% at 3, 10, 30 mg/kg/day, respectively and in females by 2%, 9%, 13% at 3, 10, 30 mg/kg/day, respectively. In the hematology studies, lower absolute monocytes and higher prothrombin time at 3 mg/kg/day, as well as higher absolute eosinophils were noted in males at 3 and 30 mg/kg/day. At termination, increased ovary weights at 3 and 30 mg/kg/day by 94% and 74%, respectively were noted. The gross pathology examinations revealed a tan nodule in the stomach and a black discoloration of the mediastinal lymph nodes in one male at 10 mg/kg/day. At the highest dose (30 mg/kg/day), one male had a red discoloration of the thymus and one had a small thymus. Cortical atrophy of the thymus was observed in two males and one female at 30 mg/kg/day. One male at 10 mg/kg had mild thymic hypoplasia. A NOAEL was 10 mg/kg/day based on thymic pathology findings.

**Study title:** A 90-Day Oral Gavage Toxicity Study with Hydrocodone in CD-1 Mice

Study no.: 22615  
 Study report location: SN 60  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: July 16, 2008  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: Hydrocodone; Lot M08546; 99.1%

**Key Study Findings**

- Body weight gain decrease of 30% was observed in HD males
- Statistically significant decreases in mean absolute and relative liver weights in HD males; no histopathological correlates
- Statistically significant decreases in mean absolute and relative adrenal gland weights in females at all doses; no histopathological correlates

- Mild vacuolization of the renal medullary tubular epithelium in two HD females
- Although the BWG decrements appear to exceed the MTD, it appears that BWG is recovering by the end of the study likely due to the development of tolerance to effects of the opioid. These transient decrements in body weight/BWG are predicted to recover over time and should not impact long-term survival of the animal. NOTE: This study was conducted as a dose range-finding study to determine doses for the 2-year bioassay.

## Methods

Doses:	5, 25, 75, 150 mg/kg
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	HC diluted in distilled water; Vehicle= distilled water
Species/Strain:	Mouse, Fischer Crl:CD-1 (ICR) BR
Number/Sex/Group:	10/sex/group
Age:	9-10 weeks
Weight:	M: 25.0-30.0 g; F: 19.3-26.6 g
Satellite groups:	TK groups: 72/sex/group
Unique study design:	HC metabolites hydromorphone and norhydrocodone were also measured in TK
Deviation from study protocol:	Age of mice slightly higher than stated in protocol

## Observations and Results

### Mortality

Two females (one low dose and one low mid dose) were found dead one hour post injection on Study Days 32 and 2, respectively. Necropsies were performed and the cause of death was not determined. The majority of the deaths were in the TK groups. Blood samples for TK were collected from the retro-orbital sinus while under CO<sub>2</sub>/O<sub>2</sub> anesthesia on Days 0, 14, and 90. Necropsies were not performed on TK group decedents and time of death post injection was not noted. Toxicokinetic group rats were subjected to anesthesia via CO<sub>2</sub>/O<sub>2</sub> prior to orbital blood draw. Combined with respiratory depression, a well-known effect of opioids, this anesthesia procedure could have contributed to the deaths in the TK groups which occurred on days when blood draws took place. The deaths in the TK group could also be attributed to an interaction of the study drug with general complications as a result of blood sampling. The Sponsor notes that some of the deaths could have been due to gavage dosing accidents especially because the females were small, however, dosing errors were not confirmed. No evidence of perforated esophagus was seen in the two main study decedents and necropsies were not performed on TK groups.

Table 8. Summary of mortality (mouse 90-day study)

		<i>Main study</i>		<i>TK</i>	
	<i>mg/kg</i>	<i>deaths</i>	<i>study day found dead</i>	<i>deaths</i>	<i>study day found dead</i>
<i>male</i>	<b>5</b>	0	-	2	7,14
	<b>25</b>	0	-	3	9,9,14
	<b>75</b>	0	-	1	14
	<b>150</b>	0	-	4	8,29,82,90
<i>female</i>	<b>5</b>	1	32*	4	8,8,15,16
	<b>25</b>	1	2*	2	8,10
	<b>75</b>	0	-	8	3,6,6,6,6,7,7,11
	<b>150</b>	0	-	7	1,1,7,8,8,11,11

\*Necropsy performed; cause of death undetermined  
TK decedents were not necropsied

### Clinical Signs

Animals were observed individually twice daily for any signs of behavioral changes, reaction to treatment or illness. Straub tail (elevated carriage of the tail) was observed in the 25, 75 and 150 dose groups for both sexes throughout the study. Straub tail is a commonly seen pharmacologic response in mice treated with some opioids and is not considered adverse. Other clinical signs were sporadic, of low incidence and not considered relevant.

### Body Weights and Food Consumption

Animals were weighed at the time of group assignment, at the start of dosing and thereafter once weekly. Throughout the study, high-dose males showed consistently lower body weights compared to controls with statistical significance being reached at Days 13, 20 and 48 (Tables 9 and 11, Figure 1). All observed decreases were less than 10%. However, a decrease in body weight gain of 30.2% was observed for the high-dose group as compared to controls (Table 13). Group mean body weights for males are shown in Table 11. Males in the mid-, high-mid-, and high-dose groups consistently consumed slightly less food than controls with males in the low-dose group consuming slightly more than controls (Figure 3). No significant differences were observed.

High-mid- and high-dose females showed a few significant decreases in body weights as compared to controls in the beginning of the study (Tables 10 and 12, Figure 2). These body weights recovered by the middle of the study and were most likely due to development of tolerance to the opioid. Decreases in body weight gain were observed for treated female groups (low, high-mid and high) but they were not dose-dependent (Table 13). Group mean body weights for females are shown in Table 12. Food consumption for high-mid and high-dose females was significantly decreased at the first

time point only. No clear differences from controls were observed throughout the remainder of the study (Figure 4).

Table 9. Mean body weights, males: percent change from control (mouse 90-day study)

<b>Dose</b>	<b>Study Day</b>												
<b>mg/kg</b>	<b>-1</b>	<b>6</b>	<b>13</b>	<b>20</b>	<b>27</b>	<b>34</b>	<b>41</b>	<b>48</b>	<b>55</b>	<b>62</b>	<b>69</b>	<b>76</b>	<b>83</b>
<b>5</b>	0.1	1.2	-0.1	-0.4	1.8	1.5	1.6	-0.1	-0.8	0.5	-0.6	-1.1	-0.7
<b>25</b>	-0.5	-1.8	-3.9	-3.5	0.2	-1.6	0.4	0.9	0.9	1.3	0.8	-0.1	-0.5
<b>75</b>	-0.3	-0.4	-3.8	-3.7	1.5	-3.7	-7.0	0.3	0.8	0.2	-0.3	-1.5	-0.7
<b>150</b>	-0.7	-3.8	<b>-8.2**</b>	<b>-8.6**</b>	-1.9	<b>-9.1**</b>	-3.6	<b>-7.3*</b>	-4.8	-3.9	-3.5	-5.8	-4.8

Statistical analysis conducted on body weight group means; \* $p \leq 0.05$ ; \*\* $p \leq 0.01$  (Dunnett's)

Table 10. Mean body weights, females: percent change from control (mouse 90-day study)

<b>Dose</b>	<b>Study Day</b>												
<b>mg/kg</b>	<b>-1</b>	<b>6</b>	<b>13</b>	<b>20</b>	<b>27</b>	<b>34</b>	<b>41</b>	<b>48</b>	<b>55</b>	<b>62</b>	<b>69</b>	<b>76</b>	<b>83</b>
<b>5</b>	1.9	-0.2	-0.5	-0.1	2.3	1.8	0.6	0.8	0.2	3.0	2.3	2.2	1.1
<b>25</b>	0.3	-1.4	-2.9	-3.4	1.7	0.2	2.3	0.7	0.6	1.5	1.9	1.0	0.8
<b>75</b>	1.8	-4.5	<b>-6.3**</b>	<b>-7.0**</b>	0.2	-4.2	0.5	-0.1	0.1	0.0	0.2	-0.3	-0.1
<b>150</b>	-1.1	<b>-4.7*</b>	<b>-8.8**</b>	<b>-8.6**</b>	-1.6	-5.8	-1.0	-3.6	-3.7	1.0	-2.8	-2.8	-2.0

Statistical analysis conducted on body weight group means; \* $p \leq 0.05$ ; \*\* $p \leq 0.01$  (Dunnett's)

Table 11. Mean body weights in grams, males (mouse 90-day study)

<b>Dose</b>	<b>Study Day</b>												
<b>mg/kg</b>	<b>-1</b>	<b>6</b>	<b>13</b>	<b>20</b>	<b>27</b>	<b>34</b>	<b>41</b>	<b>48</b>	<b>55</b>	<b>62</b>	<b>69</b>	<b>76</b>	<b>83</b>
<b>0</b>	31.3	31.7	32.7	33.4	32.9	34.2	34.3	35.0	35.1	34.8	35.3	36.1	36.3
<b>5</b>	31.3	32.1	32.7	33.2	33.5	34.7	34.9	35.0	34.9	34.9	35.1	35.7	36.0
<b>25</b>	31.1	31.2	31.5	32.2	33.0	33.7	34.5	35.4	35.4	35.2	35.5	36.1	36.1
<b>75</b>	31.2	31.6	31.5	32.2	33.4	32.9	31.9	35.2	35.4	34.8	35.2	35.5	36.0
<b>150</b>	31.1	30.5	<b>30.1**</b>	<b>30.5**</b>	32.3	<b>31.1**</b>	33.1	<b>32.5*</b>	33.5	33.4	34.0	34.0	34.5

\*p ≤ 0.05; \*\*p ≤ 0.01 (Dunnett's)

Table 12. Mean body weights in grams, females (mouse 90-day study)

<b>Dose</b>	<b>Study Day</b>												
<b>mg/kg</b>	<b>-1</b>	<b>6</b>	<b>13</b>	<b>20</b>	<b>27</b>	<b>34</b>	<b>41</b>	<b>48</b>	<b>55</b>	<b>62</b>	<b>69</b>	<b>76</b>	<b>83</b>
<b>0</b>	23.5	24.3	25.2	26.1	25.5	26.4	26.6	26.8	27.1	26.9	27.4	27.9	27.7
<b>5</b>	24.0	24.2	25.1	26.1	26.1	26.9	26.7	27.1	27.2	27.7	28.1	28.5	28.0
<b>25</b>	23.6	23.9	24.5	25.2	26.0	26.4	27.2	27.0	27.3	27.3	28.0	28.1	27.9
<b>75</b>	23.9	23.2	<b>23.6**</b>	<b>24.3**</b>	25.6	25.3	26.7	26.8	27.2	26.9	27.5	27.8	27.7
<b>150</b>	23.2	<b>23.1*</b>	<b>23.0**</b>	<b>23.9**</b>	25.1	24.9	26.3	25.9	26.1	27.2	26.7	27.1	27.2

\*p ≤ 0.05; \*\*p ≤ 0.01 (Dunnett's)

Figure 1. Mean body weights in males (mouse 90-day study)

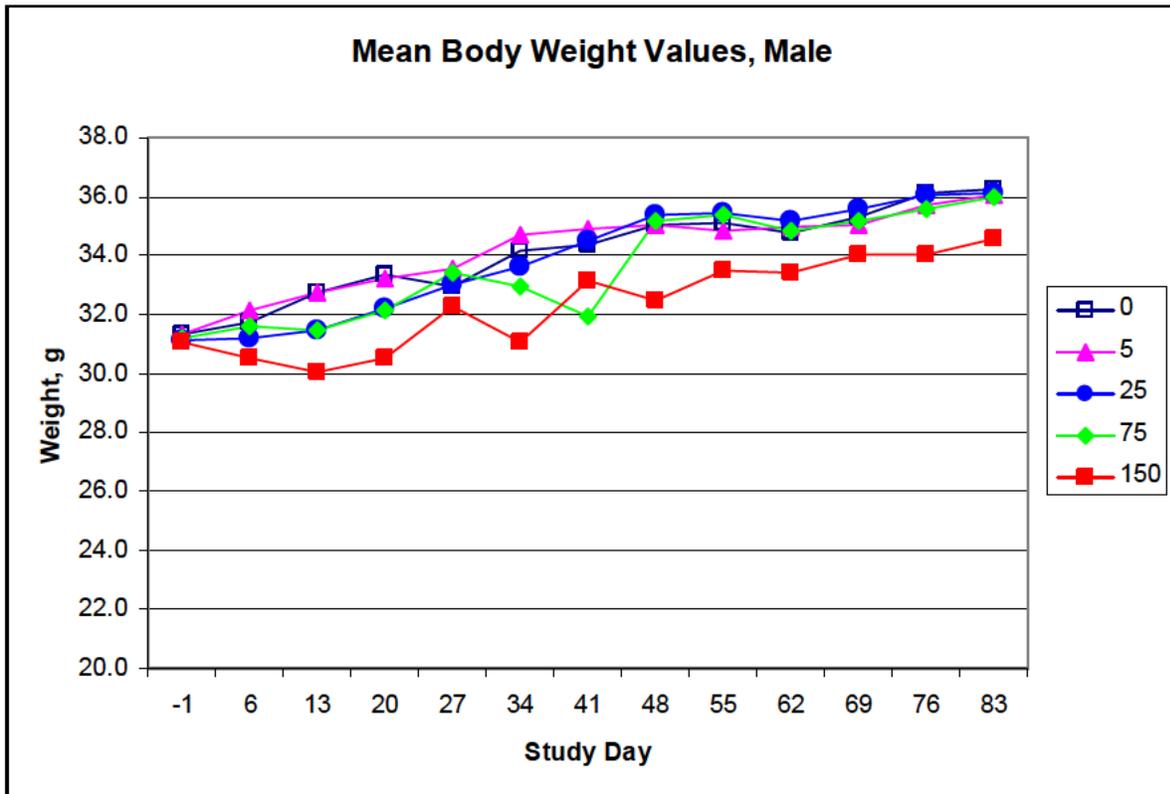


Figure 2. Mean body weights in females (mouse 90-day study)

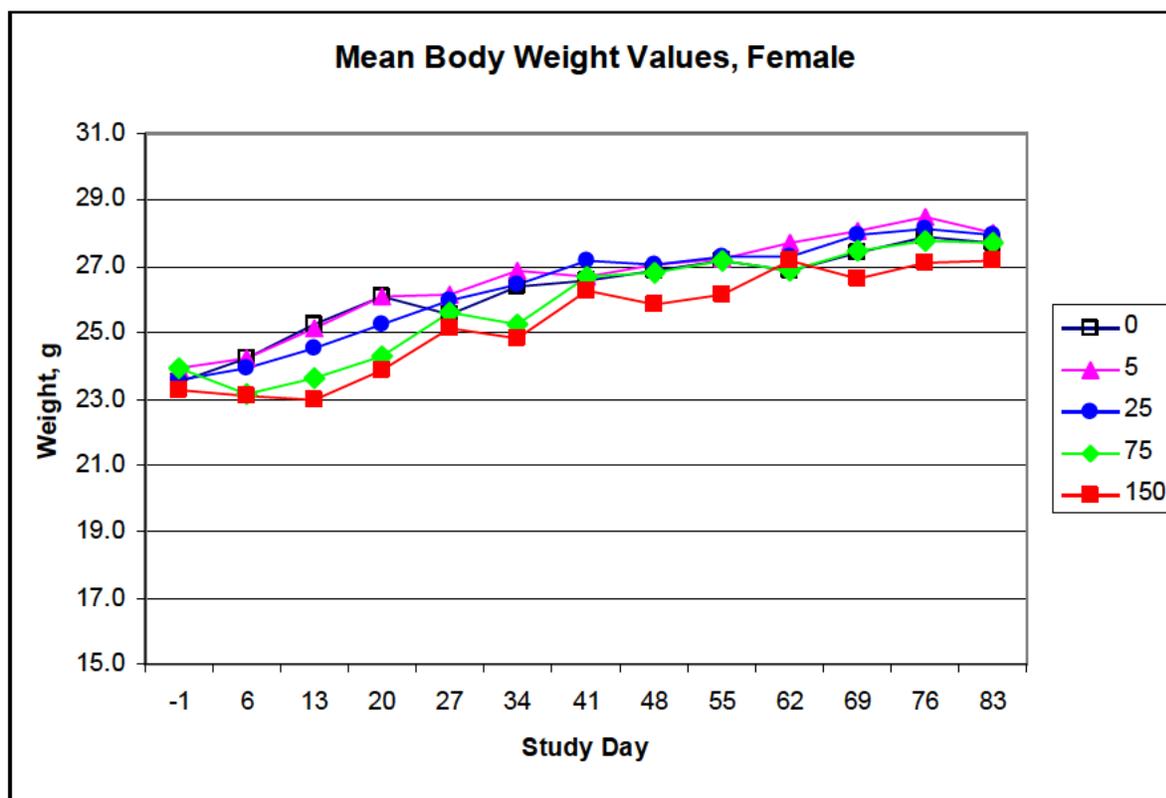


Table 13. Body weight gain (mouse 90-day study)

	<b>Dose (mg/kg)</b>	<b>Body Weight (g), Day -1</b>	<b>Body Weight (g), Day 83</b>	<b>Body Weight Gain (BWG) (g), Day -1 to Day 83</b>	<b>BWG % Change from Control</b>
<b>male</b>	<b>0</b>	31.3	36.3	5.0	-
	<b>5</b>	31.3	36.0	4.7	-5.2
	<b>25</b>	31.1	36.1	5.0	-0.2
	<b>75</b>	31.2	36.0	4.8	-3.4
	<b>150</b>	31.1	34.5	3.5	<b>-30.2</b>
<b>female</b>	<b>0</b>	23.5	27.7	4.2	-
	<b>5</b>	24.0	28.0	4.1	-3.1
	<b>25</b>	23.6	27.9	4.3	3.3
	<b>75</b>	23.9	27.7	3.8	<b>-10.7</b>
	<b>150</b>	23.2	27.2	3.9	-6.7

Figure 3. Mean food consumption, males (mouse 90-day study)

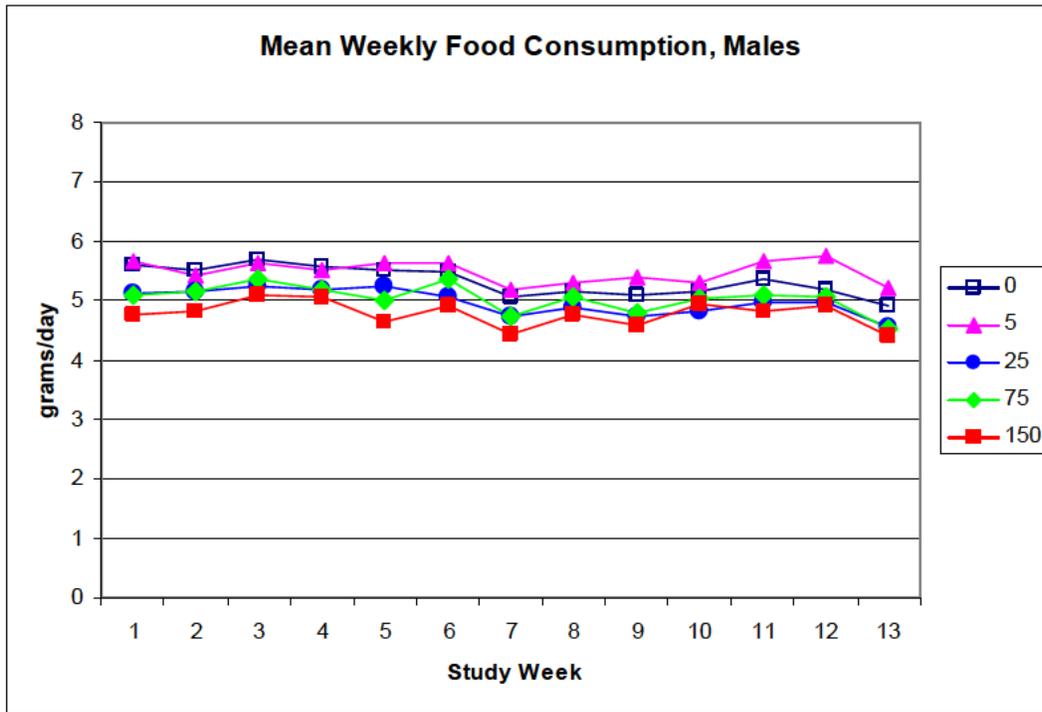
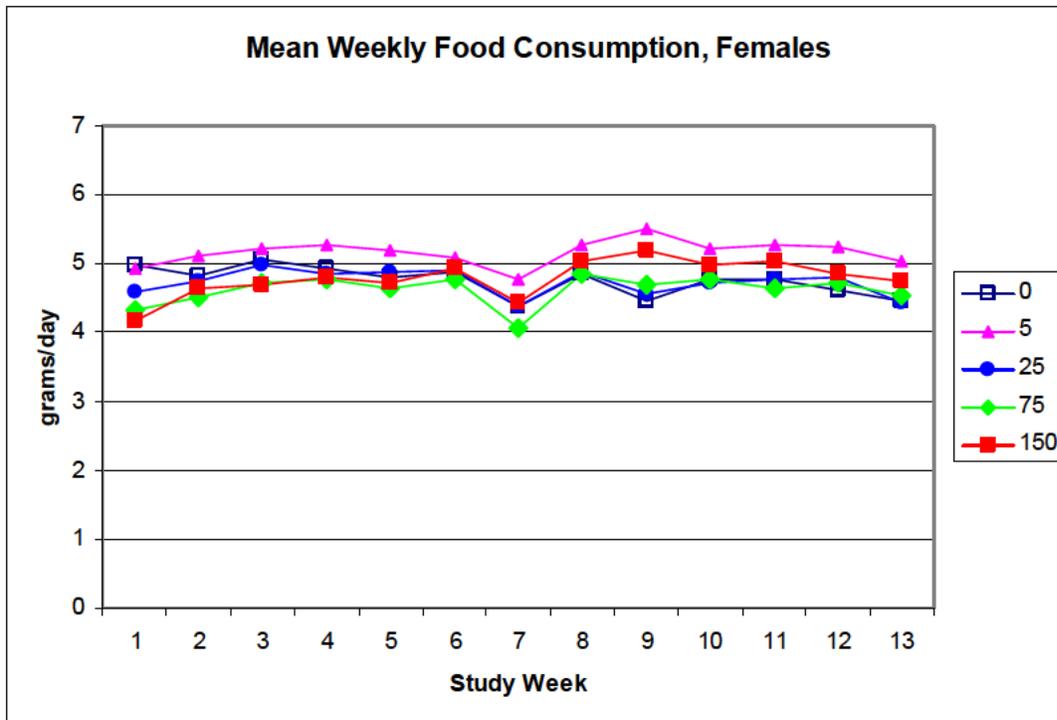


Figure 4. Mean food consumption, females (mouse 90-day study)



### Ophthalmoscopy

Ophthalmological analysis was not performed.

### Hematology

The following hematological parameters were measured at main study termination:

Hematocrit
Mean Corpuscular Volume
Mean Corpuscular Hemoglobin
Mean Corpuscular Hemoglobin Concentration
Differential Blood Count
Thromboplastin Time
Activated Partial Thromboplastin Time

No test article-related changes in hematological parameters were observed.

### Clinical Chemistry

The following clinical chemistry parameters were evaluated at main study termination:

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

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

### Urinalysis

Urinalysis was not conducted.

**Gross Pathology**

Main study mice were necropsied at the termination of the study. No test article-related macroscopic findings were observed.

**Organ Weights**

The organs weighed at the termination of the study are presented in the Histopathology Inventory. Lower mean absolute and relative liver weights were observed at the high dose in males and females with statistical significance being reached only in males (Table 14). No correlating macroscopic changes in the liver were observed. A statistically significant increase in relative brain weight only was observed in HD males. No correlating macroscopic changes in the brain were observed. Because of the lack of dose dependence, small magnitude of the decreases, and lack of histopathologic correlation, the changes in the liver and brain will not be considered toxicologically relevant or dose limiting (in terms of the carcinogenicity study). Statistically significant decreases in mean absolute and relative adrenal gland weights were observed in females at all doses (Table 14). Decreases were also observed in males at all doses although statistical significance was not reached (Table 14). No correlating macroscopic changes in the adrenals were observed in either sex. The weight changes in the adrenal glands in females will be considered test-article related.

Table 14. Absolute and relative organ weight changes (mouse 90-day study)

Group No.	1	2	3	4	5
Dose Level (mg/kg/day)	0	5	25	75	150
<b>Absolute Weights (g)</b>					
<b>Males</b>					
Final BW	35.10 ± 1.89	34.83 ± 1.83	34.46 ± 1.97	33.10 ± 1.72	31.91 <sup>1</sup> ± 1.56
Adrenals	0.01650 ± 0.00940	0.01490 ± 0.00640	0.01510 ± 0.00460	0.01520 ± 0.00430	0.01380 ± 0.00520
Brain	0.49930 ± 0.03430	0.47490 ± 0.02860	0.48310 ± 0.03710	0.51200 ± 0.03520	0.50840 ± 0.02790
Liver	1.70870 ± 0.08300	1.80610 ± 0.15190	1.74350 ± 0.18870	1.58730 ± 0.20880	1.41760 <sup>1</sup> ± 0.12290
<b>Females</b>					
Final BW	26.33 ± 1.78	26.58 ± 2.03	26.28 ± 1.17	26.11 ± 1.65	25.18 ± 1.65
Adrenals	0.02000 ± 0.00330	0.01550 <sup>2</sup> ± 0.00410	0.01450 <sup>1</sup> ± 0.00290	0.01500 <sup>2</sup> ± 0.00390	0.01420 <sup>1</sup> ± 0.00370
Brain	0.51230 ± 0.01420	0.48600 ± 0.04780	0.52460 ± 0.02670	0.50830 ± 0.03170	0.51490 ± 0.03700
Liver	1.30220 ± 0.15010	1.32760 ± 0.18160	1.22430 ± 0.16220	1.22470 ± 0.16280	1.11720 ± 0.06300
<b>Organ to Body Weight Ratios</b>					
<b>Males</b>					
Adrenals	0.4688 ± 0.2543	0.4315 ± 0.1910	0.4399 ± 0.1415	0.4597 ± 0.1232	0.4321 ± 0.1528
Brain	14.259 ± 1.197	13.672 ± 1.130	14.038 ± 1.031	15.530 ± 1.628	15.979 <sup>2</sup> ± 1.365
Liver	48.76 ± 2.58	51.98 ± 5.11	50.83 ± 6.73	47.96 ± 5.80	44.46 ± 3.63
<b>Females</b>					
Adrenals	0.7626 ± 0.1469	0.5873 <sup>2</sup> ± 0.1664	0.5542 <sup>2</sup> ± 0.1275	0.5753 <sup>2</sup> ± 0.1454	0.5652 <sup>2</sup> ± 0.1578
Brain	19.532 ± 1.367	18.378 ± 2.290	20.000 ± 1.397	19.529 ± 1.579	20.478 ± 1.218
Liver	49.48 ± 5.28	49.82 ± 4.30	46.58 ± 5.60	46.77 ± 3.80	44.53 ± 3.70
<b>Organ to Brain Weight Ratios</b>					
<b>Male</b>					
Adrenals	0.0334 ± 0.0199	0.0314 ± 0.0139	0.0318 ± 0.0116	0.0300 ± 0.0093	0.0272 ± 0.0099
Liver	3.437 ± 0.298	3.808 ± 0.298	3.633 ± 0.511	3.119 ± 0.492	2.793 <sup>1</sup> ± 0.257
<b>Females</b>					
Adrenals	0.0390 ± 0.0065	0.0327 ± 0.0113	0.0277 <sup>2</sup> ± 0.0060	0.0296 <sup>2</sup> ± 0.0082	0.0275 <sup>2</sup> ± 0.0073
Liver	2.542 ± 0.281	2.769 ± 0.541	2.337 ± 0.305	2.420 ± 0.385	2.179 ± 0.193

<sup>1</sup>Dunnett 2 Sided p < 0.01<sup>2</sup>Dunnett 2 Sided p < 0.05

**Histopathology**

<b><i>Histopathology Inventory</i></b>			
<b>Study Number</b>	22615		
<b>Species</b>	mouse		
<b><i>organ</i></b>	<b><i>assessed</i></b>	<b><i>organ</i></b>	<b><i>assessed</i></b>
Adrenal	X*	Ovary	X*
Aorta	X	Oviduct	X
Bone (femur)	X	Pancreas	X
Brain	X*	Parathyroid	X
Cecum	X	Pharynx	X
Cervix	X	Pituitary	X*
Colon	X	Prostate	X
Duodenum	X	Rectum	X
Epididymis	X	Salivary gland	X
ex-orbital lacrimal gland	X	Seminal vesicles	X
Eye	X	Skin	X
Esophagus	X	Spinal cord	X
Gross lesions	X	Spleen	X*
Harderian gland	X	Sternum	X
Heart	X*	Stomach	X
Ileum	X	Testes	X*
Jejunum	X	Thymus	X
Kidney	X*	Thyroid	X*
Larynx	X	Tongue	X
Liver	X*	Tumors, suspected tumors and associated tissues	X
Lung	X	Trachea	X
Lymph nodes, cervical	X	Urinary bladder	X
Lymph nodes mediastinal	X	Uterus	X
Mammary Gland	X	Vagina	X
Nerve	X	Zymbal gland	X
Nasal epithelium	X	Voluntary muscle	X

\*organ weighed

Adequate Battery

Yes

Peer Review

No

### Histological Findings

Mild vacuolization of the renal medullary tubular epithelium was observed in two HD females. The vacuolization was characterized by multiple small or single large intracytoplasmic vacuoles in the tubular epithelium of the outer medulla. Amorphous, faintly basophilic material was observed in some of the large vacuoles. Organ weight changes of the kidneys were not observed in either sex. The histopathological changes in the kidneys of the female HD group will be considered test-article related however due to the mild severity the finding will not be considered dose-limiting (in terms of the carcinogenicity study). No other test-article related changes in histopathology were seen.

### Special Evaluation

No special evaluations were performed.

### Toxicokinetics

Samples for TK were collected from the retro-orbital sinus (under CO<sub>2</sub>/O<sub>2</sub> anesthesia) at eight time points (predose, 1, 2, 4, 6, 8, 12, and 24 h post dose) on days 0, 14, and 90. These samples were analyzed for the concentrations of the parent drug hydrocodone as well as the major metabolite hydromorphone (Table 15). The systemic exposures of hydrocodone are roughly equal for males and females. At 25 mg/kg, systemic levels in mice were approximately equal to exposure at the human dose of 80 mg (Table 16). At 75 and 150 mg/kg, mouse exposure exceeded human exposure (80 mg) by roughly 3-fold and 5-8-fold, respectively (Table 16).

Table 15. Toxicokinetic parameters (mouse 90-day study)

**Mean TK Parameters ( $C_{max}$  and  $AUC_{0-24}$ ) for Hydrocodone, Norhydrocodone, and Hydromorphone at Days 1, 14, and 90 Following Repeated Oral Doses of Hydrocodone to CD-1 Mice**

N = 2-3/sex/time point			Hydrocodone		Norhydrocodone		Hydromorphone	
Sex	Day	Dose mg/kg/day	$C_{max}$ ng/mL	$AUC_{0-24}^*$ ng·hr/mL	$C_{max}$ ng/mL	$AUC_{0-24}^*$ ng·hr/mL	$C_{max}$ ng/mL	$AUC_{0-24}^*$ ng·hr/mL
Male	0	5	52.9	215.0	54.7	99.5	1.4	0.7
	0	25	208.0	509.0	280.0	680.0	10.4	22.7
	0	75	438.0	2025.0	641.0	2706.0	16.9	76.0
	0	150	701.0	4076.0	911.0	5779.0	27.4	199.0
	14	5	35.9	83.4	90.8	134.0	1.6	1.9
	14	25	248.0	355.0	462.0	844.0	9.4	18.3
	14	75	1955.0	5211.0	894.0	3158.0	28.6	68.7
	14	150	760.0	2827.0	2506.0	8407.0	54.6	153.0
	90	5	25.8	40.6	76.1	147.0	0.9	0.4
	90	25	296.0	466.0	612.0	1169.0	13.9	21.8
	90	75	438.0	1356.0	1634.0	4433.0	38.7	95.5
	90	150	695.0	2208.0	2288.0	8027.0	49.3	150.0
Female	0	5	50.6	112.0	56.8	108.0	3.4	4.7
	0	25	223.0	626.0	240.0	691.0	17.1	55.6
	0	75	439.0	2590.0	682.0	3121.0	38.5	183.0
	0	150	1192.0	6530.0	1771.0	9199.0	108.0	539.0
	14	5	50.2	173.0	98.2	162.0	4.6	4.9
	14	25	333.0	637.0	513.0	974.0	27.3	45.7
	14	75	725.0	1730.0	1052.0	2949.0	68.9	138.0
	14	150	1539.0	5306.0	2594.0	9760.0	125.0	303.0
	90	5	40.9	68.0	67.9	117.0	3.8	4.0
	90	25	321.0	554.0	480.0	912.0	25.6	44.1
	90	75	687.0	1846.0	1280.0	3277.0	80.5	166.0
	90	150	2307.0	5083.0	4756.0	10378.0	205.0	350.0

Table 16. Exposure ratios (plasma levels) between mouse and humans (mouse 90-day study)

	<b>Dose (mg/kg)</b>	<b>Mouse/human <math>C_{max}</math></b>	<b>Mouse/human <math>AUC_{0-24h}</math></b>
<b>Male</b>	<b>5</b>	1.0	0.1
	<b>25</b>	9.0	0.8
	<b>75</b>	20.1	3.0
	<b>150</b>	29.4	5.2
<b>Female</b>	<b>5</b>	1.1	0.1
	<b>25</b>	8.0	0.8
	<b>75</b>	19.9	2.7
	<b>150</b>	70.6	8.0

**80 mg/day** Human  $C_{max}$  = 103 ng/mL;  $AUC_{0-24h}$  = 1981 ng.h/mL (study ELN154088-203)

### Dosing Solution Analysis

The solutions utilized in the study were analyzed and found to be within acceptable concentration ranges.

### Protein Binding

The extent of hydrocodone protein binding in human plasma is not known. However, it is expected to fall in the low-to-moderate range (19 to 45%), similar to that of other opioid agents (Vicoprofen package insert).

**Study title:** A Three-Month Oral (Gavage) Toxicity Study of ELN154088 in Fischer Rats

Study no.:	214-011-02
Study report location:	SN 61
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 31, 2002
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Hydrocodone bitartrate; Lot #C16545; 99.34%

### Key Study Findings

- One HD male died on Study Day 84; cause of death was not determined
- Males showed significant body weight decreases  $\geq 10\%$  throughout the study in the HMD and HD and body weight gain decreases  $\geq 10\%$  for the MD (11.5%), HMD (21.1%) and HD (30.1%)

- Females at the HD showed a body weight gain decrease of 12.2%.
- Increases in incidences of chewing on paws, forelimbs, tails as well as increases in activity were observed in both sexes but at a higher frequency in females.
- Various changes in clinical chemistry and hematological parameters were observed but did not contribute toward the determination of the MTD.
- Although the BWG decrements appear to exceed the MTD, it appears that BWG is recovering by the end of the study likely due to the development of tolerance to effects of the opioid. These transient decrements in body weight/BWG are predicted to recover over time and should not impact long term survival of the animal. NOTE: This study was conducted as a dose range-finding study to establish doses for the 2-year rat carcinogenicity study.

## Methods

Doses:	5, 25, 50, 100 mg/kg
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	3 mL/kg
Formulation/Vehicle:	Reverse osmosis deionized water
Species/Strain:	Rat, Fischer CDF 244 CrIbr
Number/Sex/Group:	Main study: n=15
Age:	6 weeks
Weight:	M: 70 to 141 g; F: 60 to 140 g
Satellite groups:	TK: n=8 (control), n=16 (test article group)
Unique study design:	None
Deviation from study protocol:	None that compromise study

## Observations and Results

### Mortality

One HD male was found dead on Day 84. The Sponsor notes that the animal did not exhibit any unexpected clinical abnormalities prior to death and no gross necropsy or histopathological evidence to suggest the cause of mortality.

### Clinical Signs

Increases in incidences of chewing on paws, forelimbs, tails as well as increases in activity were observed in both sexes but at a higher frequency in females. Behaviors resolved in most cases by the next dosing interval. With the exception of chewing on paws, clear dose-dependency for the behaviors could not be discerned. Chewing on paws/limbs/tails did not lead to bleeding/infection/necrosis and will not be considered dose-limiting.

### Body Weights

In males, statistically significant decreases in body weights were observed at all doses. Decreases of  $\geq 10\%$  were seen throughout the study for the HMD and HD (Table 17). Group mean body weights for males are shown in Table 19. In females, statistically significant decreases in body weights were observed at all doses but none reached a magnitude of  $\geq 10\%$  (Table 18). Group mean body weights for females are shown in Table 20. Males showed body weight gain decreases  $\geq 10\%$  at the MD and above (Table 21). At the HD, most time points showed significant decreases. At all other doses, decreases were sporadic although incidence increased with dose. Females at the HD showed a body weight gain decrease of 12.2% (Table 21).

Table 17. Mean body weights, males: percent change from control (rat 90-day study)

<b>Dose</b>	<b>Study Day</b>													
	<b>0</b>	<b>7</b>	<b>14</b>	<b>21</b>	<b>28</b>	<b>35</b>	<b>42</b>	<b>49</b>	<b>56</b>	<b>63</b>	<b>70</b>	<b>77</b>	<b>84</b>	<b>89</b>
<b>5</b>	-0.8	-2.5	-2.5	-3.6	-4.8**	-3.1	-3.3	-4.5**	-4.0*	-5.4**	-6.2**	-6.1**	-7.2**	-5.6**
<b>25</b>	-1.5	-8.0**	-9.1**	-9.3**	-10.1**	-7.7**	-6.9**	-7.6**	-7.3**	-8.0**	-8.4**	-7.9**	-8.7**	-7.7**
<b>50</b>	-1.5	-11.0**	-12.1**	-13.3**	-14.1**	-12.6**	-10.2**	-11.7**	-11.3**	-12.8**	-13.1**	-12.8**	-14.0**	-13.6**
<b>100</b>	-0.8	-12.9**	-16.7**	-16.9**	-17.7**	-16.5**	-16.8**	-17.9**	-17.3**	-17.9**	-18.7**	-18.0**	-19.4**	-18.9**

Statistical analysis conducted on body weight group means; \* $p \leq 0.05$ ; \*\* $p \leq 0.01$  (Tukey-Kramer)

Table 18. Mean body weights, females: percent change from control (rat 90-day study)

<b>Dose</b>	<b>Study Day</b>													
	<b>0</b>	<b>7</b>	<b>14</b>	<b>21</b>	<b>28</b>	<b>35</b>	<b>42</b>	<b>49</b>	<b>56</b>	<b>63</b>	<b>70</b>	<b>77</b>	<b>84</b>	<b>89</b>
<b>5</b>	-1.0	-2.5	-2.2	-3.4	-3.8*	-2.5	-2.4	-3.4	-2.8	-3.3*	-2.7	-3.2*	-3.6*	-2.6
<b>25</b>	0.0	-4.1*	-3.0	-4.1*	-5.8**	-2.5	-2.4	-2.9	-1.7	-2.2	-1.6	-2.1	-3.6*	-2.1
<b>50</b>	0.0	-6.6**	-6.7**	-6.2**	-6.4**	-4.3**	-3.0	-3.4*	-3.4	-3.8**	-3.3	-3.2	-4.2**	-3.1*
<b>100</b>	0.0	-8.3**	-8.1**	-8.2**	-9.0**	-6.8**	-6.6**	-6.3**	-5.6**	-6.0**	-6.0**	-6.3**	-7.3**	-5.7**

Statistical analysis conducted on body weight group means; \* $p \leq 0.05$ ; \*\* $p \leq 0.01$  (Tukey-Kramer)

Table 19. Mean body weights in grams, males (rat 90-day study)

<b>Dose</b>	<b>Study Day</b>													
<b>mg/kg</b>	<b>0</b>	<b>7</b>	<b>14</b>	<b>21</b>	<b>28</b>	<b>35</b>	<b>42</b>	<b>49</b>	<b>56</b>	<b>63</b>	<b>70</b>	<b>77</b>	<b>84</b>	<b>89</b>
<b>0</b>	130	163	198	225	248	261	274	291	301	313	321	328	335	339
<b>5</b>	129	159	193	217	236	253	265	278	289	296	301	308	311	320
<b>25</b>	128	150	180	204	223	241	255	269	279	288	294	302	306	313
<b>50</b>	128	145	174	195	213	228	246	257	267	273	279	286	288	293
<b>100</b>	129	142	165	187	204	218	228	239	249	257	261	269	270	275

Table 20. Mean body weights in grams, females (rat 90-day study)

<b>Dose</b>	<b>Study Day</b>													
<b>mg/kg</b>	<b>0</b>	<b>7</b>	<b>14</b>	<b>21</b>	<b>28</b>	<b>35</b>	<b>42</b>	<b>49</b>	<b>56</b>	<b>63</b>	<b>70</b>	<b>77</b>	<b>84</b>	<b>89</b>
<b>0</b>	103	121	135	146	156	161	167	174	179	182	184	189	192	193
<b>5</b>	102	118	132	141	150	157	163	168	174	176	179	183	185	188
<b>25</b>	103	116	131	140	147	157	163	169	176	178	181	185	185	189
<b>50</b>	103	113	126	137	146	154	162	168	173	175	178	183	184	187
<b>100</b>	103	111	124	134	142	150	156	163	169	171	173	177	178	182

Table 21. Body weight gain (rat 90-day study)

	mg/kg	Body Weight (g), Day -1	Body Weight (g), Day 83	Body Weight Gain (BWG) (g), Day -1 to Day 83	BWG % Change from Control
<b>male</b>	<b>0</b>	130	339	209	-
	<b>5</b>	129	320	191	-8.6
	<b>25</b>	128	313	185	<b>-11.5</b>
	<b>50</b>	128	293	165	<b>-21.1</b>
	<b>100</b>	129	275	146	<b>-30.1</b>
<b>female</b>	<b>0</b>	103	193	90	-
	<b>5</b>	102	188	86	-4.4
	<b>25</b>	103	189	86	-4.4
	<b>50</b>	103	187	84	-6.7
	<b>100</b>	103	182	79	<b>-12.2</b>

Figure 5. Mean body weights in males (rat 90-day study)

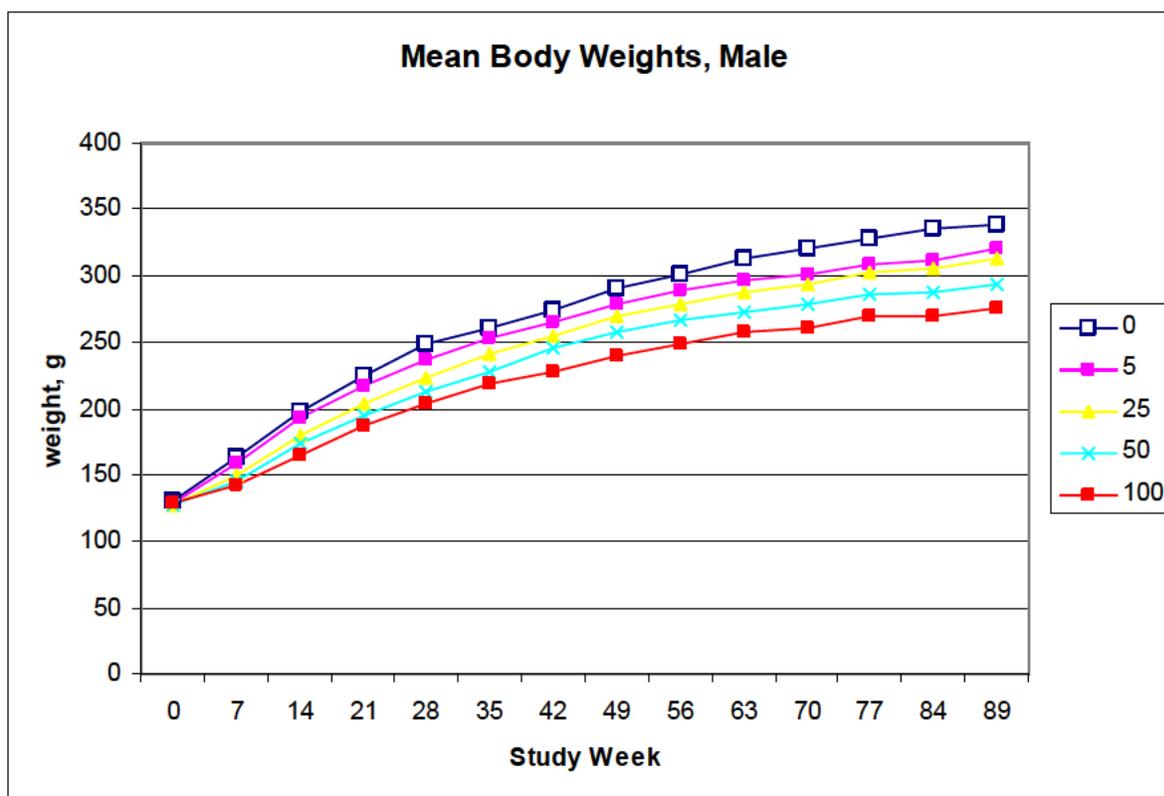
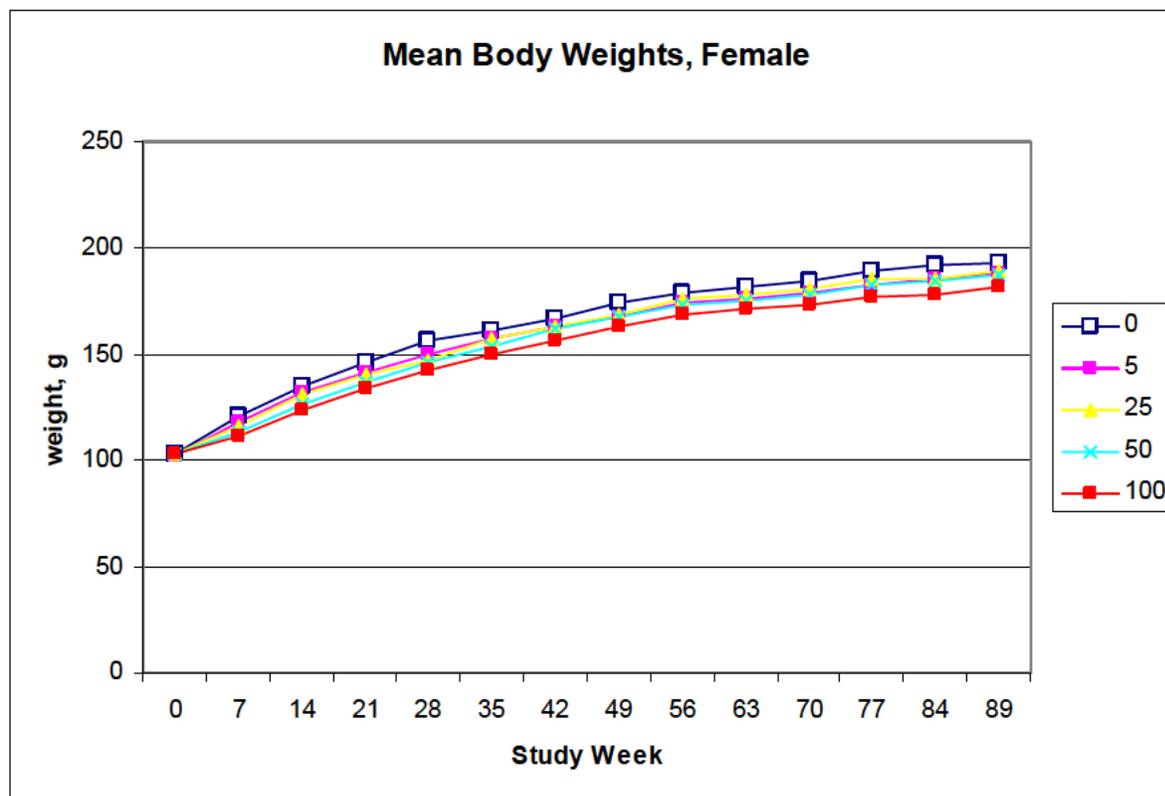


Figure 6. Mean body weights in females (rat 90-day study)



### Food Consumption

In males, decreased food consumption paralleled body weight decreases (data not shown). Overall food consumption in males at study termination was reduced 3% (LD), 3% (MD), 7% (HMD) and 11% (HD) as compared to controls. In females, decreased food consumption roughly paralleled body weight decreases but was not dose-dependent (data not shown). Overall food consumption in females at study termination was reduced 7% (LD), 2% (MD), 6% (HMD), and 8% (HD) as compared to controls.

### Ophthalmoscopy

No test article-related changes were observed.

### Hematology

In males, statistically significant dose-dependent decreases were seen in erythrocyte count and MCHC in all doses except the LD. Platelet counts at the MD and HMD were decreased and monocyte counts at the LD were decreased. Hematocrit, MCV and MCH were elevated in all dose groups except the LD. Elevations were statistically significant and dose-dependent. No changes were noted in cellular morphology.

In females, statistically significant increases were observed in hematocrit (MD and HMD) and MCV (MD, HMD, and HD). Statistically significant decreases were seen in MCHC for the MD and above. No changes were noted in cellular morphology. These changes are not thought to impact the long-term survival of the animal.

### **Clinical Chemistry**

In males, statistically significant changes included decreases in AST and ALT at the MD and higher, total protein, BUN and calcium at the HMD and HD and glucose at the MD and HD only. Significant increases included chloride and cholesterol at all doses, ALP at the MD and HMD, potassium (MD and HMD) and GGT (HMD).

In females, statistically significant decreases included total bilirubin and total protein at all doses, globulin and cholesterol at the MD and above and albumin, BUN and calcium at the HMD and HD. Sodium and ALT were decreased at the HD only. Statistically significant elevations included ALP and phosphorus in the MD and above, potassium in the HMD and HD and chloride in the MD. These changes are likely secondary to reduced food consumption and are not thought to affect the survival of the animal.

A summary of clinical pathology data is provided below.

Table 22. Significant changes in mean clinical pathology values (rat 90-day study)

Clinical Pathology	Males			Females		
	Parameter	Dose (mg/kg)	Change	Parameter	Dose (mg/kg)	Change
Clinical Chemistry	AST	25, 50, 100	↓	ALT	100	↓
	ALT	25, 50, 100	↓	Alk Phosph	25, 50, 100	↑
	Alk Phosph	50, 100	↑	Bilirubin	5, 25, 50, 100	↓
	Protein	50, 100	↓	Protein	5, 25, 50, 100	↓
	Urea N	50, 100	↓	Urea N	50, 100	↓
	Glucose	25, 100	↓	Na	100	↓
	K	25, 50	↑	K	50, 100	↑
	Cl	5, 25, 50, 100	↑	Cl	25	↑
	Ca	50, 100	↓	Ca	50, 100	↓
	Cholesterol	5, 25, 50, 100	↑	Cholesterol	25, 50, 100	↓
	Globulin	50, 100	↓	Albumin	50, 100	↓
	GGT	50	↑	Globulin	25, 50, 100	↓
				Phosphorous	25, 50, 100	↑
Hematology	Erythrocytes	25, 50, 100	↓	Hematocrit	50, 100	↑
	Hematocrit	25, 50, 100	↑	MCV	25, 50, 100	↑
	MCV	25, 50, 100	↑	MCHC	25, 50, 100	↓
	MCH	25, 50, 100	↑			
	MCHC	25, 50, 100	↓			
	Platelets	25, 50	↓			
	Monocytes	5	↓			
Urinalysis	Total Vol	100	↑	Total Vol	50, 100	↑
	pH	50, 100	↑	pH	25, 50, 100	↑

### Urinalysis

Statistically significant dose-dependent changes in urinalysis included elevated urine pH in the HMD and HD in males and MD, HMD and HD in females. Elevated total volume was observed in HD males and HMD and HD females.

### Gross Pathology

No remarkable gross findings attributed to the test article were observed in males or females.

### Organ Weights

Several changes in organ weights compared to controls were observed in males in females (Table 23). No histopathological correlates were observed in any case. These changes in organ weights do not contribute to the determination of the MTD and are not expected to effect life expectancy in a two-year bioassay.

Table 23. Significant changes in mean organ weight values (rat 90-day study)

Organ Weights	Males			Females		
	Organ	Dose (mg/kg)	Change	Organ	Dose (mg/kg)	Change
Absolute	Adrenals	25, 100	↑	Adrenals	50, 100	↓
	Heart	50, 100	↓	Ovaries	100	↓
	Kidneys	50, 100	↓	Heart	25, 50, 100	↓
	Liver	25, 50, 100	↓	Kidneys	100	↓
				Liver	50, 100	↓
Organ-to-Body	Adrenals	25, 50, 100	↑	Adrenals	50, 100	↓
	Heart	50	↓	Ovaries	100	↓
	Testes	100	↑	Heart	25, 50, 100	↓
	Brain	50, 100	↑	Kidneys	50, 100	↓
	Kidneys	50, 100	↓	Liver	25, 50, 100	↓
	Liver	5, 25, 50, 100	↓			
Organ-to-Brain	Adrenals	100	↑	Adrenals	50, 100	↓
	Heart	25, 50, 100	↓	Ovaries	100	↓
	Kidneys	25, 50, 100	↓	Heart	25, 50, 100	↓
	Liver	25, 50, 100	↓	Kidneys	100	↓
				Liver	50, 100	↓

**Histopathology**

<b><i>Histopathology Inventory</i></b>			
<b>Study Number</b>	214-011-02		
<b>Species</b>	rat		
<b><i>organ</i></b>	<b><i>assessed</i></b>	<b><i>organ</i></b>	<b><i>assessed</i></b>
Adrenal	X*	Ovary	X*
Aorta	X	Oviduct	X
Bone (femur)	X	Pancreas	X
Brain	X*	Parathyroid	X
Cecum	X	Pharynx	X
Cervix	X	Pituitary	X*
Colon	X	Prostate	X
Duodenum	X	Rectum	X
Epididymis	X	Salivary gland	X
ex-orbital lacrimal gland	X	Seminal vesicles	X
Eye	X	Skin	X
Esophagus	X	Spinal cord	X
Gross lesions	X	Spleen	X*
Harderian gland	X	Sternum	X
Heart	X*	Stomach	X
Ileum	X	Testes	X*
Jejunum	X	Thymus	X
Kidney	X*	Thyroid	X*
Larynx	X	Tongue	X
Liver	X*	Tumors, suspected tumors and associated tissues	X
Lung	X	Trachea	X
Lymph nodes, cervical	X	Urinary bladder	X
Lymph nodes mediastinal	X	Uterus	X
Mammary Gland	X	Vagina	X
Nerve	X	Zymbal gland	X
Nasal epithelium	X	Voluntary muscle	X

\*organ weighed

Adequate Battery

Yes

Peer Review

No

### Histological Findings

A dose related incidence of trace to minimal intracytoplasmic hemochrome pigment in the liver was observed in males and females including control females. No correlative changes were observed in the bone marrow or spleen. The toxicologic significance of this finding is unknown. No other test article-related findings were seen.

### Special Evaluation

Bone marrow smears were assessed in the control and HD groups. No differences in myeloid count, erythroid count and myeloid/erythroid ratio were observed.

### Toxicokinetics

Plasma samples were collected on Study Day 0 and 89 at 0.5, 1, 2, 4, 6, 12, and 24 h post dose from satellite rats. These samples were analyzed for the concentrations of the parent drug hydrocodone as well as the major metabolite hydromorphone (Table 24). The systemic exposures of hydrocodone are roughly equal for males and females. All rat exposures of hydrocodone (up to 100 mg/kg) are below human systemic exposure at the human dose of 80 mg (Table 24).

Table 24. Summary of toxicokinetic parameters (rat 90-day study)

Dose (mg/kg/day)	Sex	Hydrocodone			Hydromorphone		
		C <sub>max</sub> (ng/mL)	T <sub>max</sub> (h)	AUC <sub>(0-24)</sub> (ng•hr/mL)	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (h)	AUC <sub>(0-24)</sub> (ng•hr/mL)
Day 0							
5	Male	6.77	0.5	4.36	8.96	0.5	18.4
	Female	4.35	0.5	2.17	3.21	0.5	1.60
25	Male	18.3	0.5	26.3	28.9	0.5	120
	Female	43.0	0.5	64.0	31.1	0.5	74.4
50	Male	57.5	0.5	113	50.2	0.5	268
	Female	59.6	0.5	84.3	48.2	0.5	203
100	Male	33.5	0.5	179	29.4	1	340
	Female	18.5	0.5	96.0	17.2	0.5	216
Day 89							
5	Male	12.6	0.5	7.58	14.0	0.5	9.25
	Female	15.1	0.5	7.53	9.53	0.5	5.09
25	Male	44.7	0.5	38.9	35.5	0.5	66.2
	Female	52.4	0.5	130	30.1	0.5	26.1
50	Male	69.2	0.5	167	44.1	0.5	254
	Female	62.3	0.5	182	34.3	0.5	114
100	Male	56.4	0.5	365	48.0	0.5	337
	Female	58.0	0.5	365	39.6	0.5	215

Table 25. Exposure ratios (plasma levels) between rat and human (rat 90-day study)

	<b>Dose, mg/kg</b>	<b>Mouse/human <math>C_{max}</math></b>	<b>Mouse/human <math>AUC_{0-24h}</math></b>
<b>Male</b>	<b>5</b>	0.26	0.01
	<b>25</b>	0.78	0.05
	<b>50</b>	1.10	0.21
	<b>100</b>	1.01	0.35
<b>Female</b>	<b>5</b>	0.24	0.01
	<b>25</b>	0.80	0.08
	<b>50</b>	0.94	0.15
	<b>100</b>	0.95	0.29

**80 mg/day** Human  $C_{max}$  = 103 ng/mL;  $AUC_{0-24h}$  = 1981 ng.h/mL (study ELN154088-203)

### Dosing Solution Analysis

The solutions utilized in the study were analyzed and found to be within acceptable concentration ranges.

### Protein Binding

The extent of hydrocodone protein binding in human plasma is not known. However, it is expected to fall in the low-to-moderate range (19 to 45%), similar to that of other opioid agents (Vicoprofen package insert).

### Study title: A 28-day oral toxicity study of ELN 154088 (HC ER) capsules in beagle dogs

Study no.:	214-018-02
Study report location:	EDR 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 2, 2002
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Hydrocodone bitartrate; RD070204; 97%

### Key Study Findings

- Reductions were seen in body weight of 3%, 7% and 12% in males and 2%, 7% and 10% in females at 2.5, 10, and 30/20 mg/kg, respectively
- One 30/20 mg/kg male was euthanized on Study Day 16 because of HC-related clinical signs (exaggerated pharmacology)

- **Significant elevations in glucose were seen in 30/20 mg/kg females with minimal to mild atrophy of the acinar cells of the pancreas seen across doses in both males and females**
- **Minimal to mild increases in vacuolization of hepatocytes was seen in a few animals across doses**
- **Edema/inflammation of the gallbladder wall and a small gallbladder that lacked a normal sized lumen were observed in two 30/20 mg/kg females**
- **A nodular focus of lymphoid hyperplasia was seen in the spleen in one 20/30 mg/kg female**
- **The NOAEL for this study is the mid dose 10 mg/kg for both males and females**

Methods	
Doses:	total dose: 2.5, 10, 30/20 mg/kg
Frequency of dosing:	2 doses/day
Route of administration:	oral
Dose volume:	NA
Formulation/Vehicle:	ELN154088 (clinical formulation) strengths of 10 mg and 40 mg HC; Vehicle= placebo capsule
Species/Strain:	Dog, beagle
Number/Sex/Group:	4/sex/group
Age:	5.5 months
Weight:	5.3-7 kg
Satellite groups:	none
Unique study design:	high dose was decreased due to adverse events in several dogs*
Deviation from study protocol:	none that effect integrity of the study

\*One male and two females in the high-dose group displayed adverse events which necessitated a reduction of the dose from three capsules (30 mg/kg) to two capsules (20 mg/kg). The placebo group was also reduced from three capsules to two capsules.

## Observations and Results

### Mortality

One high dose male was euthanized on Day 16. The dog displayed clinical signs including decreased activity, unkempt appearance, labored breathing, no feces, cool to the touch, thin appearance, wobbly gait, tremors, dehydration, urine and fecal stains, rigid upon handling, vomiting and no food consumption. Other males and females at the mid and high dose exhibited similar signs (although less severe). Gross necropsy findings included dehydration, matting of the haircoat, discoloration on tongue (possibly necrosis), small thymus, brown paste-like material in the cecum, watery fecal material in the colon, reddened trachea and colon, stomach lesion, mottled kidney, distended gall bladder with thick dark green bile containing yellow particulate, tan area on lung, and pituitary cysts. None of the gross pathologic findings were observed in any other animal.

Because of the similarities of the clinical signs to the other dose groups and the typical pharmacologic effects of opioids, the death will be considered test article-related.

### Clinical Signs

Remarkable clinical observations in both males and females at the mid and high dose groups included decreased activity, wobbly gait, tremors, dehydration and vomiting. Salivation, thin appearance, no apparent food consumption and few feces were seen in the high dose group only.

### Body Weights

During the first week of dosing, there was a dose-dependent reduction in BW in both sexes at the mid and high doses (Figures 7 and 8). Following the first week the mid and high dose groups (both sexes) gained weight but as a result of the early weight loss, the groups showed lower body weights as compared to control at the termination of the study. At termination, male body weights were reduced by approximately 3%, 7% and 12% at the low, mid and high doses, respectively. Females at termination showed reductions in body weight of approximately 2%, 7% and 10% at the low, mid and high doses, respectively.

Figure 7. Mean body weights in males (28-day dog study)

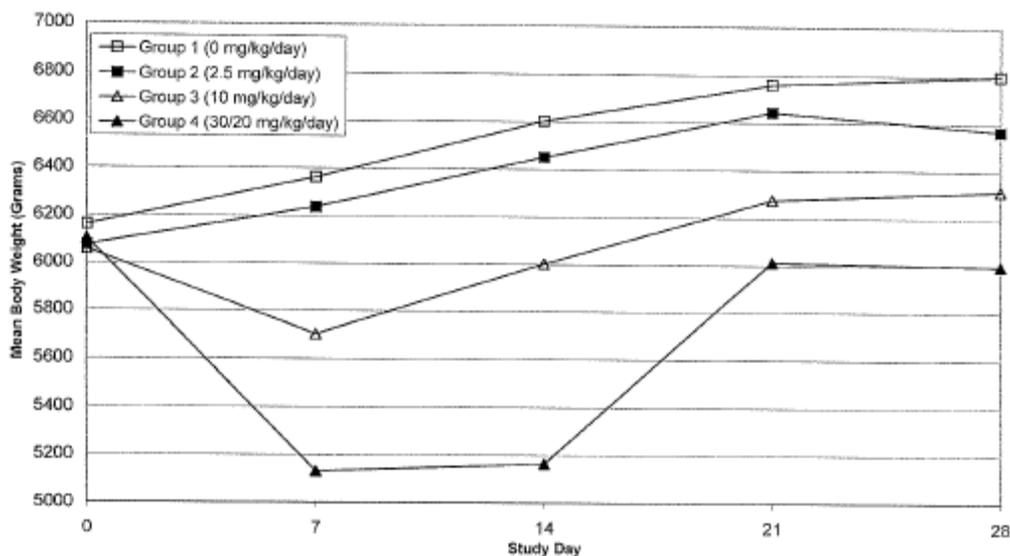
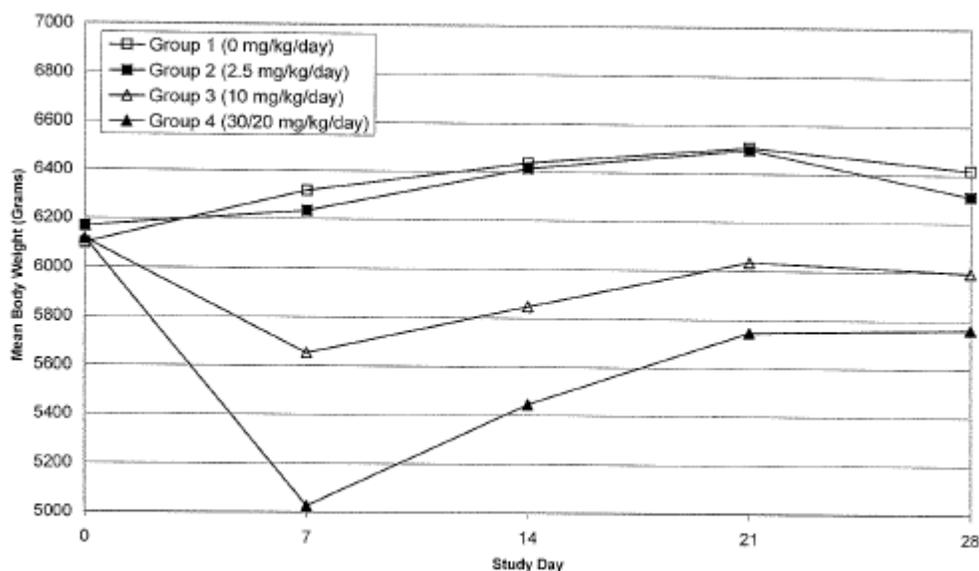


Figure 8. Mean body weights in females (28-day dog study)



**Food Consumption**

Decreased food consumption corresponded with the early reductions in BW in the first week in both sexes at the mid and high doses. By the end of the study, decreases were still observed for these groups by of a lesser magnitude than observed in the first week of the study.

**Ophthalmoscopy**

No test article-related effects on ophthalmoscopic parameters were seen.

**ECG and Blood Pressure**

No test article-related effects on ECG/BP parameters were seen.

**Hematology**

Hematocrit
Mean Corpuscular Volume
Mean Corpuscular Hemoglobin
Mean Corpuscular Hemoglobin Concentration
Differential Blood Count
Thromboplastin Time
Activated Partial Thromboplastin Time

No test article-related effects on hematology parameters were seen.

## Clinical Chemistry

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

Glucose was dose-dependently elevated in males and females and reached statistical significance in the high dose females (Table 26). The historical control mean is 102.5 mg/dL with a range of 78-125. Pancreatic changes were noted histopathologically.

Table 26. Mean glucose levels (mg/dL +/-SD) in females (28-day dog study)

	0	2.5 mg/kg	10 mg/kg	30/20 mg/kg
day -4	102 +/-7.4	110 +/-3.2	102 +/- 6.1	104 +/-1.4
days 28-30	99 +/-7.2	111 +/-2.0	122 +/-17.5	128* +/-18

\*P<sub>≤</sub>0.05

## Urinalysis

No test article-related effects on urinalysis parameters were seen.

## Gross Pathology

Hyperkeratosis of the footpads was noted in the mid and high dose groups. Necropsy findings of the one premature descendant are discussed in the mortality section. No other test article-related findings were noted.

## Organ Weights

Kidney organ to BW ratios at the high dose group in males were significantly higher than control (0.488 +/-0.048 vs 0.605 +/-0.034). Significant weight loss was noted in the high dose group and no significant differences between the kidney to brain ration was observed. No microscopic changes were noted in the kidney. The finding will not be considered toxicologically relevant.

### **Histopathology**

Mild hyperkeratosis of the footpad was observed in both males in females at doses  $\geq 10$  mg/kg. It is thought that this increase in keratin is due to inactivity and less wearing down of the keratin from contact on the cage or exercise area flooring. It is not considered toxicologically relevant. Minimal to mild atrophy of the acinar cells of the pancreas was seen in all dose groups with three female dogs in the high-dose group showing mild atrophy. Minimal to mild increases in vacuolization of hepatocytes was seen. The pancreas and liver findings will be considered test article-related but are probably secondary to decreased food consumption. Edema and inflammation of the gallbladder wall and a small gallbladder that lacked a normal sized lumen were observed in two high dose females. A nodular focus of lymphoid hyperplasia was seen in the spleen in one high dose female. This lesion has been reported before in dogs but since it occurred at the high dose it will be considered test article-related. Histopathologic lesions are summarized in Table 27.

<b>Histopathology Inventory</b>			
<b>Study Number</b>		214-018-02	
<b>Species</b>		dog	
<b>organ</b>	<b>assessed</b>	<b>organ</b>	<b>assessed</b>
Adrenal	X*	Ovary	X*
Aorta	X	Oviduct	X
Bone (femur)	X	Pancreas	X
Brain	X*	Parathyroid	X
Cecum	X	Pharynx	X
Cervix	X	Pituitary	X*
Colon	X	Prostate	X
Duodenum	X	Rectum	X
Epididymis	X	Salivary gland	X
ex-orbital lacrimal gland	X	Seminal vesicles	X
Eye	X	Skin	X
Esophagus	X	Spinal cord	X
Gross lesions	X	Spleen	X*
Harderian gland		Sternum	X
Heart	X*	Stomach	X
Ileum	X	Testes	X*
Jejunum	X	Thymus	X
Kidney	X*	Thyroid	X*
Larynx	X	Tongue	X
Liver	X*	Tumors, suspected tumors and associated tissues	X
Lung	X	Trachea	X
Lymph nodes, cervical	X	Urinary bladder	X
Lymph nodes mediastinal	X	Uterus	X
Mammary Gland	X	Vagina	X
Nerve	X	Zymbal gland	
Nasal epithelium	X	Voluntary muscle	X

\*weighed organs

Adequate Battery: Yes

Peer Review: Yes

Table 27. Histopathologic findings (28-day dog study)

organ	finding	Males, 4/group				Females, 4/group			
		0	2.5	10	30/20	0	2.5	10	30/20
footpad	hyperkeratosis,	0	0	1	1	0	0	1	2

	mild								
pancreas	acinar cell atrophy, min	0	0	1	1	0	1	1	1
	acinar cell atrophy, mild	0	1	0	1	0	0	1	3
	inflammation, min	0	0	0	0	0	1	0	0
	inflammation, chronic active, min	0	0	0	0	0	0	0	1
liver	vacuolar change, periportal, min	0	1	1	1	0	0	1	2
	vacuolar change, periportal, mild	0	0	0	0	0	0	0	1
gallbladder	edema, mod	0	0	0	0	0	0	0	1
	inflammation, mild	0	0	0	0	0	0	0	1
	malformation	0	0	0	0	0	0	0	1
spleen	nodular focus of lymphoid hyperplasia	0	0	0	0	0	0	0	1

### Toxicokinetics

Samples were collected at 0, (prior to the first dose only) 1, 2, 4, 6, 8, and 12 h after the first and second doses on Days 0 and 26. The two doses were 12 h apart. The table below summarizes TK values after the morning dose (mg/kg dose is half of the total daily dose). The  $T_{max}$  for HC ranged between 1-4 h. Generally,  $C_{max}$  and AUC values increased dose-proportionally in males and females on Study Day 0 and Study Day 26. Slight accumulation of HC was observed with repeat dosing. No gender differences were observed in this study.

Table 28. Summary of toxicokinetics (reproduced from NDA)

Dose (mg/kg/dose)	Sex	Hydrocodone				Hydromorphone			
		C <sub>max</sub> (ng/mL)	T <sub>max</sub> (h)	AUC <sub>(0-12)</sub> (ng•hr/mL)	t <sub>half</sub> (h)	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (h)	AUC <sub>(0-12)</sub> (ng•hr/mL)	t <sub>half</sub> (h)
Study Day 0 (AM)									
1.25	M	7.88	1	37.4	a	7.27	4	45.6	a
	F	10.3	2	46.7	a	7.82	4	62.0	11.4
5.0	M	36.9	4	280	4.19	37.0	6	356	9.97
	F	38.0	4	275	5.02	33.8	6	318	11.1
15.0	M	107	1	802	6.44	65.4	4	647	14.8
	F	111	1	626	6.38	56.9	6	594	22.9
Study Day 26 (AM)									
1.25	M	10.5	1	58.0	1.44	12.2	6	107	4.91
	F	12.1	2	70.9	1.46	9.76	4	78.6	2.79
5.0	M	56.5	1	324	3.80	45.9	4	456	6.95
	F	44.8	4	278	2.58	48.1	4	422	6.19
10.0 <sup>b</sup>	M	145	2	729	3.40	77.1	6	698	5.93
	F	167	1	835	2.72	109	4	1032	6.59

<sup>a</sup> Fewer than three points on a straight line in the log-linear phase.

<sup>b</sup> Dose reduced on study day 8.

## Dosing Solution Analysis

Doses used were acceptable.

## 7 Genetic Toxicology

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

#### Study title: Bacterial Reverse Mutation Assay with Hydrocodone Bitartrate

Study no.: 214-029-01 (CRO # (b) (4))  
 Study report location: EDR 4.2.3.3.1  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: August 21, 2001  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: Hydrocodone bitartrate, 1F001, 98.4%

### Key Study Findings

- Hydrocodone bitartrate is not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 and *E. coli* strain WP2uvrA 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: 75, 200, 600, 1800 and 5000 mcg +/- S9

Basis of concentration selection: initial study used 2.5 – 5000 mcg

Negative control: distilled water

Positive control: see Table 29

Formulation/Vehicle: distilled water (positive controls in DMSO)

Incubation & sampling time: 48-73 h at 37 degrees

Table 29. Positive controls (reproduced from NDA)

Strain	S9	Positive Control	Concentration ( $\mu\text{g}/\text{plate}$ )
<i>Salmonella</i> Strains	Rat	2-aminoanthracene	1.0
WP2 <i>uvrA</i>			10.0
TA98	None	2-nitrofluorene	1.0
TA100, TA1535		sodium azide	1.0
TA1537		9-aminoacridine	75.0
WP2 <i>uvrA</i>		methyl methanesulfonate	1,000.0

**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 *uvrA* in either the presence or absence of S9. The results of the confirmative assay are summarized in Table 30. 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 30).

Table 30. Summary of Ames assay results (reproduced from NDA)

S9 Activation	Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 <i>uvrA</i>
No	Vehicle	20 ± 2	222 ± 2	13 ± 3	5 ± 3	14 ± 3
	75	26 ± 1	228 ± 13	13 ± 5	6 ± 2	15 ± 3
	200	17 ± 4	242 ± 33	16 ± 5	6 ± 3	14 ± 3
	600	16 ± 9	207 ± 14	15 ± 2	4 ± 2	11 ± 2
	1800	24 ± 2	230 ± 15	21 ± 7	3 ± 2	8 ± 2
	5000	19 ± 5	195 ± 32	22 ± 5	5 ± 2	11 ± 1
	Positive	122 ± 9	687 ± 15	204 ± 15	538 ± 134	627 ± 241
	Yes	Vehicle	21 ± 3	184 ± 21	9 ± 3	5 ± 3
75		14 ± 1	211 ± 11	10 ± 1	6 ± 1	15 ± 1
200		19 ± 3	208 ± 23	10 ± 3	6 ± 3	11 ± 3
600		18 ± 3	182 ± 16	14 ± 5	6 ± 1	14 ± 2
1800		27 ± 5	209 ± 6	14 ± 3	7 ± 1	10 ± 1
5000		26 ± 4	197 ± 42	18 ± 5	6 ± 1	6 ± 2
Positive		676 ± 161	1589 ± 363	119 ± 5	105 ± 9	70 ± 12

(Average Revertants Per Plate ± Standard Deviation)

Vehicle = Vehicle Control

Positive = Positive Control

Plating aliquot: 100 µL

## 7.2 In Vitro Assays in Mammalian Cells

### Study title: In Vitro Mammalian Chromosome Aberration Test

Study no.: 214-030-01 (CRO # (b) (4))  
 Study report location: EDR 4.2.3.3.1  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: August 20, 2001  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: Hydrocodone bitartrate, 1F001, 98.4%

Table 31. Study design for in vitro chromosome aberration test

Assay	Activation	Treatment Time	Recovery Time	Positive Control (µg/mL)	Test Article (µg/mL)
1	- S9	4 hr	16 hr	MMC (0.1, 0.2)	1250, 2500, 5000
2	+S9	4 hr	16 hr	CP (10, 20)	
3	- S9	20 hr	0 hr	MMC (0.1, 0.2)	

MMC=mitomycin C; CP=cyclophosphamide

### Key Study Findings

- **Hydrocodone bitartrate is clastogenic in the presence of metabolic activation in the in vitro chromosome aberration assay. No evidence of clastogenicity was observed in the absence of metabolic activation.**

#### Methods

Cell line:	Chinese Hamster Ovary (CHO)
Concentrations in definitive study:	1250, 2500, and 5000 mcg
Basis of concentration selection:	preliminary toxicity assay using 650-5000 mcg/mL
Negative control:	distilled water
Positive control:	see Table 31
Formulation/Vehicle:	distilled water
Incubation & sampling time:	4 and 20 h without S9, 4 h with S9

#### Study Validity

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

#### Results

Hydrocodone induced chromosome breaks in CHO cells in the presence of S9 (Table 32). No increases above control were seen for structural or numerical aberrations with HC in absence of S9 or for numerical aberrations in the presence of S9 (Table 32). No toxicity (> 50% cell growth inhibition) was observed in any treatment group in either the presence or absence of S9 (Table 32). Hydrocodone was soluble up to the limit dose of 5000 mcg/mL. In presence of S9 at concentrations of 1250, 2500, and 5000 structural aberrations of 7.0%, 11.5%, and 11.5%, respectively, were observed. Although not dose dependent, these values were statistically significant and outside of the historical control range of 0.0-6.5%. The positive control, cyclophosphamide (10 mcg/mL), induced 18% numerical aberrations. It is concluded that HC induces structural chromosomal aberrations in CHO cells in the presence of S9.

Table 32. Summary of chromosomal aberration assay results (reproduced from NDA)

Treatment	S9 Activation	Treatment Time <sup>a</sup>	Cell Growth		Aberrations Per Cell <sup>b</sup> (Mean ± SD)	Cells With Aberrations		
			Inhibition (%)	Mean Mitotic Index		Numerical (%)	Structural (%)	
Water	-	4	NA	8.1	200	0.020 ±0.140	2.0	2.0
Hydrocodone Bitartrate								
1250	-	4	4	6.2	200	0.045 ±0.231	2.5	4.0
2500	-	4	4	7.0	200	0.035 ±0.184	1.0	3.5
5000	-	4	15	6.6	200	0.070 ±0.419	1.0	4.5
Mitomycin C 0.2	-	4	-33	7.1	100	0.780 ±1.397	1.5	44.0**
Water	+	4	NA	7.8	200	0.020 ±0.140	4.5	2.0
Hydrocodone Bitartrate								
1250	+	4	6	7.3	200	0.075 ±0.282	2.5	7.0*
2500	+	4	11	6.9	200	0.120 ±0.341	2.0	11.5**
5000	+	4	23	6.1	200	0.130 ±0.379	2.5	11.5**
Cyclophosphamide 10	+	4	-24	5.8	100	0.280 ±0.697	1.5	18.0**
Water	-	20		8.1	200	0.015 ±0.122	4.0	1.5
Hydrocodone Bitartrate								
1250	-	20	14	6.7	200	0.030 ±0.171	3.0	3.0
2500	-	20	20	7.1	200	0.040 ±0.196	3.0	4.0
5000	-	20	26	7.3	200	0.035 ±0.184	2.5	3.5
Mitomycin C 0.1	-	20	-11	6.8	100	0.790 ±1.149	2.5	45.0**

<sup>a</sup> Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.

<sup>b</sup> Severely damaged cells were counted as 10 aberrations.

\* p≤0.05; \*\* p≤0.01; using Fisher's exact test.

NA = Not Applicable

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

#### Study title: Mammalian Erythrocyte Micronucleus Test with Hydrocodone Bitartrate

Study no: 214-031-01 (CRO # [REDACTED] (b) (4))  
Study report location: EDR 4.2.3.3.2  
Conducting laboratory and location: [REDACTED] (b) (4)  
Date of study initiation: August 20, 2001  
GLP compliance: Yes  
QA statement: Yes  
Drug, lot #, and % purity: Hydrocodone bitartrate, 1F001, 98.4%

#### Key Study Findings

- **Hydrocodone bitartrate is not clastogenic in the in vivo bone marrow micronucleus assay under the conditions of the assay conducted.**

#### Methods

Doses in definitive study: 15, 30, 60 mg/kg  
Frequency of dosing: single dose, bone marrow collected 24 h post dose for all groups and 48 h post-dose for vehicle and 60 mg/kg group only  
Route of administration: IP  
Dose volume: 20 mL/kg  
Formulation/Vehicle: water  
Species/Strain: Mouse/ICR  
Number/Sex/Group: 5/sex/group  
Satellite groups: 5/sex/group for vehicle and dose groups for TK  
Basis of dose selection: DRF study using 1-1000 mg/kg  
Negative control: water  
Positive control: cyclophosphamide (50 mg/kg)

#### 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, and
- positive controls exhibited appropriate responses

#### Results

Dose ranging studies tested doses between 1 and 1000 mg/kg. Extensive lethality was observed at doses >100 mg/kg. Mortality was observed in one male mouse at 80 mg/kg. The observed clinical signs were typical of an opioid agonist and included hyperactivity and erect tails in both sexes of all dose groups and lethargy in males at 80 mg/kg. The MTD was determined to be 60 mg/kg. Doses used in the MN assessment were 15, 30, and 60 mg/kg. No differences in the number of polychromatic erythrocytes with micronuclei were observed between vehicle and HC treatment groups at either the 24 h or 48 h time point (Table 33). The positive control produced a clear and significant increase in the number of polychromatic erythrocytes with micronuclei compared to vehicle control (Table 33). At the doses tested, HC is not considered to be a clastogen in the MN assay. On a body surface area basis, the dose of 60 mg/kg HC in the mouse is the human equivalent to the daily dose of 300 mg per 60 kg human (5 mg/kg). At the MTDD of 3 g for HC in humans, the exposure margin based on body surface area is 0.1 fold.

Table 33. Summary of bone marrow MN assay results (reproduced from NDA)

Treatment	Sex	Time (hr)	Number of Mice	PCE/1000 Erythrocytes (Mean +/- SD)	Change from Control (%)	Micronucleated Polychromatic Erythrocytes		
						Micronuclei per 1000 PCEs (Mean +/- SD)	Micronuclei per PCEs Scored	
Water (A.C.S., 99.5%)								
20 mL/kg	M	24	5	0.445 ± 0.02	---	0.4 ± 0.22		4 / 10000
	F	24	5	0.463 ± 0.01	---	0.2 ± 0.27		2 / 10000
Hydrocodone Bitartrate								
15 mg/kg	M	24	5	0.473 ± 0.04	6	0.6 ± 0.22		6 / 10000
	F	24	5	0.475 ± 0.07	3	0.5 ± 0.00		5 / 10000
30 mg/kg	M	24	5	0.482 ± 0.02	8	0.2 ± 0.27		2 / 10000
	F	24	5	0.429 ± 0.02	-7	0.4 ± 0.22		4 / 10000
60 mg/kg	M	24	5	0.442 ± 0.03	-1	0.4 ± 0.42		4 / 10000
	F	24	5	0.476 ± 0.03	3	0.4 ± 0.22		4 / 10000
Cyclophosphamide								
50 mg/kg	M	24	5	0.342 ± 0.02	-23	22.6 ± 3.60		*226 / 10000
	F	24	5	0.340 ± 0.02	-27	26.0 ± 2.35		*260 / 10000
Water (A.C.S., 99.5%)								
20 mL/kg	M	48	5	0.460 ± 0.05	---	0.4 ± 0.42		4 / 10000
	F	48	5	0.448 ± 0.07	---	0.5 ± 0.35		5 / 10000
Hydrocodone Bitartrate								
60 mg/kg	M	48	5	0.497 ± 0.06	8	0.3 ± 0.27		3 / 10000
	F	48	5	0.458 ± 0.02	2	0.5 ± 0.00		5 / 10000

PCE = Polychromatic Erythrocyte

\* Statistically significant difference from vehicle control,  $p \leq 0.05$  (Kastenbaum-Bowman Tables)

The Applicant conducted the standard battery of genetic toxicology studies for hydrocodone bitartrate. Hydrocodone tested negative in the in vitro bacterial reverse mutation assay, the in vivo mouse micronucleus assay, and in the absence of metabolic activation in the in vitro chromosomal aberration assay. In contrast, HC tested positive for clastogenic activity in the in vitro chromosomal aberration assay in the presence of metabolic activation. Based on the results of these studies, HC is considered to have clastogenic potential. The three studies evaluated constitute the standard ICH genetic toxicology battery (a test for gene mutation in bacteria, an in vitro assessment of chromosomal damage using mammalian cells or an in vitro mouse lymphoma tk+/- assay; and an in vivo test for chromosomal damage using rodent hematopoietic cells). As outlined the January 2006 FDA Guidance document titled "Guidance for Industry and Review Staff: Recommended Approaches to Integration of Genetic Toxicology Study Results," if any of the three assays in the ICH genotoxicity standard battery are positive, the fourth test in the ICH battery should be conducted. Based on current guidance, an

appropriate fourth test in the current ICH battery that could contribute to the weight of evidence assessment of HC would be the in vitro mouse lymphoma assay. The Agency has also considered use of the in vivo comet assay as per ICH S2(R1) document. However, safety margins based on in vivo studies must take into consideration the development of tolerance to opioids.

It should be noted that the Applicant is conducting carcinogenicity assessments in mice and rats with HC. These studies are currently underway and will be submitted to the NDA as a post-marketing requirement.

**The following comment was sent to the Applicant during the course of the NDA review:**

We note that hydrocodone tested positive in the in vitro chromosomal aberration assay in the presence of S9. Consistent with ICH S2(R1), you should conduct a fourth assay in order to construct a weight-of-evidence approach. Since the positive result was seen in the presence of metabolic activation, we recommend conducting an in vivo comet assay with liver.

At the time of approval we will not have the results of the carcinogenicity studies.

#### 7.4 Other Genetic Toxicity Studies

**Study title:** (b) (4) **reverse mutation in four histidine-requiring strains of *Salmonella typhimurium* and one tryptophan-requiring strain of *Escherichia coli***

Study no.: 8243018

Study report location: EDR 4.2.3.7.6

Conducting laboratory and location: (b) (4)

Date of study initiation: March 29, 2011

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: (b) (4) batch #

1102252185, 98.3%

#### Key Study Findings

- (b) (4) is not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 and *E. coli* strain WP2uvrA 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: 156.3, 312.5, 625, 1250, 2500 and 5000 mcg/plate +/- S9

Basis of concentration selection: Initial study used 0.32, 1.6, 8, 40, 200, 1000 and 5000 mcg/plate +/- S9

Negative control: DMSO

Positive control: Table 34

Formulation/Vehicle: DMSO

Incubation & sampling time: 48-73 h at 37 degrees

Table 34. Positive controls for the Ames assay (reproduced from NDA)

Chemical	Source	Stock * concentration (µg/mL)	Final concentration (µg/plate)	Use	
				Strain(s)	S-9
2-nitrofluorene (2NF)	(b) (4)	50	5.0	TA98	-
Sodium azide (NaN <sub>3</sub> )	(b) (4)	20	2.0	TA100, TA1535	-
9-aminoacridine (AAC)	(b) (4)	500	50.0	TA1537	-
4-nitroquinoline 1-oxide (NQO)	(b) (4)	20	2.0	WP2 <i>uvrA</i>	-
Benzo[a]pyrene (B[a]P)	(b) (4)	100**	10.0	TA98	+
2-aminoanthracene (AAN)	(b) (4)	50** 150**	5.0 15.0	TA100, TA1535, TA1537 WP2 <i>uvrA</i>	+ +

## 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 WP2uvrA in either the presence or absence of S9. The results of the confirmative assay are summarized in Table 35. 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 35).

Table 35. Summary of Ames assay results

		<b>Strain</b>				
		<b>mean revertants <math>\pm</math> SD</b>				
<b>S9</b>	<b>mcg/ plate</b>	<b>TA98</b>	<b>TA100</b>	<b>TA1535</b>	<b>TA1537</b>	<b>WP2 uvrA</b>
No	Vehicle	36.4 $\pm$ 5.8	152.8 $\pm$ 8.6	16.6 $\pm$ 4.8	11.6 $\pm$ 1.5	12.2 $\pm$ 2.7
	156.3	24.3 $\pm$ 2.5	155.3 $\pm$ 4.5	18.0 $\pm$ 2.6	9.0 $\pm$ 3.0	8.7 $\pm$ 5.7
	312.5	22.7 $\pm$ 3.1	147.0 $\pm$ 15.4	14.0 $\pm$ 4.4	6.0 $\pm$ 4.6	10.7 $\pm$ 2.9
	625	26.3 $\pm$ 2.5	125.3 $\pm$ 19.9	15.7 $\pm$ 1.5	4.3 $\pm$ 2.1	16.3 $\pm$ 6.8
	1250	31.3 $\pm$ 7.1	146.3 $\pm$ 14.0	19.3 $\pm$ 7.0	8.7 $\pm$ 2.9	11.7 $\pm$ 4.6
	2500	28.0 $\pm$ 6.6	131.0 $\pm$ 5.2	22.3 $\pm$ 5.8	5.3 $\pm$ 1.2	8.7 $\pm$ 4.7
	5000	31.3 $\pm$ 1.2	152.7 $\pm$ 17.5	18.3 $\pm$ 8.0	7.3 $\pm$ 4.0	9.3 $\pm$ 4.5
	Positive	459.0 $\pm$ 110	667.0 $\pm$ 13.1	359.7 $\pm$ 14.0	204.0 $\pm$ 22.0	603.3 $\pm$ 22.0
Yes	Vehicle	26.2 $\pm$ 9.4	146.7 $\pm$ 2.7	17.0 $\pm$ 5.1	13.2 $\pm$ 1.1	14.6 $\pm$ 3.6
	156.3	38.3 $\pm$ 6.7	144.7 $\pm$ 6.1	13.0 $\pm$ 7.2	15.7 $\pm$ 3.5	16.3 $\pm$ 3.2
	312.5	43.7 $\pm$ 1.5	145.3 $\pm$ 4.5	17.7 $\pm$ 2.1	14.7 $\pm$ 6.0	15.7 $\pm$ 7.6
	625	42.0 $\pm$ 3.6	158.3 $\pm$ 13.1	11.0 $\pm$ 1.0	11.7 $\pm$ 2.1	19.0 $\pm$ 15.6
	1250	29.0 $\pm$ 6.1	137.3 $\pm$ 5.1	11.0 $\pm$ 6.6	9.7 $\pm$ 1.2	18.7 $\pm$ 2.5
	2500	31.7 $\pm$ 10.2	135.3 $\pm$ 9.3	9.7 $\pm$ 4.5	3.3 $\pm$ 2.1	21.7 $\pm$ 5.9
	5000	28.3 $\pm$ 14.6	147.3 $\pm$ 3.2	22.3 $\pm$ 4.6	2.7 $\pm$ 1.2	13.3 $\pm$ 3.2
	Positive	168.7 $\pm$ 21.6	673.3 $\pm$ 9.5	122.3 $\pm$ 12.7	65.7 $\pm$ 18.5	49.7 $\pm$ 4.0

**Study Title:** Combined Comet assay in the liver and a bone marrow micronucleus test in treated mice with (b) (4)

### Key Study Findings

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

**Study no.:** 8243025

**Volume #, and page #:** EDR 4.2.3.7.6.1

**Conducting laboratory and location:**

(b) (4)

(b) (4)

**Date of study initiation:** April 7, 2011**GLP compliance:** Yes**QA reports:** Yes**Drug, lot #, and % purity:** (b) (4) lot # 1102252185; 98.3%**Methods****Doses used in definitive study:** 185, 375, 750 mg/kg**Frequency of dosing:** Vehicle and study drug: were given at 0, 24 and 45 h and animals were sacrificed at 48 h.**Route of Administration:** oral gavage**Dose Volume:** main study: 20 mL/kg; positive control: 10 mL/kg**Dose formulation analysis:** adequate**Strains/species/cell line:** CD-1 mouse**Number/sex/group:** 6 males/group**Satellite groups:** TK: 2/group**Basis of dose selection:** MTD was determined in dose range-finding study and Main Experiment 1**Negative control:** 1% aqueous methylcellulose**Positive control:** Ethyl methanesulfonate, 150 mg/kg**Tissues to be analyzed:** Comet: liver; MN: bone marrow**Sampling times:** 3 h post last dose**Histopathology:** samples from liver were collected at sacrifice and preserved in formalin**DNA staining method:** Ethidium bromide**Scoring method:** Comet: fluorescence; MN: light microscopy**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 Table 36.

Table 36. Summary of Comet data for (b) (4)

Group/ Treatment (mg/kg/day)	Total no. cells scored	Tail Intensity			Tail Moment			Mean % clouds	Mean %Diffused cells
		Mean	SD	SEM	Mean	SD	SEM		
1 / Vehicle (b) (4)	600	0.75	0.58	0.24	0.08	0.06	0.02	1.17	0.17
2 / (b) (4)	(185)	0.68	0.48	0.20	0.08	0.06	0.02	1.67	0.67
3 / (b) (4)	(375)	1.40	1.98	0.81	0.16	0.22	0.09	1.83	0.83
4 / (b) (4)	(750)	0.54	0.30	0.12	0.07	0.04	0.01	3.83	0.50
5 / EMS (150)	600	54.64	7.76	3.17	12.29	2.47	1.01	18.00	0.67

SD Standard Deviation  
EMS Ethyl methanesulfonate

### 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 Table 37. Based on the results of this study, (b) (4) did not induce any increase in micronucleated polychromatic erythrocytes in the bone marrow.

Table 37. Summary of micronucleus data for (b) (4)

Group/ Treatment (mg/kg/day)	Cell Total	% PCE	MN PCE	Mean MN PCE/2000 PCE	% MN PCE	SD	Heterogeneity		Contingency	
							X <sup>2</sup>	S	X <sup>2</sup> C	S
1 / Vehicle (b) (4)	12000	36.30	4	0.67	0.03	0.04	5.00	NS		
2 / (b) (4)	(185)	41.28	3	0.50	0.03	0.03	3.00	NS	0.00	NS
3 / (b) (4)	(375)	33.73	6	1.00	0.05	0.03	2.00	NS	0.10	NS
4 / (b) (4)	(750)	38.40	5	0.83	0.04	0.06	8.20	NS	0.00	NS
5 / EMS (150)	12000	32.56	39	6.50	0.33	0.08			26.93	P<0.001

Linear trend: z = 0.560 NS  
NS Not significant  
MN Micronucleated  
S Significance  
SD Standard deviation  
EMS Ethyl methanesulfonate

### Conclusions

(b) (4) did not induce DNA damage in the liver of male mice following oral gavage administration at doses of 185, 375, and 750 mg/kg/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).

## 8 Carcinogenicity

As a single entity hydrocodone formulation, this drug product will yield exposures of hydrocodone much greater than seen with previous clinical experience. Therefore, carcinogenicity studies in two species have been required. The protocols for the rat and mouse 2-year bioassays have been submitted and approved by the Executive Carcinogenicity Assessment Committee and the studies are underway. The studies will be completed as a post-marketing requirement for this NDA.

## 9 Reproductive and Developmental Toxicology

### 9.1 Fertility and Early Embryonic Development

#### Study title: Oral (Gavage) Fertility and General Reproduction Toxicity Study of Hydrocodone Bitartrate in Rats

Study no.:	20007043
Study report location:	EDR 4.2.3.5.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 4, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Hydrocodone Bitartrate, Lot # 1010000552, 100.2%

#### Key Study Findings

- Dose-related decreases in body weight and food consumption were seen males and females in all dose groups.
- All doses of HC in female rats had effects on fertility parameters (no NOAEL established) which are attributed to the HC-mediated decrease in prolactin levels. Unique to rodents, prolactin is required for normal estrous cycling and the effects on fertility observed in this study are most likely rodent-specific.
- No effects on male fertility parameters (NOAEL 100 mg/kg), however decreased weights of male reproductive organs were observed at all doses.
- No effects on early embryonic development were seen in females treated with 25 mg/kg HC. The 75 and 100 mg/kg groups did not have enough pregnancies to draw any conclusions on early embryonic development.
- No changes in Cesarean-sectioning or litter parameters were observed in untreated females when mated to HC-treated males (NOAEL 100 mg/kg).

## Methods

Doses:	Males:
	Females: 25, 75, 100 mg/kg
Frequency of dosing:	Males: dosed once daily 28 days prior to cohabitation with untreated females
	Females: dosed once daily 15 days prior to cohabitation and 7 days of gestation
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Sterile water
Species/Strain:	Rat, F344/DuCrI
Number/Sex/Group:	25/sex/group
Satellite groups:	none
Study design:	see below
Deviation from study protocol:	none that compromise study

## Study Design

Because of suspected effects on fertility, treated male rats were mated with untreated female rats and treated female rats were mated with proven breeder male rats to allow for delineation of any effects on fertility that may have been limited to the male or female rats.

## Observations and Results

### Mortality

One female rat in the vehicle group was sacrificed after delivery of a full-term litter due to a mistimed mating. One female rat in the 75 mg/kg group was sacrificed due to adverse clinical signs on Study Day 39 approximately two hours post dosing. The rat showed the following clinical signs prior to sacrifice: increased motor activity (DS 15-18), excessive grooming (DS 16-26, 31-34 and 39), abrasion of forepaw (DS 17-21), hunched posture (DS 34) and red substance in cage (DS 39). The rat had no change in body weight and food consumption was comparable to vehicle group throughout the study. The Applicant states that all tissues appeared normal and attributed the "adverse observations" to the pharmacologic activity of the study drug. The Applicant also noted that the rat was not pregnant.

No test article-related mortality was observed in male rats. One 100 mg/kg male was sacrificed on Study Day 8 due to adverse clinical observations resulting from a gavage error. Necropsy of this rat revealed a perforated esophagus.

### Clinical Signs

Treatment-related findings in all treated groups for both males and females included periods of increased or decreased activity, excessive grooming, head bobbing, swollen limb(s), hunched posture, repetitive licking, and lacrimation. Effects secondary to the excessive grooming and licking included scabs and/or redness of the affected area

were observed in some animals. Chromodacryorrhea occurred in all groups but in an increased number of rats in the 100 mg/kg group.

### **Body Weight**

Body weight losses were observed in males and females throughout the study. For females, body weight decreases of 3%, 6%, and 6% during gestation were observed for the 25, 75, and 100 mg/kg dose groups, respectively. For males, body weight decreases of 4%, 11%, and 12% at the end of the study were observed for the 25, 75, and 100 mg/kg dose groups, respectively.

### **Food Consumption**

Consistent with the reductions in body weight, reductions in food consumption were observed throughout the study in males and females.

### **Toxicokinetics**

Toxicokinetics were not performed in this study. The rat exposures of 25, 75, and 100 mg/kg are equivalent to 2, 7, and 10 times the human dose of 100 mg/day of HC on a mg/m<sup>2</sup> basis.

### **Dosing Solution Analysis**

All dosing solutions used were within 3% of the targeted values and were considered acceptable.

### **Necropsy**

One 75 mg/kg female showed a diaphragmatic hernia. It is not clear whether this gross lesion is test article-related. No other test article-related necropsy findings were observed.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

#### ***Female reproductive assessments:***

Treatment-related effects were observed on estrous cycle parameters. Treated female rats (all dose groups) showed fewer estrous stages as compared to controls (Table 38). These decreases in estrous cycling correlates with the increased number of days in diestrus observed for the treated groups (>6 days in diestrus= 14-17 days) compared to control (>6 days in diestrus= 0, Table 38). The fertility index was decreased in all treated groups (Table 38). The observed effects on fertility in treated female mice can likely be attributed to the well-characterized opioid-dependent decreases in prolactin release (M Risk and G Gibory, 2001). In rodents, release of prolactin is required at the appropriate time during estrous cycling to cause release of progesterone. Sufficient amounts of progesterone are necessary for pregnancy to occur. This dependency on prolactin for fertility is specific to the rodent (Bachelot A and Binart N, 2007). No effects

on implantation were observed in the Applicant's dose-range finding study in rabbits at similar doses used in this study (see study 20007041).

Table 38. Fertility index and selected estrous cycle parameters

mg/kg HC	n	pregnant rats (fertility index)	estrous stages/ 14 days	rats with >6 consecutive days of diestrous
0	25	21 (88%)	2.7 ± 0.5	0
25	25	17 (68%)	1.9 ± 1.2*	14.0 ± 56**
75	25	2 (8%)	0.9 ± 0.6**	17 ± 68**
100	25	1 (4%)	1.1 ± 1.0**	17 ± 68**

\*p ≤ 0.05; \*\*p ≤ 0.01

In the 25 mg/kg group, no treatment-related effects were observed on the total number of corpora lutea, implantation sites, live and dead embryos, early resorptions, total number of implantation sites, and the pre- and post-implantation losses. No differences from control were observed for fertility parameters in the 75 and 100 mg/kg groups, however, the low number of pregnant rats in these groups precludes any meaningful interpretation of the findings. Selected fertility parameters are summarized in Table 39.

Table 39. Selected fertility parameters

mg/kg HC	n	Total number of corpora lutea	Total number of implantation sites	Number of live embryos
0	21	9.0 ± 1.9	7.9 ± 2.8	7.2 ± 3.0
25	17	10.2 ± 1.0	9.5 ± 0.8	8.8 ± 2.4
75*	2	7.5 ± 2.1	6.5 ± 2.1	6.5 ± 2.1
100*	1	10	9.0	9.0

n= number of pregnancies

\*note low number of pregnant rats in these groups: data presented for reference only

#### **Male reproductive assessments:**

No treatment-related effects were observed on sperm motility, count and density. In untreated females mated with treated males, no Cesarean-sectioning or litter parameters were affected by dosages of the test article up to 100 mg/kg, the highest dose tested. No differences between vehicle and dose groups were noted in litter averages for corpora lutea, implantations, preimplantation loss, viable and nonviable embryos and post-implantation loss.

Decreases in weights of male reproductive organs (epididymes, testes, seminal vesicles, prostate) were observed in all dose groups. Decreases in body weight were also observed for all dose groups. The ratio between organ weight and brain weight was not calculated.

In untreated females mated to treated males, no Cesarean-sectioning or litter parameters were affected by HC doses to males up to 100 mg/kg.

## 9.2 Embryonic Fetal Development

### Study title: Oral (Gavage) Developmental Toxicity Study of Hydrocodone Bitartrate in Rats

Study no.:	20007040
Study report location:	EDR 4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 10, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Hydrocodone bitartrate, Lot # 1010000552, 100.2%

### Key Study Findings

- Low number of pregnancies  $\geq 25$  mg/kg were observed which necessitated the inclusion of additional dose groups with lower doses
- Significant dose-dependent decreases in maternal body weight gains and food consumption were seen  $\geq 10$  mg/kg
- No effects on embryofetal development were seen at the any dose tested (developmental NOAEL = 25 mg/kg)

### Methods

Doses:	see below
Frequency of dosing:	once daily on Gestation Day (GD) 7-17, Cesarean section on GD 21
Dose volume:	10 mL/kg
Route of administration:	oral gavage
Formulation/Vehicle:	sterile water
Species/Strain:	Rat, F344/DuCrI
Number/Sex/Group:	Table 40
Satellite groups:	6/group for TK
Study design:	see below
Deviation from study protocol:	none that compromise the integrity of the study

### Study Design

The applicant originally selected 25, 75, and 100 mg/kg HC as the doses for this Embryofetal Development (EFD) study. The Fertility and Early Embryonic Development study with HC (Study 20007043) conducted by the Applicant utilized the same doses and large reductions in fertility were observed at doses  $\leq 75$  mg/kg and moderate reductions in fertility were observed at 25 mg/kg. In the current EFD study, the pregnancy rate was low for all groups *including controls* (see Table 40). The Applicant added lower dose groups (1-25 mg/kg) as well as an additional control group. The

explanation provided by the Applicant for the low pregnancy rate in the control group was that Fischer rats mature slightly later than other standard strains and the Fischer rats used in the original dosage groups were about two weeks younger than the second lower-dosed groups. They also claim that this age difference also affected the pre-implantation percentage and could not be an effect of the test article since dosage was started after implantation.

Litters from the lower-dosed groups (Groups V-IX; 1-25 mg/kg; Table 40) were evaluated for all parameters. Since the number of pregnancies/litters is low for all groups (including control) with the original selected doses (Groups I-IV) and all parameters were not evaluated for these litters, this review will only consider data from the lower dosing groups and their concomitant control group (Groups V-IX; Table 40).

Table 40. Pregnancy rates in different dose groups

Dosage group	Dose mg/kg	n	pregnancies, (fertility index)
I	0	26	15 (60%)
II	25	25	16 (64%)
III	75	25	3 (12%)
IV	100	25	4 (16%)
V	0	26	26 (100%)
VI	1	25	23 (92%)
VII	5	25	25 (100%)
VII	10	25	24 (96%)
IX	25	26	21 (81%)

Note: the approximate age at arrival for Groups I-IV and V-IX is 66 and 72 days, respectively.

## Observations and Results

### Mortality

All rats survived until scheduled sacrifice in this study.

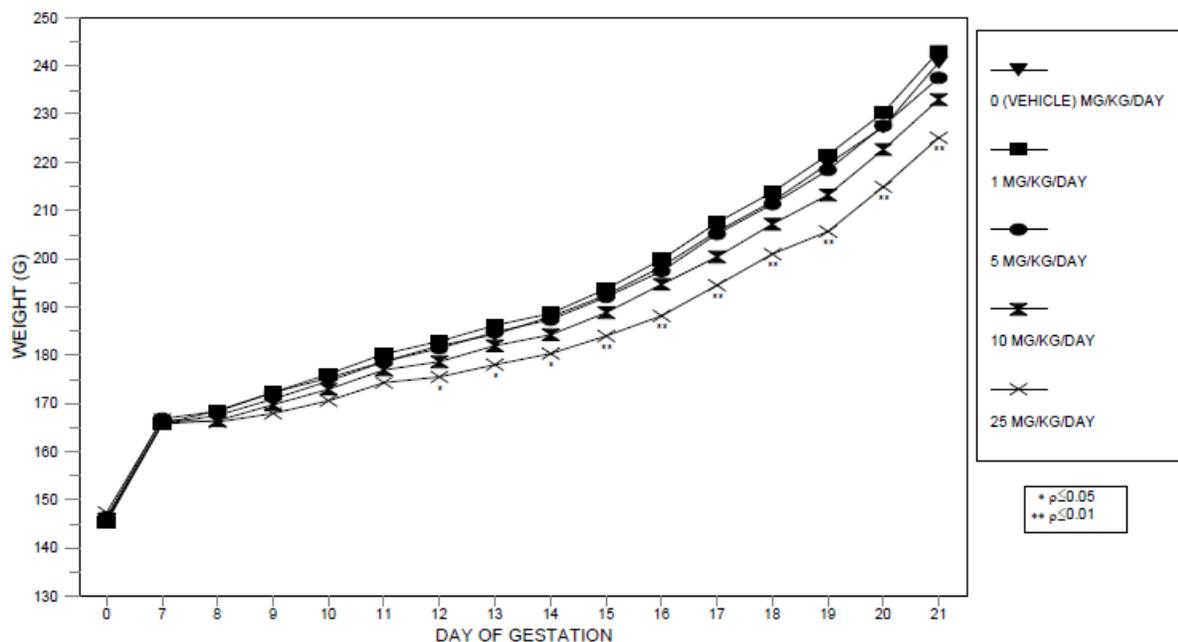
### Clinical Signs

Several clinical observations were seen in a few rats at the 10 or 25 mg/kg dose groups which included sparse hair coat, chromodacryorrhea, urine, ungroomed coat, soft or liquid feces, localized alopecia on the limbs, and red forepaw. Typical opioid agonist clinical signs were not observed, most likely because of the relatively low doses used in this study.

### Body Weight

Body weights were significantly reduced on Gestation Days 12 to 21 (GD 12 to 21) in the 25 mg/kg/day group. At the end of the dosing period (GD 18) body weights were 100.9%, 99.8%, 97.8% and 94.9% of the control group values in the 1, 5, 10 and 25 mg/kg/day groups, respectively (Figure 9). Percent decreases in body weight gains at the end of dosing (GD 18) were -4%, 1%, 7%, and 22% of the control group values in the 1, 5, 10 and 25 mg/kg/day groups, respectively. The differences in BWG reached statistical significance in the 10 and 25 mg/kg groups.

Figure 9. Maternal body weights during gestation (rat)



### Food Consumption

Food consumption was assessed in three-day intervals during the study. Food consumption was dose-dependently reduced for most time points from GD 7-21 at doses  $\geq 5$  mg/kg with statistical significance being reached for most time points in the 10 and 25 mg/kg groups.

### Toxicokinetics

After oral dosing of HC on GD7 and GD17 peak plasma concentrations were reached at 1 h post-dose. The terminal  $T_{1/2}$  for GD7 ranged between 1-2.4 h and 2.1 and 8.8 h for GD17. Several parameters were unable to be calculated due to plasma levels below the lower limit of quantitation (Table 41). The AUC values were greater-than-dose proportional at GD 7 and 17. The  $C_{max}$  at GD 17 was also greater-than-dose proportional. No appropriate human PK data are available for comparison therefore the exposure comparisons for the product label will be made using body surface area (BSA) comparisons. The rat exposures of 1, 5, 10, and 25 mg/kg are equivalent to 0.1, 0.5, 1, and 2.4 times the human dose of 100 mg/day of HC on a  $mg/m^2$  basis.

Table 41. Toxicokinetic parameters for hydrocodone in pregnant rats

Gestation Day <sup>1</sup>	Group Number	Hydrocodone Dose Level (mg/kg)	T <sub>max</sub> (hr)	Half-life (hr)	C <sub>max</sub> (ng/mL)	AUC <sub>0-∞</sub> (hr*ng/mL)	AUC <sub>0-24</sub> (hr*ng/mL)	CL <sub>F</sub> (mL/min/kg)	V <sub>d,F</sub> (L/kg)	T <sub>1/2α</sub> (hr)
DG 7	VI	1	1	NC <sup>2</sup>	0.421	0.210	NC	NC	NC	1
DG 7	VII	5	1	1.06	2.11	4.41	4.88	17,100	1,570	4
DG 7	VIII	10	1	2.37	3.14	8.55	9.90	16,800	3,450	8
DG 7	IX	25	1	2.40	11.0	38.3	43.5	9,580	1,990	8
DG 17	VI	1	1	NC	0.536	0.268	NC	NC	NC	1
DG 17	VII	5	1	NC	1.79	2.61	NC	NC	NC	2
DG 17	VIII	10	1	2.14	3.20	7.71	8.65	19,300	3,570	8
DG 17	IX	25	1	8.77	21.3	87.8	98.7	4,220	3,200	24

<sup>1</sup>DG 7 = Day 7 of presumed gestation; DG 17 = Day 17 of presumed gestation.

<sup>2</sup>NC = Not calculated, due to insufficient data points for the plasma elimination curve.

### Dosing Solution Analysis

All dosing solutions used were within an acceptable range of the targeted values.

### Necropsy

No test article-related gross findings were observed.

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

Pregnancy occurred in 26 (100.0%), 23 (92.0%), 25 (100.0%), 24 (96.0%) and 21 (80.8%) rats in the 0, 1, 5, 10 and 25 mg/kg/day dose groups, respectively. No differences from control in Cesarean-sectioning or litter parameters were observed at any dose tested. The litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights, percent resorbed conceptuses, and percent live male fetuses were comparable among the five dosage groups and did not significantly differ. One dead fetus in the control group was observed. All placentae appeared normal.

### Offspring (Malformations, Variations, etc.)

No test article-related gross external, soft tissue or skeletal malformations or variations were observed in this study. One fetus in the 1 mg/kg group had micrognathia and edema in the neck. Skeletal evaluation revealed a short mandible, extra cervical rib, bifid thoracic vertebral centra, incompletely ossified ribs and sternal centrum. Another fetus in the 1 mg/kg group showed whole body edema and cleft palate. Edema was also noted in one fetus in the control group. Moderate dilation of the pelvis of both kidneys was noted in one 25 mg/kg fetus. This alteration is considered a reversible developmental delay. These gross alterations were not dose-dependent and appeared at low incidence, within historical controls. The NOAEL for fetal toxicity in this study is the highest dose tested, 25 mg/kg.

### Conclusions

The fertility and embryonic development study conducted by the Applicant showed a reduction in fertility with dosing of  $\leq 25$  mg/kg HC (see Study 20007043). The current study began with doses of 25, 75 and 100 mg/kg which resulted in a very low number of pregnancies. The doses were subsequently dropped to 1, 5, 10, and 25 mg/kg HC. Although no clinical observations typical of opioid agonists were observed at these fairly low doses, and maternal toxicity was not technically demonstrated, reductions in body weight and body weight gain (up to 22%) as well as reductions in food consumption were observed at  $\geq 10$  mg/kg. Since the adverse effects of HC on female fertility in rat precluded higher dosing, 25 mg/kg will be considered an acceptable maximum dose for this study. No adverse effects on embryo-fetal development were observed in this study and the developmental NOAEL is considered to be the highest dose tested, 25 mg/kg.

**Study title: Oral (Stomach Tube) Developmental Toxicity Study of Hydrocodone Bitartrate in Rabbits**

Study no.:	20007042
Study report location:	EDR 4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 24, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Hydrocodone bitartrate, lot # 1103001006, 99.2%

**Key Study Findings**

- Dose-dependent decreases in maternal body weights and food consumption were seen at all doses ( $\geq 25$  mg/kg).
- Fetal body weights were significantly decreased in all treated groups ( $\geq 25$  mg/kg).
- Significant increases in the number of fetal malformations including umbilical hernia and various irregularly shaped bones (ulna, femur, tibia, fibula) were observed in the highest dose group (75 mg/kg).
- Significant decreases in the number of ossified hyoid bodies and ossified xiphoid bones were observed in the highest dose group (75 mg/kg).
- The NOAEL for teratogenic effects for this study is the mid dose, 50 mg/kg.

**Methods**

Doses:	0, 25, 50, 75 mg/kg
Frequency of dosing:	daily
Dose volume:	10 mL/kg
Route of administration:	oral (stomach tube)
Formulation/Vehicle:	sterile water for injection

Species/Strain: Rabbit, New Zealand White [Hra:(NZW)SPF]  
Number/Sex/Group: 20 F/group  
Satellite groups: 3/group for TK  
Study design: does were dosed GD 7-19, Cesarean section  
was performed on GD 29

Deviation from study protocol: none that affect integrity of the data

## **Observations and Results**

### **Mortality**

No test article-related deaths were seen in this study.

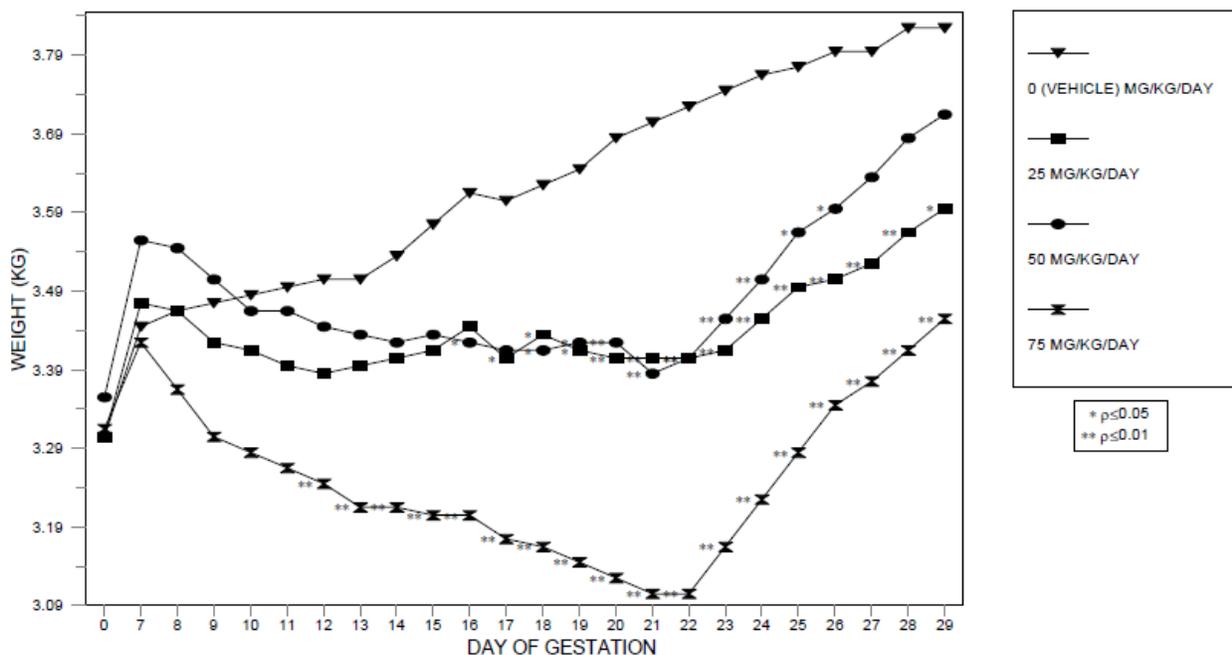
### **Clinical Signs**

Decreased motor activity was observed in the 75 mg/kg group. Decreased fecal output was observed in all treatment groups and a significant number of does in the study had no feces in the cage pan at several time points. These observations are typical signs for an opioid agonist.

### **Body Weight**

Mean maternal body weights were reduced or significantly reduced beginning on GD17, GD16 and GD12 in the 25, 50, and 75 mg/kg dose groups, respectively. These decreases were seen in all groups until the end of the dosing period. Body weights sharply recovered after the termination of dosing but statistically significant decreases compared to control were still observed for the 50 and 75 mg/kg groups through the end of the study (Figure 10).

Figure 10. Mean maternal body weights (rabbit)



### Food Consumption

Food consumption was reduced for all intervals and all doses compared to the control group.

### Toxicokinetics

Hydrocodone levels were assessed on GD7 and GD19. Exposure to hydrocodone ( $C_{max}$  and AUC) appeared to increase in a greater-than-dose-proportional manner but because of high variability between animals conclusions regarding linearity of dose proportionality could not be made. Accumulation with HC was observed. In all but one rabbit, the  $C_{max}$  and AUC values were higher on GD19 as compared to GD7. Peak plasma concentrations were reached at 1-2 h post-dose. Refer to Table 42 for a summary of toxicokinetics. No appropriate human PK data is available for comparison therefore the exposure comparisons for the product label will be made using body surface area (BSA) comparisons. The rabbit exposures of 25, 50, and 75 mg/kg are equivalent to 5, 10, and 15 times the human dose of 100 mg/day of HC on a  $mg/m^2$  basis.

Table 42. Summary of toxicokinetics

Hydrocodone Dose Level (mg/kg)	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (hr)	T <sub>last</sub> (hr)	AUC <sub>0-t</sub> (hr*ng/mL)
DG 7				
0	0.00	NC	NC	0.00
25	38.0	1	8	99.2
50	250	1	8	604
75	119	2	24	729
DG 19				
0	0.00	NC	NC	0.00
25	98.2	1	8	279
50	154	1	8	702
75	651	1	8	1870

NC = Not calculated, all samples BQL (<1 ng/mL)

### Dosing Solution Analysis

All dosing solutions used were within an acceptable range of the targeted values.

### Necropsy

No test article-related gross lesions were observed in this study.

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

Pregnancy occurred in 19 (100%), 17 (100%), 18 (100%), and 18 (100%) rabbits in the 0, 25, 50, and 75 mg/kg/day dose groups, respectively. Fetal weights in both males and females were significantly reduced in all treatment groups ( $\geq 25$  mg/kg) compared to the control group with the highest percent decreases from control of 17% in males and 23% in females seen at the high dose (Table 43). No other differences from control in Cesarean-sectioning or litter parameters were observed at any dose tested. The litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, percent resorbed conceptuses, and percent live male fetuses were comparable among the five dosage groups and did not significantly differ. No dead fetuses were observed and all placentae appeared normal.

Table 43. Mean live fetus weight

Mean live fetus weight in grams $\pm$ S.D. (% decrease from control)				
	0	25 mg/kg	50 mg/kg	75 mg/kg
litter	44.98 $\pm$ 3.52	40.36 $\pm$ 5.27* (10%)	40.06 $\pm$ 5.72* (11%)	35.72 $\pm$ 6.36** (21%)
male fetuses	45.28 $\pm$ 4.46	40.94 $\pm$ 5.45* (10%)	38.94 $\pm$ 6.14** (14%)	37.50 $\pm$ 7.16** (17%)
female fetuses	44.19 $\pm$ 3.31	40.13 $\pm$ 5.53*	40.37 $\pm$ 5.60	34.11 $\pm$ 6.80**

		(9%)	(9%)	(23%)
--	--	------	------	-------

\*p<sub>≤</sub> 0.05; \*\*p<sub>≤</sub> 0.01

### Offspring (Malformations, Variations, etc.)

Litters with fetuses which had alterations were 7 (38.9%), 8 (47.0%), 7 (39%), and 15 (83.3%) for the 0, 25, 50, and 75 mg/kg dose groups, respectively. The numbers of fetuses in which alterations were observed were 8 (5.9%), 8 (5.7%), 7 (5.0%), and 31 (20.9%) for the 0, 25, 50, and 75 mg/kg dose groups, respectively. The number of litters and fetuses with alterations in the 75 mg/kg group were both higher than control and these differences both reached statistical significance (p<sub>≤</sub>0.01).

Six fetuses from three litters in the 75 mg/kg dose group had small intestines protruding through the umbilicus. This finding was statistically significant. These rabbits were found to each have an umbilical hernia. Historical control ranges for umbilical hernias identified as a gross external alteration were 0-5.6% for litters and 0-0.7% for fetuses. Historical control ranges for intestines protruding through umbilicus identified as a soft tissue alteration were 0-9.1% for litters and 0-1.3% for fetuses. The incidences observed in this study at the high dose for both intestines protruding through umbilicus and umbilical hernia (16.7% for litters and 4.0% for fetuses) are statistically significant as well as outside the historical control ranges provided by the Applicant and will be considered test article-related. No other test article-related gross or soft tissue findings were observed.

Irregularly shaped ulna, bent femur, bent tibia and bent fibula were observed at 75 mg/kg and were significantly increased above control (Table 44). Historical control data were provided only for bent femur (litter: 0-4.3% and fetus: 0-0.5%). The incidences observed in the study for bent femur exceed the historical control ranges and are statistically increased as compared to control and will be considered test article-related (Table 44). The skeletal malformations of irregularly shaped ulna, bent tibia, and bent fibula will also be considered test article-related.

A cervical rib present at the 7<sup>th</sup> cervical vertebra and incompletely ossified sternal centra were increased above control for all dose groups but levels did not reach statistical significance. These findings are also considered to be common in rabbits and will not be considered to be test article-related (Khera, 1981). The skeletal variation of incompletely ossified pubis was observed in one fetus in the 50 mg/kg group and incompletely ossified and not ossified pubis were observed and reached statistical significance in the 75 mg/kg group. Historical control ranges provided by the Applicant pool not ossified and ossified pubis (litter: 0-10.5%; fetal: 0-1.2%). The incidences observed in the 75 mg/kg group for incompletely ossified pubis and not ossified pubis for fetal incidence are outside of the historical control litter range (for *pooled* incompletely and not ossified pubis data) as well as significantly greater than control for fetal incidence. However, these variations are common in rabbits and are associated with maternal stress and low fetal weight.

Statistically significant decreases in the number of ossified hyoid bodies and ossified xiphoid bones per fetus per litter were seen in the 75 mg/kg group (Table 44). No other test article-related changes in ossification sites were noted.

Table 44. Gross, soft tissue and skeletal variations and malformations

<i>dose group:</i>		<i>Veh</i>	<i>25 mg/kg</i>	<i>50 mg/kg</i>	<i>75 mg/kg</i>
<i>litters evaluated:</i>		18	17	18	18
<i>fetuses evaluated:</i>		135	141	139	148
<i>Malformation</i>		<i>Litter incidence N (%)</i> <i>Fetal incidence N (%)</i>			
<b>Gross malformations</b>	<b><i>Umbilical hernia</i></b>	0	0	0	3 (16.7)** 6 (4.0)**
<b>Soft tissue malformations</b>	<b><i>Intestines: protrudes through umbilicus</i></b>	0	0	0	3 (16.7)** 6 (4.0)**
<b>Skeletal variations/ malformations</b>	<b><i>Cervical vertebrae: cervical rib present at 7<sup>th</sup> cervical vertebra</i></b>	0	2 (11.8) 2 (1.4)	2 (11.1) 2 (1.4)	3 (16.7) 3 (2.0)
	<b><i>Sternal centra: incompletely ossified</i></b>	0	1 (5.9) 1 (0.7)	1 (5.6) 1 (0.7)	2 (11.1) 2 (1.4)
	<b><i>Forelimb: Ulna, irregularly shaped</i></b>	0	0	0	1 (5.6) 5 (3.4)**
	<b><i>Pelvis: pubis, incompletely ossified</i></b>	0	0	1 (5.6) 1 (0.7)	3 (16.7) 7 (4.7)**
	<b><i>Pelvis: pubis, not ossified</i></b>	0	0	0	1 (5.6) 2 (2.0)**
	<b><i>Hindlimb: femur, bent</i></b>	0	0	0	1 (5.6) 5 (3.4)**
	<b><i>Hindlimb: tibia, bent</i></b>	0	0	0	1 (5.6) 5 (3.4)**
	<b><i>Hindlimb: fibula, bent</i></b>	0	0	0	1 (5.6) 5 (3.4)**
<b>Ossification sites/fetus/litter</b>		<i>mean +/- SD</i>			
	<b><i>Hyoid</i></b>	0.99 +/- 0.02	1.00 +/- 0.00	1.00 +/- 0.00	0.94 +/- 0.13*
	<b><i>Sternum, xiphoid</i></b>	1.00 +/- 0.00	0.98 +/- 0.05	0.96 +/- 0.09	0.87 +/- 0.2**

\*p ≤ 0.05; \*\*p ≤ 0.01

### Conclusions

Does were dosed with 0, 25, 50, and 75 mg/kg HC from GD 7-19. In a dose range-finding study several deaths occurred at 100 mg/kg, therefore the highest dose tested in this study was 75 mg/kg. Significant maternal body weight loss was noted in all dose groups. Although a sharp recovery in body weights was seen after dosing ceased significant decreases were still noted in the 50 and 75 mg/kg groups throughout the duration of the study. Concomitant decreases in food consumption were also observed. Fetal body weights were significantly and dose-dependently decreased in all dose groups. Other than the fetal body weight decreases, no other changes in Cesarean section or litter parameters were observed. Test article-related increases in umbilical hernia and several skeletal malformations (irregularly shaped ulna, bent femur, tibia, fibula) were observed at the high dose. Test article-related decreases in the number of ossified hyoid bodies and ossified xiphoid bones per fetus per litter were seen in the high dose group. The NOAEL for teratogenicity is the mid dose, 50 mg/kg. Due to the maternal and fetal weight loss, no NOAEL can be determined for maternal or developmental toxicity.

### 9.3 Prenatal and Postnatal Development

#### **Study title: Oral (Gavage) Developmental and Perinatal/Postnatal Reproduction Toxicity Study of Hydrocodone Bitartrate in Rats, Including a Postnatal Behavioral/Functional Evaluation**

Study no.:	20007044
Study report location:	EDR 4.2.3.5.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 10, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Hydrocodone bitartrate, Lot # 1010000552, 100.2%

#### **Key Study Findings**

- Significant decreases in weight and number of pregnancies were observed in the F0 females at 25 mg/kg
- Significant increases in the number of stillborn pups, dams with still born pups, and number of pups dying on Lactation Day (LD) 2-4 and 5-7 were seen in the 10 and 25 mg/kg groups (F1 generation)
- Significant reductions in the number of liveborn pups as well as viability and lactation indices were seen in the 10 and 25 mg/kg groups (F1 generation)
- Body weight and food consumption were reduced in the 25 mg/kg male group throughout the study (F1 generation)
- No changes were seen in post-weaning development observations (F1 generation)

- No changes were seen in fetal body weights, sex, and gross external alterations in the F2 generation pups.
- The NOAEL for peri- and postnatal toxicity in this study is 5 mg/kg.

## Methods

Doses:	1, 5, 10, 25 mg/kg
Frequency of dosing:	once daily GD 7 through LD 20
Dose volume:	10 mL/kg
Route of administration:	oral gavage
Formulation/Vehicle:	sterile water for injection
Species/Strain:	Rat, F344/DuCrI
Number/Sex/Group:	0, 1, 25 mg/kg: 28 F/group; 5 and 10 mg/kg 27 F/group
Satellite groups:	none
Study design:	see below
Deviation from study protocol:	None that affected the integrity of the study

## Study Design

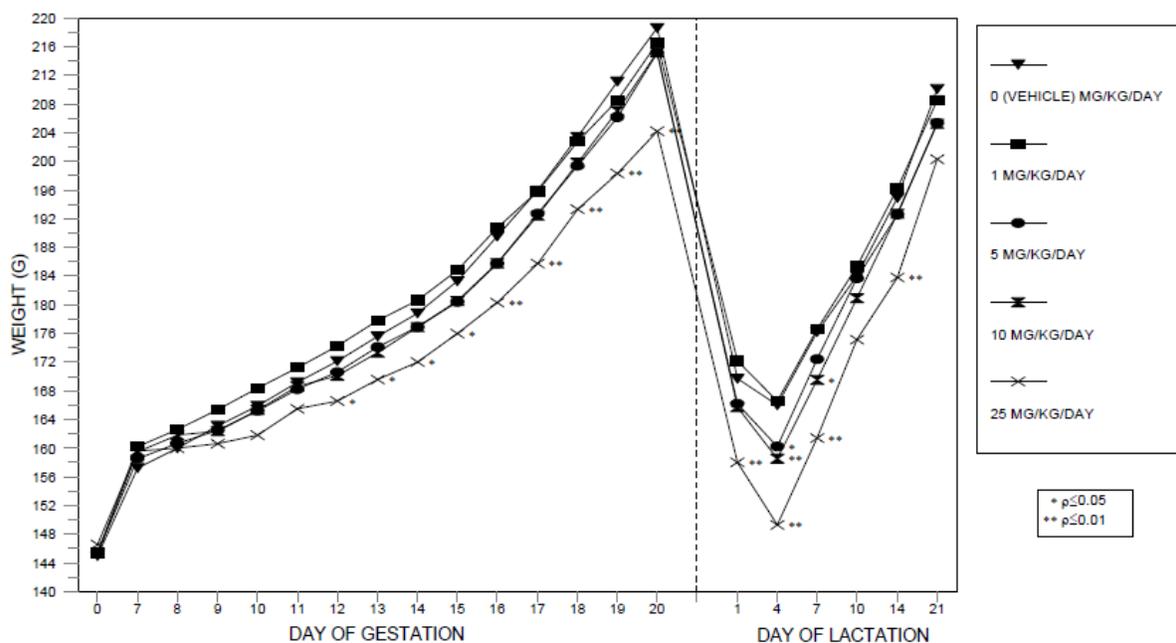
In the original study, rats were administered 0, 25, 75, and 100 mg/kg HC. The study was terminated due to insufficient number of pregnancies. The Applicant subsequently conducted a second study using 0, 1, 5, 10, and 25 mg/kg HC. Only data from the second study using the lower doses will be discussed in this review.

## Observations and Results (Optional Table)

### F<sub>0</sub> Dams

Survival:	All rats survived until scheduled euthanasia
Clinical signs:	25 mg/kg: chewing on paws, sparse hair coat on limbs
Body weight during gestation:	BW significantly reduced in 25 mg/kg groups GD 12-20 BWG significantly reduced in 10 and 25 mg/kg group on GD 7-20 and GD 0-20, respectively
Body weight during lactation:	BW significantly reduced on LD 4 and 7 for 10 mg/kg and LD 1, 4, 7, 14 for 25 mg/kg
Food consumption:	FC was dose-dependently decreased in all treated groups
Uterine content:	Pregnancy occurred in 27 (96.4%), 25 (89.3%), 27 (100.0%), 25 (92.6%) and 20 (71.4%) of the rats in the 0 (Vehicle), 1, 5, 10 and 25 mg/kg groups, respectively.
Necropsy observation:	No test article-related findings
Toxicokinetics:	TK were not conducted. The rat exposures of 1, 5, 10, and 25 mg/kg are equivalent to 0.1, 0.5, 1, and 2.4 times the human dose of 100 mg/day of HC on a mg/m <sup>2</sup> basis.
Dosing Solution Analysis	Acceptable

Figure 11. Mean maternal body weights: gestation and lactation (rat)



Pregnancy occurred in 27 (96.4%), 25 (89.3%), 27 (100), 25 (92.6%), and 20 (71.4%) of rats in the 0, 1, 5, 10, and 25 mg/kg groups. The 25 mg/kg group showed a statistically significant reduction in number of pregnant rats. This is consistent with observations from both the fertility and EFD studies. No differences between groups were seen in the male/female pup ratio. A statistically significant increase in the number of stillborn pups, dams with still born pups, and number of pups dying on Lactation Day (LD) 2-4 and 5-7 were seen in the 10 and 25 mg/kg groups. The number of liveborn pups as well as viability and lactation indices were reduced in the 10 and 25 mg/kg groups. The number of pups that were cold to the touch was significantly increased at 10 and 25 mg/kg. The number of pups that were not nursing or had no milk present in their stomach was significantly increased at the 25 mg/kg group. It is not clear whether the observed effects on nursing were due to a failure of the dams to nurse their pups or an inability of the pups to suckle. Body weights in the 25 mg/kg male rats were significantly reduced at weaning and at most scheduled weighings throughout the post-weaning period (Figure 12). Food consumption was reduced in 25 mg/kg males only. Body weights for all female test article groups were similar to control (Figure 13). In females, no changes in food consumption at any dose were observed.

No test article-related changes were seen in post-weaning development observations (passive avoidance testing, sexual maturation, water maze testing, reproductive capacity) or necropsy observations in the F1 generation pups. No test article-related changes in fetal body weights, sex and gross external alterations were noted for the F2 generation pups. The NOAEL for peri- and postnatal toxicity in this study is 5 mg/kg.

Figure 12. Mean body weights in F1 males

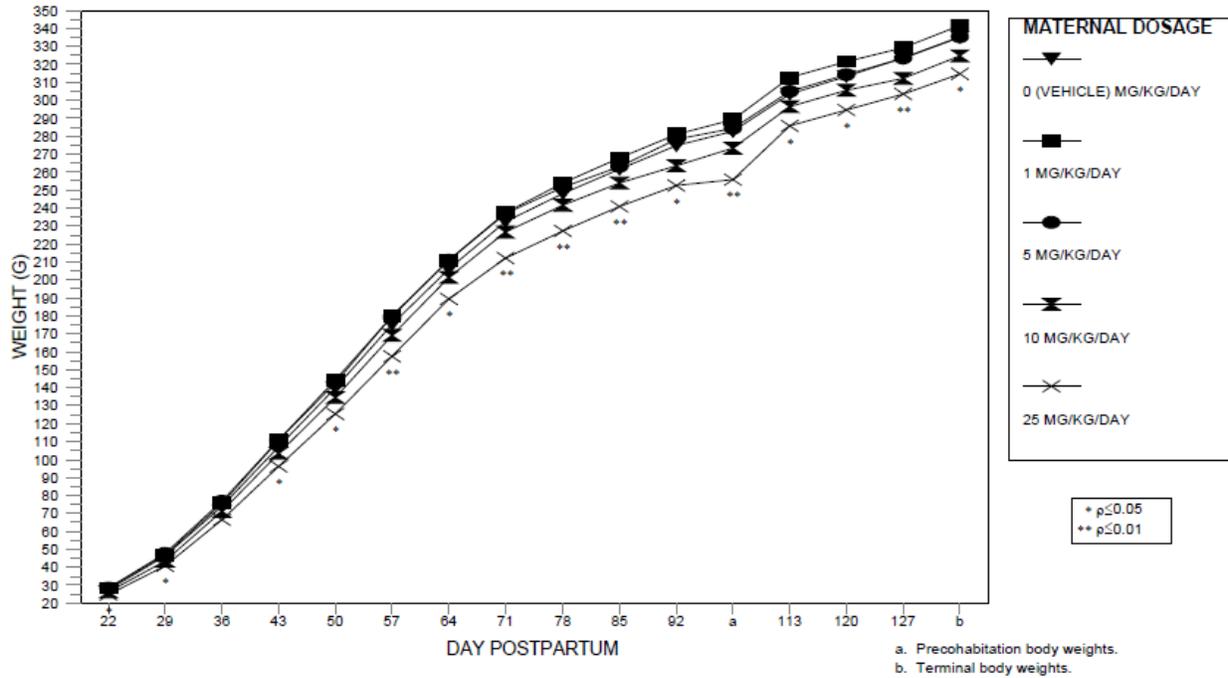
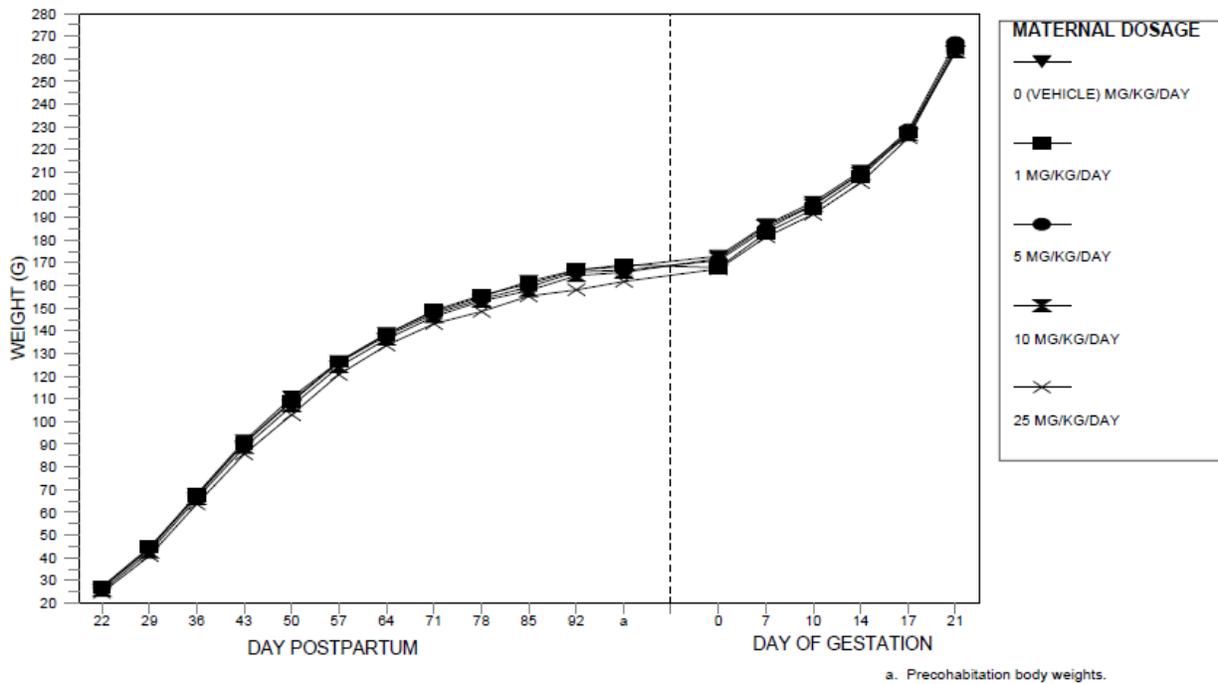


Figure 13. Mean body weights in F1 females



## 10 Special Toxicology Studies

### Repeat-Dose Toxicity

**Study title:** 13-Week Oral Gavage Toxicity and Toxicokinetic Study with Hydrocodone and (b) (4) in Rats with a 2-Week Recovery Phase

Study no.: 8243020  
 Study report location: EDR 4.2.3.6.1  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: March 29, 2011  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: Hydrocodone bitartrate (HC): Lot # 1005000871; 99.9%  
 (b) (4) (b) (4) synonym: (b) (4)  
 Lot # 1102252185; 98.3%

### Key Study Findings

- No differences between any of the groups of hydrocodone spiked (b) (4) treated rats versus the hydrocodone alone treated rats
- typical hydrocodone-related toxicities were observed
- No findings attributed to the (b) (4) were noted
- The NOEL for (b) (4) in this study is 3 mg/kg

### Methods

Doses: Veh, HC/ (b) (4) 5/0.15, 50/1.5, 100/3, 100/0 mg/kg  
 Frequency of dosing: daily  
 Route of administration: oral gavage  
 Dose volume: 10 mL/kg  
 Formulation/Vehicle: reverse osmosis water  
 Species/Strain: Rat, Fischer (F344/DuCrI)  
 Number/Sex/Group: main: 10/sex/group, recovery: 5/sex/group  
 Age: 6-7 weeks  
 Weight: M: 112-179 g; F: 102-128 g  
 Satellite groups: TK: 9/sex/group  
 Unique study design: test article is HC spiked with (b) (4)  
 Deviation from study protocol: none that affected integrity of study

### Observations and Results

#### Mortality

No test article-related mortality was seen in this study.

#### Clinical Signs

Clinical observations related to the pharmacological effects of HC were observed in all groups. Red discharge from eye, alopecia (front paws or legs), sores/scabs on front paws or legs, and/or brown or yellow haircoat of the perineal area were observed with no differences between the HC and (b) (4) groups. All observations were fully reversible after the first day of the recovery phase. No differences were noted between the 100/3 mg/kg HC/(b) (4) and the 100/0 mg/kg HC/(b) (4) groups.

**Body Weights**

In males, decreased BW and BWG was dose related in all groups compared to control (Figure 14, Table 45). Females showed the largest decrease in BWG at the lowest dose and no effects on BW or BWG were noted in the higher doses (Figure 15, Table 45). It did not appear that the inclusion of (b) (4) had any effect on body weight in males or females.

Figure 14. Mean body weights in males ( (b) (4) rat study)

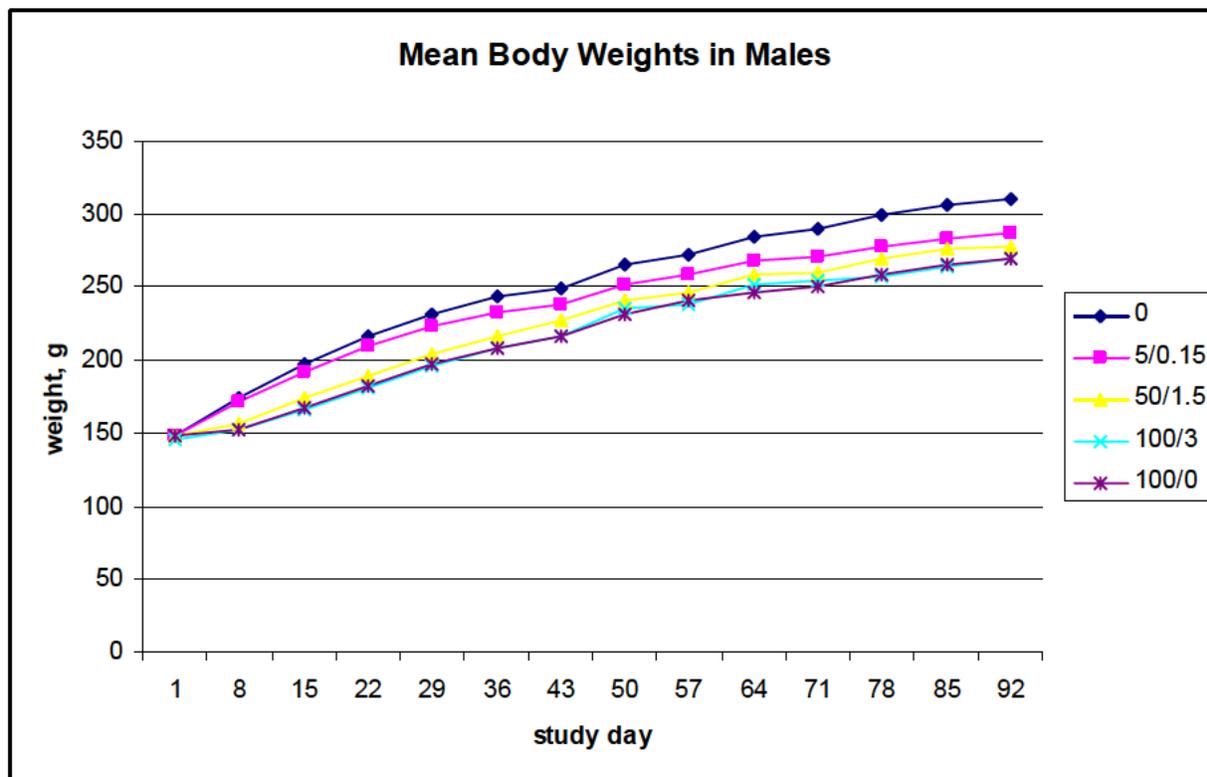


Figure 15. Mean body weights in females ( (b) (4) rat study)

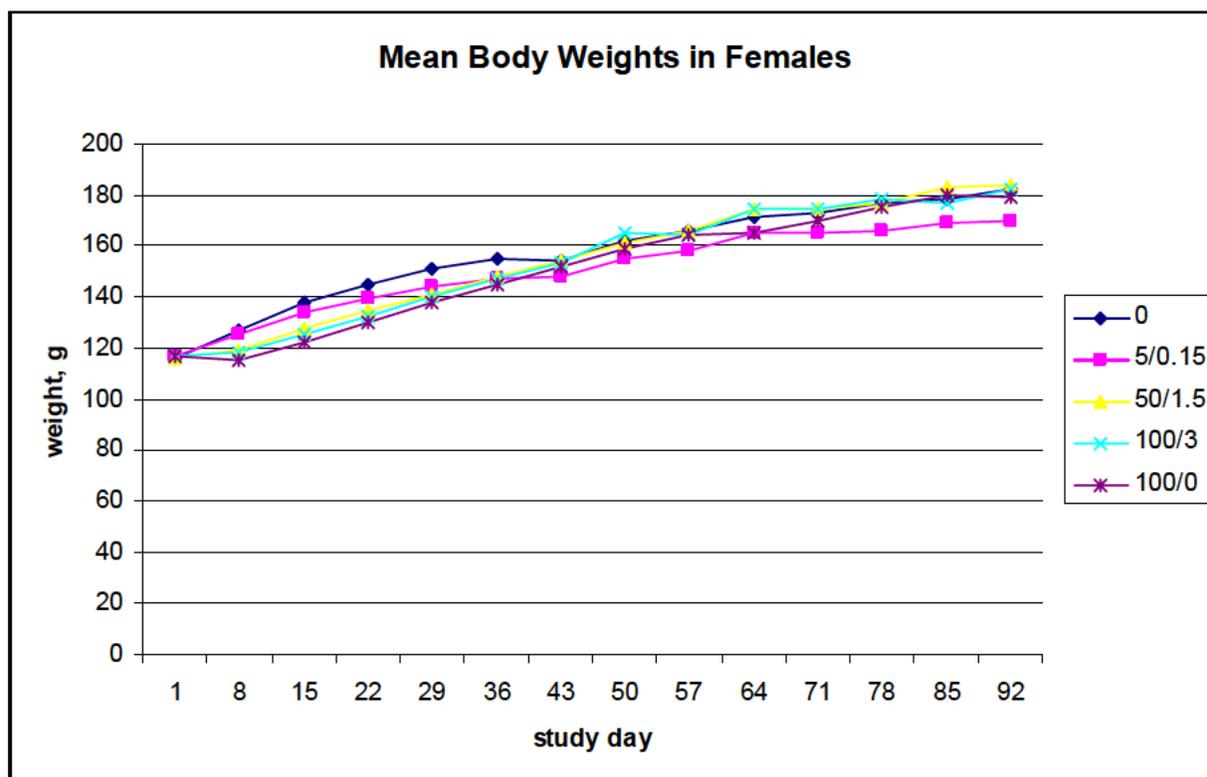


Table 45. Body weight gain ( (b) (4) rat study)

<b>Body Weight Gain</b>					
	<b>HC (b) (4) mg/kg</b>	<b>Body Weight (g), Day -1</b>	<b>Body Weight (g), Day 92</b>	<b>Body Weight Gain (BWG) (g), Day -1 to Day 92</b>	<b>BWG % Decrease from Control</b>
<b>male</b>	0	148	311	163	-
	5/0.15	148	288	140	14
	50/1.5	149	278	129	21
	100/3	146	270	124	24
	100/0	148	270	122	25
<b>female</b>	0	116	182	66	-
	5/0.15	117	170	53	20
	50/1.5	116	184	68	-3
	100/3	117	182	65	2
	100/0	117	179	62	6

### Food Consumption

Some mild decreases in food consumption were noted early in the study in the 50/1.5, 100/3, and 100/0 groups. It does not appear that the inclusion of (b) (4) influenced food consumption because no consistent differences were noted between the groups.

### Ophthalmoscopy

No changes were noted in the ophthalmoscopic exams for any group.

### Hematology, Clinical Chemistry, Gross Pathology, Organ Weights and Histopathology

No changes in any parameters were attributed to (b) (4)

### Toxicokinetics

The toxicokinetics are comparable to those seen in the previous 13-week study with HC alone. It does not appear that the inclusion of (b) (4) in the HC formulation alters the TK of HC (Table 46).

Table 46. Summary of toxicokinetics ((b) (4) rat study)

Hydrocodone (b) (4)			Hydrocodone	Hydromorphone	Norhydrocodone
Group	Dose Levels (mg/kg/day)	Sex	$C_{max}$ (ng/mL)		
7	5 (0.15)	M	5.54	8.74	16.6
		F	9.92	5.57	6.57
8	50 (1.5)	M	111	86.9	125
		F	117	63.0	75.8
9	100 (3)	M	273	93.8	251
		F	275	90.5	172
10	100 <sup>a</sup>	M	323	121	295
		F	319	91.9	193
			$AUC_{0-24}$ (ng·hr/mL)		
7	5 (0.15)	M	8.57	28.4	33.3
		F	12.8	15.4	11.5
8	50 (1.5)	M	211	279	318
		F	212	184	164
9	100 (3)	M	739	480	883
		F	681	358	501
10	100 <sup>a</sup>	M	806	508	951
		F	793	401	545

<sup>a</sup> Dose not spiked with (b) (4)

### Dosing Solution Analysis

The solutions utilized in the study were analyzed and found to be within acceptable concentration ranges.

### Conclusions

Some non-adverse clinical signs consistent with the pharmacology of HC were observed along with minor transient reductions in weight in males. It is concluded that the presence of (b) (4) in the HC formulations did not affect the toxicologic profile of HC. The NOAEL for this study is the highest dose tested, 100/3 mg/kg HC/(b) (4). The Applicant's proposed specification for (b) (4) in the drug product is (b) (4)%. At a maximum daily dose of 3 g/day of HC, the total daily intake of (b) (4) would be (b) (4). Using body surface area comparison, the 3 mg/kg rat NOAEL (18 mg/m<sup>2</sup>) provides a (b) (4)-fold safety margin over the (b) (4) mg human TDI at the MDD of HC (b) (4) mg/m<sup>2</sup>). The proposed specification of (b) (4)% for (b) (4) can be considered acceptable.

#### DEREK Assessment of (b) (4) and (b) (4)

The potential toxicities of two HC degradation products, (b) (4) and (b) (4) were assessed using the *in silico* toxicity prediction program DEREK for Windows. The structures of the compounds are shown in Figures 16 and 17.

Several endpoints were searched in the analysis, but only mutagenicity and genotoxicity endpoints will be evaluated in this review, as the other endpoints have not been validated yet. The DEREK evaluation did not indicate mutagenic/genotoxic potential for either impurity and the two impurities will be considered qualified for genotoxic potential.

(b) (4)



## 11 Integrated Summary and Safety Evaluation

The Applicant has assessed the acute and repeat-dose toxicity, genetic toxicology, and reproductive toxicology of hydrocodone. Carcinogenicity bioassays in mouse and rat are currently being conducted by the Applicant and will be submitted as a post-marketing requirement.

This formulation of extended-release hydrocodone bitartrate uses Alkermes SODAS drug delivery technology to confer controlled-release properties. The excipients, when calculated for the maximum theoretical daily dose of HC, can all be found in previously approved products and do not present any unique toxicologic concerns.

Two MFs are referenced for the HC DS (MF (b) (4) (b) (4) and MF (b) (4) (b) (4)). The specifications for all DS impurities (with the exception of (b) (4) in the (b) (4) -sourced DS) meet ICH Q3A qualification thresholds. The DS impurity (b) (4) contains a structural alert for mutagenicity but has been adequately qualified by the MF holder for genotoxic potential. It can therefore be regulated as per ICH Q3A as a typical non-genotoxic impurity. The specification set for (b) (4) in the (b) (4) DS exceeds the ICH Q3A qualification threshold and is therefore considered unacceptable. This has been communicated to the Applicant. ONDQA has deemed the (b) (4) MF unacceptable for other reasons (see review by Dr. Yong Hu) and the Applicant has stated that the (b) (4) sourced DS will not be used in the manufacture of the DP until the issues with the MF are rectified. The (b) (4) sourced DS will be used exclusively in the production of the DP. These specifications are acceptable.

The drug product contains two degradation products. The specification for (b) (4) exceeds the ICH Q3B threshold for qualification, however, the Applicant has provided adequate qualification with the appropriate genetic toxicology and repeat-dose studies. An Ames assay, combined Comet micronucleus assay and a 90-day toxicology study were conducted with (b) (4). The genetic toxicology studies with (b) (4) were both negative and the 90-day toxicology study did not reveal any unique toxicities of (b) (4) as compared to HC. The NOAEL in the 90-day toxicology study was the highest dose tested, 3 mg/kg which yields a (b) (4)-fold exposure margin if the MTDD of HC were to be consumed at the proposed specification of (b) (4)%. A DEREK evaluation conducted by the Applicant also showed that the two drug product impurities are negative for mutagenic/genotoxic potential. The specifications in the NDA for both drug product impurities are acceptable.

The standard ICH battery of genetic toxicology studies was conducted for HC. Hydrocodone tested negative in the in vitro bacterial reverse mutation assay, the in vivo mouse micronucleus assay, and the in vitro chromosome aberration assay in the absence of metabolic activation. In contrast, HC tested positive for clastogenic activity in the in vitro chromosome aberration assay in the presence of metabolic activation. Based on the results of these studies, HC is considered to have clastogenic potential. The three studies evaluated constitute the standard ICH genetic toxicology battery. As outlined the January 2006 FDA Guidance document titled "Guidance for Industry and Review Staff: Recommended Approaches to Integration of Genetic Toxicology Study

Results," if any of the three assays in the ICH genotoxicity standard battery are positive, the fourth test in the ICH battery should be conducted. It should be noted that carcinogenicity assessments in mice and rats with HC are being conducted by the applicant. These studies will be submitted to the NDA as a post-marketing requirement (PMR) but the results will not be available to the Division at the time of approval. The fourth-tier study to assess the clastogenic potential of HC will be needed as a PMR.

A full battery of developmental and reproductive toxicology studies has been conducted with HC. No NOAEL was established for female fertility parameters in the rat fertility study. However, the changes in fertility observed in the rat may be related to known opioid-mediated effects on prolactin, which is essential for estrous cycling in the rat. The clinical relevance of the fertility finding is not known. No effects on male fertility parameters (NOAEL 100 mg/kg) were noted, however decreased weights of male reproductive organs were observed at all doses. No effects on early embryonic development were seen in females treated with 25 mg/kg HC.

The effects of fertility with HC limited the dosing in the embryofetal development study in the rat. No effects on embryofetal development were seen in rat at the any dose tested (developmental NOAEL = 25 mg/kg). In rabbit, fetal body weights were significantly decreased in all treated groups. Significant increases in the number of fetal malformations including umbilical hernia and various irregularly shaped bones (ulna, femur, tibia, fibula) were observed in the highest dose group. Significant decreases in the number of ossified hyoid bodies and ossified xiphoid bones were also observed in the highest dose group. The NOAEL for teratogenic effects for this study is the mid dose, 50 mg/kg but the reductions in fetal weight were observed at all doses (no NOAEL could be established for developmental effects).

In the peri- and post-natal study, significant increases in the number of stillborn pups, dams with stillborn pups, and number of pups dying within a week after birth were seen in the 10 and 25 mg/kg groups. Significant reductions in the number of liveborn pups as well as viability and lactation indices were seen in the 10 and 25 mg/kg groups. Body weight and food consumption was reduced in the 25 mg/kg male group throughout the study. No changes were seen in post-weaning development observations or in any evaluated parameters of the F2 pups. The NOAEL for peri- and postnatal toxicity is the lowest dose tested, 5 mg/kg.

A pregnancy category C is recommended for this product and the relevant results will be described in the label. Exposure comparisons for the label will be based on body surface area because the appropriate human AUC values are not available. The highest available dose for this product will be 50 mg HC and the product is labeled to be used b.i.d., therefore, the human dose of 100 mg/day will be used as the exposure comparison to the nonclinical studies.

**Appendix/Attachments**

## Appendix 1

The studies below were reviewed by Dr. Violetta Klimek in a review dated June 8, 2002. The reviews are reproduced verbatim (with correction of any minor typographical errors).

Acute toxicity:

Acute Oral Toxicity Study of Hydrocodone Bitartrate in Sprague-Dawley Rats with a 14-Day Observation Period

- Study No. 214-021-01

Acute Oral Toxicity Study of Hydrocodone Bitartrate in Beagle Dogs with a 14-Day Observation Period

- Study No. 214-022-01

Repeated Dose Toxicity:

28-Day Oral Toxicity Study of Hydrocodone Bitartrate in Sprague-Dawley Rats

- Study No. 214-023-01

28-Day Oral Toxicity Study of Hydrocodone Bitartrate in Beagle Dogs

- Study No. 214-024-01

**Study title:           Acute Oral Toxicity Study of Hydrocodone Bitartrate in Sprague-Dawley Rats with a 14-Day Observation Period**

**Key study findings:**

- Alopecia noted in one male at 30 mg/kg/day and in one female at 3 mg/kg/day
- Dyspnea in one male at 30 mg/kg/day
- Thin appearance (one male at 3 mg/kg/day)
- Mean food consumption per day for study Day-8 and, subsequently, total food consumption was significantly higher in females at 10 mg/kg/day by 10% and 7%, respectively.
- At necropsy:
  - one male at 10 mg/kg/day had an enlarged parathyroid gland
  - one male at 30 mg/kg/day had multiple red, pin point discolorations on the pancreas
  - one female at 30 mg/kg/day had a dark nodule in the adipose tissue of the abdominal cavity and one female at the same highest dose had enlarged submandibular lymph nodes
- No definitive drug-related effects were noted at dose up to 30 mg/kg/day and up to 14 days after dosing
- A NOAEL was not identified since tissue histopathology was not performed

**Study no:** 214-021-01

**Volume #, and page #:** 3/53

**Conducting laboratory and location:**

(b) (4)

**Date of study initiation:** July 5, 2001

**GLP compliance:** Yes

**QA report:** yes ( X ) no ( )

**Drug, lot #, radiolabel, and % purity:** Batch No. 1F001, 98.42% purity

**Formulation/vehicle:** Dose formulations were based on a dose factor of 5 ml/kg body weight. The appropriate amount of test article was weighted into a beaker. Several drops of deionized water were added to the test article and it was mixed into a paste. Additional deionized water was added to achieve a final volume 1600 ml and the solution was mixed using a magnetic stir bar for approx. 5 min. Formulations were prepared once, refrigerated until use, and used for all four doses.

#### Dosing:

Species/strain: Sprague-Dawley rats  
 #/sex/group or time point (main study): 10/sex/group  
 Satellite groups used for toxicokinetics or recovery: None  
 Age: 6-8 weeks old  
 Weight: 200-225 g (males) and 150-175 g (females)  
 Doses in administered units: 0, 3, 10, 30 mg/kg/day  
 Route, form, volume, and infusion rate: Oral gavage in 4 equal doses approx. 6 hrs apart within a 24 hrs period

#### Observations and times:

Clinical signs: every 3 hrs during first 24 hrs followed by twice daily observation (once before 10 am and once after 2 pm). The observations included skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavioral patterns.

Body weights: Prior to the first dose, on study Day-8 and Day-15  
 Food consumption: Weekly on study Day-8 and Day-15  
 Ophthalmoscopy: Not performed  
 EKG: Not performed  
 Hematology: Not performed  
 Clinical chemistry: Not performed  
 Urinalysis: Not performed  
 Gross pathology: On study Day-15  
 Organs weighed: At termination on Day-15  
 Histopathology: At termination on Day-15 the tissue was collected and preserved, but was not examined microscopically.

Toxicokinetics: Not performed  
 Other: Not performed

**Results:**

Mortality: All animals survived to the scheduled termination.

Clinical signs: Clinical signs noted were considered incidental and included:

- alopecia in one male at 30 mg/kg/day and in one female at 3 mg/kg/day
- dyspnea in one male at 30 mg/kg/day
- thin appearance (one male at 3 mg/kg/day)

Body weights: There were no significant differences in body weights and body weights gains among the treated and control animals.

Food consumption: No significant difference in food consumption was observed between control and hydrocodone treated groups of males through study Day-15. Mean food consumption per day for study Day-8 and, subsequently, total food consumption, was significantly higher in females at 10 mg/kg/day by 10% and 7%, respectively. Because of lack of dose-dependency in increased food consumption in female rats, this effect is not considered test article related.

Ophthalmoscopy: Not performed

Electrocardiography: Not performed

Hematology: Not performed

Clinical chemistry: Not performed

Urinalysis: Not performed

Organ weights: There was no significant difference in organ weights among controls and test article treated groups of male and female rats.

Gross pathology: Incidental findings were noted at necropsy. One male at 10 mg/kg/day had an enlarged parathyroid gland. One male at 30 mg/kg/day had multiple red, pin point discolorations on the pancreas. One female at 30 mg/kg/day had a dark nodule in the adipose tissue of the abdominal cavity and one female at the same highest dose had enlarged submandibular lymph nodes.

Histopathology: Not performed

Toxicokinetics: Not performed

**Summary of individual study findings:** This study was designed to determine the potential acute toxicity of hydrocodone bitartrate in rats when administered orally in four equal doses within 24 hrs. The results of this study consist of some incidental clinical signs such alopecia (in one male at 30 mg/kg/day and in one female at 3 mg/kg/day), dyspnea (in one male at 30 mg/kg/day) and thin appearance (one male at 3 mg/kg/day). Increased food consumption was noted in female rats at 10 mg/kg/day. Incidental findings were noted at necropsy. One male at 10 mg/kg/day had an enlarged parathyroid gland. One male at 30 mg/kg/day had multiple red, pin point discolorations on the pancreas. One female at 30 mg/kg/day had a dark nodule in the adipose tissue

of the abdominal cavity and one female at the same highest dose had enlarged submandibular lymph nodes. Thus, no significant, definitive drug-related findings were noted at doses up to 30 mg/kg. A NOAEL cannot be identified since tissue histopathology was not performed.

**Study title: Acute Oral Toxicity Study of Hydrocodone Bitartrate in Beagle Dogs with a 14-Day Observation Period**

**Key study findings:**

- Diarrhea at 30 mg/kg/day (1 male)
- Red discharge and clear emesis (1 female), and languid behavior (1 male and 2 females) at 30 mg/kg/day; resolved by study Day-3
- Dose-dependent decrease in food consumption (significant at 30 mg/kg/day) at post-dose; resolved by study Day-4
- No significant drug-related findings were noted at doses  $\leq$  10 mg/kg/day
- A NOAEL was not identified since tissue pathology was not performed

**Study no:** 214-022-01

**Volume #, and page #:** 3/174

**Conducting laboratory and location:**

(b) (4)

**Date of study initiation:** July 5, 2001

**GLP compliance:** Yes

**QA report:** yes ( X ) no ( )

**Drug, lot #, radiolabel, and % purity:** Batch No. 1F001, 98.42% purity

**Formulation/vehicle:** The neat test article was weighted into gelatin capsules based on the study Day-1 body weights.

**Methods (unique aspects):** Capsules containing test article were stored at the room temp until use. Capsules were administered 4 times, approx. 6 hrs apart, on study Day-1. Control dogs received empty capsules.

**Dosing:**

Species/strain: Beagle Dogs

#/sex/group or time point (main study): 2/sex/group

Satellite groups used for toxicokinetics or recovery: None

Age: 6 months old

Weight: 6.5 – 7.7 kg (males) and 6.0 – 7.3 kg (females)

Doses in administered units: 0, 3, 10, 30 mg/kg/day

Route, form, volume, and infusion rate: Oral capsules in 4 equal doses approx. 6 hrs apart within a 24 hrs period

**Observations and times:**

Clinical signs: Every 3 hrs during first 24 hrs followed by twice daily observation (once before 10 am and once after 2 pm). The observations included skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavioral patterns. Detailed physical examinations were performed at randomization, prior to the first dose on study Day-1, on Day-8 and Day-15.

15	Body weights:	Prior to the first dose on study Day-1, on Day-8 and Day-15
	Food consumption:	Daily. Food was withheld for the first 3 hrs after the first dose to limit post-dose emesis.
	Ophthalmoscopy:	Not performed
	EKG:	Not performed
	Hematology:	Not performed
	Clinical chemistry:	Not performed
	Urinalysis:	Not performed
	Gross pathology:	On study Day-15
	Organs weighed:	At termination on Day-15 (histopathology inventory table)
	Histopathology:	At termination on Day-15 the tissue was collected and preserved, but was not examined microscopically
	Toxicokinetics:	Not performed
	Other:	Not performed

## Results:

Mortality: All animals survived to the scheduled termination.

Clinical signs: Animals treated with the highest dose of 30 mg/kg/day had diarrhea (1 male on study Day-1 and Day-2), red discharge and clear emesis (1 female), and languid behavior (1 male and 2 females). One female at 3 mg/kg/day had diarrhea on study Day-1. All clinical signs had resolved by study Day-3 of the 15-Day observation period.

Body weights: There were no significant differences in body weights and body weights gains between groups treated with test article and the control group.

Food consumption: Mean food consumption was reduced in a dose-dependent manner (-63%, -81%, -99% for males and -44%, -87%, -99% for females, at 3, 10 and 30 mg/kg/day, respectively) through the first 24 hrs (study Day-1 and Day-2) when compared to the control group. The statistical significance was reached at 30 mg/kg/day (2 dogs/group). For all dogs the food consumption returned to the control level by study Day-4.

Ophthalmoscopy: Not performed

Electrocardiography: Not performed

Hematology: Not performed

Clinical chemistry: Not performed

Urinalysis: Not performed

<u>Organ weights:</u>	There was no significant difference in organ weights among controls and test article-treated groups of male and female dogs.
<u>Gross pathology:</u>	Incidental findings were noted at necropsy. A cyst was present on the prostate gland of one male at 3 mg/kg/day. One male at 10 mg/kg/day had enlarged the right cervical lymph node and a discoloration of the spleen.
<u>Histopathology:</u>	Tissues from each necropsied animal were preserved in 10% neutral buffered formalin for possible future histopathology
<u>Toxicokinetics:</u>	Not performed

**Summary of individual study findings:** Acute oral administration of hydrocodone bitartrate (3, 10, and 30 mg/kg/day) to Beagle Dogs within 24 hrs period showed no test article-related adverse effects on mortality, body weights, organ weights, or gross pathology through 15-Days postdose. Clinical signs noted in dogs at the highest-dose (30 mg/kg/day) included diarrhea, red discharge, emesis and languid behavior. All signs had resolved at study Day-3. Food consumption was decreased in a dose-dependent fashion, significantly at 30 mg/kg/day, beginning on study Day-1, and returned to the control levels in all test article-treated groups by study Day-4. A NOAEL could not be identified as no tissue histopathology was performed.

**Study title: 28-Day Oral Toxicity Study of Hydrocodone Bitartrate in Sprague-Dawley Rats**

**Key study findings:**

- No test article-related deaths occurred during the study
- Clinical signs were incidental and occurred across test groups. Signs included dyspnea, hyperactivity, languid behavior, nasal discharge, pale looks, rough hair-coat, rales and thin appearance
- At termination, mean total body weight gain was significantly reduced in females at 30 mg/kg/day by approx. 34%
- Mean total food consumption at termination was lower by approx. 21% in the female rats at 30 mg/kg/day
- Increase in hemoglobin (+6%) in the male rats at 10 mg/kg/day and increase in mean corpuscular volume by approx. 4% also in males at 30 mg/kg/day
- Elevated chloride (+2%) in females at 10 and 30 mg/kg/day
- Elevated mean urine specific gravity in males at 30 mg/kg/day and decreased urine volume (-16%) in females at 10 mg/kg/day
- Lower kidney weights in males at 10 and 30 mg/kg/day by 10% and 9%, respectively
- Increased weight of thymus (39%) and decreased weight of thyroid (-33%) in males at 10 mg/kg/day
- Decreased mean weight of the liver by approx. 13% in the female rats at 30 mg/kg/day

(organ weight changes were not associated with any histological or clinical pathology observations that could be related to kidney or liver function)

- A NOAEL was identified at 10 mg/kg/day in females and at 30 mg/kg/day in males based on body weight changes

**Study no:** 214-023-01

**Volume #, and page #:** 4/1-395 and 5/1-387

**Conducting laboratory and location:**

(b) (4)

**Date of study initiation:** July 18, 2001

**GLP compliance:** Yes

**QA report:** yes ( X ) no ( )

**Drug, lot #, radiolabel, and % purity:** Batch No. 1F001, 98.42% purity

**Formulation/vehicle:** Dose formulations were based on a dose factor of 5 ml/kg body weight. The appropriate amount of test article was weighed into a beaker. Several drops of deionized water were added to the test article and it was mixed into a paste. Additional deionized water was added to achieve a final volume 1600 ml and the solution was mixed using a magnetic stir bar for approx. 5 min. Formulations were prepared once, refrigerated until use.

**Dosing:**

Species/strain:	Sprague-Dawley rats
#/sex/group or time point (main study):	10/sex/group
Satellite groups used for toxicokinetics:	18/sex/group
Age:	6-8 weeks old
Weight:	200-250 g (males) and 175-200 g (females)
Doses in administered units:	0, 3, 10, 30 mg/kg/day
Route, form, volume, and infusion rate:	Oral gavage in 4 equal doses approx. 6 hrs apart at a dose factor of 5 ml/kg/dose for 28 consecutive days.

**Observations and times:**

**Clinical signs:** Every 3 hrs during first 24 hrs followed by twice daily observation (once before 10 am and once after 2 pm). The observations included skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavioral patterns. Detailed physical examinations were performed at randomization (study Day-5 for main study males, Day-6 for main study females and Day-8 for TK animals), prior to the first daily dose on Day-1, Day-8, Day-15, Day-22, and prior to necropsy.

**Body weights:** Individual body weights were recorded at randomization, prior to the first daily dose, on study Day-1, Day-8, Day-15, Day-22, and prior to necropsy.

**Food consumption:** Weekly on study Day-1, Day-8, Day-15, Day-22, and prior to necropsy.

Ophthalmoscopy: All main study animals were examined with an ophthalmoscope and slit lamp during the week prior to the first dose and on study Day-25 (females) and Day-26 (males).

EKG: Not performed

Hematology: At necropsy on study Day-29 (animals were food fasted overnight prior to blood collection)

Clinical chemistry: At necropsy on study Day-29

Urinalysis: Urine was collected from individual animals for 18 – 24 hrs prior to necropsy on study Day-29.

Gross pathology: Conducted on all main study animals, including moribund animals and those who did not survive to the scheduled termination on study Day-29

Organs weighed: At necropsy on study Day-29 (histopathology inventory table)

Histopathology: At necropsy on study Day-29. Tissues from all animals of all groups were examined microscopically (histopathology inventory table)

Toxicokinetics: Blood samples were collected from 3 rats/sex/time point at Pre-dose 1 (0 hr) and at 0.5, 1, 2, 4, 6, 12, 18 and 24 hrs following the first daily dose on study Day-1 and Day-28. The quantification of hydrocodone and hydromorphone, a metabolite, in plasma and subsequent toxicokinetic analysis were performed. The  $T_{max}$ ,  $C_{max}$ ,  $t_{1/2}$  (hydrocodone),  $AUC_{all}$ ,  $AUC_{all/D}$  (hydrocodone),  $C_l/F$  (hydrocodone) and the  $AUC_{all}$  ratio of parent to metabolite were calculated using time points from pre-dose (plotted and analyzed as  $t=0$ ) through 6 hrs following the first daily dose.

Other: Not performed

## Results:

Mortality: One moribund male at 10 mg/kg/day was euthanized on study Day-11 and was presented with a distended, gas-filled gastrointestinal tract, including the stomach, duodenum, jejunum, ileum, cecum, and colon. One male at 30 mg/kg/day was found dead on study Day-15 and was presented with black, gas-filled stomach and one moribund control female was euthanized on study Day-10 and was presented with a small right kidney. All other animals survived to the scheduled termination.

Clinical signs: Clinical signs were incidental and occurred across test groups. Signs included dyspnea, hyperactivity, languid behavior, nasal discharge, pale looks, rough hair-coat, rales and thin appearance (See Table 1). In addition, malocclusion was noted in one male at 3 mg/kg/day on study Day-8, no feces and tremor in one male at 10 mg/kg/day on study Day-10.

Table 1. Clinical observations

Observations	Controls		3 mg/kg/day		10 mg/kg/day		30 mg/kg/day	
	Male	Female	Male	Female	Male	Female	Male	Female
<b>Dyspnea -</b>								
Number of observations		6		1	8	2	1	2
Number of animals		1		1	3	1	1	1
Days from - to		4 - 10		8 - 8	5 - 11	4 - 5	15 - 15	4 - 5
<b>Hyperactive -</b>								
Number of observations							1	
Number of animals							1	
Days from - to							12 - 12	
<b>Languid -</b>								
Number of observations		3			4	3		3
Number of animals		1			1	1		1
Days from - to		4 - 6			5 - 10	4 - 6		4 - 6
<b>Rales -</b>								
Number of observations	14	3		4	14	1	1	
Number of animals	3	1		1	3	1	1	
Days from - to	8 - 29	8 - 10		8 - 15	8 - 29	8 - 8	15 - 15	
<b>Pale -</b>								
Number of observations		4			4	3		3
Number of animals		1			1	1		1
Days from - to		10 - 10			5 - 8	4 - 6		4 - 6
<b>Thin -</b>								
Number of observations	8	10			28	5		4
Number of animals	2	1			3	2		1
Days from - to	10 - 15	6 - 21			7 - 29	6 - 9		5 - 8
<b>Nasal Discharge -</b>								
Number of observations					1		1	
Number of animals					1		1	
Days from - to					10 - 10		15 - 15	
<b>Rough Haircoat -</b>								
Number of observations	2	3			8			
Number of animals	2	1			2			
Days from - to	10 - 12	8 - 10			10 - 28			

**Body weights:** In the female rats, mean absolute body weights were reduced (statistically significant) in the 10 mg/kg/day group on study Day-15 (-7%) and in the 30 mg/kg/day group on study Day-15 and Day-29 (-6% and -5%, respectively) relative to the control group. At termination, mean total body weight gain was significantly reduced in females at 30 mg/kg/day (-34%) and not changed at 3 and at 10 mg/kg/day as compared to their control animals. In the male rats, no significant changes were observed in the mean body weights or in mean body weights gains relative to the control group.

**Food consumption:** Mean total food consumption at termination was significantly lower by approx. 21% in the female rats at 30 mg/kg/day and not changed at 3 and at 10 mg/kg/day as compared to the control females. No significant differences in mean food consumption were seen between test article-treated males and control males.

Ophthalmoscopy: No test article-related abnormalities were noted at termination.

Electrocardiography: Not performed

Hematology: The only difference between control and treatment groups was an increase in hemoglobin (+6%) in the male rats at 10 mg/kg/day and increase in mean corpuscular volume by approx. 4% also in males at 30 mg/kg/day.

Clinical chemistry: An increase in chloride by approx. 2% (statistically significant) in the female rats at 10 and 30 mg/kg/day and increased aspartate aminotransferase (+17%) were the only finding noted in this study.

Urinalysis: An increased specific gravity in males at 30 mg/kg/day and decreased total volume (-16%) in females at 10 mg/kg/day were the only findings observed in the study.

Organ weights: Significant changes in the mean organ weights were as follows:

- in the male rats, lower kidney weights at 10 and 30 mg/kg/day by 10% and 9%, respectively
- in the male rats at 10 mg/kg/day, increased weight of thymus (39%) and decreased weight of thyroid (-33%) were noted, no significant change was noted at 30 mg/kg/day
- in the female rats at 30 mg/kg/day, decreased mean weight of the liver by approx. 13% was noted

These organ weight changes were not associated with any histological or clinical pathology observations that could be related to kidney or liver function. Differences in the weights of thyroid and thymus appear to be incidental in nature as it occurred only in the mid-dose group of male rats.

Gross pathology: Findings noted in animals at scheduled sacrifice:

- Tan discoloration of the caudate lobe of the liver in one male at 3 mg/kg/day
- Small left testis in one male at 3 mg/kg/day
- An adhesion connecting the diaphragmatic lobe of the lung to the diaphragm and pericardium, an adhesion on the heart and an enlargement of the mediastinal lymph nodes in one male at 10 mg/kg/day
- Hernia of the diaphragm in one male at 10 mg/kg/day
- Small right testis in one male at 10 mg/kg/day
- Grey mass in the esophagus in one female at 10 mg/kg/day
- Tan nodule on the right kidney and an adhesion connecting the liver to the right kidney in one female at 30 mg/kg/day

Findings seem to be incidental in nature because they occur in the low and mid doses (except one female at 30 mg/kg/day) and/or in one gender only, therefore, can be considered as no test article-related.

Histopathology: The most common findings were esophageal lesions identified as mixed infiltrating cells, chronic inflammation, granuloma or fibrosis mainly as a focal lesions in the esophageal wall, that were observed in all groups of animals of the study. These lesions are most likely the result of the drug administration route, that was a 4 times a day gavage. Hyperplasia of lymphoid tissues was observed in lymph nodes and/or intestinal tracts. According to the sponsor, the hyperplasia was recognized as reactive, functional/physiological event.

Table 2. Histopathological findings after 28-Day oral hydrocodone bitartrate administration in rats

Parameters ↓	dose mg/kg/day →	Males				Females			
		0	3	10	30	0	3	10	30
<b>Thymus - # of animals</b>		10	10	9	9	9	10	10	10
→		0	0	1	0	0	0	0	0
Arteriole- inflammation, chronic		0	0	1	0	0	0	0	0
Arteriole- foreign body									
<b>Adrenal glands – # of animals →</b>		10	10	9	8	8	10	10	10
Extra adrenal tissue		0	1	0	0	0	0	0	0
Mineralization, cort.-med. junction		0	0	0	0	0	0	0	1
Mineralization, capsule		0	0	0	0	0	0	0	1
<b>Heart – # of animals →</b>		10	10	9	9	9	10	10	10
Atrium left- ectopic thyroid		0	1	0	0	0	0	0	0
Epicardium-chronic inflammation		0	0	2	0	0	0	0	0
<b>Lymph nodes, med.- #of animals→</b>		10	10	9	9	7	9	8	10
Cortex, hyperplasia, lymphocytes		0	0	1	0	0	0	0	0
Sinus, hyperplasia, histiocytosis		0	0	1	0	0	0	0	0
Sinus, hyperplasia, mast cell		0	0	0	1	0	0	0	0
<b>Thyroid – # of animals →</b>		10	10	9	9	8	10	10	10
Cyst, squamous		0	0	1	0	0	0	0	0
Capsule,- ectopic thymus		0	0	0	2	0	0	0	0
<b>Mammary gland - # of animals →</b>		10	10	9	9	9	10	10	10
Hyperplasia, mast cell		0	1	1	0	0	0	0	0
<b>Liver – # of animals →</b>		10	10	9	9	9	10	10	10
Inflammation, chronic		0	1	0	0	0	0	0	1
Necrosis		0	1	0	1	0	0	0	0
Bile duct - hyperplasia		0	0	0	1	0	0	0	0
<b>Kidneys – # of animals →</b>		10	10	9	9	9	10	10	10
Interstitial – infiltrating cell, mixed		0	1	0	0	0	0	1	0
<b>Lungs – # of animals →</b>		10	10	9	9	9	10	10	10
Artery – mineralization		0	2	0	1	0	0	0	1
Arteriole – mineralization		0	2	0	0	0	0	0	0
Inflammation, acute		0	2	0	0	0	0	0	0
Granuloma		0	0	1	0	0	0	0	0
Pleura – inflammation, chronic		0	0	1	0	0	0	0	0
Peribronchial–hyperplasia, lymphoid		0	0	0	0	0	1	0	0
<b>Ovaries - # of animals →</b>						8	10	9	10
Cyst						0	0	0	1
<b>Testes – # of animals →</b>		10	10	9	9				
Seminiferous tubule, hypoplasia		0	1	0	0				
Germinal epithelium, degeneration		0	1	1	0				
<b>Esophagus – # of animals →</b>		10	10	9	9	8	10	10	10
Muscularis –infiltrating cell, mixed		2	0	0	0	0	0	0	0
Muscularis – fibrosis		1	1	0	0	0	1	3	0
Inflammation - chronic		1	1	2	1	1	2	3	1
Granulomas -		0	0	0	0	0	0	1	0

**Toxicokinetics:** Due to the overall low levels of hydrocodone and its main metabolite, hydromorphone detected in the rat plasma at doses 0.75 and 2.5 mg/kg/dose (3 and 10 mg/kg/day, respectively), toxicokinetic parameters were

calculated only for the dose 7.5 mg/kg/dose (30 mg/kg/day). Based on mean plasma concentrations calculated at each time point, the T<sub>max</sub> for hydrocodone was determined to be 0.5 hr in both males and females on Day-1 and Day-28. The TK data from this study as summarized in Tables 3 and 4 indicate that absorption of the parent compound and biotransformation to metabolite can be gender specific. C<sub>max</sub> and AUC values for hydrocodone were approximately 2-3 fold greater in the female rats than in males on study Day-1 and Day-28, while clearance (Cl/F) remained similar between both genders.

The T<sub>max</sub> for hydromorphone was determined to be 0.5 hrs in males and females, with the exception of Day-1 males (2 hrs). The C<sub>max</sub> values for hydromorphone were similar for male and female rats and had increased approx. 2.5 times from study Day-1 to Day-28. Hydromorphone AUC values were nearly 5-fold greater in males on study Day-1 as compared to females. By study Day-28, the combined AUC values had doubled in males and gender differences were no longer apparent. The AUC ratio of hydrocodone to hydromorphone through 6 hrs after the first daily dose is less than 1.0 in males but greater than 1.0 in females, suggesting a more rapid clearance of the parent drug and/or biotransformation to the metabolite in the male rats. Generally, AUC values of hydrocodone and hydromorphone were greater on study Day-28 as compared to Day-1, indicating the possibility of test article accumulation with 28-day, 7.5 mg/kg 4 times a day, repeated oral dosing.

Table 3. Hydrocodone TK parameters in the rat (hydrocodone -7.5 mg/kg/dose)

Gender	T <sub>max</sub> (h)	C <sub>max</sub> (ng/ml)	t <sub>1/2</sub> (h)	AUC call (ng-h/ml)	AUC call/D	CL/F
<b>Day-1</b>						
Male	0.5	12.9	10.1	18.1	9.7	0.01
Female	0.5	46.8	1.4	50.2	26.8	0.03
<b>Day-28</b>						
Male	0.5	19.3	4	45.1	24.1	0.04
Female	0.5	49.6	Utd*	76.0	40.5	0.03

\* Utd = unable to determine

Table 4. Hydromorphone TK parameters in the rat (hydrocodone -7.5 mg/kg/dose)

Gender	T <sub>max</sub> (h)	C <sub>max</sub> (ng/ml)	AUC <sub>HC</sub> (ng-h/ml)	AUC <sub>HM</sub> (ng-h/ml)	AUC <sub>HC</sub> /AUC <sub>HM</sub>
<b>Day-1</b>					
Male	2.0	8.8	18.1	29.1	0.62
Female	0.5	10.4	50.2	6.4	7.8
<b>Day-28</b>					
Male	0.5	21.7	45.1	62.0	0.73
Female	0.5	26.1	76.0	58.6	1.4

### Summary of individual study findings:

No test article-related deaths occurred during the study. Clinical signs were incidental and occurred across test groups. Signs included dyspnea, hyperactivity, languid behavior, nasal discharge, pale looks, rough hair-coat, rales and thin appearance. At

termination, mean total body weight gain was significantly reduced in females at 30 mg/kg/day by approx. 34% that was correlated with lower food consumption by approx. 21% in this group of female rats.

Increases in hemoglobin (+6) and mean corpuscular volume by approx. 4% were noted in males at 10 and 30 mg/kg/day, respectively. Elevated chloride (+2%) in females at 10 and 30 mg/kg/day was also assessed. The urinalysis revealed decreased urine volume (-16%) in females at 10 mg/kg/day and increased mean urine specific gravity in males at 30 mg/kg/day. Organ weights obtained at necropsy demonstrated lower kidney weights in males at 10 and 30 mg/kg/day by 10% and 9%, respectively as well as increased weight of thymus (39%) and decreased weight of thyroid (-33%) in males at 10 mg/kg/day. The mean weight of the liver was decreased by approx. 13% in the female rats at 30 mg/kg/day. These organ weight changes were not associated with any histological or clinical pathology observations that could be related to kidney or liver function. A NOAEL was identified at 10 mg/kg/day in females and at 30 mg/kg/day in males based on body weight changes.

The PK determinations were possible only with the highest dose of hydrocodone 7.5 mg/kg/dose (30 mg/kg/day) due to undetectable or low levels of the hydrocodone and its metabolite [hydro]morphine in the rat plasma on study Day-1 and Day-28. PK data from rats receiving 7.5 mg/kg/dose, 4 times a day indicate that absorption of the parent drug and its biotransformation to the metabolite may be gender specific.  $C_{max}$  and AUC values for hydrocodone were approximately 2-3 fold greater in the female rats than in males at study Day-1 and Day-28, while clearance (Cl/F) remained similar between both genders. Male rats had less hydrocodone present relative to the metabolite (hydromorphone) through the first six hrs after the first daily dose, suggesting a more rapid clearance of hydrocodone and/or biotransformation to hydromorphone than might be predicted in female rats. Generally, AUC values of hydrocodone and hydromorphone were greater on study Day-28 as compared to Day-1, indicating the possibility of test article accumulation with 28-day, 4 times a day, repeated oral dosing in rats.

**Study title: 28-Day Oral Toxicity Study of Hydrocodone Bitartrate in Beagle Dogs**

**Key study findings:**

- Clinical observations included dose-dependent increases in signs of diarrhea, red discharge in the feces, and few or no feces, as well as emesis, salivation, thin appearance and languid behavior at 30 mg/kg/day (all resolved by study Day-19).
- Transient weight gain reduction and reduced food consumption during the first week of treatment were observed in males and females of all groups but one (male – controls). Overall body weight was reduced in males by 11%, 12%, 19% at 3, 10, 30 mg/kg/day, respectively and in females by 2%, 9%, 13% at 3, 10, 30 mg/kg/day, respectively.

- One female dog at 3 mg/kg/day was identified with a focal corneal opacity
- Lower absolute monocytes and higher prothrombin time at 3 mg/kg/day, as well as higher absolute eosinophils were noted in males at 3 and 30 mg/kg/day
- Increased ovary weights at 3 and 30 mg/kg/day by 94% and 74%, respectively
- One male at 10 mg/kg/day had a tan nodule in the stomach and a black discoloration of the mediastinal lymph nodes. At the highest dose (30 mg/kg/day), one male had a red discoloration of the thymus and one had a small thymus.
- Cortical atrophy of the thymus was observed in two males and one female at 30 mg/kg/day. One male at 10 mg/kg had mild thymic hypoplasia.
- NOAEL 10 mg/kg/day based on thymic histopathology findings and body weight changes

**Study no:** 214-024-01  
**Volume #, and page #:** 6/1-297 and 7/1-318

**Conducting laboratory and location:**

(b) (4)  
 (b) (4)

**Date of study initiation:** July 16, 2001

**GLP compliance:** Yes

**QA report:** yes ( X ) no ( )

**Drug, lot #, radiolabel, and % purity:** Batch No. 1F001, 98.42% purity

**Formulation/vehicle:** The neat test article was weighted into gelatin capsules weekly based on most recent body weights.

**Methods (unique aspects):** Capsules containing test article were stored at the room temp until use. Capsules were administered 4 times, approx. 6 hrs apart, on study Day-1 through Day-28. One capsule/group/week was retained and stored at room temp. as a reserve sample. Control dogs received empty capsules.

**Dosing:**

Species/strain:	Beagle Dogs
#/sex/group or time point (main study):	4/sex/group
Satellite groups used for toxicokinetics or recovery:	NA
Age:	6 months old
Weight:	7.4 – 9.4 kg (males) and 7.5 – 9.2 kg (females)
Doses in administered units:	0, 3, 10, 30 mg/kg/day
Route, form, volume, and infusion rate:	Oral capsules in 4 equal doses approx. 6 hrs apart within a 24 hrs period

**Observations and times:**

Clinical signs: every 3 hrs during first 24 hrs followed by twice daily observation (once before 10 am and once after 2 pm). The observations included skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavioral patterns. Detailed physical

examinations were performed at randomization, prior to the first dose on study Day-1, Day-8, Day-15, Day-22 and prior to necropsy on Day-29.

Body weights:	Prior to the first dose on study Day-1, Day-8, Day-15, Day-22 and prior to necropsy on Day-29.
Food consumption:	Daily.
Ophthalmoscopy:	Prior to the first dose and again on study Day-26 (females) and Day-27 (males).
EKG:	Prior to dosing in the control males and in one 3 mg/kg/day male, within 1 hr post-dose in all remaining test article-treated males and within 3 hrs post-dose in all females on study Day-26 (females) and Day-27 (males).
Hematology:	On study Day-1 (prior to the first dose) and on Day-26 (males) and Day-27 (females). Animals were food-fasted overnight.
Clinical chemistry:	On study Day-1 (prior to the first dose) and on Day-26 (males) and Day-27 (females). Animals were food-fasted overnight.
Urinalysis:	On study Day-1 (prior to the first dose) and on Day-26 (males) and Day-27 (females). Animals were food-fasted overnight.
Gross pathology:	At termination on Day-29
Organs weighed:	At termination on Day-29 (histopathology inventory table)
Histopathology:	At termination on Day-29 (histopathology inventory table). Tissues from all animals in all groups were examined microscopically.
Toxicokinetics:	Blood was collected on study Day-1 and Day-28 according to the schedule at Table 5.

Table 5. Toxicokinetic sampling schedule.

Time point (hour)	Collection (dose)
0.0	Pre-dose 1
0.5	0.5 hr post-dose 1
1.0	1.0 hr post-dose 1
1.5	1.5 hr post-dose 1
2.0	2.0 hr post-dose 1
3.0	3.0 hr post-dose 1
4.0	4.0 hr post-dose 1
6.0	Pre-dose 2
12.0	Pre-dose 3
18.0	Pre-dose 4
24.0	6.0 hr post-dose 4

Other: Not performed

## Results:

Mortality: All animals survived to the scheduled termination.

Clinical signs: A dose-dependent increase, both in number of observations and in number of animals presenting, was noted in males and females receiving the test article. In several males and females at 3, 10 and 30 mg/kg/day, signs of diarrhea, red discharge in the feces, and few or no feces were observed. Additional observations include emesis, salivation, thin appearance and languid behavior noted at the highest dose of 30 mg/kg/day (Table 6).

Table 6. Clinical observations in dogs treated with hydrocodone bitartrate for 28 days.

Observations	Controls		3 mg/kg/day		10 mg/kg/day		30 mg/kg/day	
	Male	Female	Male	Female	Male	Female	Male	Female
<b>Diarrhea -</b>								
Number of observations		1	1	2	6	14	3	6
Number of animals		1	1	2	3	4	2	3
Days from - to		2 - 2	4 - 4	1 - 2	2 - 7	1 - 5	6 - 7	-2 - 7
<b>Languid -</b>								
Number of observations							31	20
Number of animals							4	4
Days from - to							2 - 15	3 - 7
<b>Few feces -</b>								
Number of observations						1	1	2
Number of animals						1	1	2
Days from - to						3 - 3	4 - 4	3 - 3
<b>No feces -</b>								
Number of observations			1		5	3	22	19
Number of animals			1		3	2	3	4
Days from - to			4 - 4		4 - 6	3 - 4	4 - 15	3 - 8
<b>Salivation -</b>								
Number of observations							4	2
Number of animals							1	1
Days from - to							4 - 9	3 - 4
<b>Thin -</b>								
Number of observations							5	7
Number of animals							4	4
Days from - to							8 - 15	8 - 29
<b>Discharge -</b>								
Number of observations					1	1		5
Number of animals					1	1		1
Days from - to					4 - 4	3 - 3		15 - 19
<b>Emesis -</b>								
Number of observations							7	
Number of animals							3	
Days from - to							1-8	

Nearly all clinical observations noted in this study resolved by study Day-19 with the exception of thin appearance in females at 30 mg/kg/day.

**Body weights:** The weight gain was reduced in all animals of all but one (control males) groups during the first week of treatment (Table 7a). Following the first week of treatment, all animals treated with the test article gained weight through the study. Overall body weight gain was reduced in both males and females at 30 mg/kg/day (Table 7b). Due to the weight loss in the first week of the study the overall body weight was reduced in males by 11%, 12%, 19% at 3, 10, 30 mg/kg/day, respectively and in females by 2%, 9%, 13% at 3, 10, 30 mg/kg/day, respectively.

Table 7a. Summary of weekly body weight gains in rats treated with hydrocodone bitartrate.

Group	Males				Females			
	Day-8	Day-15	Day-22	Day-29	Day-8	Day-15	Day-22	Day-29
Control	Δ +13%	Δ -1%	Δ +1%	Δ +1%	Δ -7%	Δ +2.5%	Δ 0%	Δ +7%
3 mg/kg/day	Δ -1%	Δ +4%	Δ +1%	Δ +3%	Δ -7%	Δ +2%	Δ +3%	Δ 0%
10 mg/kg/day	Δ -7%	Δ +9%	Δ +2%	Δ +1%	Δ -16%	Δ +5%	Δ +4%	Δ 0%
30 mg/kg/day	Δ -17%	Δ +1%	Δ +8%	Δ +6%	Δ -21%	Δ +6%	Δ +4%	Δ +3%

Table 7b. Overall body weight gains and total body weights relative to the controls (100%) on Day-29 in rats treated with hydrocodone bitartrate.

Group	Males		Females	
	Body weight gain	Total body weight	Body weight gain	Total body weight
Control	Δ +15.2 %	0.0	Δ + 2.8 %	0.0
3 mg/kg/day	Δ + 6.7 %	↓ 11 %	Δ - 1.5 %	↓ 2 %
10 mg/kg/day	Δ + 5.3 %	↓ 12 %	Δ - 8.7 %	↓ 9 %
30 mg/kg/day	Δ - 4.5 %	↓ 19%*	Δ - 10.3 %	↓ 13 %

\* Significantly different than control,  $p < 0.05$ .

**Food consumption:** Mean food consumption was reduced in a dose-dependent manner for all treated groups through the first week of the study. The greatest reduction in the food consumption was observed in the male rats at 30 mg/kg/day relative to the control animals. All animals treated with test article showed increased food consumption levels near the end or after the first week of the study. Due to reduced food consumption in the first week of the study, test article-treated male rats showed a dose dependent, significant reduction in total food consumption by 29%, 31%, and 54% at 3, 10, and 30 mg/kg/day, respectively at termination relative to the controls. The reductions in total food consumption at termination were also noted in female rats at 10 and 30 mg/kg/day by 17% and 26%, respectively (statistically not significant).

**Note:** Due to significant decrease in food consumption seen in animals at 30 mg/kg/day, a Lactated Ringer's Solution was administered SC to some or all males on

study Day-4 through Day12 and females on study Day-3 through Day-8. Animals were also supplemented with wet dog food on Day-6 through Day-10 (males) and Day-5 through Day-9 (females). Two males were also given Nutrical, a high calorie dietary supplement, on Day-9 through Day-12. Supplemental food treatments were not reflected in the food consumption values.

Ophthalmoscopy: One female dog at 3 mg/kg/day was identified with a focal corneal opacity, which may have been due to trauma. All other animals had no visible ophthalmoscopic lesions. No test article-related abnormalities were identified.

Electrocardiography: ECG measurements revealed one male at 3 mg/kg/day had a ST depression of 0.2 mV, which is observed in some normal dogs, and one female at 10 mg/kg/day had a positive T wave in lead V<sub>10</sub> due to a slight lead displacement. All other ECG parameters were within normal limits, therefore, no test article-related abnormalities were observed.

Hematology: At termination a few differences in the group mean values were noted between the treatment groups and controls in the male dogs only. No significant changes were noted in the female dogs. Lower absolute monocytes (58%) and higher prothrombin time at 3 mg/kg/day, as well as higher absolute eosinophils at 30 mg/kg/day (133%) were noted at termination. These findings were not dose-dependent and noted in one gender only, therefore, they may not be test article-related.

Clinical chemistry: An increase in creatinine kinase activity in males at 3 mg/kg/day was the only significant finding at termination. However, this enzyme activity was not significantly different from its pre-dose activity. The statistical significance was obtained due to a decrease in control values at termination, therefore, the finding may not be test article-related, especially since no findings were noted at higher doses.

Organ weights: Increased ovary weights at 3 and 30 mg/kg/day by 94% and 74%, respectively, were the only significant changes noted in test article-treated animals. These changes, however, are lacking dose response and may not be test article-related.

Gross pathology: Only incidental findings were noted at necropsy. One male at 10 mg/kg/day had a tan nodule in the stomach and a black discoloration of the mediastinal lymph nodes. At the highest dose (30 mg/kg/day), one male had a red discoloration of the thymus and one had a small thymus. Thymus findings could be test article-related as they occurred at the high dose. Among the female dogs one had a tan nodule in the stomach and one had a cyst in the pituitary, both at 3 mg/kg/day. None of these findings are test article-related.

Histopathology: Cortical atrophy of the thymus was observed in two males and one female at 30 mg/kg/day. One male at 10 mg/kg had mild thymic hypoplasia. The microscopic appearance of the cortical atrophy and hypoplasia was associated with depletion and absence of lymphoid cells. Other microscopic observations in this study were minimal to moderate individual occurrences located randomly in examined tissues as it is summarized in Table 8. Thymus findings could be test article-related as 3/8 animals at the highest dose show atrophy. In addition, perivascular infiltrating cells

noted in 2/4 males at 30 mg/kg/day could potentially be test article-related. All other observed histopathological changes seem to be incidental and not test article-related.

Table 8. Histopathological findings in dogs treated with hydrocodone bitartrate for 28 days.

Parameters ↓	dose mg/kg/day →	Males				Females			
		0	3	10	30	0	3	10	30
<b>Brain –</b>									
III-ventricle choroid plexus fibrosis		0	0	0	1	0	0	0	0
<b>Pituitary –</b>									
Rathkes cleft cyst		0	0	0	1	1	0	0	0
Pars nervosa cyst		0	0	0	1	0	2	0	0
Pars distalis cyst		0	0	1	0	0	0	2	0
<b>Aorta –</b>									
Media, mucoid degeneration		0	0	0	0	0	0	1	0
<b>Adrenal glands –</b>									
Extra adrenal tissue		0	0	0	1	0	0	0	0
<b>Lungs –</b>									
Peribronchiolar, infiltrating cell mix		0	1	1	0	0	1	1	0
Pulm. artery med.- mucoid degener.		0	0	0	0	0	0	1	0
<b>Lymph nodes, mesenteric –</b>									
Sinus, erythrophagocytosis		0	0	0	0	0	0	0	1
<b>Lymph nodes, mediastinal -</b>									
Sinus, erythrophagocytosis		0	1	2	1	0	0	0	1
<b>Thyroid –</b>									
Follicular cell depletion secretory		0	0	0	1	0	0	0	0
<b>Stomach –</b>									
Lymphoid tissue, hyperplasia		0	0	0	0	0	1	0	0
<b>Rectum –</b>									
Lymphoid tissue, hyperplasia		0	0	0	0	0	1	0	0
<b>Kidneys –</b>									
Cortex, infiltrating lymphocytes		0	1	0	0	0	0	0	0
<b>Duodenum -</b>									
Mucosa, crypt - dilatation		0	0	0	1	0	0	1	0
Mucosa, epithelium – hypoplasia		0	0	1	0	0	0	0	0
Brunner's glands - hyperplasia		0	0	1	0	0	0	0	0
<b>Thymus –</b>									
Cortex, atrophy		0	0	0	2	0	0	0	1
Cortex, hypoplasia		0	0	1	0	0	0	0	0
Ectopic thyroid		0	0	0	0	0	0	0	1
<b>Ovaries –</b>									
cyst		NA	NA	NA	NA	0	2	0	0
<b>Prostate –</b>									
Infiltration, subacute		0	1	0	1	NA	NA	NA	NA
<b>Ileum –</b>									
Lymphoid tissue - hyperplasia		0	0	1	0	1	0	0	0
<b>Eyes –</b>									
Cylindric body, cyst		0	0	0	0	0	1	0	0
<b>Liver –</b>									
Perivascular infiltrating cell		0	0	0	2	0	0	0	0

**Toxicokinetics:** Plasma hydrocodone and hydromorphone indicated that the mean T<sub>max</sub> for parent and metabolite ranged from 0.5 to 2.0 hrs. C<sub>max</sub> and AUC values increased proportionally to the dose in males and females on study Day-1 and Day-28. In the highest dose group (4 X 7.5 mg/kg/day) C<sub>max</sub> and AUC values almost doubled from Day-1 to Day-28, suggesting on accumulation of parent and metabolite with repeated dosing. The AUC ratios of parent to metabolite indicate a slowing in clearance of hydrocodone and/or its biotransformation to [hydro]morphine with increasing doses of the test article. Generally, there were no gender differences in PK parameters between males and females on study Day-28.

Table 9. Hydrocodone TK parameters in the dog treated with hydrocodone bitartrate for 28 days

Gender	T <sub>max</sub> (h)	C <sub>max</sub> (ng/ml)	t <sub>1/2</sub> (h)	AUC call (ng-h/ml)	AUC call/D	CL/F
<b>Day-1 (0.75 mg/kg/dose)</b>						
Male	0.5	20.1	1.7	38.3	6.4	0.11
Female	1.0	22.8	1.2	36.0	6.0	0.12
<b>Day-28 (0.75 mg/kg/dose)</b>						
Male	0.5	14.5	1.9	23.2	3.9	0.14
Female	0.5	20.2	1.8	31.5	5.3	0.13
<b>Day-1 (2.5 mg/kg/dose)</b>						
Male	0.5	58.7 ± 37.9	1.4 ± 0.8	106.0 ± 26.5	5.3 ± 1.3	0.17 ± 0.04
Female	1.3	33.5 ± 10.5	12.9 ± 14.8	106.7 ± 18.1	5.3 ± 0.9	0.09 ± 0.07
<b>Day-28 (2.5 mg/kg/dose)</b>						
Male	1.5	63.3 ± 19.1	1.1 ± 0.3	128.7 ± 37.2	6.4 ± 1.8	0.15 ± 0.03
Female	1.5	71.9 ± 42.2	1.1 ± 0.5	124.2 ± 35.0	6.2 ± 2.0	0.16 ± 0.04
<b>Day-1 (7.5 mg/kg/dose)</b>						
Male	0.75	70.7 ± 14.6	1.3* ± 0.8	149.9 ± 61.8	2.5 ± 1.0	0.49* ± 0.3
Female	0.75	154.5 ± 108.7	1.6 ± 0.4	263.5 ± 67.5	4.4 ± 1.1	0.22 ± 0.07
<b>Day-28 (7.5 mg/kg/dose)</b>						
Male	1.3	203.6 ± 40.4	1.3 ± 0.2	495.3 ± 53.8	8.3 ± 0.9	0.12 ± 0.01
Female	1.0	186.1 ± 46.0	1.3 ± 0.5	429.3 ± 143.9	7.2 ± 2.4	0.15 ± 0.04

- \*N = 3;
- No SD for hydrocodone TK parameters at the lowest dose due to limited plasma samples

Table 10. Hydromorphone TK parameters in the dog treated with hydrocodone bitartrate for 28 days

Gender	T <sub>max</sub> (h)	C <sub>max</sub> (ng/ml)	AUC <sub>HC</sub> (ng-h/ml)	AUC <sub>HM</sub> (ng-h/ml)	AUC <sub>HC</sub> /AUC <sub>HM</sub>
					<sub>M</sub>

<b>Day-1 (0.75 mg/kg/dose)</b>					
Male	1.75	15.2 ± 1.8	38.3	60.3	0.64
Female	1.25	14.8 ± 5.0	36.0	55.7	0.65
<b>Day-28 (0.75 mg/kg/dose)</b>					
Male	1.0	10.01 ± 0.9	23.2	42.4	0.55
Female	1.5	14.1 ± 4.3	31.5	56.7	0.56
<b>Day-1 (2.5 mg/kg/dose)</b>					
Male	1.75	33.3 ± 7.9	106.0	127.5	0.83
Female	6.0	28.6 ± 4.1	106.7	109.1	0.87
<b>Day-28 (2.5 mg/kg/dose)</b>					
Male	1.5	43.2 ± 6.7	128.7	182.3	0.72
Female	2.0	46.1 ± 14.8	124.2	150.1	0.76
<b>Day-1 (7.5 mg/kg/dose)</b>					
Male	2.0	41.8 ± 19.1	149.9	142.1	1.05
Female	1.25	123.8 ± 98.8	263.5	306.0	0.86
<b>Day-28 (7.5 mg/kg/dose)</b>					
Male	1.25	128.9 ± 25.3	495.3	568.4	0.90
Female	1.25	101.3 ± 14.5	429.3	412.3	1.05

#### Summary of individual study findings:

The repeated-dose (oral) toxicity of hydrocodone bitartrate in dogs at doses up to 30 mg/kg/day for 28 days, revealed a clinical signs of diarrhea, red discharge in the feces, and few or no feces, as well as emesis, salivation, thin appearance and languid behavior at 30 mg/kg/day (all resolved by study Day-19). Transient weight gain reduction and reduced food consumption (during the first week of treatment) were observed in males and females of all groups but one (male –control). Overall body weight (relative to the control) was reduced in males by 11%, 12%, 19% at 3, 10, 30 mg/kg/day, respectively and in females by 2%, 9%, 13% at 3, 10, 30 mg/kg/day, respectively at termination.

In the hematology studies, higher absolute eosinophils were noted in males at 3 and 30 mg/kg/day. At termination, increased ovary weights at 3 and 30 mg/kg/day by 94% and 74%, respectively were noted. The gross pathology examinations revealed a tan nodule in the stomach and a black discoloration of the mediastinal lymph nodes in one male at 10 mg/kg/day. At the highest dose (30 mg/kg/day), one male had a red discoloration of the thymus and one had a small thymus. Cortical atrophy of the thymus was observed in two males and one female at 30 mg/kg/day. One male at 10 mg/kg had mild thymic hypoplasia.

A NOAEL was identified at 10 mg/kg/day based on histopathological findings in thymus. This NOAEL is associated with AUC of 38.3 ng-h/ml in males and 36.0 ng-h/ml in females.

**Toxicology summary and conclusions:** Acute oral administration of hydrocodone bitartrate at doses up to 30 mg/kg/day to Sprague Dawley rats within 24 hrs period showed no adverse effects on any of the parameters evaluated during 15 days post-dose other than minor, not dose related though incidental occurrences. Acute oral administration of hydrocodone bitartrate (3, 10, and 30 mg/kg/day) to Beagle

Dogs within 24 hrs period showed no test article-related adverse effects on mortality, body weights, organ weights, or gross pathology through 15-Days postdose. Clinical signs noted in dogs at the highest-dose (30 mg/kg/day) included diarrhea, red discharge, emesis and languid behavior. All signs had resolved at study Day-3. Food consumption was decreased in a dose-dependent fashion, significantly at 30 mg/kg/day, beginning on study Day-1, and returned to the control levels in all test article-treated groups by study Day-4. A NOAEL was not identified in the acute studies as tissue histopathology was not performed.

In the rat repeated dose toxicity studies, no test article-related deaths occurred during the study. Clinical signs were incidental and occurred across test groups. Signs included dyspnea, hyperactivity, languid behavior, nasal discharge, pale looks, rough hair-coat, rales and thin appearance. At termination, mean total body weight gain was significantly reduced in females at 30 mg/kg/day by approx. 34% that was correlated with lower food consumption by approx. 21% in this group of female rats. Increases in hemoglobin (+6) and mean corpuscular volume by approx. 4% were noted in males at 10 and 30 mg/kg/day, respectively. Elevated chloride (+2%) in females at 10 and 30 mg/kg/day was also assessed. The urinalysis revealed decreased urine volume (-16%) in females at 10 mg/kg/day and increased mean urine specific gravity in males at 30 mg/kg/day. Organ weights obtained at necropsy demonstrated lower kidney weights in males at 10 and 30 mg/kg/day by 10% and 9%, respectively as well as increased weight of thymus (39%) and decreased weight of thyroid (-33%) in males at 10 mg/kg/day. The mean weight of the liver was decreased by approx. 13% in the female rats at 30 mg/kg/day. These organ weight changes were not associated with any histological or clinical pathology observations that could be related to kidney or liver function. In rats, a NOAEL was identified at 10 mg/kg/day in females and 30 mg/kg in males based on body weight changes.

The repeated-dose (oral) toxicity of hydrocodone bitartrate in dogs, revealed clinical signs of diarrhea, red discharge in the feces, and few or no feces, as well as emesis, salivation, thin appearance and languid behavior at 30 mg/kg/day (all resolved by study Day-19). Transient weight gain reduction and reduced food consumption (during the first week of treatment) were observed in males and females of all groups but one (male –control). Overall body weight was reduced in males by 11%, 12%, 19% at 3, 10, 30 mg/kg/day, respectively and in females by 2%, 9%, 13% at 3, 10, 30 mg/kg/day, respectively. In the hematology studies, lower absolute monocytes and higher prothrombin time at 3 mg/kg/day, as well as higher absolute eosinophils were noted in males at 3 and 30 mg/kg/day. At termination, increased ovary weights at 3 and 30 mg/kg/day by 94% and 74%, respectively were noted. The gross pathology examinations revealed a tan nodule in the stomach and a black discoloration of the mediastinal lymph nodes in one male at 10 mg/kg/day. At the highest dose (30 mg/kg/day), one male had a red discoloration of the thymus and one had a small thymus.

Cortical atrophy of the thymus was observed in two males and one female at 30 mg/kg/day. One male at 10 mg/kg had mild thymic hypoplasia. In dogs, a NOAEL was 10 mg/kg/day based on thymic pathology findings.

#### Histopathology Inventory for IND # 65,111

Study	214-023-01	214-024-01
Species	Rat – 28- Day	Dog – 28 Day
Adrenals	X*	X*
Aorta	X	X
Bone Marrow smear	X	X
Bone (femur)	X	X
Brain	X*	X*
Cecum	X	X
Cervix	X	X
Colon	X	X
Duodenum	X	X
Epididymis	X	X
Esophagus	X	X
Eye	X*	X*
Gall bladder	X	X
Gross lesions	X	X
Heart	X*	X*
Ileum	X	X
Jejunum	X	X
Kidneys	X*	X*
Lachrymal gland	X	X
Liver	X*	X*
Lungs	X*	X*
Lymph nodes mandibular	X	X
Lymph nodes, mesenteric	X	X
Mammary Gland	X	X
Nasal cavity		
Optic nerves	X	X
Ovaries	X*	X*
Pancreas	X	X
Parathyroid	X*	X*
Peripheral nerve	X	X
Pituitary	X*	X*
Prostate	X	X
Rectum	X	X
Salivary gland	X	X
Sciatic nerve	X	X
Seminal vesicles	X	X
Skeletal muscle	X	X
Skin	X	X

Spinal cord	X	X
Spleen	X*	X*
Sternum	X	X
Stomach	X	X
Testes	X*	X*
Thymus	X*	X*
Thyroid	X*	X*
Tongue	X	X
Trachea	X	X
Urinary bladder	X	X
Uterus	X	X
Vagina	X	X

X, histopathology performed; \*, organ weight obtained;

## Reference List

1. York R, Hoberman A, Chambers JD and Tata P. Developmental toxicity studies in rats and rabbits with orally administered hydrocodone bitartrate (HB), USP. *Reproductive Toxicology* 17, 507. 2003.

Ref Type: Abstract

2. Hoberman A, Chambers J, Tata P and York R. Fertility and peri/postnatal toxicity studies in rats with orally administered hydrocodone bitartrate (HB). *Reproductive Toxicology* 17, 495. 2003.

Ref Type: Abstract

3. Gutstein H and Akil H (2001) Opioid Analgesics, in *Goodman and Gilman's The Pharmacological Basis of Therapeutics* (Hardman J and Limbird L eds) pp 569-620, McGraw-Hill Medical Publishing Division, New York.
4. Ko MC, Butelman ER and Woods JH (1998) The role of peripheral mu opioid receptors in the modulation of capsaicin-induced thermal nociception in rhesus monkeys. *J Pharmacol Exp Ther* **286**:150-156.
5. Bigliardi PL, Bigliardi-Qi M, Buechner S and Ruffi T (1998) Expression of mu-opiate receptor in human epidermis and keratinocytes. *J Invest Dermatol* **111**:297-301.
6. Cadet P, Bilfinger TV, Fimiani C, Peter D and Stefano GB (2000) Human vascular and cardiac endothelia express mu opiate receptor transcripts. *Endothelium* **7**:185-191.
7. 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.
8. M Risk and G Gibory (2001) Mechanism of luteal cell regulation, Kluwer Academic Publishers.
9. Bachelot A and Binart N (2007) Reproductive role of prolactin. *Reproduction* **133**:361-369.
10. Khera KS (1981) Common fetal aberrations and their teratologic significance: a review. *Fundam Appl Toxicol* **1**:13-18.

-----  
**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**  
-----

/s/  
-----

ELIZABETH BOLAN  
01/15/2013

RICHARD D MELLON  
01/15/2013

I concur with Dr. Bolan's recommendation that NDA 202880 may be approved with the recommended PMRs and labeling.

## PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

**NDA/BLA Number: 202880    Applicant: Zogenix, Inc.**

**Stamp Date: 5/1/12**

**Drug Name: Hydrocodone    NDA/BLA Type: 505(b)(2)  
Bitartrate ER**

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	X		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> 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		

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR  
NDA/BLA or Supplement**

	<b>Content Parameter</b>	<b>Yes</b>	<b>No</b>	<b>Comment</b>
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m <sup>2</sup> or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)		X	The specification for (b)(4) in the drug substance obtained from (b)(4) exceeds the ICH Q3A(R2) threshold for qualification.
11	Has the applicant addressed any abuse potential issues in the submission?			Defer to CSS
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.

As the proposed specifications may be able to be (b)(4) based on manufacturing capability this potential issue is not deemed a filing issue.

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

The specification for (b)(4) in the hydrocodone drug substance obtained from (b)(4) exceeds ICH Q3A(R2) qualification thresholds. Although adequate genetic toxicology data exist, there are no repeat-dose toxicity data to support your proposed specification. You must either (b)(4) the specification to meet ICH Q3A(R2) thresholds or provide adequate justification for the proposed level in the form of a repeat-dose toxicology study of 90-days duration in a single species.

Elizabeth A. Bolan, Ph.D. 6/18/12  
 \_\_\_\_\_  
 Reviewing Pharmacologist Date

R. Daniel Mellon, Ph.D. 6/19/12  
 \_\_\_\_\_  
 Team Leader/Supervisor Date

-----  
**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**  
-----

/s/  
-----

ELIZABETH BOLAN  
06/26/2012

RICHARD D MELLON  
06/26/2012  
I concur.