

**CENTER FOR DRUG EVALUATION AND RESEARCH**

*APPLICATION NUMBER:*  
**21-217s000**

**PHARMACOLOGY REVIEW(S)**



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

**SUPERVISOR'S SECONDARY REVIEW  
PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION**

NDA NUMBER: **21-217**  
SERIAL NUMBER: **000**  
DATE RECEIVED BY CENTER: **May 22, 2009**  
PRODUCT: **EXALGO (hydromorphone  
hydrochloride) extended release tablets**  
INTENDED CLINICAL POPULATION: **Adult patients; acute and chronic pain**  
SPONSOR: **Neuromed Pharmaceuticals, LTD.**  
REVIEW DIVISION: **Division of Anesthesia, Analgesia, and  
Rheumatology Products (HFD-170)**  
PHARM/TOX REVIEWER: **BeLinda A. Hayes, Ph.D.**  
PHARM/TOX SUPERVISOR: **R. Daniel Mellon, Ph.D.**  
DIVISION DIRECTOR: **Bob A. Rappaport, M.D.**  
PROJECT MANAGER: **Diana Walker, Ph.D.**  
Date of review: **November 10, 2009**

**Regulatory Background:** NDA 21-217 (then referred to as Dilaudid CR) was originally submitted to the FDA on December 29, 1999 (receipt date) by Knoll Pharmaceuticals as a 505(b)(1) application. The product was originally developed under IND [REDACTED] (b) (4)

[REDACTED] Dr. Kathy Haberny, the original pharmacology toxicology reviewer, reviewed a 30-day repeat-dose toxicology study in the dog with the drug product formulation, a fertility and early embryonic development study in the rat, embryo-fetal development studies in the rat and rabbit, pre- and postnatal development study in the rat, and three genetic toxicology studies for hydromorphone (the standard ICH battery). Dr. Haberny's 2000 review of NDA 21-217 notes that the pharmacology, safety pharmacology, pharmacokinetics and acute and chronic toxicity of hydromorphone were reported in the literature and cited in the original NDAs for Dilaudid (NDA 19-892 and 19-034) and referenced for the original 21-217 NDA submission (in 1999). She recommended the NDA was approvable pending agreement on the final product labeling and agreement to conduct the carcinogenicity assessment for hydromorphone hydrochloride post marketing. Dr. Dou H. Jean, the pharmacology toxicology supervisor at that time, concurred with Dr. Haberny. The NDA received an "Approvable" letter dated October 27, 2000 that listed multiple deficiencies. However, the only nonclinical deficiency listed in the Approvable letter was item 5, reproduced below:

No carcinogenicity studies of hydromorphone hydrochloride were submitted. Before the approval of the application you will have to conduct studies to evaluate the carcinogenicity of hydromorphone hydrochloride in two rodent species.

The official meeting minutes from the post action meeting held December 7, 2000 contained the following question from Knoll and response from the Agency:

**5. In several meetings prior to the December 1999 NDA submission, Knoll understood the Agency to be in agreement that carcinogenicity studies were not necessary for approval of this product. For example, the Division's 2 September minutes of the 4 August 1999 pre-NDA meeting state (p.2): "Carcinogenicity studies are not required at this time." It should also be noted that valid carcinogenicity data is a key "refusal-to-file" criteria, if not waived, for chronic indication NDAs. Knoll would appreciate clarification of the Division's rationale for this recent change in position.**

All new NDA's for chronic indication of opioids submitted after December 1999, require carcinogenicity studies data. This change in policy was implemented around the time of the Dilaudid NDA 21-217 submission, therefore the lack of carcinogenicity data was not considered a 'refusal to file' issue. The Division indicated that in the case of Dilaudid, the carcinogenicity studies have to be underway by the time of NDA submission, and that submission of final study reports could be considered a postmarketing commitment.

The approvable letter from 2000 included numerous chemistry, manufacturing, and controls (CMC) deficiencies that precluded final assessment of the drug substance and drug product impurity safety qualification.

### **Regulatory Perspective on 2009 resubmission.**

Since 2000, the ownership of the Dilaudid injections, oral solution, and immediate release tablet products transferred from Knoll to Abbott and most recently to Purdue Pharma (the current owner of these NDAs). In addition, the owner of NDA 21-217 has changed several times since the original 1999 submission (Knoll to ALZA to Neuromed).

As discussed in 2000 with Knoll/ALZA during the post action meeting and with Neuromed in 2008 prior to resubmission of this NDA, the Agency will continue to honor the agreement that the carcinogenicity studies can be completed post marketing as long as the studies are underway by the time of resubmission. The agreement with Neuromed Pharmaceuticals can be found in the meeting minutes of the August 8, 2008 meeting (Neuromed's IND 78,223). The mouse carcinogenicity study is being conducted under IND (b) (4) and the rat carcinogenicity study is being conducted under IND 78,223. Neuromed has submitted a letter of authorization from ALZA for the Agency to access the information in IND (b) (4)

On May 22, 2009, Neuromed submitted a complete response to the original Approvable letter. Dr. BeLinda Hayes completed the nonclinical pharmacology toxicology review of the second cycle submission. Although not listed as a deficiency in the approvable letter, the Applicant conducted and submitted new pharmacology, safety pharmacology, pharmacokinetic, acute and chronic toxicology studies for hydromorphone, due to the lack of right of reference to the original Dilaudid NDAs. All of the studies were evaluated; however, only those studies that were deemed pivotal for safety and labeling were formally reviewed by Dr. Hayes. Studies that were not deemed pivotal, such as dose range-finding studies and acute and subchronic toxicology studies were examined but not formally reviewed. As noted by Dr. Hayes, there were no unexpected new toxicological findings noted. The 13-week dose range-finding studies in the mouse and rat were previously reviewed to support the ongoing carcinogenicity studies. These studies have been previously reviewed in support of the carcinogenicity special protocol assessments. All studies required to support approval and labeling of a 505(b)(1) application were formally reviewed by Drs. Haberny and Hayes.

Due to a change in the referenced DMF for hydromorphone, several new drug substance impurities were identified during this cycle. However, as outlined by Dr. Hayes, the Sponsor has provided adequate safety qualification for the proposed drug substance impurities and drug product degradants.

The toxicological findings reported in the chronic repeat-dose toxicology studies for hydromorphone are expected effects of opioid drugs. There are no unexpected nonclinical findings that would preclude approval of this drug product.

### **Recommendation on approvability**

I concur with Dr. BeLinda Hayes' recommendation that, from a nonclinical pharmacology toxicology perspective, NDA 21-217 may be approved. I also concur with Dr. Hayes's proposed drug product labeling.

Based on my review of the regulatory history and the information submitted by Neuromed as part of this response to the approvable letter, Neuromed has provided all studies necessary to

ensure that this NDA submission can still be deemed a 505(b)(1) submission, even without a right of reference to the Dilaudid NDAs.

**Recommendation for nonclinical studies**

I concur that the Applicant must submit the ongoing carcinogenicity studies in rat and mouse as postmarketing requirements (PMRs) as per previous agreement with the Agency. The timelines for this submission have been confirmed with the Applicant.

Application  
Type/Number

Submission  
Type/Number

Submitter Name

Product Name

-----  
NDA-21217

-----  
ORIG-1

-----  
NEUROMED  
PHARMACEUTICA  
LS LTD

-----  
Exalgo (hydromorphone HCl)  
8/12/16/32

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**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**  
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/s/  
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RICHARD D MELLON  
02/05/2010



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

## PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 21-217  
SERIAL NUMBER: 000  
DATE RECEIVED BY CENTER: May 22, 2009  
PRODUCT: Exalgo (Hydromorphone HCl) Extended-Release  
Tablets  
INTENDED CLINICAL POPULATION: Management of moderate to severe pain for opioid  
tolerant patients  
APPLICANT: Neuromed Pharmaceuticals Ltd  
DOCUMENTS REVIEWED: Electronic Submission  
REVIEW DIVISION: Division of Anesthesia, Analgesia, and  
Rheumatology Products (HFD-170)  
PHARM/TOX REVIEWER: BeLinda A. Hayes, Ph.D.  
PHARM/TOX SUPERVISOR: R. Daniel Mellon, Ph.D.  
DIVISION DIRECTOR: Bob A. Rappaport, M.D.  
PROJECT MANAGER: Diana Walker, Ph.D.  
Date of review submission to DARRTs: November 10, 2009

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## ***EXECUTIVE SUMMARY***

### **I. Recommendations**

#### **A. Recommendation on approvability**

This NDA application is recommended for approval from a nonclinical pharmacology and toxicology perspective.

#### **B. Recommendation for nonclinical studies**

No further nonclinical studies are needed for marketing approval. The Applicant has already initiated carcinogenicity studies in both the rat and the mouse. These studies will be submitted post-marketing.

#### **C. Recommendations on labeling**

The table below contains the draft labeling submitted by the Applicant, the proposed changes and the rationale for the proposed changes. The recommended changes from the proposed labeling are in red or strikeout font.

(b) (4)



## II. Summary of nonclinical findings

### A. Brief overview of nonclinical findings

Neuromed Pharmaceuticals Ltd. has submitted a New Drug Application (NDA) under the section 505(b)(1) of the Food, Drug, and Cosmetic Act for Exalgo Extended-Release Tablets (8, 12, 16 or 32 mg of hydromorphone hydrochloride) once daily administration for the management of moderate to severe pain in opioid tolerant patients requiring continuous, around-the-clock opioid analgesia for an extended period of time. NDA 21-217 was originally submitted on December 28, 1999 by Knoll Pharmaceuticals under the trade name Dilaudid CR<sup>®</sup>. On October 27, 2000, Knoll Pharmaceuticals received an Approvable Letter from the Agency which contained five deficiencies in chemistry, non-clinical and clinical areas. Subsequently, NDA 21-217 was transferred to ALZA Corporation. Neuromed Pharmaceuticals acquired this NDA from ALZA in April 2007.

In the Approval Letter, the following non-clinical deficiency was identified: “No carcinogenicity studies of hydromorphone hydrochloride were submitted. Before the approval of the application you will have to conduct studies to evaluate the carcinogenicity of hydromorphone hydrochloride in two rodent species.” However, in the meeting minutes from the December 7, 2000 post action meeting with the sponsor, the Agency agreed that if the carcinogenicity studies were

underway by the time the NDA was resubmitted, the final study reports could be submitted post-marketing. Special Protocol Assessments- Carcinogenicity was submitted to the Agency in October of 2005 and November of 2008 for the mouse and rat proposed 2-year carcinogenicity study, respectively. During the pre-NDA meeting on August 8, 2008, the Applicant again proposed that the carcinogenicity studies be conducted as a post-marketing commitment. The Agency agreed that the studies can be conducted post-marketing as Phase 4 requirement; and that the carcinogenicity studies be underway at the time of NDA submission. Neuromed Pharmaceuticals have confirmed that the rat (Study № 1678-002) and mouse (Study № 1678-001) carcinogenicity studies were initiated on March 18, 2009 and March 24, 2009, respectively.

### **B. Pharmacologic activity**

Hydromorphone's pharmacological profile is qualitatively similar to morphine. Its principal therapeutic action is analgesic. Similar to morphine, hydromorphone is a selective  $\mu$ -opioid receptor agonist with low selectivity for both the  $\kappa$ - and  $\delta$ -opioid receptors.

Pharmacodynamic characterization of hydromorphone was conducted in three animal models of experimental pain. Results from these animal studies demonstrated that hydromorphone was an analgesic in experimental model of acute pain (e.g. hot plate test, electrical stimulation test and peritoneal pain test). Hydromorphone potency was 9- to 17-fold greater than morphine in these models employing different pain stimuli. In humans, hydromorphone is approximately 7-times more potent in producing analgesia in equianalgesic doses (Houde, 1986; Wallenstein, 1990)

### **C. Nonclinical safety issues relevant to clinical use**

There are no new nonclinical safety issues relevant to this drug product. As is well known with opioid drug products, respiratory depression, a known extension of the pharmacological action of hydromorphone, is the most prominent adverse effect of hydromorphone that is relevant to the proposed clinical use. Clinically significant respiratory depression rarely occurs with standard hydromorphone doses in the absence of underlying pulmonary dysfunction. However, at high doses or in sensitive patients, hydromorphone may produce respiratory depression or irregular breathing patterns. Gastrointestinal adverse events are another potential safety concern. Nonclinical studies in rodents have shown that the administration of hydromorphone is associated with decreasing propulsive contractions in the small and large intestine; resulting in delayed gastrointestinal emptying which can produce constipation. Caution should be used in patients with preexisting gastrointestinal conditions.

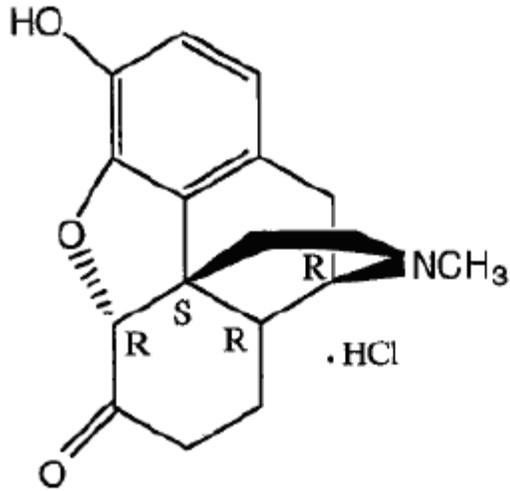
## 2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

### 2.6.1 INTRODUCTION AND DRUG HISTORY

**NDA number:** 21-217  
**Review number:** 2  
**Sequence number/date/type of submission:** 00 /May 22, 2009/Resubmission  
**Information to Applicant:** Yes ( ) No (X)  
**Applicant and/or agent:** Neuromed Pharmaceuticals  
**Manufacturer for drug substance:** (b) (4)

**Reviewer name:** BeLinda A. Hayes, Ph.D.  
**Division name:** Division of Anesthesia, Analgesia and Rheumatology Products  
**HFD #:** 170  
**Review completion date:** October 12, 2009

**Drug:**  
Trade name: Exalgo  
Generic name: Hydromorphone hydrochloride  
Code name: OROS Hydromorphone HCl  
Chemical name: Morprian-6-one,4,5-epoxy-3-hydroxy-17-methyl-, hydrochloride, 4,5a-Epoxy-3-hydroxy-17-methylmorphinan-6-one hydrochloride  
CAS registry number: 71-68-1  
Molecular formula/molecular weight: C<sub>17</sub>H<sub>19</sub>NO<sub>3</sub>.HCl/321.80  
Structure:



**Relevant INDs/NDAs/DMFs:**

INDs or NDAs	Status	Division	Indication	Stamp Date	Applicant
IND (b) (4)	Active	Anesthesia, Analgesia and Rheumatology Products	(b) (4) (b) (4) (b) (4)	(b) (4)	(b) (4) (b) (4)
78,223	Active	Anesthesia, Analgesia and Rheumatology Products	Moderate to severe pain	07/20/2007	Neuromed Pharmaceuticals Ltd

DMFs №	Subject of DMF	Holder
		(b) (4)

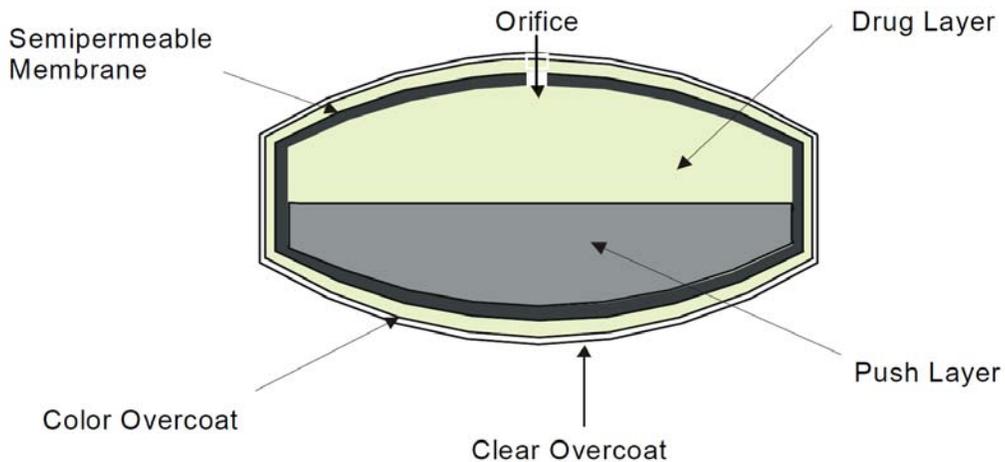
**Drug class:** Opioid Agonist  
**Intended clinical population:** Management of moderate to severe pain for opioid tolerant patients.

**Clinical formulation:** Tablet

Exaglo ER™ (OROS® Hydromorphone HCl) will be provided as extended release tablets containing 8, 12, 16, or 32 mg of hydromorphone hydrochloride for oral administration. The recommended dosage is one tablet per day. OROS® Hydromorphone HCl will be packaged in high-density polyethylene (HDPE) bottles with induction sealing and a <sup>(b) (4)</sup> child-resistant (CR) closure. The extended release property is achieved by using ALZA's OROS™ Push-Pull controlled release technology to deliver the hydromorphone hydrochloride in a controlled manner over 24 hours, thereby achieving an effective once a day treatment for chronic moderate-to-severe pain.

The design of the Exaglo ER™ (as copied from the Applicant's submission) is depicted below. The tablet is composed of a drug layer, clear overcoat, color overcoat, orifice semipermeable membrane and push layer. The drug substance is contained in the drug layer. Also, contained in the drug layer is a drug-suspending polymer that assists in the delivery of the drug substance. The semi-permeable membrane, the rate-controlling membrane, surrounds the core; it provides rate control and adds mechanical durability to the tablet. The extended delivery of hydromorphone is achieved by establishing and maintaining an osmotic activity gradient across the semipermeable membrane of the OROS® Push-Pull™ system. The osmotic gradient drives the flux of water through the membrane; the semipermeable membrane controls the rate of drug delivery from the tablet core. Delivery of the hydromorphone from the core begins when the core drug layer at the orifice erodes upon exposure to an aqueous environment. Delivery rate of the drug substance is proportional to the rate at which water permeates the membrane.

**Figure 1: Diagram of the OROS® Hydromorphone HCl Tablet**





## Hydromorphone N-oxide

Study reports for a Bacterial Reverse Mutation Assay (Ames assay), In vitro Mammalian Chromosome Aberration Test and 4-week toxicity study with hydromorphone N-oxide was submitted with the NDA. The following concerns were conveyed to the Applicant on September 29, 2009:

- 1) The proposed drug product specification for hydromorphone N-oxide of NMT (b) (4)% exceeds the impurity qualification threshold level of 0.2%. Therefore the specification of these impurity in the drug product must be reduced to NMT 0.2% or adequate safety qualification must be provided. Adequate safety qualification must include:
  - a) Minimal genetic toxicology screen (two in vitro genetic toxicology studies, e.g. point mutation assay and one chromosome aberration assay) with the isolated impurity, tested up to the limit dose for the assay.
  - b) Repeat dose toxicology of appropriate duration to support the proposed indication (90 days for a chronic indication.).
- 2) Revise the total degradation products specification accordingly.

On October 5, 2009, the Applicant responded to the Agency information request. The Applicant has agreed to lower the specification of hydromorphone N-oxide of NMT (b) (4) to (b) (4). This specification still exceeds ICHQ3B(R2) specification for drug product. The Applicant acknowledge that the new specification of hydromorphone N-oxide still exceed the acceptable level. However, the Applicant feels that this impurity has been adequately qualified for safety based on the results of the genetic toxicity studies and 4-week toxicity study in rats. The Applicant reported that there was no significant difference in response in the three test articles administered in the 4-week study; the toxicity profiles observed with hydromorphone N-oxide and hydromorphone was essentially identical. Also it was reported that the test-article effects noted in the 4-week study were similar to those observed in a 27-week study of hydromorphone in rats. Data from the genotoxicity studies did not demonstrate genetic toxicity; hydromorphone N-oxide lacked mutagenic and clastogenic effects in these studies. Based on this data, “Neuromed proposes that studies of longer duration (90 days) with hydromorphone N-oxide would be highly unlikely to yield additional useful information related to the toxicity profile and potency of this impurity and that the collective data support qualification of hydromorphone N-oxide at the proposed impurity specification levels of (b) (4)0%.”

The reviewer concurs with the Applicant that hydromorphone N-oxide has been adequately qualified. The minimal genetic toxicology screen for hydromorphone-N-oxide has been conducted; hydromorphone-N-oxide was negative for genotoxicity. The 4-week toxicity study is acceptable to qualify hydromorphone N-oxide at the (b) (4)% level. Results from the 4-week toxicity study indicated that the toxicity profile of hydromorphone N-oxide (3.5 and 14 mg/kg/day) was qualitatively similar to that of hydromorphone (14 mg/kg/day). Also, the toxicity profile of hydromorphone was not

potentiated by the co-administration of 2% (0.28 mg/kg) of hydromorphone. No significant toxicity was identified in the 4-week toxicity study performed in rats; and the toxicity profile of hydromorphone did not differ significantly in rats dosed for 4-week or 27-weeks. Thus one would not predict that hydromorphone N-oxide will not differ after 90 days of dosing. Hydromorphone N-oxide was negative for genotoxicity in the Ames and chromosomal aberration assays. In vitro data has suggested that hydromorphone N-oxide is a potential minor metabolite of hydromorphone in humans. So, one could assume that humans have been exposed to this metabolite.

**Route of administration:** Oral

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

**Studies reviewed within this submission:**

Report №	Study Title	Module/CTD Description
<b>Pharmacology</b>		
№ MPF/FG 2000/08 E	In vitro studies on the selectivity of hydromorphone hydrochloride for OP <sub>1</sub> ( $\delta$ ), OP <sub>2</sub> ( $\kappa$ ) and OP <sub>3</sub> ( $\mu$ ) opiate receptor subtypes.	<b>4.2.1.1/Primary Pharmacodynamics</b>
№ S 6985	Report on pharmacological screening tests on 3 morphine derivatives.	
<b>Safety Pharmacology</b>		
№ MPF/FE 9923	The effect of LU 138315 on cardiovascular and respiratory parameters after single oral administration in conscious, normotensive dogs.	<b>4.2.1.3/Safety Pharmacology</b>
№ 1678-003	Evaluation of the effects of hydromorphone HCl on cloned HERG channels expressed in human embryonic kidney (HEK293) cells.	
№ 1678-005	Neurobehavioral evaluation of orally administered hydromorphone HCl in rats.	
№ MPF/FS 0001	General pharmacodynamic study on the effects of hydromorphone-hydrochloride (BSF 138315) on the electrophysiological properties of the isolated guinea pig papillary muscle in vitro in comparison with morphine sulphate-pentahydrate (BSF 139513)	
№ MPF/FE 9923	The effects of LU 138315 on cardiovascular and respiratory parameters after single oral administration in conscious, normotensive dogs.	
<b>Pharmacokinetics</b>		
№ DM98/52	Plasma concentrations of hydromorphone in the dog following repeated oral administration of LU 138315 (hydromorphone hydrochloride) during a 4-week study (MPF/ET9806)	<b>4.2.2.2/Absorption</b>
№ DM98/13	Investigation of the plasma pharmacokinetics of hydromorphone in male beagle dogs after administration of a single oral (1.75 mg/kg of the active moiety) or intravenous (0.35 mg/kg of the active moiety) dose of hydromorphone.	
№ DM99/12	Plasma concentrations of hydromorphone in male	

	and female dog following repeated oral administration of BSF 138315 during a 39-week study (MPF/DT9901)	
Nº DT99025	The distribution of radiolabelled material in tissues and its persistence in the eye of male pigmented rats following a single oral dose of [ <sup>14</sup> C]hydromorphone (7 mg/kg).	<b>4.2.2.3 /Distribution</b>
Nº DM98/61	Investigation of the extent of in vitro binding of [ <sup>14</sup> C]hydromorphone to animals and human plasma proteins.	
Nº DM00/17	Investigation and characterization of the human hepatic cytochrome P450 (CYP450) isoenzymes involved in the metabolism of [ <sup>14</sup> C]hydromorphone.	<b>4.2.2.4/Metabolism</b>
Nº DM00/18	Investigation and characterization of the human hepatic glucuronyl transferases (UGTS) mediating the metabolism of [ <sup>14</sup> C]hydromorphone.	
Nº DM98/39	The metabolism of hydromorphone by rat, dog and human hepatic in vitro systems.	
Nº DM99/19	An investigation of the metabolic profile in urine, faeces and plasma of male beagle dogs after administration of a single oral dose of [ <sup>14</sup> C]hydromorphone hydrochloride (1 mg/kg of the active moiety).	
Nº MPF/DDM 9854	Excretion of radioactivity after single oral administration of [ <sup>14</sup> C]-labelled hydromorphone in dogs.	<b>4.2.2.5/Excretion</b>
<b>Toxicology</b>		
Nº TR-96-4604-057	Thirty day oral dosing study in dogs with OROS <sup>®</sup> (hydromorphone HCl) ALZA study.	<b>4.2.3.2/Repeat-Dose Toxicity</b>
Nº MPF/DT 9901	BSF 138315 – Repeated dose toxicity (at least 39-week treatment) after oral administration (Capsules) in the beagle.	
Nº MPF/DT 0010	BSF 4036516 – Toxicity study with repeated (4-week) oral administration (by gavage) to Wistar rats, using BSG 138315 as a reference item.	
Nº MPF/DT 9940	BSF 138315 – Repeated dose toxicity (at least 27 week treatment) after oral administration (gavage) in Wistar rat.	
<b>Genetic Toxicology</b>		
Nº MPF/DT 0041 E	BSF 138315- <i>Salmonella typhimurium</i> reverse mutation assay	<b>4.2.3.3/Genotoxicity</b>
<b>Other Toxicity Studies</b>		
Nº AA85YD.341 (b) (4)	In vitro mammalian chromosome aberration test.	<b>4.2.3.7.6/Impurities</b>
Nº AA85YD.503 (b) (4)	Bacterial reverse mutation assay.	
Nº MPF/DT 0012E	BSF 4036516 – <i>Salmonella typhimurium</i> reverse mutation assay.	
Nº MPF/DT 0010	BSF 4036516 – Toxicity study with repeated (4-week) oral administration (by gavage) to Wistar rats, using BSG 138315 as a reference item.	
Nº TOX8686	(b) (4) degradant: A 4-week oral toxicity study in rats followed by a 4-week recovery period.	
Nº MPF/DT 0013 E	BSF 4036516 – Chromosome aberration assay in human lymphocytes in vitro.	

**Studies not reviewed within this submission:**

Report №	Study Title	Module/CTD Description
	<b>Pharmacology</b>	
	<b>Secondary Pharmacodynamics</b>	
№ MPF/ET9820	LU 138315 – Repeated dose toxicity (4-week treatment) after oral administration (gavage) in the Wistar rat.	<b>4.2.1.2/Secondary Pharmacodynamics</b>
	<b>Safety Pharmacology</b>	
№ MPF/ET 9806	LU 138315 – Repeated dose toxicity (4-week) after oral administration to beagles.	<b>4.2.1.3/Safety Pharmacology</b>
№ MPF/DT 9940	BSF 138315 – Repeated dose toxicity (at least 27 week treatment) after oral administration (gavage) in Wistar rat.	
№ 1033-021	A 13-week oral toxicity study of hydromorphone in Han Wistar rats.	
	<b>Pharmacokinetics</b>	
№ R&D/03/694	Determination of Hydromorphone in rat or mouse plasma using a 96-Well-plates solid phase extraction system and HPLC-MS/MS in positive ESI mode.	<b>4.2.2.1/Analytical Methods and Validation Reports</b>
№ CEDRA DCN 11-A45-V3	Determination of hydromorphone in lithium heparinized CD-1 mouse plasma by LC-MS-MS.	
№ DT98020	The validation of an LC-MS method for the determination of hydromorphone in rat plasma.	
№ CEDRA DCN 11-870-VI	Determination of hydromorphone in lithium heparinized Wistar-Han rat plasma by LC-MS-MS.	
№ DT99009	The validation of an LC/MS/MS method for the determination of hydromorphone in rabbit plasma.	
№ DT98030	The validation of an LC-MS method for the determination of hydromorphone in dog plasma.	
№ DT99036	The validation of an LC/MS/MS method for the determination of hydromorphone glucuronide in human plasma.	
№ DT00002№	The stability of BTS 85 550 with respect to its potential decomposition to hydromorphone in human, rat, dog and mouse plasma.	
№ DT00008	The stability of BTS 85 550 with respect to its potential decomposition to hydromorphone in rabbit plasma.	
№ DT00011	Pharmacokinetics and plasma concentrations of hydromorphone in plasma of male Han Wistar rats after administration of a single oral (7 or 14 mg/kg of the free base) or intravenous (1 mg/kg of the free base) dose of hydromorphone.	
№ DT00018	Pharmacokinetics and plasma concentrations of hydromorphone in plasma of female Han Wistar rats after administration of a single oral (7 or 14 mg/kg of the free base) or intravenous (1 mg/kg of the free base) dose of hydromorphone.	
№ DT00027	Plasma concentrations and pharmacokinetics in female rabbit following administration of a single oral dose (12.5 mg/kg active moiety) of hydromorphone hydrochloride.	
№ R&D/03/407	Two-week oral dosage range-finding toxicity study of dilaudid in mice.	
№ R&D/03/783	Three-month oral maximum-tolerated dosage study of dilaudid (hydromorphone hydrochloride, USP) in mice.	
№ DT98050	Plasma concentrations of hydromorphone in the rat	

	following repeated oral administration of LU 138315 (hydromorphone hydrochloride) during a 4-week toxicity study (MPF/ET9820).	
Nº DT00013	Plasma concentrations of hydromorphone in male and female rats following repeated oral administration of BSF 138315 during a 27-week toxicity study (MPF/DT 9940).	
Nº DT99024	Plasma concentrations of hydromorphone in pregnant rats following oral administration of LU 138315 (hydromorphone hydrochloride) during a toxicity study.	
Nº DT99020	Plasma concentrations of hydromorphone in pregnant rabbits following repeated oral administration of LU 138315 (hydromorphone hydrochloride) during a toxicity study.	
Nº TR-96-4604-057	Thirty day oral dosing study in dogs with OROS <sup>®</sup> (hydromorphone HCl).	
Nº TR-05-5643-005	The in-vitro plasma protein binding of [ <sup>14</sup> C] hydromorphone in mouse plasma.	<b>4.2.2.3/Distribution</b>
Nº DT99033	Placental transfer of radiolabelled material in pregnant Charles River Wistar rats following administration of a single oral dose [ <sup>14</sup> C]hydromorphone hydrochloride (1.75 mg/kg; 1.56 mg/kg active moiety)	
Nº DT99034	Placental transfer of radiolabelled materials in pregnant rabbits following administration of a single oral dose (7 mg/kg) of [ <sup>14</sup> C]hydromorphone hydrochloride.	
Nº DT00049	An investigation of the metabolic profiles in the plasma, urine and faeces of male and female rats, and bile of male rats after administration of a single oral dose of [ <sup>14</sup> C]hydromorphone hydrochloride (7 mg/kg free base).	<b>4.2.2.4/Metabolism</b>
Nº DT00050	An investigation of the metabolic profiles in the urine, faeces and plasma of pregnant rabbits after administration of a single oral dose of [ <sup>14</sup> C]hydromorphone hydrochloride (7 mg/kg; 6.25 mg/kg of the active moiety).	
Nº DT99052	Investigation of the potential of hydromorphone to interact with human hepatic cytochrome P450 (CYP450).	
Nº MPF/DT 7499	BSF 1138315: Repeated dose toxicity (4-week treatment) after oral administration (gavage) in the Wistar rat (Study Nº MPF/ET 0498)- Study on liver enzyme induction.	
Nº MPF/DT 2899	BSF 1138315: Repeated dose toxicity (4-week) after oral administration to beagles (Study Nº MPF/ET 9806)- Study on liver enzyme induction.	
Nº DT99018	Excretion of radiolabelled material after the administration of a single oral dose (7 mg/kg of free base) of [ <sup>14</sup> C]hydromorphone to male rats.	<b>4.2.2.5/Excretion</b>
Nº DT99031	Excretion of radiolabelled material after the administration of a single oral dose (7 mg/kg of free base) of [ <sup>14</sup> C]hydromorphone to female rats.	
Nº DT99035	Excretion of radiolabelled material in urine and faeces of pregnant rabbits after the administration of a single oral dose (7 mg/kg of free base) of [ <sup>14</sup> C]hydromorphone.	
Nº DT00050	An investigation of the metabolic profiles in the urine, faeces and plasma of pregnant rabbits after administration of a single oral dose of [ <sup>14</sup> C]hydromorphone hydrochloride (7 mg/kg; 6.25 mg/kg of the active moiety).	
Nº DT99029	Biliary excretion of radiolabelled material in the male Charles River Wistar rat following the administration of a	

	single oral dose (7 mg/kg active moiety) of [ <sup>14</sup> C]hydromorphone.	
№ DT00049	An investigation of the metabolic profiles in the plasma, urine and faeces of male and female rats, and bile of male rats after administration of a single oral dose of [ <sup>14</sup> C]hydromorphone hydrochloride (7 mg/kg free base).	
№ DT99037	The transfer of radiolabelled material into the milk of rats following administration of a single oral dose of [ <sup>14</sup> C]hydromorphone hydrochloride (1.75 mg/kg)	
	<b>Toxicology</b>	
№ MPF/DT 9828	LU 138315-Single dose toxicity after oral administration (gavage) in the NMRI mouse.	<b>4.2.3.1/Single-Dose Toxicity</b>
№ MPF/DT 9918	BSF 138315 – Single dose toxicity after intravenous administration in the NMRI mouse.	
№ MPF/DT 9827	LU 13815 – Single dose toxicity after oral administration (gavage) in the Wistar rat.	
№ MPF/DT 9817	BSF 138315 – Single dose toxicity after intravenous administration in the Wistar rat.	
№ 1678-006	Hydromorphone HCl: An expanded oral acute toxicity study in dogs.	
№ R&D/03/407	Two-week oral dosage range finding toxicity study of Dilaudid in mice.	<b>4.2.3.2/Repeat-Dose Toxicity</b>
№ MPF/ET 9820	LU 138315 – Repeated dose toxicity (4-week treatment) after oral administration (gavage) in the Wistar rat.	
№ MPF/ET 9806	LU 138315 – Repeated dose toxicity (4-week) after oral administration to beagles.	
№ BIO-05-B045-5643	14-day oral gavage toxicity and toxicokinetic bridging study with hydromorphone HCL in mice.	
№ R&D/03/783	Three month oral maximum tolerated dosage study of Dilaudid in rats (exploratory research report).	
№ R&D/03/403	Two-week oral dosage range-finding toxicity study of Dilaudid in rats (exploratory research report).	
№ MPF/ET 0498	LU 138315 – Repeated dose toxicity (4-week treatment) after oral administration (gavage) in the Wistar rat.	
№ BIO-05-B043-5643	A 14-day oral repeat dose toxicity study with dose tolerance portion for Hydromorphone HCl in Han Wistar rats.	
№ TR-04-5643-013	A 13-week oral toxicity study of Hydromorphone HCl in Han Wistar rats.	
№ TR-05-5643-018	A 13-week oral toxicity study of Hydromorphone HCl in Han Wistar rats.	
№ MPF/ET 3997	LU 138315 – Dose range finding toxicity study after repeated (1-week treatment) oral administration (capsules) in male beagles.	
№ MPF/ET 1198	LU 138315 – Dose range finding toxicity study after repeated (2-week treatment) oral administration (capsules) in male beagles.	
№ 915-019	13-week oral toxicity study of hydromorphone HCL in Han Wistar rats.	
№ 6196-246	14-day oral gavage toxicity and toxicokinetic bridging study with hydromorphone HCl in mice.	
№ MPF/ET 3997	LU 138315 – Dose range finding toxicity study after repeated (1-week treatment) oral administration (capsules) in male beagles.	

№ MPF/ET 1198	LU 138315 – Dose range finding toxicity study after repeated (2-week treatment) oral administration (capsules) in male beagles.	
№ MPF/ET 9806	LU 138315 – Repeated dose toxicity (4-week) after oral administration beagles.	
<b>Reproductive and Development</b>		
№ MPF/DT 4898E	Dose-range-finding study to determine the dose levels for an examination of the influence of dilaudid on the pregnant rabbit and the fetus by oral administration.	<b>4.2.3.5.2/Embryo-fetal Development</b>
<b>Local Tolerance</b>		
№ MPF/ET 9451	Study to investigate the local tolerability of Diludid HP after single intravenous, intramuscular, intraarterial, paravenous and subcutaneous injection in beagle dogs.	<b>4.2.3.6/Local Tolerance</b>
№ MPF.NT 8962 E	A two-week dermal toxicity/absorption n study in rabbits with hydromorphone.	
№ MPF/PT 8783 E	Hydromorphone – A closed-patch repeated insult dermal sensitization study in guinea pigs (Modified Buehler Method).	
<b>Other Toxicity Studies</b>		
№ MPF/DT 0009	BSF 4036516 – Single dose toxicity after oral administration (gavage) in the Wistar rat.	<b>4.2.3.7.6/Impurities</b>
№ BIO-05-B026-5643	Hemolytic potential and blood compatibility testing with 200K and 2000K molecular weight polyethylene oxide in human blood.	<b>4.2.3.7.7/Other</b>
№ TR-05-5643-007	Single-dose intravenous toxicity study with 200K and 2000K molecular weight polyethylene oxide in rats.	
№ TR-05-5643-014	Two week intravenous toxicity and toxicokinetic study with 200K and 2000K molecular weight polyethylene oxide in rats with a two week recovery.	
№ MPF/DT 9951	BSF 138315 – Impact of pigmentation on ocular findings after repeated (14-day treatment) intravenous administration to male Wistar rats and male Brown Norway rats.	

## 2.6.2 PHARMACOLOGY

### 2.6.2.1 Brief summary

Hydromorphone, a semi-synthetic moriphan derivative, is an effective analgesic. Hydromorphone was first synthesized in Germany in 1924. The first FDA approved hydromorphone product was on January 11, 1984 (NDA 19-034); three strengths (1, 2, and 4 mg/mL) of injectable Dilaudid was approved for marketing in the United States as an analgesia. Currently, there are numerous hydromorphone containing products available in the United States.

Hydromorphone is marketed under the brand name Dilaudid and generic name Hydromorphone HCl. Hydromorphone is available in a variety of formulations including immediate release tablets (Dilaudid 2, 4 and 8 mg; Hydromorphone HCl 2, 4, and 8 mg), injectable (Dilaudid 1, 2, 4 and 10 mg/mL; Dilaudid-HP 10 mg/mL, 250 mg/mL;

Hydromorphone 10 mg/mL), oral solution (5 mg/5 mL) and suppositories (3 mg). Currently, there are no marketed extended release formulations of hydromorphone in the United States. Palladone, a time-release formulation of hydromorphone, was marketed for a short period in the United States; it was approved on September 24, 2004 and voluntarily withdrawn from the market on July 14, 2005. Palladone was withdrawn due to dose dumping with concomitant alcohol exposure. Extended release formulations are only marketed in the UK and Canada for once or twice a day use.

### 2.6.2.2 Primary pharmacodynamics

**Primary pharmacodynamics:** Hydromorphone is an opioid agonist with activity at the  $\mu$ -opioid receptor. Activation of  $\mu$ -opioid-receptors is associated with analgesia, respiratory depression, sedation, decreased gastrointestinal motility, euphoria and physical dependence.

**Mechanism of action:** The primary mechanism action of hydromorphone in the treatment of pain is due to mu-opioid receptor agonist effects. To characterize the mechanism of action, hydromorphone was tested in an in vitro assay using standard filtration receptor binding techniques for its affinity to various opioid receptors. A summary of these studies is discussed below.

#### **Study Title: In vitro studies on the selectivity of hydromorphone hydrochloride for OP<sub>1</sub>( $\delta$ ), OP<sub>2</sub>( $\kappa$ ) and OP<sub>3</sub>( $\mu$ ) opiate receptor subtypes. (Non-GLP compliant)**

#### **Study Report No. MPF/FG 2000/08 E**

**Objective of the study:** To assess the selectivity of hydromorphone for the opioid receptor subtypes OP<sub>1</sub>( $\delta$ ), OP<sub>2</sub>( $\kappa$ ) and OP<sub>3</sub>( $\mu$ ).

**Methods.** Using competition experiments, the IC<sub>50</sub> values were determined using membranes from rat cerebral cortex, and guinea pig cerebellum. Hydromorphone was evaluated on each opioid receptor at 10 concentrations ranging from 10<sup>-12</sup> to 10<sup>-5</sup> M in duplicate. The experimental conditions of the assay are tabulated below.

Experimental Conditions						
Receptors	Membranes	Reference compounds	Ligands	Concentrations	Nonspecific	Incubation
OP <sub>1</sub> ( $\delta$ )	Rat cerebral cortex	DPDPE	[ <sup>3</sup> H]-pCl-Phe-DPDPE	0.75 nM	Naltrexone (10 $\mu$ M)	15 hr/25°C
OP <sub>2</sub> ( $\kappa$ )	Guinea pig cerebellum	U 50488	[ <sup>3</sup> H]-U 69593	0.7 nM	Naloxone (10 $\mu$ M)	80 min/25°C
OP <sub>3</sub> ( $\mu$ )	Rat cerebral cortex	DAMGO	[ <sup>3</sup> H]-DAMGO	1 nM	Naloxone (1 $\mu$ M)	60 min/25°C

**Results.** As demonstrated in the table below, hydromorphone is a selective  $\mu$ -opioid receptor ligand. Hydromorphone demonstrated selectivity for the  $\mu$ -opioid receptor (OP) receptor with a K<sub>i</sub> value of 0.24 nM. Hydromorphone showed low selectivity for the

kappa and delta opioid receptors; compared to the OP<sub>3</sub> receptor, the K<sub>i</sub> values for the OP<sub>1</sub>(κ) and OP<sub>2</sub>(δ) receptors were 60- and 52-fold higher, respectively.

Compound	IC <sub>50</sub> and K <sub>i</sub> values at each opioid receptor subtypes								
	OP <sub>1</sub>			OP <sub>2</sub>			OP <sub>3</sub>		
	IC <sub>50</sub> (nM)	nH	K <sub>i</sub> (nM)	IC <sub>50</sub> (nM)	nH	K <sub>i</sub> (nM)	IC <sub>50</sub> (nM)	nH	K <sub>i</sub> (nM)
Hydromorphone	47.7	0.67	14.5	37.4	1.23	12.5	0.67	0.73	0.24
DPDPE	12.5	0.80	3.8	NA	NA	NA	NA	NA	NA
U 50488	NA	NA	NA	0.68	1.07	0.23	NA	NA	NA
DAMGO	NA	NA	NA	NA	NA	NA	3.1	1.25	1.11
NA: Non applicable nH: Hill coefficients									

Drug activity related to proposed indication: In vivo evaluations of hydromorphone analgesic activity were conducted in models of acute antinociception pain models. The efficacy of hydromorphone antinociception by subcutaneous administration was demonstrated after exposure to radiant heat (i.e., hot plate model), electrical stimuli and peritoneal stimuli. On the basis of the ED<sub>50</sub> values, the relative potency ratio of hydromorphone was 9- to 17-fold greater than morphine.

**Study Report №: S 6985 (non-GLP compliant). Report on pharmacological screening tests on 3 morphine derivatives.**

Test	Species	Test Article	ED <sub>50</sub> (mg/kg)	Potency relative to morphine
Hot-plate Test	Mouse	Morphine	5.5 (SC)	1-fold
		Dicodid	2.8 (SC)	2-fold
		Dilaudid	0.45 (SC)	12-fold
Electrical Stimulation Test	Mouse	Morphine	2.50 (SC)	1-fold
		Dicodid	0.55 (SC)	5-fold
		Dilaudid	0.15 (SC)	16.7-fold
Peritoneal Pain Test	Mouse	Morphine	0.8 (SC)	1-fold
		Dicodid	6.5 (SC)	↓ 8-fold
		Dilaudid	0.09 (SC)	8.9-fold
NOTE: Dicodid = hydrocodone				

### 2.6.2.3 Secondary pharmacodynamics

The Applicant did not conduct formal secondary pharmacodynamics studies. As with other opioid analgesics, hydromorphone secondary pharmacological effects include dysphoria, euphoria, sedation, respiratory depression, decreased gastrointestinal motility and physical dependence.

#### 2.6.2.4 Safety pharmacology

**Neurological effects:** The neurological effects of hydromorphone were characterized in several studies. A summary of these studies is discussed below.

**Study Title: Neurobehavioral evaluation of orally administered hydromorphone HCl in rats. (GLP compliant)**

**Study Report №: 1678-005.**

**Objective of the study:** To evaluate the potential neurobehavioral toxicity of hydromorphone HCl in rats.

**Methods.** The neurobehavioral effects of hydromorphone were assessed in Wistar [CrI:WI] rats using the Functional Observational Battery (FOB) evaluations. Rats (n = 10/sex/group) were dosed orally (gavage) with 0, 5 or 10 mg/kg of hydromorphone. Battery of neurological parameters examined included, but not limited to: evaluation of activity and arousal, posture, rearing, bizarre behavior, clonic and tonic movements, gait, mobility, stereotypy, righting reflex, response to stimulus (approach, click, tail pinch, and touch), palpebral closure, pupil response, piloerection, exophthalmus, lacrimation, salivation, and respiration. Behavioral parameters were assessed predose (Day -1) and at 1.5, 4 and 12 hours after treatment. Other evaluations included: forelimb and hindlimb grip strength, hindlimb splay, pain perception (hot plate assay), clinical observations, body temperature and body weight measurements.

#### **Results.**

Hydromorphone was not shown to produce neurobehavioral toxicity in the condition of the FOB evaluation. However, some statistically significant effects were observed during the FOB evaluation following the acute administration of hydromorphone. These findings are noted in the table below. Stereotypic behavior (i.e., head weaving and circling) were noted in the high-dose male group at 1.5 hours after dosing and in the high-dose females at 1.5 and hours after dosing. Male rats in the high-dose group exhibited rearing behavior 4-hours after dosing; decreased hindlimb splay was noted 1.5 and 4-hours after dosing in the high-dose male group. These effects are consistent with the pharmacological effects of hydromorphone and are not considered to be adverse neurological effects.

FOB Evaluation : Males			Mean Score ( $\pm$ SD)		
			Dose (mg/kg)		
Category	Observation	Observation Time (Hr)	0	5	10
Neuromuscular	Mean Hindlimb Splay	1.5	127.60 (9.677)	124.43 (14.606)	111.27 (17.487) <sup>A</sup>
		4	126.53 (11.689)	123.63 (17.033)	104.57 (18.888) <sup>B</sup>
Activity/Arousal	Stereotypy	4.0	1.2	1.0	1.0
	Rearing	4	2.9 (3.07)	6.7 (4.47)	7.8 (4.83) <sup>A</sup>
Autonomic	Palpebral Closure (Home Cage)	4	0.8	0.0 <sup>A</sup>	0.0 <sup>A</sup>
	Defecation	4	2.1 (2.08)	0.4 (1.26)	0.0 (0) <sup>A</sup>
		12	2.2 (3.16)	0.6 (1.26)	0 (0) <sup>A</sup>
Physiological	Body Temperature, °C	4	36.52 (0.865)	37.2 (0.433) <sup>A</sup>	37.60 (0.745) <sup>B</sup>
	Body Weight, g	4	257.3 (8.17)	265.8 (10.08) <sup>A</sup>	266.4 (7.50) <sup>A</sup>

A: Statistically significant when compared to control at p<0.05  
B: Statistically significant when compared to control at p<0.01

**Cardiovascular effects:** Four in vivo and/or in vitro studies were performed to evaluate the effects of hydromorphone on cardiovascular functions. The cardiovascular effects of hydromorphone were characterized in Study Reports № MPF/FE 9923, MPF/ET 9806, MPF/DT 9901, MPF/FS 0001 and 1678-003. A summary of these studies are discussed below. The key findings from these studies are:

- **Cardiac Action Potential:** Hydromorphone had no effects on cardiac action potential. Using guinea pig papillary muscle it was shown that hydromorphone had no effects on amplitude of action potential and duration of action potential. Also, hydromorphone had no effects on resting membrane potential or maximum depolarization velocity.
- **hERG Channels:** Hydromorphone did not possess potassium channel blocking properties. Results demonstrated that hydromorphone was not a direct hERG channel blocker.

**Study Title:** The effects of LU 138315 on cardiovascular and respiratory parameters after single oral administration in conscious, normotensive dogs.

**Study Report №:** MPF/FE 9923

**Objective of the study:** To characterize the cardiovascular and respiratory effects of LU 138315 (hydromorphone hydrochloride) in conscious dogs following a single oral administration.

**Method:** Male beagle dogs (n = 6) were orally administered LU 138315 at dose levels of 3.5, or 7.0 mg/kg (dose refers to active moiety).

The effects of LU 138315 on hemodynamic and electrocardiographic parameters were measured in conscious dogs by telemetry. Male beagle dogs (n = 6) were treated with oral doses of 3.5, and 7.0 mg/kg of LU 138315. There was at least 7 days between each dosing. Baseline values for cardiovascular parameters were obtained prior to dosing; and the cardiovascular parameters were monitored continuously for 6 hours after dosing. Hemodynamic parameters included: heart rate, systolic, diastolic and mean blood pressure, left ventricular systolic and end-diastolic pressure, and the maximal left ventricular pressure development. Electrocardiographic parameters included: PQ, QT, and QTc intervals.

**Results:** Treatment-induced retching was observed in 3 dogs following the oral administration of 7.0 mg/kg of LU 138315. As a result of the observed overt signs, the cardiovascular data of the 7.0 mg/kg group was not analyzed due to disturbance of data collection.

Results from this study suggested that LU 138315 had no clinically relevant hemodynamic effects under these study conditions. LU 138315, at 3.5 mg/kg, had no significant effects on heart rate, systolic, diastolic and mean blood pressure, left ventricular systolic and end-diastolic pressure, and maximal left ventricular pressure during any of the post-dosing interval periods. No treatment-related ECG effects were observed following LU 138315 at dose level of 3.5 mg/kg.

Treatment-induced retching was observed in 3 dogs following the oral administration of 7.0 mg/kg of LU 138315. As a result of the observed overt signs, the cardiovascular data of the 7.0 mg/kg group was not analyzed due to disturbance of data collection.

### **Effects on hERG channels.**

**Study Title:** Evaluation of the effects of hydromorphone HCl on cloned hERG channels expressed in human embryonic kidney (HEK293) cells. (GLP compliant)

**Study Report № 1678-003.**

**Objective of the study:** To evaluate the effects of hydromorphone hydrochloride on the rapidly activating inward rectifying potassium current (IKr) conducted by hERG channels.

**Methods:** The potential of hydromorphone to inhibit potassium current in cardiac action potential duration and QT interval was studied electrophysiologically in vitro using human embryonic kidney cell line (HEK293) that stably expressed human-ether-a-go-go-related (hERG) gene encoded potassium channel on hERG-mediated potassium current were evaluated in voltage-clamped human embryonic kidney (HEK293 cells). Cisapride (positive control) and hydromorphone were evaluated at perfusion concentrations of 0.1  $\mu$ M 10.0  $\mu$ M, respectively.

**Results:** Results indicated hydromorphone was not a direct hERG channel blocker. Relative to the reference drug cisapride, hydromorphone was a weak inhibitor of hERG-mediated potassium channel current. Hydromorphone elicited a potassium current block of 2.7% (n=3). Cisapride elicited a potassium current block of 68.9% (n=3).

**Effects on cardiac action potential parameters.**

The effects of hydromorphone on evoked action potential characteristics and conduction were evaluated in vitro in papillary muscles isolated from guinea pigs. Results showed that hydromorphone had no effects on action potential duration in guinea pig papillary muscles.

**Study Title: General pharmacodynamic study on the effects of hydromorphone-hydrochloride (BSF 138315) on the electrophysiological properties of the isolated guinea pig papillary muscle in vitro in comparison with morphine sulphate-pentahydrate (BSF 139513). (GLP compliant)**

**Study Report №: MPF/FS 0001**

**Objective of the study:** To investigate the potential cardiovascular side effects of hydromorphone by examining its effects on the action potential of guinea pig papillary muscle.

**Method:** Isolated papillary muscles from the right ventricle were perfused with carbogen (95% O<sub>2</sub>; 5% CO<sub>2</sub>) in Tyrode's solution and allowed to equilibrate. After a 60-minute equilibration period, baseline action potentials were recorded at a stimulation rate of 1 and 0.5 Hz. Subsequently, papillary muscles (n = 6/group) were exposed to BSF 138315 (hydromorphone HCl) or BSF 139513 (morphine sulphate-pentahydrate) cumulatively at 30-minutes interval at concentrations of 1 x10<sup>-7</sup>, 1 x10<sup>-6</sup> and 1x10<sup>-5</sup> mol/L. After completion of the evaluation of the test article BSF 138315 and BSF 139513, the reference antiarrhythmic agent BSF 46208 (dl-sotalol hydrochloride) was tested at a concentration of 3x10<sup>-5</sup> mol/L at both stimulation frequencies. Following a 30-minute incubation period, the action potential was recorded at a stimulation rate of 1 and 0.5 Hz. Electrophysiological parameters monitored were: resting potential (RP), amplitude of action potential (AP), duration of action potential at 30%, 60% and 90% repolarization (APD<sub>30</sub>, APD<sub>60</sub> and APD<sub>90</sub>, respectively) and maximum upstroke velocity (V<sub>max</sub>).

**Results:** Results indicate that both hydromorphone and morphine do not affect cardiac action potential characteristics. As can be seen in the Applicant's tables below, hydromorphone and morphine had no apparent effect on the shape of the papillary muscles action potential at concentrations of 1 x10<sup>-7</sup>, 1 x10<sup>-6</sup> and 1x10<sup>-5</sup> mol/L at a stimulation rate of 1 and 0.5 Hz; that is neither test article caused a prolongation of action potential duration. BSF 46208 (3x10<sup>-5</sup> mol/L) produced effects as expected, increase in action potential duration. APD<sub>30</sub>, APD<sub>60</sub> and APD<sub>90</sub> was significantly (p<0.05) increased from baseline. Hydromorphone had no effects on maximum upstroke velocity, resting

potential at the tested concentrations of  $1 \times 10^{-7}$ ,  $1 \times 10^{-6}$  and  $1 \times 10^{-5}$  mol/L at a stimulation of 1 and 0.5 Hz.

**Table 1** Effect of dilaudid (BSF 138315) on action potential parameters in guinea pig papillary muscle at a stimulation rate of 1 Hz

Difference to baseline in %

Concentration [mol/l]	RP [mV]	AP [mV]	APD <sub>30</sub> [ms]	APD <sub>60</sub> [ms]	APD <sub>90</sub> [ms]	V <sub>max</sub> [V/s]
0 <sup>a</sup>	-91 ± 0.2	126 ± 0.8	125 ± 5.6	165 ± 5.8	182 ± 6.0	260 ± 19
1 • 10 <sup>-7</sup>	-0.2 ± 0.7	-0.1 ± 0.1	-0.5 ± 1.1	-0.1 ± 0.9	-0.4 ± 0.8	1.9 ± 1.6
1 • 10 <sup>-6</sup>	-0.4 ± 0.8	-0.4 ± 0.2	-0.9 ± 0.9	0.1 ± 0.7	-0.1 ± 0.7	2.6 ± 1.1
1 • 10 <sup>-5</sup>	0.0 ± 0.3	-0.5 ± 0.3	-1.6 ± 0.5	-0.4 ± 0.3	-0.5 ± 0.3	1.1 ± 1.6
<hr/>						
3 • 10 <sup>-5</sup>						
dl-sotalol	0.0 ± 0.9	-0.5 ± 0.3	8.4 ± 1.4*	15 ± 1.6*	14 ± 1.6*	1.6 ± 2.4

\* p < 0.05 vs baseline (Bonferroni-Holm)

Mean ± SEM; n = 6

0<sup>a</sup> = baseline

**Table 2** Effect of dilaudid (BSF 138315) on action potential parameters in guinea pig papillary muscle at a stimulation rate of 0.5 Hz

Difference to baseline in %

Concentration [mol/l]	RP [mV]	AP [mV]	APD <sub>30</sub> [ms]	APD <sub>60</sub> [ms]	APD <sub>90</sub> [ms]	V <sub>max</sub> [V/s]
0 <sup>a</sup>	-90 ± 0.2	129 ± 1.6	133 ± 5.2	174 ± 6.5	194 ± 6.8	269 ± 16
1 • 10 <sup>-7</sup>	-0.4 ± 0.6	0.7 ± 1.0	-1.2 ± 1.1	-0.6 ± 1.0	-0.3 ± 1.1	2.4 ± 1.6
1 • 10 <sup>-6</sup>	0.6 ± 0.8	-0.2 ± 1.4	-2.0 ± 1.1	-1.0 ± 1.3	-1.2 ± 1.3	-4.9 ± 2.6
1 • 10 <sup>-5</sup>	1.5 ± 1.5	0.8 ± 1.0	-1.3 ± 0.8	-0.1 ± 1.1	-0.2 ± 1.0	-2.7 ± 2.0
<hr/>						
3 • 10 <sup>-5</sup>						
dl-sotalol	2.6 ± 1.1	0.4 ± 1.1	10 ± 2.1*	18 ± 2.7*	17 ± 2.5*	-2.0 ± 2.3

\* p < 0.05 vs baseline (Bonferroni-Holm)

Mean ± SEM; n = 6

0<sup>a</sup> = baseline

**Table 3** Effect of morphine sulphate (BSF 139513) on action potential parameters in guinea pig papillary muscle at a stimulation rate of 1 Hz

Difference to baseline in %

Concentration [mol/l]	RP [mV]	AP [mV]	APD <sub>30</sub> [ms]	APD <sub>60</sub> [ms]	APD <sub>90</sub> [ms]	V <sub>max</sub> [V/s]
0 <sup>a</sup>	-90 ± 0.3	125 ± 1.7	123 ± 5.7	158 ± 5.5	176 ± 6.0	241 ± 16
1 • 10 <sup>-7</sup>	-1.3 ± 0.3	0.3 ± 0.3	0.9 ± 1.2	1.1 ± 1.0	0.6 ± 0.8	1.6 ± 2.0
1 • 10 <sup>-6</sup>	-0.2 ± 1.0	0.8 ± 0.5	-1.6 ± 1.0	-0.5 ± 0.9	-0.6 ± 0.8	1.9 ± 2.1
1 • 10 <sup>-5</sup>	-1.1 ± 0.9	1.0 ± 0.7	-1.7 ± 1.1	-0.1 ± 0.9	-0.4 ± 0.9	-1.7 ± 1.7
3 • 10 <sup>-5</sup>						
dl-sotalol	-0.7 ± 1.0	0.8 ± 0.7	8.1 ± 2.0*	17 ± 2.9*	16 ± 2.9*	1.4 ± 1.9

\* p < 0.05 vs baseline (Bonferroni-Holm)

Mean ± SEM; n = 6

0<sup>a</sup> = baseline

**Table 4** Effect of morphine sulphate (BSF 139513) on action potential parameters in guinea pig papillary muscle at a stimulation rate of 0.5 Hz

Difference to baseline in %

Concentration [mol/l]	RP [mV]	AP [mV]	APD <sub>30</sub> [ms]	APD <sub>60</sub> [ms]	APD <sub>90</sub> [ms]	V <sub>max</sub> [V/s]
0 <sup>a</sup>	-90 ± 0.6	128 ± 0.9	127 ± 4.0	164 ± 4.3	183 ± 5.1	272 ± 29
1 • 10 <sup>-7</sup>	-0.4 ± 0.7	0.4 ± 0.3	0.7 ± 1.0	1.4 ± 0.8	1.4 ± 0.7	-0.1 ± 1.6
1 • 10 <sup>-6</sup>	-0.5 ± 0.7	0.0 ± 0.7	0.0 ± 1.0	0.7 ± 0.8	0.5 ± 0.8	-0.8 ± 1.4
1 • 10 <sup>-5</sup>	0.2 ± 1.0	-0.1 ± 0.7	-0.9 ± 1.2	0.6 ± 0.9	0.5 ± 0.8	-1.8 ± 1.1
3 • 10 <sup>-5</sup>						
dl-sotalol	-0.7 ± 1.0	-0.9 ± 0.8	9.3 ± 2.4*	17 ± 2.6*	16 ± 2.7*	-4.3 ± 1.8

\* p < 0.05 vs baseline (Bonferroni-Holm)

Mean ± SEM; n = 6

0<sup>a</sup> = baseline

**Pulmonary effects:** The effects of hydromorphone on respiratory parameters were evaluated in vivo in beagle dogs at an oral dose of 3.5 and 7.0 mg/kg. Results showed that hydromorphone had a biphasic effect on respiratory rate. Relative to baseline, hydromorphone initially increased respiratory rate from 0.5 to 2 hours after dosing followed by a decrease in respiratory rate from 3 to 6 hours after dosing. Hydromorphone (7.0 mg/kg) decreased blood oxygen levels and increased carbon levels.

**Study Title: The effects of LU 138315 on cardiovascular and respiratory parameters after single oral administration in conscious, normotensive dogs.**

**Study Report №:** MPF/FE 9923

**Objective of the study:** To characterize the respiratory effects of LU 138315 (hydromorphone hydrochloride) in conscious dogs following a single oral administration.

**Method:** Male beagle dogs (n = 6) were orally administered LU 138315 at dose levels of 3.5, or 7.0 mg/kg (dose refers to active moiety).

The effects of LU 138315 on respiratory function (respiratory rate), blood gas parameters (pO<sub>2</sub>, O<sub>2</sub> saturation, pCO<sub>2</sub>, HCO<sub>3</sub> and pH) and body temperature were measured in conscious dogs. Male beagle dogs (n = 6) were treated with a single oral doses of 3.5, and 7.0 mg/kg of LU 138315; there was at least 7 days between dosing. Baseline values for cardiovascular parameters were obtained prior to dosing; and the respiratory and blood gas parameters and body temperature were monitored for 6 hours after dosing.

As depicted in the Applicant's tables below, at a dose of 3.0 mg/kg, LU 138315 significantly (p<0.05) increased respiratory rate at 3, 4 and 5 hours post-dosing. Relative to baseline, respiratory rate was increased 8.8/min, 3.8/min and 2.2/min at 3, 4 and 5 hours post-dosing, respectively. LU 138315 at a dose of 7.0 mg/kg also increased respiratory rate. Compared to baseline, the observed increase was not statistically significant. Compared to baseline, blood oxygen levels were significantly (p<0.05) decrease in the 3.5 mg/kg group at 2, 4 and 5 hours after dosing.

**Table 2:**

Evaluation of the respiratory activity of LU 138315 (3.5 mg/kg po) in conscious, normotensive dogs (n = 6); values represent MEAN  $\pm$  S.E.M. and are reported as change to the initial value, \* p < 0.05 vs initial value

Parameters measured: body temperature (temp), respiratory rate (Resp. Rate), partial pressure of oxygen and carbondioxide (pO<sub>2</sub> and pCO<sub>2</sub>), oxygen saturation (O<sub>2</sub>-sat), bicarbonate ions (HCO<sub>3</sub><sup>-</sup>) and blood pH.

Parameter	Initial value	Change after administration							
		30 min	60 min	90 min	2 h	3 h	4 h	5 h	6 h
Temp [°C]	39.6 $\pm$ 0.3	0.3 $\pm$ 0.2	0.0 $\pm$ 0.3	-0.3 $\pm$ 0.4	-0.6 $\pm$ 0.4	-1.1 $\pm$ 0.5	-1.4 $\pm$ 0.5*	-1.5 $\pm$ 0.5*	-1.7 $\pm$ 0.5*
Resp. Rate [min <sup>-1</sup> ]	13.7 $\pm$ 1.0	17.3 $\pm$ 17	18.5 $\pm$ 14.8	22.3 $\pm$ 13.1	29 $\pm$ 15.1	8.8 $\pm$ 3.1*	3.8 $\pm$ 0.8*	2.2 $\pm$ 0.4*	2.2 $\pm$ 1.2
pO <sub>2</sub> [mmHg]	101.5 $\pm$ 2.8	-17.1 $\pm$ 15.7	-1.7 $\pm$ 1.8	-3.0 $\pm$ 1.5	-6.7 $\pm$ 1.5*	-4.2 $\pm$ 2.3	-8.0 $\pm$ 2.4*	-8.8 $\pm$ 4	-7.3 $\pm$ 4.3
O <sub>2</sub> -Sat [%]	99.8 $\pm$ 0.2	-0.5 $\pm$ 0.6	-0.3 $\pm$ 0.4	-0.3 $\pm$ 0.4	-0.8 $\pm$ 0.5	-0.5 $\pm$ 0.5	-0.8 $\pm$ 0.7	-0.8 $\pm$ 0.5	-0.8 $\pm$ 0.7
pCO <sub>2</sub> [mmHg]	32.6 $\pm$ 0.8	2.2 $\pm$ 1.2	0.6 $\pm$ 1.5	1.6 $\pm$ 1.2	2.1 $\pm$ 1.3	0.5 $\pm$ 1.3	1.4 $\pm$ 1.7	1.5 $\pm$ 1.4	2.0 $\pm$ 1.4
HCO <sub>3</sub> <sup>-</sup> [mM]	16.1 $\pm$ 0.5	-0.1 $\pm$ 0.3	-0.5 $\pm$ 0.6	-0.1 $\pm$ 0.4	0.0 $\pm$ 0.5	-0.8 $\pm$ 0.4	0.3 $\pm$ 0.7	-0.1 $\pm$ 0.5	0.6 $\pm$ 0.6
pH	7.32 $\pm$ 0.0	-0.01 $\pm$ 0.01	-0.01 $\pm$ 0.01	-0.01 $\pm$ 0.01	-0.02 $\pm$ 0.02	-0.07 $\pm$ 0.06	-0.01 $\pm$ 0.03	-0.02 $\pm$ 0.02	-0.01 $\pm$ 0.02

**Table 4:**

Evaluation of the respiratory activity of LU 138315 (7 mg/kg po) in conscious, normotensive dogs (n = 3); values represent MEAN  $\pm$  S.E.M. and are reported as change to the initial value, \* p < 0.05 vs initial value

Parameters measured: body temperature (temp), respiratory rate (Resp. Rate), partial pressure of oxygen and carbondioxide (pO<sub>2</sub> and pCO<sub>2</sub>), oxygen saturation (O<sub>2</sub>-sat), bicarbonate ions (HCO<sub>3</sub><sup>-</sup>) and blood pH.

Parameter	Initial value	Change after administration							
		30 min	60 min	90 min	2 h	3 h	4 h	5 h	6 h
Temp [°C]	38.8 $\pm$ 0.3	0.5 $\pm$ 0.3	0.4 $\pm$ 0.3	0.1 $\pm$ 0.3	-0.2 $\pm$ 0.3	-0.5 $\pm$ 0.3	-0.7 $\pm$ 0.2	-0.8 $\pm$ 0.3	-1.0 $\pm$ 0.3
Resp. Rate [min <sup>-1</sup> ]	16 $\pm$ 1	8.7 $\pm$ 8.2	6.7 $\pm$ 6.2	20 $\pm$ 11.6	37.7 $\pm$ 30.2	12.7 $\pm$ 10.7	4.0 $\pm$ 3.5	6.0 $\pm$ 3.1	4.7 $\pm$ 5.2
pO <sub>2</sub> [mmHg]	98 $\pm$ 2.9	-0.8 $\pm$ 2.1	-4.8 $\pm$ 1.6	-4.8 $\pm$ 0.8	-5.2 $\pm$ 4.7	-4.1 $\pm$ 5.4	-3.2 $\pm$ 5.4	-5.8 $\pm$ 3.6	-1.6 $\pm$ 3.4
O <sub>2</sub> -Sat [%]	100 $\pm$ 0	0.0 $\pm$ 0.0	-1.1 $\pm$ 0.6	-1.1 $\pm$ 0.6	-1.1 $\pm$ 0.6	-0.9 $\pm$ 0.5	-0.3 $\pm$ 0.3	-0.8 $\pm$ 0.4	-0.1 $\pm$ 0.1
pCO <sub>2</sub> [mmHg]	34.1 $\pm$ 0.8	0.5 $\pm$ 1.0	1.6 $\pm$ 1.1	1.4 $\pm$ 0.5	-0.7 $\pm$ 2.2	1.5 $\pm$ 1.8	-1.2 $\pm$ 0.5	1.4 $\pm$ 0.6	-1.8 $\pm$ 1.0
HCO <sub>3</sub> <sup>-</sup> [mM]	17.8 $\pm$ 0.7	-0.1 $\pm$ 0.9	-0.3 $\pm$ 0.9	-0.5 $\pm$ 1.0	-1.4 $\pm$ 1.1	-0.9 $\pm$ 1.3	-1.5 $\pm$ 1.0	-0.3 $\pm$ 1.0	-0.5 $\pm$ 0.8
pH	7.35 $\pm$ 0.01	0.00 $\pm$ 0.01	-0.02 $\pm$ 0.02	-0.03 $\pm$ 0.02	-0.02 $\pm$ 0.01	-0.04 $\pm$ 0.01	-0.02 $\pm$ 0.02	-0.02 $\pm$ 0.02	0.00 $\pm$ 0.01

**Renal effects:** The Applicant did not conduct formal safety pharmacology studies to evaluate potential renal safety concerns with hydromorphone administration. A review of the literature did not identify any animal studies that specifically addressed hydromorphone-related renal effects.

**Gastrointestinal effects:** The Applicant did not conduct formal safety pharmacology studies to evaluate potential gastrointestinal safety concerns with hydromorphone administration. The Applicant submitted numerous articles from the published literature.

The following discussion was obtained from some of the references submitted by the Applicant or from the literature search performed by the reviewer.

Practically all commonly used opioids produce gastrointestinal adverse effects by a combination of actions on opioid receptors within the central nervous system and opioid receptors located within the enteric nervous system. Inhibition of gastrointestinal motility (i.e., propulsive peristalsis) is a long-known classical effect of morphine and morphine-like opiates. In addition to this effect, opioid drugs exert a wide spectrum of other effects on the mammalian intestinal function. These effects include reduction in secretions (pancreatic, biliary, and electrolyte/fluid) and increases in intestinal fluid absorption.

Knoll and colleagues (1974) reported that hydromorphone elicited qualitatively similar gastrointestinal effects in mice and guinea pigs as morphine. The effects of hydromorphone, morphine and several other opioids on intestinal propulsions (i.e., GI transit time) were evaluated in male and female mice. Mice were administered single subcutaneous doses (doses evaluated were not described) of hydromorphone, codeine, morphine, oxymorphone, methadone and pethidine 1 to 3 hours prior to the oral administration of charcoal solution (10% charcoal in saline). The distance covered by charcoal in the intestine in the opioid treated mice was compared to control mice. As depicted in the table below, relative to morphine, hydromorphone was more potent than morphine.

<b>Inhibition of propulsive contractions in mice</b>		
<b>Opioid</b>	<b>ED<sub>50</sub> (mg/kg)</b>	<b>ED<sub>50</sub> morphine/ ED<sub>50</sub> (opioid)</b>
Morphine	4.6 x 10 <sup>-6</sup>	1.0
Hydromorphone	2.4 x 10 <sup>-7</sup>	1.90
Codeine	6.2 x 10 <sup>-7</sup>	0.07
Oxymorphone	1.4 x 10 <sup>-6</sup>	3.30
Methadone	1.3 x 10 <sup>-5</sup>	0.35
Pethidine	1.8 x 10 <sup>-5</sup>	0.26

King et al. (1935) reported that hydromorphone elicited qualitatively similar effects as morphine on the intestinal muscular of a Thirty-Vella loop of canine ileum. Hydromorphone and morphine at a dose of 0.01 and 0.1 mg/kg, respectively, decreased the frequency of segmentation while increasing the amplitude.

Gruber and Brundage (1935) compared the effects of hydromorphone and morphine on the Thirty-Vella loop of the jejunum and ileum in dogs. The effects of dilaudid and morphine on intestinal tone, rhythmic contraction and peristaltic contraction were evaluated in female dogs. Dogs were administered a single intravenous dose of hydromorphone (0.00005 to 5 mg/kg). Hydromorphone was qualitatively similar to morphine in its effects on the intestinal musculature of the Thirty-Vella loop of the dog ileum and jejunum. Both hydromorphone and morphine had a biphasic effect on musculature tone. At low doses, they increased musculature tone and higher doses, they decreased musculature tone. Hydromorphone was 10 times more potent than morphine in its effects on the general musculature tonus; maximum increase in the degree and

duration maximum changes in general musculature tone and amplitude of tone were noted at 0.1 mg/kg and 1.0 mg/kg of dilaudid and morphine, respectively. Both morphine and hydromorphone decreased the jejunum of the rhythmic contraction of the amplitude.

**Abuse liability:** As with most opiates, hydromorphone is a highly addictive substance; it has a high abuse and dependence potential and produces tolerance. Hydromorphone immediate release is a DEA Schedule II controlled substance. The incidence and rates of prescription opioid abuse have increased in the United States over the past several years (Compton and Volkow, 2006). In a recent study, Walsh and colleagues (2008) compared the abuse liability of hydromorphone to that of hydrocodone and oxycodone following oral administration in non-dependent sporadic prescription opioid abusers in a double-blind, randomized, within-in subject, placebo-controlled outpatient study. All three opioids produced the typical  $\mu$  opioid agonist profile of subjective (i.e., increased ratings of liking, good effects, high and opiate symptoms). Results indicated that the abuse potential/liability of hydromorphone did not differ substantially from oxycodone and hydrocodone in ratings of measures related to euphoria-like responses (i.e., liking for the drug, good effects, high, and MBG scores) and sedation (i.e., PCAG scores, sleepy and nodding).

**Other:** None applicable.

#### **2.6.2.5 Pharmacodynamic drug interactions**

#### **2.6.3 PHARMACOLOGY TABULATED SUMMARY**

**Primary Pharmacodynamic Effects.** Summary tables were not provided by Applicant.

**Safety Pharmacology:**

## 2.6.3.4 Safety Pharmacology Findings, Pharmacology and Toxicology Studies

Test Article: Hydromorphone HCl

Organ Systems Evaluated	Species/ Strain	Method of Admin.	Doses (mg/kg)	No. per Group/Sex	Noteworthy Findings	GLP	Report Number	Module
Cardiovascular	hERG	<i>In vitro</i>	10 <sup>-5</sup> M	NA	-2.7% mean inhibition at 10 µM, 1.9 mean inhibition PSS vehicle Cisapride positive control 68.9% at 0.1 µM	Yes	1678-003	4.2.1.3
Cardiovascular	Guinea pig/papillary muscle	<i>In vitro</i>	10 <sup>-7</sup> , 10 <sup>-6</sup> , 10 <sup>-5</sup> M	NA	No noteworthy effects on action potential duration, maximum upstroke velocity, resting potential or amplitude of action potential.	Yes	MPF/FS 0001	4.2.1.3
Cardiovascular Respiratory	Dog/beagle	Oral capsule	3.5, 7.0	4-6M	Single death at 7.0 mg/kg 3.5 mg/kg: No effects on cardiovascular parameters. Increased respiratory rate: 17.3/min 30 min post, 29.0/min 2 h, 8.8/min 3 h, 2.2/min 6 h, relative to baseline. Decreased body temperature: 1.7°C, 30 min post dosing. Decreases in blood oxygen: -7.3 mmHg 6 h. Increases in pCO <sub>2</sub> : 2.0, 6 h. No cardiovascular data analysis of 7.0 mg/kg data due to data disturbances due to emesis. 7.0 mg/kg respiratory effects: increased respiration rate: 37.7/min 2 h, 4.7/min 6 h Decreased blood oxygen: increased CO <sub>2</sub>	No	MPF/FE 9923	4.2.1.3

Organ Systems Evaluated	Species/ Strain	Method of Admin.	Doses (mg/kg)	No. per Group/Sex	Noteworthy Findings	GLP	Report Number	Module
Cardiovascular	Dog/beagle	Oral capsule	1.75, 3.5, 7.0 daily 4-week	3-4M/3-4F	7 mg/kg: decrease in mean arterial pressure 18-21 mm Hg, compensatory increase in heart rate 17-40 bpm.	Yes	MPF/ET 9806	4.2.3.2
Cardiovascular	Dog/beagle	Oral capsule	1.75, 4.0, 9.0 daily 39-week	7M/7F	No effect on QRS, PQ, QT parameters.	Yes	MPF/DT 9901	4.2.3.2
CNS	Rat/Wistar	Oral gavage	5, 10	10M/10F	Rearing observations: Increased, M 10 mg/kg 4 h Hindlimb splay: Decreased, M 10 mg/kg 1.5 and 4 h Stereotypic movements: M 10 mg/kg, 1.5 h, F 1.5 and 4 h	Yes	1678-005	4.2.1.3
CNS	Rat/Wistar	Oral gavage	3.5, 7, 14 daily 27-week	20M/20F	All doses affected. From week 1: Dose-related sedation, lassitude, mydriasis, compulsive gnawing, hyperactivity, exophthalmus, self-mutilation, rough coat From week 4: aggressive behavior	Yes	MPF/DT 9940	4.2.3.2

## 2.6.4 PHARMACOKINETICS/TOXICOKINETICS

## 2.6.4.1 Brief summary

The absorption, distribution, metabolism and elimination profiles of hydromorphone were investigated in nonclinical toxicity and/or pharmacokinetic studies conducted in mice (CD-1), rats (Sprague-Dawley, Han Wistar), rabbits (Himalayan) and dogs (Beagle). The key findings of these studies are listed below:

- Hydromorphone is well absorbed following oral administration.
- Oral bioavailability of hydromorphone is low; 12% and 21% in male and female rats, respectively, at a 14 mg/kg dose. At a dose of 1.75 mg/kg, hydromorphone bioavailability was 10 in male dogs.
- Urine is the principal route of  $^{14}\text{C}$  excretion following [ $^{14}\text{C}$ ]-hydromorphone administration in rats, rabbits and dogs. The majority of the excretion occurred within 48 hours.
- [ $^{14}\text{C}$ ]-Hydromorphone is excreted in milk of lactating rats following the administration of a single oral dose of [ $^{14}\text{C}$ ]-hydromorphone.
- The in vivo biotransformation of hydromorphone was characterized following oral administration in rats, rabbits and dogs. Metabolism of hydromorphone was similar in all three species and similar as in humans. The conjugated metabolite hydromorphone-3-glucuronide is the primary metabolite of hydromorphone identified in pregnant rabbits, rats and dogs following oral administration. The metabolites were found in both the circulation and in urine in all three species. In the dog, hydromorphone-3-glucuronide was also excreted in feces.
- Hydromorphone itself does not appear to be an inducer of cytochrome P450 enzymes at concentrations in the range of 10 – 10,000  $\mu\text{M}$  when tested in vitro. Hydromorphone did not induce significant activity of CYP 450 isoforms CYP1A2, CYP2C9, CYP2C19, CYP4A11 and CYP3A4.

#### **2.6.4.2 Methods of Analysis**

[see under individual study reviews]

### 2.6.4.3 Absorption

Single-dose and repeat-dose absorption studies were conducted in rodents, rabbits and dogs following oral or intravenous administration. Only the absorption studies conducted in dogs were reviewed since the pivotal toxicology studies were performed in dogs.

**Study Title: Study Title: Investigation of the plasma pharmacokinetics of hydromorphone in male beagle dogs after administration of a single oral (1.75 mg/kg of the active moiety) or intravenous (0.35 mg/kg of the active moiety) dose of hydromorphone. (GLP)**

**Study №: DM98/13 (Report №: DT00020)**

Male beagle dogs (n = 6) were administered a single oral (1.75 mg/kg, gelatin capsule,) or intravenous (0.35 mg/kg) dose of hydromorphone free base. On day 1 of the study, the dogs (fasted) were administered hydromorphone (free base) intravenously. On day 13 of the study, the same dogs (fasted) were administered hydromorphone (free base) orally. Blood samples were taken by venipuncture of the cephalic vein for quantification of hydromorphone (free base) plasma concentration. Blood samples were collected as following for the two treatment groups:

Treatment Group (dose)	Blood Sampling prior to dosing	Blood Collection Time (hrs) after dosing
Oral (1.75 mg/kg)	Pre-dose	0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and 24
Intravenous (0.35 mg/kg)	Day-1 and pre-dosing	0.08, 0.25, 0.5, 1, 1.5, 2, 2.5, 4, 6, 8, and 10

Plasma samples were evaluated using a validated liquid chromatography-mass spectrometry analytic method. The limit of quantification was 1 ng/mL.

*Deviation from the protocol.* Due to four dogs (№ 2, 3 and 6) vomiting after the oral dose of hydromorphone (1.75 mg/kg), these dogs (fasted) were re-tested on study day 28 and blood was drawn and quantified for plasma level of hydromorphone. The blood was drawn as described in the table above.

#### Key study findings:

- Hydromorphone was rapidly absorbed following oral administration; mean maximum concentration was observed at 1 hour following an oral dose of 1.75 mg/kg and at 5 minutes (0.08 hours) after the intravenous dose of 0.35 mg/kg.

Time after dosing (hrs)	Mean Plasma Concentration of Hydromorphone (ng/mL) ± SD	
	Route: Dose (mg/kg)	
	Oral: 1.75 <sup>a</sup>	Intravenous: 0.35 <sup>b</sup>
0.08	NS	112 ± 21.6

Time after dosing (hrs)	Mean Plasma Concentration of Hydromorphone (ng/mL) ± SD	
	Route: Dose (mg/kg)	
	Oral: 1.75 <sup>a</sup>	Intravenous: 0.35 <sup>b</sup>
0.25	NS	63.5 ± 14.8
0.50	6.8 ± 5.2	35.9 ± 16.0
1.00	12.5 ± 7.1	18.3 ± 9.1
1.50	9.0 ± 3.8	11.2 ± 5.7
2.00	6.0 ± 1.7	7.7 ± 5.4
2.50	5.2 ± 3.0	5.6 ± 5.0
3.00	4.2 ± 1.8	NS
4.00	2.7 ± 1.1	1.7 ± 2.2
6.00	1.7 ± 1.5	<1
8.00	2.3 ± 2.7	<1
10.00	1.7 ± 1.1	<1
24.00	<1	<1

a: means of 5 dogs  
b: means of 6 dogs  
NS: No sample  
Limit of Quantification: 1 ng/mL

- Following a single oral dose of hydromorphone, the mean maximum concentration of hydromorphone in plasma was 12.5 ng/mL in male dogs. The corresponding T<sub>max</sub> was 1.0 hour.
- As indicated by the AUC, systemic exposure to hydromorphone was higher following intravenous compared to oral administration. AUC<sub>0-10</sub> was 70.6 and 36.4 ng·h/mL following intravenous and oral administration, respectively.
- The primary elimination of hydromorphone following oral and intravenous administration was 1.4 and 0.9 hours, respectively.
- The bioavailability of the hydromorphone following oral administration. The bioavailability of the oral dose was 10%.

Pharmacokinetic Parameters of hydromorphone following oral and intravenous administration		
PK Parameters	Route: Dose (mg/kg)	
	Oral: 1.75	Intravenous: 0.35
C <sub>max</sub> (ng/mL)	12.5	112
AUC <sub>0-t</sub> (ng·h/mL) <sup>d</sup>	36.4	70.7
AUC <sub>0-inf</sub> (ng·h/mL)	52.3	72.8
T <sub>max</sub> (hrs)	1	0.08
Primary T <sub>1/2</sub> (hrs)	1.4 <sup>a</sup>	0.9 <sup>b</sup>
Secondary T <sub>1/2</sub> (hrs)	6.7 <sup>c</sup>	NC
K <sub>el</sub> (1/hrs)	0.10	0.8
Cl <sub>tot/f</sub> (ml/in/kg)	NC	80.1
V <sub>d/f</sub> (l/kg)	NC	6.18
Bioavailability (%)	10	100

a: Measured between 1.5 and 3 hours after dosing  
b: Measured between 1 and 4 hours after dosing  
c: Measured between 3 and 10 hours after dosing  
d: t -10 hours after dosing for oral and 4 hours after dosing for IV  
NC: Not calculated

**Study Title: Plasma concentrations of hydromorphone in the dog following repeated oral administration of LU 138315 (Hydromorphone Hydrochloride) during a 4-week toxicity study. (MPF/RT9806). (GLP)**

**Study №: DM98/52 (Report № DT98053)**

Male and female dogs were administered oral doses of LU 138315 (hydromorphone, formulated in gelatin capsules for 4 weeks at dose levels of 1.75 (n=3/sex), 3.5 (n=3/sex) and 7 mg/kg/day (n = 4/sex). Blood samples were collected on day 1 and day 28 to 29 for quantification of hydromorphone (free base) plasma concentration. Plasma samples were evaluated using a validated high performance liquid chromatography-mass spectrometry analytic method. The limit of quantification was 1 ng/mL.

**Key study findings:**

- As depicted in the figures below, after a single dose, hydromorphone was rapidly absorbed following an oral administration with detectable levels measured in plasma of both males and females at the first sampling time.

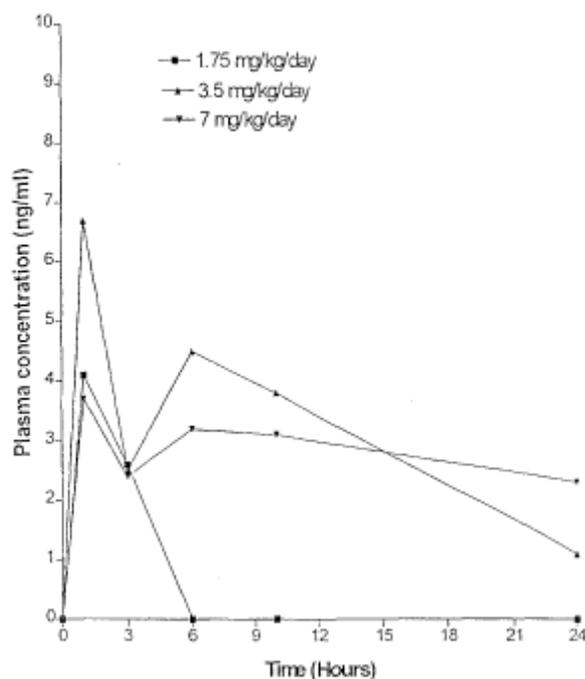


Figure 1 (from Applicant's submission). Mean plasma concentrations of hydromorphone in plasma of male dogs after administration of a single oral dose of LU 138315.

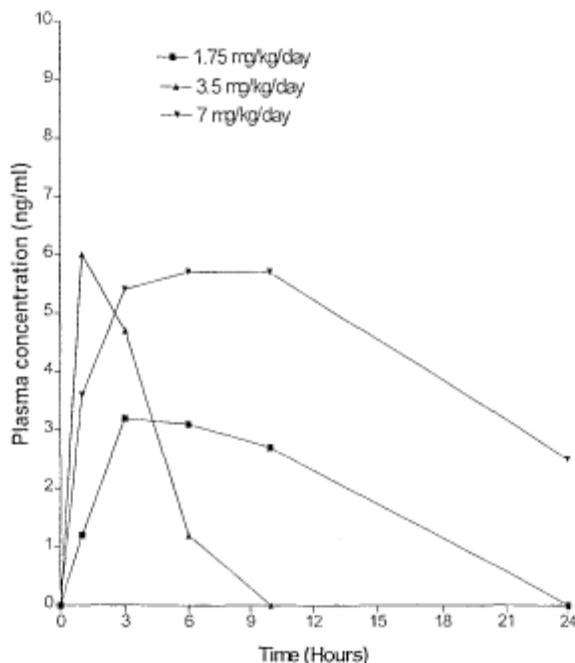


Figure 2 (from Applicant’s submission). Mean plasma concentrations of hydromorphone in plasma of female dogs after administration of a single oral dose of LU 138315.

- As depicted in the table below, exposure to hydromorphone was dose dependent in both males and females.
- As suggested by  $C_{max}$ , there was large inter-individual variability in plasma levels of hydromorphone on day 1 and day 28.

Day	Dose	Hydromorphone Toxicokinetic Parameters					
		$C_{max}$ (ng/mL)			AUC <sub>0-24</sub> (ng·h/mL)		
		M	F	M+F	M	F	M+F
1	1.75	4.1 ± 4.4	3.4 ± 1.1	3.7 ± 2.9	14.1 ± 13.9	48.1 ± 16.8	31.1 ± 23.2
	3.5	7.7 ± 6.9	6.8 ± 3.1	7.3 ± 4.8	74.1 ± 43.6	29.0 ± 24.9	51.5 ± 40.2
	7.0	5.6 ± 0.6	6.8 ± 3.2 ±	6.2 ± 2.2	67.2 ± 35.4	107 ± 64.0	87.2 ± 52.5
28	1.75	9.0 ± 3.8	10.0 ± 5.1	9.5 ± 4.1	22.0 ± 8.1	71.1 ± 23.8	46.6 ± 31.3
	3.5	12.4 ± 0.8	12.4 ± 1.4	12.4 ± 1.0	62.8 ± 20.7	143 ± 76.6	103 ± 66.8
	7.0	28.4 ± 10.4	32.7 ± 13.2	30.5 ± 11.2	234 ± 50.7	243 ± 112	239 ± 80.7

**Study Title: Plasma concentrations of hydromorphone in male and female dogs following repeated oral administration of BSF 138315 during a 39-week toxicity study (MPF/DT 9901). (GLP)**

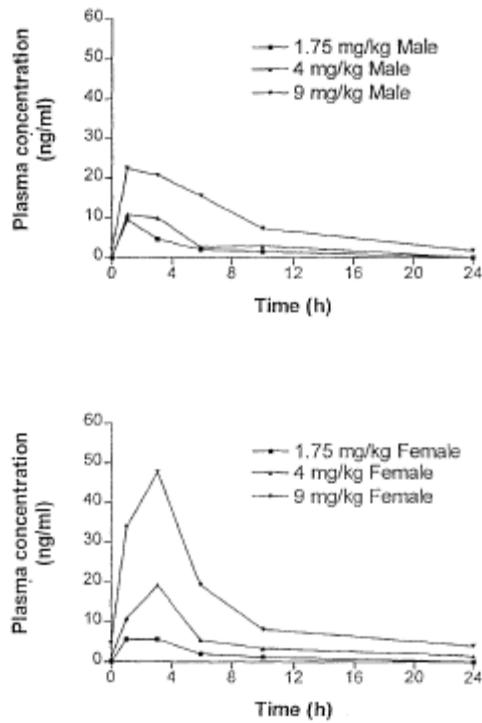
**Study №: DM99/12 (Report №: DT00001)**

Male beagle dogs (n = 6) were administered an oral dose of BSF 138315 (hydromorphone,) once daily (gavage in gelatin capsules) for 39-weeks at free dose levels of 1.75, 4.0 and 9.0 mg/kg/day. On day 1 of the study, the dogs (fasted) were administered hydromorphone (free base) intravenously. Blood samples were taken by venipuncture of the antebrachial cephalic vein for quantification of hydromorphone (free base) plasma concentration. Blood samples were collected prior to the first dose and at pre-dose, 3, 6, 10 and 24 hours after dosing in weeks 11, 25 and 39. Due to severe clinical signs in several dogs during week 4, blood was not drawn at 1-hr and 24-hrs from males in groups 2, 3 and 4. Plasma concentrations (free base equivalents) of hydromorphone were evaluated using a validated LC-MS-MS analytic method. The limit of quantitation was 1 ng/mL.

**Key study findings:**

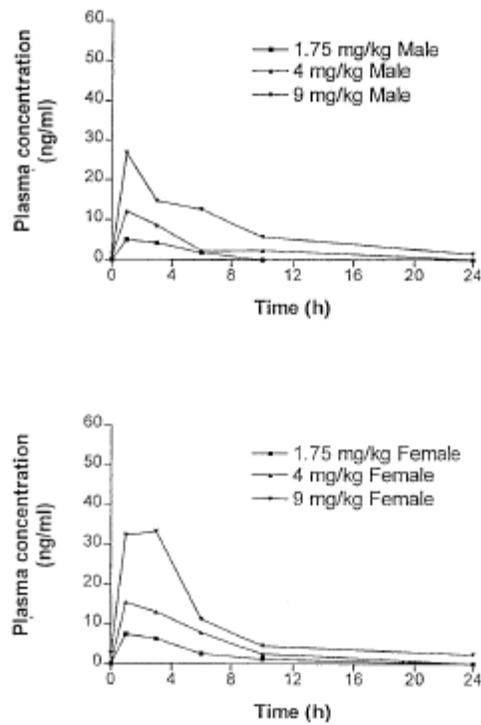
- As depicted in the Applicant's figure below, hydromorphone was rapidly absorbed following oral administration during week 11.

FIGURE 1 Plasma profiles of hydromorphone (free base) in plasma of male and female dogs during week 11 after daily oral doses of BSF 138315

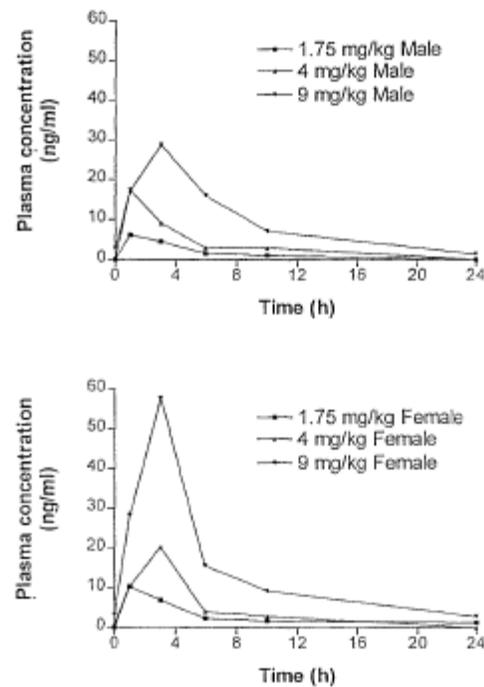


- Exposure was also apparent at weeks 25 and 39.
- Consistent with the results observed at week 11, exposure was slightly higher in females in the high dose group.

**FIGURE 2** Plasma profiles of hydromorphone (free base) in plasma of male and female dogs during week 25 after daily oral doses of BSF 138315



**FIGURE 3** Plasma profiles of hydromorphone (free base) in plasma of male and female dogs during week 39 after daily oral doses of BSF 138315



- Exposure increased in a linear fashion. Compared to males, exposure was slightly higher in females at the mid- and high-dose during weeks 11, 25 and 39.

FIGURE 4 AUC (0-24 h) of hydromorphone (free base) in male and female dogs versus dose during week 11 after daily oral dose of BSF 138315

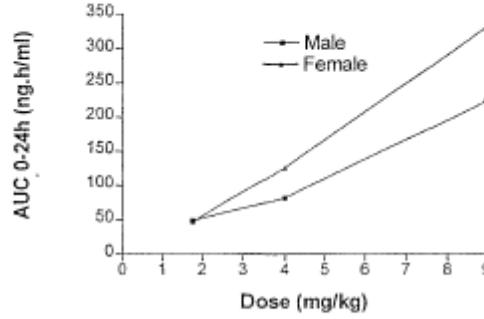


FIGURE 5 AUC (0-24 h) of hydromorphone (free base) in male and female dogs versus dose during week 25 after daily oral doses of BSF 138315

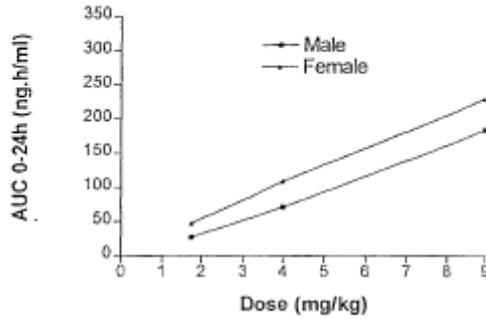
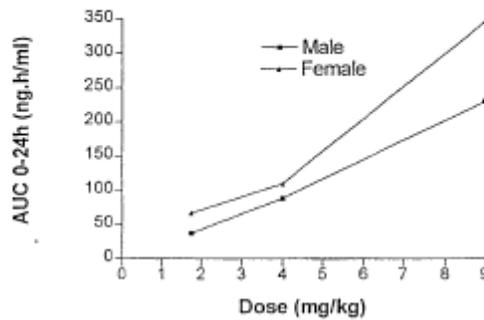


FIGURE 6 AUC (0-24 h) of hydromorphone (free base) in male and female dogs versus dose during week 39 after daily oral doses of BSF 138315



#### 2.6.4.4 Distribution

Tissue distribution of hydromorphone was evaluated in male rats following oral administration.

**Study Title: The distribution of radiolabelled material in tissues and its persistence in the eye of male pigmented rats following a single oral dose of [<sup>14</sup>C]hydromorphone (7 mg/kg). (GLP compliance)**

#### **Study №: DT99025**

The distribution of [<sup>14</sup>C]-hydromorphone following a single oral dose of 7 mg/kg (free base) was characterized in male pigmented rats. One rat was euthanized by carbon dioxide asphyxia at 1, 4, 8, 72 and 168 hours after dosing. Immediately after euthanasia, the right eye of each animal was removed. The bodies were rapidly frozen in a hexane/solid carbon dioxide bath at approximately -70°C. All rats were prepared for quantitative whole-body autoradiography to assess tissue distribution in selected tissues. Concentration of radiolabelled material in the eyes was analyzed by tissue combustion followed by liquid scintillation counting of the trapped combustion products.

#### **Key Study Findings:**

- Concentrations of radioactivity in select tissues are presented in the Applicant's table below. Distribution was extensive; all tissues were exposed to radioactivity at the first time point (i.e., 1 hour) after dosing.
- At one hour post-dosing, [<sup>14</sup>C]-hydromorphone-derived radioactivity concentrations in all tissues were at their maximum. The highest concentrations of [<sup>14</sup>C]-hydromorphone-derived radioactivity at 1 hour was measured in the kidney (3.08 µg equiv/g) followed by the liver (2.96 µg equiv/g), pancreas (1.17 µg equiv/g) and spleen (1.03 µg equiv/g).
- Levels of [<sup>14</sup>C]-hydromorphone-derived radioactivity in the tissues decreased with time; but were still detectable in all tissues at 168-hours after dosing. In most of the tissues, the measured [<sup>14</sup>C]-hydromorphone-derived radioactivity represented less than 0.02% of the administered [<sup>14</sup>C]-hydromorphone.

**TABLE 1**      The concentration of radiolabelled material in tissues from male pigmented rats after administration of a single oral dose of [<sup>14</sup>C]hydromorphone (7 mg/kg of the free base)

Tissue	Concentration of radiolabelled material (µg equiv/g)					
	1 h	4 h	8 h	24 h	72 h	168 h
Adrenal gland	(b) (4)					
Blood (in heart)						
Bone marrow						
Brain						
Brown fat						
Heart muscle						
Kidney						
Liver						
Lung						
Muscle (skeletal)						
Pancreas						
Spleen						
Testes						
Thymus						
Thyroid gland						

< : Less than LOQ for that film.

(%) : % of dose administered to that animal.

- [<sup>14</sup>C]-Hydromorphone-derived radioactivity was measured in the eye. As shown in the Applicant's table below, the highest levels (0.53 µg equiv/g) of [<sup>14</sup>C]-hydromorphone-derived radioactivity were measured at 8-hours after dosing. According to the Applicant, the [<sup>14</sup>C]-hydromorphone-derived radioactivity was associated with the uveal tract of the eye and may represent reversible binding to melanin.

**TABLE 2** The concentration of radiolabelled material in eye from male pigmented rats after administration of a single oral dose of [<sup>14</sup>C]hydromorphone (7 mg/kg of the free base)

Tissue	Concentration of radiolabelled material (µg equiv/g)								
	1 h	4 h	8 h	24 h	72 h	168 h	360 h	672 h	1200 h
Eye	(b) (4)								

Results expressed as mean (n=2).

(%) : % of dose administered to animal which remained in the tissue.

**Study Title: Investigation of the extent of in vitro binding of [<sup>14</sup>C]hydromorphone to animal and human plasma proteins.**

**Study №: DM98/61 (Report № DT99021)**

The extent of [<sup>14</sup>C]-hydromorphone binding to plasma proteins were evaluated in vitro in rat, dog and human. The binding of [<sup>14</sup>C]-hydromorphone to plasma protein was determined at nominal concentrations of 10 to 2000 ng/mL; 200, 1000 and 2000 ng/mL for rat, 30, 150 and 300 ng/mL for dog and 10, 50 and 100 ng/mL for human. In vitro binding of [<sup>14</sup>C]-hydromorphone to plasma proteins was determined at nominal initial drug concentrations of 10 to 2000 ng/mL, 200, 1000 and 2000 ng/mL for rat, 30, 150 and 300 ng/mL for dog and 10, 50 and 100 ng/mL for human, using the equilibrium dialysis technique. Dialysis was carried out at 37°C for three hours.

To further characterize the extent of [<sup>14</sup>C]-hydromorphone binding to human plasma protein, human albumin (5%) and α<sub>1</sub>-acid glycoprotein (0.07% solution) were incubated with [<sup>14</sup>C]-hydromorphone at nominal initial concentrations of 10, 50 and 100 ng/mL.

**Key Study Findings:**

The Applicant’s tabulated results were reproduced in the table below.

- The overall mean plasma protein binding was low in the rat, dog and human. Percent of drug bound to plasma proteins was 17%, 12%, and 27% in rat, dog and human plasma, respectively.
- The highest degree of plasma protein binding was observed in human.
- In humans, the binding of hydromorphone to plasma was mediated by albumin with minimal binding to α<sub>1</sub>-acid glycoprotein

<b>Extent of binding of [<sup>14</sup>C]-Hydromorphone to the plasma protein of rat, dog and human.</b>			
Species	[ <sup>14</sup> C]Hydromorphone Active Moiety Concentration at equilibrium (ng/mL)	% Bound*	Mean % Bound
Rat (pooled blood)	102	18	17
	446	17	
	898	17	
Dog (pooled plasma)	15	12	12
	74	12	
	144	12	
Human (mean of 4 subjects)	8	30	27
	28	24	
	55	27	

\*: Mean of duplicate measurements  
% Bound = (dpm/g plasma –dpm/g buffer} x 100/dpm/g plasma

### 2.6.4.5 Metabolism

The metabolism of hydromorphone was characterized in in vivo, in vitro and ex vivo studies. In vitro metabolism of hydromorphone was investigated in liver microsomes (rat, dog and human), hepatocytes (mouse, rat, rabbit, dog and human) and kidney microsomes (rat and human). The in vivo biotransformation of hydromorphone was characterized following oral administration in rats, rabbits and dogs.

**Study Title: An investigation of the metabolic profile in urine, faeces and plasma of male beagle dogs after administration of a single oral dose of [<sup>14</sup>C]hydromorphone hydrochloride (1mg/kg). (GLP)**

**Study №: DM99/19 (Report № DT00043)**

The objective of the study was to identify the metabolites of hydromorphone in plasma, urine and feces extract samples obtained from male dog (n = 3) in after a single oral dose of [<sup>14</sup>C]-hydromorphone hydrochloride (1 mg/kg). Plasma samples were collected at 2 hours post-dosing; the plasma samples were pooled. Urine (pooled) and feces (pooled) were collected up to 48 hours after dosing. To investigate whether or not the metabolites were conjugated, pooled samples of plasma, feces and urine were mixed an enzyme preparation containing β-glucuronidase and sulphatase activities. Metabolite profiling was done with high performance liquid chromatography (HPLC) and mass spectrometry.

#### **Key Study Findings:**

- Metabolite profiling indicated that metabolites were qualitatively similar in plasma, urine and feces. In the absence of enzyme hydrolysis, the parent compound, hydromorphone (DM3), accounted for 16.6%, 32.7% and 2.8% of the radioactivity in plasma, feces and urine, respectively. The metabolites hydromorphone-3-glucuronide (DM1) and hydromorphone-3-sulphate (DM2) were detected in plasma, urine and feces before enzyme hydrolysis. Hydromorphone-3-glucuronide, metabolite was the major metabolite detected before enzyme hydrolysis; it accounted for 64%, 83.6%

and 47.8% in plasma, urine and feces, respectively. DM2 represents a minor metabolite in plasma (19.5%), urine (13.6%) and feces (19.6%) before enzyme hydrolysis.

- After enzyme hydrolysis, only DM1 and DM3 were detected in plasma, feces and urine; DM2 was not detected.

Percent of Radiolabeled Material in Sample							
Sample	Before Enzyme Hydrolysis				After Enzyme Hydrolysis		
	DM1	DM2	DM3		DM1	DM2	DM3
Plasma	64.0	19.5	16.6		15.2	ND	84.8
Urine	83.6	13.6	2.8		36.1	ND	63.9
Feces	47.8	19.6	32.7		17.5	ND	82.6
DM1: Hydromorphone-3-glucuronide DM2: Hydromorphone-3-sulphate DM3: [ <sup>14</sup> C]hydromorphone ND: Not detected							

- The major route of hydromorphone metabolism involved glucuronidation of hydromorphone to hydromorphone-3-glucuronidation.

**Study Title: The metabolism of hydromorphone by rat, dog and human hepatic in vitro systems.**

**Study №: DM98/39 (Report № DT99032)**

The objective of this study was to identify and compare the metabolites of hydromorphone produced when incubated with hepatic microsomal preparations from rats, dogs and humans. Liver microsomes from male rats, female rats, male dogs and humans (pooled male and female) were incubated with [<sup>14</sup>C]-hydromorphone at a final concentration of 200 µM for 30 and 90 minutes. Microsomes were incubated with: 1) the NADPH co-factor to characterize phase 1 metabolism of hydromorphone, or 2) the NADPH co-factor and uridine diphosphoglucuronic acid (UDPGA) to assess phase 1 and 2 metabolism of hydromorphone. In addition, the metabolism of [<sup>14</sup>C]-hydromorphone by flavin monooxygenases (FMOs) was investigated using baculovirus expression systems expressing human FMO 1, FMO 3 and FMO 5. Metabolite profiling was done with high performance liquid chromatography (HPLC) with radiochemical detection ultraviolet (UV) absorption or mass spectrometry (HPLC/MS) methods.

**Key Study Findings:**

**Phase 1 Metabolism:**

- As depicted in the table below, limited phase 1 metabolism of hydromorphone was observed. Norhydromorphone was the primary metabolite formed in hepatic microsomes isolated from rat (male), humans and dogs. Formaldehyde was also detected in male rats, humans and dogs. According to the Applicant, formaldehyde was formed by N-[<sup>14</sup>C]-demethylation of hydromorphone, also forming

norhydromorphone. No phase 1 metabolism was noted in female rats; neither norhydromorphone nor formaldehyde was detected in female rats.

Percent of Radiolabelled Material in Sample					
	Formaldehyde <sup>A</sup>			Norhydromorphone <sup>B</sup>	
	Incubation period (minutes)			Incubation period (minutes)	
Species	30	90		30	90
Rat (male)	11.5	10.5		12.2	16.1
Rat (female)	ND	ND		ND	ND
Dog	ND	0.4		0.7	ND
Human	ND	1.4		2.7	ND

A: Results expressed as % radiolabelled peaks for formaldehyde  
 B: Results expressed as % UV peaks for norhydromorphone assuming the UV response of hydromorphone and norhydromorphone to be identical  
 ND: Not detected

### **Combined Phase 1 and 2 Metabolism:**

- After 90 minutes of incubation, the primary metabolite observed in rats, dogs and humans was hydromorphone-3-glucuronide in the absence of enzyme hydrolysis. Hydromorphone-3-glucuronide represented 74%, 100% and 41% of the radiolabelled peak in rats, dogs and humans, respectively.
- In the presence of  $\beta$ -glucuronidase, hydromorphone-3-glucuronide was hydrolyzed to hydromorphone. Hydromorphone represented 85%, 77% and 95% of the radiolabelled peak in rats, dogs and humans, respectively.

Percent Radiolabelled Peak					
	Before Enzyme Hydrolysis			After Enzyme Hydrolysis	
	DM1	DM3		DM1	DM3
Rat (male)	74	26		15	85
Dog	100	ND		23	77
Human	41	59		5	95

DM1: Hydromorphone-3-glucuronide  
 DM3: Hydromorphone  
 ND: Not detected

### **FMO Expression System:**

- Incubation with the enzyme expressing FMO 1 activity produced trace levels of hydromorphone-N-oxide.
- No metabolites of hydromorphone were detected in the microsomes incubated with enzyme expressing FMO 3 and FMO 5 activity.

FMO Enzyme	Percent Radiolabelled Peak
	Mean % hydromorphone-N-oxide
FMO 1	0.9
FMO 3	ND
FMO 5	ND

ND: Not detected

Reference marker	Metabolite Profiling			
	Identification	Rat (male)	Dog	Human
<b>Phase 1 Metabolism</b>				
BSF 410036	Hydromorphone-3-glucuronide	ND	ND	ND
BSF 138315	Hydromorphone	+	+	+
BSF 410088	Norhydromorphone	+	+	+
BSF 4036516	Hydromorphone-N-oxide	ND	ND	ND
	Formaldehyde dinitrophenyl-hydrazone	+	+	+
<b>Combined Phase 1 and 2</b>				
BSF 410036	Hydromorphone-3-glucuronide	+	+	+
BSF 138315	Hydromorphone	+	+	+
BSF 410088	Norhydromorphone	+	+	+
BSF 4036516	Hydromorphone-N-oxide	ND	ND	ND
	Formaldehyde dinitrophenyl-hydrazone	NA	NA	NA

**Study Title: Investigation of the potential of hydromorphone to interact with human hepatic cytochrome P450 (CYP450). (GLP)**

**Study №: DM98/44 (Report № DT99052)**

The objective of this study was to investigate the potential of hydromorphone to interact with cytochrome P450 in human hepatic microsomes obtained from male and female donors. The potential of hydromorphone to interact with the cytochrome P450 isoforms CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP4A11 was investigated using specific probe substrates for the CYP450 isoforms. Human hepatic microsomes (pooled from 10 subjects) were incubated with 0, 50, 100, 1000, 5000 and 10000 µM of hydromorphone in the presence of the following probe substrates: phenacetin (CYP1A2), testosterone (CYP3A4), tolbutamide (CYP2C9), S-mephenytoin (CYP2C19), dextromethorphan (CYP2D6) and lauric acid (CYP4A11). After the appropriate incubation period, analysis of radioactivity was measured by HPLC. The effects of hydromorphone on the specific CYP450 isoforms activity were assessed by comparing the amount of metabolite produced by metabolism of the probe substrate in the absence and presence of hydromorphone.

**Key Study Findings:**

- As depicted in the table below, hydromorphone decreased CYP3A4 activity in a concentration-dependent manner.
- As depicted in the table below, hydromorphone over 10 to 1000 µM concentration range had minimal modulatory effect on CYP1A2, CYP2C9, CYP2C19, CYP3A4 and CYP4A11.
  - **CYP1A2:** In the concentration range of 10-5000 µM, a slight induction of CYP1A2 was observed; ranging from 107.5% to 139.4%. Maximum enzyme activity was observed at 10 µM of hydromorphone, CYP1A2 activity was increased to 139.4% of control activity.
  - **CYP2C9:** In the concentration range of 10-10000 µM, a slight inhibition of CYP2C9 activity was observed; ranging from 85.6% to 93.4%.

- **CYP2C19:** In the concentration range of 10-5000  $\mu\text{M}$ , a slight induction of CYP2C19 activity was observed; ranging from 103.2% to 127.2%. At the highest concentration evaluated, a 44.0% inhibition of CYP2C19 was inhibited.

Hydromorphone Conc. ( $\mu\text{M}$ )	Metabolite Level expressed as % Control Value <sup>+</sup>					
	CYP450 Isoforms					
	CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4	CYP4A11
0 (control)	100	100	100	100	100	100
10	139.4	87.4	103.2	96.3	108.0	112.0
50	122.4	93.4	122.8	56.5	112.6	96.6
100	132.9	83.9	120.0	25.4	87.0	88.0
1000	119.0	87.0	127.2	13.7	106.1	96.4
5000	107.5	87.9	104.0	ND	60.4	88.3
10000	65.0	85.6	44.0	ND	41.1	107.9

+: % Metabolite (in presence of hydromorphone)/% metabolite (control)

**Study Title: Investigation and characterisation of the human hepatic cytochrome P450 (CYP450) isoenzymes involved in the metabolism of [<sup>14</sup>C]hydromorphone. (GLP)**

**Study №: DM00/17 (Report № DT00053)**

The objective of this study was to investigate the role of human hepatic CYP450 isoenzymes in the metabolism of hydromorphone. Baculovirus expressing specific humans CYP1A2, CYP3A4, CYP2D6, CYP2C9 and CYP2C19 were incubated with [<sup>14</sup>C]-Hydromorphone (500  $\mu\text{M}$ ) and evaluated at a concentration of 100 pmol/mL. After 30, 60 or 90 minutes of incubation, aliquots of supernatants were removed and examined. Supernatants were examined by high performance liquid chromatography (HPLC) and liquid chromatography mass spectrometry (LC/MS) methods.

**Key Study Findings:**

- Under the experimental condition, hydromorphone was not metabolized by the CYP450 isoenzymes CYP1A2, CYP3A4, CYP2D6, CYP2C9 and CYP2C19. Thus suggesting that the potential of hydromorphone to be involved in drug interactions at the level of CYP450 may be minimal.

**Study Title: Investigation and characterization of the human hepatic glucuronyl transferases (UGTs) mediating the metabolism of [<sup>14</sup>C]hydromorphone. (GLP)**

**Study №: DM00/18 (Report № DT00051)**

The objective of this study was to investigate the role of human hepatic glucuronyl transferase isoenzymes in the metabolism of hydromorphone. Human glucuronyl transferase (UGT) microsomes expression systems 1A1, 1A4, 1A6, 1A9 and human UGT supersome expression systems 1A3, 2B7, and 2B15 were incubated with [<sup>14</sup>C]-hydromorphone and evaluated at a concentration of 0.5 mg/mL. Hydromorphone was tested at a final concentration of 200 µM. After 90 and 240 minutes of incubation, aliquots (250 µL) were removed and centrifuged. Supernatants were examined by high performance liquid chromatography (HPLC) and liquid chromatography mass spectrometry (LC/MS) methods.

**Key Study Findings:**

- Only the isoenzymes UGT2B7 was shown to be involved in the metabolism of hydromorphone. The metabolite hydromorphone-3-glucuronide was identified in both the 90 and 240 minutes incubation period with UGT2B7.
- The isoenzymes UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A9, UGT1A10 and UGT2B15 were shown not to be involved in the metabolism of hydromorphone. No metabolites were identified when hydromorphone was incubated with these isoenzymes.

**2.6.4.6 Excretion**

The excretion profile of radioactivity in urine and feces following oral administration of [<sup>14</sup>C]-hydromorphone was investigated in rabbits, rats and dogs. Recovery of total radioactivity was examined at intervals up to 168 hours. Further the excretion pattern of [<sup>14</sup>C]-hydromorphone-derived radioactivity in rat milk was examined. Only excretion study performed in dogs were reviewed since the pivotal toxicology studies were performed in dogs.

**Study Title: Excretion of radioactivity after single oral administration of [<sup>14</sup>C]-labelled hydromorphone in dogs.**

**Study №: MPF/DDM 9854**

The excretion of [<sup>14</sup>C]-hydromorphone-derived radioactivity was determined in urine and feces in male dogs (n = 3) following single oral administration at a dose of 1 mg/kg (33 mCi/g). Urine and fecal samples were collected at the following intervals after dosing: 0-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 hours. [<sup>14</sup>C]-hydromorphone-derived radioactivity was measured using liquid scintillation counting.

The Applicant's tables below show the percentage of administered dose of [<sup>14</sup>C]-hydromorphone-derived radioactivity excreted in urine and feces following oral.

**Table 2:**  
Renal and faecal excretion of <sup>14</sup>C-radioactivity after po dosing of 1 mg <sup>14</sup>C-hydromorphone/kg bwt (% of dose)

Sampling period [h]	Urine			Faeces		
	Animal no. 1	Animal no. 2*	Animal no. 3	Animal no. 1	Animal no. 2*	Animal no. 3
0 - 24	44.46	24.16	40.00	38.71	31.82	0.00
24 - 48	5.49	9.94	13.11	4.90	11.91	33.29
48 - 72	2.52	2.24	4.97	0.43	0.98	3.26
72 - 96	0.40	0.94	0.91	0.40	0.55	3.00
96 - 120	0.12	0.22	0.36	0.12	0.53	0.34
120 - 144	0.05	0.15	0.28	0.16	0.38	0.10
144 - 168	0.04	0.08	0.07	0.07	0.12	0.04
0 - 168	53.1	37.7	59.7	44.8	46.3	40.0

\* animal no. 2 vomited 10 min after administration with neglecting amount of radioactivity

### Key Study Findings:

- In the condition of this study, one route of excretion was not favored. [<sup>14</sup>C]-Hydromorphone-derived radioactivity was excreted in both urine and feces.
- Excretion was rapid; approximately 90% of the renally and fecally excreted [<sup>14</sup>C]-hydromorphone-derived radioactivity was excreted within 48-hours after dosing.
- Mean total radioactivity recovered within 168 hours after dosing was  $93.7 \pm 8.6\%$ ;  $50.2\% \pm 11.3\%$  and  $43.7 \pm 3.3\%$  of the dose was recovered in urine and feces, respectively.

#### 2.6.4.7 Pharmacokinetic drug interactions

No new data was submitted.

#### 2.6.4.8 Other Pharmacokinetic Studies

None applicable

#### 2.6.4.9 Discussion and Conclusions

Hydromorphone, in dogs, is rapidly absorbed following oral administration with an absolute oral bioavailability in the range of 10%. Hydromorphone is extensively distributed in tissues following oral administration and crosses the blood brain barrier and

placenta. The compound is weakly bound to plasma proteins *in vitro*; in rat, dog and human; percent of drug bound to plasma proteins was 17%, 12% and 27%, respectively. Phase 2 metabolism is the primarily mode of hydromorphone metabolism; Phase 1 metabolism is limited. Hydromorphone undergoes metabolism primarily via first pass glucuronidation in all species evaluated (rats, dogs and pregnant rabbits). Hydromorphone-3-glucuronide is the primary metabolite and is common in rats, dog and rabbit. Norhydromorphone was formed in male rats via Phase 1 metabolism. *In vitro* incubation of hydromorphone with human P450 isoforms CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP4A11 indicated that Phase 1 metabolism involvement in the metabolism of hydromorphone was limited. Hydromorphone over a 1000-fold concentration range had minimal modulatory effect on P450 isoforms. Hydromorphone was shown to be a weak inducer on P450 isoenzymes in dogs at doses as low as 1.75 mg/kg/day.

Following oral administration, hydromorphone is rapidly excreted primarily in both urine and feces. Rats, dogs and rabbit (pregnant) displayed similar pattern of excretion of hydromorphone; unchanged hydromorphone and the glucuronide metabolite hydromorphone-3-glucuronide were identified as the principal component in urine.

#### 2.6.4.10 Tables and figures to include comparative TK summary

##### Toxicokinetics parameters of hydromorphone after repeated doses.

**Table 5: Comparative C<sub>max</sub> and AUC Values for Hydromorphone in Mouse, Rats, Rabbits and Dogs at Termination of Oral Repeat Dose Toxicity Studies**

Species Report Number	Dosing Day	Dose (mg/kg/day)	Hydromorphone	
			C <sub>max</sub> (ng/ml)	AUC (ng•h/ml)
<u>Mouse</u> R&D/03/783	87/88 <sup>A</sup>	15	77.6	162
<u>Rat</u> DT00013	168 <sup>A</sup>	14	439	1,410
<u>Pregnant rat</u> DT99024	12 <sup>B</sup>	7	35.8	135
<u>Pregnant rabbit</u> DT99020	15 <sup>C</sup>	28	16.9	141
<u>Dog</u> DT00001	273 <sup>A</sup>	9	44.3	288

A: Values presented are from males and females combined

B: Dosing Day 12 = Day 17 of pregnancy

C: Dosing Day 15 = Day 20 of pregnancy

Species/Strain: Mouse/CD-1  
 GLP: No  
 Test Article: Hydromorphone HCl  
 CTD Module: 4.2.3.2  
 Report Number: R&D/03/407

Number of Animals/Sex (M/F)	18M/18F	18M/18F	18M/18F	18M/18F	
Vehicle/Formulation	H <sub>2</sub> O	H <sub>2</sub> O	H <sub>2</sub> O	H <sub>2</sub> O	
Method of Administration	PO gavage	PO gavage	PO gavage	PO gavage	
Dose, hydromorphone free base (mg/kg/day)	15	35	75	150	
Treatment day	13	13	13	13	
Sample	Plasma	Plasma	Plasma	Plasma	
Analyte	Free base	Free base	Free base	Free base	
Assay	HPLC-MS	HPLC-MS	HPLC-MS	HPLC-MS	
<b>TK Parameters: Day 12 (Mean)</b>					
Males:	C <sub>max</sub> (ng/mL)	52.6	84.9	164.1	877.0
	AUC <sub>0-24</sub> (ng•hr/mL)	96.8	254.9	749.4	uc
	T <sub>max</sub> (hr)	0.5	0.5	0.5	0.5
Females:	C <sub>max</sub> (ng/mL)	56.4	100.2	480.8	1856.0
	AUC <sub>0-24</sub> (ng•hr/mL)	114.1	279.7	922.8	uc
	T <sub>max</sub> (hr)	0.8	0.5	0.5	0.8
Combined:	C <sub>max</sub> (ng/mL)	54.5	92.5	322.5	1366.5
	AUC <sub>0-24</sub> (ng•hr/mL)	105.5	267.9	821.4	uc
	T <sub>max</sub> (hr)	0.7	0.5	0.5	0.7

Additional Information: uc= unable to calculate

Species/Strain: Dog/Beagle  
 GLP: Yes  
 Test Article: Hydromorphone HCl  
 CTD Module: 4.2.2.2  
 Report Number: DT98053

Number of Animals/Sex (M/F)	3M/3F	3M/3F	4M/4F	3M/3F	3M/3F	4M/4F	
Vehicle/Formulation	Gelatin	Gelatin	Gelatin	Gelatin	Gelatin	Gelatin	
Method of Administration	PO capsule	PO capsule	PO capsule	PO capsule	PO capsule	PO capsule	
Dose, hydromorphone free base (mg/kg/day)	1.75	3.5	7	1.75	3.5	7	
Treatment day	1	1	1	28	28	28	
Sample	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	
Analyte	Free base	Free base	Free base	Free base	Free base	Free base	
Assay	HPLC-MS	HPLC-MS	HPLC-MS	HPLC-MS	HPLC-MS	HPLC-MS	
<b>TK Parameters: (Mean)</b>							
Males:	C <sub>max</sub> (ng/mL)	4.1	7.7	5.6	9.0	12.4	28.4
	AUC <sub>0-24</sub> (ng•hr/mL)	14.1	74.1	67.2	22.0	62.8	234
Females:	C <sub>max</sub> (ng/mL)	3.4	6.8	6.8	10.0	12.4	32.7
	AUC <sub>0-24</sub> (ng•hr/mL)	48.1	29.0	107	71.1	143	243
Combined:	C <sub>max</sub> (ng/mL)	3.7	7.3	6.2	9.5	12.4	30.5
	AUC <sub>0-24</sub> (ng•hr/mL)	31.1	51.5	87.2	46.6	103	239

**Additional Information:**

There was large inter-animal variation, especially in C<sub>max</sub>  
 An approximately proportional increase in exposure was seen with increasing dose levels  
 There was an increase in exposure on Day 28 versus Day 1, especially at 7 mg/kg (i.e. 4-fold)

Species/Strain: Dog/Beagle  
 GLP: Yes  
 Test Article: Hydromorphone HCl  
 CTD Module: 4.2.3.2  
 Study Number: TR-96-4604-057

Number of Animals/Sex (M/F)	4M/4F	4M/4F	4M/4F	4M/4F	4M/4F	4M/4F
Vehicle/Formulation	OROS <sup>®</sup> hydromorphone	OROS <sup>®</sup> hydromorphone	Dilaudid IR	OROS <sup>®</sup> hydromorphone	OROS <sup>®</sup> hydromorphone	Dilaudid IR
Method of Administration	PO capsule	PO capsule	PO capsule	PO capsule	PO capsule	PO capsule
Dose, hydromorphone free base (mg/animal)	8	64	64 <sup>3</sup>	8	64	64 <sup>3</sup>
Treatment day	1	1	1	30	30	30
Sample	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	Free base	Free base	Free base	Free base	Free base	Free base
Assay	HPLC-MS	HPLC-MS	HPLC-MS	HPLC-MS	HPLC-MS	HPLC-MS
<b>TK Parameters: (Mean)</b>						
Males:						
C <sub>max</sub> (ng/mL)	NR	NR	NR	NR	NR	NR
AUC <sub>0-24</sub> (ng•hr/mL)	8.7	10.3	NR	22.7	11.0	NR
Females:						
C <sub>max</sub> (ng/mL)	NR	NR	NR	NR	NR	NR
AUC <sub>0-24</sub> (ng•hr/mL)	12.5	11.6	NR	12.6	9.4	NR
Combined:						
C <sub>max</sub> (ng/mL)	0.9	5.2	8.7	0.8	7.2	17.1
AUC <sub>0-24</sub> (ng•hr/mL)	8.9	68.1	96.9	17.7	81.6	115.9
T <sub>max</sub> (h)	6.9	7.8	8.3	9.3	9.3	6.8

Additional Information:  
 NR= data not reported.  
<sup>3</sup> Dosed as 32 mg twice daily

## 2.6.5 PHARMACOKINETICS TABULATED SUMMARY

### Absorption:

**Table 4: Hydromorphone Pharmacokinetic Parameters in Rats, Rabbits and Dogs Following Intravenous or Oral Dosing**

Parameter	Male rat			Female rat			Female rabbit	Male dog	
	DT00011			DT00018			DT00027	DT00020	
Report	IV	PO	PO	IV	PO	PO	PO	IV	PO
Dose (mg/kg)	1	7	14	1	7	14	12.5	0.35	1.75
C <sub>max</sub> (ng/ml)	231	51.0	95.6	217	33.5	130	14.8	112	12.5
T <sub>max</sub> (h)	0.25 <sup>A</sup>	0.5	0.5	0.25 <sup>A</sup>	1.0	0.5	1	0.08 <sup>A</sup>	1
Half life (h)	0.9 <sup>B</sup>	1.7 <sup>B</sup>	1.5 <sup>B</sup>	1.1 <sup>B</sup>	1.0 <sup>B</sup>	2.2 <sup>B</sup>	-	0.9 <sup>B</sup>	1.4 <sup>B</sup>
AUC (ng•h/mL)	-	8.9	7.5	-	14.2	10.2	3.2	-	6.7
Clearance (ml/min/kg)	268	219	459	235	282	681	63.1	72.8	52.3
Bioavailability (%)	62.2	-	-	71.0	-	-	-	80.1	-
		12	12		17	21	-		10

<sup>A</sup> : First time point measured

<sup>B</sup> : measurable only up to 6 h and therefore this is a primary, and not a terminal, half life

<sup>C</sup> : AUC calculated as 0-inf

-: not calculated

**Pharmacokinetics parameters of hydromorphone after repeated doses.**

Species/Strain: Rat/Wistar  
 GLP: Yes  
 Test Article: Hydromorphone HCl  
 CTD Module: 4.2.2.2  
 Report Number: DT98050

Number of Animals/Sex (M/F)	3M/3F	3M/3F	3M/3F	3M/3F	3M/3F	3M/3F
Vehicle/Formulation	H <sub>2</sub> O					
Method of Administration	PO gavage					
Dose, hydromorphone free base (mg/kg/day)	3.5	7	14	3.5	7	14
Treatment day	1	1	1	15	15	15
Sample	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	Free base					
Assay	HPLC-MS	HPLC-MS	HPLC-MS	HPLC-MS	HPLC-MS	HPLC-MS
<b>PK Parameters: (Mean)</b>						
<b>Males:</b>						
C <sub>max</sub> (ng/mL)	15.8	16.1	39.5	33.3	55.5	211
AUC <sub>0-24</sub> (ng•hr/mL)	52.8	105	311	97.8	184	533
T <sub>max</sub> (hr)	-	-	-	-	-	-
<b>Females:</b>						
C <sub>max</sub> (ng/mL)	16.3	29.5	57.9	21.4	84.11	204
AUC <sub>0-24</sub> (ng•hr/mL)	72.7	125	330	104	260	785
T <sub>max</sub> (hr)	-	-	-	-	-	-
<b>Combined:</b>						
C <sub>max</sub> (ng/mL)	14.1	21.1	48.7	27.4	69.8	207
AUC <sub>0-24</sub> (ng•hr/mL)	62.8	115	321	101	222	659
T <sub>max</sub> (hr)	-	-	-	-	-	-

**Additional Information:**  
 -: not calculated  
 There was large inter-animal variation, especially in C<sub>max</sub>  
 An approximately proportional increase in exposure was seen with increasing dose levels, except in females where there was a small supra-proportional increase  
 There was a small increase in exposure on Day 15 versus Day 1

**Pharmacokinetics parameters of hydromorphone in pregnant or nursing animals.**

Report No.	DT99033		DT99034	
GLP	Yes		Yes	
Species/Strain	Rat/Wistar		Rabbits/New Zealand White	
Placental Transfer				
Gestation Day/Number of Animals:	Day 17 / 3 animals per time point	Day 17 / 3 animals per time point	Day 19 / 3 animals per time point	Day 19 / 3 animals per time point
Feeding Condition:	Fasted	Fasted	Fed	Fed
Vehicle/Formulation:	dH <sub>2</sub> O	dH <sub>2</sub> O	dH <sub>2</sub> O	dH <sub>2</sub> O
Method of Administration:	PO gavage	PO gavage	PO gavage	PO gavage
Dose, [ <sup>14</sup> C] hydromorphone free base (mg/kg/day):	1.75	1.75	6.25	6.25
Analyte:	Total radioactivity	Total radioactivity	Total radioactivity	Total radioactivity
Assay:	Liquid Scintillation Counting	Liquid Scintillation Counting	Liquid Scintillation Counting	Liquid Scintillation Counting
Time (hr)	2	24	2	72
Concentration (mcg equiv/g ± SD)				
Maternal plasma	0.60±0.10	0.03±0.01	2.69±0.41	0.02±0.01
Fetus	0.05±0.01	0.02±<0.01	0.18±0.03	0.04±<0.01
Amount (% of dose)				
Fetus	0.05	0.04	0.01	0.01

**Additional Information:**  
 Rat, fetal to plasma ratio was 0.08 at 2 h and 0.67 at 24 h.  
 Fetal levels of radiolabelled material decreased with time indicating reversible distribution across the placenta into the fetus.

**Metabolism:**

**Metabolism: In vivo - Interspecies comparison.**

Species/Strain	Rat/Han Wistar	Rabbit/New Zealand White	Dog/Beagle
Number of Animals/Sex (M/F):	17M/10F	6F pregnant	3M
Feeding Condition:	Fasted	Fed	Fed
Vehicle/Formulation:	dH <sub>2</sub> O	dH <sub>2</sub> O	Gelatin capsule
Method of Administration:	PO gavage	PO gavage	PO
Dose, hydromorphone free base (mg/kg):	7	6.25	1 <sup>b</sup>
Radionuclide:	<sup>14</sup> C	<sup>14</sup> C	<sup>14</sup> C
Specific Activity:	502–1236 MBq/g	561 MBq/g	1236 MBq/g

Species/ Sex	Sample	Sampling Time/ Period (hr)	% of Dose in Sample	% Compound in Sample					Report Number	GLP	CTD Module	
				Parent	M1	M2	M3	M4				M5
Rat Male	Plasma	1	–	1.21	93.54	ND	–	–	–	DT00049	Yes	4.2.2.5
	Urine	0–48	48.3±2.9	10.42	85.09	ND	–	–	–			
	Feces	0–48	20.8±6.1	61.32	ND	38.68	–	–	–			
	Bile <sup>a</sup>	0–24	33.3±17.3	2.36	97.64	ND	–	–	–			
Rat Female	Plasma	1	–	1.56	94.37	ND	–	–	–	DT00049	Yes	4.2.2.5
	Urine	0–48	58.6	7.88	81.47	ND	–	–	–			
	Feces	0–72	16.9	61.19	ND	38.81	–	–	–			
Rabbit Pregnant	Plasma	2	–	Trace	100	ND	–	ND	ND	DT00050	Yes	4.2.2.4
	Urine	0–24	60.5±3.0	0.13	88.4	ND	–	8.5	3.0			
	Feces	0–24	17.6±5.6	28.7	ND	71.3	–	ND	ND			
Dog Male	Plasma	2	–	16.6	64.0	–	19.5	–	–	DT00043	Yes	4.2.2.4
	Urine	0–48	45.7±10.2	2.8	83.6	–	13.6	–	–			
	Feces	0–48	40.2±6.0	32.7	47.8	–	19.6	–	–			

**Additional Information:**  
M1: hydromorphone-3-glucuronide, M2: dihydromorphone, M3: hydromorphone-3-sulphate, M4: dihydromorphone-3-glucuronide, M5: hydromorphone-N-oxide glucuronide  
ND not detected; – not assessed  
<sup>a</sup>Bile excretion data (parent) presented in Study DT99029  
<sup>b</sup>Dosing occurred on Day 19 of gestation

**Metabolism: In vitro - Interspecies comparison.**

**Study System:** [<sup>14</sup>C] hydromorphone was incubated with microsomal samples of rat, dog and human liver supplemented with NADPH (Phase 1 metabolism) or uridine diphosphoglucuronic acid (UDPGA; Phase 1 and 2 metabolism combined).  
Investigation of [<sup>14</sup>C] hydromorphone metabolism by flavin monooxygenases using baculovirus expression systems expressing human FMO1, FMO3 and FMO5

Phase 1 metabolism	Species/Sex	% formaldehyde		% norhydromorphone		
		30 minutes	90 minutes	30 minutes	90 minutes	
	Rat/Male	11.5	10.5	12.2	16.1	
	Rat/Female	ND	ND	ND	ND	
	Dog/Male	ND	0.4	0.7	ND	
	Human/Pooled	ND	1.4	2.7	ND	
Phase 1 and 2 metabolism combined	Species/Sex	Pre-enzyme hydrolysis		Post-enzyme hydrolysis		
		Hydromorphone-3-glucuronide	Hydromorphone	Hydromorphone-3-glucuronide	Hydromorphone	
		Rat/Male	74	26	15	85
		Dog/Male	100	ND	23	77
	Human/Pooled	41	59	5	95	
FMO metabolism	Species/Sex	FMO Enzyme		Mean % hydromorphone-N-oxide		
		Human/Pooled	FMO1	0.9		
		Human/Pooled	FMO3	ND		
		Human/Pooled	FMO5	ND		

ND: not detected

**Metabolism: In vitro – Human.**

Test Article: Hydromorphone HCl  
 CTD Module: 4.2.2.4  
 Report Number: DT00053  
 GLP: Yes

Study System:	[ <sup>14</sup> C]hydromorphone was incubated with specific human CYP450s, 1A2, 3A4, 2D6, 2C9 and 2C19 enzymes expressed in commercial baculovirus preparations. Expression systems supplemented with NADPH to investigate phase I metabolism
Results:	No phase I metabolism products, as radiolabelled components or by mass spectrometry, were observed compared with controls. Potential for interaction at CYP450 level is limited.

Test Article: Hydromorphone HCl  
 CTD Module: 4.2.2.4  
 Report Number: DT99052  
 GLP: Yes

Study System:	Incubation of hydromorphone (0 to 10,000 µM) with human hepatic microsomes in presence of probe substrates with specificity for CYP450 human isoforms					
	CYP (% metabolite [presence of hydromorphone]/% metabolite [control])					
Hydromorphone (µM)	1A2	2C9	2C19	2D6	3A4	4A11
0	100	100	100	100	100	100
10	139.4	87.4	103.2	96.3	108.0	112.0
50	122.4	93.4	122.8	56.5	112.6	96.6
100	132.9	83.9	120.0	25.4	87.0	88.0
1,000	119.0	87.0	127.2	13.7	106.1	96.4
5,000	107.5	87.9	104.0	ND	60.4	88.3
10,000	65.0	85.6	44.0	ND	41.1	107.9

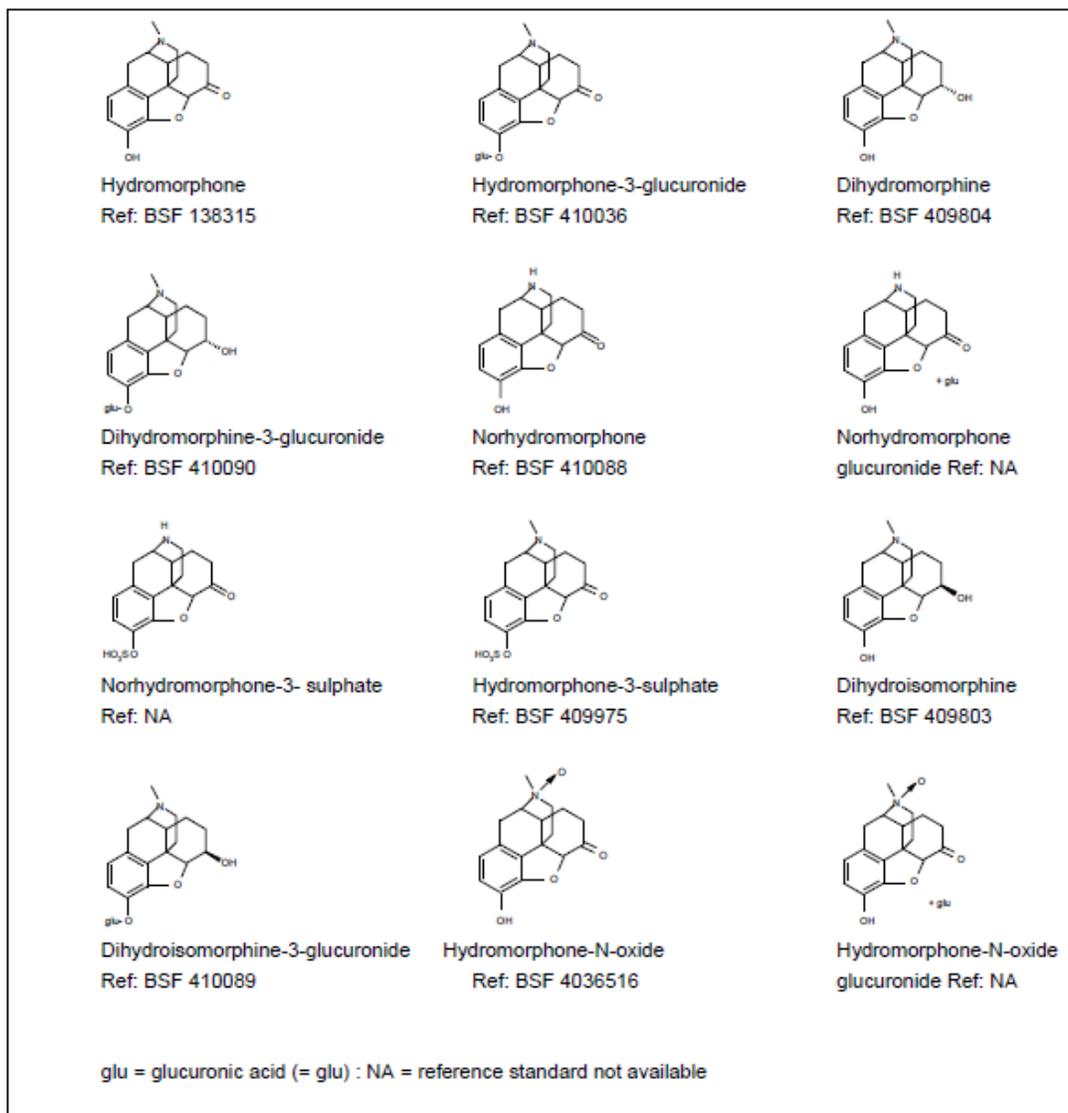
ND: not detected

Test Article: Hydromorphone HCl  
 CTD Module: 4.2.2.4  
 Report Number: DT00051  
 GLP: Yes

Study System:	Incubation of [ <sup>14</sup> C]hydromorphone with human hepatic expression systems of specific glucuronyl transferases (UGTs)	
Result:	No metabolism of [ <sup>14</sup> C]hydromorphone with UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A9, UGT1A10, UGT2B15	
	% hydromorphone-3-glucuronide	
UGT2B7	90 minutes 1.9	240 minutes 4.4

### Metabolism: Proposed Metabolites of Hydromorphone

Structures of metabolites and proposed metabolites of hydromorphone



**Distribution**

Test Article: Hydromorphone HCl  
 CTD Module: 4.2.2.3  
 Report Number: DT99025  
 GLP: Yes

Species/Strain: Rat / pigmented (PVG)  
 Number of Animals/Sex (M/F): 20 M  
 Feeding Condition: Fasted  
 Vehicle/Formulation: dH<sub>2</sub>O  
 Method of Administration: PO gavage  
 Dose, hydromorphone free base (mg/kg/day): 7  
 Radionuclide: <sup>14</sup>C  
 Specific Activity: 1236 MBq/g  
 Assay: Quantitative whole-body autoradiography  
 Sampling Time: 1, 4, 8, 24, 72, 168 hr – measured by quantitative, whole-body autography

Concentration of radiolabelled material (mcg equiv/g)					
Time (hr)	1	168	Time (hr)	1	168
Tissue/Organ			Tissue/Organ		
Blood (in heart)	0.73	0.10	Muscle (skeletal)	0.44	0.18
Plasma	Not measured	Not measured	Heart muscle	0.55	0.15
Brain	0.63	0.13	Thymus	0.61	0.19
Lung	0.76	0.12	Thyroid gland	0.65	0.15
Liver	2.96	0.45	Eye <sup>a,b</sup>	0.45	0.29
Kidney	3.08	0.22	Spleen	1.03	0.17
Pancreas	1.17	0.19	Adrenal gland	0.85	0.15
Testes	0.54	0.23	Brown fat	0.63	0.17

Additional Information:  
 C<sub>max</sub> for most tissues was 1 hr  
 Distribution from tissues was slow  
<sup>a</sup> Radioactivity in eye measured by tissue combustion liquid scintillation counting  
<sup>b</sup> Eye C<sub>max</sub> was 0.52 mcg equiv/g at 8 hr

**Excretion: Excretion into urine, feces, bile and expired air**

**Table 10: Proportions of Oral Hydromorphone Dose Recovered in Urine, Feces, Bile and Expired Air in Rats, Rabbits or Dogs**

Species Report	Route	Dose (mg/kg of [ <sup>14</sup> C] hydromorphone)	% of dose recovered in			
			urine	feces	bile	expired air
<a href="#">Male rat DT99018</a>	oral	7	51.1	23.7	NM	16.3
<a href="#">Female rat DT99031</a>	oral	7	63.1	18.7	NM	<1
<a href="#">Male rat<sup>A</sup> DT99029</a>	oral	7	58.0	2.7	33.4	NM
<a href="#">Pregnant rabbit DT99035</a>	oral	6.25	66.7	19.0	NM	NM
<a href="#">Male dog MPF/DDM 9854</a>	oral	1	50.2	43.7	NM	NM

*Rat<sup>A</sup>* : Bile duct cannulated to collect secreted bile  
 NM : not measured

**Excretion: Excretion into milk**

2.6.5.13B Pharmacokinetics: Excretion

Species/Strain Rat/Wistar  
 GLP: Yes

Test Article: Hydromorphone HCl  
 CTD Module: 4.2.2.5  
 Report Number: DT99037

Excretion into Milk	
Lactating Day/Number of Animals	Day 12 / 3 animals per time point
Feeding Condition	Fed
Vehicle/Formulation	dH <sub>2</sub> O
Method of Administration	PO gavage
Dose, hydromorphone free base (mg/kg/day)	1.56
Analyte	<sup>14</sup> C
Assay	Liquid Scintillation Counting

Time (hr)	1	3	6	24
Concentration (mcg equiv/g ± SD)				
Milk	0.06±<0.01	0.08±<0.01	0.07±0.02	0.02±<0.01
Maternal Plasma	0.65±0.20	0.28±0.04	0.11±0.02	0.01±<0.01
Milk/Plasma	0.10±0.04	0.28±0.06	0.65±0.31	1.80±0.28

Additional Information:  
 T<sub>max</sub> was 3 h in milk and 1 h in plasma  
 <1% of the dose was secreted into the milk in the 24 h period post dosing

**2.6.6 TOXICOLOGY**

**2.6.6.1 Overall toxicology summary**

**General toxicology:**

In support of the chronic indication, the systemic toxicity of hydromorphone was studied in mice, rats and dogs. The key repeat-dose toxicology studies to support the chronic indication were conducted in dogs up to 39-weeks and in rats up to 6 months. Only, the 30-days and 39-weeks repeat-dose study in dogs and 27-weeks repeat-dose study in rats were reviewed because they were pivotal studies for the safety assessment for the intended route and treatment duration.

In the 30-day repeat dose dog study (Report № TR-96-4604-057), beagle dogs were administered OROS hydromorphone orally at doses of 0, 8, and 64 mg/kg/day and IR hydromorphone at a dose of 64 mg/kg/day. Dose levels were based on the findings of the 5-day preliminary study that was performed to determine if dogs could tolerate repeated daily dosing of 64 mg/kg/day of hydromorphone. In the 5-day study, 2 dogs were orally administered 32 mg/day of Dilaudid (16 mg x 2/day for 2 days; 32 mg x 1/day for 3 days) and another 2 dogs were dosed with 64 mg/day (32 mg x 1/day for 2 days; 64 mg x 1/day for 3 days) of Dilaudid. Clinical signs of toxicity were consistent with opiate-like pharmacological effects and included excessive salivation, vomiting, diarrhea, decreased activity and tremors. Treatment-related effect decrease in food consumption and food

consumption were noted during week 1. There were no treatment-related effects observed on organ weight, hematology and urinalysis parameters. No target organ of toxicity was identified. Based on the observed clinical signs, not considered to be safety issues, the 64 mg/kg (Day 30 AUC = 115.9 ng·hr/mL) was identified as the NOAEL.

In the 39-week repeat dose dog study (Report No MPF/DT 9901), beagle dogs were administered hydromorphone orally at doses of 0, 1.75, 4.0 and 9.0 mg/kg/day. Treatment-related clinical signs, consistent with opiate-related pharmacological effects, included sedation, tremors, ventral recumbency, lateral position, vomiting and weak hind limbs. There were no deaths. Statistically significant decreases in body weight were noted in the hydromorphone-treated groups and control during the study. Body weights were significantly decreased for males at 9 mg/kg/day beginning on day 2 and continued through study day 143 (week 21). A target organ of toxicity was not identified; histological and gross pathology evaluation showed no evidence of toxicologically-relevant effects. The NOAEL was 9 mg/kg/day based on clinical signs not considered to be significant safety issues. This NOAEL was associated with a mean AUC of 230 and 346 ng·h/mL in males and females, respectively; and  $C_{max}$  of 39 (males) and 58 (females) ng/mL.

In the 27-week repeat dose rat study (Report No MPT/DT 9940), Wistar rats were administered BSF 138315 (hydromorphone, active moiety) orally at doses of 0, 3.5, 7.5 and 14 mg/kg/day. Treatment-related clinical signs, consistent with opiate-related pharmacological effects typically observed in rats included compulsive gnawing, hyperactivity, self-mutilation, sedation, mydriasis, exophthalmos and diarrhea. Two animals were sacrificed *in extremis* due to poor condition. Statistically significant and dose-dependent decrease in body weight was noted in the hydromorphone-treated group. The effects of BSF 138315 on mean body weight was more pronounced in the male than the female rats. A target organ of toxicity was not identified; histological and gross pathology evaluation showed no evidence of toxicologically relevant effects. The ocular findings (i.e., retinal atrophy and corneal opacities) observed in the hydromorphone treated rats are known to be associated with morphine-like drugs and are rat-specific. The NOAEL was 3.5 mg/kg/day (HED = 0.567 mg/kg). This corresponds to AUC<sub>0-24 hr</sub> value of 322 and 219 ng·h/mL in males and females, respectively, during week 24.  $C_{max}$  values at this dose during week 24 were 103 and 66.2 ng/mL in males and females, respectively.

Genetic toxicology: The Applicant submitted one genetic toxicology study performed with hydromorphone. The Applicant conducted an *in vitro* bacterial reverse mutation assay; this study included the *Salmonella typhimurium* tester strain TA 102 which was not evaluated in the earlier Ames test. The Applicant referred the reviewer to the original NDA submission for the *in vitro* bacterial reverse mutation assay and *in vivo* micronucleus test study reports; these studies were reviewed by Dr. Kathleen Haberny in June of 2000. Specific details of these studies can be found in NDA 21-217 review from Dr. Haberny. Additionally, the Applicant conducted genotoxicity studies with the (b) (4) impurity (b) (4) and the degradant impurities (b) (4) and hydromorphone N-oxide.

Hydromorphone tested negative in the in vitro bacterial reverse mutation assay (Ames test) at concentrations of 33, 100, 333, 1000, 2500 and 5000 µg/plate in the presence and absence of metabolic activation. Hence, hydromorphone is considered non-mutagenic in the Ames test under the condition of the study. The genotoxic potential of hydromorphone was also evaluated in the in vitro chromosomal aberration assay in human lymphocytes from healthy female donors. There were no increases in the numbers of structural chromosome aberrations after incubation with hydromorphone hydrochloride at up to 3200 µg /mL in the presence and absence of metabolic activation with S9 mix. Ethylmethane sulfonate and cyclophosphamide, the positive controls, significantly increased the frequency of structural chromosomal aberrations. Therefore, hydromorphone hydrochloride was considered to be non-clastogenic in human lymphocytes under the conditions of this study.

Hydromorphone tested negative in the in vivo mouse micronucleus test. No induction of micronuclei in polychromatic erythrocytes of bone marrow cells by hydromorphone hydrochloride administered at up to 100 mg/kg PO (approximately 7.5x the human dose of 64 mg/day in a 60 kg patient on a mg/m<sup>2</sup> basis).

(b) (4) tested negative in the in vitro bacterial reverse mutation assay (Ames test) at concentrations of 15, 50, 150, 500, 1500 and 5000 µg/plate in the presence and absence of metabolic activation. Hence, under the condition of the study, (b) (4) is considered non-mutagenic in the Ames test under the condition of the study.

Both degradant impurities, hydromorphone N-oxide and (b) (4) was shown not to be mutagenic. (b) (4) was evaluated in the bacterial reverse mutation assay using *Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* tester strain WP2 *uvrA* in the presence and absence of metabolic S9 activation. (b) (4) tested negative at concentrations up to 5000 µg/plate.

The genotoxic potential of hydromorphone N-oxide was evaluated in the in vitro chromosomal aberration assay in human lymphocytes from a healthy male donor. There was no increase in the number of structural chromosome aberrations after incubation with hydromorphone N-oxide at up to 5000 µg /mL in the presence and absence of metabolic activation with S9 mix. Ethylmethane sulfonate and cyclophosphamide, the positive controls, significantly increased the frequency of structural chromosomal aberrations. Therefore, hydromorphone N-oxide was not clastogenic in human lymphocytes under the conditions of this study. The genotoxic potential of hydromorphone N-oxide was also evaluated in an in vitro bacterial reverse mutation assay. Hydromorphone N-oxide tested negative at concentrations of 33, 100, 333, 1000, 2500 and 5000 µg/plate in the presence and absence of metabolic activation. Hence, under the condition of the study, hydromorphone N-oxide is considered non-mutagenic in the Ames test under the condition of the study.

Carcinogenicity: Studies to evaluate the carcinogenic potential of hydromorphone hydrochloride are on going. The rat (Study № 1678-002) and mouse (Study № 1678-

001) carcinogenicity studies were initiated on March 18, 2009 and March 24, 2009, respectively.

**Reproductive toxicology:** No formal reproductive toxicity studies were submitted with the current NDA. However, studies to evaluate the potential effects of hydromorphone on fertility and early embryonic development, embryo-fetal development and pre-natal and post-natal development were submitted in the original NDA. Dr. Haberny reviewed these studies; specific details of these studies can be in NDA 22-217 review from Dr. Haberny. In brief, hydromorphone had no effects on female and male fertility, and was not teratogenic in either rats or rabbits.

**Special toxicology:** The Applicant conducted local tolerance studies in dogs, guinea pigs and rabbits. Since these studies were not relevant to the proposed route of administration, these studies were not reviewed.

#### **2.6.6.2 Single-dose toxicity**

Single-dose toxicology studies were conducted in mice, and rats following oral and intravenous administration, and in dogs following oral administration. These studies were not considered pivotal in the safety assessment of OROS hydromorphone; therefore, these studies were not reviewed.

#### **2.6.6.3 Repeat-dose toxicity**

Repeat-dose toxicology studies were conducted in mice, rats and dogs following oral administration for 2-weeks to 39-weeks. The Applicant considered the three studies performed in rats (i.e., 4-, 13- and 27-weeks) and dogs (30-day, 4-week and 39-week) to be the pivotal repeat-dose toxicity studies. However, only the 30-days and 39-weeks repeat-dose study in dogs and 27-weeks repeat-dose study in rats were reviewed because they were pivotal studies for the safety assessment for the, intended delivery system (i.e., OROS), intended route and treatment duration.

#### **Study title: Thirty day oral dosing study in dogs with OROS<sup>®</sup> (hydromorphone HCl) ALA study.**

**Key study findings:** Hydromorphone was orally administered to dogs for 30 days in the OROS delivery system (0 mg OROS, 8 mg OROS or 64 mg OROS) or as the immediate release tablets (32 mg/day Dilaudid tablets). The results were:

1. Mean peak plasma level was generally obtained between 7 hours and 9 hours for the OROS system. There were no signs of accumulation upon repeat dosing.
2. Treatment-related clinical signs include vomiting, excessive salivation, decreased activity, loose stools and mydriasis.
3. No treatment-related macroscopic or microscopic changes in the gastrointestinal system were observed.

4. Based on the observed clinical signs not considered to be a significant safety issues, NOAEL is 64 mg/kg/day (HED = 34.6 mg/kg). This corresponds to AUC<sub>0-24</sub> value of 81.6 and 115.881 ng•h/mL for OROS and IR formulation, respectively, on day 30. C<sub>max</sub> values on day 30 were 7.2 (OROS) and 17.1 (IR) ng/mL.

**Study no.:** TR-96-4604-057  
**Volume #, and page #:** eCTD submission  
**Conducting laboratory and location:** (b) (4)  
**Date of study initiation:** November 8, 1996  
**GLP compliance:** Yes  
**QA report:** Yes ( X ) No ( )  
**Drug, lot #, and % purity:**  
 OROS® (Hydromorphone HCl) - 8 mg: Code № 0003092, % purity not stated  
 OROS® (Hydromorphone HCl) - 64 mg: Code № 0003095, % purity not stated  
 Immediate Release Hydromorphone HCl, Lot № 10900116, % purity not stated  
 OROS® Placebo, Code № 0003804

**Methods**

Doses: 0, 8 and 64 mg/kg/day (OROS); 64 mg/kg/day (IR Hydromorphone HCl)  
 Based on the results from the 5-day preliminary study, the IR dose of 64 mg/kg/day was administered as 32 mg twice a day was selected.  
 Species/strain: Dog/Beagle  
 Number/sex/group or time point (main study): 4/sex/group  
 Route, formulation, volume, and infusion rate: Oral, gelatin capsule (appropriate numbers of OROS systems or IR Dilaudid tablets were packed in gelatin capsule)

			Number of Animals <sup>A</sup>									
Group	Test Article	Dosage Level (mg/kg/day)	Initial		Clinical Laboratory Studies <sup>B</sup>		Toxicokinetic Samples <sup>C</sup>		Necropsy		Microscopic Pathology	
			F	M	F	M	F	M	F	M	F	M
1	OROS® (Placebo) System	0	4	4	4	4	4	4	4	4	4	4
2	OROS® - Hydromorphone HCL System	8	4	4	4	4	4	4	4	4	4	4
3	OROS® - Hydromorphone HCL System	64	4	4	4	4	4	4	4	4	4	4
4	IR Hydromorphone <sup>D</sup>	64	4	4	4	4	4	4	4	4	4	4

A: All animals received clinical observations pretest and daily throughout the study  
 B: Hematology, clinical chemistry and urinalysis parameters were examined pretest and termination  
 C: Blood was collected for toxicokinetic evaluations on test days 1, 7, 14, 21 and 28  
 D: IR = Immediate Release tablets (Dilaudid). Doses were administered at 32 mg twice per day (once in the morning and once in the afternoon), approximately 4 hours apart.

Satellite groups used for toxicokinetics or recovery: 4/sex/group as noted above.

Age: Approximately 5 months

Weight: Males: 9.0 kg (8.2 kg – 10.0 kg); Females: 8.0 kg (7.3 kg – 8.9 kg)

Sampling times: Plasma concentrations of hydromorphone were assessed in all animals on test days 1, 7, 21, and 28.

Unique study design or methodology (if any): None

**Observations and times: (these parameters can be captured separately here or described in connection with each endpoint under the results section.)**

The observation parameters were captured under the results section.

**Results**

**Toxicokinetics:**

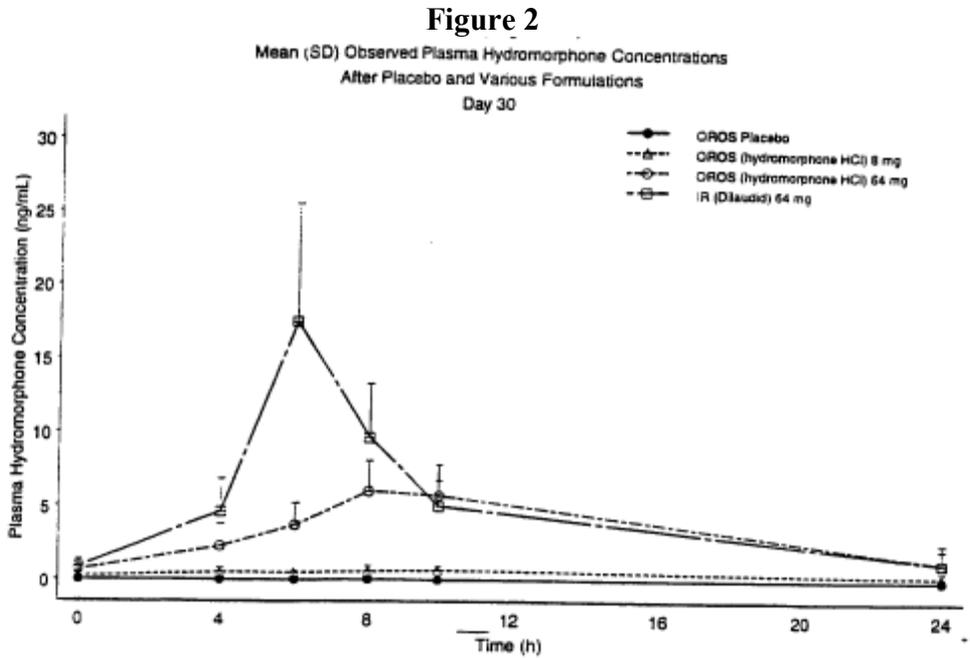
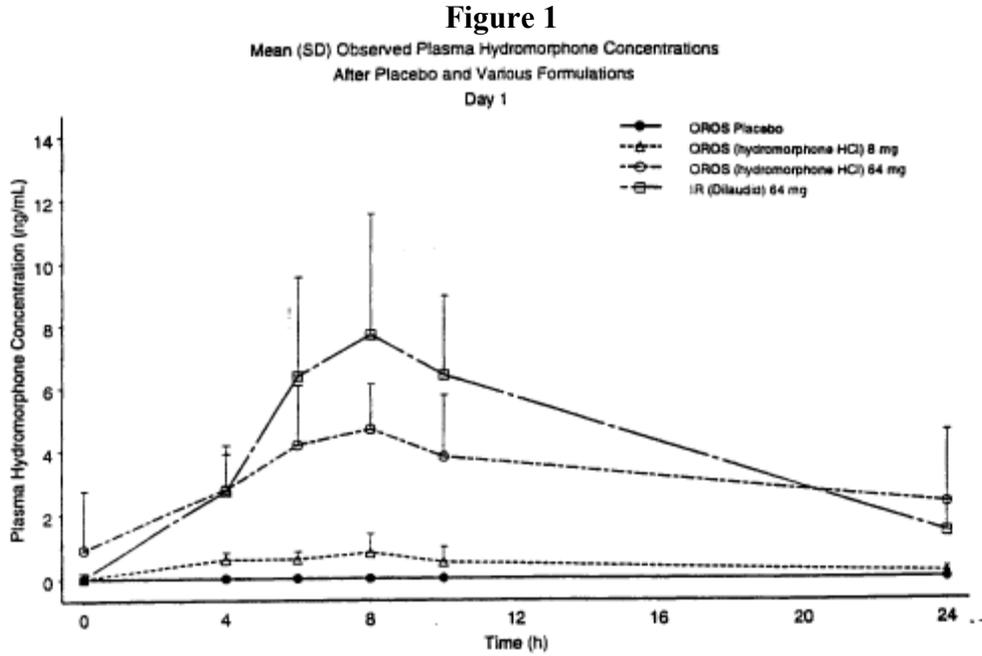
*Blood (approx. 5 mL) were collected via jugular and/or cephalic venipuncture from all animals (4/sex/group) for toxicokinetic evaluation according to the schedule below.*

*Plasma concentration of hydromorphone was determined by LC/MS/MS*

<i>Blood Sampling Day</i>	<i>Time Point</i>
<i>Days 1 and 30</i>	<i>Pre-dosing, 4, 6, 8, 10 and 24 hours post-dosing</i>
<i>Days 7, 14, and 21</i>	<i>Pre-dosing and 6 hours post-dosing</i>

*Fecal samples were collected on days 15, 16 and 17 to collect tablet remnants.*

Toxicokinetic analysis is presented in the figures and table below. As depicted in the figure 1 below,  $C_{max}$  levels of hydromorphone increased with increased dosage of OROS hydromorphone on both day 1 and day 30. Compared to the 64 mg/kg dose of extended release formulation of hydromorphone, the 64 mg/kg dose of immediate release formulation of hydromorphone reached a higher plasma level.



Exposure increased in a dose-dependent manner but not quite dose-proportionally (variable). On Day 1 of dosing, the  $C_{max}$  increased in a sub-proportional manner by 5.8-fold instead of the 8-fold following oral administration of OROS hydromorphone. The AUC increased in an approximate dose-proportionate manner following oral administration of OROS hydromorphone. On day 30 of dosing, AUC increased in a less than dose-proportional manner (4.6-fold instead of an 8-fold) and  $C_{max}$  increased in a dose-proportional manner. On day 1 of dosing,  $T_{max}$  range was 6.9 and 7.8 hours

following 8 and 64 mg/kg OROS hydromorphone, respectively. On day 30 of dosing,  $T_{max}$  was 9.3 hours following both 8 and 64 mg/kg OROS hydromorphone.

On Day 1 of dosing, the  $C_{max}$  increased in a sub-proportional manner by 5.8-fold instead of the 8-fold following oral administration of OROS hydromorphone. The AUC increased in an approximate dose-proportionate manner following oral administration of OROS hydromorphone. On day 30 of dosing, AUC increased in a less than dose-proportional manner (4.6-fold instead of an 8-fold) and  $C_{max}$  increased in a dose-proportional manner. On day 1 of dosing,  $T_{max}$  range was 6.9 and 7.8 hours following 8 and 64 mg/kg OROS hydromorphone, respectively. On day 30 of dosing,  $T_{max}$  was 9.3 hours following both 8 and 64 mg/kg OROS hydromorphone.

**Table. Toxicokinetic parameters for hydromorphone on study days 1 and 30 following daily oral doses of hydromorphone in dogs for 30 days.**

Sampling period	TK Parameter	Dose (mg/kg/day)			
		Hydromorphone OROS System			Immediate Release Dilauidid® Tablet
		0	8	64	64
1	$C_{max}$ (ng/mL)	0 ± 0.0	0.906 ± 0.527	5.221 ± 1.412	8.653 ± 3.326
	$T_{max}$ (hr)	-	6.9 ± 2.3	7.8 ± 1.7	8.3 ± 1.7
	AUC <sub>0-24</sub> (ng*h/mL)	0 ± 0.0	8.948 ± 5.924	68.069 ± 31.545	96.867 ± 25.526
	AUC <sub>inf</sub> (ng*h/mL)	0 ± 0.0	10.6 ± 6.7	87.6 ± 56.6	96.867 ± 25.526
30	$C_{max}$ (ng/mL)	0.0228 ± 0.0559	0.8435 ± 0.3106	7.2475 ± 1.2713	17.1325 ± 7.2766
	$T_{max}$ (hr)	8.0 ± 0	9.3 ± 6.6	9.3 ± 1.0	6.8 ± 1.0
	AUC <sub>0-24</sub> (ng*h/mL)	0.046 ± 0.112	17.674 ± 14.846	81.613 ± 22.561	115.881 ± 23.183
	k (h <sup>-1</sup> )	-	0.1147 ± 0.0148	0.1368 ± 0.0676	0.1142 ± 0.0338
	$t_{1/2}$ (hr)	-	6.12 ± 0.81	8.28 ± 8.89	6.76 ± 2.76

### **Mortality:**

No treatment-related deaths were observed during the 30-day study.

### **Clinical signs:**

*The animals were evaluated for emesis, abnormal stools, other discharges, signs of poor health or toxic or pharmacologic effects (e.g. assessed prior to dosing, and at several time intervals post-dosing. On the first day of testing, observations were performed at 15 and 30 minutes post-dosing and every 30 minutes thereafter for 8-hours post-dosing. Test day 2 and through day 30, the animals will be examined in the morning prior to dosing and twice in the afternoon (post-dosing and PM observation). During the recovery period, behavior and general health assessment was performed once daily.*

As shown in the Applicant's table below, treatment-related clinical signs include vomiting, excessive salivation (Group 4 only), decreased activity, increased incidence of unformed stool, and mydriasis. These effects were dose-dependent, gender-dependent and dosage form-dependent. Compared to the IR formulation of hydromorphone, the incidence of the observed clinical signs was lower in the dogs that received the OROS formulation of hydromorphone.

**Most Prevalent Clinical Observations  
Males**

Animal No.	Vomiting	Excessive Salivation	Unformed Stool <sup>a</sup>	No Stool	Decreased Activity <sup>b</sup>	Tremors	Eye Effects <sup>c</sup>
<b>Group I<sup>d</sup></b>							
1225	1	0	0	0	0	0	0
1226	0	0	8	0	0	0	0
1227	0	0	0	0	0	0	0
1228	1	0	1	0	0	0	0
<b>Total</b>	<b>2</b>	<b>0</b>	<b>9</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
<b>Group II<sup>e</sup></b>							
2225	0	0	5	0	0	0	0
2226	0	0	13	0	0	0	0
2227	0	0	5	0	0	0	0
2228	0	0	3	0	0	0	1
<b>Total</b>	<b>0</b>	<b>0</b>	<b>26</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1</b>
<b>Group III<sup>f</sup></b>							
3225	0	0	6	0	0	0	0
3226	0	0	11	0	1	0	0
3227	3	0	12	0	9	0	5
3228	1	0	6	1	7	1	4
<b>Total</b>	<b>4</b>	<b>0</b>	<b>35</b>	<b>1</b>	<b>17</b>	<b>1</b>	<b>9</b>
<b>Group IV<sup>g</sup></b>							
4225	9	6	9	0	16	0	3
4226	10	22	14	0	9	0	3
4227	2	2	9	1	24	1	3
4228	2	2	19	0	29	0	8
<b>Total</b>	<b>23</b>	<b>32</b>	<b>51</b>	<b>1</b>	<b>78</b>	<b>1</b>	<b>17</b>

<sup>a</sup> Includes watery stool  
<sup>b</sup> Includes lethargy  
<sup>c</sup> Includes constriction, dilation, and lacrimation.  
<sup>d</sup> OROS<sup>®</sup> Placebo  
<sup>e</sup> 8 mg/day OROS<sup>®</sup> (hydromorphone HCl) System  
<sup>f</sup> 64 mg/day OROS<sup>®</sup> (hydromorphone HCl) System  
<sup>g</sup> 64 mg/day (2 x 32) immediate-release tablets Dialudid<sup>®</sup>

**Most Prevalent Clinical Observations  
Females**

Animal No.	Vomiting	Excessive Salivation	Unformed Stool <sup>a</sup>	No Stool	Decreased Activity <sup>b</sup>	Tremors	Eye Effects <sup>c</sup>
<b>Group I<sup>d</sup></b>							
1725	0	0	10	0	0	0	0
1726	0	0	1	0	0	0	0
1727	0	0	3	0	0	0	0
1728	0	0	1	0	0	0	0
<b>Total</b>	<b>0</b>	<b>0</b>	<b>15</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
<b>Group II<sup>e</sup></b>							
2725	0	0	4	0	0	0	0
2726	1	0	1	0	1	0	0
2727	1	0	2	0	0	0	0
2728	0	0	1	0	0	0	0
<b>Total</b>	<b>2</b>	<b>0</b>	<b>8</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>
<b>Group III<sup>f</sup></b>							
3725	1	0	11	3	9	2	4
3726	6	0	3	1	8	0	4
3727	3	0	2	0	8	0	4
3728	5	0	1	0	10	0	2
<b>Total</b>	<b>15</b>	<b>0</b>	<b>17</b>	<b>4</b>	<b>35</b>	<b>2</b>	<b>14</b>
<b>Group IV<sup>g</sup></b>							
4725	5	27	18	0	23	2	3
4726	5	9	2	6	36	7	12
4727	3	14	4	2	25	1	2
4728	10	55	7	0	32	6	5
<b>Total</b>	<b>23</b>	<b>105</b>	<b>31</b>	<b>8</b>	<b>116</b>	<b>16</b>	<b>22</b>

<sup>a</sup> Includes watery stool

<sup>b</sup> Includes lethargy

<sup>c</sup> Includes constriction, dilation, and lacrimation.

<sup>d</sup> OROS<sup>®</sup> placebo

<sup>e</sup> 8 mg/day OROS<sup>®</sup> (hydromorphone HCl) System

<sup>f</sup> 64 mg/day OROS<sup>®</sup> (hydromorphone HCl) System

<sup>g</sup> 64 mg/day (2 x 32) immediate-release tablets Dialudid<sup>®</sup>

**Body weights:**

*Body weights were recorded twice prior to testing, weekly during the treatment period and just prior to necropsy (terminal fasted body weight).*

As depicted in the tables and Applicant's figures below, the effects of hydromorphone on mean and mean body weight change were more pronounced in the males than in the females. Apparent treatment-related decreases in body weight were noted in males. Mean body weight change were significantly lower in male dogs treated with either 64 mg/kg/day of OROS hydromorphone or 64 mg/kg/day immediate release dilaudid tablets during the first week of the treatment period. The lost in body weight during week 1 of treatment is correlated to the decreased food consumption during this week. As depicted

in the figure and table below, the male dog mean body weight gain during weeks 2 through 4 were significantly lower than the control group.

Hydromorphone-related effects on body weight gain were less pronounced in the females. During week 1, body weight gain was significant lower in females in 64 mg/kg/day OROS hydromorphone group. At the end of the treatment period, the mean body weight of all female dogs dosed with hydromorphone was considered comparable to control values.

**Mean Body Weight (kg) and Body Weight Gain (kg) Changes in Male Dogs treated with Hydromorphone for 30 days.**

	Males Body Weight Data			
	Hydromorphone OROS System			Immediate Release Dilaudid® Tablet
	0	8	64	64
Dose (mg/kg/day) →	0	8	64	64
Pre-dosing: Week -1	9.0 ± 0.8	9.1 ± 0.6	9.0 ± 0.6	9.0 ± 0.7
<b>Week 1</b>				
Mean Body Weight (kg) ± SD (% change of control)	9.4 ± 0.7	9.2 ± 0.6 (-2.1%)	8.5 ± 1.1 (9.6%)	8.5 ± 0.9 (-9.6%)
Mean Body Weight Changes from pre-dosing (kg)	0.5 ± 0.1	0.1 ± 0.3	<b>-0.6 ± 0.5**</b>	<b>-0.4 ± 0.2*</b>
<b>Week 2</b>				
Mean Body Weight (kg) ± SD (% change of control)	9.7 ± 0.9	9.6 ± 0.5 (-1.0%)	8.9 ± 1.0 (-8.2%)	9.0 ± 0.8 (-7.2%)
Mean Body Weight Changes from pre-dosing (kg)	0.8 ± 0.1	0.6 ± 0.1	<b>-0.1 ± 0.5**</b>	<b>0.0 ± 0.1**</b>
<b>Week 3</b>				
Mean Body Weight (kg) ± SD (% change of control)	10.0 ± 0.8	9.8 ± 0.6 (-2.0%)	9.4 ± 1.1 (-6.0%)	9.3 ± 0.8 (-7.0%)
Mean Body Weight Changes from pre-dosing (kg)	1.0 ± 0.1	0.8 ± 0.3	<b>0.4 ± 0.5*</b>	<b>0.4 ± 0.1*</b>
<b>Week 4</b>				
Mean Body Weight (kg) ± SD (% change of control)	10.3 ± 0.9	10.0 ± 0.7 (-2.9%)	9.7 ± 1.0 (-5.8%)	9.6 ± 0.8 (-6.8%)
Mean Body Weight Changes from pre-dosing (kg)	1.3 ± 0.2	0.9 ± 0.5	0.7 ± 0.4	<b>0.7 ± 0.1*</b>
*: Statistically significant when compared to control at p ≤ 0.05				
**: Statistically significant when compared to control at p ≤ 0.01				

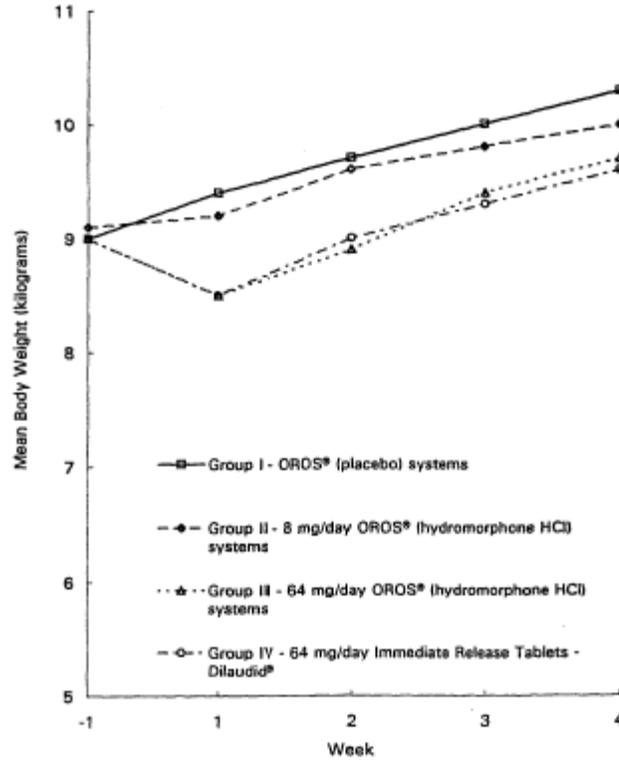
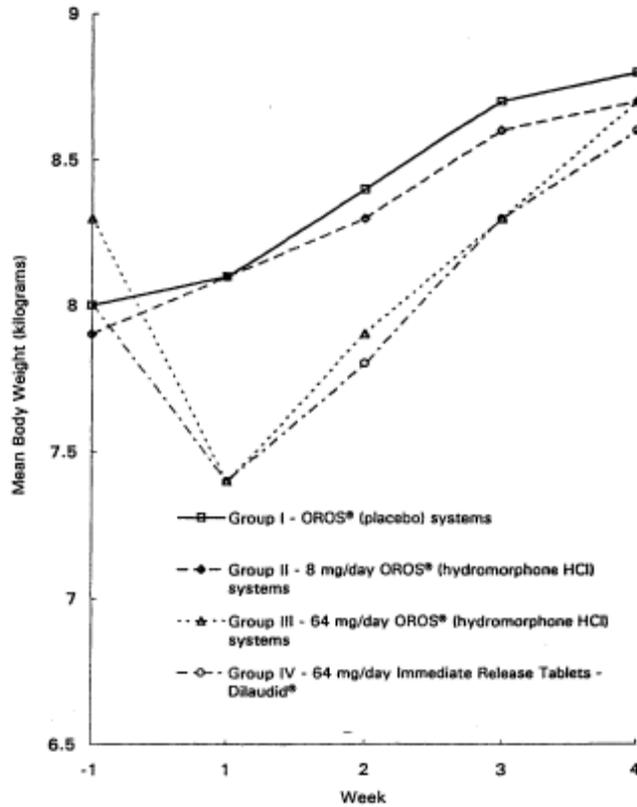


Figure: Males – Mean Body Weight

**Mean Body Weight (kg) and Body Weight Gain (kg) Changes in Female Dogs treated with Hydromorphone for 30 days.**

	Females Body Weight Data			
	OROS System			Immediate Release Dilaudid® Tablet
Dose (mg/kg/day) →	0	8	64	64
Pre-dosing: Week -1	8.0 ± 0.4	7.9 ± 0.5	8.3 ± 0.5	8.0 ± 0.5
<b>Week 1</b>				
Mean Body Weight (kg) ± SD (% change of control)	8.1 ± 0.5	7.9 ± 0.5 (-2.4%)	7.4 ± 0.8 (-8.6%)	7.4 ± 0.5 (-8.6%)
Mean Body Weight Changes from pre-dosing (kg)	0.2 ± 0.4	0.2 ± 0.2	<b>-1.0 ± 0.4*</b>	-0.6 ± 0.7
<b>Week 2</b>				
Mean Body Weight (kg) ± SD (% change of control)	8.4 ± 0.5	8.1 ± 0.5 (-3.6%)	7.9 ± 1.1 (-6.0%)	7.8 ± 0.8 (-7.1%)
Mean Body Weight Changes from pre-dosing (kg)	0.5 ± 0.4	0.4 ± 0.2	-0.5 ± 0.6	-0.1 ± 0.9
<b>Week 3</b>				
Mean Body Weight (kg) ± SD (% change of control)	8.7 ± 0.4	8.3 ± 0.6 (-4.6%)	8.3 ± 1.1 (-4.6%)	8.3 ± 0.5 (-4.6%)
Mean Body Weight Changes from pre-dosing (kg)	0.7 ± 0.2	0.7 ± 0.2	0.0 ± 0.6	0.4 ± 0.6
<b>Week 4</b>				
Mean Body Weight (kg) ± SD (% change of control)	8.8 ± 0.5	8.7 ± 0.6 (-1.1%)	8.7 ± 1.0 (-1.1%)	8.6 ± 0.4 (-2.3%)
Mean Body Weight Changes from pre-dosing (kg)	0.9 ± 0.3	0.8 ± 0.2	0.4 ± 0.5	0.7 ± 0.6

\*: Statistically significant when compared to control at p ≤ 0.05



**Figure: Females – Mean Body Weight**

**Food consumption:**

*Food consumption was recorded once a week prior to initiation of treatment and daily during the study.*

Treatment-related effects on food consumption were observed in the high dose groups. Compared to control group food intake, males treated with either 64 mg/kg/day OROS hydromorphone or 64 mg/kg/day of immediate release dilaudid tablets significantly ( $p \leq 0.05$ ) consumed less food during week 1. Similarly, the food consumption of females treated with 64 mg/kg/day OROS hydromorphone was significantly lower ( $p \leq 0.05$ ) relative to controls. After week 1, food consumption returned to control range or was slightly greater than the control group, thereafter in males in the immediate release dilaudid treatment group and females in the OROS hydromorphone group. Food consumption in males treated with 8 mg/kg/day OROS hydromorphone was comparable to control values.

	Mean Food Consumption (g/kg/day) ± SD (% change of control)			
	OROS System			Immediate Release Dilaudid® Tablet
Dose (mg/kg/day) →	0	8	64	64
<b>Males</b>				
Pre-dosing	27.1 ± 2.0	27.9 ± 3.9	27.5 ± 5.4	28.5 ± 5.2
<b>Week 1</b>				
Week 1	38.7 ± 2.5	38.0 ± 3.1 (-1.8%)	<b>21.8 ± 12.1*</b> <b>(-43.7%)</b>	<b>21.6 ± 7.5*</b> <b>(-44.1%)</b>
Week 2	33.0 ± 3.2	34.0 ± 3.3 (+3%)	37.3 ± 2.6 (+13.0%)	32.9 ± 7.5 (-0.3%)
Week 3	31.6 ± 3.0	35.5 ± 3.9 (+12.3%)	<b>39.3 ± 2.1*</b> <b>(+24.4%)</b>	36.1 ± 33.4 (+14.2%)
Week 4	30.9 ± 3.1	32.9 ± 5.1 (6.5%)	38.2 ± 3.5 (+23.6%)	33.4 ± 4.2 (+8.1%)
<b>Females</b>				
Pre-dosing: Week -1	26.8 ± 4.9	27.1 ± 4.6	26.3 ± 6.3	28.2 ± 5.9
Week 1	39.3 ± 7.1	37.3 ± 4.5 (+2.8%)	<b>15.6 ± 8.1*</b> <b>(-60.3%)</b>	20.2 ± 18.5 (-48.6%)
Week 2	36.2 ± 5.5	34.5 ± 1.3 (+8.2%)	36.3 ± 6.8 (+2.7%)	37.1 ± 10.2 (+2.5%)
Week 3	31.9 ± 4.1	32.3 ± 5.2 (+1.3%)	37.8 ± 6.0 (+18.5%)	38.4 ± 8.3 (+20.4%)
Week 4	31.9 ± 3.1	33.1 ± 4.5 (+3.8%)	33.3 ± 4.5 (+4.4%)	38.3 ± 5.0 (+20.0%)
*: Statistically significant when compared to control at p ≤ 0.05				

### **Ophthalmoscopy:**

*Eyes of all animals were examined before the first dose and on day 29 (termination). During the examination of the eye, the following ocular structures were examined by indirect ophthalmoscopy: cornea, anterior chamber, lens, iris, vitreous humor, retina and optic disc. Also, the lids, lacrimal apparatus and conjunctiva were examined grossly. Mydrucyl (1%) was used to induce mydriasis.*

No treatment-related changes were noted in the eye or the following ocular structures: cornea, anterior chamber, iris, lens, vitreous humor, retina and optic disc.

### **EKG:**

*Not performed*

### **Hematology:**

*Blood was collected from the jugular vein from unanesthetized dogs (4/sex/group) that were fasted overnight prior to each blood collection interval. Blood was collected pretest and on day 29 (termination). The following parameters were examined:*

Hematology Parameters	
White Blood Cell Parameters	Red Blood Cell Parameters
Total Leukocyte count (WBC)	Erythrocyte count (RBC)
Differential leukocyte count (Absolute) - Segmented Neutrophils - Lymphocyte (LYM)	Red Cell Morphology
Leukocyte count (WBC)	Hemoglobin (HGB)
White Blood Cells	Hematocrit (HCT)
	Mean corpuscular volume (MCV)
	Mean corpuscular hemoglobin (MCH)
	Mean corpuscular hemoglobin concentration (MCHC)
	Platelet Count (PLT)

No treatment-related changes were noted in the hematology parameters measured.

**Coagulation:** Blood was collected from the jugular vein from unanesthetized dogs (4/sex/group) that were fasted overnight prior to each blood collection interval. Blood was collected pretest and on day 29 (termination). The following parameters were examined:

Coagulation Parameters
Activated Partial Thromboplastin Time (ATPT)
Prothrombin Time (PT)

No treatment-related changes were noted in the coagulation parameters measured.

### **Clinical chemistry:**

Blood was collected from the jugular vein from unanesthetized dogs (4/sex/group) that were fasted overnight prior to each blood collection interval. Blood was collected pretest and on day 29 (termination). The following parameters were examined:

Serum Chemistry Parameters	
Alanine aminotransferase (ALT)	Inorganic Phosphorus
Albumin (ALB)	Potassium (K)
Aspartate aminotransferase (AST/GOT)	Sodium (Na)
Albumin/globulin ratio (AGR)	Total bilirubin (TBIL)
Calcium (Ca)	Blood Urea Nitrogen (BUN)
Chloride (Cl)	Total Protein (TP)
Creatinine (CREA)	
Glucose (GLU)	
Globulin (GLOB)	

Occasional statistically significant ( $p \leq 0.05$  or  $p \leq 0.01$ ) changes in clinical chemistry parameters in hydromorphone treated animals compared to the vehicle control treated animals were observed in females and males. A slight increase in glucose level was observed in both females and males treated with immediate release hydromorphone

compared to vehicle control animals. Slight increase in chloride level was observed in the 8.0, and 64.0 mg/kg/day (IR) male treatment groups. Due to a lack of a dose-dependent pattern, these findings are considered to be of little toxicological significant.

Parameter	Study Day	Gender	Dose (mg/kg/day) (% change of control)			
			OROS System			Immediate Release Dilaudid® Tablet
			0	8	64	64
Glucose (mg/dL)	Pretest	Males	104 ± 13.0	104 ± 6.0	107 ± 4.0	103 ± 9.0
	29	Males	97 ± 0.2	104 ± 10.0 (-5.0%)	<b>110.0 ± 4.0<sup>#</sup></b> <b>(+13.4%)</b>	<b>121.0 ± 7.0<sup>**</sup></b> <b>(+24.7%)</b>
	Pretest	Females	109 ± 8.0	109 ± 10.0	105 ± 5.0	110 ± 8.0
	29	Females	108 ± 7.0	113 ± 5.0 (+4.6%)	117 ± 10.0 (+8.3%)	<b>125 ± 5.0<sup>*</sup></b> <b>(+15.7%)</b>
K <sup>+</sup> (mEq/L)	Pretest	Males	4.8 ± 0.3	4.8 ± 0.1	4.9 ± 0.6	4.7 ± 1.0
	29	Males	5.0 ± 0.4	4.7 ± 0.4 (-6.0%)	4.6 ± 0.2 (-8.0%)	<b>4.3 ± 0.14<sup>*</sup></b> <b>(-1.4%)</b>
Chloride (mEq/L)	Pretest	Males	106 ± 1.0	105 ± 1.0	104 ± 1.0	106 ± 1.0
	29	Males	108 ± 1.0	<b>111 ± 1.0<sup>**</sup></b> <b>(+2.8%)</b>	109 ± 1.0 (+0.92%)	<b>111 ± 1.0<sup>**</sup></b> <b>(+2.8%)</b>
Sodium (mEq/L)	Pretest	Females	146 ± 2.0	144 ± 2.0	144 ± 1.0	144 ± 1.0
	29	Females	147 ± 1.0	<b>151 ± 3.0<sup>*</sup></b> <b>(+2.7%)</b>	147 ± 1.0 NC	148 ± 2.0 (+0.68%)

\*: Statistically significant (at p≤0.05) differences compared to controls  
 \*\*: Statistically significant (at p≤0.01) differences compared to controls  
 #: Significantly different (at p≤0.05) difference compared to immediate release dilaudid treatment group

**Urinalysis:**

*Freshly voided urine was collected while the animals (4 animals/sex/group) were fasting and water deprived (approximately 5 hours) prior to dosing and on study day 29. The following parameters were evaluated:*

Urine Parameters
Urinalysis
Appearance: Color
Bilirubin
Occult Blood (semi-quantitatively)
Glucose
Ketones
Microscopy of sediment
pH
Protein
Specific gravity

There were no statistically significant changes in urine parameters in the hydromorphone-treated animals compared to the vehicle treated animals.

**Gross pathology:**

*Surviving animals (fasted overnight prior to the scheduled sacrifice) were anesthetized with sodium pentobarbital and exsanguinated. Terminal sacrifice was performed on day 31 of the study. The animals were sacrificed in the following order: one animal was selected from group I, group IV, group III and group II; this sequence was repeated until all animals were sacrificed. Macroscopic evaluation was performed. Macroscopic evaluation was performed on all orifices, external surface of the body, cranial cavity, external surfaces of the brain and spinal cord, cervical tissues and organs, tissues of the thoracic, abdominal, and pelvic cavities and neck. Also, the gastrointestinal tract was examined for the presence of tablets or tablets remnants.*

No drug-related macroscopic changes were observed.

**Organ weights:**

*The absolute and relative weights (organ-to-body weight and organ-to-brain weight ratio) of the following organs were measured at necropsy in all terminally sacrificed animals: Paired organs were weighed separately.*

Adrenal glands (2)	Pancreas
Brain (medulla/pons, cerebrum & cerebellum)	Pituitary gland
Heart	Prostrate
Kidneys (2)	Spleen
Liver	Testes/epididymides
Lungs (with main bronchi)	Thyroid/parathyroids
Ovaries (2)	Uterus (body/horns) with cervix

Compared to control, there were no treatment-related effects on absolute or relative organ weight.

**Histopathology:**

*Adequate Battery: yes ( ), no ( x )— While the standard battery of organs were collected and preserved; they were not examined microscopically. Only organs of the gastrointestinal system were examined microscopically.*

*Peer review: yes ( x ), no ( ) Dr. Ward Richter performed the microscopic pathology evaluation of this study. Dr. C. Fred Morris peer-reviewed the small intestine slides.*

*The following tissues were collected from all animals. However, only organs of the gastrointestinal system were examined microscopically. The other tissues collected were preserved and not evaluated.*

	Main Study			
	OROS System			IR Hydromorphone
	0	8.0	64.0	64.0
Dose (mg/kg) →				
Species	Dogs			
<b>Collected - Examined Microscopically</b>				
Cecum	X	X	X	X
Colon	X	X	X	X
Duodenum	X	X	X	X
Esophagus	X	X	X	X
Ileum	X	X	X	X
Jejunum	X	X	X	X
Oral Pharynx	X	X	X	X
Rectum (including anus)	X	X	X	X
Stomach	X	X	X	X
Tissues with macroscopic findings including gross lesions	X	X	X	X
<b>Collected and preserved - Not Examined Microscopically</b>				
Adrenal glands	X	X	X	X
Aorta (abdominal)	X	X	X	X
Bone (sternum/femur with articular surfaces)	X	X	X	X
Bone Marrow (sternum/femur)	X	X	X	X
Brain (medulla/pons, cerebrum & cerebellum)	X	X	X	X
Eyes with optic nerve	X	X	X	X
Gallbladder	X	X	X	X
Heart	X	X	X	X
Kidneys	X	X	X	X
Lacrimal gland	X	X	X	X
Liver	X	X	X	X
Lungs (with mainstem bronchi)	X	X	X	X
Mammary gland	X	X	X	X
Nerve (sciatic)	X	X	X	X
Ovaries	X	X	X	X
Pancreas	X	X	X	X
Pituitary gland	X	X	X	X
Prostrate	X	X	X	X
Salivary gland (submandibular)	X	X	X	X
Skeletal muscle (Biceps femoris)	X	X	X	X
Spinal cord (cervical, thoracic, lumbar)	X	X	X	X
Spleen	X	X	X	X
Testes (with epididymides)	X	X	X	X
Thymic region	X	X	X	X
Thyroid/parathyroid glands	X	X	X	X
Tongue	X	X	X	X
Trachea	X	X	X	X
Urinary bladder	X	X	X	X
Uterus (body/horns) with cervix	X	X	X	X

All tissues were fixed in 10% neutral buffered formalin with the exception of the eyes that were initially placed in glutaraldehyde/paraformaldehyde initially and then retained in 10% formalin. The lungs and urinary bladder were infused with formalin prior to being immersed in a large volume of formalin. Tissue selected for microscopic analysis were processed and stained with hematoxylin and eosin stain and examined by light microscopy.

No treatment-related histological findings were observed.

Other: OROS systems that were excreted in fecal material was collected and analyzed for drug content. Seventeen and nineteen OROS systems were retrieved from the dogs in the low- and high-dose OROS groups, respectively. Males and females in the low-dose group had similar mean delivery amounts of hydromorphone; 76% and 73% in males and females, respectively. In contrast, the mean delivery amount of hydromorphone differed in the males and females in the high-dose group. The mean delivery amounts of hydromorphone were 45% and 66% in the males and females, respectively. The difference in the delivery of hydromorphone may be a reflection of a difference in GI transient time.

**Percentage of Drug Delivered  
8 mg OROS® (hydromorphone HCl)**

Percentage Drug Delivered				
Males				
Animal Number	Day 16	Day 17	Day 18	Mean delivery (%)
2225	a	73	73	73 <sup>b</sup>
2226	a	82	77	80 <sup>b</sup>
2227	a	84	a	84 <sup>c</sup>
2228	a	72	72	72 <sup>b</sup>
Total Mean/Group				76
Females				
Animal Number	Day 16	Day 17	Day 18	Mean Delivery (%)
2725	a	87	66	76 <sup>c</sup>
2726	63	87	76	75
2727	73	54	79	69
2728	a	66	75	71 <sup>b</sup>
Total Mean/Group				73

<sup>a</sup> No OROS® system found in feces  
<sup>b</sup> n = 2  
<sup>c</sup> n = 1

**Percentage of Drug Delivered  
64 mg OROS® (hydromorphone HCl)**

Percentage Drug Delivered				
Males				
Animal Number	Day 16	Day 17	Day 18	Mean delivery (%)
3225	83	59	52	65
3226	38	24	28	30
3227	80	47	a	64 <sup>b</sup>
3228	34	28	23	28
Total Mean/Group				45

Females				
Animal Number	Day 16	Day 17	Day 18	Mean Delivery (%)
3725	a	100	a	100
3726	46	68	a	57 <sup>b</sup>
3727	90	74	a	82 <sup>b</sup>
3728	61	37	50	49
Total Mean/Group				66

\* No OROS® system found in feces  
<sup>b</sup>n = 2  
<sup>c</sup>n = 1

**Study title: BSF- Repeated dose toxicity (at least 39-week treatment) after oral administration (Capsules) in the beagle.**

**Key study findings:** BSF 138315 [hydromorphone hydrochloride] (0.0, 1.75, 4.0, and 9.0 mg/kg/day) was orally administered to dogs for 39 weeks with the following results:

1. BSF 138315 (hydromorphone hydrochloride) was rapidly absorbed. Mean peak plasma level was generally obtained between 1.9 hours and 3.3 hours after dosing. Increase in the exposure was noted with increasing doses. There were no signs of accumulation upon repeat dosing; but there was evidence of slightly higher exposure in the females than in the males.
2. Treatment-related clinical signs include sedation, tremors, ventral recumbency, lateral position, vomiting and weak hind limbs.
3. Statistically significant alterations of uncertain toxicological significance were observed in some of the hematology and clinical chemistry parameters in both male and female dogs.
4. Post-mortem examination revealed increased relative pituitary gland weight in males at  $\geq 4.0$  mg/kg/day. The observed increased relative pituitary gland weight was not correlated with any microscopic changes. The change in

relative pituitary gland weight was reversible. Organ weight for females in all treatment groups was comparable to the control animals.

5. No treatment-related microscopic or macroscopic findings were observed.
6. The NOAEL was identified by the Applicant and to which this reviewer is in agreement, as 9.0 mg/kg/day (HED = 4.9 mg/kg). This corresponds to AUC value of 230 and 346 ng\*h/mL in males and females, respectively, during week 39. C<sub>max</sub> values at this dose during week 39 were 30.6 (males) and 58 (females) ng/mL.

**Study no.:** MPF/DT 9901  
**Volume #, and page #:** eCTD submission  
**Conducting laboratory and location:** Knoll AG Research and Development, 67061 Ludwigshafen Germany  
**Date of study initiation:** February 16, 1999  
**GLP compliance:** Yes  
**QA report:** Yes ( X ) No ( )  
**Drug, lot #, and % purity:** Hydromorphone, Batch № L0002652, 99.2% purity

**Methods**

Doses: 0, 1.75, 4.0 and 9.0 mg active moiety/kg/day  
 Doses were selected based on the clinical and pharmacokinetic results from a 4-week toxicity study in beagle (Knoll Study Report № 9806), a 2-week dose range-finding study (Knoll Study Report № 1198) and on the results of a 7-day preliminary toxicity study (Knoll Study Report № 3997)  
 Species/strain: Dog/Beagle  
 Number/sex/group or time point (main study): 4/sex/group  
 Route, formulation, volume, and infusion rate: Oral, hard gelatin capsules

Group	Test Article	Dosage Level (mg active moiety/kg/day)	Dosage Level (mg hydrochloride/kg)	Total Number of Animals		Sacrificed after treatment period		Sacrificed after 8-wk recovery period	
				Females	Males	F	M	F	M
<b>Main Study</b>									
<b>1</b>	BSF 138315	0	0	7	7	4	4	3	3
<b>2</b>	BSF 138315	1.75	1.96	7	7	4	4	3	3
<b>3</b>	BSF 138315	4.0	4.48	7	7	4	4	3	3
<b>4</b>	BSF 138315	9.0	10.08	7	7	4	4	3	3

Satellite groups used for toxicokinetics or recovery: Satellite group were included for toxicokinetic analysis; separate animals were included for recovery groups in this study.

Age: 8.5 to 9.5 months  
 Weight:

Females: 7.7 kg (6.5 kg – 8.8 kg) at pre-treatment  
Males: 8.9 kg (7.7 kg – 9.9 kg) at pre-treatment  
Sampling times:  
Unique study design or methodology (if any): None

**Observations and times: (these parameters can be captured separately here or described in connection with each endpoint under the results section.)**

The observation parameters were captured under the results section.

## Results

### Toxicokinetics:

*Blood samples (1 – 3 mL) were collected via puncture of the antebrachial cephalic vein from all animals/sex/group (including control animals) for toxicokinetic evaluation at pre-dose, 3, 6, 10 and 24 hours after dosing in weeks 4, 11, 25 and 39. During week 4, blood was not collected (24-hours post-dosing) from males in groups 2, 3 and 4 due to severe clinical signs observed in the animals. Also, it was not possible to collect blood at 1 hour post-dosing in 7 males in group 2 (№ 1008 and 1012), group 3 (№ 1017 and 1018) and group 4 (№ 1025, 1026 and 1028).*

*Deviation from the protocol. As a result of these problems in the male groups, blood sampling was discontinued in both the males and females during week 4. Thus, blood determination of plasma levels and toxicokinetic evaluation was only assessed in weeks 11, 25 and 39.*

Toxicokinetic analysis is presented in the table below. BSF 138315 (hydromorphone hydrochloride) was rapidly absorbed. Mean peak plasma level were generally obtained between 1.9 hours and 3.3 hours after dosing. Systemic exposure was apparent at all dose levels. Exposure increased in a dose-dependent manner but not quite dose-proportionally (variable). A 2-fold increase in dose (1.75 - 4.0 mg/kg/day) resulted in a 1-fold and 2.7-fold increase in  $C_{max}$  on repeat dosing in males (10.2 vs 12 ng/mL) and females (7.4 vs 19.8 ng/mL), respectively, during week 11. During week 25 and 39, a 2-fold increase in dose (1.75-4.0 mg/kg/day or 4.0-9.0 mg/kg/day) resulted in an increase in  $C_{max}$  in a dose proportional manner in both males and females. A 5-fold increase in dose (1.75 - 9.0 mg/kg/day) resulted in a 3-fold and 4-fold increase in  $C_{max}$  in males during week 11 (10.2 vs 35.4 ng/mL) and 39 (8.1 vs 30.6 ng/mL), respectively. In females, a 5-fold increase in dose (1.75 vs 9.0 mg/kg/day) resulted in a 7-, 4.4- and 4.5-fold increase  $C_{max}$  during weeks 11 (7.4 vs 49.0 ng/mL), 25 (9.2 vs 40.7 ng/mL) and 39 (12.9 vs 58 ng/mL), respectively.

Between LD and MD, change in BSF 138315 mean  $AUC_{(0-24)}$  levels increased in an approximate dose proportional manner for males and females during weeks 11 and 39 and weeks 25 and 39, respectively. During week 11 and week 25, a 2-fold increase in

dose (between LD and MD), AUC<sub>(0-24)</sub> levels increased in a greater than dose-proportional manner in males and females, respectively.

Between MD and HD (2-fold increase in dose), change in BSF 138315 mean AUC<sub>(0-24)</sub> levels increased in a slightly greater than dose proportional manner in both males and females during weeks 11 and 39. During week 11, a 2.7-fold (81.4 vs 223 ng\*h/mL) and 2.8-fold (126 vs 332 ng\*h/mL) increase in AUC<sub>(0-24)</sub> was noted in males, respectively. During week 25, AUC<sub>(0-24)</sub> increased in slightly greater than dose-proportional manner and dose-proportional manner between MD and HD in females and in males, respectively. A 3-fold increase (71.5 vs 184 ng\*h/mL) and a dose-proportional increase (110 vs 230 ng\*h/mL) in AUC<sub>(0-24)</sub> was noted in males and females, respectively. In females and males, a 2-fold increase in dose resulted in a 3.1-fold increase (110 vs 346 ng\*h/mL) and 2.6-fold (88.4 vs 230 ng\*h/mL), respectively, during week 39. Female rats were generally exposed to slightly greater levels of BSF 138315. Also, the data indicate little reduction in exposure to BSF 138315 with repeated dosing over 39 weeks.

**Table 1. Toxicokinetic parameters for hydromorphone during weeks 11, 25 and 39 following oral administration of daily oral doses of BSF 138315 in dogs.**

Mean ± SD value for hydromorphone *free base)					
Study Week	Dose (mg/kg/day)	Sex	T <sub>max</sub> (hr)	C <sub>max</sub> (ng/mL)	AUC (ng*h/mL)
11	1.75	M	2.3 ± 3.4	10.2 ± 4.8	48.2 ± 9.7
	1.75	F	2.9 ± 1.7	7.4 ± 2.9	46.5 ± 14.5
	1.75	Combined	2.6 ± 2.6	8.8 ± 4.1	47.4 ± 11.9
	4.0	M	1.6 ± 1.0	12.0 ± 4.9	81.4 ± 34.4
	4.0	F	3.1 ± 1.5	19.8 ± 12.6	126 ± 47.0
	4.0	Combined	2.4 ± 1.4	15.9 ± 10.1	104 ± 43.5
	9.0	M	3.3 ± 2.3 <sup>a</sup>	35.4 ± 19.3 <sup>a</sup>	223 ± 47.0 <sup>a</sup>
	9.0	F	2.1 ± 1.1	49.0 ± 16.8	332 ± 86.2
9.0	Combined	2.7 ± 1.8 <sup>b</sup>	42.8 ± 18.6 <sup>b</sup>	282 ± 88.3 <sup>b</sup>	
25	1.75	M	2.6 ± 1.8	6.8 ± 1.7	27.6 ± 12.3
	1.75	F	2.3 ± 1.9	9.2 ± 3.3	47.4 ± 12.8
	1.75	Combined	2.4 ± 1.8	8.0 ± 2.8	37.5 ± 15.9
	4.0	M	1.6 ± 1.0	13.3 ± 7.3	71.5 ± 24.3
	4.0	F	2.7 ± 2.4	21.3 ± 12.9	110 ± 34.8
	4.0	Combined	2.1 ± 1.8	17.3 ± 10.9	90.5 ± 34.9
	9.0	M	3.7 ± 2.3	34.2 ± 42.3	184 ± 65.4
	9.0	F	1.9 ± 1.1	40.7 ± 8.7	230 ± 42.5
9.0	Combined	2.8 ± 2.0	37.4 ± 29.6	207 ± 58.0	
	1.75	M	1.9 ± 1.1	8.1 ± 2.6	37.2 ± 13.4
	1.75	F	1.9 ± 1.1	12.9 ± 4.6	66.7 ± 32.7
	1.75	Combined	1.9 ± 1.0	10.5 ± 4.4	51.9 ± 28.5
	4.0	M	1.6 ± 1.0	19.0 ± 10.3	88.4 ± 30.4

Mean $\pm$ SD value for hydromorphone *free base)					
Study Week	Dose (mg/kg/day)	Sex	T <sub>max</sub> (hr)	C <sub>max</sub> (ng/mL)	AUC (ng*h/mL)
Week 39	4.0	F	3.0 $\pm$ 0	20.3 $\pm$ 3.9	110 $\pm$ 10.6
	4.0	Combined	2.3 $\pm$ 1.0	19.6 $\pm$ 7.5	99.1 $\pm$ 24.5
	9.0	M	3.9 $\pm$ 1.5	30.6 $\pm$ 8.7	230 $\pm$ 68.8
	9.0	F	2.7 $\pm$ 0.8	58.0 $\pm$ 17.7	346 $\pm$ 57.4
	9.0	Combined	3.3 $\pm$ 1.3	44.3 $\pm$ 19.5	288 $\pm$ 85.7
	a: n = 6 and for the combined males and females are mean $\pm$ SD (n=14) b: n = 13				

**Mortality:**

*Assessed prior to dosing, and at several time intervals post-dosing.*

No treatment-related deaths occurred during the course of the study.

**Clinical signs:**

*The behavior and general health of the animals were assessed prior to dosing and several times after dosing (depending on the findings). The behavior and general health of the recovery animals were examined once daily during the dosing period.*

*Deviation from the protocol. The status and reflex of the pupils were assessed in all dogs on day 16 (prior to dosing and 3-hrs post-dosing) and in weeks 5 and 13 (3-hrs post-dosing).*

As depicted in the Applicant's table below, apparent test article-related clinical observations included, sedation, foamy salivation, ventral recumbency, lateral position, weakness of hindlimbs, vomiting, diarrhea and tremor. The following clinical signs persisted throughout the dosing period: sedation, foamy salivation, and ventral recumbency. Sedation and foamy salivation were observed in all treatment groups throughout the 39-weeks treatment period. Occurrence of ventral recumbency in both females and males was dose-dependent and was observed throughout the treatment period in the mid- and high-dose groups.

While lateral position, weakness of hindlimbs, vomiting, diarrhea and tremor also persisted throughout most of the dosing period in all treatment groups; however, the highest incidence of these clinical signs were noted during the first week of dosing. Lateral position was observed in both males and females in a dose-dependent manner. The occurrence of weak hindlimbs, diarrhea, and vomiting were not dose-dependent. Tremors were observed in both males and females with the highest incidence noted in the first week of dosing. In females, the incidence of tremors was dose-dependent; whereas in males, the incidence of tremors was higher in the mid- and high-dose groups. Incoordinate gait, imbalance and tachypnoea were other treatment-related clinical signs

noted. These observed clinical signs were transient; noted during the initial treatment days only.

**Table 8 Clinical signs (selected data; for complete data see appendix 1, pp 1 – 148)**

Clinical sign	1.75 mg/kg BW				4.0 mg/kg BW				9.0 mg/kg BW			
	Male		Female		Male		Female		Male		Female	
	Week	Score	Week	Score	Week	Score	Week	Score	Week	Score	Week	Score
<b>PERSISTENT, NO SIGNIFICANT CHANGES IN INCIDENCE DURING TREATMENT</b>												
Sedated	1-39	A	1-39	A	1-39	A	1-39	A	1-39	A	1-39	A
Foamy salivation	1-39	A	1-39	A	2-39	A	1-39	A	1-39	A	2-39	A
Incompl. food cons.	1-39	A	1-39	A	1-39	A	1-39	A	1-39	A	1-39	A
Ventral recumbency	1-24	1	1-37	3	1-39	4	1-39	6	1-39	6	1-39	9
Clinical sign	1.75 mg/kg BW				4.0 mg/kg BW				9.0 mg/kg BW			
	Male		Female		Male		Female		Male		Female	
	Week	Score	Week	Score	Week	Score	Week	Score	Week	Score	Week	Score
<b>PERSISTENT, HIGHEST INCIDENCE IN INITIAL TREATMENT WEEK(S)</b>												
Vomiting	1-34	7	1-36	6	1-38	7	1-39	7	1-38	9	1-39	7
Weak hind limbs	1-30	6	1-33	7	1-39	6	1-39	9	1-39	7	1-39	9
Lateral position	1-31	1	1-27	3	1-39	3	1-37	3	1-39	6	1-37	9
Diarrhoea	2-39	1	3-30	1	1-39	4	1-26	6	2-39	4	1-30	4
Tremor	1	1	1	1	1-39	4	1-39	3	1-39	3	1-39	9
Clinical sign	1.75 mg/kg BW				4.0 mg/kg BW				9.0 mg/kg BW			
	Male		Female		Male		Female		Male		Female	
	Week	Score	Week	Score	Week	Score	Week	Score	Week	Score	Week	Score
<b>TRANSIENT, IN INITIAL TREATMENT DAYS ONLY</b>												
Incoordinate gait	1	4	1	1	1	6	1-2	4	1	3	1	4
Abnormal behaviour	-	-	-	-	-	-	-	-	1	1	-	-
Imbalance	1	1	1	1	1	1	1	3	1	1	1	4
Tachypnoea	1	1	-	-	1	3	1	3	1	1	1	3
Vomit with blood trace	-	-	-	-	-	-	2	1	2	1	-	-
Diarrhoea with blood trace	-	-	-	-	1	1	2	1	1	1	1-2	1
Clinical sign	1.75 mg/kg BW				4.0 mg/kg BW				9.0 mg/kg BW			
	Male		Female		Male		Female		Male		Female	
	Week	Score	Week	Score	Week	Score	Week	Score	Week	Score	Week	Score
<b>TRANSIENT, OCCASIONAL</b>												
Vomit (white mucus)	-	-	-	-	-	-	-	-	34	1	-	-
Aggressive	-	-	-	-	-	-	-	-	11-16	1	-	-
Abnormal posture	-	-	-	-	-	-	-	-	-	-	28	1

Week = Treatment interval during which clinical sign was observed  
 Score = Maximum clinical score corresponding to % of affected animals as follows: 1 = between 5% and 15%, ...etc.; A = more than 95%)

**Body weights:**

*Body weights were recorded on the day prior to initiation of treatment and twice weekly thereafter. Also, body weight was measured on the day of dissection.*

Statistically significant decreases in body weight were noted between BS 138315-treated groups and control during the study. Body weights were significantly decreased (9% - 16%) for male dogs at 9 mg/kg/day beginning on study day 3 (week 1) and continued

through study day 143 (week 21). Body weights were also significantly decreased in male dogs in the 4.0 mg/kg/day but only on study days 7, 10 and 17. Bodyweight by the end of the study was not affected by BSF 138315 when compared with body weight of vehicle treated animals. However, during the recovery period, the body weight was statistically significantly decreased in male dogs in all treatment groups from recovery week 1 (study day 7) thru week 8.

No treatment-related effects on body weight were observed in the female dogs. Body weight gain was unaffected by treatment, except for a statistically significant decrease on study day 10 (↓15%).

**Food consumption:**

*Food consumption was recorded daily.*

The Applicant stated that food consumption was completed but did not provide any data on food consumption.

**Ophthalmoscopy:**

*Eyes of all animals were examined before the first dose (week 0), treatment weeks 10, 24 and 38. During the examination of the eye, the following ocular structures were examined: cornea, iris, lens, vitreous body, and ocular fundus (photographic documentation). All recovery animals were examined in week 8 of the recovery period.*

*Deviation from the protocol. Exemplary photo documentation of cornea lesions in animal № 1018, 1020, 1042 and 1045 was done in week 1 of the recovery period.*

No treatment-related findings were observed for male and female dogs.

Although several ophthalmologic findings (tabulated below), such as unilateral lesions of the cornea and punctuate opacities in the corneal stroma opacities were observed, none appeared to be test article related as they were found in control groups and/or did not demonstrate dose-dependent increases in incidence or severity.

Dose	Gender	Animal №	Ophthalmologic finding (s)	Treatment Week Occurrence
<b>Treatment Period</b>				
0	M	1004	Lesions of the left cornea (fluoresceine positive)	10
	F	1032	Unilateral lesions of the cornea (fluoresceine positive)	10
1.75	F	1037	Unilateral lesions of the cornea (fluoresceine positive)	10
			Pinhead opacity in the corneal stroma (right posterior subcapsular)	10, 24 and 37
4.0	F	1043	Single Pinhead opacity in the left corneal stroma	10, 24 and 37

Dose	Gender	Animal №	Ophthalmologic finding (s)	Treatment Week Occurrence
			Single Pinhead opacity in the right corneal stroma	37
<b>Recovery Phase</b>				
1.75	F	1042	Unilateral punctuate opacities	
4.0	M	1018	Unilateral punctuate opacities	
		1020	Unilateral punctuate opacities	

**EKG:**

*Electrocardiography recordings were performed before initiation of treatment, and during treatment weeks 6, 12, 26 and 37 prior to dosing and 3 hours after dosing. EKG was also performed on the recovery animals in week 8 of the recovery period. The standard bipolar limb leads (I, II and III), augmented leads aVR, aVL and aVF were recorded. Heart rate, PQ, QRS and QT intervals were measured.*

There were no statistically significant differences in EKG parameters in the BSF 138315 treated animals compared to the vehicle treated animals.

Compared to the vehicle treated dogs, statistically significant decrease in mean arterial blood pressure (MAP) was observed 3-hours after dosing during weeks 6 and 37 in the mid- and high-dose group males and mid-dose males during week 12. The Applicant stated that these changes were within physiological range of variation. The reviewer concurs with the Applicant and does not consider these differences to be of biological or toxicological significant. There were no statistically significant differences in MAP in the BSF 138315 treated females compared to the vehicle control females.

As depicted in the Applicant's table below, lower heart rates were measured in the females in the 4.0 and 9.0 mg/kg/day during weeks 12 and 37. During week 6, heart rate was moderately higher in females in the mid- and high-dose groups. These changes were within normal physiological range; thus are not considered to be toxicologically relevant.

**Mean heart rate data in male and female dogs administered BSF 138315 for 39 consecutive weeks.**

Weeks	Control		1.75 mg/kg BW			4.0 mg/kg BW			9.0 mg/kg BW		
	a.a	3 h p.a.	a.a	3 h p.a.	a.a.	3 h p.a.	a.a.	3 h p.a.	a.a	3 h p.a.	
<b>Males</b>											
0	101	-	97	-	96	-	93	-			
6	87	89 +2	108	92 -16	106	91 -15	124*	87 -37			
12	100	89 -11	99	87 -12	96	89 -7	109	90 -19			
26	97	92 -5	101	83 -18	91	81 -10	102	88 -14			
37	86	85 -1	98	79 -19	91	75 -16	94	92 -2			
Recovery week 8	115	-	85	-	91	-	89	-			
<b>Females</b>											
0	110	-	100	-	91	-	105	-			
6	91	89 -2	115*	119 +4	90	113 +23	93	126* +33			
12	96	95 -1	100	107 +7	103	80 -23	119	91 -28			
26	99	96 -3	100	99 -1	90	92 +2	105	90 -15			
37	91	85 -6	105	103 -2	97	78 -19	114	78 -36			
Recovery week 8	91	-	103	-	79	-	77	-			

+/- = Deviation from a.a. value indicated in right column of each dose group; \* = p ≤ 0.05 (Dunnett's test)

**Hematology:**

Blood was collected from the antebrachial cephalic vein in treatment weeks 8, 18, 28 and 38. Blood was collected week 8 of the recovery period from all the recovery animals. The following parameters were examined:

<b>Hematology Parameters</b>	
<b>White Blood Cell Parameters</b>	<b>Red Blood Cell Parameters</b>
Leukocyte count (WBC)	Erythrocyte count (RBC)
White Blood Cells	
Differential leukocyte count (Absolute)	Red Cell Morphology
- Juvenile Neutrophil (NEUT JU)	- Normal
- Band Neutrophil (NEU BA)	- Abnormal
- Segmented Neutrophils (NEUTR SE)	- Normoblasts (NORMOBL)
- Monocyte (MONO)	- Anisocytosis (ANS)CYT)
- Lymphocyte (LYM)	- Polychromasia (POLYCHR)
- Eosinophil (EOS)	
- Basophil (BASO)	
- Atypical Cells (ATYP CE)	
Leukocyte count (WBC)	Hemoglobin (HGB)
White Blood Cells	Hematocrit (HCT)
	Mean corpuscular volume (MCV)
	Mean corpuscular hemoglobin (MCH)
	Mean corpuscular hemoglobin concentration (MCHC)
	Reticulocyte Count (RETI)
	Reticulocyte-high fluorescence ratio (HFR)

Hematology Parameters	
White Blood Cell Parameters	Red Blood Cell Parameters
	reticulocyte-middle fluorescence ratio (MFR)
	reticulocyte-low fluorescence ratio (LFR)
	Reticulocytes (RET; % of the erythrocytes)
	Platelet Count (PLT)

**Coagulation:** Blood was collected from the antebrachial cephalic vein in treatment weeks 8, 18, 28 and 38. Blood was collected week 8 of the recovery period from all the recovery animals. The following parameters were examined:

Coagulation Parameters
Thromboplastin Time (TPT)
Prothrombin Time (PT)

The results indicated some significant changes in hematology parameters when examined during weeks 8, 18, 28, and 38. Significant changes noted are summarized in the table below. A 59% and 42% increase in platelet counts were observed in females in the 9.0 mg/kg/day group during week 8 and 28, respectively, but not in males. A significant increase (+72%) in reticulocytes (MFR) count in female dogs treated with 9.0 mg/kg/day was also noted during week 8. During weeks 18 and 28, the monocytes count was significantly higher (+60%) in females and males (+60%), respectively, in the high-dose group compared to controls. Some minor changes in hematology parameters were observed in male dogs. None of these changes should have biological significance. Minimal but statistically significant changes from control mean were observed in the coagulation parameters partial thromboplastin time (PTT) and thromboplastin time. None of these changes should have biological significance.

Parameter	Study Week	Percent Change in Hematology					
		Males (mg/kg)			Females (mg/kg)		
		1.75	4.0	9.0	1.75	4.0	9.0
Platelets (giga/L)	8	NSC	NSC	NSC	NSC	NSC	↑59%*
	28	NSC	NSC	NSC	NSC	NSC	↑42%*
Reti_MFR (%)	8	NSC	NSC	NSC	NSC	NSC	↑72% <sup>+</sup>
Reti_LFR (%)	8	NSC	NSC	NSC	NSC	NSC	↓10% <sup>+</sup>
<b>Differential White Blood Cell Count</b>							
Lymphocytes (%)	18	NSC	NSC	NSC	NSC	NSC	↓20% <sup>+</sup>
Monocytes (%)	18	NSC	NSC	NSC	NSC	NSC	↑60% <sup>+</sup>
	28	NSC	NSC	↑60% <sup>+</sup>	NSC	NSC	NSC
Segmented Neutrophil (%)	38	NSC	NSC	↑16% <sup>+</sup>	NSC	NSC	NSC
MCHC (mmol/L)	28	NSC	NSC	NSC	↓3%*	NSC	↓2%*
	38	↑2%	NSC	NSC	NSC	NSC	NSC
MCV (fL)	8	↓5%*	NSC	NSC	NSC	NSC	NSC
	18	↓4%*	NSC	NSC	NSC	NSC	NSC
	38	↓4%	NSC	NSC	NSC	NSC	NSC
MCH (fmol)	8	↓4%*	NSC	NSC	NSC	NSC	NSC
RBC (tera/L)	38	↑10%	NSC	NSC	NSC	NSC	NSC
<b>Coagulation Parameters</b>							

Parameter	Study Week	Percent Change in Hematology					
		Males (mg/kg)			Females (mg/kg)		
		1.75	4.0	9.0	1.75	4.0	9.0
PTT (sec)	28	↑6%	NSC	NSC	NSC	NSC	NSC
Thromboplastin time (sec)	18	NSC	NSC	NSC	NSC	↑10%*	NSC
	28	↑13%*	↑7%*	NSC	NSC	NSC	NSC
<b>Recovery Period</b>							
RBC (tera/L)	8	NSC	NSC	↓15%*	NSC	NSC	NSC
HCT (l/L)	8	NSC	NSC	↓13%*	NSC	NSC	NSC
HGB (mmol/L)	8	NSC	NSC	↓15%*	NSC	NSC	NSC
MCH (fmol)	8	NSC	NSC	↓6%*	NSC	NSC	NSC
PTT (sec)	8	NSC	NSC	NSC	↓8%*	↓6%*	↓7%**
*: Significant different from control value (Dunnett-test based on pooled variance sign.), $p \leq 0.05$ +: Significant different from control value (Steel-test), $p \leq 0.05$ NSC: No statistically significant change LFR: Low fluorescence ratio MFR: Middle fluorescence ratio HFR: High fluorescence ratio							

**Clinical chemistry:**

*Blood was collected from the cephalic vein in treatment weeks 8, 18, 28 and 38. Blood was collected during week 8 of the recovery period from all the recovery animals. The following parameters were measured:*

Serum Chemistry Parameters	
Alanine aminotransferase (ALT)	Inorganic Phosphate
Albumin (ALB)	Potassium (K)
Aspartate aminotransferase (AST/GOT)	Sodium (Na)
Albumin/globulin ratio (AGR)	Total bilirubin (TBIL)
Alkaline phosphatase (ALP)	Cholesterol (CHOL)
Calcium (Ca)	Urea (BUN)
Chloride (Cl)	Total Protein (TP)
Cholinesterase	
Creatinine (CREA)	
Glucose (GLU)	
Globulin (GLOB)	

Significant changes noted at weeks 8, 18, 28 and 38 blood draws are summarized in the table below. Male and female dogs demonstrated a significant ( $p \leq 0.05$ ) decrease in AST levels. Male dogs in the high-dose group exhibited a decrease in AST levels during all blood draw weeks. Partial recovery was apparent after an 8-week recovery period; compared to control group, a 10% decrease in AST values was noted. In females, AST levels were significantly ( $p \leq 0.05$ ) lower than controls during weeks 8, 28 and 38. The lower serum AST level is possibly a result of nutritional imbalance; food intake was reported to be low during the treatment period. After the 8-week recovery period, the AST level was comparable to the control females. During week 28, male dogs in all treatment-groups exhibited a significant increase ( $p \leq 0.05$ ) in bilirubin levels. Only mid-dose females exhibited a significant ( $p \leq 0.05$ ) decrease (30%) in serum bilirubin levels. Bilirubin levels were comparable to controls after recovery period.

BSF 138315-treated males and females dogs exhibited significantly ( $p \leq 0.05$ ) higher glucose values. Glucose level was increased dose-dependently in all males groups during week 38 (max  $\uparrow 37\%$ ). During weeks 18 and 28, compared to control, male dogs in the high-dose group glucose level was significantly higher by 24% and 39%, respectively. Females in all treatment groups demonstrated a significant increase in glucose levels during week 38. During weeks 8 and 18, mid- and high-dose serum glucose level was significantly higher than the control females.

$\gamma$ -Globulin levels were significantly ( $p \leq 0.05$ ) decreased in BSF 138315-treated females and males.  $\gamma$ -Globulin levels were reduced in the mid- and high-dose males during weeks 8, 28 and 38. Females demonstrated significant decrease at all dose levels during week 28. All other alterations of statistical significance involving blood chemistry parameters were not dose-related or only seen in one gender. Changes in  $\gamma$ -Globulin were reversible at the end of the recovery period.

Parameter	Study Week	Percent from control Change in Clinical Chemistry					
		Males (mg/kg)			Female (mg/kg)		
		1.75	4	9	1.75	4	9
AST	8	–	–	$\downarrow 22\%^*$	–	$\downarrow 28\%^*$	$\downarrow 31\%^*$
	18	–	–	$\downarrow 21\%^*$	–	$\downarrow 25\%^*$	–
	28	–	–	$\downarrow 23\%^*$	–	$\downarrow 30\%^*$	$\downarrow 29\%^*$
	38	–	–	$\downarrow 25\%^*$	$\downarrow 25\%^*$	$\downarrow 31\%^*$	$\downarrow 28\%^*$
	Recovery (Wk 8)	–	–	$\downarrow 10\%$	–	–	–
Bilirubin	8	–	$\uparrow 83\%^*$	$\uparrow 49\%^*$	–	–	–
	18	–	–	–	–	$\downarrow 30\%^*$	–
	28	$\uparrow 161\%^*$	$\uparrow 181\%^*$	$\uparrow 130\%^*$	–	–	–
	38	–	–	–	–	–	–
	Recovery (Wk 8)	–	–	–	–	–	–
Calcium	8	–	–	$\downarrow 5\%^*$	–	–	$\downarrow 4\%^*$
	18	–	–	–	–	–	–
	28	–	–	–	–	–	–
	38	–	–	$\downarrow 5\%^*$	–	–	–
	Recovery (Wk 8)	–	–	–	$\uparrow 5\%^*$	–	–
Chloride	8	–	$\downarrow 3\%^*$	–	–	$\uparrow 4\%^*$	–
	18	–	–	–	$\uparrow 3\%^*$	–	$\uparrow 3\%^*$
	28	–	–	–	$\downarrow 5\%^*$	$\downarrow 5\%^*$	$\downarrow 5\%^*$
	38	–	$\uparrow 3\%^*$	$\uparrow 3\%^*$	–	–	–
	Recovery (Wk 8)	$\uparrow 4\%^*$	–	–	–	–	–
Cholesterol	8	–	–	–	–	–	–
	18	$\downarrow 18\%^*$	–	–	–	$\downarrow 31\%^*$	–
	28	$\downarrow 19\%^*$	–	$\downarrow 18\%^*$	–	–	–
	38	$\downarrow 20\%^*$	–	–	–	–	–
	Recovery (Wk 8)	–	–	–	–	–	–
Glucose	8	–	–	–	–	$\uparrow 27\%^*$	$\uparrow 17\%^*$
	18	–	–	$\uparrow 24\%^*$	–	$\uparrow 32\%^*$	$\uparrow 28\%^*$
	28	–	–	$\uparrow 39\%^*$	–	–	–
	38	$\uparrow 19\%^*$	$\uparrow 23\%^*$	$\uparrow 37\%^*$	$\uparrow 16\%^*$	$\uparrow 24\%^*$	$\uparrow 18\%^*$
	Recovery (Wk 8)	$\downarrow 3\%^*$	$\uparrow 8\%^*$	$\uparrow 19\%^*$	–	–	–
$A_1$ -Globulin	8	–	$\downarrow 19\%^*$	$\downarrow 19\%^*$	–	–	–
	18	$\downarrow 16\%^*$	$\downarrow 16\%^*$	$\downarrow 12\%^*$	–	–	–
	28	–	–	–	–	–	–
	38	–	–	$\downarrow 12\%^*$	–	–	–
	Recovery (Wk 8)	–	–	–	–	–	–
$\Gamma$ -Globulins	8	–	$\downarrow 20\%^*$	$\downarrow 24\%^*$	–	–	–
	18	–	–	–	–	–	–

Parameter	Study Week	Percent from control Change in Clinical Chemistry					
		Males (mg/kg)			Female (mg/kg)		
		1.75	4	9	1.75	4	9
	28	–	↓16%*	↓18%*	↓19%*	↓22%*	↓19%*
	38	–	↓20%*	↓20%*	–	–	–
	Recovery (Wk 8)	–	–	–	–	–	–

\*: Significant different from control value (Dunnett-test based on pooled variance sign.), p ≤ 0.05  
 -: No statistically significant change

Urinalysis:

Urine was collected from all animals during treatment weeks 8, 18, 28 and 38. Urine was collected from all recovery animals during week 8 of the recovery period. The following parameters were evaluated:

Urine Parameters
Urinalysis
Appearance: Color and transparency
Bilirubin
Erythrocytes/hemoglobin
Glucose
Ketones
Microscopy of sediment
Nitrite
pH
Protein
Specific gravity
Urobilinogen
Total volume
White blood cells

No treatment-related alterations in urinalysis parameters were observed with analysis of urine or urine sediment in male dogs orally treated with BSF 138315 for 39 weeks. However, in females, significant changes in urine parameters were noted. Hemoglobin values in the high-dose female group differed from the control group with statistical significance at Week 18. Specific gravity in the high-dose female group differed from the control group with statistical significant (+2%) at Week 8. These changes are considered to be incidental and should have biological significant.

**Gross pathology:**

*Surviving and moribund animals were sacrificed by exsanguination under sodium pentobarbital (Narcoren<sup>®</sup>, 120 mg/kg) anesthesia. Macroscopic evaluation was performed.*

Sporadic or isolated changes were noted in some animals; however there did not appear to be a clear pattern suggesting macroscopic pathology associated with test article (see table below).

Macroscopic Findings								
Organ System	Males (n = 4/group)				Females (n = 4/group)			
	0	1.75	4.0	9.0	0	1.75	4.0	9.0
<b>Terminal Sacrifice</b>								
<b>Cardiovascular</b>								
Heart: Tricuspid Valve Thickening	0	1	1	0	4	0	0	0
Heart: Mitral Valve-Single Hemorrhage	0	0	1	0	0	0	0	0
<b>Gastrointestinal System</b>								
Gastric Mucosa: Yellow discoloration	0	0	1	0	0	0	1	0
Gastric Mucosa: focal reddening	0	0	1	0	0	0	0	0
Jejunum: Focal reddening of the mucosa	0	1	1	1	0	1	1	2
Ileum: Focal hemorrhaging in the mucosa	0	1	0	0	0	0	0	0
Ileum: Moderate reddening of the mucosa	0	0	0	1	0	1	0	0
Pancreas: Single pale focus	0	0	1	0	0	0	0	0
Rectum: Grayish foci in the mucosa	0	1	1	1	0	0	1	0
Gallbladder: Whitish foci in the mucosa	0	0	1	0	0	0	0	0
<b>Endocrine</b>								
Adrenal Gland (left):	0	1	0	0	0	0	0	0
Kidneys: Cortical yellowish discoloration	0	0	0	0	0	0	1	0
<b>Reproductive</b>								
Prostate: Enlarged	0	1	0	0	-	-	-	-
Uterus: Contained opaque fluid	-	-	-	-	0	0	0	1
Uterus: Single cyst	-	-	-	-	0	0	0	1
<b>Lymphatic System</b>								
Lymph Node (mediastinal): Enlarged	0	0	0	0	0	1	0	0
Bone Marrow: Pale and discoloration	0	0	0	1	0	0	0	0
<b>Recovery Sacrifice</b>								
	Males (n=3)				Females (n=3)			
	0	1.75	4.0	9.0	0	1.75	4.0	9.0
<b>Cardiovascular</b>								
Heart: Tricuspid Valve Thickening	0	0	1	0	0	0	0	0
<b>CNS</b>								
Pituitary Gland: Diminished sizes	0	1	0	1	0	0	0	1
<b>Gastrointestinal System</b>								
Gastric Mucosa: Yellowish discoloration & focal reddening	0	0	0	0	0	0	1	0
Gastric Mucosa: Grayish-white discoloration	0	0	0	0	0	0	0	1

Macroscopic Findings								
Organ System	Males (n = 4/group)				Females (n = 4/group)			
	0	1.75	4.0	9.0	0	1.75	4.0	9.0
Colon: Grayish foci in the mucosa	0	0	0	1	0	0	0	0
Colon: Brown to black discoloration in the mucosa	0	0	0	0	0	0	0	1
Jejunum: Focal reddening of the mucosa	0	1	0	0	0	0	0	2
Jejunum: Raised surface of Peyer's patches	0	0	0	0	0	1	1	0
Ileum: Focal hemorrhaging in the mucosa	0	0	0	1	0	0	0	0
Rectum: Brown to black discoloration	0	0	0	0	0	0	0	1
Gallbladder: Brownish discoloration of the mucosa	0	0	0	0	0	0	0	1
<b>Excretory</b>								
Kidney (Left): Depressed area in the caudal pole	0	0	0	1	0	0	0	0
Urinary Bladder: Reddish foci in the mucosa	0	0	1	0	0	0	0	0
<b>Endocrine</b>								
Thyroid Gland: Enlarged	0	0	1	0	0	0	0	0
Adrenal Gland: Cyst	0	0	0	0	0	0	0	1
<b>Reproductive</b>								
Prostrate: Irregular appearance	0	0	0	1	-	-	-	-
	1	0	0	0	-	-	-	-
Bone marrow: Pale discoloration	0	1	1	0	0	0	1	0

**Organ weights:**

*The absolute and relative weights of the following organs were measured at necropsy in all terminally sacrificed animals and recovery animals: Paired organs were weighed separately.*

Adrenals (2)	Ovaries (2)
Brain	Pituitary gland
Heart (emptied)	Prostrate
Kidneys (2)	Spleen
Liver	Testes (2)

Statistically significant changes noted in organ weight are summarized in the table below. Probable test article-related organ weight changes were identified in the pituitary gland. Mean relative pituitary gland weight was moderately increased at the mid-and high-dose male groups. There were no histological correlates to the organ weight change in the pituitary gland.

Relative (to body weight) Organ Weight: % Change vs Control					
Organ		Dose mg/kg/day			
		0	3.5	7	14
Pituitary Gland	Terminal Sacrifice	M: - F: -	M: NSC F: NSC	M: ↑27%* F: NSC	M: ↑25%* F: NSC
	Recovery Sacrifice	M: - F: -	M: NSC F: NSC	M: NSC F: NSC	M: NSC F: NSC
	Recovery Sacrifice	M: -	M: NSC	M: NSC	M: NSC

\*: Statistically significant at p≤ 0.05 (Dunn's)  
NSC: No statistically significant change

**Histopathology:**

*Adequate Battery:* yes (x), no ( )—explain

*Peer review:* yes (x), no ( )

*The following tissues were collected from all main study animals and all recovery animals at necropsy; and those that were sacrificed prematurely during the study:*

Dose (mg/kg) →	Main Study				Recovery Group			
	0	1.75	4.0	9.0	0	1.75	4.0	9.0
<b>Species</b>	<b>Dogs</b>							
Adrenals	X	X	X	X	X	X	X	X
Aorta (thoracic)	X	X	X	X	X	X	X	X
Bone Marrow smear	X	X	X	X	X	X	X	X
Bone (left femur)	X	X	X	X	X	X	X	X
Brain	X	X	X	X	X	X	X	X
Cecum	X			X	X	X	X	X
Cervix								
Colon	X	X	X	X	X	X	X	X
Duodenum	X	X	X	X	X	X	X	X
Epididymis	X	X	X	X	X	X	X	X
Esophagus (middle part)	X	X	X	X	X	X	X	X
Eye	X	X	X	X	X	X	X	X
Fallopian tube								
Gall bladder	X	X	X	X	X	X	X	X
Gross lesions	X			X	X	X	X	X
Harderian gland								
Heart	X	X	X	X	X	X	X	X
Ileum	X	X	X	X	X	X	X	X
Injection site								
Jejunum	X	X	X	X	X	X	X	X
Joint (left knee)								
Kidneys	X	X	X	X	X	X	X	X
Lachrymal gland								
Larynx								

	Main Study				Recovery Group			
Dose (mg/kg) →	0	1.75	4.0	9.0	0	1.75	4.0	9.0
<b>Species</b>	<b>Dogs</b>							
Liver (Lt and Rt lateral lobe; caudal lobe)	X	X	X	X	X	X	X	X
Lungs	X	X	X	X	X	X	X	X
Lymph nodes, cervical	X	X	X	X	X	X	X	X
Lymph nodes mandibular	X	X	X	X	X	X	X	X
Lymph nodes, mesenteric	X	X	X	X	X	X	X	X
Mammary Gland (caudal complexes)	X	X	X	X	X	X	X	X
Nasal cavity (incl. paranasal cavity)								
Optic nerves								
Ovaries (and oviduct)	X	X	X	X	X	X	X	X
Pancreas (left lobe)	X	X	X	X	X	X	X	X
Parathyroid	X			X	X	X	X	X
Peripheral nerve								
Pharynx								
Pituitary	X	X	X	X	X	X	X	X
Prostate ( ventral, lateral and dorsal)	X	X	X	X	X	X	X	X
Rectum	X	X	X	X	X	X	X	X
Rib	X	X	X	X	X	X	X	X
Salivary gland (submandibular)	X			X	X	X	X	X
Sciatic nerve	X	X	X	X	X	X	X	X
Seminal vesicles								
Skeletal muscle	X	X	X	X	X	X	X	X
Skin (Inguinal region)								
Spinal cord								
Spleen	X			X	X	X	X	X
Sternum	X			X	X	X	X	X
Stomach (cardia, fundus & pylorus)	X	X	X	X	X	X	X	X
Testes	X	X	X	X	X	X	X	X
Thymus	X	X	X	X	X	X	X	X
Thyroid	X	X	X	X	X	X	X	X
Tongue	X			X	X	X	X	X
Trachea (middle part)	X			X	X	X	X	X
Urinary bladder	X			X	X	X	X	X
Uterus (Lt and Rt horn and body)	X	X	X	X	X	X	X	X
Vagina	X	X	X	X	X	X	X	X
Vena Cava (caudal)								
Zymbal gland								

*All tissues were fixed in phosphate-buffered neutral 4% formaldehyde solution with the exception of the eyes and optic nerves that were fixed in formalin (5%)-glutaraldehyde (0.5%) solution (terminally sacrificed animals) or in Davisson's fixative (recovery sacrificed animals) mixture. Tissues were stained with hematoxylin and eosin stain and examined histologically.*

No treatment-related histological findings were observed.

**Other:**

BSF 138315 administration was associated with lower respiratory rates. In females, at all dose levels, the respiratory rate was marginally but consistently lower. BSF 138315 administration in males at 4.0 and 9.0 mg/kg/day during weeks 2, 6 and 12 resulted in a lowering of respiratory rates. LD males also were observed having a lower respiratory rate during weeks 6 and 12. The observed changes in the treated males were within the control range; thus are not considered to be toxicological relevant.

**Study title: BSF-138315 – Repeated dose toxicity (at least 27-week treatment) after oral administration (gavage) in the Wistar rat.**

**Key study findings:** BSF 138315 (0, 3.5, 7 and 14 mg/kg/day) was orally administered to rats for 27-weeks followed by a 4-week recovery period with the following results:

1. The primary BSF 138315 related clinical signs included compulsive gnawing, lassitude, self-mutilation, mydriasis and exophthalmos.
2. BSF 138315 reduced liver size in both males and females; the effect occurred at a greater incidence in males. This reduction in liver size was correlated with hepatocellular atrophy.
3. BSF 139315 had no direct effects on both hematological and clinical chemistry parameters.
4. The NOAEL for hydromorphone was identified by the sponsor and to which the reviewer is in agreement as 3.5 mg/kg/day (HED = 0.567 mg/kg). This corresponds to AUC<sub>0-24 hr</sub> value of 322 and 219 ng\*h/mL in males and females, respectively, during week 24. C<sub>max</sub> values at this dose during week 24 were 103 and 66.2 ng/mL in males and females, respectively.

<b>Study no.:</b>	MPF/DT 9940
<b>Volume #, and page #:</b>	eCTD submission
<b>Conducting laboratory and location:</b>	Knoll AG Research and Development 67061 Ludwigshafen Germany
<b>Date of study initiation:</b>	May 4, 1999 (beginning of treatment)
<b>GLP compliance:</b>	Yes
<b>QA report:</b>	yes ( X ) no ( )
<b>Drug, lot #, and % purity:</b>	BSF 138315, Batch No L0002652, 99,2% purity

**Methods**

Doses: 0, 3.5, 7.0 and 14.0 mg/kg (active moiety)

Species/strain: Rats/Wistar

Number/sex/group or time point (main study): 20/sex/group

Route, formulation, volume, and infusion rate: Oral (gavage), solution, 5 mL/kg

Group	Test Article	Dose (mg active moiety/kg/day)	Concentration of active moiety (mg/mL)	Total Number of Animals		Sacrificed after treatment period		Sacrificed after 8-wk recovery period	
				Females	Males	F	M	F	M
<b>Main Study</b>									
<b>1</b>	Vehicle	0	0	20	20	13	13	7	7
<b>2</b>	BSF 138315	3.5	0.7	20	20	13	13	7	7
<b>3</b>	BSF 138315	7.0	1.4	20	20	13	13	7	7
<b>4</b>	BSF 138315	14.0	2.8	20	20	13	13	7	7

Satellite groups used for toxicokinetics or recovery: Recovery group

Age: Approximately 4 weeks (at delivery)

Weight: Males: 197 g (169g -216 g; Females: 168 g (147-187 g)

Sampling times:

Unique study design or methodology (if any):

**Observations and times: (these parameters can be captured separately here or described in connection with each endpoint under the results section.)**

The observation parameters were captured under the results sections.

**Results****Toxicokinetics:**

*Toxicokinetic evaluation was performed on study day 1 and in treatment weeks 11 and 24 91 from 3 animals/sex/group/time point. Blood samples (sufficient to obtain approx. 300  $\mu$ L of plasma/sample) were collected by retro- orbital puncture under isoflurane anesthesia prior to dosing and at 0.5 hr, 1 hr, 4 hr, 8 hr and 24 hr after dosing. Also blood was obtain from moribund animals prior to sacrifice.*

Toxicokinetic analysis is presented in the Applicant's table below. BSF 138315 was rapidly absorbed. Mean peak plasma level were generally obtained between 0.5 and 4 hours after dosing. Systemic exposure, as assessed with the  $C_{max}$  and  $AUC_{0-24 hr}$ , was apparent at all dose levels. Exposure increased in a dose-dependent manner but not quite dose-proportionally (variable). There was a 2- to 5-fold increase in exposure on repeat dosing.

**Toxicokinetic data**

Dose (as active moiety)	3.5 mg/kg BW/day		7.0 mg/kg BW/day		14.0 mg/kg BW/day	
	Male	Female	Male	Female	Male	Female
Day 1						
C <sub>max</sub> [ng/ml]	12.1	10.2	17.9	16.4	23.3	41.1
AUC <sub>0-24 h</sub> [h • ng/ml]	71.9	83.6	170	192	333	456
t <sub>max</sub> [h]	0.5	1	1	1	4	4
Week 11						
C <sub>max</sub> [ng/ml]	96.5	44.8	146	132	416	326
AUC <sub>0-24 h</sub> [h • ng/ml]	187	173	433	946	1030	1290
t <sub>max</sub> [h]	0.5	0.5	0.5	0.5	0.5	0.5
Week 24						
C <sub>max</sub> [ng/ml]	103	66.2	254	129	481	397
AUC <sub>0-24 h</sub> [h • ng/ml]	322	219	678	582	1650	1160
t <sub>max</sub> [h]	0.5	0.5	0.5	0.5	0.5	0.5

**Mortality:**

There were two unscheduled deaths, two males in the recovery group. These animals were sacrificed *in extremis* due to poor condition. The observed clinical signs and histological findings observed in these animals are tabulated below. The Applicant contributed the poor conditions observed in rat № 40 and 70 to esophageal perforation and inflammation and bronchopneumonia, respectively.

<b>Unscheduled Sacrifice <i>in extremis</i></b>				
Treatment Group: Dose	Animal №	Week of Unscheduled sacrifice	Clinical Observations	Macroscopic finding(s)
Group 2: 3.5 mg/kg/day	40	18	- Emaciation - Paleness - Lassitude - dehydration	- Marked esophagitis - Esophageal perforation
Group 4: 14 mg/kg/day	72	12	- Acute respiratory sounds - Rough coat - Salivation	- Moderate inflammation of the heart - Moderate bronchopneumonia. <u>Note:</u> attributed to misgavage of BSF 138315.

**Clinical signs:**

*The behavior and general health of the animals were assessed prior to dosing and several times after dosing.*

As indicated in the Applicant table below, hyperactivity, compulsive gnawing, self-mutilation, lassitude, sedation, mydriasis, exophthalmos, rigid posture, diarrhea and alopecia were the primary treatment-related clinical signs.

- Hyperactivity and compulsive gnawing were observed in all animals in the LD, MD and HD test article groups.

- Self-mutilation was observed in all treatment groups with no clear dose-related trend. Self-mutilation was mainly noted in the tail and front paw (s).
- Dose-dependent lassitude was predominantly observed in the first three weeks of treatment only in all treatment groups. As depicted in the table, the incidence of lassitude was greater in males than females.
- Dose-dependent sedation was observed in the first week of treatment only in all male treatment groups and in the high-dose female group.
- Dose-dependent mydriasis was only observed during the first three weeks of treatment in all male treatment groups. In contrast, a low incidence of mydriasis was noted in the LD and MD female treatment groups.
- Dose-dependent exophthalmos was observed from the onset of treatment in all treatment groups. While exophthalmos was observed in the MD and HD groups throughout the treatment, it was only noted in the LD group up to week 8 of treatment.
- Dose-dependent diarrhea was observed in all treatment groups from week 11 to end of the treatment period.

**Table (Applicant Table 11). Incidence of relevant clinical signs during 27-week treatment period (20 animals/sex/group).**

Clinical signs	Control		3.5 mg/kg BW		7.0 mg/kg BW		14.0 mg/kg BW	
	Male	Female	Male	Female	Male	Female	Male	Female
<b>BEHAVIOUR</b>								
Hyperactivity	0	0	20	20	20	20	20	20
Compulsive gnawing	0	0	20	20	20	20	20	20
Self-mutilation	0	0	18	10	16	18	16	16
• Tail	0	0	12	5	15	12	12	7
• Front paw(s)	0	0	11	9	11	14	8	15
• Hind paw(s)	0	0	2	0	3	1	1	1
Lassitude	0	0	15	12	17	11	20	16
Sedation	0	0	1	0	10	0	20	5
Aggressiveness	0	0	0	4	4	6	8	8
Rigid posture	0	0	0	0	7	3	15	8
<b>EYES</b>								
Exophthalmos	0	0	16	19	19	19	20	20
Mydriasis	0	0	2	1	3	3	6	0
<b>SKIN / FUR</b>								
Rough coat	0	0	1	0	10	3	16	10
Alopecia	2	2	12	15	16	18	17	19
• Chin	0	0	9	13	15	18	16	19
<b>SECRETION / EXCRETION</b>								
Soiled nasolabial area	4	1	12	5	12	12	12	13
Nasal red discharge	3	0	16	8	18	7	13	10
Diarrhoea	0	0	6	4	18	15	16	19

**Body weights:**

*Body weights were recorded three times a week from week 1 to week 13 and then weekly thereafter.*

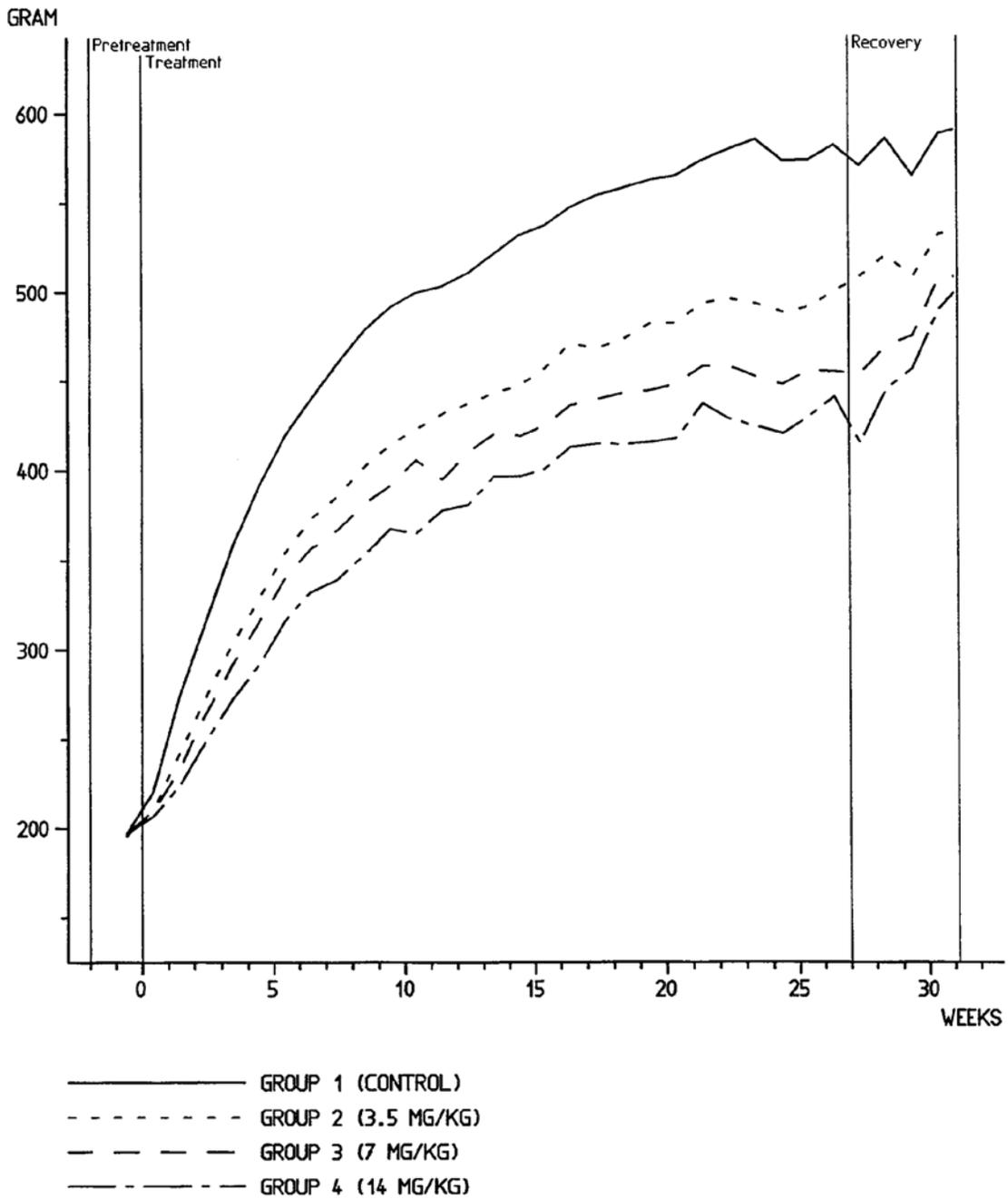
Body weight data as copied from the Applicant's submission is shown below. As depicted in the Applicant's figures below, the effects of BSF 138315 on mean body weight was more pronounced in the males than the females. Apparent dose-dependent treatment-related decreases in body weight were noted in all male treatment groups; mean body weight was statistically significant ( $p \leq 0.05$ ) lower in all treatment groups throughout the entire 27-week treatment period. At the end of the treatment period, the mean body weight of males in the low-, mid- and high-dose groups were 20.9%, 32.9% and 36.6% lower than that of the control males, respectively. The body weight deficit was still evident during the recovery period; compared to control males, mean body weight was significantly ( $p \leq 0.05$ ) lower throughout the 4-week recovery period.

The observed treatment-related effects on body weight were less discernible in the females. Relative to the control, the mean body weight was significantly ( $p \leq 0.05$ ) lower during weeks 2 thru 4 in the low- and mid-dose groups. The mean body weight in the high dose female group was statistically significant ( $p \leq 0.05$ ) lower than the controls from week 2 to week 27-week treatment period. At the end of the treatment period, the mean body weight of females in the low-, mid- and high-dose groups were 0.9%, 0.5% and 8.6% lower than that of the control females, respectively. The observed body weight deficit observed in the high-dose group reversible; during the recovery period the mean body weight was comparable to the controls.

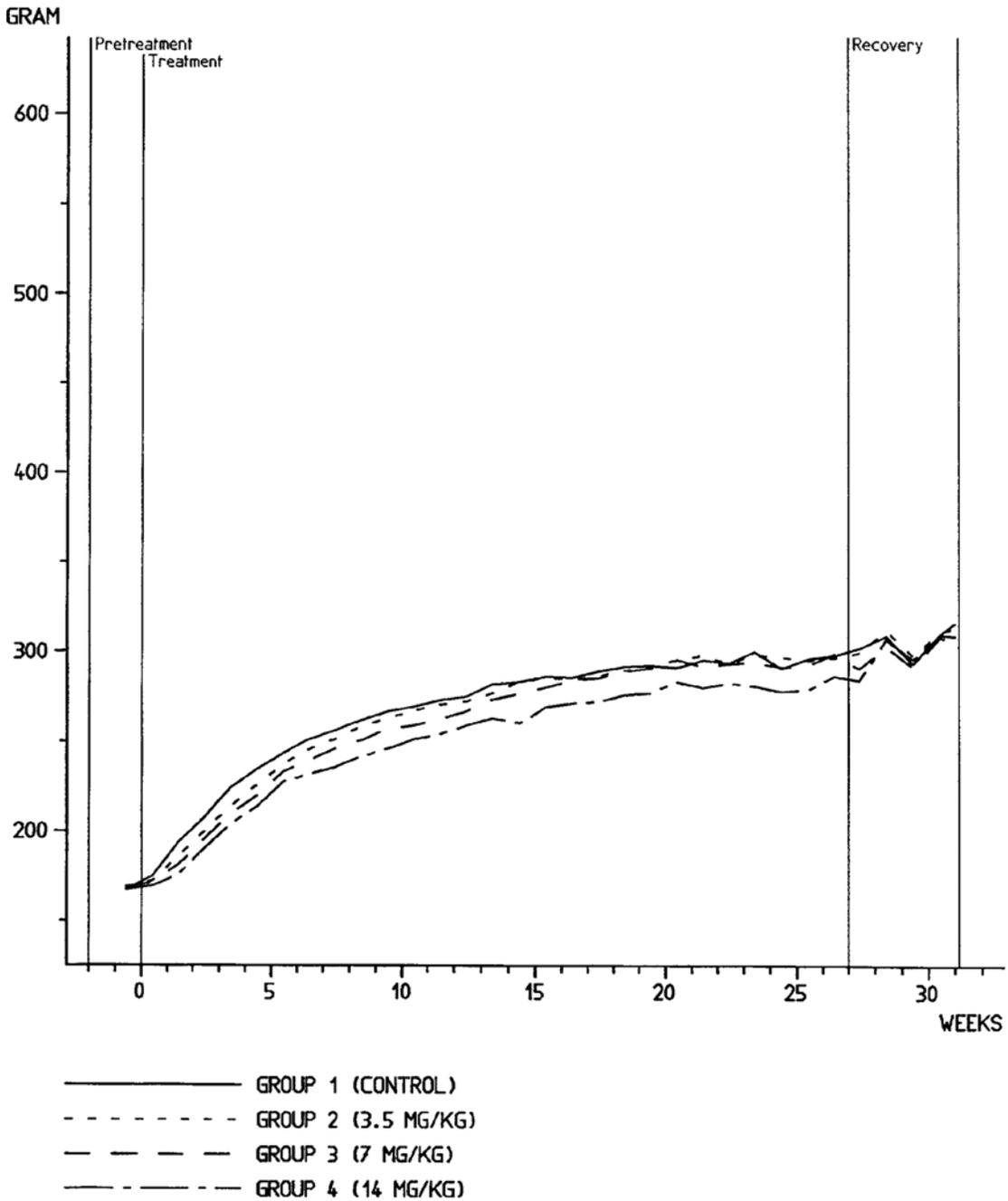
**Table 12** Body weight gain in grams (data in parentheses indicate weight gain as a percentage of control weight gain); (selected data; for complete data see appendix 1, pp 269 - 302)

Treatment period (weeks)	Control		3.5 mg/kg BW		7.0 mg/kg BW		14.0 mg/kg BW	
<b>Males</b>								
0 - 4	161.3	(100)	108.4	(67.2)	95.3	(59.1)	75.9	(47.1)
0 - 13	313.3	(100)	240.9	(76.9)	212.8	(67.9)	183.8	(58.7)
0 - 27	384.0	(100)	303.6	(79.1)	257.5	(67.1)	243.6	(63.4)
Recovery	19.9	(100)	25.7	(129.1)	54.9	(275.9)	84.2	(423.1)
<b>Females</b>								
0 - 4	56.3	(100)	46.1	(81.9)	40.1	(71.2)	36.2	(64.3)
0 - 13	107.3	(100)	104.8	(97.7)	97.5	(90.9)	91.2	(85.0)
0 - 27	130.0	(100)	128.8	(99.1)	129.3	(99.5)	118.8	(91.4)
Recovery	13.9	(100)	13.8	(99.3)	15.6	(112.2)	24.8	(178.4)

# BODY WEIGHTS MALES



# BODY WEIGHTS FEMALES



## Food consumption:

*Food consumption and water consumption was recorded weekly.*

Treatment-related effects on food consumption were observed in both males and females. Food consumption was significantly lower in the all BSF 138315 groups sporadically throughout the treatment periods. No dose-related trend was observed.

**Ophthalmoscopy:**

*Ophthalmological examination was performed during weeks 0, 3, 6, 12 and 25. Table below detail the animals evaluated. During the examination of the eye, the following ocular structures were examined: cornea, iris, lens, vitreous body, and ocular fundus. A photo slip lamp was used to document all relevant ocular findings.*

<b><i>Ophthalmological Examination</i></b>	
<i>Week of Examination</i>	<i>Animals evaluated</i>
0	- 10/animals/sex/group - All substitute animal: o Males № 161-180 o Females: № 181-200
3	- 10 animals/sex/group, except low-dose males
6	10 animals/sex/group
12	10 animals/sex/group
25	10 animals/sex/group, except 1 male each of groups 2 and 4 which had to be sacrificed prematurely

*Deviation from the protocol. As a result of the eye examination conducted in week 25, three animals/sex/group that were initially assigned to the recovery group were sacrificed at the end of the treatment period for to clarify ocular findings.*

As depicted in the Applicant’s table below, prominent or opaque lenticular sutures and lens opacities were the primary ocular changes observed. However, the reviewer does not consider these changes to be treatment-related since they were also noted in some animals in the control groups and/or did not demonstrate dose-dependent increases in incidence or severity.

**Table 13 No. of affected animals versus No. of examined animals for relevant lenticular changes (selected parameters; for complete data see appendix 1, pp 535 - 569)**

Week	Control		3.5 mg/kg BW		7.0 mg/kg BW		14.0 mg/kg BW	
	Male	Female	Male	Female	Male	Female	Male	Female
<b>Prominent or opaque lenticular suture</b>								
0	0/10	0/10	1/10	0/10	1/10	0/10	0/10	1/10
3	1/10	0/10	nd	0/10	1/10	3/10	1/10	2/10
6	3/10	1/10	1/10	2/10	3/10	6/10	2/10	5/10
12	5/10	5/10	6/10	7/10	7/10	9/10	4/10	7/10
25	7/10	6/10	5/9	8/10	7/10	9/10	6/9	7/10
Recovery	4/7	5/7	3/6	5/7	4/7	7/7	4/6	5/7
<b>Lens opacities</b>								
0	0/10	1/10	0/10	2/10	0/10	2/10	0/10	0/10
3	0/10	1/10	0/10	2/10	0/10	6/10	2/10	2/10
6	1/10	2/10	1/10	3/10	3/10	7/10	4/10	3/10
12	3/10	3/10	5/10	2/10	6/10	9/10	5/10	4/10
25	2/10	2/10	4/9	3/10	6/10	9/10	5/9	4/10
Recovery	2/7	3/7	3/6	3/7	5/7	7/7	4/6	2/7

nd = Not determined

**EKG:**

*Not performed.*

**Hematology:**

*Blood was collected from 10 animals/sex/group during treatment weeks 4, 13 and 26, and from all the recovery animals at the end of the recovery period (week 31). Blood sample was collected via retro-orbital under isoflurane anesthesia. The following parameters were examined.*

<b>Hematology Parameters</b>	
<b>White Blood Cell Parameters</b>	<b>Red Blood Cell Parameters</b>
Total Leukocyte count (WBC)	Erythrocyte count (RBC)
<u>Differential leukocyte count (Absolute)</u>	<u>Red Cell Morphology</u>
- Juvenile Neutrophil (NEUT JU)	- Normal
- Band Neutrophil (NEU BA)	- Abnormal
- Segmented Neutrophils (NEUTR SE)	- Normoblasts (NORMOBL)
- Monocyte (MONO)	- Anisocytosis (ANS)CYT
- Lymphocyte (LYM)	- Polychromasia (POLYCHR)
- Eosinophil (EOS)	
- Basophil (BASO)	
- Atypical Cells (ATYP CE)	

Hematology Parameters	
White Blood Cell Parameters	Red Blood Cell Parameters
	Hemoglobin (HGB)
	Hematocrit (HCT)
	Mean corpuscular volume (MCV)
	Mean corpuscular hemoglobin (MCH)
	Mean corpuscular hemoglobin concentration (MCHC)
	Reticulocyte count ratio (RETI)
	Reticulocyte-high fluorescence ratio (HFR)
	reticulocyte-middle fluorescence ratio (MFR)
	reticulocyte-low fluorescence ratio (LFR)
	Platelet Count (PLT)

Coagulation Parameters
Thromboplastin Time (TPT)
Partial Thromboplastin Time (PTT)

The results revealed some sporadic statistically significant changes in some hematology parameters. No dose-related trend was observed in the hematology parameters changes. Overall, the results indicated that there were no apparent biologically significant test article effects on hematological parameters under the tested conditions.

**Clinical chemistry:**

*Blood was collected from 10 animals/sex/group during treatment weeks 4, 13 and 26, and from all the recovery animals at the end of the recovery period (week 31). Blood sample was collected via retro-orbital under isoflurane anesthesia. The following parameters were examined.*

Serum Chemistry Parameters	
Alanine aminotransferase (ALT)	Potassium (K)
Alkaline phosphatase (ALP)	Sodium (Na)
Calcium (Ca)	Total bilirubin (TBIL)
Chloride (Cl)	Urea (BUN)
Cholesterol (CHOL)	Total Protein (TP)
Cholinesterase	Triglycerides
Glucose (GLU)	
Inorganic Phosphate	

Significant changes noted at weeks 4, 13 and 26 blood draws are summarized in the table below. No direct BSF 138315-related effects were evidence. As shown in the table below, significant changes were noted in some of the electrolytes parameters in males. Plasma potassium and calcium were reduced while plasma chloride was elevated. The Applicant suggested that the change in electrolyte was possibly due to a nutritional imbalance and BSF-138315-induced diarrhea. The reviewer concurs. A slight to moderate reduction in total proteins, triglycerides and urea were noted in males  $\geq 7$

mg/kg/day. At the end of recovery, decreased protein and elevated chloride levels were still present at the end of the recovery period; although evidence of recovery was noted.

Parameter	Study Week	Percent from control Change in Clinical Chemistry		
		Males (mg/kg/day)		
		3.5	7.0	14.0
Potassium	4	↓7%*	↓10%*	↓12%*
	13	↓9%*	↓7%*	↓13%*
	26	↓7%*	↓12%*	↓16%*
	Recovery (Wk 31)	-	-	-
ALT	4	↓25%*	-	-
	13	-	-	-
	26	-	-	-
	Recovery (Wk 31)	-	-	-
Calcium	4	-	↓4%*	↓5%*
	13	-	↓4%*	↓4%*
	26	-	-	-
	Recovery (Wk 31)	-	-	-
Chlorine	4	↑44%*	-	↑99%*
	13	↑57%*	↑41%*	↑98%*
	26	↑49%*	-	↑33%*
	Recovery (Wk 31)	-	-	-
Chloride	4	-	-	-
	13	-	-	-
	26	↑3%*	-	↑4%*
	Recovery (Wk 31)	-	↑3%*	↑3%*
Triglycerides	4	↓38%*	↓39%*	↓56%*
	13	↓41%*	↓51%*	↓75%*
	26	↓45%*	↓48%*	↓63%*
	Recovery (Wk 31)	-	-	-
Urea	4	-	↓26%*	↓28%*
	13	-	↓18%*	↓25%*
	26	-	↓14%*	-
	Recovery (Wk 31)	-	-	-
Inorganic Phos	4	↑8%*	-	-
	13	↑13%*	-	-
	26	-	↑22%*	↑18%*
	Recovery (Wk 31)	-	↑14%*	↑10%*
Total Protein	4	-	↓5%*	↓5%*
	13	↓4%*	↓5%*	↓5%*
	26	↓6%*	↓9%*	↓10%*
	Recovery (Wk 31)	-	↓4%*	↓6%*
Creatine	4	-	-	↑45%*
	13	-	-	↓10%*
	26	-	-	-
	Recovery (Wk 31)	-	-	-
*: Significant different from control value (Dunnett-test based on pooled variance sign.), p ≤ 0.05				
-: No statistically significant change				

Parameter	Study Week	Percent from Control Change in Clinical Chemistry		
		Females (mg/kg/day)		
		3.5	7.0	14.0
Bilirubin	4	-	↑32%*	↑43%*
	13	-	↓38%*	↓35%*
	26	-	↓39%*	↓28%*
	Recovery (Wk 31)	-	-	-

Parameter	Study Week	Percent from Control Change in Clinical Chemistry		
		Females (mg/kg/day)		
		3.5	7.0	14.0
Potassium	4	-	-	-
	13	-	-	-
	26	-	↓13%*	↓11%*
	Recovery (Wk 31)	-	-	-
ALT	4	↓26%*	-	↓16%*
	13	↓36%*	↓23%*	↓26%*
	26	↓51%*	↓56%*	↓64%*
	Recovery (Wk 31)	↓53%*	↓57%*	↓65%*
Calcium	4	-	-	-
	13	-	-	↑3%*
	26	-	-	-
	Recovery (Wk 31)	-	-	-
Cholesterol	4	-	↓20%*	↓26%*
	13	↓22%*	↓23%*	↓29%*
	26	↓21%*	↓28%*	↓40%*
	Recovery (Wk 31)	-	-	-
Chlorine	4	-	-	-
	13	-	-	-
	26	-	-	↓29%*
	Recovery (Wk 31)	-	↓26%*	↓27%*
Chloride	4	-	↑3.2*	↑3%*
	13	↑4%*	-	-↑3%*
	26	-	-	-
	Recovery (Wk 31)	-	↑3%*	↑5%*
Triglycerides	4	-	↓27%*	↓32%*
	13	-	-	-
	26	-	↓36%*	↓52%*
	Recovery (Wk 31)	-	-	-
Urea	4	-	↓24%*	↓17%*
	13	-	-	↓18%*
	26	-	-	-
	Recovery (Wk 31)	-	-	↑16%*
Inorganic Phos	4	-	-	-
	13	-	↑13%*	↑11%*
	26	↑12%*	↑18%*	↑24%*
	Recovery (Wk 31)	-	-	-
Creatine	4	-	↓68%*	↑64%*
	13	-	-	-
	26	-	-	↑9%*
	Recovery (Wk 31)	-	-	-

\*: Significant different from control value (Dunnett-test based on pooled variance sign.), p ≤ 0.05  
 -: No statistically significant change

**Urinalysis:**

Urinalysis was performed during treatment weeks 3, 12 and 25, and during week 3 of the recovery period. Freshly voided urine was collected while the animals were fasting (fasted for about 18 hours) and with free access to drinking water. Animals were placed in a stainless steel metabolism cages for 18 hours to collect urine. The following parameters were collected:

Urine Parameters	
Urinalysis	
Appearance: Color and transparency	
Bilirubin	
Erythrocytes/haemoglobin	
Glucose	
Ketones	
Microscopic examination of urine sediment (organized components)	
Nitrite	
pH	
Protein	
Urobilinogen	
Total Volume/18 hour	
White blood cells	

Significant urinalysis findings are presented in a table below. A dose-dependent increase in urine pH was observed in both males and females. Males demonstrated significant increase at all dose levels during weeks 12 and 25. Females demonstrated significant increase at most dose levels during weeks 12 and 25. This pH effect was reversible; the pH was comparable to controls after the 4-week recovery period. Also, BSF 138315 significantly increases urine volume at most time points in the treatment periods.

**Summary of significant urinalysis findings following oral administration of BSF 138315.**

Parameter	Study Week	Percent Control Change in Urinalysis			
		0	3.5	7.0	14.0
<b>Males</b>					
pH	3	6.8	-	-	↑10%*
	12	6.9	↑14%*	↑13%*	↑12%*
	25	6.7	↑22%*	↑22%*	↑22%*
	Recovery (Wk 30)	6.7	-	-	-
Urine Volume (mL)	3	11.04	-	-	-
	12	9.56	-	↑29%	-
	25	7.09	-	-	↑124%*
	Recovery (Wk 30)	7.4	-	-	-
<b>Females</b>					
pH	3	6.8	-	-	-
	12	6.3	-	↑19%*	↑21%*
	25	5.9	↑17%*	↑20%*	↑32%*
	Recovery (Wk 30)	5.9	-	-	-
Urine Volume (mL)	3	12.25	↓41%*	↓31%*	-
	12	5.67	-	-	↑216%*
	25	6.87	-	↑89%	↑124%*
	Recovery (Wk 30)	5.91	-	-	-
*: Significant different from control value (Dunnett-test based on pooled variance sign.), p ≤ 0.05					
-: No statistically significant change					

**Gross pathology:**

*All moribund and surviving animals were anesthetized with Narcoren (sodium pentobarbital, 120 mg/kg, i.p.) and exsanguinated. Macroscopic examination was performed.*

Sporadic or isolated macroscopic findings were observed in some animals; the type and incidence of these findings were similar in test-article treated and control rats. Hence these macroscopic pathology were not considered to be test-article related. However, macroscopic findings observed in the liver, eyes and musculoskeletal system (including skin) was possibility treatment-related. Reduced liver size was dose-dependent and primarily observed in male rats. Only three females in the high-dose group presented with a reduced liver size. The reduced liver was partially reversible. Evidences of self-mutilation were noted in both male and female rats, being more discernible in females.

Macroscopic Findings	Sacrifice	№ of affected animals/Total № of animals							
		BSF 138315 (mg/kg/day)							
		0		3.5		7.0		14.0	
		M	F	M	F	M	F	M	F
Reduced Liver Size	Terminal	0/13	0/13	7/13	0/13	13/13	0/13	13/13	3/13
	Recovery	0/7	0/7	0/7	0/7	1/7	0/7	1/7	0/7
Unilateral focal corneal opacities	Terminal	0/13	0/13	0/13	0/13	2/13	0/13	0/13	1/13
	Recovery	0/7	0/7	0/7	0/7	1/7	0/7	1/7	0/7
Corneas, striate alterations	Terminal	0/13	0/13	1/13	0/13	1/13	0/13	0/13	1/13
	Recovery	0/7	0/7	2/7	0/7	2/7	0/7	0/7	1/7
Injuries to limbs and/or tails	Terminal	0/13	0/13	0/13	2/13	0/13	3/13	2/13	3/13
	Recovery	0/7	0/7	0/7	0/7	0/7	2/7	0/7	1/7

**Organ weights:**

*The absolute and relative weights (organ-to body weight and organ-to-brain weight) of the following organs were measured at necropsy in all terminally sacrificed animals and recovery animal. Paired organs were weighed separately.*

Adrenals (2)	Ovaries (2)
Brain	Pituitary gland
Heart	Prostrate
Kidneys (2)	Spleen
Liver	Testes (2)
Mesenteric lymph node	Thymus

Statistically significant changes noted in organ weights are summarized in the table below. A statistically significant and dose-dependent decrease in liver absolute, relative-to-body weight and relative-to-brain weight were noted in males at all dose levels of BSF 138315. The decreased liver weight was correlated with the decreased liver size. After

the 4-week recovery period, the observed liver weight deviations were partially reversible. Decreased liver weight-relative-to-body-weight was completely reversible; however statistically significant decrease in absolute liver weight and weight relative-to-brain was still noted in the mid- and high-dose male groups. In females a statistically significant decrease in liver relative-to-brain weight was observed only in the high-dose group. After a 4-week recovery period, the relative-to-body weight and relative-to-brain weight was observed in the mid-dose groups only.

A statistically significant and dose-dependent decrease in kidney absolute, relative-to-body weight and relative-to-brain was also observed in males at all doses of BSF 138315. These kidney weight deviations were reversible following the 4-week recovery period. A dose-dependent decreased adrenal gland absolute, relative-to-body weight and relative-to-brain weight were noted in males at all dose levels of BSF 138315. These adrenal gland weight deviations were partially reversible following the 4-week recovery period. The other significant weight changes did not show a dose-dependent relationship.

Organ Weight: Percent of Control					
	Dose mg/kg/day →	0	3.5	7.0	14.0
Organ	Parameter				
<b>Males</b>					
<b>Terminal Sacrifice</b>					
Adrenal Males	Absolute wt.	0.0.54	-3.7%*	+7.4%*	+16.7%*
	Relative-to body weight(%)	0.010	0%	+3.0%*	+4.0%*
	Relative-to-brain weight (%)	0.002	-50%	-50%	-50%
Liver-	Absolute wt.	16.76 g	-27.9%*	-41.5%*	-44%*
	Relative-to body weight(%)	2.90	-17%*	-27%*	-26.9%*
	Relative-to-brain weight (%)	760.99	-28.2%*	-41.7%*	-44.8%*
Mesenteric Lymph Node	Absolute wt.	0.294	-14.3%	-16.7%*	-35.7%*
	Relative-to body weight (%)	0.051	-2.0%	-20.3%*	-28.8%*
	Relative-to-brain weight (%)	13.355	-14.5%	-16.2%	-36.9%*
\ Heart	Absolute	1.596 g	-7.5%	-12.4%*	-14.3%*
	Relative-to-body weight (%)	0.277	+6.1%	+9.8%*	+11.9%*
	Relative-to-brain weight (%)	72.346	-8.0%	-12.4%*	-15.6%*
Kidney	Absolute wt.	3.219	-14.0%*	-20.5%*	-23.7%*
	Relative-to body weight(%)	0.560	-1.6%*	-8.9%*	-5.4%*
	Relative-to-brain weight (%)	145.980	-14.3%*	-20.5%*	-24.8%*
Spleen	Absolute wt.	1.041 g	-12.0%*	-22.1%*	-19.1%*
	Relative-to body weight (%)	0.180	+5.5%	-22.2%	+6.1%
	Relative-to-brain weight (%)	47.273	-12.7%	-22.1%*	-20.3%*
<b>Recovery Group</b>					
	Absolute wt.	0.054	-3.7%	-1.9%	+11.1%

Organ Weight: Percent of Control					
		BSF 138315			
		0	3.5	7.0	14.0
Organ	Parameter				
Adrenal	Relative-to body weight(%)	0.009	-11.1%	-11.15	+33.3%*
	Relative-to-brain weight (%)	2.380	-1.3%	+3.9%	+12.6%
Liver	Absolute wt.	17.55 g	14.0%	-17.9%*	-22.9%*
	Relative-to body weight (%)	2.97	-5.38%	-4.7%	-9.0%
	Relative-to-brain weight (%)	779.48	-13.4%	-16.3%*	-22.7%*
Mesenteric Lymph Node	Absolute wt.	0.281 g	-1.4%	-15.3%	-18.9%
	Relative-to body weight (%)	0.048	8.3%	-2.0%	-4.2%
	Relative-to-brain weight (%)	12.485	-6.4%	-13.7%	-18.8%
Heart	Absolute	1.581 g	-5.1%	-20.2%	-6.3%
	Relative-to-body weight (%)	0.268	+5.6%	+6.0%	+10.8%
	Relative-to-brain weight (%)	70.211	-4.2%	-6.8%	-6.0%
Kidney	Absolute	3.134 g	-10.25	-6.9%	-4.8%
	Relative-to-body weight (%)	0.532	-5.6%	+7.9%	+12.6%*
	Relative-to-brain weight (%)	139.139	-9.4%	-5.1%	-4.4%
Spleen	Absolute	1.065 g	+0.2%	-10.9%	-14.2%
	Relative-to-body weight (%)	0.151	-60.3%	+26.5%	-40.4%
	Relative-to-brain weight (%)	47.288	+1.2%	-9.2%	-14.0%
Females					
Terminal Sacrifice					
Liver	Absolute wt.	8.51 g	-1.8%	-2.2%	-9.3%
	Relative-to body weight (%)	2.85	-1.4%	-3.9%	-5.6%
	Relative-to-brain weight (%)	423.90	-3.2%	-4.6%	-10.3%*
Recovery Group					
Liver	Absolute wt.	9.55 g	1.4%	11.4%	8.6%
	Relative-to body weight (%)	3.02	0%	-9.3%*	-6.02%
	Relative-to-brain weight (%)	468.49	-2.6%	-14.0%*	-9.9%

\*: Significant different from control value (Dunnett-test based on pooled variance sign.), p ≤ 0.05

**Histopathology:** Adequate Battery: yes ( X ), no ( )—explain  
 Peer review: yes ( X ), no ( )

*The following tissues were collected from all animals (schedule sacrificed and unscheduled necropsy).*

<b>Main Study and Recovery Animals</b>	
<b>Tissues collected for histological analysis</b>	
<b>Species</b>	<b>Rats</b>
Adrenals (2)	Pancreas: left lobe
Aorta (thoracic)	Parathyroid glands (2)
Bone: left femur and sternum	Pituitary
Bone marrow, sternum, left femur	Prostate: ventral, dorsal lateral lobe
Brain - Planes I: lateral olfactory tract and optic nerve - Plane II: Infundibulum of pituitary - Plane III: mammillary body lane IV: cerebellum (vermis, parallocculus)	Rectum
Cecum	Rib: bone-cartilage-transitional zone
Colon	Salivary gland: left submandibular gland with mandibular lymph node
Duodenum	Sciatic nerve (left and right)
Epididymis (2)	Seminal vesicles left and right with coagulating glands
Esophagus (middle portion)	Skeletal muscle: left and right semitendinosus, semimembraneous
Eyes (2)	Skin: lingual region
Gross lesions	Spinal cord: cervical, thoracic and lumbar
Harderian gland	Spleen
Heart	Stomach: forestomach, fundus, pylorus
Ileum	Testes (2)
Jejunum	Thymus
Joint: left knee	Thyroid (2)
Kidneys (2)	Trachea (middle portion)
Liver: let and right lateral lobes, caudal lobe	Trachea (middle portion)
Lungs (left and right phrenic lobes, cranial lobe)	Tongue
Lymph nodes, mesenteric	Urinary bladder
Mammary Gland: caudal complexes	Uterus: left and right horn, body of uterus
Nasal and paranasal cavity	Vagina
Ovaries (2) and oviduct	Vena cava (caudal)

*All tissues were fixed in 4% formaldehyde solution with the exception of the eyes including optic nerves and Harderian glands that were fixed in Heidenhain’s Susa’s fixative mixture. Tissues were stained with hematoxylin and eosin stain and examined histologically. The liver was stain with oil red staining according to LILLIE. Tissues from animals in the control and high-dose treatment groups, animals sacrificed prematurely and all animals from the recovery groups were examined microscopically by (b) (4) (b) (4). Also, the livers and eyes (including Harderian gland) from animals in the low- and mid-dose test article groups were examined microscopically.*

Noteworthy microscopic findings are summarized in the table below.

**Treatment-related hepatocellular atrophy of the liver** was primarily observed in males at doses  $\geq 3.5$  mg/kg/day. Severity ranged from minimal to moderate. The pathologist described the affected hepatocytes as having a “morphologic appearance consistent with a reduction in glycogen content and an appearance observed in animals following a substantial reduction in food intakes. This effect was reversible; it was not observed in male in the LD and MD groups or female rats in any of the dose level groups of BSF 138315 after the 4-week recovery period; this liver change was still observed in one male in the HD group.

Increased incidence of retinal atrophy was observed in both males and females at doses of  $\geq 3.5$  mg/kg/day. This effect is not considered to be a toxic effect of BSF 138315 but a secondary effect of its known, mydriasis, pharmacological activity on eyes. This ocular change was reversible.

		<b>Microscopic Findings</b>			
		<b>Dose (mg/kg/day)</b>			
		<b>0</b>	<b>3.5</b>	<b>7</b>	<b>14</b>
Organ	No of animals examined	Terminal: 13 Recovery: 7	Terminal: 13 Recovery: 7	Terminal: 13 Recovery: 7	Terminal: 13 Recovery: 7
<b>Males</b>					
<b>Liver</b>					
Reduced size/hepatocellular atrophy	Terminal	0	8	13	13
	Recovery	0	0	0	1
Inflammatory cell focus/foci	Terminal	13	12	13	13
	Recovery	7	7	7	7
<b>Eyes</b>					
Retinal atrophy (reduction/loss of outer nuclear layer)	Terminal	1	5	4	5
	Recovery	0	0	0	0
<b>Females</b>					
<b>Liver</b>					
Reduced size/hepatocellular atrophy	Terminal	0	0	0	3
	Recovery	0	0	0	0
Inflammatory cell focus/foci	Terminal	13	13	12	12
	Recovery	7	7	7	7
<b>Eyes</b>					
Retinal atrophy (reduction/loss of outer nuclear layer)	Terminal	4	7	10	7
	Recovery	0	0	0	1

**Study title: BSF 4036516 – Toxicity study with repeated (4-week oral administration (by gavage) to Wistar rats, using BSF 138315 as a reference item.**

**Key study findings:** Hydromorphone N-oxide (0, 3.5, 7 and 14 mg/kg/day), hydromorphone (14 mg/kg/day) and hydromorphone (14 mg/kg/day) plus hydromorphone N-oxide (2 % equivalent to 0.28 mg/kg/day) was orally administered to rats for 4-weeks with the following results:

1. Hydromorphone N-oxide (BSF 4036516) and hydromorphone (BSF 138315) elicited qualitatively similar clinical signs (i.e., compulsive gnawing, hyperactivity, self-mutilation and exophthalmos).
2. The co-administration of hydromorphone N-oxide with hydromorphone did not potentiate the severity and incidence of the hydromorphone-induced clinical signs.
3. Both hydromorphone and hydromorphone N-oxide reduced liver size in both males and females. This reduction in liver size.
4. The NOAEL for hydromorphone was identified by the sponsor and to which the reviewer is in agreement as 3.5 mg/kg/day.

**Study no.:** MPF/DT 0010  
**Volume #, and page #:** eCTD submission  
**Conducting laboratory and location:** Knoll AG Research and Development  
67061 Ludwigshafen Germany  
**Date of study initiation:** Unspecified (approximately May 2000)  
**GLP compliance:** Yes  
**QA report:** Yes ( X ) No ( )  
**Drug, lot #, and % purity:**  
BSF 138315 (Dilaudid), Batch № L0001974, % purity not stated  
BSF 4036516 (Hydromorphone-N-oxide), Batch № 10010, % purity not stated

**Methods**

Doses:

BSF 4036516: 0, 3.5, 7 and 14 mg/kg/day

BSF 138315: 14 mg/kg (+ 2% BSF 4036516= 0.28 mg/kg/day),  
14 mg/kg/day (without BSF 4036516)

Doses were selected based on the results from a 4-week toxicity study in rats (Knoll Report № MPF/ET 9820). In this study, rats were administered BSF 138315 orally a doses of 3.5, 7 and 14 mg/kg. The NOAEL was identified by the Applicant to be 3.5 mg/kg. In order to make a comparative assessment of hydromorphone-N-oxide toxicity profile to that of hydromorphone, the same doses were selected for this study.

Species/strain: Rats/Wistar

Number/sex/group or time point (main study): 10/sex/group

Route, formulation, volume, and infusion rate: Oral, solution, 5 mL/kg/day

Group	Test Article	Dosage Level (mg active moiety/kg/day)	Dosage Level (mg active moiety/m <sup>2</sup> /day)	Total Number of Animals		Sacrificed after treatment period		Sacrificed after 8-wk recovery period	
				Females	Males	F	M	F	M
<b>Main Study</b>									
1	Vehicle	0	0	10	10	4	4	3	3
2	BSF 4036516	3.5	21	10	10	4	4	3	3
3	BSF 4036516	7.0	42	10	10	4	4	3	3
4	BSF 4036516	14.0	84	10	10	4	4	3	3
5	BSF 138315/ BSF 4036516#	14.0	84	10	10				
6	BSF 138315	14.0	84	10	10				
#: Additionally containing 2% BSF 4036516 equivalents to 0.28 mg/day, S = Body Surface area									

Satellite groups used for toxicokinetics or recovery: see table above

Age: approx. 4 weeks

Weight:

Females: 155 g (132 – 172 g) at end of pretest

Males: 196 g (174 – 217 g) at end of pretest

Sampling times:

Unique study design or methodology (if any):

**Observations and times: (these parameters can be captured separately here or described in connection with each endpoint under the results section).**

The observation parameters were captured under the results section.

## Results

### Toxicokinetics:

*Not performed*

**Mortality:** There was one unscheduled death, one male (№ 56) in the BSF 13815 group (14 mg/kg/day). This animal was sacrificed in a moribund state on Day 7. Clinical signs observed in this animal included a mutilated right forepaw (missing toes and phalanges), emaciation and signs of anemia. The reviewer considers these observed clinical signs to be treatment-related.

### Clinical signs:

*The behavior and general health of the animals were assessed prior to dosing and several times after dosing.*

BSF 4036526-related clinical signs were dose-dependent. As indicated in the Applicant's table below, apathy, lassitude, self-mutilation, hyperactivity and exophthalmos were the primary treatment-related clinical signs. Compulsive gnawing was observed in all treatment groups. Males appeared to be more sensitive to the effects of BSF 4036526. The incidence of these clinical signs did not differ between the two treatment groups.

**Table 7 Treatment-related clinical signs**

Clinical signs	Sex	No. of affected animals / Total No. of animals				
		BSF 4036516 (mg/kg/day)			BSF 138315 (mg/kg/day)	
		3.5	7	14	14#	14
<b>Behavior</b>						
Apathy	M	0/10	4/10	9/10	10/10	10/10
	F	0/10	0/10	2/10	9/10	9/10
Lassitude	M	1/10	7/10	10/10	10/10	10/10
	F	1/10	0/10	3/10	10/10	10/10
Hyperactivity	M	3/10	6/10	7/10	6/10	9/10
	F	2/10	0/10	8/10	7/10	7/10
Self-mutilation	M	5/10	6/10	9/10	3/10	8/10
	F	5/10	7/10	7/10	8/10	7/10
• Tail	M	4/10	3/10	5/10	1/10	6/10
	F	5/10	6/10	5/10	0/10	3/10
• Front paw(s)	M	1/10	3/10	6/10	3/10	4/10
	F	0/10	1/10	3/10	8/10	6/10
• Hind paw(s)	M	0/10	0/10	1/10	0/10	0/10
	F	0/10	0/10	0/10	0/10	1/10
Compulsive Gnawing	M	10/10	10/10	10/10	10/10	10/10
	F	10/10	10/10	10/10	10/10	10/10
<b>Eyes</b>						
Exophthalmos	M	3/10	6/10	9/10	10/10	10/10
	F	0/10	4/10	5/10	10/10	9/10
<b>Skin / fur</b>						
Pale	M	0/10	0/10	1/10	2/10	1/10
	F	0/10	1/10	2/10	5/10	1/10
Rough coat	M	0/10	1/10	2/10	1/10	5/10
	F	0/10	0/10	1/10	0/10	3/10
Alopecia (chin)	M	0/10	0/10	0/10	2/10	3/10
	F	0/10	0/10	2/10	1/10	0/10
<b>Secretion / excretion</b>						
Diarrhea	M	0/10	0/10	1/10	4/10	3/10
	F	1/10	3/10	4/10	5/10	6/10
<b>Posture</b>						
Rigid	M	0/10	0/10	0/10	10/10	10/10
	F	0/10	0/10	0/10	10/10	10/10

# Additionally containing 2% BSF 4036516 equivalent to 0.28 mg/kg/day

**Body weights:**

*Body weights were recorded three times a week and once in week 5.*

Body weight data are presented in the figures (copied from Applicant's submission) and table below. Statistically significant decreases in body weight were noted between BSF 4036536-treated groups and control during the study. Body weights were significantly

decreased for male rats in all treatment groups at the end of weeks 2, 3 and 4. Body weights were also significantly decreased in female rats in the 14.0 mg/kg/day at the end of week 3 and 4 dosing period, but only on study days 7, 10 and 17.

Mean body weight gain was reduced in both males and females administered BSF 4036516 throughout the dosing period. Table below presents the percent body weight gains relative to the control. The reduction in body weight gain was dose-dependent. A gender difference on the effects of BSF 4036536 was noted; BSF 4036516 effects were more marked in the males than the females. In males the greatest reduction in body weight compared to the control was noted at the end of the first week of dosing. The percent body weight gains in the males administered BSF 4036526 at 3.5, 7 and 14 mg/kg, at the end of the end of the first week of dosing were 62.7%, 52.5% and 43.2% of the percent weight gain in the controls, respectively. The percent body weight gains in the females administered BSF 4036526 at 3.5, 7 and 14 mg/kg, at the end of the end of the first week of dosing were 78.8%, 77.5% and 57.5% of the percent weight gain in the controls, respectively. At the end of the 4-week dosing period, the mean body weight gains of the BSF 4036516-treated animals were reduced compared to the control. The body weight gains were 39.6%, 39.9% and 42.6% reduced relative to the control in males administered 3.5, 7 and 14 mg/kg/day, respectively. The body weight gains were 12.1%, 14.7% and 30.5% reduced relative to the control in females administered 3.5, 7 and 14 mg/kg/day, respectively.

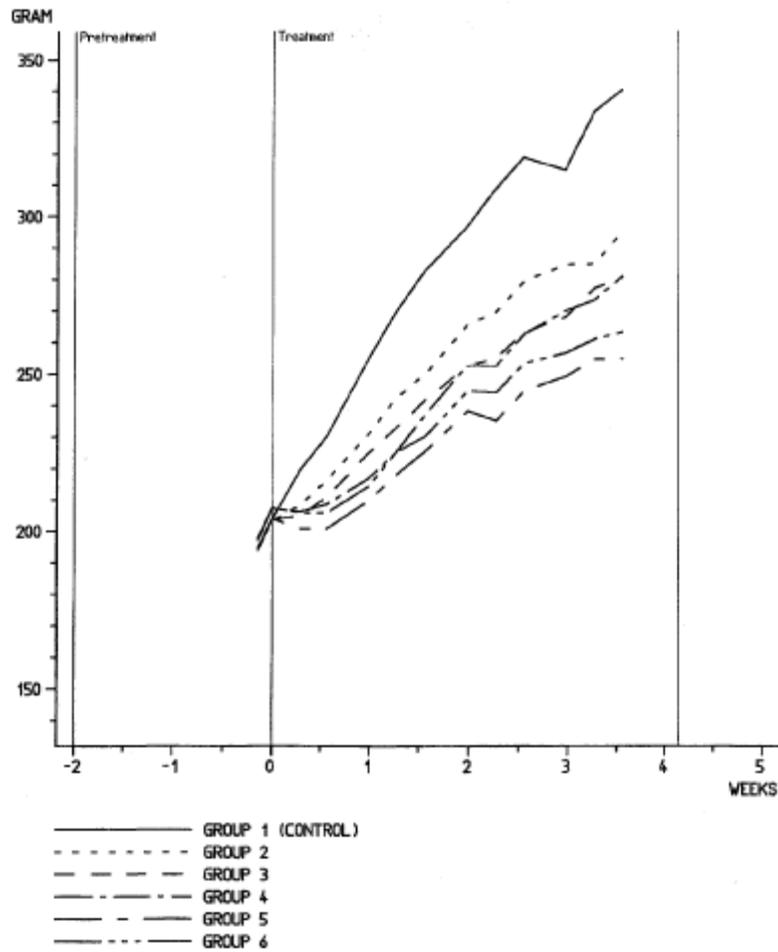
Treatment-related effects in group mean body weights gains in males and females treated with 14 mg/kg of BSF 138315 were compared to vehicle treated control animals. The mean body weight gains were 63.5%, 66.0%, 56.3%, and 53.7% reduced relative to control in males at the end of the dosing week 1, 2, 3 and 4, respectively. The mean body weight gains were 32.5%, 53.4%, 49.7%, and 43.9% reduced relative to control in females at the end of the dosing week 1, 2, 3 and 4, respectively. Compared to BSF 4036526, BSF 138315 had a more pronounced effect on body weight.

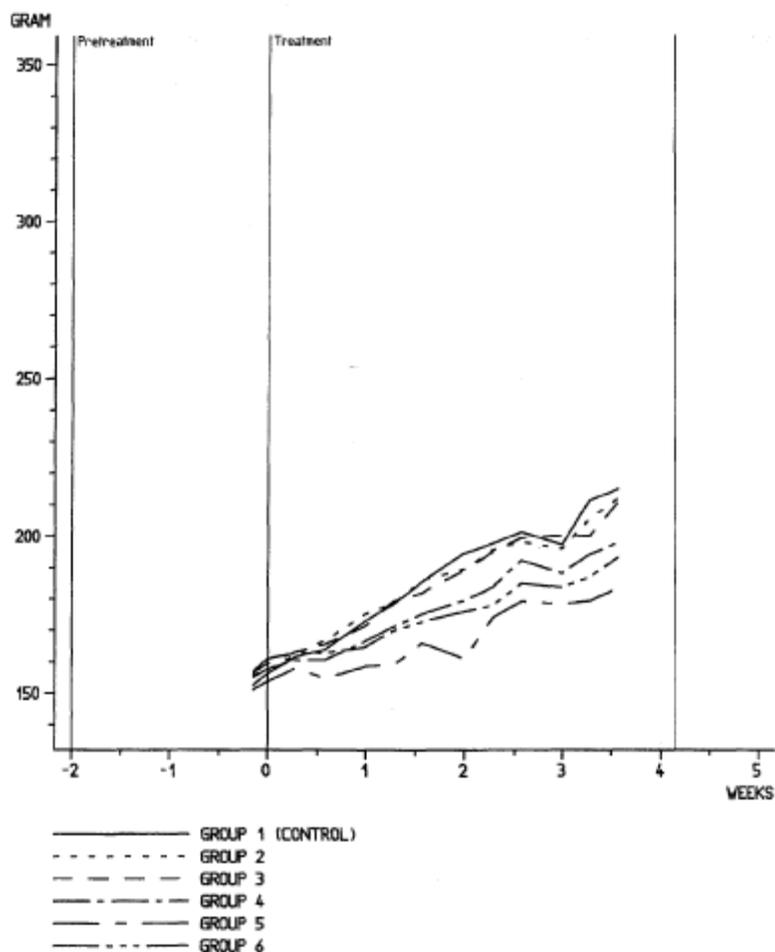
Week	Sex	Mean Body Weight Gain (grams) (as a % of control)					
		BSF 4036526 (mg/kg)				BSF 138315 (mg/kg)	
		0	3.5	7	14	14 <sup>A</sup>	14
0-1	M	23.6 (100%)	14.8 (62.7%)	12.4 (52.5%)	10.2 (43.2%)	7.2 (30.5%)	8.6 (36.4%)
	F	8.0 (100%)	6.3 (78.8%)	6.2 (77.5%)	4.6 (57.5%)	4.4 (55.0%)	5.4 (67.5%)
0-2	M	74.5 (100%)	46.5 (62.4%)	38.0 (52.3%)	29.1 (39.1%)	22.9 (30.7%)	25.3 (34.0%)
	F	26.2 (100%)	24.0 (91.6%)	21.0 (80.2%)	15.8 (60.3%)	10.0 (38.2%)	12.2 (46.6%)
0-3	M	113.4 (100%)	76.8 (67.7%)	62.7 (55.3%)	58.7 (51.8%)	44.6 (39.3%)	49.5 (43.7%)
	F	45.1 (100%)	38.6 (85.6%)	37.9 (84.0%)	30.0 (66.5%)	20.2 (44.8%)	22.7 (50.3%)
0-4	M	134.8 (100%)	93.5 (69.4%)	81.0 (60.1%)	77.4 (57.4%)	58.0 (43.0%)	62.4 (46.3%)
	F	55.2 (100%)	48.5 (87.9%)	47.1 (85.3%)	38.3 (69.4%)	29.2 (52.9%)	31.0 (56.2%)

Week	Sex	Mean Body Weight Gain (grams) (as a % of control)					
		BSF 4036526 (mg/kg)			BSF 138315 (mg/kg)		
		<b>0</b>	<b>3.5</b>	<b>7</b>	<b>14</b>	<b>14<sup>A</sup></b>	<b>14</b>
		(100%)	(87.9%)	(85.3%)	(69.4%)	(52.9%)	(56.2%)

A: Additionally containing 2% BSF 4036516 equivalents to 0.28 mg/day

**BODY WEIGHTS  
MALES**



**BODY WEIGHTS  
FEMALES****Food consumption:**

*Food and water consumption were recorded weekly.*

Compared to controls a significant difference ( $p < 0.05$ ) in food intake was noted in males administered BSF 4036536 at dose levels of 3.5, 7 and 14 mg/kg/day throughout the 4-weeks dosing period. Compared to controls food intake a significant difference ( $p < 0.05$ ) was noted in HD females at the end of the second week of dosing.

**Ophthalmoscopy:**

*Eyes of all animals were examined before the first dose (pre-test), treatment week 4/5 at 1 hour on all animals and at 4-6 hours post-dosing in all animals in groups 3-6. During the examination of the eye, the following ocular structures were examined: cornea, iris, lens, vitreous body, and fundus. Findings were documented with a photo slit lamp.*

Treatment-related findings in the cornea were observed in males in the MD BSF 4036516 group and both males and females in the BSF 138315 groups. As depicted in the table below, macular corneal opacities and corneal dehydration were the observed ophthalmologic changes. Macular corneal opacity was observed in 1/10 (10%), 7/10 (70%) and 4/10 (40%) males 1-hour after the administration of 3.5, 7.0 and 14 mg/kg/day of BSF 44936515, respectively. The incidence of macular corneal opacity in females was lower. Macular corneal opacity was observed in 0/10 (0%), 0/10 (0%) and 3/10 (30%) females 1-hour after the administration of 3.5, 7.0 and 14 mg/kg/day of BSF 44936515, respectively. Corneal dehydration was observed in 0/10 (0%), 6/10 (60%) and 3/10 (30%) males 1-hour after the administration of 3.5, 7.0 and 14 mg/kg/day of BSF 44936515, respectively. Corneal dehydration was reversible; it was not observed in either male or female animals 4- to 5-hours post-dosing. Both macular corneal opacity and corneal dehydration was more pronounced in rats administered BSF 138315 at a dose of 14 mg/kg/day.

Ophthalmologic Change	Sex	No of Affected Animal/Total № of Animals					
		BSF 44936516 (mg/kg/day)				BSF 138315 (mg/kg/day)	
		0	3.5	7.0	14	14 <sup>A</sup>	14
<b>Macular Corneal Opacity</b>							
Pretest	M	0/10	0/10	0/10	0/10	0/10	0/10
	F	0/10	0/10	0/10	0/10	0/10	0/10
Week 4/5 (1 hr post-dosing)	M	0/10	1/10	7/10	4/10	10/10	10/10
	F	0/10	0/10	0/10	3/10	5/10	8/10
Week 4/5 (4-6 hr post-dosing)	M	NE	NE	2/10	4/10	9/10	5/9
	F	NE	NE	0/10	0/10	1/10	5/10
<b>Corneal Dehydration</b>							
Pretest	M	0/10	0/10	0/10	0/10	0/10	0/10
	F	0/10	0/10	0/10	0/10	0/10	0/10
Week 4/5 (1 hr post-dosing)	M	0/10	0/10	6/10	3/10	8/10	9/9
	F	0/10	0/10	0/10	5/10	8/10	9/10
Week 4/5 (4-6 hr post-dosing)	M	NE	NE	0/10	0/10	0/10	0/9
	F	NE	NE	0/10	0/10	0/10	3/10
A: Additionally containing 2% BSF 4036516 equivalents to 0.28 mg/day NE: Not examined							

**EKG:**

*Not performed.*

**Hematology:**

Blood was collected by retro-orbital puncture in treatment week 5. The following parameters were examined:

Hematology Parameters	
White Blood Cell Parameters	Red Blood Cell Parameters
Leukocyte count (WBC)	Erythrocyte count (RBC)
Differential leukocyte count (Absolute)	Red Cell Morphology
- Juvenile Neutrophil (NEUT JU)	- Normal
- Band Neutrophil (NEU BA)	- Abnormal
- Segmented Neutrophils (NEUTR SE)	- Normoblasts (NORMOBL)
- Monocyte (MONO)	- Anisocytosis (ANSCYT)
- Lymphocyte (LYM)	- Polychromasia (POLYCHR)
- Eosinophil (EOS)	
- Basophil (BASO)	
- Atypical Cells (ATYP CE)	
	Hemoglobin (HGB)
	Hematocrit (HCT)
	Mean corpuscular volume (MCV)
	Mean corpuscular hemoglobin (MCH)
	Mean corpuscular hemoglobin concentration (MCHC)
	Reticulocyte Count (RETI)
	Reticulocyte-high fluorescence ratio (HFR)
	reticulocyte-middle fluorescence ratio (MFR)
	reticulocyte-low fluorescence ratio (LFR)
	Reticulocytes ratio (RETI)
	Platelet Count (PLT)

The results indicated some significant changes in hematology parameters when examined during week 5. Significant changes noted are summarized in the table below. A significant decrease in RBC count in both males (-8.7%) and female (-9.6%) rats treated with 14.0 mg/kg/day of BSF 4036516 was noted. During week 5, the reticulocytes count was significantly lower (-35.2%) in males and higher (+ 20.6%) in females administered BSF 138315 (plus 2% BSF 4036516) compared to controls. Some minor changes in hematology parameters were observed in female and male rats. None of these changes should have biological significance.

Parameter	Sex	BSF 4036516 (mg/kg/day)				BSF 138315 (mg/kg/day)	
		0	3.5	7	14	14 <sup>A</sup>	14
<b>Erythrocyte Parameters</b>							
RBC (tera/L)	M	8.38	NSC	NSC	↓8.7%*	NSC	NSC
	F	7.97	NSC	NSC	↓9.6%*	NSC	NSC
HGB (mmol/L)	M	9.5	↑5.3%*	NSC	NSC	NSC	NSC
	F	9.2	NSC	NSC	NSC	NSC	NSC

Parameter	Sex	BSF 4036516 (mg/kg/day)				BSF 138315 (mg/kg/day)	
		0	3.5	7	14	14 <sup>A</sup>	14
RETI(%)	M	30.5	NSC	NSC	NSC	NSC	NSC
	F	28.7	NSC	NSC	NSC	↓35.2%+	NSC
RETI-HFR (%)	M	5.8	NSC	NSC	NSC	NSC	NSC
	F	3.6	NSC	NSC	NSC	↑20.6%+	NSC
MCV (%)	M	52.8	NSC	NSC	↑7.0%*	↑5.7%*	NSC
	F	52.5	NSC	NSC	NSC	NSC	NSC
MCH (fmol)	M	1.130	NSC	NSC	↑7.3%*	↑4.6%*	NSC
	F	NSC	NSC	NSC	NSC	NSC	NSC
MCHC (mmol/L)	M	21.4	↑3.7%*	↑2.3%*	NSC	NSC	NSC
	F	NSC	NSC	NSC	NSC	NSC	NSC
<b>Leukocyte Parameters</b>							
WBC (giga/L)	M	9.5	NSC	NSC	NSC	↓27.4%*	NSC
	F	5.9	NSC	NSC	NSC	NSC	NSC
LYMPHO (%)	M	89	NSC	NSC	NSC	↓6.7%+	NSC
	F	87	NSC	NSC	NSC	↓10.3%+	NSC
SEGM.N (%)	M	8	NSC	NSC	NSC	↑62.5%+	NSC
	F	NSC	NSC	NSC	NSC	NSC	NSC
<b>Platelets Parameters</b>							
Platelet (giga/L)	M	1045	NSC	NSC	↓17.1%*	↓18.6%*	↓15.7%*
H-QUICK (sec0)	M	38.0	NSC	↑6.8%*	↑5.8%*	↑14.0%*	↑13.9%*
A: Additionally containing 2% BSF 4036516 equivalents to 0.28 mg/day *: Significant different from control value (Dunnnett-test), p ≤ 0.05 +: Significant different from control value (Steel-test), p ≤ 0.06							

**Coagulation:**

Blood was collected by retro-orbital puncture in treatment week 5. The following parameters were examined:

Coagulation Parameters
Thromboplastin Time (TPT)
Prothrombin Time (PT)

No treatment-related changes were noted in the coagulation parameters measured.

**Clinical chemistry:**

Blood was collected by retro-orbital puncture in treatment week 5. The following parameters were measured:

Serum Chemistry Parameters	
Alanine aminotransferase (ALT)	Potassium (K)
Alkaline phosphatase (ALP)	Sodium (Na)
Calcium (Ca)	Total bilirubin (TBIL)
Chloride (Cl)	Urea (BUN)
Cholesterol (CHOL)	Total Protein (TP)

Cholinesterase	Triglycerides
Glucose (GLU)	
Inorganic Phosphate	

As depicted in the Applicant’s table below, statistically significant differences in the select clinical chemistry parameters were observed in the test-article animals compared to the vehicle treated animals.

**Table 13 Selected clinical biochemistry parameters (mean values) in week 5**

Parameter	Sex	BSF 4036516 (mg/kg/day)			BSF 138315 (mg/kg/day)		
		0	3.5	7	14	14.#	14
Potassium (mmol/l)	M	5.35	4.99	4.90*	4.74*	4.77*	4.85*
	F	4.64	4.54	4.29	4.46	4.43	4.32
Sodium (mmol/l)	M	142.6	140.8	143.8	142.9	146.6*	146.6*
	F	140.6	141.4	143.4*	143.8*	142.4	141.9
Calcium (mmol/l)	M	2.71	2.68	2.65	2.64	2.57*	2.61*
	F	2.80	2.63*	2.66*	2.75	2.53*	2.58*
Chloride (mmol/l)	M	101.5	102.0	105.3*	103.9*	106.7*	106.7*
	F	103.4	102.6	105.4*	105.3*	103.8	104.1
Phosphate (mmol/l)	M	2.53	2.89*	2.75*	2.72	2.74*	2.89*
	F	2.29	2.45	2.28	2.48	2.13	2.36
ALT (u/l)	M	21.6	22.7	21.9	23.5	58.3*	58.3*
	F	30.1	26.0	23.1*	31.5	23.8*	22.5*
AP (u/l)	M	278.2	261.0	219.2*	251.3	233.6*	231.7*
	F	186.7	208.6	193.9	161.2	193.3	189.9
Glucose (mmol/l)	M	7.46	6.81	7.02	7.36	7.09	7.39
	F	7.15	7.33	8.12*	7.40	7.64	7.55
Protein (g/l)	M	56.6	52.9	50.9*	50.6*	54.2	54.0
	F	61.2	56.6*	58.4	58.4	53.6*	57.1*
Urea (mmol/l)	M	6.81	6.02	5.67	5.06*	4.91*	4.83*
	F	6.68	6.49	6.70	5.35*	5.39*	5.36*
Cholesterol (mmol/l)	M	1.69	1.76	1.72	1.65	1.86	1.92
	F	1.68	1.51	1.44*	1.57	1.21*	1.29*
Bilirubin (µmol/l)	M	1.61	1.32	1.74	1.98	1.56	1.63
	F	1.49	1.75	1.94*	1.57	1.22	2.06*
Cholinesterase (u/l)	M	553	614	614	649	767*	732*
	F	1387	1203	1412	1100	1288	1413
Triglycerides (mmol/l)	M	3.00	1.75*	0.84*	0.92*	0.92*	0.90*
	F	2.32	2.26	1.55	0.95*	1.62	1.28*
Creatinine (µmol/l)	M	34.4	31.7	33.6	34.7	35.0	34.9
	F	41.0	36.5*	36.7*	39.4	34.2*	35.7*

# Additionally containing 2% BSF 4036516 equivalent to 0.28 mg/kg/day;

\* = p ≤ 0.05 (Dunnett's test).

**Urinalysis:**

*Urine, collected using a metabolism cage, was collected from 8 animals/sex/group in treatment week 4.*

Urine Parameters
Urinalysis
Appearance: Color and transparency
Bilirubin
Erythrocytes/hemoglobin
Glucose
Ketones
Microscopy of sediment
Nitrite
pH
Protein
Urobilinogen
Total volume
White blood cells

Treatment-related effects on urine chemistry were observed. As depicted in the Applicant’s table below, statistically BSF 4036516 and BSF 138315 caused a significant increase in mean value for urine volume, and/or pH. BSF 138315 in the presence and absence of BS 4036516 significantly ( $p \leq 0.05$ ) increased urine volume in males. In contrast, urine volume was significantly increased in females treated with BSF 138315 (14 mg/kg/day) only. In males treated with the MD and HD of BSF 4036516 and BSF 138315 in the absence and presence of BS 4036516, urine pH was significantly increased compared to control males. Urine pH was significantly increased only in the HD BSF 4036516 and 14 mg/kg/day BSF 138315 group only.

**Table 14 Selected urinalysis parameters (mean values) in week 4**

Parameter	Sex	BSF 4036516 (mg/kg/day)				BSF 138315 (mg/kg/day)	
		0	3.5	7	14	14 #	14
Volume (ml)	M	7.58	8.20	11.01	10.29	23.41*	24.20*
	F	4.25	7.73	9.08	6.20	8.80	10.53*
pH	M	6.4	6.6	6.9*	6.9*	7.2*	7.1*
	F	6.3	6.6	6.7	6.9*	6.8	7.3*
Triple phosphate (score)	M	1	2	2	1	1	0
	F	1	2	2	3+	2	2
Protein (score)	M	2	2	1	1	1	0+
	F	1	1	1	1	1	1
White cells (score)	M	1	1	1	1	0	0+
	F	0	0	0	0	0	0

# Additionally containing 2% BSF 4036516 equivalent to 0.28 mg/kg/day;  
 \*  $p \leq 0.05$  (Dunnett's test); +  $p \leq 0.05$  (Steel test)

**Gross pathology:**

*All moribund and surviving animals were anesthetized with Narcoren (sodium pentobarbital, 120 mg/kg, i.p.) and exsanguinated. Macroscopic examination was performed.*

Macroscopic findings are presented in the Applicant's table below. BSF 4036515-related macroscopic findings were observed in the liver and limb/tail of both male and female rats. Reduction in liver organ weight was dose-dependent and correlated to the reduced liver size. BSF 4036516 effects were more discernible in the males than the females. Evidence of self-mutilation was noted in both males and females. Results observed in the BSF 138315 group were qualitatively similar to that of BSF 4036515.

**Table 17 Macroscopic findings**

Macroscopic Findings	No. of affected animals / Total No. of animals									
	BSF 4036516						BSF 138315			
	3.5 mg/kg		7 mg/kg		14 mg/kg		14 mg/kg #		14 mg/kg	
	M	F	M	F	M	F	M	F	M	F
Reduced liver size	9/10	1/10	8/10	3/10	10/10	7/10	10/10	7/10	9/9	4/10
Forepaw injuries	0/10	0/10	0/10	0/10	3/10	1/10	1/10	2/10	1/9	5/10
Tail injuries	3/10	1/10	1/10	4/10	2/10	2/10	0/10	0/10	1/9	0/10
Thickened ribs	0/10	0/10	0/10	0/10	0/10	1/10	2/10	0/10	1/9	0/10

**Organ weights:**

*The absolute and relative weights of the following organs were measured at necropsy in all terminally sacrificed animals and recovery animal. Paired organs were weighed separately.*

Adrenals (2)	Ovaries (2)
Brain	Pituitary gland
Heart	Prostrate
Kidneys (2)	Spleen
Liver	Testes (2)
Mesenteric lymph node	Thymus

Treatment-related decrease in liver absolute, relative-to-body weight and relative-to-brain weight were noted in both males and females at all dose levels of BSF 4036516. This reduction in liver weight is correlated with the reduced liver size.

		Organ Weight: Percent of Control					
		BSF 4056516				BSF 138315	
Dose mg/kg/day →		0	3.5	7.0	14.0	14 <sup>A</sup>	14
Organ	Parameter						
Liver- Males	Absolute wt.	17.00 g	-21%*	-32%*	-40%*	-44%*	-41%*
	Relative-to body weight(%)		-16%*	-20%*	-29%*	-30%*	-28%*
	Relative-to-brain weight (%)		-26%*	-30%*	-39%*	-43%*	-40%*
Liver - Females	Absolute wt.	9.97	-11%*	-16%*	-19%*	-23%*	-19%*
	Relative-to body weight (%)		-9%*	-13%*	-11%*	-13%*	-12%*
	Relative-to-brain weight (%)		-12%*	-19%*	-20%*	-25%*	-20%*

\*: Statistically significant at p≤ 0.05 (Dunnett's test)

**Histopathology:**

Adequate Battery: yes (x), no ( )—explain

Peer review: yes (x), no ( )

The following tissues were collected from all animals at necropsy. These tissues were examined histologically from animals in groups 1 (0 mg/kg BSF 4036516), 4 (14 mg/kg BSF 4036516), 5 (BSF 138315 + BSF 4036516) and 5 (14 mg/kg BSF 138315). Due to changes observed at the highest dosage (Group 4), histopathological examination was extended to include the liver, lungs, skin and spleen from all animals administered 3.5 or 7 mg/kg/day BSF 4036516.

	BSF 4036516				BSF 138315 + BSF 4036516	BSF 138315
Dose (mg/kg) →	0	3.5	7.0	14.0	14 <sup>#</sup>	14
<b>Species</b>	<b>Rats</b>					
Adrenals	X	X	X	X	X	X
Aorta (thoracic)	X	X	X	X	X	X
Bone Marrow smear Sternum and Lt. humerus)	X	X	X	X	X	X
Bone (left humerus)	X	X	X	X	X	X
Brain	X	X	X	X	X	X
Cecum	X			X	X	X
Colon	X	X	X	X	X	X
Duodenum	X	X	X	X	X	X
Epididymis	X	X	X	X	X	X
Esophagus (middle part)	X	X	X	X	X	X
Eye (including optic nerves)	X	X	X	X	X	X
Gross lesions	X	X	X	X	X	X
Harderian gland	X	X	X	X	X	X
Heart (Atria with auricles)	X	X	X	X	X	X

	BSF 4036516				BSF 138315 + BSF 4036516	BSF 138315
Dose (mg/kg) →	0	3.5	7.0	14.0	14 <sup>#</sup>	14
Species	Rats					
and Lt. & Rt. ventricles)						
Ileum	X	X	X	X	X	X
Jejunum	X	X	X	X	X	X
Joint (elbow)	X	X	X	X	X	X
Kidneys	X	X	X	X	X	X
Liver (Lt and Rt lateral lobe; caudal lobe)	X	X	X	X	X	X
Lungs (Lt. & Rt. phrenic & cranial lobes)	X	X	X	X	X	X
Lymph nodes mandibular	X	X	X	X	X	X
Lymph nodes, mesenteric	X	X	X	X	X	X
Mammary Gland (caudal complexes)	X	X	X	X	X	X
Nasal cavity (incl. paranasal cavity)	X	X	X	X	X	X
Ovaries (and oviduct)	X	X	X	X	X	X
Pancreas (left lobe)	X	X	X	X	X	X
Parathyroid gland	X	X	X	X	X	X
Pituitary gland	X	X	X	X	X	X
Prostate (ventral, lateral and dorsal)	X	X	X	X	X	X
Rectum	X	X	X	X	X	X
Rib (bone-cartilage transitional zone)	X	X	X	X	X	X
Salivary gland (submandibular)	X			X	X	X
Sciatic nerve	X	X	X	X	X	X
Seminal vesicles	X	X	X	X	X	X
Skeletal muscle (Lt. & Rt. Semitendinosus; semimembranosus)	X	X	X	X	X	X
Skin (Inguinal region)	Xx	X	X	X	X	X
Spinal cord (Cervical, lumbar & thoracic)	X	X	X	X	X	X
Spleen	X	X	X	X	X	X
Sternum	X	X	X	X	X	X
Stomach (fore stomach, fundus & pylorus)	X	X	X	X	X	X
Testes	X	X	X	X	X	X
Thymus	X	X	X	X	X	X
Thyroid gland	X	X	X	X	X	X
Tongue	X	X	X	X	X	X
Urinary bladder	X			X	X	X
Uterus (Lt and Rt horn and body)	X	X	X	X	X	X
Vagina	X	X	X	X	X	X
Vena Cava (caudal)	X	X	X	X	X	X

*All tissues were fixed in phosphate-buffered neutral 4% formaldehyde solution with the exception of the eyes including optic nerves and Harderian glands that were fixed in Susa's fixative, and epididymides and testes were fixed in Bouin's fixative mixture. Tissues were stained with hematoxylin and eosin stain and examined histologically.*

**Macroscopic Findings. Macroscopic findings are tabulated below. Reduced liver size was observed in the all BSF 4036516 treatment groups, BSF 40366 plus BSF 4036516 and BSF 138315 only groups.**

Macroscopic Findings	№ of Affected Animals/Total № of Animals									
	BSF 4036516 (mg/kg/day)						BSF 138315 (mg/kg/day)			
	3.5		7.0		14.0		14.0A		14.0	
	M	F	M	F	M	F	M	F	M	F
Reduced Liver size	9/10	1/10	8/10	3/10	10/10	7/10	10/10	7/10	9/9	4/10
Forepaw Injuries	0/10	0/10	0/10	0/10	3/10	1/10	1/10	2/10	1/9	5/10
Tail Injuries	3/10	1/10	1/10	4/10	2/10	2/10	0/10	0/10	1/9	0/10
Thickened ribs	0/10	0/10	0/10	0/10	0/10	1/10	2/10	0/10	1/9	0/10

Liver. Hepatocellular glycogen was the primary finding observed in the liver of males, scored as minimal to moderate severity, with dosing  $\geq 3.5$  mg/kg/day. According to the Applicant, there was no significant difference in respect of hepatocellular glycogen levels between animals given BSF 4036516 alone and those given BSF 4036516 combined with BSF 138315 or those given BSF 138315 alone. The incidence of hepatocellular glycogen in females was minimal. It was also noted that females administered HD of BSF 4036516, BSF 4036516 combined with BSF 138315 or BSF 138315 alone had significantly lower amount of periportal fat compared to controls. These microscopic findings correlate with the reduced relative liver weight.

Microscopic Findings		№ of Animals/Total № of Animal					
		BSF 4036516 (mg/kg/day)				BSF 138315 (mg/kg/day)	
		0	3.5	7.0	14.0	14A	14.0
Liver							
Hepatocellular Glycogen Content	Minimal	M: 1/10 F: 6/10	M: 3/10 F: 0/10	M: 6/10 F: 0/10	M: 3/10 F: 1/10	M: 0/10 F: 0/10	M: 0/10 F: 1/10
	Slight	M: 5/10 F: 3/10	M: 2/10 F: 0/10	M: 1/10 F: 0/10	M: 0/10 F: 0/10	M: 0/10 F: 0/10	M: 0/10 F: 0/10
	Moderate	M: 3/10	M: 1/10	M: 1/10	M: 0/10	M: 0/10	M: 0/10
Periportal Fat Vacuolation	Minimal	F: 6/10	F: 7/10	F: 4/10	F: 5/10	F: 5/10	F: 6/10

**Tail.** Ulceration, amputated tip, epithelial crust formation or focal dermatitis was observed in the tail of both males and females in the HD group of BSF 4036516 or males in the BSF 138315 alone group.

**Study title:** (b) (4) **degradant: A 4-week oral toxicity study in rats followed by a 4-week recovery period (Study TOX8686)**

**Key study findings:** (b) (4) degradant (0, 0.274, 1.37 and 2.74 mg/kg/day) was orally administered to rats for 4 weeks with the following results.

1. (b) (4) was detected in the serum as early as 15 minutes and as late as 240-minutes after dosing.
2. (b) (4) degradant had no effects on clinical signs, eye examination, and clinical pathology parameters.
3. No treatment-related macroscopic or microscopic findings were observed.
4. The NOAEL was identified by the Applicant and to which the reviewer is in agreement as 2.74 mg/kg/day.

**Study №:** 1033-036  
**Volume #, and page #:** eCTD submission  
**Conducting laboratory and location:** (b) (4)  
**Date of study initiation:** December 26, 2007  
**GLP compliance:** Yes  
**QA report:** yes ( x ) no ( )  
**Drug, lot #, and % purity:** (b) (4) Batch № CS07-05-45, 95.2% purity

**Methods**

Doses: 0, 0.274, 1.37 and 2.74 mg/kg/day  
 Species/strain: Rat/CD® [CrI:CD®(SD)]  
 Number/sex/group or time point (main study): 10/sex/group  
 Route, formulation, volume, and infusion rate: Oral, solution, 10 mL/kg

Group	Test Article	Dosage Level (mg/kg/day)	Dosage Concentration (mg/mL)	Dosage Volume (mL/kg)	Total Number of Animals		Sacrificed after treatment period		Sacrificed after 4-wk recovery period	
					Females	Males	F	M	F	M
<b>Main Study</b>										
<b>1</b>	(b) (4)	0	0	10.0	15	15	10	10	5	5
<b>2</b>	(b) (4)	0.274	0.0274	10.0	10	10	-	-	-	-

Group	Test Article	Dosage Level (mg/kg/day)	Dosage Concentration (mg/mL)	Dosage Volume (mL/kg)	Total Number of Animals		Sacrificed after treatment period		Sacrificed after 4-wk recovery period	
					Females	Males	F	M	F	M
	(b) (4)									
3	(b) (4)	1.37	0.137	10.0	10	10	-	-	-	-
4	(b) (4)	2.74	0.274	10.0	15	15	10	10	5	5
<b>TK Study</b>										
5	(b) (4)	0	0	10	6	6				
6	(b) (4)	0.274	0.0274	10.0	12	12				
7	(b) (4)	1.37	0.137	10.0	12	12				
8	(b) (4)	2.74	0.274	10.0	12	12				

Satellite groups used for toxicokinetics or recovery: Satellite group were included for toxicokinetic analysis; separate recovery animals included for recovery groups in this study.

Age: 7 weeks of age (initiation of dosing)

Weight: Females: 149-213 g; Males: 174-223 g (initiation of dosing)

Sampling times:

Unique study design or methodology (if any): None

**Observations and times: (these parameters can be captured separately here or described in connection with each endpoint under the results section.)**

The observation parameters were captured under the results section.

**Results**

**Toxicokinetics:**

Blood samples (approx. 0.5 to 1.0 mL) were collected via the orbital sinus after carbon dioxide/oxygen inhalation for toxicokinetic evaluation at pre-dose, 15, 30, 60, 120 and 240 minutes after dosing on days 1 and 28. Plasma was collected from the vehicle group prior to dosing and at 15 minutes post-dosing on days 1 and 28. Three animals were examined per time point. An additional plasma sample was collected from three extra toxicokinetic animals/sex/group at 24-hours post-dosing on days 1 and 28. Plasma concentration of (b) (4) was determined by high performance liquid chromatography coupled to a Tandem Mass Spectrometric Detector (LC/MS/MS). The lower limit of quantitation was BLQ, <1 ng/mL.

Two hundred and seventy-five samples were analyzed; only 55 of these samples had detectable serum levels of (b) (4). The number of samples with detectable levels of (b) (4) increased with increasing dose. Mean plasma concentration of (b) (4) is presented in the tables below. (b) (4) was detected in the serum within 15-minutes after dosing and still detectable 240-minutes after dosing in several animals. Toxicokinetic parameters were not calculated.

Mean ( $\pm$ SD)		(b) (4) Degradant Serum Concentrations						
Dose (mg/kg/day)		Post-dosing Time (hrs)						
		0	0.25	0.50	1	2	4	24
<b>Day 1</b>								
0	M	0 $\pm$ 0	0 $\pm$ 0	-	-	-	-	-
	F	0 $\pm$ 0	0 $\pm$ 0	-	-	-	-	-
0.274	M	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
	F	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
1.37	M	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0.747 $\pm$ 0.648	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
	F	0 $\pm$ 0	0.367 $\pm$ 0.635	0 $\pm$ 0	0.377 $\pm$ 0.652	0.357 $\pm$ 0.618	0 $\pm$ 0	0 $\pm$ 0
2.74	M	0 $\pm$ 0	1.51 $\pm$ 0.690	1.15 $\pm$ 0.101	1.28 $\pm$ 0.197	1.44 $\pm$ 0.304	0.730 $\pm$ 0.638	0 $\pm$ 0
	F	0 $\pm$ 0	1.25 $\pm$ 0.208	1.08 $\pm$ 0.0794	1.48 $\pm$ 0.346	0.750 $\pm$ 0.650	0.680 $\pm$ 0.589	0 $\pm$ 0

Mean ( $\pm$ SD)		(b) (4) Degradant Serum Concentrations						
Dose (mg/kg/day)		Post-dosing Time (hrs)						
		0	0.25	0.50	1	2	4	24
<b>Day 28</b>								
0	M	0 $\pm$ 0	0 $\pm$ 0	-	-	-	-	-
	F	0 $\pm$ 0	0 $\pm$ 0	-	-	-	-	-
0.274	M	0 $\pm$ 0	0 $\pm$ 0	0.893 $\pm$ 1.55	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
	F	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
1.37	M	0 $\pm$ 0	1.52 $\pm$ 1.83	0 $\pm$ 0	0 $\pm$ 0	0.823 $\pm$ 0.745	0 $\pm$ 0	0 $\pm$ 0
	F	0 $\pm$ 0	1.24 $\pm$ 2.15	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
2.74	M	0 $\pm$ 0	0.903 $\pm$ 0.826	0.687 $\pm$ 0.595	0.850 $\pm$ 0.744	0.903 $\pm$ 0.782	0.357 $\pm$ 0.618	0 $\pm$ 0
	F	0 $\pm$ 0	3.22 $\pm$ 1.99	1.54 $\pm$ 0.295	0.350 $\pm$ 0.606	0.417 $\pm$ 0.722	0 $\pm$ 0	0 $\pm$ 0

### **Mortality:**

*All animals were observed for morbidity and mortality at least twice daily throughout the duration of the study.*

No treatment-related deaths occurred during the course of the study. However, one female in the MD (1.37 mg/day) toxicokinetic group was found dead on day 2 following the TK blood draw (prior to dosing). This death was considered to be related to the blood collection procedure (the reviewer concurs with the Applicant).

### **Clinical signs:**

*The animals were examined at 15 minutes post-dosing on day 1 and at least once during each study week. During the recovery period, behavior and general health assessment was performed weekly. The assessment included, but were not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, and nervous system effects including tremors, convulsions, reactivity to handling, and bizarre behavior.*

No treatment-related clinical signs occurred during the course of the study.

### **Body weights:**

*Body weights were recorded prior to randomization and weekly during the study for all animals. Body weights were measured and recorded weekly during the recovery period.*

There were no statistically significant differences in group mean body weights in males or females treated with (b) (4) compared to vehicle treated control animals. However a statistically significant decrease in body weight was noted between the high dose (b) (4) group and control at week 6 during the recovery phase. Relative to control, body weights were significantly decreased (7.4%) for female rats at 2.74 mg/kg/day

### **Food consumption:**

*Food consumption was recorded weekly during the study. Food consumption was not conducted on toxicokinetic animals.*

There were no treatment related effects on food consumption in (b) (4) the treated groups compared to the vehicle treated group. However a statistically significant decrease in food consumption was noted between the high dose (b) (4) group and control during weeks 6 and 7 of the recovery phase. Relative to control, food consumption was significantly decreased by 14.4% and 11.4% during week 6 and week 7, respectively, for female rats at 2.74 mg/kg/day

### **Ophthalmoscopy:**

*Eyes of all animals were examined at the terminal sacrifice. Details of the ophthalmological examination were not described.*

No treatment-related effects were observed.

**EKG:**

*Not performed.*

**Hematology:**

*Blood (approx. 4 mL) was collected from the vena cava after carbon dioxide inhalation. Animals were fasted overnight prior to blood collection. Blood was collected prior to the terminal and recovery necropsies. The following hematology parameters were examined from the main study (n=10/sex/group) and recovery animals (5/sex/group; control and HD groups):*

Hematology Parameters	
White Blood Cell Parameters	Red Blood Cell Parameters
Leukocyte count (WBC)	Erythrocyte count (RBC)
Differential leukocyte count (Absolute)	Red Cell Morphology
- Neutrophil (NEUT)	- Normal
- Monocyte (MONO)	- Microcytosis
- Lymphocyte (LYM)	- Macrocytosis
- Eosinophil (EOS)	- Anisocytosis
- Basophil (BASO)	- Hypochromic
- Large Unstained cells	
	Hemoglobin (HGB)
	Hematocrit (HCT)
	Mean corpuscular volume (MCV)
	Mean corpuscular hemoglobin (MCH)
	Mean corpuscular hemoglobin concentration (MCHC)
	Absolute reticulocyte Count (RETI)
	Percent reticulocyt
	Platelet Count (PLT)

No treatment-related effects were observed on hematology parameters at termination or recovery. However a statistically significant decrease in neutrophils was noted between the high dose (b) (4) group and control at the terminal sacrifice. Relative to control, the mean neutrophil count was significantly decreased by 41.6% in males at 1.37 mg/kg/day. The effect on neutrophils appeared not to be toxicologically relevant; a dose-dependent relationship was not demonstrated.

**Coagulation:**

*Blood (approx. 4 mL) was collected from the vena cava after carbon dioxide inhalation. Animals were fasted overnight prior to blood collection. Blood was collected prior to the terminal and recovery necropsies. The following coagulation parameters were examined were examined in the main study (n=10/sex/group) and recovery animals (5/sex/group; control and HD groups):*

Coagulation Parameters	
Activated partial thromboplastin Time (APTT)	
Prothrombin Time (PT)	
Fibrinogen	

There were no statistically significant differences in the coagulation parameters assessed in the (b) (4) treated animals compared to the vehicle treated animals.

**Clinical chemistry:**

*Blood was collected from the vena cava after carbon dioxide inhalation. Animals were fasted overnight prior to blood collection. Blood was collected prior to the terminal and recovery necropsies. The following parameters were examined*

Serum Chemistry Parameters	
Alanine aminotransferase (ALT)	Phosphorus
Albumin (ALB)	Potassium (K)
Aspartate aminotransferase (AST)	Sodium (Na)
Albumin/globulin ratio (AGR)	Sorbitol Dehydrogenase
Alkaline phosphatase (ALP)	Total bilirubin (TBIL)
Calcium (Ca)	Urea nitrogen
Chloride (Cl)	Total Protein (TP)
Cholesterol (CHOL)	Triglycerides
Creatinine (CREA)	
Gamma Glutamyltransferase (GGT)	
Glucose (GLU)	
Globulin (GLOB)	

There were no statistically significant differences in the clinical chemistry parameters assessed in the (b) (4) treated animals compared to the vehicle treated animals.

**Urinalysis:**

*Urine was collected from main study animals prior to the terminal and recovery necropsies (10/sex/group and 5/sex/group, respectively). The animals were housed in stainless steel metabolism cages and urine was collected for at least 16 hours. The following parameters were examined:*

Urine Parameters	
Urinalysis	
Appearance: Color and appearance	
Bilirubin	
Glucose	
Ketones	
Microscopy of spun deposit	
Occult blood	
pH	

Urine Parameters	
Urinalysis	
Protein	
Specific gravity	
Total volume	
Urobilinogen	

There were no statistically significant differences in the urine parameters assessed in the (b) (4) treated animals compared to the vehicle treated animals.

**Gross pathology:**

*Surviving animals were euthanized by carbon dioxide inhalation followed by exsanguinations via the abdominal vena cava. Macroscopic evaluation was performed on all orifices, external surface of the body, cranial cavity, and organs, thoracic, abdominal, and pelvic cavities and viscera.*

There were no drug-related macroscopic changes in the (b) (4) treated animals compared to the vehicle control animals.

**Organ weights:**

*The absolute and relative weights of the following organs were measured at necropsy in all terminally sacrificed animals and recovery animals. Paired organs were weighed together. The thyroid gland with the bilateral parathyroid glands was weighed together. Also, the right mandibular/sublingual salivary glands were weighed together. The thyroid/parathyroid gland and pituitary gland were weighed following fixation.*

Adrenals (2)	Ovaries (2)
Brain	Pituitary glands
Epididymis	Salivary gland, mandibular/sublingual
Heart	Spleen
Kidneys (2)	Testes (2)
Liver	Thymus
Lung with bronchi	Thyroid/parathyroid (2)

Statistically significant changes noted in organ weight are summarized in the table below. These changes in organ weight at terminal necropsy are not considered to be treatment-related. These statistically significant changes are <20%; thus they are considered incidental/not toxicologically significant.

		Percent Control Changes in Organ Weight			
		Males: Terminal Sacrifice			
Organ	Dose mg/kg/day	0	0.274	1.37	2.74
Thymus	Parameters				
	Absolute	0.506 ± 0.119	+7.9%	+3.6%	+25.5%*
	Relative-to-body weigh (Thymus g/Bwt %)	0.1448 ± 0.0343	+6.8%	+4.21%	+24.8%*

Percent Control Changes in Organ Weight					
Males: Terminal Sacrifice					
Organ	Dose mg/kg/day	0	0.274	1.37	2.74
	Relative-to-brain weight (Thymus g/Br Wt ratio)	0.2760 ± 0.0992	+4.0%	-5.0%	+18.2%
Females: Terminal Sacrifice					
Brain	Absolute	1.734 ± 0.100	+4.6%	+4.7%	+6.6%*
	Relative-to-body weigh (Brain g/Bwt %t)	0.7411 ± 0.0491	+5.3%	+6.1%	+6.0%
*: Significant different from control value, p ≤ 0.05					

**Histopathology:**

*Adequate Battery:* yes (X), no ( )—explain

*Peer review:* yes (X), no ( )

*The following tissues (Applicant’s table) were collected from all main study animals and recovery animals at necropsy. Histopathological evaluation was conducted on all main study animals at the end of the treatment period. According to the Applicant, all tissues from at least 3 male and 3 female rats were randomly selected from the control groups and 3 females and 3 males were randomly selected from the high dose group to be peer reviewed. The mandibular and sublingual salivary glands and the thymus were examined from all animals in all dose groups.*

- 
- 
- |  |   |
|--|---|
| <ul style="list-style-type: none"> <li>- Adrenal (2)*</li> <li>- Aorta</li> <li>- Bone with marrow [femur]</li> <li>- Bone with marrow [sternum]</li> <li>- Bone marrow smear [2 collected]<sup>a</sup></li> <li>- Brain [cerebrum, midbrain, cerebellum, medulla/pons]*</li> <li>- Epididymis (2)*</li> <li>- Eye including optic nerve (2)</li> <li>- Gastrointestinal tract:             <ul style="list-style-type: none"> <li>esophagus</li> <li>stomach [glandular and nonglandular]</li> <li>duodenum</li> <li>jejunum</li> <li>ileum</li> <li>cecum</li> <li>colon</li> <li>rectum</li> </ul> </li> <li>- Gonads:             <ul style="list-style-type: none"> <li>ovary* with oviduct (2)</li> <li>testis (2)*</li> </ul> </li> <li>- Gross lesions</li> <li>- Heart*</li> <li>- Joint, tibiofemoral</li> <li>- Kidney (2)*</li> <li>- Lacrimal gland, exorbital (2)</li> <li>- Larynx</li> </ul> | <ul style="list-style-type: none"> <li>- Liver [3 sections collected; 2 examined]*</li> <li>- Lung with bronchi [collected whole; 2 sections examined]*</li> <li>- Lymph nodes: mandibular, mesenteric and popliteal</li> <li>- Mammary gland</li> <li>- Pancreas</li> <li>- Peyer's patch</li> <li>- Pituitary*</li> <li>- Prostate with seminal vesicle (2)</li> <li>- Salivary gland, mandibular/sublingual [2 collected; 1 examined]*<sup>b</sup></li> <li>- Salivary gland, parotid [2 collected; 1 examined]</li> <li>- Sciatic nerve (2)</li> <li>- Skeletal muscle, biceps femoris</li> <li>- Skin</li> <li>- Spinal cord [cervical, thoracic, and lumbar]</li> <li>- Spleen*</li> <li>- Thymus*</li> <li>- Thyroid/parathyroid (2)*</li> <li>- Tongue</li> <li>- Trachea</li> <li>- Ureter (2)</li> <li>- Urinary bladder</li> <li>- Uterus [both horns]/Cervix</li> <li>- Vagina</li> </ul> |
|--|---|
- 

<sup>a</sup>Bone marrow smears were collected at the scheduled necropsy and held.

<sup>b</sup>The combined weight of the right mandibular/sublingual salivary gland was obtained.

(2) Paired organ

\*Weighed organ

All tissues were fixed in neutral buffered formalin with the exception of the eyes (including optic nerve) and testes that were fixed in a modified Davidson's fixative. The lung via the trachea and the urinary bladder were infused with formalin. The pituitary was fixed in situ. Tissues were stained with hematoxylin and eosin stain and examined histologically.

No treatment-related findings were observed at end of the 4-week dosing period. However, as described below, some microscopic findings were noted in the liver, lungs and thymus.

**Hepatocellular vacuolation** compatible with lipid overload was observed in female rats at all doses including controls, without dose-relationship. The incidence in females was 3/10, 5/10, 8/10 and 6/10 at dose levels of 0, 0.274, 1.37 and 2.74 mg/kg/day, respectively. Severity ranged from minimal to mild. This observed microscopic change in female rats was considered incidental and not treatment-related or toxicologically relevant because a dose-dependent relationship was not demonstrated.

**Microscopic findings were observed in the lungs** in both males and female rats. Pulmonary hemorrhage associated with alveolar histiocytosis and acute inflammation was observed in female rats at 1.37 and 2.74 mg/kg/day. In males, pulmonary hemorrhage associated with alveolar histiocytosis and acute inflammation or only with an alveolar histiocytosis was observed in males at 0.274 and 2.74 mg/kg/day. These pulmonary microscopic changes were considered incidental and not treatment-related.

**Thymic lymphoid depletion** was observed in both male and female rats at all doses including controls without dose-relationship. The incidence in males was 3/10, 5/10, 2/10 and 7/10 at dose levels of 0, 0.274, 1.37 and 2.74 mg/kg/day, respectively. In females, the incidence was 3/10, 5/10, 2/10 and 7/10 at dose levels of 0, 0.274, 1.37 and 2.74 mg/kg/day, respectively. Severity of thymic lymphoid depletion was minimal in both females and males at all dose groups. This observed microscopic change was considered incidental and not treatment-related or toxicologically relevant because a dose-dependent relationship was not demonstrated and the microscopic finding was not associated with a decrease in organ weight.

Observation	№ of Animals Examined →	Dose (mg/kg/day)			
		0 10	0.274 10	1.37 10	2.74 10
<b>Females</b>					
<b>Liver</b>					
Within Normal Limits		2	2	0	0
Hepatocellular Vacuolation	Total	3	5	8	6
	Minimal	3	5	5	6
	Mild	2	2	0	1
<b>Lungs</b>					
Within Normal Limit		10	9	6	6
Hemorrhage	Minimal	0	1	1	4
Histocytosis, alveolar	Minimal	0	0	4	4
Inflammation, lymphoid, perivascular	Minimal	0	0	0	2
Inflammation, acute	Minimal	0	0	1	2
<b>Thymus</b>					
Within Normal Limit		6	5	8	3
Depletion, lymphoid	Minimal	3	5	2	7
Cyst		1	0	0	0
<b>Male</b>					
<b>Lungs</b>					
Within Normal Limit		7	5	8	7
Inflammation, acute	Minimal	0	1	1	0
<b>Thymus</b>					
Within Normal Limit		5	5	7	4
Depletion, lymphoid	Minimal	5	4	3	6

**2.6.6.4 Genetic toxicology**

**Study title: Bacterial reverse mutation assay.**

**Key findings:** (b) (4) was evaluated in the Ames Reverse Mutation Assay at concentrations of 15, 50, 150, 500, 1500 and 5000 µg/plate. Under the conditions of the study, (b) (4) was negative in the bacterial reverse mutation assay.

**Study №:** AA85YD.503 (b) (4)  
**Volume #, and page #:** eCTD submission  
**Conducting laboratory and location:** (b) (4)  
**Date of study initiation:** January 15, 2004  
**GLP compliance:** Yes  
**QA reports:** yes ( x ) no ( )  
**Drug, lot #, and % purity:** (b) (4) Lot № B5586P44-R, 98% purity

**Methods**

Strains/species/cell line: *Salmonella typhimurium* TA100, TA1535, TA98, and TA1537 and *Escherichia coli* WP2uvrA

Doses used in definitive study: 15, 50, 150, 500, 1500 and 5000 µg/plate.

Basis of dose selection: A preliminary dose-range finding toxicity-mutation assay was conducted to establish the (b) (4) doses to be used for the definitive experiments. Using the test strains TA98, TA100, TA1535, TA1537 and WP2uvrA in the presence and absence of S9 mix, 8 concentrations of (b) (4) (2.5, 7.5, 25, 75, 200, 600, 1800, 1000, and 5000 µg/plate) were assayed. No precipitation of the drug was observed up to 5000 µg/plate. (b) (4) was toxic in the preliminary assay at dose levels ≥ 600 µg/plate in the TA1535 strain in the presence of S9 mix, at ≥ 1800 µg/plate in TA98, T100, TA1535, TA1537 and WP2uvrA strains in the absence of S9 mix. In the presence of S9 mix, toxicity was observed at ≥ 1800 µg/plate in TA1535, TA1537 and WP2uvrA.

Negative controls: Vehicle (sterile distilled water)

Positive controls: 2-nitrofluorene (2-NF), sodium azide (NaAZ), methyl methane sulfonate (MMS), 9-aminoacridine (9AA), and 2-aminoanthracene (2AA) were selected for positive controls based on the bacterial strain as indicated in the following table:

Test Strain	Positive Control Substance (µg/plate)	
	-S9	+S9
TA100	NaAZ (1.0)	2AA (10.0)
TA1535	NaAZ (1.0)	2AA (10.0)
TA98	2-NF (1.0)	2AA (10.0)

<b>TA1537</b>	9AA (75.0)	2AA (10.0)
<b>WP2uvrA</b>	MMS (1,000.0)	2AA (10.0)

Incubation and sampling times: The mutagenicity test was performed according to the plate-incorporation procedures. The S9 metabolic activator, the tester strain, and the test article were combined in molten agar which is overlaid onto the minimal agar plate. The agar plate was incubated at 37°C for 48 to 72 hours. The vehicle control, positive control and all doses of the test article were plated in triplicate.

Analysis:

- Mutation frequencies: Expressed as mean number and standard deviation of revertants per plates.
- Cytotoxicity: Background lawn was observed using a dissecting microscope. A dose level is considered toxic if it causes a >50% reduction in the mean number of revertants per plate relative to the mean vehicle control value. The reduction in mean revertants must be accompanied by an abrupt dose-dependent drop in the revertant count.

Criteria for positive results: Criteria for positive results included:

- a reproducible 2-fold increase for strains TA98, TA100 and WP2uvrA or 3-fold increase for strains TA1535 and TA1537 in the number of revertants compared with the vehicle controls, in any strain at any dose-level and/or evidence of a dose-relationship and reference to historical control data was taken into consideration when evaluating the study results.
- a dose-dependent increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations.

## Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The study appears to be valid for the following reasons: 1) the appropriate strains were tested, 2) the appropriate controls were used, and 3) the positive control substances produced reliable results. The assay methods, positive and negative controls, and the concentrations of drug used for the definitive study were adequate.

Study outcome: (b) (4) was evaluated at the following concentrations: 15, 50, 150, 500, 1500 and 5000 µg/plate in the mutagenicity assay. In the mutagenicity assay, the positive controls induced mutation frequencies as expected; the mean revertants per plate were within historical control data for the laboratory. Compared to the vehicle control groups, (b) (4) did not increase the number of revertants for the *Salmonella typhimurium* strains (TA987, TA100, TA1535 or TA1537) or the *Escherichia coli* test strain (WP2uvrA) in the presence or absence of the metabolic activator. (b) (4) did not precipitate out of the media or interfere with colony counting. At a concentration of 1500 µg/plate, toxicity was observed in TA1535 (reduction of colonies by 58%) and WP2uvrA (reduction of colonies by 70%) in the absence of metabolic activation and in TA98 (reduction of colonies by 58%) in the presence of metabolic activation. Relative to

the vehicle control, 100% reduction of colonies were observed at 5000 µg/plate for test strains TA98, TA100, TA1536, TA1537 and WP2<sup>uvrA</sup> in the presence and absence of S9 mix.

**Study title: In vitro mammalian chromosome aberration test.**

**Key findings:** Under the conditions of this assay, (b) (4) was not clastogenic in the human lymphocyte cultures in vitro in the presence or absence of metabolic activation.

**Study no.:** AA85YD.341 (b) (4)  
**Volume #, and page #:** eCTD submission  
**Conducting laboratory and location:** (b) (4)  
**Date of study initiation:** January 15, 2004  
**GLP compliance:** Yes  
**QA reports:** yes ( x ) no ( )  
**Drug, lot #, and % purity:** (b) (4), Lot No B5586P44-R, 92% purity

**Methods**

Strains/species/cell line: Peripheral blood lymphocytes were obtained from healthy non-smoking 45 years old adult females for the preliminary toxicity assay. For the definitive assay, peripheral blood lymphocytes were obtained from healthy non-smoking adult females.

Doses used in definitive study:

Treatment Condition	Treatment Time (hr)	Recovery Time (hr)	Dose (µg/mL)
Non-activate	4	16	1.25, 2.5, 5, 35, 50, 65, 75
	20	0	1.25, 2.5, 5, 10, 15, 20, 25
S9 activated	4	16	5, 10, 20, 35, 50, 65, 75

Basis of dose selection: Dose selection was based on the results from the preliminary toxicity assay. It was based on reduction of mitotic index relative to solvent control at (b) (4) concentrations of 0.32, 0.96, 3.2, 9.6, 32, 96, 320, 960 and 3200 µg/mL. Substantial toxicity, as defined by at least 50% growth inhibition (i.e., reduction in mitotic index) relative to the solvent control, was observed at dose levels ≥ 32 µg/mL in both the 4 and 20 hour exposure condition without metabolic activation; and at dose levels ≥96 µg/mL in the 4 hour exposure condition in the presence of metabolic activation. The highest concentration selected for the chromosomal aberration test was the dose that caused at least 50% inhibition of mitotic inhibition relative to the solvent control.

Negative controls: Water

Positive controls: Mitomycin C (MMC) and cyclophosphamide (CP) were positive controls for the chromosomal aberration test. In both the 4 and 20 hour exposure condition without metabolic activation, MMC was used as positive control after dissolution in sterile distilled water to a final concentration of 0.3 and 0.6 µg/mL. CP served as the positive control for the 4 hour exposure condition with metabolic activation after dissolution in sterile distilled water to a fixed concentration of 200 and 400 µg/mL.

Incubation and sampling times: For the definitive chromosome aberration assay cells were exposed to metabolic activation for 4 hours or no metabolic activation for 4 or 20 hours. In the non-metabolic activated study, the cells were exposed to solvent alone and (b) (4) at concentrations of 1.25, 2.5, 5, 35, 50, 65 and 75 µg/mL for 4 hours and at concentrations of 1.25, 2.5, 5, 10, 15, 20 and 25 µg/mL for 20 hours at 37°C in a humidified atmosphere of 5±1% CO<sub>2</sub> in air. In the metabolic activated study, the cells were exposed to solvent vehicle and (b) (4) at concentrations of 5, 10, 20, 35, 50, 65 and 75 µg/mL for 4 hours at 37°C in a humidified atmosphere of 5±1% CO<sub>2</sub> in air. After the 4 exposure period, the treatment medium was removed, the cells washed with calcium and magnesium-free phosphate buffered saline (CMF-PBS), refed with complete medium containing 2% PHA and returned to the incubated for an additional 16 hours. In the 20 hour exposure group, treatment was continuous until the time of cell collection. Two hours prior to the scheduled cell harvest at 20 hours after treatment initiation, Colcemid<sup>®</sup> (0.1 µg/mL) was added to the cultures. After the 2 hour exposure to Colcemid<sup>®</sup>, metaphase cells were harvested by centrifugation. Cells were collected by centrifugation, fixed, stained and the number of mitosis per 500 cells scored was determined. Isolated cells were fixed on slides for analysis.

## Results

Study validity: According to the Applicant, the results were judged positive if: 1) the percentages of cells with aberrations were increased in a dose-dependent manner with one or more concentrations being statistical significantly higher in the test article treatment groups relative to the solvent control group ( $p \leq 0.05$ ), and 2) dose-dependency or reproducibility could be conformed. *Counting Method* – Under blind condition, 100 well-spread metaphases per slide (200 metaphase per dose) were analyzed. Chromosomal aberrations were recorded as chromatid-type (i.e., chromatid and isochromatid breaks and exchanges figures) or chromosome-type aberrations (chromosome breaks and exchanges such as dicentrics and rings). *Validity* – the study appears valid as the appropriate positive controls were used, an acceptable number of mitotic cells were evaluated, duplicate negative and positive controls were used, approximately 200 metaphase spreads per treatment were scored, the frequency of cells with structural chromosome aberrations in the solvent controls were within the historical control range for solvent control, and the protocol was consistent with those described in the OECD Guideline 473: *In vitro* Mammalian Chromosome Aberration Test.

Study outcome: Results from the definitive chromosomal aberration tests are presented in the Applicant's table 7 reproduced below. In comparison to the solvent control, (b) (4) was void of significant clastogenic activity in the 4 hour exposure condition

in both the absence and presence of metabolic activation (Applicant's table 5) and the 20 hour exposure condition in the absence of metabolic activation. There were no statistically significant increases in the percentage of cells with aberrations over the concurrent controls in either study condition. At the highest concentration evaluated, (b) (4) was cytotoxic as suggested by a mitotic index of just below 5 (50%). The positive controls induced statistically significant increases in structural aberrations over the solvent control in the presence and absence of S9 mix.

TABLE 7  
SUMMARY

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations	
				Numerical	Structural	Numerical (%)	Structural (%)		
Water	-S9	4	9.1	200	200	0.000	±0.000	0.0	0.0
(b) (4)									
1.25	-S9	4	8.7	200	200	0.005	±0.071	0.0	0.5
2.5	-S9	4	7.0	200	200	0.000	±0.000	0.0	0.0
5	-S9	4	4.5	200	200	0.000	±0.000	0.0	0.0
MMC, 0.6	-S9	4	5.9	200	100	0.230	±0.468	0.0	21.0**
Water	+S9	4	9.5	200	200	0.000	±0.000	0.0	0.0
(b) (4)									
5	+S9	4	8.9	200	200	0.000	±0.000	0.0	0.0
10	+S9	4	7.7	200	200	0.000	±0.000	0.5	0.0
20	+S9	4	4.6	200	200	0.010	±0.100	0.0	1.0
CP, 20	+S9	4	6.6	200	200	0.155	±0.402	0.0	14.0**
Water	-S9	20	10.5	200	200	0.000	±0.000	0.0	0.0
(b) (4)									
2.5	-S9	20	8.9	200	200	0.000	±0.000	0.0	0.0
5	-S9	20	8.5	200	200	0.010	±0.100	0.0	1.0
10	-S9	20	5.0	200	200	0.010	±0.100	0.0	1.0
MMC, 0.3	-S9	20	6.2	200	100	0.290	±0.518	0.0	26.0**

**Treatment:** Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.

**Aberrations per Cell:** Severely damaged cells were counted as 10 aberrations.

**Percent Aberrant Cells:** \*, p≤0.05; \*\*, p≤0.01; using the Fisher's exact test.

**Study title: BSF 138315 – *Salmonella typhimurium* reverse mutation assay.**

**Key findings:** Hydromorphone (BSF 138315) was evaluated in the Ames Reverse Mutation Assay at concentrations of 33, 100, 333, 1000, 2500 and 5000 µg/plate. Under the conditions of the study, hydromorphone was negative in the bacterial reverse mutation assay.

**Study no.:** MPF/DT 0041 E

**Volume #, and page #:** eCTD submission

**Conducting laboratory and location:** [REDACTED] (b) (4)

**Date of study initiation:** December 13, 2000

**GLP compliance:** Yes

**QA reports:** yes ( x ) no ( )

**Drug, lot #, and % purity:** BSF 138315 (Hydromorphone-HCl), Batch No L0001974, 99.3%

**Methods**

Strains/species/cell line: *Salmonella typhimurium* TA100, TA102, TA1535, TA98, and TA1537

Doses used in definitive study: 33, 100, 333, 1000, 2500 and 5000 µg/plate.

Basis of dose selection: Doses were selected based on the results obtained in Knoll study report No MPF/WT 9352.

Negative controls: water (solvent)

Positive controls: Sodium azide (NaN<sub>3</sub>), 4-nitro-phenylene-diamine (4-NOPD), methyl methane sulfonate (MMS), and 2-aminoanthracene (2AA) were selected for positive controls based on the bacterial strain as indicated in the following table:

Test Strain	Positive Control Substance (µg/plate)	
	-S9	+S9
TA100	NaN <sub>3</sub> (10.0)	2AA (2.5)
TA1535	NaN <sub>3</sub> (10.0)	2AA (2.5)
TA98	4-NOPD (10.0)	2AA (2.5)
TA1537	4-NOPD (50)	2AA (2.5)
TA102	MMS (5.0)	2AA (10.0)

Incubation and sampling times: The mutagenicity test was performed according to the plate-incorporation procedures. The S9 metabolic activator, the tester strain, and the test article were mixed in a test tube and incubated at 37°C for 60 minutes. After pre-

incubation, 2.0 mL overlay agar (45°C) was added to each test tube; the mixture was then poured on minimal agar plates. After solidification the agar plates were incubated at 37°C for 48 hours. The vehicle control, positive control and all doses of the test articles were plated in triplicate.

Analysis:

- Mutation frequencies: Expressed as mean number of revertants per plates.
- Cytotoxicity: Background lawn was observed macroscopically or with a stereoscope (x 40).
- Counting Method: The number of revertant colonies was counted using the AUTOCOUNT. To check the proper function of the counter, parts of the colonies were counted manually.

Criteria for positive results: “The results were judged positive (+) if

- a biologically relevant and dose-dependent increase in the number of revertant colonies is induced.
- the number of reversions is at least twice the spontaneous reversion rate in strains TA98, TA100 and TA102 or thrice in strains TA1535 and TA1537.
- the results showed a dose-dependent and reproducible increase in the number of revertants is regarded as an indication of possibly existing mutagenic potential of the test article regardless whether the highest dose increased the number of revertant colonies.”

## Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The assay methods, positive and negative controls, and the concentrations of drug used for the definitive study were adequate. The study appears to be valid for the following reasons: 1) the appropriate strains were tested, 2) the appropriate controls were used, and 3) the positive control substances produced reliable results.

Study outcome: Results from the plate incorporation assay (experiment I) and the pre-incubation assay (experiment II) are presented in the Applicant's table reproduced below. The positive controls induced mutation frequencies as expected; the mean revertants per plate were within historical condition data for the laboratory. Hydromorphone did not induce mutation frequencies in any of the *Salmonella typhimurium* strains (TA98, TA100, TA102, TA1535 or TA1537) in the presence or absence of the metabolic activator; no increase in revertants was observed at any dose. Slight toxicity was observed in TA100 at 5000 µg/plate in the absence of S9 mix (reduction of cell count by 61%) in experiment I and in TA1537 at 5000 µg/plate in the absence of S9 mix (reduction of cell count by 55%) in experiment II.

Concentration µg/plate	Revertants/plate (mean from three plates)									
	TA 1535		TA 1537		TA 98		TA 100		TA 102	
	I	II	I	II	I	II	I	II	I	II
Without S9 mix										
Negative control	10	12	6	9	17	21	119	105	161	249
Solvent control	10	14	7	9	15	19	108	128	163	233
Positive control	587	854	59	49	210	243	503	821	603	1012
33	10	14	7	9	12	16	122	124	167	246
100	6	9	8	7	13	16	127	146	147	226
333	8	12	6	6	10	13	117	131	162	237
1000	9	11	6	6	12	15	120	141	166	264
2500	5	9	5	5	12	16	117	129	146	235
5000	9	11	7	4	16	19	47	128	136	262
With S9 mix										
Negative control	10	13	6	8	15	20	117	141	229	345
Solvent control	12	12	6	9	16	22	120	145	234	344
Positive control	64	97	69	164	693	800	833	741	1049	1282
33	7	10	5	8	16	18	152	133	227	348
100	6	13	8	9	17	17	139	155	241	386
333	12	12	7	6	19	20	157	146	298	400
1000	9	11	5	7	17	23	148	151	251	325
2500	8	10	6	9	15	23	163	173	217	332
5000	7	12	5	9	15	21	160	158	239	323

**Study title:** BSF 4036516 – *Salmonella typhimurium* reverse mutation assay.

**Key findings:** Hydromorphone-N-oxide, a degradant product of hydromorphone, was evaluated in the Ames Reverse Mutation Assay at concentrations of 33, 100, 333, 1000, 2500 and 5000 µg/plate. Under the conditions of the study, hydromorphone-N-oxide was negative in the bacterial reverse mutation assay.

**Study №.:** MPF/DT 0012E

**Volume #, and page #:** eCTD submission

**Conducting laboratory and location:** [REDACTED] (b) (4)

**Date of study initiation:** May 2, 2000

**GLP compliance:** Yes

**QA reports:** yes ( x ) no ( )

**Drug, lot #, and % purity:** BSF 4036516 (Hydromorphone-N-oxide, Batch № 10010, 98.9%)

**Methods**

**Strains/species/cell line:** *Salmonella typhimurium* TA100, TA102, TA1535, TA98, and TA1537

Doses used in definitive study: 33, 100, 333, 1000, 2500 and 5000 µg/plate.

Basis of dose selection: A dose-range finding study was conducted to select the appropriate doses to use in the mutagenicity assay.

Negative controls: DMSO

Positive controls: Sodium azide (NaN<sub>3</sub>), 4-nitro-phenylene-diamine (4-NOPD), methyl methane sulfonate (MMS), and 2-aminoanthracene (2AA) were selected for positive controls based on the bacterial strain as indicated in the following table:

Test Strain	Positive Control Substance (µg/plate)	
	-S9	+S9
TA100	NaN <sub>3</sub> (10.0)	2AA (2.5)
TA1535	NaN <sub>3</sub> (10.0)	2AA (2.0)
TA98	4-NOPD (10.0)	2AA (2.5)
TA1537	4-NOPD (50)	2AA (2.5)
TA102	MMS (5.0)	2AA (10.0)

Incubation and sampling times: The mutagenicity test was performed according to the plate-incorporation procedures. The S9 metabolic activator, the tester strain, and the test article were mixed in a test tube and incubated at 37°C for 60 minutes. After pre-incubation, 2.0 mL overlay agar (45°C) was added to each test tube; the mixture was then poured on minimal agar plates. After solidification the agar plates were incubated at 37°C for 48 hours. The vehicle control, positive control and all doses of the test articles were plated in triplicate.

Analysis:

- Mutation frequencies: Expressed as mean number of revertants per plates.
- Cytotoxicity: Background lawn was observed macroscopically or with a stereoscope (x 40)
- Counting Method: The number of revertant colonies was counted using the AUTOCOUNT. To check the proper function of the counter, parts of the colonies were counted manually.

Criteria for positive results: “The results were judged positive (+) if

- a biologically relevant and dose-dependent increase in the number of revertant colonies is induced.
- the number of reversions is at least twice the spontaneous reversion rate in strains TA98, TA100 and TA102 or thrice in strains TA1535 and TA1537.
- the results showed a dose-dependent and reproducible increase in the number of revertants is regarded as an indication of possibly existing mutagenic potential of the test article regardless whether the highest dose increased the number of revertant colonies.”

## Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The assay methods, positive and negative controls, and the concentrations of drug used for the definitive study were adequate. The study appears to be valid for the following reasons: 1) the appropriate strains were tested, 2) the appropriate controls were used, and 3) the positive control substances produced reliable results.

Study outcome: BSF 4036516 (hydromorphone-N-oxide) was evaluated at the following concentrations: 33, 100, 333, 1000, 2500 and 5000 µg/plate in the mutagenicity assay. In the mutagenicity assay, the positive controls induced mutation frequencies as expected; the mean revertants per plate were within historical control data for the laboratory. BSF 4036516 did not induce mutation frequencies in any of the *Salmonella typhimurium* strains (TA98, TA100, TA102, TA1535 or TA1537) in the presence or absence of the metabolic activator; no increase in revertants was observed at any dose. Slight toxicity was observed in TA1535 at 1000 µg/plate in the presence of S9 mix (reduction of cell count by 45%) and in TA98 at 5000 µg/plate in the presence of S9 mix (reduction of cell count by 55%).

### **Study title: BSF 4036516 – Chromosome aberrations assay in human lymphocytes in vitro.**

**Key findings**: Under the conditions of this assay, hydromorphone-N-oxide was not clastogenic in the human lymphocyte cultures in vitro in the presence or absence of metabolic activation.

<b>Study №:</b>	MPF/DT 0013 E
<b>Volume #, and page #:</b>	eCTD submission
<b>Conducting laboratory and location:</b>	 (b) (4)
<b>Date of study initiation:</b>	April 12, 2000
<b>GLP compliance:</b>	Yes
<b>QA reports:</b>	Yes ( X ) No ( )
<b>Drug, lot #, and % purity:</b>	Hydromorphone-N-oxide, Batch № 10010, 98.9% purity

## Methods

Strains/species/cell line: Peripheral blood lymphocytes were obtained from a healthy non-medicating 36 years old male donor.

Doses used in definitive study:

Treatment Condition	Exp.	Preparation Interval (hr)	Exposure Period (hr)	Dose ( $\mu\text{g}/\text{mL}$ )
Non-activate	1	22	4	1250, 2500, 5000
	1	46	22	1250, 2500, 5000
	11	22	22	1250, 2500, 5000
S9 activated	1	22	4	1250, 2500, 5000
	1	46	4	1250, 2500, 5000
	11	22	4	1250, 2500, 5000

Basis of dose selection: Dose selection was based on the results from the preliminary toxicity assay. For selection of BSF 4036516 concentrations for the definitive experiment, any toxicity that was indicated by a reduction of the mitotic index was used to adjust the BSF 4036516 doses.

Negative controls: solvent (medium)

Positive controls: In both the 4 and 22 hours exposure condition, ethylmethane sulfonate (EMS) and cyclophosphamide (CPA) were positive controls, in the absence and presence of metabolic activation, respectively. In both the 4 and 22 hours exposure conditions without metabolic activation, EMS was used as positive control after dissolution in culture medium (DMEM/F12) to a final concentration of 330 and 440  $\mu\text{g}/\text{mL}$ . CPA served as the positive control for the 4 hours exposure condition with metabolic activation after dissolution in culture medium (DMEM/F12) to a final concentration of 30  $\mu\text{g}/\text{mL}$ .

Incubation and sampling times: For the definitive chromosome aberration assay cells were exposed to metabolic activation for 4 hours or no metabolic activation for 4 or 22 hours. In the non-metabolic activated study, the cells were exposed to solvent alone and BSF 4036516 at concentrations of 1250, 2500 and 5000  $\mu\text{g}/\text{mL}$  for 4 hours or 22 hours at 37°C in a humidified atmosphere of 15% CO<sub>2</sub> (85% air). In the metabolic activated study, the cells were exposed to solvent vehicle and BSF 4036516 at concentrations of 1250, 2500 and 5000  $\mu\text{g}/\text{mL}$  for 4 hours at 37°C in a humidified atmosphere of 15% CO<sub>2</sub> (85% air). Three hours prior to the scheduled cell harvest at 22 and 44 hours after treatment initiation, Colcemid<sup>®</sup> (0.2  $\mu\text{g}/\text{mL}$ ) was added to the cultures. After the 2 hour exposure to Colcemid<sup>®</sup>, metaphase cells were harvested by centrifugation. Cells were collected by centrifugation, fixed, stained and the number of mitosis per 500 cells scored was determined. Isolated cells were fixed on slides for analysis.

## Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): According to the Applicant, the results were classified as mutagenic if: 1) “the number of induced structural chromosome aberrations are not in the range of our historical control data (0-4% aberrant cells exclusive gaps)”, and 2) “either a

concentration-related or a significant increase of the number of structural chromosome aberrations are observed. The results were classified as non-mutagenic if: 1) “the number of structural chromosome aberrations in all evaluated dose groups are in the range of our historical control data (0-4% aberrant cells exclusive gaps)”, 2) “no significant increase of the number structural chromosome aberrations are observed.”

*Counting Method* – Under blind condition, 100 well-spread metaphases per slide (200 metaphase per dose) were analyzed. Chromosomal aberrations were recorded as chromatid-type (i.e., chromatid and isochromatid breaks, fragment, deletion and chromatid type exchange) or chromosome-type aberrations (iso-break, iso-fragment, iso-deletion and chromosome type exchanges), gaps (gap and iso-gap), other (multiple aberration and chromosomal disintegration). *Validity* – the study appears valid as the appropriate positive controls were used, an acceptable number of mitotic cells were evaluated, duplicate negative and positive controls were used, approximately 200 metaphase spreads per treatment were scored, the frequency of cells with structural chromosome aberrations in the solvent controls were within the historical control range for solvent control, and the protocol was consistent with those described in the OECD Guideline 473: *In vitro* Mammalian Chromosome Aberration Test.

Study outcome: Results from the definitive chromosomal aberration tests are presented in the Applicant’s table reproduced below. In comparison to the solvent control, BSF 4036516 was void of significant clastogenic activity in the 4 hour exposure condition in both the absence and presence of metabolic activation and the 22 hour exposure condition in the absence of metabolic activation. There were no statistically significant increases in the percentage of cells with aberrations over the concurrent controls in either study condition. At the highest concentration evaluated, BSF 4036516 was cytotoxic as in the absence of metabolic activation suggested by 31% reduction in mitotic index relative to control. The positive controls induced statistically significant increases in structural aberrations over the solvent control in the presence and absence of S9 mix. Under these study conditions, BSF 4036516 did not induce chromosomal aberrations in cultured human lymphocytes.

## Summary of results of the chromosomal aberration study with BSF 4036516

Exp.	Preparation interval	Test item concentration (µg/ml)	Polyploid cells (%)	Mitotic index (% of control)	Aberrant cells (%)		
					incl. gaps	excl. gaps*	exchanges
<b>Exposure period 4 h without S9 mix</b>							
I	22 h	solvent control <sup>1</sup>	0.5	100.0	0.5	0.5	0.0
		positive control <sup>2</sup>	0.0	86.1	9.5	9.5 <sup>S</sup>	0.5
		1250	0.0	116.5	2.5	1.5	0.0
		2500	0.0	108.2	0.0	0.0	0.0
		5000	1.0	91.8	0.5	0.5	0.0
<b>Exposure period 22 h without S9 mix</b>							
II	22 h	solvent control <sup>1</sup>	1.0	100.0	0.5	0.0	0.0
		positive control <sup>2</sup>	0.0	29.5	11.5	10.5 <sup>S</sup>	3.5
		1250	0.5	73.2	0.5	0.5	0.5
		2500	0.0	52.6	0.0	0.0	0.0
		5000	0.0	31.5	0.0	0.0	0.0
<b>Exposure period 22 h without S9 mix</b>							
I	46 h	solvent control <sup>1</sup>	0.5	100.0	2.5	2.0	0.0
		positive control <sup>3</sup>	0.5	27.3	13.5	12.5 <sup>S</sup>	4.0
		1250	0.0	72.7	1.5	1.0	0.0
		2500	0.0	79.8	2.5	1.5	0.0
		5000	0.0	64.2	1.0	0.0	0.0
<b>Exposure period 4 h with S9 mix</b>							
I	22 h	solvent control <sup>1</sup>	0.5	100.0	1.0	1.0	0.0
		positive control <sup>4</sup>	0.0	18.7	11.5	9.5 <sup>S</sup>	2.5
		1250	0.0	60.3	0.5	0.5	0.0
		2500	0.5	94.4	1.5	1.0	0.0
		5000	0.0	76.0	2.5	1.5	0.0
II	22 h	solvent control <sup>1</sup>	0.0	100.0	1.0	0.5	0.0
		positive control <sup>4</sup>	0.0	16.5	11.0	10.0 <sup>S</sup>	1.0
		1250	0.0	95.1	1.5	1.5	0.0
		2500	0.5	90.6	1.5	0.5	0.0
		5000	0.5	86.1	0.0	0.0	0.0
I	46 h	solvent control <sup>1</sup>	0.0	100.0	2.0	1.0	0.0
		positive control <sup>4</sup>	0.0	32.1	11.5	11.0 <sup>S</sup>	2.5
		1250	0.0	93.3	1.0	0.0	0.0
		2500	0.0	79.8	1.0	0.5	0.0
		5000	0.0	100.8	0.5	0.5	0.0

\* inclusive cells carrying exchanges

<sup>S</sup> aberration frequency statistically significant higher than corresponding solvent control values<sup>1</sup> medium<sup>2</sup> EMS 440 µg/ml<sup>3</sup> EMS 330 µg/ml<sup>4</sup> CPA 30 µg/ml

**Study title: Bacterial Reverse Mutation Assay.**

**Key findings:** (b) (4) was evaluated in the Ames Reverse Mutation Assay at concentrations of 50, 150, 500, 1500 and 5000 µg/plate. Under the conditions of the study, (b) (4) was negative in the bacterial reverse mutation assay.

**Study no.:** TOX8695  
**Volume #, and page #:** eCTD submission  
**Conducting laboratory and location:** (b) (4)  
**Date of study initiation:** December 20, 2007  
**GLP compliance:** Yes  
**QA reports:** Yes ( X ) No ( )  
**Drug, lot #, and % purity:** (b) (4) Degradant, Batch No CS07-05-45, 95.2% purity

**Methods**

Strains/species/cell line: *Salmonella typhimurium* TA100, TA1535, TA98, and TA1537 and *Escherichia coli* WP2 uvrA

Doses used in definitive study: 50, 150, 500, 1500 and 5000 µg/plate

Basis of dose selection: A preliminary dose-range finding toxicity study was conducted to establish the (b) (4) doses to be used for the definitive experiments. Using the test strains TA98, TA 100, TA1535, TA1537 and WP2 *uvrA* in the presence and absence of S9 mix, 8 concentrations of (b) (4) (1.5, 5.0, 15, 150, 500, 1000, 1500 and 5000 µg/plate) were assayed. No precipitation of the drug was observed up to 5000 µg/plate. (b) (4) was not toxic in the preliminary assay at dose levels up to 5000 µg/plate in the in the absence or presence of S9 mix.

Negative controls: Vehicle (Dimethyl sulfoxide (DMSO))

Positive controls: Sodium azide (NaN<sub>3</sub>), 42-nitrofluorene (2-NF), methyl methane sulfonate (MMS), 9-aminoacridinium chloride (9-AAC) and 2-aminoanthracene (2AA) were selected for positive controls based on the bacterial strain as indicated in the following table:

Test Strain	Positive Control Substance (µg/plate)	
	-S9	+S9
TA100	NaN <sub>3</sub> (1.0)	2AA (1.0)
TA1535	NaN <sub>3</sub> (1.0)	2AA (2.0)
TA98	2-NF (1.0)	2AA (1.0)
TA1537	9-AAC (75.0)	2AA (1.0)
WP2uvra	MMS (1000.0)	2AA (10.0)

Incubation and sampling times: The mutagenicity test was performed according to the plate-incorporation procedures. In the definitive study, plates were exposed to either the negative or positive controls or drug treatment in the presence or absence of S9 metabolic activation for 48 to 72 hr and incubated at 37°C. At the end of the treatment period, plates were counted with an automatic plate reader.

Analysis:

- Mutation frequencies: Expressed as mean number and standard deviation of revertants per plates.
- Cytotoxicity: Background lawn was observed using a dissecting microscope. A dose level is considered toxic if it causes a >50% reduction in the mean number of revertants per plate relative to the mean vehicle control value. The reduction in mean revertants must be accompanied by an abrupt dose-dependent drop in the revertant count

Criteria for positive results: Criteria for positive results included:

- a reproducible 2-fold increase for strains TA98, TA100 and WP2uvrA or 3-fold increase for strains TA1535 and TA1537 in the number of revertants compared with the vehicle controls.
- a dose-dependent increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations

## Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The study appears to be valid for the following reasons: 1) the appropriate strains were tested, 2) the appropriate controls were used, and 3) the positive control substances produced reliable results. The assay methods, positive and negative controls, and the concentrations of drug used for the definitive study were adequate.

Study outcome: (b) (4) was evaluated at the following concentrations: 50, 150, 500, 1500 and 5000 µg/plate in the mutagenicity assay. Applicant's table is copied below. In the mutagenicity assay, the positive controls induced mutation frequencies as expected; the mean revertants per plate were within historical control data for the laboratory. Compared to the vehicle control groups, (b) (4) did not increase the number of revertants for the *Salmonella typhimurium* strains (TA987, TA100, TA1535 or TA1537) or the *Escherichia coli* test strain (WP2uvrA) in the presence or absence of the metabolic activator. (b) (4) did not precipitate out of the media or interfere with colony counting. No cytotoxicity was observed. (b) (4) did not induce bacterial mutagenicity under the conditions tested.

Bacterial Mutation Assay  
 Summary of Results – Confirmatory Mutagenicity Assay  
 Table 22

Test Article Id : (b) (4) Degradant (aka (b) (4))  
 Study Number : AC05BT.503.(b) (4)  
 Experiment No : B2

Average Revertants Per Plate ± Standard Deviation

Activation Condition	None									
Dose (µg/plate)	TA98		TA100		TA1535		TA1537		WP2 <i>uvrA</i>	
Vehicle	10±	3	148±	19	15±	2	8±	3	11±	4
50	12±	4	152±	12	18±	1	6±	3	11±	4
150	13±	3	163±	15	19±	4	11±	7	13±	2
500	12±	3	185±	10	20±	7	9±	4	10±	3
1500	12±	2	161±	23	17±	3	10±	2	10±	3
5000	12±	2	136±	34	17±	8	6±	2	9±	5
Positive	128±	44	595±	58	450±	26	790±	166	141±	23

Activation Condition	Rat Liver S9									
Dose (µg/plate)	TA98		TA100		TA1535		TA1537		WP2 <i>uvrA</i>	
Vehicle	22±	8	170±	13	16±	0	9±	1	19±	1
50	17±	1	194±	25	12±	2	4±	1	17±	6
150	20±	7	169±	14	19±	7	10±	1	15±	2
500	20±	6	160±	37	13±	2	9±	3	13±	6
1500	16±	9	179±	18	14±	5	6±	2	10±	3
5000	18±	2	192±	16	12±	2	4±	1	8±	3
Positive	220±	116	619±	9	64±	19	70±	20	167±	21

Vehicle = Vehicle Control  
 Positive = Positive Control (50 µL plating aliquot)  
 Plating aliquot = 100 µL

**Study title:** (b) (4): Reverse mutation assay “Ames Test” screening method using *Salmonella Typhimurium* TA100, TA98 and TA102.

**Key findings:** (b) (4) was evaluated in the Ames Reverse Mutation Assay at concentrations of 0.5, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate. Under the conditions of the study, (b) (4) was negative in the bacterial reverse mutation assay. As the assay did not include all of the appropriate strains, it is not a valid assessment of the mutagenic potential of (b) (4).

**Study no.:** 2033/0055

**Volume #, and page #:** eCTD submission

**Conducting laboratory and location:** (b) (4)

**Date of study initiation:** Performed between Sept. 8 and Sept. 14, 2004

**GLP compliance:** No GLP statement submitted

**QA reports:** Yes ( ) No ( x )

**Drug, lot #, and % purity:** (b) (4) Batch No BDR17-205a, % purity not stated.

## Methods

Strains/species/cell line: *Salmonella typhimurium* TA100, TA98, and TA102

Doses used in definitive study: 0.5, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate

Basis of dose selection: Applicant did not describe basis for selecting the doses employed in the definitive study.

Negative controls: Vehicle (dimethyl sulphoxide)

Positive controls: N-ethyl-N'-nitro-N-nitrosoguanidine (EENG), 4-nitroquinoline-1-oxide (4NQO), Mitomycin C (MMC), 2-aminoanthracene (2AA), benzo(a)pyrene (BP) and 1,8-dihydroxyanthraquinone (DAN) were selected for positive controls based on the bacterial strain as indicated in the following table:

Test Strain	Positive Control Substance (µg/plate)	
	-S9	+S9
TA100	EENG (3.0)	2AA (1.0)
TA98	4NQO (0.2)	BP (5.0)
TA102	MMC (0.5)	DAN (10.0)

Incubation and sampling times: The mutagenicity test was performed according to the plate-incorporation procedures. In the definitive study, plates were exposed to either the negative or positive controls or drug treatment in the presence or absence of S9 metabolic activation. Details of the incubation procedure and sampling times were not described. The procedure was performed in triplicate for each bacterial strain and concentration of the test material in the absence and presence of S9 metabolic activation.

Analysis:

- Mutation frequencies: Expressed as mean number of revertants per plates.
- Cytotoxicity: Cytotoxicity was not defined.

Criteria for positive results: Criteria for positive results were not defined by the Applicant.

## Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The assay methods, positive and negative controls, and the concentrations of drug used for the definitive study were adequate. The appropriate controls were used, and the positive control substances produced reliable results. However, the study is not valid for the following reasons: 1) the appropriate strains were not. The Applicant only evaluated the *Salmonella typhimurium* TA100, TA98, and TA102 strains. TA1535 and TA1537 were not evaluated.

Study outcome: (b) (4) was evaluated at the following concentrations in the mutagenicity assay: 0.5, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate. Results are presented in the Applicant's tables below. There was no increase in the number of revertants compared to the vehicle control groups for test strains TA98, TA100 and TA102 in the presence and absence of S9 mix. The number of revertant colonies (b) (4)-treated plates was comparable to those of the control at all concentrations tested up to 5000 µg/plate. A clear increase was seen in the positive control plates. (b) (4) did not induce bacterial mutagenicity under the conditions tested.

**Table 2 Test Results: Main Experiment – Without Metabolic Activation**

Test Period		From: 14 September 2004				To: 17 September 2004	
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)					
		Base-pair substitution type				Frameshift type	
		TA100		TA102		TA98	
-	0	67	(70)	317	(325)	25	(17)
		72	2.9#	318	12.4	14	7.0
		72		339		12	
-	0.5	61	(62)	339	(325)	9	(15)
		62	0.6	319	11.8	22	6.7
		62		318		13	
-	1.5	64	(66)	338	(320)	16	(15)
		68	2.1	297	20.8	14	1.0
		65		324		15	
-	5	76	(65)	317	(319)	12	(15)
		62	9.8	321	2.0	23	6.7
		57		319		11	
-	15	62	(64)	309	(311)	15	(15)
		61	3.8	309	4.0	14	0.6
		68		316		15	
-	50	66	(67)	295	(293)	17	(16)
		57	11.1	293	2.0	19	3.6
		79		291		12	
-	150	52	(70)	294	(295)	9	(14)
		94	21.6	303	7.1	21	6.2
		64		289		12	
-	500	73	(70)	243	(287)	17	(16)
		72	4.9	303	38.9	17	1.2
		64		316		15	
-	1500	81	(74)	180	(245)	22	(20)
		75	7.5	277	56.6	21	2.6
		66		279		17	
-	5000	89	(93)	255	(269)	22	(23)
		89	6.9	294	21.7	15	9.1
		101		258		33	
Positive controls S9-Mix	Name Concentration (µg/plate) No. colonies per plate	ENNG		MMC		4NQO	
		3		0.5		0.2	
		428	(469)	1488	(1522)	204	(197)
-	-	501	37.4	1554	33.0	198	8.1
-	-	479		1524		188	

**Table 2 Test Results: Main Experiment – Without Metabolic Activation**

Test Period		From: 14 September 2004				To: 17 September 2004	
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)					
		Base-pair substitution type				Frameshift type	
		TA100		TA102		TA98	
-	0	67	(70)	317	(325)	25	(17)
		72	2.9#	318	12.4	14	7.0
		72		339		12	
-	0.5	61	(62)	339	(325)	9	(15)
		62	0.6	319	11.8	22	6.7
		62		318		13	
-	1.5	64	(66)	338	(320)	16	(15)
		68	2.1	297	20.8	14	1.0
		65		324		15	
-	5	76	(65)	317	(319)	12	(15)
		62	9.8	321	2.0	23	6.7
		57		319		11	
-	15	62	(64)	309	(311)	15	(15)
		61	3.8	309	4.0	14	0.6
		68		316		15	
-	50	66	(67)	295	(293)	17	(16)
		57	11.1	293	2.0	19	3.6
		79		291		12	
-	150	52	(70)	294	(295)	9	(14)
		94	21.6	303	7.1	21	6.2
		64		289		12	
-	500	73	(70)	243	(287)	17	(16)
		72	4.9	303	38.9	17	1.2
		64		316		15	
-	1500	81	(74)	180	(245)	22	(20)
		75	7.5	277	56.6	21	2.6
		66		279		17	
-	5000	89	(93)	255	(269)	22	(23)
		89	6.9	294	21.7	15	9.1
		101		258		33	
Positive controls S9-Mix	Name Concentration (µg/plate) No. colonies per plate	ENNG		MMC		4NQO	
		3		0.5		0.2	
		428	(469)	1488	(1522)	204	(197)
-		479	37.4	1524	33.0	188	8.1

**Study title:** (b) (4): Screening chromosome aberration test in human lymphocytes in vitro.

**Key findings:** Under the conditions of this assay, (b) (4) was not clastogenic in the human lymphocyte cultures in vitro in the presence or absence of metabolic activation.

**Study no.:** 2033/006

**Volume #, and page #:** eCTD submission

**Conducting laboratory and location:** (b) (4)

**Date of study initiation:** Not stated

**GLP compliance:** No GLP statement submitted

**QA reports:**

**Drug, lot #, and % purity:** (b) (4) yes ( ) no ( x ), Batch No - not stated, ≈ 100%

**Methods**

Strains/species/cell line: Human peripheral blood lymphocyte.

Doses used in definitive study:

Treatment Condition	Treatment Time (hr)	Expression Period (hr)	Dose (µg/mL)
Non-activate	4	20	750, 1500, 2250
	24	0	187.5, 375, 562.5
S9 activated	4	20	750, 1500, 2250

Basis of dose selection: Dose selection was based on the results from the preliminary toxicity assay. For selection of (b) (4) concentrations for the definitive experiment, any toxicity that was indicated by a reduction of the mitotic index was used to adjust the (b) (4) doses.

Negative controls: solvent (dimethyl sulphoxide (DMSO))

Positive controls: Mitomycin C (MMC) and cyclophosphamide (CPA) were positive controls for assay.

Treatment Condition	Treatment Time (hr)	Expression Period (hr)	Positive Control (µg/mL)
Non-activate	4	20	MMC (0.4)
	24	0	MMC (0.2)
S9 activated	4	20	CP (7.5)

Incubation and sampling times: Details of the study design was not provided.

**Results**

Study validity (comment on replicates, counting method, criteria for positive results, etc.):

Replicates: Concentrations were performed in duplicates.

Criteria for positive results: Not defined

Counting Method: Not described

Study outcome: Results from the definitive chromosomal aberration tests are presented in the Applicant's table reproduced below. In comparison to the solvent control, (b) (4) was void of significant clastogenic activity in the 4 hour exposure condition in both the absence and presence of metabolic activation and the 24 hour exposure condition in the absence of metabolic activation. There were no statistically significant increases in the percentage of cells with aberrations over the concurrent controls in either study condition. The positive controls induced statistically significant

increases in structural aberrations over the solvent control in the presence and absence of S9 mix. Under these study conditions, (b) (4) did not induce chromosomal aberrations in cultured human lymphocytes.

**Table 2 Results of Chromosome Aberration Test – 4(20)-Hour Exposure Without Metabolic Activation (S9)**

Exposure Group	Replicate	Mitotic Index (%)	Number of Cells Scored	Number of Cells with Aberrations						Number of Polyploids	Total Number of Aberrations		Frequency of Aberrant Cells (%)	
				Total Gaps	Chromatid		Chromosome		Others X		(+ Gaps)	(-Gaps)	(+Gaps)	(-Gaps)
Vehicle Control	A	4.25	100	0	0	0	0	0	0	0	0	0	0	0
	B	4.55												
	Total	4.40 (100)	100 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
750 µg/ml	A	4.85	100	0	0	0	1	0	0	0	2	2	1	1
	B	4.65												
	Total	4.75 (108)	100 (100)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.0)	2 (2.0)	1 (1.0)	1 (1.0)
1500 µg/ml	A	2.90	100	1	0	0	1	0	0	0	2	1	2	1
	B	3.40												
	Total	3.15 (72)	100 (100)	1 (1.0)	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.0)	1 (1.0)	2 (2.0)	1 (1.0)
2250 µg/ml	A	2.25	100	0	1	0	1	0	0	0	3	3	2	2
	B	1.80												
	Total	2.03 (46)	100 (100)	0 (0.0)	1 (1.0)	0 (0.0)	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (3.0)	3 (3.0)	2 (2.0)	2 (2.0)
Positive Control MMC 0.4 µg/ml	A	1.45	50*	16	7	13	2	0	0	0	52	36	23	19
	B	1.50												
	Total	1.48 (34)	50 (100)	16 (32.0)	7 (14.0)	13 (26.0)	2 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)	52 (104.0)	36 (72.0)	23 (46.0)	19 (38.0)

\*\*\* = p ~ 0.001

**Table 3 Results of Chromosome Aberration Test – 4(20)-Hour Exposure With Metabolic Activation (S9)**

Exposure Group	Replicate	Mitotic Index (%)	Number of Cells Scored	Number of Cells with Aberrations						Number of Polyploids	Total Number of Aberrations		Frequency of Aberrant Cells (%)	
				Total Gaps	Chromatid		Chromosome		Others X		(+ Gaps)	(-Gaps)	(+Gaps)	(-Gaps)
Vehicle Control	A	4.75	100	1	0	0	0	0	0	0	1	0	1	0
	B	3.45												
	Total	4.10 (100)	100 (100)	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)	1 (1.0)	0 (0.0)
750 µg/ml	A	4.10	100	0	0	0	0	0	0	0	0	0	0	0
	B	4.05												
	Total	4.08 (99)	100 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
1500 µg/ml	A	3.40	100	1	0	0	0	0	0	0	1	0	1	0
	B	3.25												
	Total	3.33 (81)	100 (100)	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)	1 (1.0)	0 (0.0)
2250 µg/ml	A	2.75	100	2	2	1	1	0	0	0	6	4	5	3
	B	2.00												
	Total	2.38 (58)	100 (100)	2 (2.0)	2 (2.0)	1 (1.0)	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (6.0)	4 (4.0)	5 (5.0)	3 (3.0)
Positive Control CP 7.5 µg/ml	A	1.10	100	1	3	1	1	0	0	0	7	6	5	4
	B	0.70	100	1	16	4	3	1	0	0	7	6	37	23
	Total	1.45 (35)	200 (100)	2 (1.0)	19 (9.5)	5 (2.5)	4 (2.0)	1 (0.5)	0 (0.0)	0 (0.0)	14 (7.0)	12 (6.0)	42 (21.0)	27 (13.5)

\*\*\* = p ~ 0.001

**Table 4 Results of Chromosome Aberration Test – 24-Hour Exposure Without Metabolic Activation (S9)**

Exposure Group	Replicate	Mitotic Index (%)	Number of Cells Scored	Number of Cells with Aberrations						Number of Polyploids	Total Number of Aberrations		Frequency of Aberrant Cells (%)	
				Total Gaps	Chromatid Breaks	Exchanges	Chromosome Breaks	Exchanges	Others X		(+ Gaps)	(-Gaps)	(+Gaps)	(-Gaps)
Vehicle	A	6.10	100	0	0	0	0	0	0	0	0	0	0	
	B	5.30												
Control	Total	5.70 (100)	100 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
	A	5.50	100	0	0	0	0	0	0	0	0	0	0	
187.5 µg/ml	B	5.90												
	Total	5.70 (100)	100 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
375 µg/ml	A	3.80	100	0	0	0	0	0	0	0	0	0	0	
	B	3.55												
562.5 µg/ml	Total	3.68 (64)	100 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
	A	2.30	100	2	4	0	0	0	0	0	8	4	7	4
Positive Control MMC 0.2 µg/ml	B	1.55												
	Total	1.93 (34)	100 (100)	2 (2.0)	4 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	8 (8.0)	4 (4.0)	7 (7.0)	4 (4.0)
Positive Control	A	1.35	50 <sup>a</sup>	7	11	19	1	0	0	0	50	40	26	25
	B	1.05												
MMC 0.2 µg/ml	Total	1.20 (21)	50 (100)	7 (14.0)	11 (22.0)	19 (38.0)	1 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	50 (100.0)	40 (80.0)	26 (52.0)	25*** (50.0)

\*\*\* = p ~ 0.001

**2.6.6.5 Carcinogenicity**

Carcinogenicity studies are currently being conducted by the Applicant.

**2.6.6.6 Reproductive and developmental toxicology**

The Applicant submitted results from the dose-range finding study (№ MPF/DT 4898) and referred the reviewer to original NDA submission for the pivotal reproductive and developmental toxicity studies. These studies were reviewed by Dr. Kathleen Haberny in June of 2000. Specific details of these studies can be found in NDA 22-217 review from Dr. Haberny. In her review, Dr. Haberny concluded the following:

- Hydromorphone hydrochloride (1.56, 3.13 and 6.25 mg/kg/day) had no effects on male and female fertility in rats under the conditions of the study.
- Hydromorphone hydrochloride (6.25, 12.5 and 25 mg/kg/day, p.o.) was not teratogenic in fetal rabbits at up to 25 mg/kg on gestation days 6-20 under the conditions of the teratogenicity study conducted in pregnant Himalayan/CHR rabbits.
- Hydromorphone hydrochloride (1.56, 3.13, and 6.25 mg/kg/day, p.o.) was not teratogenic in rats at up to 6.25 mg/kg/day (approximately 1x the high human doses of 64 mg in a 60 kg patient on an AUC basis) given during gestation days 6-17 under the conditions of the study.

**2.6.6.7 Local tolerance**

The Applicant submitted three study reports (b) (4)



(b) (4)

These studies were not reviewed because they were not considered pivotal in the safety assessment of OROS hydromorphone.

#### 2.6.6.8 Special toxicology studies

Not applicable

#### 2.6.6.9 Discussion and Conclusions

Neuromed has submitted NDA 21-217 for EXAGLO, an extended release hydromorphone product. NDA 21-217 is a resubmission in response to the Agency October 27, 2000 Approvable letter.

#### 2.6.6.10 Tables and Figures

Not applicable

### 2.6.7 TOXICOLOGY TABULATED SUMMARY

Not applicable

## OVERALL CONCLUSIONS AND RECOMMENDATIONS

**Conclusions:** The proposed drug product does not present any unique toxicology concerns compared to the already approved drug products and based on current practice in OND, no further studies are required to support this NDA application.

**Unresolved toxicology issues (if any):** The Applicant has the following postmarketing study requirements to honor. The following should be conveyed to the Applicant.

We remind you of your postmarketing study requirements in your submission dated October 20, 2009. These requirements are listed below.

1. Carcinogenicity study in mouse, Report 1678-001 (currently ongoing per August 8, 2008, agreement)

Protocol Submitted:	October 6, 2005
Study Start:	March 24, 2009
Final Report Submission:	by November 2011

2. Carcinogenicity study in rat, Report 1678-002 (currently ongoing per August 8, 2008, agreement)

Protocol Submitted	November 21, 2008
Study Start:	March 18, 2009

Final Report Submission: by November 30, 2011

Recommendations: From the nonclinical pharmacology and toxicology perspective, the NDA may be approved pending agreement on the labeling.

Suggested labeling: See executive summary.

Signatures (optional):

Reviewer Signature \_\_\_\_\_

Supervisor Signature \_\_\_\_\_ Concurrence Yes \_\_\_ No \_\_\_

**REFERENCES:**

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Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-21217	ORIG-1	NEUROMED PHARMACEUTICA LS LTD	Exalgo (hydromorphone HCl) 8/12/16/32

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/s/

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BELINDA A HAYES  
11/10/2009

RICHARD D MELLON  
02/05/2010

## REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA

Division of Anesthetic, Critical Care, and Addiction Drug Products

HFD 170 / Kathleen Haberny, Ph.D.

**Key Words:** Hydromorphone hydrochloride; Dilaudid CR; Reproductive toxicity; Genotoxicity

**NDA 21-217** Serial number 000/ December 29, 1999 / Original New Drug Application

**Information to sponsor:** Yes ( x ) No ( )

**Review Completion Date:** June 26, 2000

**Sponsor:** Knoll Pharmaceutical Company  
3000 Continental Drive - North  
Mount Olive, NJ 07828

**Manufacturer:** Knoll Pharmaceutical Co., 30 North Jefferson Road, Whippany, NJ 07981

### Drug:

**Generic Name:** Hydromorphone hydrochloride

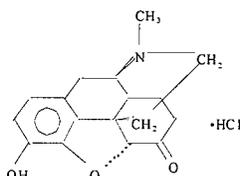
**Trade Name:** Dilaudid CR™

**Chemical Name:** 4,5alpha-epoxy-3-hydroxy-17-methylmorphinan-6-one hydrochloride

**CAS Registry Number:** 71-68-1

**Molecular Formula/ Molecular Weight:** C<sub>17</sub>H<sub>19</sub>NO<sub>3</sub>·HCl / 321.80

**Structure:**



### Relevant INDs/NDAs/DMFs:

IND

(b) (4)

IND

(b) (4)

DMF

(b) (4)

DMF

DMF

DMF

DMF

**Drug Class:** Semisynthetic phenanthrene-derivative opiate agonist

**Indication:** Management of moderate to severe pain

**Route of Administration:** Oral

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**Proposed Clinical Use:** This product is intended for once per day oral administration at 8, 16, 32 and 64 mg/day, to provide 24-hour relief from moderate to severe pain in patients for whom the use of an opioid analgesic is appropriate for more than a few days.

**Clinical Formulation:**

Component	Reference	Role	8 mg	16 mg	32 mg	64 mg
Weight of Component (mg)						
(b) (4)						(b) (4)
Hydromorphone HCl	USP	Active				
Polyethylene Oxide, (b) (4)	NF					(b) (4)
Povidone, (b) (4)	USP/Ph Eur					
Magnesium Stearate	NF/Ph Eur					
Ferric Oxide, (Yellow)	NF					
Butylated Hydroxytoluene, (b) (4)	FCC					
(b) (4)						
Polyethylene Oxide, (b) (4);	NF					
Sodium Chloride, (b) (4)	USP/Ph Eur					
(b) (4)	USP/Ph Eur					
Iron Oxide Black (b) (4)	DMF (b) (4)					
Magnesium Stearate	NF/Ph Eur					
Butylated Hydroxytoluene (b) (4)	FCC					
(b) (4) <sup>1</sup>						(b) (4)
Cellulose Acetate (b) (4)	NF					(b) (4)
Polyethylene Glyco (b) (4)	NF					
(b) (4)						
	DMF (b) (4)					
	DMF (b) (4)					
	DMF (b) (4)					
	DMF (b) (4)					
(b) (4) <sup>2</sup>						
(b) (4)	DMF (b) (4)					
(b) (4)						
	DMF (b) (4)					
(b) (4)						
	USP/Ph Eur					
	NF					
<b>Total System Weight</b>			<b>181.0</b>	<b>333.5</b>	<b>342.7</b>	<b>434.3</b>
(b) (4)						

**Note:** Portions of this review were excerpted directly from the sponsor's submission.

**Introduction/Drug History:** Hydromorphone HCl, a hydrogenated ketone of morphine, has been used in the treatment of surgical, cancer and trauma pain since 1926. Available dosage forms include immediate-release oral tablet, controlled-release capsule, oral liquid, rectal suppository, powder, cough syrup, and solutions for intravenous, subcutaneous and intramuscular injection. The safety and efficacy of hydromorphone are well established in clinical use. Controlled release Hydromorphone Contin® (Purdue Frederick, 3 and 6 mg) is commercially marketed in Canada. However, Dilaudid CR™ (hydromorphone HCl) Controlled-Release Tablets have not been marketed in any country. The Oral Osmotic System (OROS®), also called Gastrointestinal Therapeutic System (GITS) by ALZA Corporation is a sustained release, once-daily hydromorphone HCl formulation that is being developed jointly by ALZA Corporation under (b) (4) and Knoll Pharmaceutical Company under IND (b) (4).

**Previous Clinical Experience:** The safety and efficacy of hydromorphone HCl in the treatment of pain have been demonstrated during nearly 80 years of clinical use since its discovery in 1921. Hydromorphone has been approved and marketed in several dosage forms including immediate-release tablets (Dilaudid®), oral liquid, rectal suppositories, powder, cough syrup, and intravenous, subcutaneous and intramuscular injection forms. Hydromorphone is available in controlled-release capsule form in Canada. This NDA is for an oral dosage form using an oral osmotic system (OROS®) for 24-hour controlled-release delivery. For the NDA, pharmacokinetic parameters were studied in eight Phase I clinical studies in healthy volunteers, one Phase II study in patients and in one long-term Phase III study in patients. Four phase III studies were conducted in 558 cancer and non-cancer patients with moderate to severe chronic pain. The patients received 8-64 mg/day for 1-392 days (mean treatment duration 84.5 days). The results showed pain relief that was comparable between the immediate release form and controlled release form, with mild to moderate adverse events that are typical of those induced by opioid analgesic drugs including nausea, constipation, headache, sweating, pruritis, asthenia, insomnia, dry mouth, vomiting, dizziness and somnolence. There were no deaths or serious adverse events.

**Studies reviewed within this submission:**

Examination of the Influence of Dilaudid on the Fertility and Early Embryonic Development to Implantation of Sprague-Dawley Rats by Oral Administration to the Animals of Fo-Generation in Accordance with ICH Guideline 4.1.1 - Segment I Study (Report MPF/DT 9858E)

Examination of the Influence of Dilaudid on the Pregnant Rat and the Foetus by Oral Administration - Embryotoxicity Study/Segment II Study (Report MPF/DT 9856E)

Examination of the Influence of Dilaudid on the Pregnant Rabbit and the Foetus by Oral Administration - Embryotoxicity Study/Segment II Study (Report MPF/DT 9857E)

Examination of Dilaudid on the Pre- and Postnatal Development (Including Maternal Function) of Sprague-Dawley Rats Following Oral Administration to the Dams of the Fo-Generation in Accordance with ICH Guideline 4.1.2 - Segment III Study (Report MPF/DT 9859E)

Salmonella/Microsome Test (Ames Test) With Hydromorphone HCl (Study Report MPF/WT 9352)

Dilaudid (Hydromorphone Hydrochloride) - Cytogenic Test After Single Oral Administration in Mice (Micronucleus Test) (Study Report MPF/DT 9855E)

Chromosome Aberration Assay in Human Lymphocytes in Vitro With Dilaudid (Hydromorphone Hydrochloride) (Study Report MPF/DT 9854E)

**Studies not reviewed within this submission:** None

## TOXICOLOGY

**General Comments:** A GLP subacute toxicity study entitled "Thirty Day Oral Dosing Study in Dogs with OROS® (hydromorphone HCl)" (ALZA Study # TR-96-4604-057) was conducted under IND (b) (4) (serial number (b) (4)). The purpose of this study was to evaluate local gastrointestinal effects and systemic toxicity of repeated dose OROS® administration at up to 64 mg/d for 30 consecutive days.

### Methods:

#### Dosing:

- **species/strain:** Beagle dogs (b) (4)
- **#/sex/group or time point:** 4
- **age:** 4 months
- **weight:** 8.2-10.0 kg males, 7.3-8.9 kg females
- **dosage groups in administered units:** 0 (GITS placebo), 8 and 64 mg OROS, 64 mg IR Dilaudid
- **route, form, volume, and infusion rate:** solid oral tablets
- **drug, lot#:** placebo #848096, GITS hydromorphone HCl 8 and 64 mg # 834696 and 836596, IR hydromorphone HCl # 10900116

#### Observations and times:

- **Mortality:** twice daily
- **Clinical signs:** three times daily
- **Body weights:** weekly
- **Food consumption:** once daily
- **Ophthalmoscopy:** baseline and at termination
- **Hematology:** baseline and at termination
- **Clinical chemistry:** baseline and at termination
- **Urinalysis:** baseline and at termination
- **Organ weights:** post-mortem

- **Organs examined:**

<b>Organ (Macroscopic examination conducted)</b>	<b>#/animal</b>	<b>Weighed</b>	<b>Examined Microscopically</b>
Adrenal glands	2	X	
Aorta (abdominal)	1		
Bone (sternum/femur w/ articular surfaces)	2		
Bone marrow (sternum/femur)	2		
Brain (medulla/pons, cerebrum, cerebellum)	3	X	
Esophagus	1		X
Eyes with optic nerve	4		
Gallbladder	1		
Heart	3	X	
Kidneys	2	X	
Large intestine (cecum, colon, rectum, anus)	3		X
Lacrimal gland	2		
Liver	2	X	
Lungs (with mainstem bronchi)	2	X	
Lymph nodes (mesenteric, mediastinal)	1		
Mammary gland	1		
Nerve (sciatic)	1		
Oral pharynx	1		X
Ovaries	2	X	
Pancreas	1	X	
Pituitary gland	1	X	
Prostate	1	X	
Salivary glands (submandibular)	2		
Skeletal muscle (biceps femoris)	1		
Small intestine (duodenum, ileum, jejunum)	3		X
Spinal cord (cervical, thoracic, lumbar)	3		
Spleen	1	X	
Stomach	3		X
Testes (w/ epididymides)	2	X	
Thymic region	1		
Thyroid/parathyroid glands	2	X	
Tongue	1		
Trachea	1		
Urinary bladder	1		
Uterus (body/horns) w/ cervix	3	X	
Tissues with macroscopic findings including gross lesions	1		X

- **Gross pathology:** post-mortem
- **Histopathology:** post-mortem
- **Toxicokinetics:** blood sampled at pre-dose, 4, 6, 8 and 24 hours on days 1 and 30 and at predose and 6 hours on days 7, 14 and 21

**Results:**

- **Mortality:** No deaths
- **Clinical signs:**

	Males				Females			
	Placebo	8 mg OROS®	64 mg OROS®	64 mg IR Dilaudid	Placebo	8 mg OROS®	64 mg OROS®	64 mg IR Dilaudid
Vomiting	2	0	4	23	0	2	15	23
Excessive Salivation	0	0	0	32	0	0	0	105
Unformed Stool	9	26	35	51	15	8	17	31
No Stool	0	0	1	1	0	0	4	8
Decreased Activity	0	0	17	78	0	1	35	116
Tremors	0	0	1	1	0	0	2	16
Eye Effects <sup>1</sup>	0	1	9	17	0	0	14	22

\*The data represent total number of observations within the group over the 30 day dosing period.

<sup>1</sup>Eye effects were constriction, dilation, lacrimation.

These effects were attributed to the pharmacologic activity of hydromorphone. The clinical effects occurred with greater frequency during the first 2 weeks of the study and with greater frequency after administration of the immediate release formulation. Tolerance developed to all clinical effects except excessive salivation, during weeks 3 and 4.

- **Body weights:** Mean body weights lower than pre-dose weights in males and females given OROS® at 64 mg/d and hydromorphone IR at 64 mg/d during week one only
- **Food consumption:** Decreased in male dogs given hydromorphone IR at 64 mg/d and in male and female dogs given OROS® at 64 mg/d compared to placebo-treated dogs during week one only; Increased in male dogs given OROS® at 64 mg/d compared to controls during week 3
- **Ophthalmoscopy:** No treatment-related effects
- **Hematology:** No treatment-related effects
- **Clinical chemistry:** No treatment-related effects
- **Urinalysis:** No treatment-related effects
- **Organ Weights:** No treatment-related effects
- **Gross pathology:** No treatment-related effects
- **Histopathology:** No treatment-related effects
- **Toxicokinetics Key Study Findings:** The C<sub>max</sub> and T<sub>max</sub> for OROS® and immediate release hydromorphone could not be directly compared due to different dosing schedules in the beagle dogs. The half-life and AUC were not provided for immediate release hydromorphone. The C<sub>max</sub> values increased proportionally with increasing OROS® dose, and were comparable on days 1 and 30, at 0.91 ng/ml (d1) and 0.84 ng/ml (d30) for 8 mg/d and 5.22 ng/ml (d1) and 7.25 ng/ml (d30) for 64 mg/d. The T<sub>max</sub> values were similar across doses for 8 mg/d and 64 mg/d OROS® on day 1 (6.9 h and 7.8 h respectively) and on day 30 (9.3 h at both doses). There was no significant effect of OROS® dose on half-life (6.1 h and 8.3 h at 8 mg/d and 64 mg/d respectively). The AUC showed dose proportionality, increasing from 10.6 to 87.6 ng.h/ml at 8 mg/d to 64 mg/d respectively on day 1, and from 17.7 to 81.6 ng.h/ml at 8 mg/d to 64 mg/d respectively on day 30. The AUC results did not show significant differences on days 1 and 30: there was no evidence of accumulation.

The NOAEL can be considered at 8 mg/d (approximately 1 mg/kg/d) OROS®, and the MTD at 64 mg/d (approximately 9 mg/kg/d) OROS®.

The results of drug content analysis of the recovered OROS® systems indicated that drug delivery was incomplete. Approximately 75% of the hydromorphone in the 8 mg systems was delivered in the male and female dogs, and 45% and 66% of the hydromorphone in the 64 mg systems was delivered in male and female dogs respectively.

### CARCINOGENICITY

Studies to evaluate the carcinogenic potential of hydromorphone HCl were not conducted, and will be requested as a phase IV commitment.

### REPRODUCTIVE TOXICOLOGY

**Study title:** *Examination of the Influence of Dilaudid on the Fertility and Early Embryonic Development to Implantation of Sprague-Dawley Rats by Oral Administration to the Animals of Fo-Generation in Accordance with ICH Guideline 4.1.1 - Segment I Study*

**Study Report No:** MPF/DT 9858E

**Volume # 27, Page #s 23-217**

**Site and testing facility:** [REDACTED] (b) (4)

**Study Dates:** August 6, 1998 - July 26, 1999

**GRP compliance:** Yes (  ) No (  )

**QA- Reports:** Yes (  ) No (  )

**Lot and batch numbers:** L 000 1974. Certificate of Analysis provided.

**Protocol reviewed by Division** Yes (  ) No (  ): Study conducted according to GLP regulations (EC and FDA), ICH Guideline 4.1.1 on Study of Fertility and Early Embryonic Development to Implantation, and EC Document III/3387/93 on Detection of Toxicity to Reproduction for Medicinal Products. Historical data provided.

**Methods:**

- **Species/strain:** Rat/Sprague-Dawley/Crt: CD®BR, [REDACTED] (b) (4)  
[REDACTED] Ages 8 weeks, weights 252-273 g males and 168-187 g females.
- **Doses employed:** Active Moiety: 0, 1.56, 3.13 and 6.25 mg/kg/day. Corresponding to hydromorphone salt at 0, 1.75, 3.5 and 7 mg/kg/day. In 5 ml/kg bwt/day sterile water (Batch 8223 A21, [REDACTED] (b) (4))
- **Route of Administration:** Oral by gavage
- **Study Design:** Males: daily administration of placebo or test substance from 4 weeks before mating through the end of the mating period. Females: daily administration from 2 weeks before mating through the 7th day of gestation. Oestrus stages and positive mating verified by daily vaginal lavage. Day 0 of gestation determined by presence of copulatory plug *in situ*.
- **Number of animals/sex/dosing group:** 20

- **Parameters and endpoints evaluated:** Daily mortality and clinical signs; weekly body weight, food and water consumption; on gestation day 13 fertility (number of fetuses and placental, number and size of resorptions, corpora lutea, implantations and location of fetuses in ovaries and uterine horns respectively), sperm number, motility and viability, early embryonic development, necropsy findings (after mating in males, gestation day 13 in females), and weights of uterus, ovary, testis, seminal vesicle, coagulating gland, prostate gland and epididymis. The following parameters were evaluated: resorption rate 100% (resorptions/implantations x 100), fertility index female % (#pregnant rats/number rats used x 100), fertility index male % (number siring litter/number paired x 100), pre-implantation loss % (corpora lutea-implantations/corpora lutea x 100), and post-implantation loss % (implantations-living fetuses/implantations x 100).
- **Statistical evaluations:** Bartlett chi-square test for homogeneity of variances, DUNNETT test (if variances homogenous) or Student's t-test (if heterogeneity of variance) comparing experimental and control groups. Fisher's exact test or chi<sup>2</sup> test with Yates' correction for continuity for comparison of classification measurements.

### Results:

- **Clinical signs:** Restlessness and increased motor activity at 1.56, 3.13 and 6.25 mg/kg/day PO from pre-mating (all males and females) through gestation period (all females), with decreased incidence during gestation. Self-mutilation of tail, paws, limbs, chest (including hairloss, eschar formation, hemorrhagic canthus and snout) observed occasionally (1-7/dose in all active drug groups). Convulsions were observed in 2/20 male rats at 1.56 mg/kg/day during mating.
- **Mortality:** No premature deaths in male or female parent rats.
- **Body weight:** Dose related decrease at 3.13 mg/kg/day (-3% to -7% in males during pre-mating, -7% to -8% in females during pre-mating and -6% to -10% in females during gestation) and at 6.25 mg/kg/day (-6% to -7% in males and -7% to -8% in females during pre-mating, -8% to -14% in females during gestation).
- **Food consumption:** Decreased at 6.25 mg/kg/day in males (-5%) and females (-5% to -9%) during pre-mating compared to controls. No effect on food consumption during gestation period and no effect on water consumption throughout study.
- **Fertility in Males**
  - **In-life observations:** No treatment-related effects.
  - **Terminal and Necroscopic evaluations:** No treatment-related pathology. No effects on testicle and epididymis weights. No effects on number, motility and viability of spermatozoa/spermatids.
- **Fertility and Early Embryonic Development in Females**
  - **In-life observations:** No treatment-related effects.
  - **Terminal and Necroscopic evaluations:** No effects on uterus weight. At 6.26 mg/kg/day decreased number of implantation sites and viable fetuses. No effects on number of corpora lutea, resorption rate, pre-implantation loss and post-implantation loss.
- **Terminal and Necroscopic Evaluations:**
  - **Dams:** No treatment-related effects.
  - **Males:** Self-mutilation indicated by hair loss (forepaws and chest) and eschar formation in 1 male at 1.56 mg/kg/day, 5 males at 3.13 mg/kg/day and 2 males at 6.25 mg/kg/day.
  - **Offspring:** No dead fetuses, no fetal malformations on gross examination. No effects on number of placentae relative to number of fetuses.

Hydromorphone HCl dose (mg/kg/day)	Mating Index (%)	Fertility Index (%)	Resorption Rate (%)	Pre-Implantation Loss (%)	Post-Implantation Loss (%)
0	95	85	4.9	10.4	4.9
1.56	100	95	7.0	13.6	7.0
3.13	100	90	6.1	8.8	6.1
6.25	100	95	4.6	9.3	4.6

**Summary and Evaluation:** The numbers of implantation sites and viable fetuses were decreased at 6.25 mg/kg/d PO but hydromorphone hydrochloride had no effect on male and female fertility under the conditions of this study. In the fetal rats, the NOEL was 3.13 mg/kg/day PO. The high dose in this study (6.25 mg/kg PO) was shown to result in an AUC of 135 ng.h/ml (slightly less than 1x the exposure after the high proposed human dose of 64 mg in a 60 kg patient, AUC 156 ng.h/ml) in another study in rats.

There was no maternal or paternal toxicity at 1.56 mg/kg PO hydromorphone hydrochloride. At 1.56 mg/kg/day PO, pharmacological effects of the drug, restlessness, self-mutilation and increased motor activity were observed. Hydromorphone toxicity was observed at 3.13 mg/kg/day PO (reduced body weight) and 6.25 mg/kg/day PO (reduced body weight and during pre-mating reduced food consumption).

**Study title: Examination of the Influence of Dilaudid on the Pregnant Rat and the Foetus by Oral Administration - Embryotoxicity Study/Segment II Study**

**Study No:** MPF/DT 9856E

**Volume # 28, Page #s 7-359**

**Site and testing facility:** [REDACTED] (b) (4)

**Study Dates:** October 21, 1998 - January 27, 1999

**GRP compliance:** Yes ( x ) No ( )

**QA- Reports:** Yes ( x ) No ( )

**Lot and batch numbers:** L 000 1974. Certificate of Analysis provided

**Protocol reviewed by Division Yes ( ) No ( x ): Study conducted according to GLP regulations (EC and USFDA), ICH Guideline on Detection of Toxicity to Reproduction for Medicinal Products (EC-Doc. III/3387/93) and EC Guidelines 'Teratogenicity Test' - rodent and non-rodent -, Official Journal of the European Communities L 133/24, May 30th, 1988.**

**Methods:**

- **Species/strain:** Pregnant Female Rat/Sprague-Dawley/Crl: CD@BR, [REDACTED] (b) (4)  
[REDACTED] Ages 7 weeks, weights 184-229 g.
- **Doses employed:** Active Moiety: 0, 1.56, 3.13 and 6.25 mg/kg/day. Corresponding to hydromorphone salt: at 0, 1.75, 3.5 and 7 mg/kg/day. In 5 ml/kg bwt/day sterile water (Batch 8223 A21, [REDACTED] (b) (4)).
- **Route of Administration:** Oral by gavage
- **Study Design:** Dosed daily from gestation days 6-17 for a total of 12 doses. Dams laparotomised on gestation day 20.

- **Number of animals/dosing group:** 20 (teratogenicity study) + 3 (toxicokinetics study)
- **Parameters and endpoints evaluated:** Maternal mortality (daily), clinical signs (daily), body weight (daily), food and water consumption (daily) and gross necropsy. In the reproductive toxicity evaluation, number of corpora lutea, number and location of implantation sites, number and size of resorptions, gross evaluation and weight of placenta, fetal weights and sex, number of live and dead fetuses, placental weights, external fetal examination, skeletal anomalies, malformations, variations and retardations.
- **Statistical evaluations:** Homogeneity of variances by Bartlett chi-square test. When homogeneous, Dunnett test comparing experimental and control group. When heterogeneous, Student's t-test. For comparison of classification measurements, Fisher's exact test.

### Results:

- **Clinical signs:** No effects at 1.56 mg/kg/day. Pharmacodynamic effects consistent with known opioid effects at 3.13 and 6.25 mg/kg/d: restlessness, increased motor activity from dosing days 8-12 (gestation days 13-17) in 12/20 mid-dose (3.13 mg/kg/day) and 20/20 high dose (6.25 mg/kg/day) dams, from 5-20 minutes after dosing and lasting 1-2 hours. Also, hair loss in 1/20 mid-dose dams and 6/20 high dose dams. Self mutilation indicated by red tail in 2/20 high dose dams.
- **Mortality:** No deaths.
- **Body weight:** No effects at 1.56 mg/kg/day. Mean body weight increases were 74% at 1.56, 67% at 3.13 and 57% at 6.25 mg/kg/d compared to 80% in the control group during gestation (days 0-19). Mean maternal body weight on day 20 significantly lower in high dose group (326.3 g) compared to control group (361.9 g).
- **Food consumption:** No effects at 1.56 mg/kg/day. Food consumption reduced 12% at 3.13 and 18% at 6.25 mg/kg/d compared to controls. No effects on water consumption.
- **Toxicokinetics:**

Gestation Day	Number of Doses	Hydromorphone HCl Dose (mg/kg/day)	Mean Cmax (ng/ml)	Mean Tmax (h)	Mean AUC <sub>0-24h</sub> (ng.h/ml)
6	1	1.56	5.6 ± 0.9	1.3 ± 1.4 <sup>b</sup>	53.2 ± 12.9
		3.13	7.6 ± 4.4	0.5 ± 0	42.2 ± 9.2
		6.25	13.8 ± 1.3	0.5 ± 0	89.6 ± 33.0
17	12	1.56	7.6 ± 2.5	0.3 ± 0.3 <sup>a</sup>	55.5 ± 30.8
		3.13	12.7 ± 6.0	0.5 ± 0	67.4 ± 16.6
		6.25	35.8 ± 1.3	0.5 ± 0	135.2 ± 36.6

<sup>a</sup> Due to contamination of sample in one animal showing a Cmax at the 0 hour collection point.

<sup>b</sup> Due to maximum of 6.5 ng/ml at 3 hours in one animal that had a concentration of 6.2 ng/ml at 0.5 hours.

### - Terminal and Necroscopic Evaluations:

**Dams:** No findings at 1.56 mg/kg/day. Hair loss in 3/20 dams at 3.13 and 5/20 dams at 6.25 mg/kg/day. Unilateral dilatation of renal pelvis in 1/20 dams at 6.25 mg/kg/day (within historical range). No effects on reproductive parameters in the dams except slight increase in number of corpora lutea, number of implants and number of resorptions at the high dose.

	Hydromorphone Dose (mg/kg/day)							
	0		1.56		3.13		6.25	
Females with sperm	25		25		25		25	
Pregnant females	25		25		24		24	
Pregnant females evaluated	20		20		20		20	
Corpora lutea	total	290	total	301	total	301	total	310
	per dam	14.5	per dam	15.1	per dam	15.1	per dam	15.5
Implantation sites	total	286	total	291	total	291	total	294*
	per dam	14.3	per dam	14.6	per dam	14.6	per dam	14.7*
Live fetuses	total	276	total	280	total	282	total	279
	per dam	13.8	per dam	14.0	per dam	14.1	per dam	14.0
Dead fetuses (litter mean)	0		0		0		0	
Resorptions	total	10	total	11	total	9	total	15
	per dam	0.5	per dam	0.6	per dam	0.5	per dam	0.8
Early resorptions	total	9	total	9	total	8	total	9
	per dam	0.5	per dam	0.5	per dam	0.4	per dam	0.5
Late resorptions	total	1	total	2	total	1	total	6
	per dam	0.1	per dam	0.1	per dam	0.1	per dam	0.3
Pre-implantation loss (mean%)	1.3		2.6		3.0		4.9	
Post-implantation loss (mean%)	3.4		3.8		3.0		5.2	
	Males	Females	Males	Females	Males	Females	Males	Females
Mean weight of fetuses (g)	3.5	3.3	3.6	3.4	3.5	3.3	3.4	3.2
Total number of fetuses	137	139	142	138	148	134	136	143

### Embryo-fetal Development

No effects on sex distribution of the fetuses and placental weights.

External examination: no fetal malformations and variations, and no dead fetuses at the time of laparotomy. Four runts (1 at 3.13 mg/kg/day and 3 at 6.25 mg/kg/day) in 4 different litters. Runt weights 2.0 g compared to a mean of 3.45 g in the 3.13 mg/kg/day group and 3.29 g in the 6.25 mg/kg/day group. No significant differences in mean fetal weights as an effect of dose.

Skeletal examination: No skeletal malformations. Skeletal variations observed: reduced or shortened ribs, wavy accessory 14th ribs and bipartite, dumbbell-shaped or misaligned sternbrae in all groups including the controls. Skeletal retardations observed in all groups including the controls: incomplete ossification of frontal, parietal, interparietal and supraoccipital regions of the skull, missing ossification of the hyoid, incomplete or missing ossification or reduced size of the sternbrae, dumbbell-shaped or bipartite or missing ossification of the thoracic or caudal vertebral bodies, and reduced size of lumbar vertebral arches and pubis and os ischii. All skeletal retardations within historical range (see Vol. 28, pages 332-339) and similar in incidence in all groups except for significant decrease in ossification of the sternbrae at 1.56 mg/kg/day.

Soft tissue examination: No malformed fetal organs. Variations were unilateral/bilateral dilated renal pelvis, misplaced kidney, hemorrhage or hematoma in liver, thoracic cavity or brain without relation to dose compared to controls.

		Dose (mg/kg/day)				
		0	1.56	3.13	6.25	
<b>Malformations</b>	Absolute	0	0	0	0	
	Per dam	0.0	0.0	0.0	0.0	
	Malformation rate (mean %)	0.0	0.0	0.0	0.0	
<b>Skeletal Variations</b>	Fetal incidence	N	7	9	5	4
		%	5.1	6.4	3.5	2.9
	Litter incidence	N	4	6	4	3
		%	20.0	30.0	20.0	15.0
<b>Skeletal Retardations</b>	Fetal incidence	N	137	135	140	138
		%	99.3	96.4	99.3	98.6
	Litter incidence	N	20	20	20	20
		%	100.0	100.0	100.0	100.0
<b>Soft Tissue Variations</b>	Fetal incidence	N	10	4	17	11
		%	7.2	2.9	12.1	7.9
	Litter incidence	N	8	3	11	8
		%	40.0	15.0	55.0	40.0

**Summary and Evaluation:** The NOEL for developmental toxicity was 3.13 mg/kg/day. Developmental toxicity was shown by the occurrence of 3 runts in a total of 279 fetuses at 6.25 mg/kg/day. Hydromorphone hydrochloride was not teratogenic at up to 6.25 mg/kg/day (AUC 135 ng.h/ml) given on gestation days 6 through 17 in rats. The high dose in this study was slightly less than 1x the high dose of 64 mg/day in a 60 kg adult patient (AUC 156 ng.h/ml).

No maternal toxicity was observed at 1.56 mg/kg/day (AUC 55.5 ng.h/ml) hydromorphone hydrochloride. Pharmacodynamic effects of restlessness, increased motor activity and self-mutilation, and maternal toxicity indicated by reduced maternal body weights and food consumption were observed at 3.13 and 6.25 mg/kg/day.

In the toxicokinetic analysis, absorption was rapid with maximum plasma levels observed at approximately 0.5 hours except in the low dose group on dosing day 1 (1.3 h). Total exposure (AUC) generally increased with dose and was greater on gestation day 17 (135 ng.h/ml) than on gestation day 6 (89.6 ng.h/ml) in the high dose (6.25 mg/kg/day) group.

**Study title: Examination of the Influence of Dilaudid on the Pregnant Rabbit and the Foetus by Oral Administration - Embryotoxicity Study/Segment II Study**

**Study No:** MPF/DT 9857E

**Volume # 29, Page #s 2-266**

**Site and testing facility:** In life-phase evaluation: (b) (4)  
 ; Study protocol, statistical evaluation, report: (b) (4)

**Study Dates:** November 19, 1998 - March 16, 1999

**GRP compliance:** Yes ( x ) No ( )

**QA- Reports:** Yes ( x ) No ( )

**Lot and batch numbers:** L 000 1974. Certificate of Analysis provided

**Protocol reviewed by Division:** Yes ( ) No ( x ): Study conducted according to GLP regulations (EC and USFDA), ICH Guideline on Detection of Toxicity to Reproduction for

Medicinal Products (EC-Doc. III/3387/93) and EC Guidelines 'Teratogenicity Test' - rodent and non-rodent -, Official Journal of the European Communities L 133/24, May 30th, 1988.

#### Methods:

- **Species/strain:** Pregnant Rabbit/Himalayan (b) (4)  
Ages 4-5 months, weights 2.13-2.80 kg.
- **Doses employed:** Active Moiety: 0, 6.25, 12.5 and 25 mg/kg/day. Corresponding to hydromorphone salt: at 0, 7, 14 and 28 mg/kg/day. In 5 ml/kg bwt/day sterile water (Batch 8223 A21, (b) (4)).
- **Route of Administration:** Oral by gavage
- **Study Design:** Dosed daily from gestation days 6-20 for a total of 15 doses. Dams laparotomised on gestation day 29.
- **Number of animals/dosing group:** 20 (teratogenicity study) + 3 (toxicokinetics study)
- **Parameters and endpoints evaluated:** Maternal mortality (daily), clinical signs (daily), body weight (daily), food and water consumption (daily) and gross necropsy. In the reproductive toxicity evaluation, number of corpora lutea, number and location of implantation sites, number and size of resorptions, gross evaluation and weight of placenta, fetal weights and sex, number of live and dead fetuses, placental weights, external fetal examination, skeletal anomalies, malformations, variations and retardations.
- **Statistical evaluations:** Homogeneity of variances by Bartlett chi-square test. When homogeneous, Dunnett test comparing experimental and control group. When heterogeneous, Student's t-test. For comparison of classification measurements, Fisher's exact test or chi<sup>2</sup>-test with Yates' correction for continuity.

#### Results:

- **Clinical signs:** No effects at 6.25 mg/kg/day. Reduced motility at 12.5 mg/kg/day in 17/20 dams during the first 4 dosing days, and at 25 mg/kg/day in 20/20 dams throughout the dosing period. Additional effects at 25 mg/kg/day were sedation, abdominal position, and mydriasis. Onset of effects 5-60 minutes after dosing, duration 1-2 hours.
- **Mortality:** No maternal deaths during dosing period
- **Body weight:** Body weight gain lower in the high dose (25 mg/kg/day) rabbits (+7%) than in controls (+13%).
- **Food consumption:** Reduced food intake at the high dose (-68%) during dosing. No effects on water consumption.
- **Toxicokinetics:**

Gestation Day	Number of Doses	Hydromorphone HCl Dose (mg/kg/day)	Mean Cmax (ng/ml)	Mean Tmax (h)	Mean AUC <sub>0-24h</sub> (ng.h/ml)
6	1	6.25	7.1	0.5	51.6
		12.5	9.7	0.8	91.0
		25	53.2	0.7	237.2
20	15	6.25	8.9	0.8	51.8
		12.5	10.0	0.8	103.9
		25	16.9	0.7	141.1

#### - Terminal and Necroscopic Evaluations:

**Dams:** No treatment-related effects in the gross examination.

	Hydromorphone Dose (mg/kg/day)							
	0		6.25		12.5		25	
Females with sperm	24		24		24		24	
Pregnant females	22		21		22		22	
Pregnant females evaluated	20		20		20		20	
Corpora Lutea	total	140	total	138	total	151	total	134
	per dam	7.0	per dam	6.9	per dam	7.6	per dam	6.7
Implantation sites	total	125	total	124	total	129	total	118
	per dam	6.3	per dam	6.2	per dam	6.5	per dam	5.9
Live fetuses	total	121	total	118	total	126	total	113
	per dam	6.1	per dam	5.9	per dam	6.3	per dam	5.7
Dead fetuses (total)	0		0		0		0	
Resorptions	total	4	total	6	total	3	total	5
	per dam	0.2	per dam	0.3	per dam	0.2	per dam	0.3
Early Resorptions	total	4	total	4	total	3	total	4
	per dam	0.2	per dam	0.2	per dam	0.2	per dam	0.2
Late Resorptions	total	0	total	2	total	0	total	1
	per dam	0.0	per dam	0.1	per dam	0.0	per dam	0.1
Pre-implantation loss	mean%	10.4	mean%	10.5	mean%	14.9	mean%	13.0
Post-implantation loss	mean%	3.4	mean%	4.8	mean%	2.8	mean%	2.8
	Males	Females	Males	Females	Males	Females	Males	Females
Mean weight of fetuses (g)	39.3	39.3	40.2	40.2	40.1	38.7	40.9	40.6
Total number of fetuses	54	67	53	65	55	71	64*	49*

\* Significantly different from control at p[0.05.

**Embryo-fetal Development:** No treatment-related effects on placenta or fetal weights.

**External examination:** One externally malformed fetus with hyperflexion of the paws at 6.25 mg/kg/day. No external variations and no dead fetuses. Three runts (2 at 12.5 mg/kg/day and 1 control).

**Skeletal examination:** No skeletal malformations. Skeletal variations were fused ribs, bipartite or fused or misaligned sternum and fused thoracic vertebral bodies. Skeletal retardations were incomplete or missing ossification or reduced size of the sternbrae, dumbbell-shaped thoracic vertebral bodies. Numbers of variations and retardations within historical range at all doses (see Vol. 29, pages 240-249).

**Internal examination:** No soft-tissue malformations.

			Dose (mg/kg/day)			
			0	1.56	3.13	6.25
<b>Malformations</b>	Absolute		0	1	0	0
	Per dam		0.0	5.0	0	0
	Malformation rate (mean %)		0.0	0.7	0	0
<b>Skeletal Variations</b>	Fetal incidence	N	7	12	10	9
		%	5.8	10.2	7.9	8.0
	Litter incidence	N	6	10	6	6
		%	30.0	50.0	30.0	30.0
<b>Skeletal Retardations</b>	Fetal incidence	N	81	88	85	71
		%	66.9	74.6	67.5	62.8
	Litter incidence	N	19	19	18	19
		%	95.0	95.0	90.0	95.0

<b>Soft Tissue Variations</b>	Fetal incidence	N	0	0	0	0
		%	0	0	0	0
	Litter incidence	N	0	0	0	0
		%	0	0	0	0

**Summary and Evaluation:** Hydromorphone HCl was not teratogenic in rabbits at up to 25 mg/kg/day (AUC 150 ng.h/ml, approximately 1x the human exposure of 156 ng.h/ml at 64 mg/d in a 60 kg patient on an AUC basis) when given orally from gestation days 6 through 20. The ratio of male to female fetuses was higher in the high dose (25 mg/kg/d) group than in the control group. The NOEL for developmental toxicity was 25 mg/kg/day in this study, as there was no evidence of embryotoxicity.

No maternal toxicity was observed at 6.25 mg/kg/day (AUC 51.8 ng.h/ml). The LOAEL for maternal toxicity was 12.5 mg/kg/day. Reduced motility, sedation, abdominal position and mydriasis were observed in the female rabbits at 12.5 and 25 mg/kg/day, and reduced body weight and food consumption at 25 mg/kg/day.

In the toxicokinetic analysis, absorption was rapid and peak plasma levels occurred in less than one hour at all doses (6.25-25 mg/kg/day) on both gestation days 6 and 20. Exposure increased linearly on day 6 and was comparable at the low (6.25 mg/kg/day) and mid (12.5 mg/kg/day) doses on both days, but was considerably lower at the high (25 mg/kg/day) dose on day 20 compared to day 6.

**Study title: Examination of Dilaudid on the Pre- and Postnatal Development (Including Maternal Function) of Sprague-Dawley Rats Following Oral Administration to the Dams of the Fo-Generation in Accordance with ICH Guideline 4.1.2 - Segment III Study**

**Study No:** MPF/DT 9859E

**Volume # 30, Page #s 6-459**

**Site and testing facility:** [REDACTED] (b) (4)

**Study Dates:** August 26, 1998 - March 5, 1999

**GRP compliance:** Yes (x) No ( )

**QA- Reports:** Yes (x) No ( )

**Lot and batch numbers:** L 000 1974

**Protocol reviewed by Division:** Yes ( ) No ( x ): Study conducted according to GLP regulations (EC and USFDA), EC Guideline on Detection of Toxicity to Reproduction for Medicinal Products (EC-Doc. III/3387/93) and ICH Guideline 4.1.2 Study for Effects on Pre- and Postnatal Development, Including Maternal Function.

**Methods:**

- **Species/strain:** Rat/Sprague Dawley/Crl: CD BR, [REDACTED] (b) (4)
- **Doses employed:** Dilaudid (hydromorphone HCl) at 0, 1.56, 3.13 and 6.25 mg/kg/day corresponding to hydromorphone salt at 0, 1.75, 3.5 and 7 mg/kg/day in 5 ml/kg/day water for injection (batch 8223 A21, [REDACTED] (b) (4))
- **Route of Administration:** Oral
- **Study Design:** Fo generation dams dosed only: daily by gavage from gestation day 6 through 21st day of lactation (weaning)
- **Number of animals/sex/dosing group:** 20

- **Parameters and endpoints evaluated:** Clinical signs, food and water consumption, body weight, deficiencies in Fo generation maternal care (inadequate construction/cleaning of nest, physical abuse of pups, inadequate lactation), necropsy of Fo dams, F<sub>1</sub> pups and parents, and F<sub>2</sub> pups, F<sub>1</sub> postnatal physical and functional development (morphological landmarks including pinna detachment, opening of ears, eyes and vagina, incisor eruption, cleavage of balanopreputial gland, functional tests including righting reflex, pupillary reflex, auditory startle reflex, open-field, passive avoidance learning and memory tests). The following indices were determined: gestation index (# bearing live pups/# pregnant), birth index (# live + dead pups born/# implantation scars), live birth index (# pups live on day 0 of lactation / total # pups born), viability index (# pups on day 4 of lactation/# live on day 0), lactation index (# pups live on lactation day 21/# pups live on day 4) and overall survival index (# pups live on lactation day 21/total # pups born).
- **Statistical evaluations:** Homogeneity of variances by Bartlett chi-square test. When homogeneous, Dunnett test ( $p \leq 0.01$ ) comparing experimental and control group. When heterogeneous, Student's t-test ( $p \leq 0.01$ ). For comparison of classification measurements, Fisher's exact test or chi<sup>2</sup>-test with Yates' correction for continuity.

## Results:

### - Clinical signs:

**Fo females:** Increased motor activity (7/20 dams at 1.56, 20/20 at 3.13 and 20/20 at 6.25 mg/kg/day), self-mutilation, loss of hair and eschar formation (6/20 dams at 1.56, 9/20 at 3.13 and 7/20 at 6.25 mg/kg/day), and wounds (2/20 at 3.13 and 1/20 at 6.25 mg/kg/day)

F<sub>1</sub> animals: No effects

F<sub>2</sub> animals: No effects

- **Mortality:** No effects in Fo, F<sub>1</sub> and F<sub>2</sub> generations at any dose

### - Body weight:

**Fo females:** Reduced at 1.56 (-4% to -6% compared to controls [not significant], 3.13 (-3% to -9%) and 6.25 (-4% to -13%) mg/kg/day, reduced pup weights at birth at 6.25 mg/kg/day (8% decreased in females and 11% decrease in male pups). Dam body weights normalized after 3 weeks lactation.

F<sub>1</sub> animals: Reduced in F1 pups during pre- and post-weaning period at 3.13 (-4% to -10% compared to controls) and 6.25 mg/kg/day (-2% to -19%), reduced in F1 dams at 3.13 (-2% to -6%) and 6.25 (-5% to -12%) mg/kg/day during pre-mating.

F<sub>2</sub> animals: No effects

### - Food consumption:

**Fo females:** Reduced at 1.56 (-3% to -15% compared to controls), 3.13 (-6% to -17%) and 6.25 (-5% to -32%) mg/kg/day

F<sub>1</sub> animals: No effects

F<sub>2</sub> animals: No effects

-**Water consumption:** No effects

### - Prenatal and postnatal development, including maternal function

#### - In-life observations:

-At 6.26 mg/kg/day, extensive loss of blood during littering (1 dam), reduced number of pups alive from lactation day 4 onward, decreased overall survival and viability indices, decreased maternal brood care (decreased suckling) in Fo dams

-Morphological landmarks including time-points of pinna detachment, upper incisor eruption, ear and eye opening, cleavage of the balanopreputial gland and vaginal

opening not affected except for delayed ear opening (by a mean of  $0.8 \pm 0.6$  days) at 6.25 mg/kg/day

-No effects on functional test and open-field test at 1.56 and 3.13 mg/kg/day. Auditory startle reflex reduced on day 14 at 6.25 mg/kg/day. In open-field test, decreased rearings and sectors entered at 6.25 mg/kg/day.

#### Peri- and Postnatal Data: Fo Generation of Mothers

		Dose (mg/kg/day)							
		0		1.56		3.13		6.25	
Parents	Females with sperm	24		24		24		24	
	Pregnant females	24		22		23		22	
	Females with delivery	20		20		20		21	
	Mean duration of gestation (d)	21.4		21.3		21.3		21.3	
Litter Means	Implantations	16.1		15.8		15.1		16.5	
	Live births	14.8		14.4		14.0		15.2	
	Stillbirths	0.3		0.0		0.1		0.0	
	Survivors at day 4 pp	14.8		13.6		12.6		11.0*	
	Survivors at weaning	14.5		13.5		12.4		11.3*	
		Males	Females	Males	Females	Males	Females	Males	Females
	Weight at birth (g)	6.7	6.5	6.8	6.4	6.6	6.3	6.2*	5.8*
	Weight at weaning (g)	41.7	40.7	41.4	39.7	41.7	40.5	40.6	40.0
	Number of live newborns	143	153	134	154	143	136	173	145

\* Significantly different from control at  $p \leq 0.01$  (Dunnett's test)

#### Peri- and Postnatal Data: F<sub>1</sub> Generation of Mothers

		Dose (mg/kg/day) given the Fo females							
		0		1.56		3.13		6.25	
Parents	Females with sperm	20		20		20		19	
	Pregnant females	16		19		17		17	
	Evaluated pregnant females	16		19		16		17	
Litter Means	Corpora lutea	Not evaluated							
	Implantations	16.6		17.0		17.0		16.2	
	Live fetuses	15.2		15.3		15.0		15.0	
	Dead fetuses	0.4		0.4		0.3		0.2	
	Resorptions	Not evaluated							
		Males	Females	Males	Females	Males	Females	Males	Females
	Weight of fetuses (g)	6.5	6.1	6.8	6.4	6.8	6.3	6.5	6.0
	Number of live newborns	149	144	119	123	136	119	122	132

#### - Terminal and Necroscopic Evaluations:

- Dams: No effects.
- Offspring: No effects.

**Summary and Evaluation:** There were no effects on gestation duration or reproduction parameters. Parturition was influenced in one high dose dam showing severe blood loss during parturition. There were no effects on numbers of live pups prior to lactation, but live pups were decreased on day 4 of lactation in the high dose group (6.25 mg/kg/d, approximately 1x the human exposure on an AUC basis based on the toxicokinetic analysis of another reproductive toxicity study in rats). There were no effects on birth index, live birth index, viability index, lactation index and overall survival index at the low dose, but the viability and survival indices were decreased at 6.25 mg/kg/day and a trend toward a decrease in lactation index at that dose. There were no effects on sex distribution of the F<sub>1</sub> generation. The NOAEL for

developmental toxicity was 1.56 mg/kg/day. At 3.13 and 6.25 mg/kg/day, reduced overall survival index and mean pup weight were observed for the F<sub>1</sub> generation through sexual maturity, but no developmental toxicity was shown in the F<sub>2</sub> generation. Decreased maternal brood care in the Fo dams at 6.25 mg/kg/day was indicated by absence of milk in the pup stomachs on lactation days 1 and 2. Altered morphological landmarks and functional tests in the F<sub>1</sub> pups were observed at the high dose. There was a delay in ear opening, reduced auditory startle reflex and reduced rearings and sectors entered by the pups at that dose. The NOAEL in the F<sub>2</sub> generation was > 6.25 mg/kg/day.

Maternal toxicity, including reduced body weight and food consumption was observed at all doses (1.56-5.25 mg/kg/d) administered the Fo dams in this study.

## GENETIC TOXICOLOGY

**Study Title:** *Salmonella/Microsome Test (Ames Test) With Hydromorphone HCl*

**Study No:** MPF/WT 9352

**Study Type:** *In vitro* mutagenicity test: Induction of gene mutations in bacteria

**Volume # 31, Page #s 30-71**

**Conducting Laboratory:** Knoll AG, Research and Development, Department of Drug Toxicology, Knollstr. 50, 67061 Ludwigshafen/Germany

**Date of Study Initiation/completion:** August 31, 1993/ September 3, 1993

**GLP Compliance:** Yes (x) No ( )

**QA- Reports:** Yes (x) No ( )

**Drug Lot Number:** W 61571

**Study Endpoint:** Reverse mutations indicated by increase in revertant count in histidine-deficient mutant *Salmonella typhimurium* strains

### Methodology:

- **Strains/Species/Cell line:** Source: (b) (4)

	TA 98	TA 100	TA 1535	TA 1537
Histidine mutation	his D3052	his G 46	his G 46	his C3076
LPS (deep rough)	rfa	rfa	rfa	rfa
Repair	< uvrB	< uvrB	< uvrB	< uvrB
R factor (pKM 101)	+R	+R	-	-

- **Basis of dose selection:** 5000 mcg/plate highest dose required by OECD Guideline # 471 and the EEC Guideline 92/69/EEC, no precipitation or bacteriotoxic effects up to 5000 mcg/plate in preliminary test
- **Test Agent Stability:** Evaluated January 20, 1992 and January 31, 1994, determined to be stable.
- **Metabolic Activation System:** S9 mix from male rat liver enzyme induction using Aroclor 1254, 20 and 50 mcl per plate, mix composed of S9 fraction, co-factors NADP and glucose-6-phosphate, buffer and salts.
- **Controls:**
  - **Vehicle:** Water for injection, sterile and apyrogenic, 100 mcl/plate
  - **Negative Controls:** Vehicle
  - **Positive Controls:** 2-aminoanthracene (2-AA, 2.5 or 5 mcg/plate): all strains, 4-nitro-orthophenylene diamine (4-NOPD, 10 mcg/plate): TA 98, and 9-aminoacridinium chloride (9-AAC, 75 mcg/plate):TA 1537, in dimethyl sulfoxide

(DMSO) (b) (4); and Sodium azide (2.5 mcg/plate):TA 100 and TA 1535, in water for injection (b) (4)

**- Exposure Conditions:**

- **Incubation and sampling times:** 48 hours at 37°C
- **Doses used in definitive study:** 0 (solvent), 100, 316, 1000, 2500 and 5000 mcg/plate
- **Study design:** Suspensions of histidine-deficient bacteria incubated on minimal agar plates without histidine, with the test solution and in the presence or absence of S9 mix. Base-pair substitution (in TA 100 and TA 1535) or frame shift mutation (in TA 98 and TA 1537) would allow the bacteria to synthesize histidine and form colonies. Colonies counted and compared to number of colonies in the positive and negative control plates.

**- Analysis:**

- **No. plates/replicate:** 2 plates/experimental point, 2 replications
- **Counting method:** Artec automatic colony counter
- **Cytotoxic endpoints:** Not provided.
- **Genetic toxicity endpoints/results:** Increased revertant colony count compared to negative control

**Results:**

**Mean Number of Revertants/Plate**

Strain	Substance	Dose (mcg/plate)	wo/S9 Exp. 1	wo/S9 Exp. 2	w/s9 Exp. 1	w/S9 Exp. 2
TA 98	Solvent	0	22.50	21.50	32.00	39.75
	Hydromorphone	100	24.75	20.50	31.50	35.00
	Hydromorphone	316	21.75	21.50	29.25	33.75
	Hydromorphone	1000	21.50	19.50	34.75	39.00
	Hydromorphone	2500	20.25	21.25	35.00	41.50
	Hydromorphone	5000	23.50	23.50	37.00	44.75
	2-AA	2.5	-	-	1034.00	690.25
	4-NOPD	10	620.25	435.50	-	-
TA 100	Solvent	0	96.50	111.75	110.75	106.50
	Hydromorphone	100	96.50	100.50	103.75	85.50
	Hydromorphone	316	100.50	118.25	109.00	88.50
	Hydromorphone	1000	86.75	111.50	106.75	95.25
	Hydromorphone	2500	98.25	101.75	112.75	97.50
	Hydromorphone	5000	98.25	98.00	108.25	98.75
	2-AA	2.5	-	462.25	1060.50	1179.25
	Na Azide	2.5	482.00	-	-	-
TA 1535	Solvent	0	11.25	10.50	12.25	13.75
	Hydromorphone	100	11.75	8.75	11.75	11.75
	Hydromorphone	316	9.50	10.50	12.75	11.50
	Hydromorphone	1000	10.00	11.75	10.00	11.75
	Hydromorphone	2500	10.75	10.00	13.50	14.25
	Hydromorphone	5000	12.25	11.00	11.25	10.75
	2-AA	5	-	-	105.75	120.50
	Na Azide	2.5	341.25	346.75	-	-
TA 1537	Solvent	0	9.25	9.50	8.25	10.00
	Hydromorphone	100	7.75	8.75	8.00	12.00
	Hydromorphone	316	8.50	8.50	8.25	12.25
	Hydromorphone	1000	8.50	10.25	9.76	12.25
	Hydromorphone	2500	8.00	10.75	10.25	11.50
	Hydromorphone	5000	10.25	10.25	7.25	11.00
	2-AA	5	-	-	300.00	412.50
	9-AAC	75	431.75	365.25	-	-

**Summary:** No increase in revertant count was observed at up to 5000 mcg/plate hydromorphone hydrochloride, with and without metabolic activation with S9. Therefore, the test substance is considered non-mutagenic in the Salmonella/microsome test (Ames test) under the conditions of this study.

**Study Title: Dilaudid (Hydromorphone Hydrochloride) - Cytogenic Test After Single Oral Administration in Mice (Micronucleus Test)**

**Study No:** MPF/DT 9855E

**Study Type:** *In vivo* mutagenicity test: induction of micronuclei in polychromatic erythrocytes of mouse bone marrow

**Volume #31, Pages #72-106**

**Conducting Laboratory:** [REDACTED] (b) (4)

**Date of Study Initiation/completion:** August 18, 1998 - December 7, 1998

**GLP Compliance:** Yes (x) No ( )

**QA- Reports:** Yes (x) No ( )

**Drug Lot Number:** L 0001974

**Study Endpoint:** Induction of micronuclei in polychromatic erythrocytes of mouse bone marrow

**Methodology:**

- **Strains/Species:** Male and female NMRI mice, [REDACTED] (b) (4) ages 8-12 weeks, mean weights 33.3 g males and 27.0 g females
- **Dose Selection Criteria:**
  - **Basis of dose selection:** Toxicity range-finding study found maximum tolerated dose at 100 mg/kg PO
  - **Range finding studies:** 2 male and 2 female mice per dose received hydromorphone hydrochloride at up to 100, 150 and 200 mg/kg PO; evaluated at 1, 6, 24 and 48 hours after dosing
- **Test Agent Stability:** Shown stable at 1% in H<sub>2</sub>O (pH 4.8, room temperature, brown glass) at least 14 days, analyzed August 12, 1998
- **Metabolic Activation System:** *In vivo* system; not applicable
- **Controls:**
  - **Vehicle:** Physiological (0.9%) NaCl solution at 10 ml/kg
  - **Negative Controls:** Physiological (0.9%) NaCl solution at 10 ml/kg
  - **Positive Controls:** Cyclophosphamide [REDACTED] (b) (4) at 40 mg/kg PO in 10 ml/kg deionized water
- **Exposure Conditions:**
  - **Doses used in definitive study:** 0, 10, 33.3 and 100 mg/kg
  - **Study design:** Hydromorphone hydrochloride, positive control or vehicle control substances administered to mice in a single oral dose; animals sacrificed by cervical dislocation at 24 (all doses) or 48 hours (high dose) and femurs removed; femoral bone marrow isolated, suspended in calf serum, mounted on slides and stained with May-Grunwald [REDACTED] (b) (4); ≥2000 polychromatic erythrocytes (PCE) analyzed per slide for micronuclei and ratio between polychromatic and normochromatic erythrocytes determined
- **Analysis:**
  - **No. animals analyzed:** 6/sex/dose at 24 hours and 6/sex given 100 mg/kg at 48 hours after dosing

- **Counting method:** NIKON microscopes with 100x oil immersion objectives used to observe at least 2000 polychromatic erythrocytes per animal.
- **Cytotoxic endpoints:** Increase in ratio of polychromatic to normochromatic erythrocytes
- **Genetic toxicity endpoints:** Increase in rate of micronucleated PCEs
- **Statistical methods:** Nonparametric Mann-Whitney test ( $p < 0.05$ )
- **Comments:** Scoring of preparations replicated for verification of results.
- **Criteria for Positive Results:** Dose-related increase in number of micronucleated polychromatic erythrocytes or statistically significant positive response.

#### Results:

- **Study Validity:** Vehicle controls in range of historical control data (0.03 - 0.26% PCEs with micronuclei), positive controls showed statistically significant increase in values, and more than 80% animals evaluable.

#### - Study Outcome

Pre-Experiment for Toxicity: Spontaneous activity reduced at all doses (100, 150 and 200 mg/kg PO) at 1, 6, 24 and 48 hours, eyelid closed in one female given 200 mg/kg at 24 hours, apathy observed in one male and one female given 100 mg/kg at 1 hour, and one female given 200 mg/kg at 24 hours, and deaths in one male given 150 mg/kg at 1 hour and one male given 200 mg/kg at 1 hour.

Main Study Toxicity/Cytotoxicity: Deaths in 3/24 mice at 100 mg/kg. Increase in NCE/NCE ratio at 100 mg/kg at 48 hours.

Genotoxicity: No significant increase in rate of micronucleated PCEs in negative control mice and in mice given up to 100 mg/kg hydromorphone hydrochloride. Micronucleated PCEs increased 1.335% in mice given positive control article cyclophosphamide.

Group	Dose (mg/kg)	Sampling Time (h)	PCEs with micronuclei (%)
Vehicle (first scoring)	0	24	0.030
Vehicle (second scoring)	0	24	0.095
Vehicle (total)	0	24	0.063
Hydromorphone HCl	10	24	0.025
Hydromorphone HCl	33.3	24	0.025
Hydromorphone HCl (first scoring)	100	24	0.095
Hydromorphone HCl (second scoring)	100	24	0.120
Hydromorphone HCl (total)	100	24	0.108
Cyclophosphamide	40	24	1.335
Hydromorphone HCl	100	48	0.015

**Summary:** Hydromorphone hydrochloride did not induce micronuclei in mouse bone marrow cells at up to 100 mg/kg PO (approximately 7.5x the human dose of 64 mg/day in a 60 kg patient on a mg/m<sup>2</sup> basis). This dose induced systemic toxicity including premature deaths and cytotoxicity with an increase in the ratio of polychromatic to normochromatic erythrocytes at 48 hours after dosing. In conclusion, hydromorphone was not clastogenic under the conditions of this study.

#### **Study Title: Chromosome Aberration Assay in Human Lymphocytes in Vitro With Dilaudid (Hydromorphone Hydrochloride)**

**Study No:** MPF/DT 9854E

**Study Type:** *In vitro* test for induction of chromosome aberrations in human lymphocytes

**Volume #31, Pages #107-141****Conducting Laboratory**

(b) (4)

**Date of Study Initiation/completion:** October 21, 1998 - February 23, 1999**GLP Compliance:** Yes (x) No ( )**QA- Reports:** Yes (x) No ( )**Drug Lot Number:** L 0001974**Study Endpoint:** Induction of concentration-related increase in number of structural chromosomal aberrations or significant and reproducible positive response**Methodology:**

- **Cell line:** Lymphocytes isolated from blood collected from healthy 30 (for experiment I with S9 and experiment II) and 45 (for experiment I without S9) year old female donors.

- **Dose Selection Criteria:**

- **Basis of dose selection:** Results of pre-test on cytotoxicity

- **Range finding studies:** Experiment I: Cell cultures incubated with 8 concentrations of hydromorphone hydrochloride (800-3200 mcg/ml (10 mM), and negative, solvent (in presence of 5-bromodeoxyuridine 6 mcg/ml) and positive controls (EMS 400 mcg/ml with S9 and CPA 37.6 mcg/ml without S9) for 4 hours with 18 hour recovery period, with and without S9 mix. Cytotoxicity indicated by percentages of mitotic suppression compared to controls in 1000 cells/culture. Performed in duplicate. No toxicity observed at up to 3200 mcg/ml.

- **Test Agent Stability:** Analyzed August 12, 1998, found stable in H<sub>2</sub>O at least 14 days at 1%, pH 4.8, room temperature, brown glass

- **Metabolic Activation System:** S9 liver microsomal fraction from livers of male Wistar rats induced with 3 applications of phenobarbital and B-naphthoflavone. S9 mix prepared with NgCl<sub>2</sub>, KCl, glucose-6-phosphate and NADP in sodium-orthophosphate buffer.

- **Controls:**

- **Vehicle:** Deionised water

- **Negative Controls:** Culture medium

- **Positive Controls:** Without metabolic activation: Ethylmethane sulfonate (b) (4) at 3.56 mM (experiment I) and 2.67 mM (experiment II). With metabolic activation: Cyclophosphamide (b) (4) at 0.131 and 0.105 mM (experiment I), 0.105, 0.131 and 0.158 mM (experiment II)

- **Exposure Conditions:**

- **Incubation and sampling times, doses used:**

Exp.	Prep. interval	Exposure period	Concentration (mcg/ml)							
			Without S9 mix							
I	22h	4h	25	50	100	200	400	800	1600	3200
II	22.5h	22h			100	200	400	800	1600	3200
II	46h	46h					400	800	1600	3200
With S9 mix										
IA*	23h	4h	25	50	100	200	400	800	1600	3200
IB-ID*	22h	4h				200	400	800	1600	3200
II	46h	4h				200	400	800	1600	3200

\*Experiment I repeated 4 times. ID cultures evaluated for cytogenetic damage because IA and IB showed low responses in positive controls, and IC solvent control not scoreable.

- **Study design:** Isolated lymphocytes cultured 48-72 hours before treatment at 37°C in 15% CO<sub>2</sub>. Cultures then exposed to test article, negative control or positive control agents for 4 hours with and without S9 mix in Experiment I and with S9 mix in Experiment II. Exposure was 22.5 and 46 hours without S9 mix in experiment II. Toxicity was indicated by reduced mitotic index (<5% control) at 3200 mcg/ml without S9 mix in the Cytogenic Experiment; therefore highest concentration evaluated without S9 was 1600 mcg/ml. Recovery times 18 hours without S9 and 19 hours with S9 mix in Experiment I and 42 hours in Experiment II. Cells were harvested, suspended on microscope slides and stained with Giemsa (b) (4) for evaluation of structural chromosome aberrations.

- **Analysis:**

- **No. slides evaluated:** ≥100 metaphase plates per culture
- **Counting method:** According to standard protocol of Arbeitsgruppe der Industrie, Cytogenetik (Engelhardt, 1987) using NIKON microscope with 100x oil immersion objective.
- **Cytotoxic endpoints:** Mitotic index (% cells in mitosis) and number of polyploid cells (% polyploid metaphases). Percentage of mitotic suppression compared to controls in 1000 cells per culture in duplicate.
- **Genetic toxicity endpoints:** Aberrations recorded: Breaks, fragments, deletions, exchanges and chromosomal disintegrations.
- **Statistical methods:** Fisher's exact test
- **Criteria for Positive Results:** Concentration-related increase in number of structural chromosomal aberrations, significant and reproducible positive response.

**Results:**

- **Study Validity:** Numbers of chromosomal aberrations in negative control cultures within laboratory historical control data range of 0.0%-4.0%. Positive control articles produced significant increase in frequencies of aberrations.

- **Study Outcome:**

Exp.	Prep interval	Dilaudid ccn. (mcg/ml)	Polyploid cells (%)	Mitotic index (% control)	Aberrant cells (%)		
					Incl. gaps	Excl. gaps*	Exchanges
<b>Exposure period 4 hours without S9 mix</b>							
I	22h	negative control	0.0	100.0	0.5	0.0	0.0
		solvent control	1.0	100.0	1.0	1.0	0.0
		positive control	0.0	79.6	11.0	<b>10.0<sup>a</sup></b>	0.5
		800	0.5	115.1	1.5	1.0	0.0
		1600	0.0	101.4	3.5	3.0	0.0
		3200	0.0	76.7	1.5	1.0	0.0
<b>Exposure period 22.5 hours without S9 mix</b>							
II	22.5h	negative control	1.0	100.0	2.0	1.5	0.0
		solvent control	0.0	100.0	2.0	2.0	0.0
		positive control	1.0	39.7	12.0	<b>9.0<sup>a</sup></b>	1.5
		400	0.5	85.3	0.5	0.5	0.0
		800	0.0	57.4	0.5	0.0	0.0
		1600	0.0	49.6	1.0	1.0	0.0
<b>Exposure period 46 hours without S9 mix</b>							
II	46h	solvent control	1.0	100.0	2.5	1.5	0.0
		1600	0.0	57.6	5.5	3.5	0.0
<b>Exposure period 4 hours with S9 mix</b>							
I	23h	negative control	0.0	100.0	0.5	0.5	0.0
		solvent control	0.0	100.0	4.5	3.5	0.5
		positive control	1.0	61.5	15.0	<b>14.0<sup>a</sup></b>	7.5
		800	0.0	121.1	1.0	1.0	0.0
		1600	0.5	103.3	3.5	3.0	0.0

		<b>3200</b>	0.0	107.3	0.5	0.0	0.0
II	46h	negative control	0.0	100.0	1.0	1.0	0.0
		solvent control	0.0	100.0	1.0	1.0	0.5
		positive control	0.5	27.4	9.5	<b>9.0<sup>a</sup></b>	2.0
		800	0.5	105.4	1.0	1.0	0.0
		1600	0.5	103.2	2.0	2.0	0.0
		3200	0.0	108.6	0.0	0.0	0.0

\* inclusive cells carrying exchanges

<sup>a</sup> Aberration frequency statistically significant higher than corresponding solvent control values

**Summary:** There were no increases in the numbers of human lymphocytes with structural chromosome aberrations after *in vitro* treatment with hydromorphone hydrochloride at concentrations up to 3200 mcg/ml in the presence and absence of metabolic activation with S9 mix under the conditions of this study. The positive controls ethylmethane sulfonate and cyclophosphamide significantly increased the frequency of structural chromosomal aberrations.

## OVERALL SUMMARY AND EVALUATION

**Introduction:** Hydromorphone hydrochloride is a semisynthetic phenanthrene derivative that differs from morphine in two structural alterations; the 6-hydroxyl group of morphine is replaced with an oxygen and the 7-8 double-bond is hydrogenated. Hydromorphone is a strong analgesic that has been used in the treatment of moderate to severe pain associated with surgery, cancer and trauma since 1926. This drug is approved and marketed in several dosage forms to include immediate-release oral tablet, controlled-release capsule, oral liquid, rectal suppository, powder, cough syrup, and solutions for intravenous, subcutaneous and intramuscular injection. The safety and efficacy of hydromorphone are well established in clinical use. A controlled release form (Hydromorph Contin®; Purdue Frederick, 3 and 6 mg) is commercially marketed in Canada, but Dilaudid CR™ (hydromorphone HCl) Controlled-Release Tablets have not been marketed in any country. The hydromorphone controlled release delivery system proposed in this application, called Oral Osmotic System (OROS®) and Gastrointestinal Therapeutic System (GITS) for once-daily administration has been developed jointly by ALZA Corporation and Knoll Pharmaceutical Company under separate INDs (see IND (b) (4)).

The pharmacology, safety pharmacology, pharmacokinetics and acute and chronic toxicity of hydromorphone were reported in the literature and cited in the original NDA for Dilaudid (NDA 19-892). The sponsor conducted studies on reproductive toxicology and genotoxicology for this submission, and a bridging toxicology study on controlled release hydromorphone in dogs under IND (b) (4) (ALZA, serial number (b) (4)) (b) (4).

**Pharmacology:** In comparison to morphine, hydromorphone has a more rapid onset and a shorter duration of action. The potency is approximately 4x-5x that of morphine in cat. In humans, hydromorphone is approximately 7x more potent in producing analgesia but produces less drowsiness than does morphine in equianalgesic doses (Remington's, 1990; Wallenstein et al., 1990; Houde, 1986). The onset of analgesia is approximately 15-30 minutes, depending on the route of administration. Analgesia is usually maintained for 4-5 hours when given in immediate-release form. Hydromorphone also causes circling, catalepsy and stereotypical behavior in rats, due to actions at opioid receptors that interact with dopaminergic and GABA-ergic neurons in the substantia nigra and striatum (Gilman et al., 1985). Intraperitoneal and

intracerebro-ventricular hydromorphone injections decreased hepatic glutathione concentrations in male and female ICR mice, suggesting a potential for increased toxicity of compounds such as acetaminophen that are dependent on GSH for detoxification, when given in combination with hydromorphone (Skoulis et al., 1989).

The mechanism of action of hydromorphone is primarily due to binding of opiate receptors in the central nervous system and intestines. The highest concentrations of binding sites are in the limbic system, thalamus, striatum, hypothalamus, midbrain and spinal cord.

Hydromorphone binds several opiate receptor subtypes, including mu-receptors in brain and spinal cord involved in pain modulation, kappa-receptors in deep layers of the cerebral cortex, delta-receptors in the limbic regions and sigma-receptors involved in producing dysphoria and psychotomimetic effects. Analgesia is believed to result from alteration of the perception of pain and the emotional response to pain by interaction with receptors in the spinal cord and in the substantia gelatinosa, spinal trigeminal nucleus, periaqueductal gray, periventricular gray, medullary raphe nuclei and hypothalamus.

Interaction with non-opiate receptor systems by hydromorphone is expected to occur to an extent similar to that of the opioid drugs as a class. For example, opioid drugs decrease peripheral acetylcholine release, increase brain acetylcholine levels and antagonize hemicholinium depletion of acetylcholine. Morphine was shown to antagonize serotonin (5-hydroxytryptamine) effects in some studies, but increase its release in the gut and serotonin turnover in brain.

**Safety Pharmacology:** The side effects of hydromorphone are characteristic of those produced by opiate mu-receptor agonists as a class, and include nausea, vomiting, respiratory depression, somnolence, hypotension, constipation, constricted pupils and euphoria. The gastrointestinal effects are mediated by stimulation of the chemoreceptor trigger zone in medulla oblongata (nausea and vomiting) and effects on smooth muscle tone (constipation). Hydromorphone can also decrease gastric, biliary and pancreatic secretions. A study in dogs showed that hydromorphone induces less nausea, emesis and other gastrointestinal disturbances than does morphine (Booth and McDonald, 1982). Respiratory depression is a result of effects on respiratory centers in the brain stem that decrease sensitivity to an increase in serum carbon dioxide tension. This can result in cerebral vasodilation and increased cerebral blood and cerebrospinal fluid pressure. Pupil constriction results, in part from excitatory action in the nucleus of the oculomotor nerve.

Endocrinologic effects of hydromorphone include stimulation of vasopressin, epinephrine and corticotropin release, and inhibition of gonadotropin release in the pituitary and thyrotropin from the adenohypophysis leading to decreased release of thyroid hormone. Hydromorphone may alter immunologic function due to high numbers of opioid receptors on lymphocytes, neutrophils, monocytes and mast cells.

Potential of the depressant effects of hydromorphone is expected with central nervous system depressants including alcohol, anesthetics, other opiate agonists, tranquilizers, sedatives and hypnotics, phenothiazines, tricyclic antidepressants, and monoamine oxidase inhibitors.

**Pharmacokinetics:** Hydromorphone is well absorbed orally (American Hospital Formulary Service, 1989) with a bioavailability of approximately 60%. The apparent volume of distribution is approximately 1.2 l/kg. The drug is distributed into skeletal muscle, kidneys, intestinal tract,

liver, spleen, lungs, and brain. Hydromorphone also crosses the placenta and can be detected in milk. The metabolic profile is similar in rats, rabbits, dogs and humans. Metabolism occurs primarily in the liver by conjugation with glucuronic acid. Hydromorphone is excreted in urine (30%) as the glucuronic acid conjugate. The metabolites dihydroisomorphine and dihydromorphine have also been identified in urine. Some metabolism also occurs in the central nervous system, kidneys, lungs and placenta. Approximately 6% of the drug is excreted unchanged and trace amounts as the 6-hydroxymetabolite. Excretion of hydromorphone and its metabolites occurred within the first 24 hours in a study comparing excretion patterns in rats, dogs, guinea pigs, rabbits and man (Cone et al., 1977). The percent dose excreted in urine on the first day after dosing was 70.7%, 27.7%, 60.5%, 21.8% and 36.6% in guinea pigs, rats, dogs, rabbits and humans respectively.

**Toxicology:** Preclinical studies reported in the literature have addressed hydromorphone acute, subchronic, and chronic toxicity, reproductive toxicity, cytotoxicity, immunotoxicity, tolerance and withdrawal. Overall, hydromorphone HCl is approximately 5x more potent than morphine, but the side effect profile shows lower toxicity, suggesting a higher therapeutic index or margin of safety. The following information on single-dose and repeated-dose toxicology, from the published literature (Blumberg et al., 1954; Blumberg et al., 1961; Buchwald and Eadie, 1941; Cone et al., 1977; Eddy and Reid, 1934; Emerson and Phatak, 1938; Ercoli and Lewis, 1945; Geber, 1970; Geber et al., 1975; Geber and Schramm, 1975; Giarman and Condouris, 1954; Haas et al., 1953; Huggins et al., 1950; King et al., 1935; Krueger et al., 1943; Lewis, 1989; Needham et al., 1981; Rice and Gerber, 1978; Seki et al., 1964; Skoulis et al., 1989) was cited for the original NDA #19-034 (Dilaudid-HP™) and referenced for this NDA submission.

**Single Dose Toxicology** The LD50 values reported in the literature were 61-104 mg/kg IV and 84-124 mg/kg SC in the mouse (1/5 the morphine LD50 in mouse), 51 mg/kg SC in the rat (1/5 the morphine LD50), 150-200 mg/kg SC in the rabbit (1/3 the morphine LD50), 200-250 mg/kg SC in guinea pig (1/2 the morphine LD50 in this species). The acute lethal dose in cats was 5 mg/kg SC and in dogs 50 mg/kg SC. There was an effect of age on the lethal dose in dogs, with a 10x increase in younger animals.

The clinical signs of acute hydromorphone toxicity in mice were circling, prostration, muscle twitching and convulsions. In rats, sedation, catalepsy, decreased temperature and respiration, immobility, excitement and convulsions were observed with increasing severity as the dose increased. Rabbits showed decreased activity and postural reflexes, somnolence, respiratory depression (at 0.1 mg/kg SC), decreased intestinal peristalsis, increased blood sugar, hyperexcitation, muscle twitching and convulsions at high doses (200 mg/kg SC). The clinical signs of acute toxicity in guinea pigs were depression, muscle twitching, increased activity, decreased respiration (30-100 mg/kg SC), decreased intestinal tone and intestinal contractions, and convulsions. In studies in cats, unusual behavior, reduced respiration, catalepsy, emesis, excitation, and clonic and tonic convulsions were observed after initial dosing at 2 mg/kg SC. Dogs responded to high doses with emesis, depression, reduced heart and respiratory rates (2-5 mg/kg SC) and body temperature, abdominal muscle contractions, increased gastrointestinal transit time and convulsions in some animals at doses of 2-20 mg/kg SC. Hydromorphone produced less nausea, emesis and GI disturbances than did morphine in the dog. In all species, the clinical signs of toxicity were dose-related in severity and incidence. The acute toxic signs in monkeys were depression and decreased heart rate. Similarly, decreased heart and respiration rates were observed in horses at 60 mg/kg but not 20-40 mg/kg by single injection. Horses also showed decreased body temperature and intestinal

sounds, behavioral changes, tail rigidity, tail extension, stamping, tail switching, apprehension, excessive salivation and excitation.

Laboratory analyses showed increased (10x) serum glutamateoxaloacetate transaminase (SGOT) in mice given hydromorphone at 100 mg/kg IP. Serum interferon was reduced 32% at 20 mg/kg IP and 86% at 50 mg/kg IP compared to saline control levels. In comparison, serum interferon was reduced by morphine by 20% at 1.4 mg/kg IP and 81% at 113 mg/kg IP in the mice. GSH concentrations were decreased significantly at 100 mg/kg IP and 40 mcg/kg ICV in mice.

In the histopathology examinations, Dilaudid administration by intraperitoneal injection produced fatty infiltration of the liver in mice, that was reversible after 36 hours.

**Repeat Dose Toxicology** Repeated dosing with 100 mg/kg IP hydromorphone HCl increased SGOT and produced fatty infiltration of the liver in mice. Dogs given hydromorphone at 2-5 mg/kg SC (6 injections/week) for 18 weeks showed cardiac and respiratory depression. Body weights were reduced (7%) at 4 mg/kg/d SC for 10 weeks in monkeys.

In the histopathology examinations, microscopic hemorrhages in the kidney and especially Bowman's capsule, dilated canaliculi and calcareous cylinders in the uriniferous tubules were observed in rabbits treated for 23 days with hydromorphone at 5 mg/kg. However, when examined after a 10 day recovery period, rabbits treated for 49 days at up to 7.5 mg/kg/d SC showed no renal changes, but hyperemic livers and small splenic follicles lacking germinal centers were observed. Body weight loss was seen in monkeys at 10 mg/kg SC for 10 weeks.

Tolerance to many of the acute toxic effects of hydromorphone was observed with repeated dosing in all species. However, effects such as delayed growth and maturation, cessation of menstrual bleeding, decreased pulse and respiratory rates were not attenuated after as much as 14 weeks of treatment with 25-30 mg/kg/d SC in monkeys. Withdrawal symptoms were similar to those observed in withdrawn morphine-treated dogs, but induced more severe symptoms than seen in morphine withdrawal in monkeys.

**Special Studies** In a study on cytotoxicity, there was no effect on DNA, RNA or protein biosynthesis at concentrations below 1.0 nM, and no effect on cell growth in KB cells from epidermal carcinoma cell lines when exposed to hydromorphone hydrochloride at 320 nM (Nassiri et al., 1991). Immunotoxicity was studied because opioid receptors are known to be present on immune cells including monocytes, neutrophils, mast cells and lymphocytes. Decreased serum interferon levels were observed in mice given 50 mg/kg SC hydromorphone.

### **Safety Evaluation:**

A GLP toxicity study was conducted in dogs and submitted under IND (b) (4) (serial number (b) (4) and (b) (4) ("Thirty Day Oral Dosing Study in Dogs with OROS® (hydromorphone HCl)", ALZA Study # TR-96-4604-057). This investigation evaluated local gastrointestinal effects and systemic toxicity of repeated dose OROS® administration at up to 64 mg/d (approximately 3x the estimated AUC at the same dose in a clinical study) for 30 consecutive days. The results showed no deaths and no serious adverse effects of OROS® at up to 64 mg/d for 30 consecutive days. Mean body weights and food consumption were lower in the dogs given 64 mg/kg/d OROS® and 64 mg/kg/d IR Dilaudid during the first week only. Clinical observations included vomiting, excessive salivation,

decreased activity, eye effects (pupil constriction), and unformed stool. These effects were dose-related, more frequent after immediate release than after sustained release hydromorphone, and more frequent in female than in male dogs. There were no treatment-related effects in the ophthalmoscopy examination, hematology, clinical chemistry, and urinalysis. There was no target organ toxicity observed in the gross pathology and histopathology examinations and in the organ weight evaluations. The observed signs were considered to be pharmacological effects of hydromorphone HCl. The NOAEL was considered to be 8 mg/d (approximately 1 mg/kg/d) and the MTD 64 mg/d (approximately 9 mg/kg/d). Analysis of drug content of the recovered OROS® systems showed that drug delivery was incomplete with approximately 75% hydromorphone delivered in the 8 mg systems and 45%-66% delivered in the 64 mg systems. In the toxicokinetic analysis, the C<sub>max</sub> values increased proportionally with increasing OROS® dose and were comparable on dosing days 1 and 30. The T<sub>max</sub> (approximately 7-9 h) and half-life values (6.1-8.3 h) were similar across doses and on dosing days 1 and 30. The AUC showed dose-proportionality, increasing from 10.6 to 87.6 ng.h/ml at 8 and 64 mg respectively on Day 1 and from 17.7 to 81.6 ng.h/ml at the same doses on day 30. There was no apparent accumulation.

**Reproductive Toxicology:** Studies to evaluate the potential effects of hydromorphone HCl on fertility and early embryonic development (Segment I), teratogenicity (Segment II) and pre- and post-natal development (Segment III) were conducted for this NDA submission. All studies were GLP compliant and quality assurance reports were signed and present. Certificates of analysis were provided.

In the segment I reproductive toxicology study, eight week-old male and female rats were administered hydromorphone at 1.56, 3.13 and 6.25 mg/kg/d from 2 weeks (females) or 4 weeks (males) before mating through the end of the mating period, and dosing continued in the females through gestation day 7. There were no treatment-related effects on testicle and epididymis weights or the number, motility and viability of spermatozoa/spermatids in the males. There were no treatment-related effects on uterine weights, number of corpora lutea, resorption rate, pre-implantation loss and post-implantation loss in the females. In the fetal rats, the NOEL was 3.13 mg/kg/day PO. The number of implantation sites and viable fetuses were decreased at 6.25 mg/kg/d PO (approximately 1x the human exposure at the high clinical dose of 64 mg in a 60 kg patient on an AUC basis, based on the results of toxicokinetics analysis in the Segment II study in rats). These effects were probably related to maternal and paternal toxicity. There were no treatment-related adverse effects in the male and female adult rats at 1.56 mg/kg PO. However, pharmacological effects of the drug were observed, including restlessness, self-mutilation and increased motor activity. Hydromorphone toxicity was observed in the adult rats at 3.13 mg/kg/day PO (reduced body weight) and 6.25 mg/kg/day PO (reduced body weight and food consumption). In conclusion, hydromorphone had no effects on male and female fertility in rats under the conditions of this study.

Teratogenicity (Segment II reproductive toxicology) was studied in rats and rabbits. Maternal Sprague-Dawley rats were administered hydromorphone at 1.56, 3.13 and 6.25 mg/kg/day by oral gavage, daily from gestation days 6-17 for a total of 12 doses. There were no treatment-related effects on sex distribution of the fetuses, placental and fetal weights, and no fetal malformations or variations, dead fetuses, skeletal malformations or malformed fetal organs. There were four runts in four different litters, at doses that were maternally toxic (1/282 fetuses at 3.13 mg/kg/d and 3/279 fetuses at 6.25 mg/kg/d). The runts weighed 2.0 g compared to mean weights of 3.45 g in the mid dose and 3.29 g in the high dose fetuses. Skeletal and soft tissue variations and retardations were without relation to dose, observed in the control groups

with comparable frequency, or within historical range. No maternal toxicity was observed at 1.56 mg/kg/day hydromorphone hydrochloride. Pharmacodynamic effects including restlessness, increased motor activity and self-mutilation, and maternal toxicity indicated by decreased maternal body weight gain (67% and 57% in the mid- and high dose groups compared to 80% in the control group) and food consumption were observed at 3.13 and 6.25 mg/kg/day. In the toxicokinetic analysis, absorption was rapid with maximum plasma levels observed at approximately 0.5 hours in all groups on both days except in the low dose group on dosing day 1 (1.3 h). The C<sub>max</sub> was dose proportional on both gestation days 6 and 17, ranging from 5.6-7.6 ng/ml at 1.56 mg/kg/d (gestation days 6 and 17 respectively) to 13.8-35.8 ng/ml (gestation days 6 and 17 respectively) after 6.25 mg/kg/d. Total exposure (AUC) generally increased with dose and was greater on gestation day 17 (135 ng.h/ml) than on gestation day 6 (89.6 ng.h/ml) in the high dose (6.25 mg/kg/day) group. The AUC values were similar on both days in the low (53-55 ng.h/ml) and mid (42-67 ng.h/ml) dose groups. In conclusion, hydromorphone hydrochloride was not teratogenic in rats at up to 6.25 mg/kg/d (approximately 1x the high human dose of 64 mg in a 60 kg patient on an AUC basis) given during gestation days 6-17 under the conditions of this study.

In the second teratogenicity study, pregnant Himalayan/CHR rabbits were administered hydromorphone by oral gavage at 6.25, 12.5 and 25 mg/kg/d, daily from gestation days 6 through 20. The NOEL for developmental toxicity was 25 mg/kg/day (AUC 150 ng.h/ml, approximately 1x the human exposure of 156 ng.h/ml at the maximum recommended human dose (MRHD) of 64 mg in a 60 kg patient) in this study, as there was no evidence of embryotoxicity. However, the ratio of male to female fetuses was higher in the high dose group than in the control group. The observed skeletal variations were within historical range. There was no evidence of teratogenicity in the fetal rabbits at up to 25 mg/kg/day. Maternal toxicity, observed at the mid-dose (12.5 mg/kg/d) and high dose (25 mg/kg/d), included reduced motility, sedation, abdominal position and mydriasis. Decreased maternal body weight gain (7% compared to 13% in the controls) and food consumption were observed at 25 mg/kg/day. In the toxicokinetic analysis, absorption was rapid and peak plasma levels occurred in less than one hour at all doses (6.25-25 mg/kg/day) on both gestation days 6 and 20. Exposure increased linearly on day 6 and was comparable at the low (51.6 and 51.8 ng.h/ml at 6.25 mg/kg/day) and mid (91.0 and 103.9 ng.h/ml at 12.5 mg/kg/day) doses on both days, but was considerably lower at the high (25 mg/kg/day) dose on day 20 (141.1 ng.h/ml) compared to day 6 (237.2 ng.h/ml). In conclusion, hydromorphone was not teratogenic in fetal rabbits at up to 25 mg/kg/d by oral gavage on gestation days 6-20 under the conditions of this study.

Pre- and postnatal development (Segment III reproductive toxicology) were studied in Sprague-Dawley rats dosed daily with hydromorphone at 1.56, 3.13 and 6.26 mg/kg/d by oral gavage from gestation day 6 through lactation day 21 (weaning). There were no effects on gestation duration or reproduction parameters except a potential parturition effect, shown by severe blood loss in one of 24 high dose dams during parturition. There were no effects on numbers of live pups prior to lactation, but live pups were decreased on day 4 of lactation in the high dose group. There were no effects on birth index, live birth index, viability index, lactation index and overall survival index at the low dose. The viability and survival indices were decreased at 6.25 mg/kg/day and a trend toward a decrease in lactation index at that dose. There were no effects on sex distribution of the F<sub>1</sub> generation. The NOAEL for developmental toxicity was 1.56 mg/kg/day. At 3.13 and 6.25 mg/kg/day, reduced overall survival index and mean pup weight (-4% to -10% at 3.13 mg/kg/d and -2% to -19% at 6.25 mg/kg/d in the pups, and -2% to -6% at 3.13 mg/kg/d and -5% to -12% at 6.25 mg/kg/d in the F<sub>1</sub> dams) were observed for the F<sub>1</sub> generation through sexual maturity, but no developmental toxicity was shown in the F<sub>2</sub>

generation. Decreased maternal brood care in the Fo dams at 6.25 mg/kg/day was indicated by absence of milk in the pup stomachs on lactation days 1 and 2. Altered morphological landmarks and functional tests in the F<sub>1</sub> pups were observed at the high dose. There was a delay in ear opening, reduced auditory startle reflex and reduced rearings and sectors entered by the pups at that dose. Maternal toxicity (Fo dams) was observed at all doses, indicated by reduced body weight (-4% to -6% at 1.56 mg/kg/d, -3% to -9% at 3.13 mg/kg/d and -4% to -13% at 6.25 mg/kg/d compared to controls) and food consumption. There were no treatment-related effects in the F<sub>2</sub> generation at up to 6.25 mg/kg/day.

Segment II studies reported in the literature showed increased incidence of cranioschisis and exencephaly in hamsters given a single dose of hydromorphone at 125 mg/kg SC on gestation day 8 (Geber, 1970; Geber and Schramm, 1975). However in those studies, a dose relationship was not observed and the effects of opioid-induced toxicity in the dams (e.g., decreased food and water intake, altered motor activity) could not be determined. Fetal brain lesions were also demonstrated in hamsters given hydromorphone at 150 mg/kg SC (single dose) and 121, 82 and 82 mg/kg (triple dose) (Rice and Geber, 1978). Mice administered hydromorphone at 5 mg/kg SC (single injection) during organogenesis produced offspring with increased incidence of minor skeletal defects and soft tissue anomalies (undescended testes) (Behm et al., 1985).

**Genetic Toxicology:** Potential genotoxicity of hydromorphone hydrochloride was evaluated in three GLP studies including the Ames test, the Mouse Micronucleus test and the Chromosome Aberration Assay in human lymphocytes. There was no increase in the mean number of revertants per plate at up to 5000 mcg/plate hydromorphone hydrochloride compared to the negative controls, with and without metabolic activation with S9 mix in histidine-deficient mutant *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537. The test was validated using appropriate positive controls. Therefore, the test substance is considered non-mutagenic in the *Salmonella*/microsome test (Ames test) under the conditions of this study.

In the *in vivo* study in mice, there was no induction of micronuclei in polychromatic erythrocytes of bone marrow cells by hydromorphone hydrochloride administered at up to 100 mg/kg PO (approximately 7.5x the human dose of 64 mg/day in a 60 kg patient on a mg/m<sup>2</sup> basis). This dose induced systemic toxicity including premature deaths and cytotoxicity with an increase in the ratio of polychromatic to normochromatic erythrocytes at 48 hours after dosing. The study was validated using appropriate negative and positive controls. In conclusion, hydromorphone was not clastogenic in the Mouse Micronucleus Test under the conditions of this study.

The Chromosome Aberration Assay, conducted in human lymphocytes from healthy female donors, there were no increases in the numbers of structural chromosome aberrations after incubation with hydromorphone hydrochloride at up to 3200 mcg/ml in the presence and absence of metabolic activation with S9 mix. The negative control cultures showed chromosomal aberrations within the laboratory historical control data range. The positive controls ethylmethane sulfonate and cyclophosphamide significantly increased the frequency of structural chromosomal aberrations. Therefore, hydromorphone hydrochloride was not mutagenic in human lymphocytes under the conditions of this study.

**Carcinogenicity:** Studies to evaluate the carcinogenic potential of hydromorphone HCl were not conducted, and will be requested as a phase IV commitment.

**Excipients:** All excipients in the proposed drug product, Polyethylene Oxide NF, Povidone USP, Magnesium Stearate NF, Ferric Oxide NF, Butylated Hydroxytoluene (b) (4) Sodium Chloride USP, (b) (4) USP, iron oxide (b) (4) Cellulose Acetate NF, Polyethylene Glycol NF, (b) (4) and (b) (4) are approved and Generally Recognized as Safe (GRAS).

Polyethylene Oxide NF was evaluated for carcinogenic and mutagenic potential in mice, rats and dogs. There was no evidence of increased neoplasm incidence after daily administration at 5% dietary intake for two years and polyethylene oxide was nonmutagenic in the Ames test, the micronucleus assay and in an *in vitro* cytogenetics assay. Povidone USP is approved for ingestion at 50 mg/kg/day. Povidone was negative for teratogenicity in rabbits at 500 mcg injected into the yolk sac horn, negative in a carcinogenicity evaluation in rats given up to 5 g/kg/day for 2 years in the diet, and nonmutagenic in the Ames test in *Salmonella typhimurium* (TA 1530 and TA 1538), the *in vitro* mammalian lymphoma assay and transformation assay, the *in vivo* dominant lethal assay and Chinese hamster bone marrow test. Povidone was mutagenic in the Ames test in *Escherichia coli* and in *S. typhimurium* (TA 1530) when the assay was conducted at 4°C rather than 37°C. Magnesium Stearate NF is approved for use as a food additive and was negative in tests on carcinogenicity in mice, teratogenicity in rabbits at up to 2.5 mg/kg) and mutagenicity in the Ames test. Ferric Oxide NF, approved at up to 5 mg/day, was non-mutagenic in the Ames test with and without metabolic activation with S9.

Butylated hydroxytoluene is approved as a food additive at not more than 0.02% of fat or oil content. In carcinogenicity studies, butylated hydroxytoluene increased the incidence of benign liver tumors in male mice at approximately 4 g/kg/d in the diet for 104 weeks, but was negative for carcinogenicity in mice at 1100 mg/kg/d and rats at 500 mg/kg/d. Segment II reproductive toxicity studies showed no increase in fetal malformations in mice at 250 mg/kg/d (gavage) for 7 days, in mice at 1 g/kg by single dose on different gestation days (gavage), in rats at up to 759 mg/kg/d PO on gestation days 6-12 or 1-22, and in hamsters at up to 280 mg/kg/d (gavage) on gestation days 6-10. Rabbits given 3.2-320 mg/kg/d (gavage) on gestation days 6-18 did show increased fetal deaths at all doses and decreased maternal growth at doses of 69.1 mg/kg and greater. There were no reproductive effects of butylated hydroxytoluene in monkeys administered 50 mg/kg/d for 2 years in the diet, mated one year into the dosing period. No mutagenicity was observed in the Ames test, and *in vivo* mammalian cell assays including rat cells and mouse bone marrow; however, butylated hydroxytoluene induced chromosome damage and inhibition of DNA synthesis without mutations in an *in vitro* mammalian cell assay using human cells.

Sodium Chloride USP had adverse effects (unspecified) on newborn rats when exposed at 145 g/kg/d for 7 days throughout gestation. Although sodium chloride was nonmutagenic in the Ames test and in Chinese hamster ovary cells with metabolic activation, mutagenicity was observed in the mouse lymphoma assay with metabolic activation with S9.

(b) (4)

Polyethylene Glycol NF (PEG) administration produced no evidence of

carcinogenicity in mice given 0.3 ml/wk PO (PEG 400) for 30 weeks and rats given 0.25 ml/wk SC (PEG 400) for 20 weeks, although one hepatoma was seen in one of 25 rats given PEG at 0.25 ml/wk IP. PEG induced testicular tubule degeneration and degenerated sperm in rats fed PEG at 0.55-16% (0.04-19 g/kg/d) but had no reproductive effects on three generations of rats given 0.85-62 mg/kg/d in drinking water in another study. In mutagenicity studies, PEG was nonmutagenic in the Chinese hamster ovary mutation test with and without metabolic activation with S9. There was a significant increase in sister chromatid exchange (SCE) at 0.05% PEG with metabolic activation only. (b) (4)

Based on the results of the studies conducted by the sponsor, and on the known pharmacology and toxicology of hydromorphone hydrochloride and the excipients in the proposed drug product, this NDA is approvable from a pharmacology and toxicology point of view. Recommended changes to the proposed label are described below. Studies to evaluate the carcinogenic potential of hydromorphone hydrochloride will be needed and can be conducted as a Phase IV commitment.

## CONCLUSIONS

### Key Study Findings:

- **In dogs:** no serious adverse gastrointestinal and systemic effects of repeated daily dosing with OROS® for 30 days at up to 64 mg/d PO (AUC 81.6 ng.h/ml on day 30, approx. 1/2x human exposure at the clinical dose of 64 mg/d [AUC 156 ng.h/ml] in a 60 kg patient on an AUC basis); pharmacological effects of hydromorphone (vomiting, excessive salivation, decreased activity, pupil constriction, unformed stool) occurred with lower frequency than after immediate release formulation and decreased with repeated dosing
- **Segment I in Rats:** No effect on male and female fertility at up to 6.25 mg/kg/d PO (<1x the human dose of 64 mg/d in a 60 kg patient on an AUC basis)
- **Segment II in Rats:** Not teratogenic at up to 6.25 mg/kg/d PO (<1x the highest proposed clinical dose of 64 mg/d on an AUC basis) given on gestation days 6-17; developmental toxicity shown by 3 runts in 279 fetuses at that dose.
- **Segment II in Rabbits:** Not teratogenic at up to 25 mg/kg/d PO (<1x the human dose of 64 mg/d on an AUC basis) given on gestation days 6-20; Ratio of male to female fetuses higher at 25 mg/kg/d.
- **Segment III in Rats:** Severe blood loss in one high dose (6.25 mg/kg/d PO) dam during parturition; decreased number of live pups on lactation day 4 in high dose group; decreased viability and survival indices at 6.25 mg/kg/d; reduced pup weight at 3.13 mg/kg/d in F1 generation; decreased maternal brood care in F0 dams at 6.25 mg/kg/d; altered morphological landmarks and functional tests in F1 pups (delayed ear opening, reduced auditory startle reflex, reduced rearings and sectors entered) at 6.25 mg/kg/d
- **Non-mutagenic in the Ames test**

- **Non-clastogenic in the micronucleus test**
- **No increase in numbers of human lymphocytes with structural chromosome aberrations**
- **Carcinogenicity not studied; to be requested as Phase IV commitment**
- **Dilaudid CR™ (hydromorphone HCl) Controlled-Release Tablets approvable from a pharmacology and toxicology viewpoint with changes to the proposed label to reflect the results of the reproductive toxicology and genotoxicology studies.**

### **LABELING REVIEW**

The proposed Nonclinical Pharmacology and Toxicology sections of the Package Insert for Dilaudid CR™ are presented below:



(b) (4)

The Nonclinical Pharmacology and Toxicology sections of the label should be revised as follows:

*Mutagenicity/Carcinogenicity/Impairment of Fertility*

Hydromorphone was not found to be mutagenic or clastogenic in the Ames Test, Mouse Micronucleus Test and the Human Chromosomal Aberration Assay. Animal studies on hydromorphone to evaluate the carcinogenic potential have not been conducted. Hydromorphone HCl had no effect on male and female fertility and early embryonic development to implantation in the rat (highest dose tested 6.25 mg/kg/day by oral gavage, active moiety, < 1 times the highest recommended human dose of 64 mg/day on an AUC basis) when administered daily from 2 weeks before mating through gestation day 7 in females and 4 weeks before mating through the end of the mating period in males.

*Pregnancy - Pregnancy Category C*

*Teratogenic Effects*

Teratogenicity testing has been conducted in pregnant rats and rabbits. The rats were administered 1.56, 3.13 and 6.25 mg/kg/day of hydromorphone by oral gavage on gestation days 6-17, and the rabbits 6.25, 12.5 and 25 mg/kg/day by gavage on gestation days 6-20. No teratogenic effects were observed in these studies. Developmental toxicity was shown by the occurrence of 3 runts in a total of 279 fetuses at 6.25 mg/kg/d in the rats. The highest doses given the rats and rabbits were approximately equal to the highest recommended human dose of 64 mg/day on an AUC basis. In the pre- and post-natal effects study in rats, neonatal viability was reduced at the highest dose tested (6.25 mg/kg/day; active moiety). This finding appears to be a class effect of an opioid analgesic and therefore not a new finding.

Teratology studies reported in the literature showed increased incidence of cranioschisis and exencephaly in hamsters given a single dose of hydromorphone at 20 mg/kg subcutaneously on gestation day 8. A maximal effect (50% fetuses affected) was observed at a subcutaneous dose of 125 mg/kg. A dose relationship was not observed in those studies and the effects of opioid-induced toxicity in the dams (e.g., decreased food and water intake, altered motor activity) could not be determined. Fetal brain lesions were also demonstrated in hamsters given hydromorphone subcutaneously at 150 mg/kg (single dose) and 121, 82 and 82 mg/kg (triple dose). Mice administered hydromorphone by continuous infusion with a micro-osmotic minipump at 5 mg/kg/day during organogenesis produced offspring with increased incidence of minor skeletal defects and soft tissue anomalies including split supraoccipital, checkerboard and split sternbrae, delayed ossification of the paws, ectopic ossification sites, cryptorchidism, cleft palate, and malformed ventricles and retina.

*Nonteratogenic Effects*

Neonates born to mothers who have been taking opioids regularly before delivery may exhibit withdrawal symptoms, including irritability and excessive crying, tremors, hyperactive reflexes, increased respiratory rate, increased stools, sneezing, yawning, vomiting, and fever. The onset, duration, and severity of the syndrome do not always correlate with the duration of maternal opioid use or dose. Approaches to the treatment of this syndrome have included supportive care and, when indicated, drugs such as paregoric or phenobarbital.

Dilaudid CR Tablets should only be used during pregnancy if the potential benefit justifies the potential risk to the fetus (see *Labor and Delivery* and DRUG ABUSE AND DEPENDENCE).

## RECOMMENDATIONS:

### Recommendations to the Sponsor:

It is recommended that the results of the studies on hydromorphone reproductive toxicity and genotoxicity be included in the product label, as described under the Labeling Review, using exposure data for comparison between animals and humans.

Studies to evaluate the carcinogenic potential of hydromorphone hydrochloride will be needed as a Phase IV commitment.

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Kathleen Haberny, Ph.D.

  
Pharmacology Team Leader: Dou H. Jean, Ph.D.

cc: NDA 21-217 Arch.  
HFD 170/Division File  
HFD 170/J Milstein  
HFD 170/ K Haberny

**45 DAY MEETING CHECKLIST**

**FILEABILITY:**

On initial overview of the NDA application:

YES NO

**PHARMACOLOGY:**

(1) On its face, is the pharmacology section of the NDA organized in a manner to allow substantive review to begin? ✓

(2) Is the pharmacology section of the NDA indexed and paginated in a manner to allow substantive review begin? ✓

(3) On its face, is the pharmacology section of the NDA legible so that substantive review can begin? ✓

(4) Are all required(\*) and requested IND studies completed and submitted in this NDA (carcinogenicity, mutagenicity, teratogenicity\*, effects on fertility\*, juvenile studies, acute adult studies\*, chronic adult studies\*, maximum tolerated dosage determination, dermal irritancy, ocular irritancy, photocarcinogenicity, animal pharmacokinetic studies, etc.)? ✓

(5) If the formulation to be marketed is different from the formulation used in the toxicology studies, has the sponsor made an appropriate effort to either repeat the studies using the marketed product or to explain why such repetition should not be required? ✓

(6) Are the proposed labeling sections relative to pharmacology appropriate (including human dose multiples expressed in either mg/m<sup>2</sup> or comparative serum/plasma levels) and in accordance with 201.57? ✓  
will revise

(7) Has the sponsor submitted all special studies/data- requested by the Division during pre-submission discussions with the sponsor? ✓

(8) On its face, 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 sponsor submitted rationale to justify the alternative route? ✓

**PHARMACOLOGY:**

YES NO

(9) Has the sponsor submitted a statement(s) that all of the pivotal pharm/Tox studies been performed in accordance with- the GLP regulations (21 CFR 58 or an explanation for any significant deviations? ✓

(10) Has the sponsor submitted a statement(s) that the pharm/Tox studies have been performed using acceptable, state-of-the-art protocols which also reflect agency animal welfare concerns? ✓

(11) From a pharmacology perspective, is this NDA fileable? If "no", please state below why it is not. ✓

Note: Carcinogenicity studies will be required as a Phase IV commitment.

Kathleen G. Hoberg 1/6/2000  
Reviewing Pharmacology Officer Date

D.H. (Lynn) Jean 1/6/2000  
Supervisory Pharmacology Officer Date