

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**022396Orig1s000**

**PHARMACOLOGY REVIEW(S)**



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

**PHARMACOLOGY/TOXICOLOGY NDA MEMO TO FILE**

Application number:	NDA 22-396
Supporting document/s:	46, 48
Applicant's letter date:	October 31, 2014 (response to CR) December 9, 2014 (PMR and response to request for information)
Product:	Dyloject® (diclofenac sodium) intravenous injection
Indication:	Treatment of acute moderate to severe pain
Applicant:	Hospira, Inc.
Review Division:	Division of Anesthesia, Analgesia, and Addiction Products
Reviewer:	Armaghan Emami, PhD
Team leader:	Jay Chang, PhD
Supervisor:	Dan Mellon, PhD
Division Director:	Sharon Hertz, MD
Project Manager:	Swati Patwardhan

## **1 Executive Summary**

### **1.1 Introduction**

The Applicant submitted an NDA for Dyloject (administered as a 37.5 mg intravenous bolus injection) on December 2, 2009, referencing the Agency's prior findings of efficacy and safety for diclofenac potassium (Cataflam, NDA 20142). The Applicant received a Complete Response Letter (CR) on October 1, 2010. The deficiencies cited in the CR letter were related to both the clinical and the chemistry, manufacturing, and controls (CMC) disciplines, as well as labeling. In response to the CR letter, the Applicant submitted adequate information and data to demonstrate the safety and effectiveness of the product. However, the inspection of the manufacturing facilities identified significant

issues that precluded approval of this application during the second review cycle and the Applicant received the second CR letter on December 23, 2013.

On October 31, 2014, the Applicant submitted this resubmission in response to the second CR letter that included a proposal for a pediatric development plan. During this third review cycle, we accepted the Applicant's request to defer conduct of pediatric studies for patients ages 1 to <17 until after approval of the NDA as post-marketing requirement (PMR) studies. Additionally, we waived pediatric studies for patients from birth to <12 months of age (see medical officer review for rationale).

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

There were no new nonclinical data submitted during the third review cycle. Note that there were no pharmacology toxicology issues that precluded approval during the first and second review cycle.

## **1.3 Recommendations**

### **1.3.1 Approvability**

From the nonclinical pharmacology toxicology perspective, NDA 22396 may be approved. We do recommend a post-marketing requirement for a juvenile animal study (see below).

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

In a teleconference with the Applicant on December 4, 2014, we informed the Sponsor that a juvenile animal toxicology study would be required as a post-marketing requirement (PMR) to support the safety of clinical dosing in a pediatric population aged 1-2 years old. As noted above, the clinical pediatric studies will be completed as PMRs and will include children from 1 year <17 years of age. Note that pediatric clinical studies will evaluate the pharmacokinetics, safety, and efficacy of an age-appropriate formulation of Dyloject in pediatric patients in a tiered manner beginning first with children of ages 2 to less than 17 years and lastly with children aged 1 to less than 2 years. The nonclinical juvenile toxicology study is required prior to clinical studies in the younger pediatric cohort (1-< 2 years of age) to address the safety of the drug product for this age group as the kidney is not fully formed until approximately 1 year of age and is potentially a uniquely vulnerable target organ of toxicity for both diclofenac and the hydroxypropyl- $\beta$ -cyclodextrin (HP $\beta$ CD). Note that the drug substance, diclofenac sodium, has not been approved for pediatric use. In addition, HP $\beta$ CD, an excipient, is in FDA-approved intravenous drug products, but none of these products have been approved for pediatric use. In general toxicology studies, this excipient showed kidney

findings in mature rats (mild renal tubular vacuolation) and mature monkeys (mild granular appearance of the renal tubular cells in the medullary rays), but was not associated with impairment of renal function or progressive renal disease, and was cleared with full histological reversibility. However, there is no safety information available that addresses the potential effects of this excipient on a developing kidney of the pediatric population aged 1-2 years old and given the lack of full maturity of this and several other organs, a juvenile toxicity study is warranted. We recommend that the study include full histopathology with specialized assessment of the kidney function, including validated rat kidney biomarkers. The study should test the clinical formulation as diclofenac and the HP $\beta$ CD both could impact kidney function.

The Agency did indicate in the initial conversation that, if they feel there are adequate data to justify not conducting the juvenile animal study, the Applicant may submit a literature-based justification for the safety of their ultimate pediatric formulation and request release from the PMR for Agency review.

On December 9, 2014, the applicant submitted their timeline for the conduct of a juvenile animal study as a PMR to evaluate the general toxicology of the Dyloject pediatric formulation prior to initiation of the clinical study in pediatric patients  $\geq 1$  through  $< 2$  years of age. Below is the proposed timeline for juvenile animal study which is acceptable in the context of the full pediatric development program. (b) (4)



Protocol Submission: May 2017

Study Completion: March 2018

Final Study Report Submission: July 2018

### **1.3.3 Labeling**

From the nonclinical perspective, there are no labeling issues that need to be addressed prior to approval. The labeling recommendations were finalized in the last review cycle. Although the Pregnancy Labeling and Lactation Final Rule was just published, there is inadequate time remaining in this review cycle to consider including these labeling revisions at this time.

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/s/  
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ARMAGHAN EMAMI  
12/12/2014

JAY H CHANG  
12/12/2014

RICHARD D MELLON  
12/12/2014

I concur with Dr. Emami's recommendation that NDA 22396 may be approved from a nonclinical pharmacology toxicology perspective and with the recommended post-marketing requirement and labeling.



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**PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION**

Application number: 22-396  
Supporting document/s: 000  
Applicant's letter date: December 2, 2009  
CDER stamp date: December 3, 2009  
Product: Dyloject® (diclofenac sodium) Intravenous injection  
Indication: Treatment of acute moderate to severe pain in adult  
Applicant: Hospira, Inc.  
Review Division: Division of Anesthesia and Analgesia Products  
Reviewer: Armaghan Emami, Ph.D.  
Supervisor/Team Leader: Adam Wasserman, Ph.D.  
Division Director: Bob Rappaport, M.D.  
Project Manager: Kathleen Davies

*Template Version: December 7, 2009*

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## TABLE OF CONTENTS

<b>1</b>	<b>EXECUTIVE SUMMARY .....</b>	<b>4</b>
1.1	RECOMMENDATIONS .....	4
1.2	BRIEF DISCUSSION OF NONCLINICAL FINDINGS .....	6
<b>2</b>	<b>DRUG INFORMATION .....</b>	<b>9</b>
2.1	DRUG .....	9
2.2	RELEVANT IND/s, NDA/s, AND DMF/s .....	9
2.3	CLINICAL FORMULATION .....	10
2.4	PROPOSED CLINICAL POPULATION AND DOSING REGIMEN .....	13
2.5	REGULATORY BACKGROUND .....	13
<b>3</b>	<b>STUDIES SUBMITTED.....</b>	<b>15</b>
<b>4</b>	<b>PHARMACOLOGY .....</b>	<b>16</b>
4.1	PRIMARY PHARMACOLOGY .....	16
4.2	SECONDARY PHARMACOLOGY .....	16
4.3	SAFETY PHARMACOLOGY .....	17
<b>5</b>	<b>PHARMACOKINETICS/ADME/TOXICOKINETICS .....</b>	<b>18</b>
5.1	PK/ADME.....	18
5.2	TOXICOKINETICS .....	19
<b>6</b>	<b>GENERAL TOXICOLOGY.....</b>	<b>19</b>
6.1	SINGLE-DOSE TOXICITY .....	19
6.2	REPEAT-DOSE TOXICITY .....	19
6.2.1	NON-PIVOTAL REPEAT-DOSE TOXICITY .....	20
6.2.1.1	RATS.....	20
6.2.1.2	MONKEYS .....	21
6.2.2	PIVOTAL REPEAT-DOSE TOXICITY .....	23
6.2.2.1	RATS.....	23
6.2.2.2.1	MONKEY .....	35
6.2.2.2.2	MONKEY .....	48
<b>7</b>	<b>GENETIC TOXICOLOGY .....</b>	<b>50</b>
7.1	<i>IN VITRO</i> REVERSE MUTATION ASSAY .....	50
7.2	<i>IN VITRO</i> MOUSE LYMPHOMA MUTATION ASSAY .....	58
7.3	<i>IN VITRO</i> MOUSE LYMPHOMA MUTATION ASSAY (VOLTAROL) .....	65
7.4	<i>IN VIVO</i> MOUSE MICRONUCLEUS TEST.....	72
7.5	OTHER GENETIC TOXICITY STUDIES (FOR AN IMPURITY) .....	75
7.5.1	<i>IN VITRO</i> REVERSE MUTATION ASSAY IN BACTERIAL CELLS.....	75
7.5.2	<i>IN VITRO</i> CHROMOSOMAL ABERRATION ASSAY .....	82
<b>8</b>	<b>CARCINOGENICITY .....</b>	<b>86</b>
<b>9</b>	<b>REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY .....</b>	<b>87</b>

<b>10</b>	<b>SPECIAL TOXICITY STUDIES</b> .....	<b>87</b>
<b>11</b>	<b>LOCAL TOLERANCE STUDIES</b> .....	<b>87</b>
11.1	INTRAVENOUS (IV) LOCAL TOLERANCE STUDY .....	88
11.2	INTRAMUSCULAR (IM) LOCAL TOLERANCE STUDY .....	92
<b>12</b>	<b>INTEGRATED SUMMARY AND SAFETY EVALUATION</b> .....	<b>97</b>
<b>13</b>	<b>REFERENCES</b> .....	<b>103</b>
<b>14</b>	<b>APPENDIX/ATTACHMENTS</b> .....	<b>105</b>

# 1 Executive Summary

## 1.1 Recommendations

**1.1.1 Approvability:** From the non-clinical pharmacology toxicology perspective, this NDA may be approved.

**1.1.2 Additional Non Clinical Recommendations:** None

### 1.1.3 Labeling

Note the Applicant uses language from the Listed Drug Cataflam® with adjusted dose margins based on the higher total daily dose with Dyloject. The final label may differ based on negotiations with Applicant and further internal discussion.

Sponsor's Proposed Labeling	Recommended Labeling	Rationale/Comment
<p><b>8.1 Pregnancy</b>                      Teratogenic Effects - Pregnancy Category C prior to 30 weeks gestation; Category D starting at 30 weeks gestation.</p> <p>Starting at 30 weeks gestation, (b) (4) and other NSAIDs, should be avoided by pregnant women as premature closure of the ductus arteriosus in the fetus may occur. Diclofenac can cause fetal harm when administered to a pregnant women starting at 30 weeks gestation.</p> <p>There are no adequate and well-controlled studies in pregnant women. Prior to 30 weeks gestation, Dyloject should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.</p> <p>Reproductive studies have been performed in mice given diclofenac sodium (up to 20 mg/kg/day or 60 mg/m2/day) and in rats and rabbits given diclofenac sodium (up to 10 mg/kg/day or 60 mg/m2/day for rats, and 80 mg/m2/day for rabbits, 0.75-fold and 1-fold (b) (4) (b) (4)), and have revealed no evidence of teratogenicity despite the induction of maternal toxicity and fetal toxicity. In rats maternally toxic doses were associated with dystocia, prolonged gestation, reduced fetal weights and growth, and</p>	<p>No changes recommended</p>	

<p>reduced fetal survival. Diclofenac has been shown to cross the placental barrier (b) (4).</p> <p>(b) (4)</p>		
<p><b>8.2 Labor and Delivery</b></p> <p>(b) (4)</p> <p>In rat studies, maternal exposure to NSAIDs, as with other drugs known to inhibit prostaglandin synthesis, increased the incidence of dystocia and delayed parturition, and decreased pup survival.</p>	<p>No changes recommended</p>	
<p><b>8.3 Nursing Mothers</b></p> <p>(b) (4)</p> <p>(b) (4)</p>	<p>No changes recommended</p>	
<p><b>13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility</b></p> <p>(b) (4)</p> <p>Carcinogenesis: Long-term carcinogenicity studies in rats given diclofenac sodium up to 2 mg/kg/day (b) (4) have revealed no significant increase in tumor incidence. A 2-year carcinogenicity study conducted in mice employing diclofenac sodium at doses up to 0.3 mg/kg/day (b) (4) in males and 1mg/kg/day (b) (4) in females did not reveal any oncogenic potential.</p> <p>Mutagenesis: Diclofenac sodium did</p>	<p>(b) (4)</p> <p>(b) (4) 0.01-fold (b) (4) of 150 mg/day) in males and 1mg/kg/day (b) (4) 0.04-fold (b) (4) of 150 mg/day) in females did not reveal any oncogenic potential.</p>	<p>(b) (4)</p> <p>Correcting margin calculations as appropriate.</p>

<p>not show mutagenic activity in <i>in vitro</i> point mutation assays in mammalian (mouse lymphoma) and microbial (yeast, Ames) test systems and was non mutagenic in several mammalian <i>in vitro</i> and <i>in vivo</i> tests, including dominant lethal and male germinal epithelial chromosomal aberration studies in Chinese hamsters.</p> <p>Impairment of Fertility: Diclofenac sodium administered to male and female rats at 4 mg/kg/day (b) (4) did not affect fertility.</p>		
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## 1.2 Brief Discussion of Nonclinical Findings

### Background

Dyloject is a complex of diclofenac sodium [non-steroidal anti-inflammatory drug] and hydroxypropyl- $\beta$ -cyclodextrin (b) (4). Dyloject (diclofenac sodium 37.5 mg, HP $\beta$ CD 333mg in 1ml vial), also known as DIC075V in this submission, is an injectable formulation intended for the treatment of acute moderate to severe pain. The applicant proposes DIC075V may be administered 4 times a day (b) (4). The maximum recommended daily human dose (MRHD) is therefore 150 mg diclofenac and 1332 mg HP $\beta$ CD. DIC075V is intended to be administered IV (b) (4).

Dyloject was approved by the Medicines and Healthcare products Regulatory Agency (MRHA) in October 2007 and is currently marketed in the United Kingdom (UK) for both IM and IV administration. According to the Applicant, from approval to April 29, 2009, an estimated (b) (4) patients in the UK have been treated with DIC075V based on cumulative sales of (b) (4) 75 mg/2 mL vials. A similar drug, Voltarol® (diclofenac sodium, 75 mg/3 mL), is currently manufactured and marketed by Novartis Pharmaceuticals in Europe for both IM and IV injections however, it does not contain the excipient HP $\beta$ CD. A parenteral formulation of diclofenac is not currently available in the U.S.

### Overview of nonclinical findings

The Applicant has provided single and repeat dose IV toxicity studies, genotoxicity and parenteral local tolerance studies with diclofenac formulations containing HP $\beta$ CD (DF-HP $\beta$ CD) to support the safety of DIC075V for human use. The Applicant also relies upon the Agency's previous finding of safety and efficacy for Cataflam® (diclofenac potassium, NDA 20-142) 50 mg Tablets and has conducted several bridging studies as well as providing literature references to support this NDA. The Applicant has obtained a Letter of Authorization to NDA 20-966 for Sporanox® (itraconazole) to support the safety of the excipient, hydroxypropyl- $\beta$ -cyclodextrin (HP $\beta$ CD).

Notably, the daily dosage and duration of use of Dyloject is within the listed drug (LD) but the route of administration is different. Also, human AUC and Cmax values are not covered by LD exposure at the MRHD. Therefore the Applicant has conducted the nonclinical studies with an IV formulation to support both systemic exposure and local tolerance.

At the MRHD, the total daily intake of 1.3 g/day HP $\beta$ CD (333 mg QID) is 12-fold lower for Dyloject as compared to Sporanox®, 16 g/day (8000 mg BID). However, the administered concentration is (b) (4) Dyloject (333 mg/mL) compared to Sporanox (b) (4) and the rate of administration is more rapid (IV bolus vs. 60 min-IV infusion, respectively).

The applicant conducted a 7-day IV (1ml/min) local tolerance in rats with DIC075V (the to-be-marketed product) to support the safety of the drug product concentration at the injection site and the rate of administration. The results suggested a mild to moderate irritation when injected IV but the injection site findings were relatively absent after a 7-day recovery period. The data indicate that inadvertent perivascular injection can cause local muscle irritation or necrosis. The microscopic changes from 7-day IV study indicate that DIC075V may be mildly irritating when injected intravascularly or when extravasated into the surrounding perivascular tissue. However, local tolerance was not a significant issue in clinical trials. According to the medical officer review clinical studies reports of local injection site reactions were infrequent and mild to moderate in severity.

Also the applicant conducted a 7-day intramuscular local tolerance studies in rabbits with DIC075V. The results suggested a moderate to marked irritation when injected IM. However the changes were reversible after 14 days of the recovery period. (b) (4)

Two pivotal 28-day intravenous repeat-dose toxicology studies of an earlier formulation of Dyloject (DIC075U; lower diclofenac and HP $\beta$ CD concentration, some excipient differences) were conducted in the rat and monkey. The toxicology findings were either in response to diclofenac which demonstrated classical NSAID-related toxicities (gastrointestinal toxicity with secondary regenerative anemia in rats and monkeys and also some evidence of impaired wound healing in monkey tail lesions) or to HP $\beta$ CD (histopathological changes in the kidney). There is evidence that 533 mg/kg/day of HP $\beta$ CD given IV in monkeys for 4 weeks caused only minor granular changes to renal tubular epithelial cells, reflective of cyclodextrin uptake and intracellular breakdown, and no evidence for renal dysfunction while providing exposures of approximately 3363  $\mu$ g·h/mL. It has been shown that human subjects administered DIC075V at the MRHD have an exposure approximating 265  $\mu$ g·h/mL for HP $\beta$ CD. This exposure is 12.7 times lower than that which causes very minimal histopathologic changes with no evidence for functional renal deficits in monkeys. These two studies demonstrated a NOAEL in rat and acceptable LOAEL in monkey that provide an acceptable exposure margin for the maximum daily intravenous dose of diclofenac and both studies along with prior approved use in Sporanox support the level of HP $\beta$ CD.

Neither diclofenac nor HP $\beta$ CD are genotoxic. Diclofenac was sufficiently evaluated in carcinogenicity studies as described in the listed drug Cataflam label and though

exposure equivalent to the human exposure was not attained, this is not of significance due to the proposed acute duration.

### Pharmacology

Extensive published data have demonstrated that diclofenac possesses analgesic, anti-inflammatory and antipyretic properties as a result of relatively non-selective COX inhibition and decreased eicosanoid biosynthesis. The most prominent adverse clinical effect reported for diclofenac use is gastrointestinal toxicity, which is considered to be related to its pharmacological mechanism of action [Goodman & Gilman, 2007; Cryer et al, 1998].

In view of the well understood pharmacological properties of diclofenac, the applicant did not conduct further pharmacology studies with DIC075V.

### Non-clinical safety issues relevant to clinical use:

In the local toxicity study in rats microscopic evaluation of tails (the site of injection) indicate that DIC075V may be mildly irritating when injected intravascularly or when extravasated into the surrounding perivascular tissue. The incidence and severity of changes were increased in multiple daily dose groups compared to single dose groups, suggesting that injection sites in human need to be rotated if administered as multiple daily doses.

HPβCD may be delayed in its clearance from urinary epithelium. However it has been shown in animals and humans to not interfere with renal function, lead to progressive renal disease, and in animal models is cleared with full histological reversibility.

As with other non-selective NSAIDs, gastrointestinal toxicity, effects on wound healing, and fetal risk of premature closure of the ductus arteriosus are considered the most relevant toxicological concerns for the assessment of the clinical safety of DIC075V.

The excipients in the proposed drug product are acceptable and the impurities and degradants are within both ICH Q3A and Q3B limits, respectively. The Applicant did not initially provide adequate justification for the proposed specifications of (b) (4) a drug product degradant. While two *in vitro* genotoxicity assays were conducted for this (b) (4) with the negative results, repeat dose toxicity study with isolated impurity was not provided. The Sponsor provided data from two repeat dose toxicity studies to support the safety of maximal human intake of the impurity using the proposed specification. However, as noted in impurity section below, these data do not adequately qualify the (b) (4) impurity. Therefore the Applicant has (b) (4) to comply with ICHQ3B limits.

## 2 Drug Information

### 2.1 Drug

Dyloject™

#### 2.1.1 CAS Registry Number (Optional)

15307-79-6

#### 2.1.2 Generic Name

Diclofenac sodium

#### 2.1.3 Code Name

DIC075V

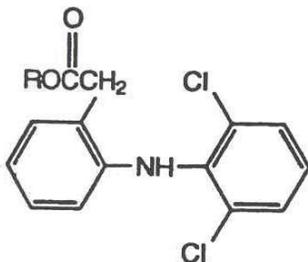
#### 2.1.4 Chemical Name

2-[(2, 6-dichlorophenyl) amino] benzeneacetic acid, monosodium

#### 2.1.5 Molecular Formula/Molecular Weight

C<sub>14</sub>H<sub>10</sub>Cl<sub>2</sub>NNaO<sub>2</sub> / 318.13

#### 2.1.6 Structure



#### 2.1.7 Pharmacologic class

Non-steroidal anti-inflammatory drug (NSAID)

## 2.2 Relevant IND/s, NDA/s, and DMF/s

Drug Name and FDA Application Number	Active Ingredients	Dosage Form/Route	Strength	Marketing Status	Company	Approval date
<b>VOLTAREN (NDA # 019201)</b>	diclofenac sodium	tablet, delayed release; oral	75MG	Prescription	Novartis	1988
<b>VOLTAREN (NDA # 020037)</b>	diclofenac sodium	solution/drops; ophthalmic	0.1%	Prescription	Novartis	1991

<b>VOLTAREN</b> (NDA # 022122)	diclofenac sodium	gel; topical	1%	Prescription	Novartis	2007
<b>VOLTAREN- XR</b> (NDA # 020254)	diclofenac sodium	tablet, extended release; oral	100MG	Prescription	Novartis	1996
<b>CATAFLAM</b> (NDA # 020142)	diclofenac potassium	tablet, oral	50MG	Prescription	Novartis	1993
<b>ZIPSOR</b> (NDA #022202)	diclofenac potassium	capsule, oral	25MG	Prescription	Xanodyne Pharm	2009
<b>PENNSAID</b> (NDA #020947)	diclofenac sodium	solution; topical	1.5%	Prescription	Mallinckrodt	2009
<b>FLECTOR</b> (NDA # 021234)	diclofenac epolamine	patch; topical	1.3%	Prescription	Inst Biochem	2007

## 2.3 Clinical Formulation

### 2.3.1 Drug Formulation

The drug product, Dyloject Injection (37.5 mg/mL) is a sterile, aqueous solution presented in 1 mL fill volume. (b) (4)

The commercial formulation used in the clinical program has been designated as DIC075V. Two formulations used in earlier clinical and preclinical trials are designated as DIC075T and DIC075U.

**Table 3 Investigational Formulations for Preclinical and Clinical Studies**

Ingredient	DIC075T	DIC075U <sup>1</sup>	DIC075V <sup>2</sup>
Diclofenac sodium	75 mg	75 mg	75 mg
Hydroxypropyl betadex	(b) (4)		
Monothioglycerol	(b) (4)		
Hydrochloric acid (pH adjuster)	(b) (4)		
Sodium hydroxide (pH adjuster)	(b) (4)		
Water for injection	(b) (4)		

<sup>1</sup>also designated as DRG0003

<sup>2</sup> DIC075V is the intended “to be marketed” formulation

### 2.3.2 Comments on Novel Excipients

The formulation excipients are hydroxypropyl betadex NF (hydroxypropyl-β-cyclodextrin, HPβCD), monothioglycerol (MTG) NF, hydrochloric acid NF, sodium hydroxide NF, and water for injection USP

Component	Function	w/v
Diclofenac sodium USP	Active ingredient	37.5 mg
Hydroxypropyl betadex NF	(b) (4)	333 (b) (4)mg
Monothioglycerol NF	(b) (4)	5.0 mg
Hydrochloric acid NF	pH adjuster	as needed
Sodium hydroxide NF	pH adjuster	as needed
Water for Injection USP	(b) (4)	(b) (4)

The total daily exposure to the excipients was less than the maximum potency limits as listed in the IIG.

- At the MRHD, the total daily intake of 1.3 g/day HPβCD is 12-fold lower for Dyloject as compared to Sporanox® (16 g; NDA 20-966);
- MTG concentration is 0.5% compared to maximum potency of 1% in IIG. Also at the MRHD, the total daily intake (b) (4) (MTG) is (b) (4) for Dyloject as compared to Lusedra® (NDA 22-244)

### 2.3.3 Comments on Impurities/Degradants of Concern

During pharmaceutical development the only identifiable impurity was (b) (4)

(b) (4) Based on this development work, the specification limit (b) (4) is set at not more than (b) (4) % w/w.

The maximum amount observed in the stability program to date is (b) (4) % w/w. This exceeds ICH Q3B threshold level for qualification (for (b) (4) maximum daily dose, the qualification threshold would be (b) (4) % or (b) (4) TDI which ever is lower).

Impurity	Structure
(b) (4)	(b) (4)

Two *in vitro* genotoxicity assays were conducted for a drug product degradant (b) (4) to qualify the proposed specification and the results were negative. However repeat dose toxicity study with isolated impurity was not provided. However the Sponsor believes the

repeat-dose studies previously conducted in two species, qualifies this degradant without further investigations (see below, the Sponsor justification from toxicology written summary page 46-48).

4-week (to 15 mg/kg/day) IV toxicity studies in rats and a 4-week (to 60 mg/kg/day) IV toxicity study in monkeys with diclofenac sodium injection, which contained reduced concentrations (b) (4) (0.06% for rats and 0.07% for monkeys) to that potentially provided to human subjects were conducted.

**Table 25** Diclofenac and (b) (4) Exposure Ratio Data

(b) (4)



The Sponsor conclusion: (b) (4)



This reviewer does not agree with the Sponsor (b) (4)



The Applicant revised the (b) (4) specification to NMT (b) (4) % to comply with ICHQ3B limits and proposed 18-month expiration dating for the product to ensure meeting the NMT (b) (4) % (b) (4) specification.

The compound (b) (4) was determined to be a leachable during CMC review (by Dr. Martin Haber). This compound is (b) (4)



(b) (4) The total daily intake with 4 vials/day maximum would represent (b) (4) µg. The compound was reported to be negative in two published genetic toxicity studies (Ames assay and Mouse Lymphoma assay) and has a high oral LD50 in rat of 8000

mg/kg which suggests low (oral) toxicity. This reviewer believes that the acute nature of the treatment with Dyloject reduces concern.

## 2.4 Proposed Clinical Population and Dosing Regimen

Dyloject Injection (37.5mg/mL), a parenteral formulation intended for the management of acute moderate to severe pain in adults. The dosage regimen is 37.5 mg every six hours (not to exceed 150 mg/day) (b) (4)

## 2.5 Regulatory Background

- Diclofenac is the same active ingredient in Dyloject, Voltaren®, Cataflam®, Voltarol®, Zipsor® and Pennsaid®. Diclofenac may be supplied as either the sodium or potassium salt. Diclofenac has been available in the U.S. in immediate-release oral preparations, delayed release enteric-coated tablets, sustained-release tablets, ophthalmic drops and topical gels. Diclofenac is available as a generic drug in a number of formulations. However a parenteral formulation of diclofenac is not available in the U.S. Only two parenteral NSAIDs; Ketorolac (Toradol®) and Ibuprofen (Caldolor®) have been approved in the U.S.
- Voltarol® (diclofenac sodium, 75 mg/3 mL) and Dyloject (diclofenac sodium, 75 mg/2 mL) for IM and IV injections are available outside of the U.S. Voltarol Ampoules were first approved in the United Kingdom (UK) on 6 August 1981 for IM and 30-minute IV infusion administrations. Voltarol Ampoules 75 mg/3 mL which is currently manufactured and marketed by Novartis Pharmaceuticals in Europe, was used as an active comparator in certain nonclinical studies in this submission and does not contain the excipient HPβCD. Dyloject was approved by the Medicines and Healthcare products Regulatory Agency (MHRA) in October 2007 and is currently marketed in the United Kingdom (UK). From approval to April 29, 2009, an estimated (b) (4) patients in the UK have been treated with DIC075V based on cumulative sales of (b) (4) 75 mg/2 mL vials (see 3rd Periodic Safety Update Report (CTD 5.3.6.3 from NDA submission).
- Related IND 65,048 is active since 06/14/2002
- From EOP2 meeting on April 21, 2006
  - Provide evidence/data which indicate that Hydroxypropyl-β-cyclodextran (HPβCD) does not interfere or trap any other chemicals or biological materials in the body
  - The Sponsor agreed to provide qualification data for their IIG.
  - For the NDA, you may need to complete nonclinical pharmacokinetic bridging studies in order to compare exposures obtained in the listed drug product with those obtained with your drug product for the product labeling.
  - The Division commented that because there are different Cmax values between the listed drug and the proposed drug, the Sponsor should ensure that adequate preclinical coverage is available to cover all doses
- From Pre-NDA meeting on March 10, 2008

- Establishing a cutoff for meaningful HP $\beta$ CD complexation of likely coadministered drugs in the intended patient population based on in vitro experiments and the derived stability constants is likely to be more informative.
- The nonclinical studies outlined are sufficient to support submission of the NDA. However, before submission of the NDA, in vivo non-clinical data may be needed to adequately assess the potential for drug-drug interactions between diclofenac sodium injection and commonly used drugs to confirm the report provided by the consultant in the briefing package.
- Cataflam® (oral diclofenac potassium, 50 mg immediate release tablets), manufactured by Novartis Pharmaceuticals Corporation, East Hanover, New Jersey, is the listed product for this 505(b)(2) NDA.
- Reference also made to Sporanox® (itraconazole, 60-minute IV infusion) to support the safety of the excipient HP $\beta$ CD. Sporanox, NDA 20-966, was approved on March 30, 1999 and was withdrawn on December 4, 2009 due to commercial reasons

### 3 Studies Submitted

#### 3.1 Studies Reviewed:

Study Type and Duration	Route of Administration	Species	GLP Compliant	Study Number
<b>Single Dose Toxicity</b> Dose range finding for micronucleus test	Intravenous injection	CD-1 Mouse	Yes	NC-DFC-012
<b>Repeat Dose Toxicity</b> <b>Non-Pivotal Studies</b>				
2 week dose range finding	Intravenous slow bolus injection	Sprague-Dawley Rat	Yes	NC-DFC-001
Maximum tolerated dose study	Intravenous slow bolus injection	Cynomolgus Monkey	Yes	NC-DFC-002
<b>Pivotal Studies</b>				
4 week study including 9 week recovery	Intravenous slow bolus injection	Sprague-Dawley Rat	Yes	NC-DFC-004
4 week study including 13 week recovery	Intravenous slow bolus injection	Cynomolgus Monkey	Yes	NC-DFC-010
4 week study including 13 week recovery	Intravenous slow bolus injection	Cynomolgus Monkey	Yes	NC-DFC-011
<b>Genotoxicity</b> <b>In vitro</b>				
Ames test	<i>in vitro</i>	S. Typhimurium and E. Coli	Yes	NC-DFC-007
Mouse lymphoma mutation assay	<i>in vitro</i>	L5178Y mouse lymphoma cells	Yes	NC-DFC-009
Mouse lymphoma mutation assay (Voltarol®)	<i>in vitro</i>	L5178Y mouse lymphoma cells	Yes	NC-DFC-008
<b>In vivo</b>				
Micronucleus test	Intravenous injection	CD-1 Mouse	Yes	NC-DFC-012
<b>Local Tolerance</b>				
7 day study including 7 day recovery	Intravenous slow bolus injection	Sprague-Dawley Rat	Yes	NC-DFC-013
7 day study	Intramuscular injection	New Zealand White Rabbit	Yes	NC-DFC-005
7 day study	Intramuscular injection	New Zealand White Rabbit	Yes	NC-DFC-006
7 day study including 14 day recovery	Intramuscular injection	New Zealand White Rabbit	Yes	NC-DFC-014
<b>Other Toxicity Studies</b>				
	(b) (4) Impurity			
Ames test	<i>in vitro</i>	S. Typhimurium and E. Coli	Yes	NC-DFC-016
Chromosomal Aberration Test	<i>in vitro</i>	Cultured human lymphocytes	Yes	NC-DFC-017
<b>Safety pharmacology</b>				
Cardiovascular		HEK 293 cells transfected with hERG	<i>In vitro</i>	NC-DFC-018 4.2.1.3.1

**3.2 Studies not reviewed:** below study was not fully reviewed. However summarized in PK section

Type of study	Test System or Species/Strain	Method of Admin.	Duration of Dosing	Concentrations or Doses (mg/kg)	GLP Compliance	Testing Facility	Study Number	Location
<b>Other pharmacokinetic studies</b>								
Validation of an Experimental Drug-HP $\beta$ CD Complexation Module following Administration of DIC075V (Diclofenac Sodium Injection) 37.5 mg/mL	<i>In vitro</i>	NA	NA	The concentration of drugs was limited by their solubilities, and depended on the HPLC detection threshold of dialysis and varied from $0.7 \cdot 10^{-5}$ to $2.6 \cdot 10^{-3}$ M	no	University of Iceland <sup>b</sup>	NC-DFC-015	4.2.2.7.1

**3.3 Previous Reviews Referenced:** This is a 505(b)(2) application and reference is made to the listed drug Cataflam® (diclofenac potassium) 50 mg Tablets, marketed by NOVARTIS, NDA 20-142, approved November 24, 1993. Also for HP $\beta$ CD (excipient) reference is made to Sporanox®, owned by Ortho McNeil Janssen, NDA 20-966, approved March 30, 1999.

## 4 Pharmacology

### 4.1 Primary Pharmacology

Primary pharmacodynamic studies were not conducted with DIC075V. Extensive references on the pharmacology of diclofenac are summarized below.

Diclofenac is a potent non-selective inhibitor of cyclooxygenase isoforms 1 and 2 (COX-1 and COX-2). Diclofenac is a slightly stronger inhibitor of COX-2 (the inducible isoform of cyclooxygenase involved in inflammatory responses) than of COX-1 (the constitutive isoform of cyclooxygenase that is important in the maintenance of functional homeostasis) (Cryer et al, 1998 and Kato et al, 2001). Through the inhibition of cyclooxygenase, diclofenac reduces the formation of prostaglandins, prostacyclins and thromboxanes. Diclofenac also decreases the formation of products of the lipoxygenase pathways, specifically the leucotrienes and 5-hydroxyeicosatetraenoic acid. This decrease is not due to a direct inhibition of lipoxygenase, but is related to a reduction of the availability of the substrate, arachidonic acid (Scholer, 1986).

Analgesic, anti-inflammatory and antipyretic activities have been seen in several animal models (Menasse et al., 1978).

### 4.2 Secondary Pharmacology

Secondary pharmacodynamic studies were not conducted with DIC075V. High doses of diclofenac (100 mg/kg) inhibit adenosine diphosphate or thrombin-induced platelet aggregation in rats (Todd et al., 1988).

### 4.3 Safety Pharmacology

The applicant conducted only one safety pharmacology study (hERG assay) which is summarized below.

Study title: **Evaluation of the Effect of Diclofenac, DIC075V and HP $\beta$ CD on the Human Potassium Channel using Human Embryonic Kidney 293 Cells Transfected with a Human Ether-a-go-go-related Gene**

Study no.: NC-DFC-018 (eCTD 4.2.1.3)

Conducting laboratory and location: (b) (4)

Date of study initiation: March 06, 2008

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: Diclofenac sodium, lot # 0701, purity: 99.9%  
HP $\beta$ CD, lot # CH2071650

After recording baseline current levels, patched cells (n=7) were sequentially exposed to increasing diclofenac concentrations (1.58, 15.8, 158, 474  $\mu$ g/mL), DIC075V concentrations (containing 1.58, 15.8, 158, 474  $\mu$ g/mL diclofenac and 14, 140, 1400, 4200  $\mu$ g/mL HP $\beta$ CD) or HP $\beta$ CD concentrations (14, 140, 1400, 4200  $\mu$ g/mL). The concentrations selected for this study reflect a range calculated to exceed the therapeutic exposure to each constituent (diclofenac and HP $\beta$ CD) of DIC075V, and provide as safety margin exceeding 50 times the anticipated clinical exposure.

The 3 highest concentrations of DIC075V tested respectively caused 45.3, 47.8 and 41% of inhibition of the hERG tail current. None of the concentrations of diclofenac tested caused an inhibition of the hERG tail current. The four concentrations of HP $\beta$ CD tested respectively caused 22.5, 39.3, 43.5 and 36.5% of inhibition of the hERG tail current.

This suggests that HP $\beta$ CD, in the specific experimental context of this study, interacts with the protein encoded by the hERG gene, and is responsible for the effects on I<sub>Kr</sub> current at concentrations of 14 $\mu$ g/mL and greater. The Sponsor stated that the absence of clinical effect of HP $\beta$ CD suggests that the effect observed in this study represents one of the confounding aspects of HP $\beta$ CD use in patch-clamp experiments, as reported by the two authors (Himmel H. 2007 and Mikhail A. 2007).

## 5 Pharmacokinetics/ADME/Toxicokinetics

### 5.1 PK/ADME

Nonclinical ADME studies with diclofenac in the HP $\beta$ CD containing formulations, DIC075U or DIC075V, were not conducted as extensive data on the ADME characteristics of diclofenac have been published.

Several published studies indicate near complete oral absorption of diclofenac. The acylglucuronide metabolite of diclofenac is subject to enterohepatic recirculation in rat and dog, which is thought to contribute to the increased gastrointestinal toxicity of diclofenac in those species (Peris-Ribera et al, 1991; Tabata, et al. 1995; Tsuchiya, et al. 1980; John, 1979) compared with human.

The FDA approved labeling for Cataflam (2006) provides the following information:

The apparent volume of distribution (V/F) of diclofenac potassium is 1.3 L/kg. Diclofenac is more than 99% bound to human serum proteins, primarily to albumin. Serum protein binding is constant over the concentration range (0.15-105  $\mu$ g/mL) achieved with recommended doses. Diclofenac diffuses into and out of the synovial fluid. Diffusion into the joint occurs when plasma levels are higher than those in the synovial fluid, after which the process reverses and synovial fluid levels are higher than plasma levels. It is not known whether diffusion into the joint plays a role in the effectiveness of diclofenac.

Five diclofenac metabolites have been identified in human plasma and urine. The metabolites include 4'-hydroxy-, 5-hydroxy-, 3'-hydroxy-, 4',5-dihydroxy- and 3'-hydroxy-4'-methoxy diclofenac. In patients with renal dysfunction, peak concentrations of metabolites 4'-hydroxy- and 5-hydroxy-diclofenac were approximately 50% and 4% of the parent compound after single oral dosing compared to 27% and 1% in normal healthy subjects. However, diclofenac metabolites undergo further glucuronidation and sulfation followed by biliary excretion. One diclofenac metabolite 4'-hydroxy-diclofenac has very weak pharmacologic activity.

Diclofenac is eliminated through metabolism and subsequent urinary and biliary excretion of the glucuronide and the sulfate conjugates of the metabolites. Little or no free unchanged diclofenac is excreted in the urine. Approximately 65% of the dose is excreted in the urine and approximately 35% in the bile as conjugates of unchanged diclofenac plus metabolites. Because renal elimination is not a significant pathway of elimination for unchanged diclofenac, dosing adjustment in patients with mild to moderate renal dysfunction is not necessary. The terminal half-life of unchanged diclofenac is approximately 2 hours.

HP $\beta$ CD is added to diclofenac [REDACTED] (b) (4) HP $\beta$ CD is a cyclic oligosaccharide comprised of 7 glucopyranose units with 4.06 to 5.11 2-hydroxypropyl groups per molecule of cyclodextrin (Janssen, SPC of Sporanox IV, 2004). [REDACTED] (b) (4)

[REDACTED] After single IV administration, plasma concentrations of HP $\beta$ CD decline rapidly, although in dogs and humans, there is a biphasic elimination curve, with half-lives of 0.8 hours in dogs and 1.7 hours in humans. The volumes of distribution in rats and dogs are very similar to that of the human, indicating limited tissue distribution. Total plasma clearance in rats is about 4-times that seen in dogs and

1.4-times higher than in humans. Clearance rates correspond well with glomerular filtration rates for the various species. After IV administration of  $^{14}\text{C}$ -HP $\beta$ CD in rats and dogs, plasma levels and total radioactivity are similar for 4-8 hours post-dose and then decline gradually. After repeat dosing (during a day), steady state plasma concentrations of HP $\beta$ CD were achieved in rats and dogs within the first day. Plasma concentrations demonstrate dose linearity and no accumulation occurs, even following 3 months of daily dosing in rats with 400 mg/kg/day and in dogs with 825 mg/kg/day. Tissue distribution is very limited, likely due to the size of the molecule. However, renal tissue concentrations, especially cortical, exceed those of plasma and remain for an extended period following drug discontinuation due to known uptake into tubular epithelium as a means of elimination. Peak concentrations in the lung and liver are 3 times lower and 8 times lower than plasma, respectively. Levels of hepatic HP $\beta$ CD concentrations were lower than levels of plasma radioactivity, indicating there was some hepatic metabolism of HP $\beta$ CD. After intravenous dosing in pregnant rats, concentrations of total radioactivity in fetal blood, whole fetuses and amniotic fluid are 10-25-times lower than the peak maternal and tissue concentrations. Metabolism of HP $\beta$ CD after IV dosing is minimal. Most of the dose is excreted intact. Rats eliminate only 3% of a radiolabeled dose via the feces and 0.6% by expired air. Dogs excrete only 0.2% of total radioactivity via the feces. In humans, 80-90% of an IV dose is eliminated rapidly via urine.

## 5.2 Toxicokinetics

See repeat dose toxicity studies

## 6 General Toxicology

### 6.1 Single-Dose Toxicity

See section 7.4 *In Vivo* mouse micronucleus test

### 6.2 Repeat-Dose Toxicity

Study Type and Duration	Route of Administration	Species	GLP Compliant	Study Number
<b>Non-Pivotal Studies</b>				
2 week dose range finding	Intravenous slow bolus injection	Sprague-Dawley Rat	Yes	NC-DFC-001
Maximum tolerated dose study	Intravenous slow bolus injection	Cynomolgus Monkey	Yes	NC-DFC-002
<b>Pivotal Studies</b>				
4 week study including 9 week recovery	Intravenous slow bolus injection	Sprague-Dawley Rat	Yes	NC-DFC-004
4 week study including 13 week recovery	Intravenous slow bolus injection	Cynomolgus Monkey	Yes	NC-DFC-010
4 week study including 13 week recovery	Intravenous slow bolus injection	Cynomolgus Monkey	Yes	NC-DFC-011

## 6.2.1 Non-Pivotal Repeat-Dose Toxicity

### 6.2.1.1 Rats

Groups of 3 or 5 male and 3 or 5 female Sprague-Dawley rats received daily intravenous bolus injections of solutions of the test material (DIC075U) via the tail vein, at a constant rate of 1 ml/min, for a maximum of two weeks.

The animals treated at HD were only dosed for 4 days and then sacrificed prematurely due to poor clinical condition. The animals treated at 15 mg/kg/day (a replacement group) were only dosed for the last 8 days of the 2 week dosing period.

Species/ strain	Method of administration (vehicle/ formulation)	Duration of dosing	Doses <sup>a</sup> (mg/kg)	Gender and no. per group	NOAEL <sup>b</sup> (mg/kg)	Noteworthy findings	Study number
<b>Rat</b>	Intravenous Bolus						NC-DFC-001
Sprague- Dawley	Control (Sterile Water for Injection USP)	15	0	5 M	10		
				5 F			
	Diclofenac DF-HPβCD	15	3	5 M			
				5 F			
	(DIC075U)	15	10	5 M			
				5 F			
	8	15	3 M	<u>15 mg/kg</u> Occasional poor condition, slight decrease in body weight gain, reduction of food consumption over first few days, dark feces (no occult blood), slight increase leucocytes and platelets count in females, very mild nephropathy			
			3 F				
		4	30	5 M	<u>30 mg/kg</u> Not tolerated. Sacrifice for humane reasons after 4 administrations (poor general condition, dark feces with occult blood, signs of anemia)		
				5 F			

Toxicity at HD was swollen/firm abdomen with the production of black feces (positive fecal occult blood analysis); general signs of poor condition, body weight loss and reduced food consumption. Necropsy findings confirmed abnormalities of the gastrointestinal tract in those animals most severely affected. Additional findings considered to be related to treatment at this level included decreased red blood cells and associated parameters and increased mean cell volume, white blood cells and platelets (males only). Clinical chemistry investigations revealed decreases in plasma enzyme activity, urea, protein parameters, bilirubin and cholesterol.

At 15 mg/kg/day black feces was observed in 2 animals, (but both were negative for fecal occult blood); a slight decrease in weight gain and a reduction in food consumption was also noted over the first few days of dosing. Other treatment related findings included marginal decreases in red blood cells in females accompanied by slight increases in white blood cells and platelets in both sexes. Note that these animals were only dosed for 8 days whereas the others were dosed for 2 weeks.

There were no obvious effects of treatment revealed at either 10 mg/kg /day or 3mg/kg/day levels.

### 6.2.1.2 Monkeys

A non-pivotal, IV, maximum-tolerated dose study NC-DFC-002 was performed in male and female Cynomolgus monkeys (n = 1 per sex per group) to establish dose levels for a subsequent 4-week toxicity study. The study consisted of 2 parts, Part A and Part B. In Part A of the study, HPβCD (group 1) or DIC075U (group 2) was administered by slow (1 mL/minute) IV bolus injection at increasing dose levels for a total period of 40 to 42 days. In Part B of the study, HPβCD (group 1) or DIC075U (group 3) was given by IV bolus injection at a fixed dose level daily for 14 days.

Species/ strain	Method of administration (vehicle/ formulation)	Duration of dosing	Doses <sup>a</sup> (mg/kg)	Gender and no. per group	NOAEL <sup>b</sup> (mg/kg)	Noteworthy findings	Study number
<b>Monkey</b>	Intravenous Slow Bolus						NC-DFC-002
Cynomolgus	<u>Group 1</u>	3	28.9		na	Very mild to mild granular appearance of renal tubular cells in medullary rays	
	Escalating + Fixed <sup>c</sup> Dose Study	3	55.5	1 M			
	HPβCD (222 mg/mL Water for Injection USP)	10	222	1 F			
		7	355				
		30	533				
	<u>Group 2</u>	3	3.25		na		
	Escalating Dose Study <sup>d</sup>	3	6.25				
	Diclofenac	10	25				
	DF-HPβCD (DIC075U)	7	40	1 M		60 mg/kg: Some body weight reduction. Decreased hematocrit. Decrease in total protein, albumin, albumin/globulin ratio, aspartate aminotransferase and alanine aminotransferase	
		7	60	1 F			
		7	100				100 mg/kg/day: Hunched, subdued, unsteady on feet, impaired coordination immediately after dosing (recovery within 30 minutes). Body weight reduction. Decrease in red blood cell parameters. Decrease in clinical chemistry parameters as for the 60 mg/kg dose group

Species/ strain	Method of administration (vehicle/ formulation)	Duration of dosing	Doses <sup>a</sup> (mg/kg)	Gender and no. per group	NOAEL <sup>b</sup> (mg/kg)	Noteworthy findings	Study number
<b>Monkey</b>							NC-DFC-002
Cynomolgus	<u>Group 3</u>	14	60	1 M 1 F	na	Decreases in red blood cell parameters, increase in reticulocytes and white blood cell count. Decrease in total protein, albumin, albumin/globulin ratio, aspartate aminotransferase and alanine aminotransferase. Trace of blood pigments in the urine (male). Very mild to mild granular appearance of renal tubular cells in medullary rays	
	Fixed Dose Study						
	Diclofenac						
	DF-HPβCD (DIC075U)						

DF- HPβCD: (b) (4) Diclofenac Sodium + (b) (4) hydroxypropyl-β-cyclodextrin/mL Water for Injection USP; (b) (4) per vial; coded DIC075U

HPβCD: hydroxypropyl-β-cyclodextrin

M: male

F: female

na: not applicable due to escalating dose and fixed dose design

<sup>a</sup> Dose levels expressed as mg Diclofenac/kg/day for the DF-HPβCD-treated group (group 2) and as mg HPβCD/kg/day for the HPβCD -treated group (group 1)

<sup>b</sup> No Observed Adverse Effect Level

<sup>c</sup> For the HPβCD -treated group, the fixed dose part of the study was performed in the same animals as the escalating dose part of the study

<sup>d</sup> Histopathological examination not performed

There were no premature deaths in the study. In Part A of the study, a DIC075U dose level of 100 mg/kg/day was associated with clinical signs (unsteady on feet, impaired coordination, hunched appearance, subdued) immediately after dosing, body weight reduction, and decreases in red blood cell parameters, total protein, albumin, albumin/globulin ratio, AST, and ALT. At DIC075U 60 mg/kg/day, body weight reduction, and decreased hematocrit, albumin and albumin/globulin ratio were observed. There were no adverse treatment-related clinical signs, body weight, food consumption, hematology, clinical chemistry, urinalysis, organ weight, or gross necropsy changes following treatment with HP $\beta$ CD alone at any dose tested. At necropsy, the male that had received DIC075U had 2 depressed grey dermal foci at or near the injection site, whereas the female that received the same treatments demonstrated SC reddening at the injection site.

In Part B of the study, DIC075U at 60 mg/kg/day IV caused decreases in red blood cell parameters, an increase in reticulocytes and white blood cell count, decreases in total protein, albumin, albumin/globulin ratio and sodium, a slight increase in spleen weight, and a very mild to mild granular appearance of renal tubular cells in the medullary rays in the kidney. The latter finding was also observed in monkeys treated with HP $\beta$ CD alone, but did not appear to be associated with any functional deficits in renal function. At necropsy, the female (but not the male) demonstrated a black/red SC area around the injection site vein. This reviewer agrees with the Sponsor's statement that this finding was not considered to be related to the test article and was not evaluated histologically since it was not dose dependent and was not seen in the control groups.

There were no treatment-related alterations in organ weights or macroscopic pathology in any monkey at any phase of the study. Based on these findings, a DIC075U dose level of 60 mg/kg/day was established as the maximum tolerated dose in Cynomolgus monkeys.

## 6.2.2 Pivotal Repeat-Dose Toxicity

### 6.2.2.1 Rats

#### Study title: **DF-HP $\beta$ CD 4 Week Toxicity Study in Rats with Administration by the Intravenous Route with Recovery Period**

Study no.: NC-DFC-004 (CTD 4.2.3.2.2)

Study report location: (b) (4)

Conducting laboratory and location: (b) (4)

Date of study initiation: 10 January, 1997

GLP compliance: Yes

QA statement: Yes

Drug: DF-HP $\beta$ CD; coded **DIC075U**  
Lot # R &D 227  
% purity: 96.4%

#### Key Study Findings

A 4-week IV toxicity study including a 9-week recovery period was performed in rats testing DIC075U doses of 3, 7 and 15 mg/kg/day, with respective HP $\beta$ CD doses of 27, 62, and 133 mg/kg/day.

One HD male died on Day 6 and 1 HD female was sacrificed on Day 18. Both deaths were associated with poor general condition and peritonitis.

In HD females, slight decrease in red blood cell parameters, slight increase in white blood cell count and statistically significant increase in reticulocytes in HD rats were noted (regenerative anemia).

In only females at HD, slight decreases in total protein (12 % $\downarrow$ ) and albumin (14 % $\downarrow$ ) was observed.

In HD female increased spleen weights associated with extramedullary hematopoiesis were noted.

There was increased extramedullary hemopoiesis in spleen for all doses and increase of incidence was dose dependent. However these findings were reversible after recovery period.

There were gastro-intestinal (stomach, cecum, colon, duodenum, ileum and Jejunum) histology findings ( e.g, peritoneal inflammatory cell infiltration or peritonitis) in HD animals which severity of most of the findings was very mild to mild. However these differences were absent after recovery period.

There were very mild to mild renal tubular vacuolation LD, MD and HD. These changes did not completely disappear after recovery period; however there was evidence of reversibility since only very mild renal tubular vacuolation was observed at the end of

recovery period. This renal tubular vacuolation was an expected observation of intravenous administration of HPβCD and was not seen amongst the water treated control animals.

Consistent with higher systemic exposure to diclofenac, female rats appeared more sensitive than male rats to GI toxicity.

Due to histopathology, organ weight and hematology findings a dose level of 7 mg/kg/day was considered to be NOAEL. At 7 mg/kg/day on day 28, the exposure (AUC<sub>0-t</sub>) was 9,496 and 13,408 ng.hr/mL and C<sub>max</sub> was 49,015 and 54,643 ng/mL in males and females, respectively.

#### **METHODS**

DOSES:	DICLOFENAC: 0, 3, 7 AND 15 MG/KG HPβCD: 0, 27, 62 AND 133 MG/KG
FREQUENCY OF DOSING:	DAILY
ROUTE OF ADMINISTRATION:	INTRAVENOUS (SLOW BOLUS, 1ML/MIN)
DOSE VOLUME:	SEE BELOW THE SPONSOR'S TABLE
FORMULATION/VEHICLE:	<b>DIC075U</b> ( (b) (4) ) DICLOFENAC SODIUM + (b) (4) HPβCD) (b) (4) STERILE WATER FOR INJECTION USP
SPECIES/STRAIN:	SPRAGUE-DAWLEY RATS
NUMBER/SEX/GROUP:	MAIN GROUP: 10/SEX/GROUP RECOVERY GROUP: 5/SEX/GROUP
DURATION OF DOSING:	4 WEEKS
DURATION OF POST DOSE:	9 WEEKS
AGE:	6 WEEKS
WEIGHT:	MALES: 79-88 G FEMALES: 54-61 G
SATELLITE GROUPS:	12/SEX/TEST ARTICLE GROUP FOR TOXICOKINETICS
UNIQUE STUDY DESIGN:	NONE
DEVIATION FROM STUDY PROTOCOL:	NO DEVIATIONS IN THE STUDY PROTOCOL WERE DESCRIBED BY THE APPLICANT.

The main and recovery groups consisted of 15 male and 15 female rats which received daily intravenous bolus injections of solutions of DIC075U at a nominal concentration of 25 mg diclofenac sodium/mL via the tail vein, at a constant rate of 1 ml/min (slow bolus). All animals were monitored for mortality (twice daily), for adverse clinical signs (daily), for body weight (weekly), food and water consumption (weekly), ophthalmic changes (Week 4 treatment and recovery), hematology, clinical chemistry, urinalysis changes (Week 4 treatment and recovery), and pathologic changes at and after necropsy. All rats were given a detailed post mortem examination and major organs were weighed and preserved. Histological examination was performed on all tissues from the control and HD main study animals and on the kidney, spleen and gastro- intestinal tract from all LD and MD group main study animals and control and high group recovery study animals. Additionally kidney sections were examined from LD and MD group recovery study animals.

The TK groups consisted of 12 males and 12 females were dosed with DIC075U at levels of 3, 7 and 15 mg/kg/day. Blood samples were collected on Day 0 (first day of dosing) and Day 28 of the study. All these rats (with the exception of premature decedents) were killed without necropsy.

Below table copied from the IND submission:

**Table 8 Experimental Design of the 4-Week Intravenous Toxicity Study Including Recovery Period with DIC075U in Sprague-Dawley Rats**

Test Article	Dose (mg/kg/day)		Volume (mL/kg)	Day of Sacrifice and No. of Animals	
	diclofenac	HP $\beta$ CD		Day 28/29 <sup>c</sup>	Day 91 <sup>c</sup>
Control <sup>a</sup>	0	0	0.6	10 M + 10 F	5 M + 5 F
DIC075U <sup>b</sup>	3	26.6	0.12	10 M + 10 F	5 M + 5 F
DIC075U <sup>b</sup>	7	62.1	0.28	10 M + 10 F	5 M + 5 F
DIC075U <sup>b</sup>	15	133	0.6	10 M + 10 F	5 M + 5 F

HP $\beta$ CD: hydroxypropyl- $\beta$ -cyclodextrin; M: males; F: females.

<sup>a</sup> Control: Sterile Water for Injection USP.

<sup>b</sup> DIC075U: (b)(4)diclofenac + (b)(4)HP $\beta$ CD/mL Water for Injection USP.

<sup>c</sup> Day 28/29: end of treatment; Day 91: end of recovery period.

Source: NC-DFC-004, Section: Experimental Procedure: Treatment (CTD 4.2.3.2.2)

## Observations and Results

### Mortality:

One HD male died on Day 6 and 1 HD female was sacrificed on Day 18. Both deaths were associated with poor general condition and peritonitis (see Appendix 3, histopathologic finding in detail for both animals). Also there were two female premature deaths at HD in TK satellite groups (see below table from the NDA submission). These deaths are considered to be related to treatment. Two control males died during the bleed for laboratory investigations on Day 24.

APPENDIX 34

DF-HPBCD  
4 Week Toxicity Study in Rats with Administration  
by the Intravenous Route with Recovery Period  
Individual Clinical Observations and Necropsy Findings:  
Premature Decedents (Toxicokinetic Study)

Group/Dose Level (mg DF-HPBCD. kg <sup>-1</sup> .day <sup>-1</sup> )	Animal Number	Clinical Observations	Day(s) Recorded	Necropsy Findings
7 (15)	181	Piloerection, abdomen appears firm and irregular, hunched appearance, weight loss, subdued behaviour, whole body appears pale, dark eyes, black faeces, unscheduled kill.	8	Pancreas, spleen and small intestine adhered; abdomen contains cream gelatinous material; thickened mucosal wall of jejunum appears to cause a blockage in lumen.
	186	Black faeces Piloerection, abdomen appears firm and irregular, hunched appearance, spine prominent, subdued behaviour, whole body cold to touch, dark eyes, black faeces, rolling gait, unscheduled kill	11-15 16	Spleen pale, abdomen contains fluid, small intestine firmly adhered to mesentery tissue and surrounding organs, abdomen contains white caseous material

**Clinical Signs:**

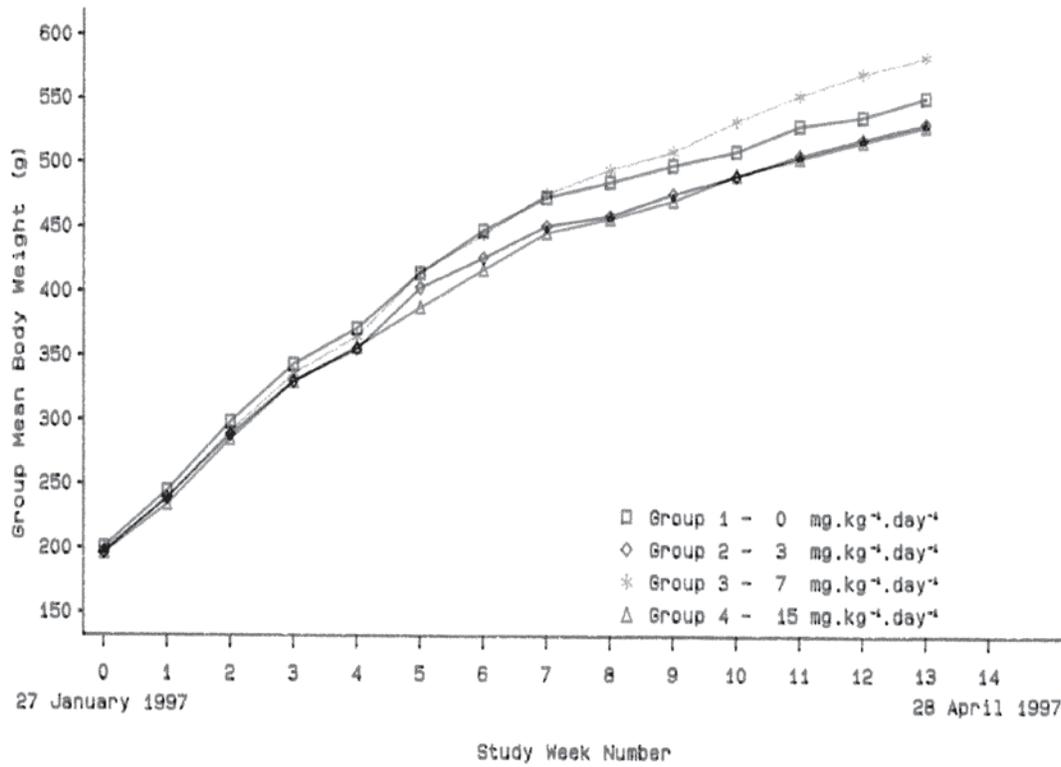
At HD, abnormal colored feces (dark, probably blood) were recorded for the majority of animals (22/28). There were no other treatment related findings in animals during the study.

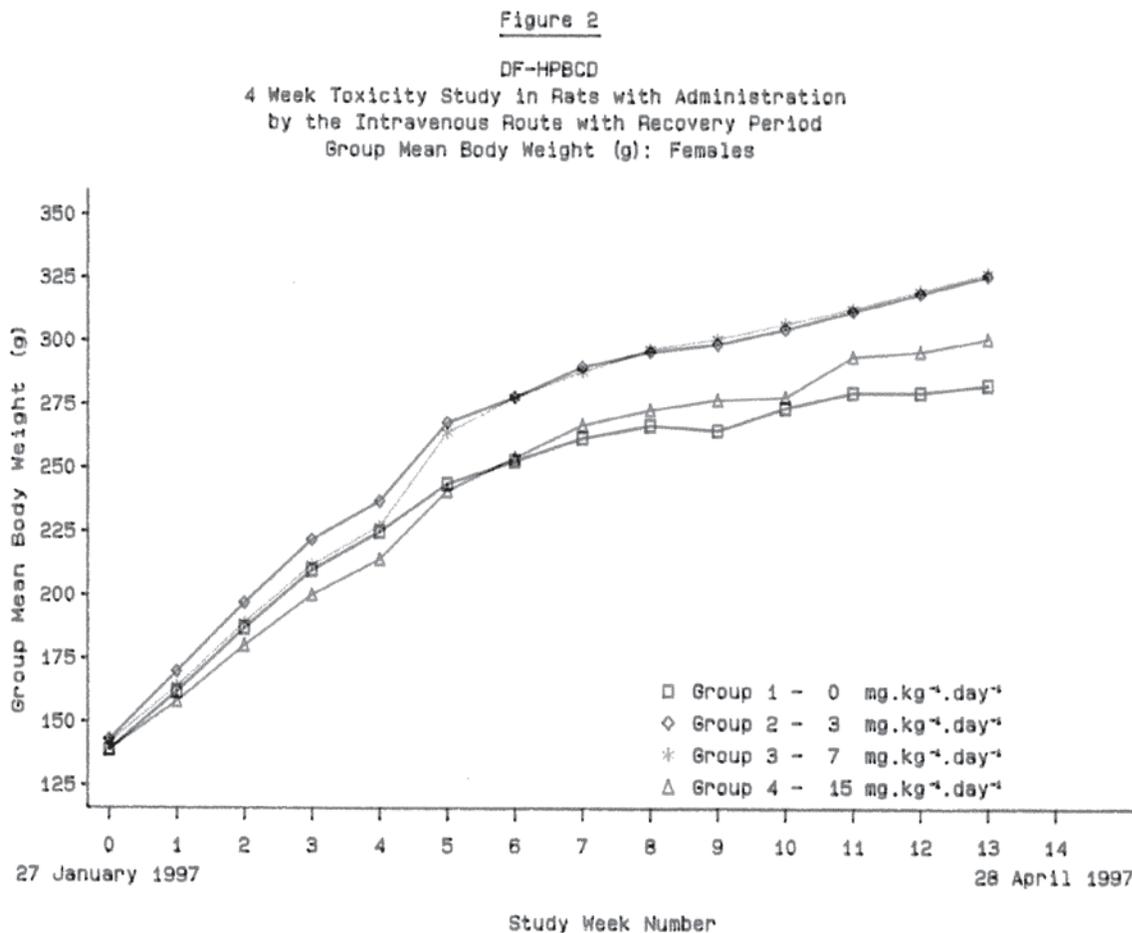
**Body Weights:**

There was a decrease (especially in females at HD group) in mean body weight gain over the first few days of dosing and thereafter, weight gain appeared to be comparable to control (see below figures).

Figure 1

DF-HPBCD  
4 Week Toxicity Study in Rats with Administration  
by the Intravenous Route with Recovery Period  
Group Mean Body Weight (g): Males





**Feed Consumption:** No treatment related findings

**Ophthalmoscopy:** No treatment related findings

**Hematology:**

In HD females, slight decrease in red blood cell parameters and slight increase in white blood cell count, primarily driven by increased neutrophils, was noted (see below table from the IND submission).

Daily dose of Diclofenac (mg/kg)	0 (SWFI Control – Group 1)		3 (Group 2)		7 (Group 3)		15 (Group 4)	
Daily dose of HPβCD (mg/kg)	0 (SWFI Control – Group 1)		26.6		62.1		133	
Daily Volume (mL/kg)	0.60		0.12		0.28		0.60	
No. of Animals – Evaluated at End of Dosing	M: 14	F: 15	M: 15	F: 15	M: 14	F: 14	M: 12	F: 14
<b>Hematology</b>								
Hemoglobin (g/dL)	14.2	13.5	14.8	14.4	14.6	14.5	14.4	13.1*
Red Blood Cells (x10 <sup>12</sup> /L)	7.2	6.8	7.6	7.3	7.5	7.4	7.3	6.6**
Mean Cell Hb Concentration (g/dL)	33.3	38.3	33.4	34.3	33.3	34.3	33.2	33.5***
White Blood Cells (x10 <sup>9</sup> /L)	15.1	12.6	15.2	12.4	15.4	11.8	15.9	19.0***
Neutrophils (x10 <sup>9</sup> /L)	3.2	2.6	3.2	2.3	3.5	2.3	3.7	6.3***
Monocytes (x10 <sup>9</sup> /L)	0.35	0.29	0.40	0.29	0.40	0.26	0.37	0.58**
Eosinophils (x10 <sup>9</sup> /L)	0.17	0.16	0.16	0.16	0.21	0.16	0.19	0.41***
Basophils (x10 <sup>9</sup> /L)	0.04	0.03	0.04	0.03	0.04	0.03	0.04	0.05*

While there was an increase in reticulocytes (40 %↑) in HD female in the main study there was a decrease in reticulocytes (35 %↓) in HD recovery female group when compared with female controls which suggests this effect is reversible.

**Clinical Chemistry:**

In HD females, slight decreases in total protein (12 %↓) and albumin (14 %↓) were noted. Very slight increased creatinine (7 %↑) in HD males was observed. There were no other treatment related findings in clinical chemistry parameters at any of the dose levels administered. There were no differences between HD and controls at recovery.

**Urinalysis:** no treatment-related findings

**Gross Pathology:** At HD, after 4 weeks of treatment, the findings of note were reddening of the jejunum in one male animal, enlarged spleen in one female and abdomen containing fluid in the same female in the main study as an unscheduled kill due to poor condition. There were no other treatment related necropsy findings after 4 weeks of dosing and, after the recovery period.

**Organ Weights:**

Selected organ weight findings of DIC075U in 28-day repeat dose toxicity in rats: (reviewer's table).

TARGET TISSUE (WEIGHT)	DOSE MG/KG/DAY							
	0		3		7		15	
	M	F	M	F	M	F	M	F
<b>LIVER</b>								
ABSOLUTE (G)	16.4	9.31	14.6 11%↓	9.32	15.4 6%↓	9.04	14.1 14%↓	9.2 9
RELATIVE TO BW	0.049	0.041	0.042 14%↓	0.039	0.042 14%↓	0.041	0.039 20%↓	0.0 44
<b>SPLEEN</b>								
ABSOLUTE (G)	0.84	0.62	0.78	0.64	0.82	0.6	0.85	0.8 2 32 %↑

RELATIVE TO BW	0.002 2	0.0027	0.0022	0.0027	0.0022	0.0027	0.0023	0.0 039 44 %↑
<b>ADRENAL</b>								
ABSOLUTE (G)	0.058 6	0.0658	0.0487 17%↓	0.0651	0.0568	0.0625	0.0529	0.0 563 15 %↓
RELATIVE TO BW	0.00026	0.00029	0.00014 12%↓	0.00028	0.00015	0.00028	0.00015	0.00 027

TARGET TISSUE (WEIGHT)	RECOVERY DOSE (MG/KG/DAY)			
	0		15	
	M	F	M	F
<b>LIVER</b>				
ABSOLUTE (G)	22.7	9.81	21.06 8%↓	10.82 10%↑
RELATIVE TO BW	0.041	0.035	0.042	0.036
<b>SPLEEN</b>				
ABSOLUTE (G)	0.98	0.59	0.98	0.63
RELATIVE TO BW	0.0018	0.0021	0.0019	0.0021
<b>ADRENAL</b>				
ABSOLUTE (G)	0.064	0.075	0.064	0.0695
RELATIVE TO BW	0.00027	0.00027	0.00023 15%↓	0.00023 15%↓

In HD female increased spleen weights associated with extramedullary hematopoiesis were noted. Mean absolute liver weights and relative to body weight were lower in HD male rats compared to the controls. However, these changes seem less affected in recovery groups.

**Histopathology:**

**Adequate Battery:**

Tissues from the gastro-intestinal tract, kidneys, liver and urinary bladder were processed and examined histological from the control and high dose animals in the main study as high priority. Subsequently all other specified tissues from the control and high dose animals were examined. The data obtained from all tissues in control and

high dose animals were reviewed and the decision was taken to examine kidney, spleen and the gastro-intestinal tract in the low and intermediate main study animals and subsequently in the control and high recovery study animals. Finally, kidneys were examined in the low and intermediate recovery study animals.

**Peer Review:**

In addition to the routine internal peer review, a second set of kidney sections were examined [REDACTED] (b) (4)

**Histological Findings**

Table below summarizes the microscopic findings of DIC075U in this 28-day repeat dose toxicity in rats.

*Note: The protocol stated that the first 10 animals of each group would be for main study animals. However, following the premature death of several animals, the animals were redistributed into main and recovery study animals.*

TARGET TISSUE	SEVERITY	DOSE MG/KG/DAY								RECOVERY DOSE (MG/KG/DAY)			
		0		3		7		15		0		15	
		M	F	M	F	M	F	M	F	M	F	M	F
<b>ABDOMEN</b> PERITONITIS	VERY MILD	-	-	-	-	-	-	1/1	1/1	-	-	-	-
<b>STOMACH</b> PERITONEAL INFLAMMATORY CELL INFILTRATION	VERY MILD	0/11	0/10	0/10	0/10	0/10	0/10	0/11	2/10	0/4	0/5	0/4	0/4
HYPERKERATOSIS	VERY MILD	0/11	0/10	0/10	0/10	0/10	0/10	0/11	1/10				
AUTOLYSED		0/11	0/10	0/10	0/10	0/10	0/10	1/11	0/10				
<b>RECTUM</b> PERITONEAL INFLAMMATORY CELL INFILTRATION	VERY MILD	0/11	0/10	0/10	0/10	0/10	0/10	0/11	1/10	0/4	0/5	0/4	0/4
<b>CECUM</b> PERITONEAL INFLAMMATORY CELL INFILTRATION	VERY MILD	0/11	0/10	0/10	0/10	0/10	0/10	0/10	2/11	0/4	0/5	0/4	0/4
INFLAMMATOION IN LAMINA PROPRIA	VERY MILD	0/11	0/10	0/10	0/10	0/10	0/10	0/11	1/11				
AUTOLYSED		0/11	0/10	0/10	0/10	0/10	0/10	1/11	0/11				
<b>COLON</b> PERITONEAL INFLAMMATORY CELL INFILTRATION	VERY MILD	0/11	0/10	0/10	0/10	0/10	0/10	0/11	1/11	0/4	0/5	0/4	0/4
AUTOLYSED		0/11	0/10	0/10	0/10	0/10	0/10	1/11	0/11				
<b>DUODENUM</b> PERITONEAL INFLAMMATORY CELL INFILTRATION	VERY MILD	0/11	0/10	0/10	0/10	0/10	0/10	0/11	1/11	0/4	0/5	0/4	0/4
	MODERATE	0/11	0/10	0/10	0/10	0/10	0/10	1/11	0/11				
AUTOLYSED		0/11	0/10	0/10	0/10	0/10	0/10	1/11	0/11				
<b>ILEUM</b> PERITONEAL INFLAMMATORY CELL INFILTRATION	VERY MILD	0/11	0/10	0/10	0/10	0/10	0/10	0/11	3/11	0/4	0/5	0/4	0/4
	MILD	0/11	0/10	0/10	0/10	0/10	0/10	0/11	1/11				
	MODERATE	0/11	0/10	0/10	0/10	0/10	0/10	1/11	1/11				
INFLAMMATION IN LAMINA PROPRIA	VERY MILD	0/11	0/10	0/10	0/10	0/10	0/10	0/11	1/11				
INFLAMMATION IN MUSCLE LAYER	VERY MILD	0/11	0/10	0/10	0/10	0/10	0/10	0/11	1/11				
AUTOLYSED	VERY MILD	0/11	0/10	0/10	0/10	0/10	0/10	1/11	0/11				
<b>JEJUNUM</b> PERITONEAL INFLAMMATORY CELL	VERY MILD	0/11	0/10	0/10	0/10	0/10	0/10	0/11	5/11	0/4	0/5	0/4	0/4

INFILTRATION	MODERATE	0/11	0/10	0/10	0/10	0/10	0/10	0/10	1/11	0/11				
AUTOLYSED		0/11	0/10	0/10	0/10	0/10	0/10	0/10	1/11	0/11				
KIDNEY TUBULAR VACUOLATION	VERY MILD	0/11	0/10	4/10	6/10	3/10	6/10	2/11	1/11	1/4	1/5	4/4	3/4	
	MILD	0/11	0/10	0/10	0/10	2/10	1/10	2/11	8/11					
BASOPHILIC TUBULES	VERY MILD	6/11	6/10	5/10	2/10	6/10	3/10	3/11	6/11	3/4	2/5	3/4	2/4	
	MILD	0/11	1/10	0/10	0/10	0/10	0/10	0/11	1/11					
NEPHROPATHY	VERY MILD	1/11	3/10	0/10	1/10	2/10	0/10	3/11	2/11	2/4	1/5	1/4	0/4	
	MILD	0/11	0/10	0/10	0/10	0/10	0/10	0/11	1/11					
MEDULLARY MINERAL DEPOSIT	VERY MILD & MILD	0/10	10/10	0/10	10/10	0/10	7/10	1/11	10/10	0/4	5/5	0/4	3/4	
CAPSULAR ADHESION	VERY MILD	0/11	0/10	0/10	0/10	0/10	0/10	0/11	1/11	0/4	0/5	0/4	0/4	
UROTHELIAL HYPERPLASIA	VERY MILD	0/11	0/10	1/10	0/10	0/10	0/10	1/11	1/11	0/4	0/5	0/4	0/4	
UROTHELIAL PAPILLARY EDEMA	VERY MILD	0/11	0/10	0/10	0/10	0/10	0/10	0/11	1/11	0/4	0/5	0/4	0/4	
BILATERAL PAPILLARY EDEMA	VERY MILD	0/11	0/10	0/10	0/10	0/10	0/10	0/11	1/11	0/4	0/5	0/4	0/4	
PELVIC DILATATION	MODERATE	0/11	0/10	0/10	0/10	0/10	0/10	1/11	0/11	0/4	0/5	0/4	0/4	
FOCAL TUBULAR DILATION	VERY MILD	0/11	0/10	0/10	0/10	0/10	0/10	0/11	1/11	0/4	0/5	0/4	0/4	
AUTOLYSED		0/11	0/10	0/10	0/10	0/10	0/10	1/11	0/11	0/4	0/5	0/4	0/4	
SPLEEN INCREASED EXTRAMEDULLAR Y HEMOPOIESIS	VERY MILD	0/11	0/10	0/10	1/10	0/9	0/10	0/11	1/11	0/4	0/5	0/4	0/4	
	MILD	1/11	3/10	1/10	3/10	1/9	5/10	0/11	3/11					
	MODERATE	1/11	1/10	2/10	1/10	1/9	2/10	1/11	5/11					
PANCREAS PERITONEAL INFLAMMATORY CELL INFILTRATION	VERY MILD	0/11	0/10	-	-	-	-	0/11	3/11	-	-	-	-	
	MILD	0/11	0/10	-	-	-	-	0/11	1/11	-	-	-	-	
LIVER FOCAL INFLAMMATORY CELL INFILTRATION	VERY MILD	9/11	7/10	-	-	-	-	5/11	11/11	-	-	-	-	
LUNG ALVEOLAR FOAMY CELL PROLIFERATION	VERY MILD	0/11	0/10	-	-	-	-	0/11	2/11	-	-	-	-	
GRANULOMA	VERY MILD	0/11	3/10	-	-	-	-	2/11	3/11	-	-	-	-	
	MODERATE	0/11	0/10	-	-	-	-	0/11	1/11	-	-	-	-	
TESTIS FOCAL TUBULAR ATROPHY	VERY MILD	1/11	-	-	-	-	-	1/11		-	-	-	-	
	MILD	0/11	-	-	-	-	-	2/11		-	-	-	-	
ATOLYSED		0/11	-	-	-	-	-	1/11		-	-	-	-	
(-) NOT EXAMINED														

There was gastro-intestinal (stomach, cecum, colon, duodenum, ileum and jejunum) toxicity in HD animals which was considered to be consistent with the administration of a non-steroidal anti-inflammatory such as diclofenac sodium. There were no similar findings at the lower dose levels and the findings are considered to be reversible within a 9 week recovery period.

In addition, very mild to mild renal tubular vacuolation was observed at 3, 7 and 15 mg/kg/day of DF-HPβCD. There was a greater incidence of the mild changes with increasing dosage and also among the females. These changes did not completely disappear after recovery period in HD animals; however there was evidence of reversibility since only very mild renal tubular vacuolation was observed at the end of recovery period.

This renal tubular vacuolation was an expected observation of intravenous administration of HPβCD (as described with unsubstituted β-cyclodextrin by Frank et al, 1976) and was not seen amongst the water treated control animals. It was confirmed as

an expected finding by an external pathologist (b) (4) experienced in the evaluation of tissues from HPβCD treated animals.

Note: also in repeat IV dose toxicity in monkey very mild to mild granular appearance of the renal tubular cells in the medullary rays was observed at all dose levels of DIC075U and in animals given HPβCD only in study NC-DFC-011. As noted in the literature (Gould and Scott, 2005; Coussement, W. et al, 1990), these findings are considered an expected consequence of IV administration of HPβCD.

There was increased extramedullary hemopoiesis (which may link to decreased in RBC, increased reticulocytes and increased spleen weight) for all doses and increase in incidence was dose-dependent. However these findings were reversible after recovery period.

Also there were findings in liver, lung, pancreas and testis (see above table). However these organs in the main study (LD and MD groups) and recovery groups were not evaluated and it is not clear weather these findings were treatment related.

**Special Evaluation:** None

### Toxicokinetics:

Intravenous diclofenac sodium has a large volume of distribution, a rapid clearance and a relatively short half-life (0.418 to 2.55 hours). The rate of clearance was higher in males but decreased with increasing dosage and the volume of distribution decreased with exposure between Day 0 (first day of dosing) and Day 28.  $C_{max}$  and AUC values were dose dependent. AUC values were consistently higher (mild) in females compared to males. Some evidence of accumulation was noted in the low dose group. Below table copied from NDA submission.

**Table 9 Mean Toxicokinetic Data of Diclofenac from Rats Dosed 4 Weeks with DIC075U**

Treatment	$T_{max}$ (h)	$C_{max}$ (ng/mL)	$T_{1/2}$ (h)	Cl (ml/h.kg)	Vd (mL/kg)	AUC <sub>0-t</sub> (ng.h/mL)
<b>3 mg/kg/day</b>						
Males-Day 1	0.00	5294	0.742	1479.9	1584.3	1965
Females-Day 1	0.00	13462	1.120	742.6	1200.2	3607
Males-Day 28	0.00	17920	0.418	950.4	573.0	3144
Females-Day 28	0.00	13866	0.689	620.7	616.8	4736
<b>7 mg/kg/day</b>						
Males-Day 1	0.00	55010	1.913	733.9	2025.1	8510
Females-Day 1	0.00	46877	0.822	463.2	549.2	14340
Males-Day 28	0.00	49015	1.605	694.6	1608.0	9496
Females-Day 28	0.00	54643	1.251	481.0	868.1	13408
<b>15 mg/kg/day</b>						
Males-Day 1	0.00	100389	1.755	545.2	1380.0	23932
Females-Day 1	0.00	94282	2.553	405.8	1494.7	28255
Males-Day 28	0.00	108172	0.826	508.2	605.3	28872
Females-Day 28	0.00	127082	1.287	432.9	803.2	32757

Source: NC-DFC-004, Section: Appendix 35, Toxicokinetic Studies (CTD 4.2.3.2.2)

**Stability and Homogeneity:**

Routine analysis of samples of the dosing solutions during the course of the study indicated satisfactory stability of the vials of test material.

The analyzed concentration of the dosing solution was within  $\pm 6\%$  of the nominal at each analysis occasion.

DF-HP $\beta$ CD  
4 Week Toxicity Study in Rats with Administration  
by the Intravenous Route with Recovery Period  
Analysis of Dosing Solution (Batch R&D 227)

Date of Analysis (Week Beginning)	Nominal Concentration of Sodium Diclofenac (mg.ml <sup>-1</sup> )	Calculated Concentration of Sodium Diclofenac (mg.ml <sup>-1</sup> )	$\frac{[\text{Calculated}]}{[\text{Nominal}]} \times 100$ (%)
25 November 1996	24.1	25.50	105.8
10 February 1997	24.1	23.84	98.9
28 April 1997	24.1	24.44	101.4

Dosing on this study was conducted between 27 January 1997 and 24 February 1997

**6.2.2.2.1 Monkey**

**Study title: DF-HP $\beta$ CD and HP $\beta$ CD alone, 4 Week Intravenous Toxicity Study in Cynomolgus Monkeys with a 3 Month Recovery Period**

Study no.: NC-DFC-010 (CTD 4.2.3.3.1.1)

Study report location: (b) (4)

Conducting laboratory and location: (b) (4)

Date of study initiation: February 6, 1997

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: DF-HP $\beta$ CD; coded DIC075U

Lot # R &D 227, % purity: 96.4%

HP $\beta$ CD; Lot # R & D225

**Key Study Findings**

A 4-week IV toxicity study including a 13-week recovery period was performed in monkeys testing DIC075U doses of 3, 15 and 60 mg/kg/day, with respective HP $\beta$ CD doses of 27, 133, and 533 mg/kg/day. There were two control groups including water and HP $\beta$ CD (533 mg/kg.day) control in this study.

- HD animals died or were sacrificed moribund prior to scheduled termination. Resolution of the toxicology findings could not be assessed in the male and female at HD dose (both main and recovery groups), due to the need for premature sacrifice.
  - At HD, two monkeys were sacrificed at the end of week 1 and two additional monkeys were sacrificed at the end of week 2 due to poor clinical condition and tail lesions. The remaining animals were sacrificed during Week 3 of the study.
  - Most animal at HD produced dark feces and soft/liquid feces in the first week of dosing and a number of these also had red mucoidal material (assumed to be blood) in the feces. Some of the animals in this group appeared hunched, subdued and lethargic.
- Dark feces and soft/liquid feces at MD groups were observed in the first week of dosing but not in LD or control groups.
- The majority of clinical signs were skin lesions, primarily of the tail but also on the limbs and thorax. Tail skin lesions were observed in a number of animals from all groups. The number and severity of these lesions increased as the study progressed and notably in MD animals.
- Animals at HD showed body weight loss (~ 6%) and reduced food consumption.
- HD animals showed reduced levels of hemoglobin, red blood cells and hematocrit and elevated reticulocytes, white blood cell and platelet counts. At MD, there were slightly lower levels of hemoglobin and red blood cells and higher levels of reticulocytes and platelets in both sexes in week 4 compared with the control values.
- HD animals showed reduced levels of albumin, the albumin-globulin ratio and alkaline phosphatase. Albumin levels and albumin-globulin ratios were reduced at week 4 in both sexes dosed at MD compared with control.
- Thymus weights were lower in animals of all groups (only males) including those receiving HPβCD alone (vehicle control) in both sexes. However after 13 weeks recovery, thymus weights were higher in vehicle control group compared to water control groups. Spleen weights were higher in all groups compared to vehicle control. After 13 weeks recovery spleen weights were higher in vehicle control groups (only males) compared to water controls.
- In one **HD** female severe gastrointestinal lesions, including multifocal mucosal necrosis and submucosal edema was seen. Also pyloric mononuclear-cell infiltrate in stomach was seen in one HD male. Other histopathological findings in this group included thymus atrophy, polymorphonuclear leukocyte infiltration, and ulceration of tail skin lesions, and a very mild to mild granular appearance of the renal tubular cells in the medullary rays.

Histopathological findings in **MD** groups included mild inflammatory cell infiltration in the colon in two animals, moderate thymus atrophy in 1 male, polymorphonuclear leukocyte infiltration and ulceration of tail skin, and a very mild to mild granular appearance of the renal tubular cells in the medullary rays.

In **LD** groups the only finding was a very mild to mild granular appearance of the renal tubular cells in the medullary rays.

In the vehicle control (HP $\beta$ CD), histopathology of the kidneys identified a very mild to mild granular appearance of the tubular epithelium, which was reversible after 3 months of drug discontinuation. These findings were considered an expected consequence of IV administration of HP $\beta$ CD.

- Premature sacrifice of HD groups prior to study conclusion and absence of recovery animals for MD and LD groups based on study design does not allow a conclusion as to the reversibility of observed toxicity.
- Due to microscopic findings, organ weight and hematology findings a dose level of 3 mg/kg/day for diclofenac is considered to be the NOAEL. At 3 mg/kg/day, the exposure (AUC<sub>0-t</sub>) on day 28 was 6054 and 6493 ng•hr/mL and C<sub>max</sub> was 32,500 and 33,151 ng/mL in males and females, respectively.
- A dose level of 533 m/kg for HP $\beta$ CD is considered to be the NOAEL, since the mild changes in kidney were reversible after the recovery period.

## METHODS

Doses:	Diclofenac: 0, 3, 15 and 60 mg/kg HP $\beta$ CD: 0, 27, 133 and 533 mg/kg
Frequency of dosing:	Daily
Route of administration:	Intravenous (Slow bolus, 1ml/min)
Dose volume:	See below the Sponsor's table
Formulation/Vehicle:	( (b) (4) Diclofenac Sodium + (b) (4) HP $\beta$ CD) (b) (4) Water for Injection USP
Species/Strain:	Cynomolgus monkeys/Macaca fascicularis
Number/Sex/Group:	Main group: 3/Sex/Group Recovery group: 2/sex/group (see below table)
Age:	14-19 months
Weight:	1.7-2.1 kg
Satellite groups:	N/A
Unique study design:	N/A
Deviation from study protocol:	No deviations in the study protocol were described by the applicant.

DIC075U and HP $\beta$ CD were administered by slow bolus injection (1 mL/min) via the saphenous or brachial vein to male and female cynomolgus monkeys according to the experimental design (see below the Sponsor's table)

**Table 11 Experimental Design of the 4-Week Intravenous Toxicity Study including Recovery Period with DIC075U and HPβCD in Cynomolgus Monkeys**

Test Article	Dose (mg/kg/day)		Volume (mL/kg)	Day of Sacrifice and No. of Animals	
	diclofenac	HPβCD		Day 28/29 <sup>c</sup>	Day 119 <sup>c</sup>
Control <sup>a</sup>	0	0	2.4	3 M + 3 F	2 M + 2 F
HPβCD <sup>b</sup>	0	533	2.4	3 M + 3 F	2 M + 2 F
DIC075U <sup>b</sup>	3	26.6	0.12	3 M + 3 F	-
DIC075U <sup>b</sup>	15	133	0.6	3 M + 3 F	-
DIC075U <sup>b</sup>	60	533	2.4	3 M + 3 F <sup>d</sup>	2 M + 2 F <sup>d</sup>

M: males; F: females.

<sup>a</sup> Control: Sterile Water for Injection USP.

<sup>b</sup> HPβCD: (b) (4) HPβCD /mL Water for Injection USP; DIC075U: (b) (4) diclofenac + (b) (4) HPβCD)/mL Water for Injection USP.

<sup>c</sup> Day 28/29: end of treatment; Day 119: end of recovery period.

<sup>d</sup> All animals given DIC075U at the dose level of 60 mg diclofenac per kg were sacrificed prematurely.

Source: NC=DFC-010, Section: Experimental Procedure: Treatment (CTD 4.2.3.2.4)

Monkeys were evaluated twice daily for survival and moribundity and at multiple times each treatment day for signs of toxicity. Body weights were recorded twice weekly, commencing at least 2-weeks prior to Day 1 and continuing until prior to necropsy. Food consumption was estimated and recorded daily, commencing 2 weeks prior to dosing, and reported on a weekly basis. Ophthalmoscopic examinations were conducted prior to dosing and near the end of dosing, with recovery monkeys being examined again during Weeks 5, 9, and 13 during the recovery period. Electrocardiograms were examined from all monkeys, once pretrial and during Weeks 1 and 4 during treatment (approximately 3 hours post-dose) and Weeks 5, 9, and 13 during recovery. Post-fasting laboratory specimens were obtained once pre-trial and on Weeks 4, 5, 9, and 13 for hematology, clinical chemistries, and urinalyses. Special blood samples were collected from the highest dose group during week 2. Blood sampling to define the toxicokinetics of DIC075U were collected from all monkeys on Days 1 and 28 of treatment. All monkeys were weighed and underwent a comprehensive necropsy examination at the end of the treatment or recovery periods. Femoral bone marrow smears were collected and preserved but were not evaluated, owing to the absence of hematologic toxicity, as per protocol. Macroscopic tissue changes were recorded, select organs were weighed, and a comprehensive list of tissues was preserved, processed, and evaluated by light microscopy for histopathologic changes from all monkeys.

## Observations and Results

### Mortality:

At 60 mg/kg/day (HD) group, dosing was discontinued at the end of week 1 in females, following the premature sacrifice on Day 6 of 1 female (no. 40) due to poor general condition and the observation of severe clinical signs in several other monkeys in this group. Dosing in males was discontinued on Day 8. Two additional males (no. 19 & 18) were sacrificed in Week 2 and the remaining animals were sacrificed during Week 3 of the study.

*Note: At HD more severe clinical finding were elicited in the first week of dosing than had been anticipated from preliminary work (Inveresk Project No. 564147).*

**Clinical Signs:**

Most animal in the HD group produced dark feces and soft/liquid feces in the first week of dosing and a number of these also had red mucoidal material (assumed to be blood) in the feces. Some of the animals in this group appeared hunched, subdued and lethargic. Dark feces and soft/liquid feces at MD groups were observed in the first week of dosing.

The majority of clinical signs recorded throughout the study were skin lesions, primarily of the tail but also on the limbs and thorax. Tail skin lesions were observed in a number of animals from all groups. However the Incidence and the severity of such lesions were greater at HD and MD compared to controls. Some lesions were seen pre-trial and some animals even had tail lesions before delivery, but they were mild by the start of dosing. The number and severity of these lesions increased as the study progressed and notably in MD animals (high dose animals were sacrificed early in the study). There was no exacerbation of skin lesions at the low dose level or in the group receiving HP $\beta$ CD alone. However, tail skin lesions were observed in HP $\beta$ CD alone recovery groups (see appendix 4) which shows some effects on tail skin.

*Note: A combination of the observed tail lesions and the clinical condition of the animals treated at HD led to further animals being sacrificed prematurely. This group was considered no longer to be viable.*

## From Toxicology Tabulated Summary:

Daily dose of Diclofenac (mg/kg)	0 (SWFI Control – Group 1)	0 (HPβCD Control – Group 2)	3 (Group 3)	15 (Group 4)	60 (Group 5)
Daily dose of HPβCD (mg/kg)	0 (SWFI Control – Group 1)	533	26.6	133	533

No. of Animals – Evaluated during Dosing Period	M: 5	F: 5	M: 5	F: 5	M: 3	F: 3	M: 3	F: 3	M: 5 <sup>b</sup>	F: 5 <sup>b</sup>
No. Died or Sacrificed Moribund	0	0	0	0	0	0	0	0	5	5
Clinical observations <sup>c</sup>										
Dark feces (no fecal occult blood)	0	0	0	0	0	0	15.5	2.4	8.0	10.3
Soft/Liquid feces	0	0	0	0	0	0	7.1	0	14.7	7.4
Red mucoid material in the feces	0	0	0	0	0	0	0	1.2	13.3	8.8
Hunched	0	0	0	0	0	0	0	0	4.0	1.5

Eroded areas on tail	Small	28.6	33.6	20.7	32.1	15.5	16.7	17.9	19.0	4.0	0
	Large	5.0	0	5.7	11.4	7.1	0	14.3	44.0	5.3	13.2
Subdued		0	0	0	0	0	0	0	0	12.0	5.9
Pustules, raised nodules, swellings and /or ulcerated areas on tail and/or limbs		3.6	3.6	2.1	4.8	4.8	3.6	17.9	14.3	9.3	14.7

<sup>b</sup> prematurely sacrificed in weeks 1-3

<sup>c</sup> data are given as incidence (%) of the maximum number of observations (No. of observation days \* No. of animals alive)

**Note:**

Maximum incidence of observation was 5 or 3 (animals) X 28 (days). However for HD male and female the number of observations was 75 and 68 respectively since they were sacrificed in week 1-3.

**Body Weights:**

Body weight was reduced (~ 6%) in animals dosed at HD compared with the vehicle controls but was unaffected by treatment with either HPβCD or DIC075U at LD or MD.

**Feed Consumption:** was reduced in animals dosed at HD compared with the vehicle controls but not in other groups.

**Ophthalmoscopy:** No treatment related findings

**ECG:** No treatment related findings

**Hematology:**

Group mean values for main study are presented in below table:

DAILY DOSE	0 MG/KG (WATER CONTROL)		0 MG/KG (HPβCD CONTROL)		3 MG/KG		15 MG/KG		60 MG/KG*	
	M	F	M	F	M	F	M	F	M	F
HEMOGLOBIN (G/DL)	12.4	12.4	13.2	12.7	12.6	13.0	11.8	12.1	9.7	10.8
RBC (x10 <sup>12</sup> /L)	6.09	6.07	6.50	6.45	6.48	6.51	5.82	6.09	5.10	5.65
HEMATOCRIT (L/L)	0.407	0.414	0.430	0.425	0.419	0.444	0.390	0.417	0.349	0.370
NEUT (x10 <sup>9</sup> /L)	6.33	4.05	5.17	6.88	3.87	4.92	6.65	5.91	17.33	13.41
WBC (x10 <sup>9</sup> /L)	12.53	9.23	11.03	11.62	10.97	10.93	12.00	11.22	23.79	19.74
RETICULOCYTES (%RBC)	0.9	1.0	0.6	0.7	1.1	1.0	1.7	1.5	3.9	2.1
PLATELETS (x10 <sup>9</sup> /L)	327	366	419	400	371	350	517	526	663	530

\* DATA PRESENTED OBTAINED DURING WEEK 2 (PREMATURELY SACRIFICED)

HD animals showed reduced levels of hemoglobin, red blood cells and hematocrit and elevated reticulocytes, white blood cell and platelet counts.

*Note: These RBC parameter findings are not unexpected of parenteral administration of a non-steroidal anti-inflammatory drug and are considered to be related to the observed gastro-intestinal bleeding. The observed elevated white blood cell counts at HD may have been related to the tail lesions.*

At MD, there were slightly lower levels of hemoglobin and red blood cells and higher levels of reticulocytes and platelets in both sexes in week 4 compared with the control values. The differences from the water control were significant for reticulocytes and platelet levels but the differences from HPβCD controls were significant for hemoglobin, red blood cells and hematocrit in males.

Group mean values for week 13 recovery (note this only compares vehicle to water):

DAILY DOSE	0 MG/KG (WATER CONTROL)		0 MG/KG (HPβCD CONTROL)	
	M	F	M	F
HEMOGLOBIN (G/DL)	12.7	12.8	13.6	13.7
RBC (x10 <sup>12</sup> /L)	6.5	6.18	6.97	6.64
HEMATOCRIT (L/L)	0.426	0.414	0.432	0.455
NEUT (x10 <sup>9</sup> /L)	0.27	0.81	0.46	0.71
WBC (x10 <sup>9</sup> /L)	9.08	11.34	9.57	11.67
RETICULOCYTES (%RBC)	0.7	1.0	0.8	0.8
PLATELETS (x10 <sup>9</sup> /L)	324	359	290	339

**Clinical Chemistry:**

Group mean values are presented in below table:

DAILY DOSE	0 MG/KG (WATER CONTROL)		0 MG/KG (HP $\beta$ CD CONTROL)		3 MG/KG		15 MG/KG		60 MG/KG*	
	M	F	M	F	M	F	M	F	M	F
ALBUMIN (G/L)	49	50	50	50	47	52	41	42	33	35
ALBUMIN-GLOBULIN RATIO	1.4	1.5	1.4	1.4	1.3	1.4	1.0	1.1	0.8	0.8
ALKALINE PHOSPHATASE (IU/L)	2114	1773	1645	1463	1104	1642	1002	1371	1080	1022
SODIUM (MMOL/L)	151	150	150	151	151	153	151	157	152	156
POTASSIUM (MMOL/L)	3.7	3.9	3.8	4.0	4.1	3.6	4.3	4.6	4.5	5.3
PHOSPHATE (MMOL/L)	2.08	1.89	2.01	2.00	2.22	1.70	2.14	2.30	1.55	1.52
CREATININE( $\mu$ MOL/L)	65	64	68	63	69	71	69	70	61	62
TOTAL BILIRUBIN ( $\mu$ MOL/L)	3.4	3.0	3.1	2.6	2.5	2.3	2.5	1.5	1.9	2.1

\* DATA PRESENTED OBTAINED DURING WEEK 2 (PREMATURELY SACRIFICED)

Compared to control, animals treated for one week at HD, showed reduced levels of albumin, the albumin-globulin ratio and alkaline phosphatase. These are considered to be effects of treatment. Creatinine, organic phosphate and total bilirubin levels were also lower.

Albumin levels and albumin-globulin ratios were reduced at week 4 in both sexes dosed at MD compared with vehicle control.

Significant findings at week 4 compared with the water control included lower alkaline phosphatase (AP) activity for males at LD and males and females at MD, higher potassium levels for males at LD and males and females at MD, and higher levels of sodium and inorganic phosphate in females at MD.

**Urinalysis and fecal occult blood:** No treatment related findings

**Gross Pathology:** One HD female dosed for one week had red mucosa of the stomach and the entire length of the small intestines was red with watery contents. One HD male, one MD male and two males treated with HP $\beta$ CD alone (at the end of recovery) showed reddened gastric mucosa. Three HD animals had spongy lungs which were not seen in control groups. One MD animal had a small thymus. Tail lesions were observed in a number of animals from all groups, including the controls and the number and severity of these lesions increased as the study progressed in MD and HD animals.

**Organ Weights:**

Selected organ weight findings of DIC075U in 28-day repeat dose toxicity in monkeys: (reviewer's table, group mean values).

TARGET TISSUE (WEIGHT)	DOSE (MG/KG/DAY)									
	0 (WATER CONTROL)		0** (HPβCD CONTROL)		3		15		60 *	
	M	F	M	F	M	F	M	F	M	F
<b>KIDNEYS</b>										
ABSOLUTE (G)	11.39	10.93	12.7	12.42	13.54	11.81	11.89	12.35	11.15	11.36
RELATIVE TO BW	5.7	5.5	6.0	6.5	6.1	5.9	5.9	6.2	5.6	6.3
			11%↑	14%↑						
			5%↑	18%↑						
<b>SPLEEN</b>										
ABSOLUTE (G)	4.99	5.65	5.77	3.23	6.15	4.37	6.36	5.30	6.56	4.92
RELATIVE TO BW	2.5	2.7	2.7	1.7	2.8	2.18	3.18	2.6	3.3	2.7
			16%↑	43%↓			27%↑	6%↓	31%↑	13%↓
				37%↓			27%↑	10%↓	32%↑	0%
<b>THYMUS GLAND</b>										
ABSOLUTE (G)	3.28	2.58	2.50	1.68	1.93	2.06	1.25	2.23	1.06	1.02
RELATIVE TO BW	1.6	1.2	1.2	0.88	0.88	1.03	0.62	1.1	0.53	0.56
			23%↓	35%↓	41%↓		62%↓		68%↓	61%↓
			25%↓	27%↓	45%↓		62%↓		67%↓	53%↓
<b>LUNG</b>										
ABSOLUTE (G)	12.13	11.94	11.54	9.76	10.79	11.71	10.65	10.14	11.12	12.82
RELATIVE TO BW	6.1	5.7	5.5	5.1	5.0	5.8	5.3	5.1	5.6	7.1

\* PREMATURELY SACRIFICED IN WEEKS 1-3  
 \*\* (%) COMPARED WITH WATER CONTROL

Group mean values for week 13 recovery:

TARGET TISSUE (WEIGHT)	DOSE (MG/KG/DAY)			
	0 (WATER CONTROL)		0 (HPβCD CONTROL)	
	M	F	M	F
<b>KIDNEYS</b>				
ABSOLUTE (G)	10.29	12.38	11.53	10.58
RELATIVE TO BW	4.9	5.9	5.2	5.0
<b>SPLEEN</b>				
ABSOLUTE (G)	4.29	4.36	6.00	4.76
RELATIVE TO BW	2.0	2.1	2.7	2.3
			40%↑	
<b>THYMUS GLAND</b>				
ABSOLUTE (G)	2.77	3.26	4.13	4.13
RELATIVE TO BW	1.3	1.5	1.9	1.9
<b>LUNG</b>				
ABSOLUTE (G)	10.33	10.43	10.95	11.39
RELATIVE TO BW	4.9	5.0	5.0	5.4

Thymus weights were lower in animals of all groups (only males) including those receiving HPβCD alone in both sexes. However after 13 weeks recovery, thymus weights were higher in vehicle control group compared to water control groups. After 13 weeks recovery, spleen weights were higher in vehicle control groups (only males) compared to water controls.

**Histopathology:****Adequate Battery: Yes**

- A full histological examination of the below listed tissues was undertaken for all animals on the study. Recovery groups included water control and vehicle control (HP $\beta$ CD alone). The designated HD recovery groups were sacrificed prior to scheduled termination due to deaths and poor condition.

Tissues	Weigh	Examine	Comments
Abnormal Tissue		x	-
Adrenal Glands x 2	x	x	-
Aortic Arch		x	-
Brain	x	x	The brain was sectioned at three levels (cerebral cortex, mid-brain and cerebellum with medulla).
Eye x 2		x	Both eyes were fixed in Davidson's fluid. One eye only was processed.
Gall Bladder		x	Weighed drained with the liver.
Gastro-intestinal Tract:			Opened at necropsy and mucosa examined.
Oesophagus		x	Sections of stomach body and pylorus were produced.
Stomach		x	
Duodenum		x	
Ileum		x	
Jejunum		x	
Colon		x	
Caecum		x	
Rectum		x	
Heart	x	x	-
Injection Sites		x	-
Kidney x 2	x	x	An extra formalin fixed, frozen section was stained with Oil Red O to ascertain the presence of fat. Section for E.M. (see below).
Liver	x	x	Weighed with drained gall bladder. An extra formalin fixed, frozen section was stained with Oil Red O to ascertain the presence of fat. Section for E.M. (see below).
Lungs	x	x	One lobe was perfusion fixed. Gross lesions in other lobes were immersion fixed.
Marrow Smear			Femoral marrow smears were taken from all animals, air-dried and fixed in methanol.
Mesenteric Lymph Node		x	-
Optic Nerve x 2		x	Fixed in Davidson's fluid. Only one was processed.
Ovary x 2	x	x	-
Pancreas	x	x	-
Pituitary Gland	x	x	-
Prostate Gland	x	x	-

Tissues	Weigh	Examine	Comments
Sciatic Nerve		x	-
Seminal Vesicles		x	-
Skeletal Muscle		x	-
Skin and Mammary Gland		x	-
Spinal Cord		x	Cervical, thoracic and lumbar samples taken.
Spleen	x	x	-
Sternum		x	To include bone marrow.
Submandibular Lymph Node		x	-
Submaxillary Salivary Gland		x	-
Tattoos			-
Testes x 2	x	x	Including epididymides. Fixed in Bouin's fluid, trimmed after 24 h then returned to Bouin's for a further 3-4 h. Trimmed tissue then transferred to absolute alcohol and processed, or returned to 10% formalin.
Thymus Gland	x	x	-
Thyroid with Parathyroid Glands x 2	x	x	Weighed together (unfixed).
Tongue		x	-
Trachea		x	-
Urinary Bladder		x	-
Uterus with Cervix	x	x	-
Vagina		x	-

**Peer Review:** In addition to the routine internal peer review, sections of kidney and bladder from Groups 1 and 2 in the main study were reviewed (b) (4)

Agreement on terminology was reached between (b) (4) and the study pathologist and the results reported reflect this consensus.

**Histological Findings:**

Table below summarizes the microscopic findings of DIC075U in at the end of treatment as well as at the end of a 9-week treatment-free recovery period in monkey.

TARGET TISSUE	SEVERITY	DOSE MG/KG/DAY										RECOVERY DOSE (MG/KG/DAY)				
		0 (WATER CONTROL)		0 (HPβCD CONTROL)		3		15		60*		0 (WATER CONTROL)		0 (HPβCD CONTROL)		
		M	F	M	F	M	F	M	F	M	F	M	F	M	F	
<b>STOMACH</b>																
HEMORRHAGE IN LAMINA PROPRIA	MODERATE	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/5	1/5	0/2	0/2	0/2	0/2
PYLORIC MONONUCLEAR-CELL INFILTRATE IN LAMINA PROPRIA	MILD	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/5	1/5	1/2	0/2	0/2	0/2
<b>ILEUM</b>																
NECROTISING ILEITIS	SEVERE	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/5	1/5	0/2	0/2	0/2	0/2
SUBMUCOSAL OEDEMA	SEVERE	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/5	1/5	0/2	0/2	0/2	0/2
<b>JEJUNUM</b>																
MUCOSAL NECROSIS	SEVERE	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/5	1/5	0/2	0/2	0/2	0/2
<b>KIDNEY</b>																
CYTOPLASMIC GRANULARITY (IN MEDULLARY RAYS)	VERY MILD MILD	0/3	0/3	0/3	2/3	2/3	1/3	0/3	3/3	3/5	3/5	0/2	0/2	0/2	0/2	0/2
		0/3	0/3	3/3	0/3	1/3	2/3	3/3	0/3	0/5	1/5	0/2	0/2	0/2	0/2	0/2
<b>COLON</b>																
INFLAMMATORY CELL INFILTRATION IN LAMINA	MILD	0/3	0/3	0/3	0/3	0/3	0/3	1/3	1/3	0/5	0/5	0/2	0/2	0/2	0/2	0/2
<b>HEART</b>																
FOCAL INFLAMMATORY CELL INFILTRATION	VERY MILD	1/3	0/3	0/3	2/3	1/3	2/3	1/3	2/3	4/5	4/5	0/2	1/2	1/2	0/2	0/2
<b>THYMUS</b>																
ATROPHY	VERY MILD MILD MODERATE	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/5	0/3	0/2	0/2	0/2	0/2
		0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	4/5	0/2	0/2	0/2	0/2
		0/3	0/3	0/3	0/3	0/3	0/3	1/3	0/3	1/5	0/3	0/2	0/2	0/2	0/2	0/2
<b>TAIL SKIN LESIONS</b>																
ULCERATION WITH INFLAMMATION/NECROSIS		1/2	2/2	1/2	2/2	2/2	1/2	3/3	3/3	5/5	4/4	2/2	0/2	1/2	0/2	0/2
POLYMORPHONUCLEAR LEUKOCYTIC		1/2	0/2	1/2	2/2	1/2	1/2	3/3	3/3	4/5	2/4	1/2	0/2	1/2	0/2	0/2
<b>LIVER</b>																
OIL RED -O STAIN POSITIVE FOR FAT		0/3	1/3	2/3	0/3	0/3	0/3	0/3	0/3	0/3	2/5	2/5	0/2	0/2	2/2	2/2
* PREMATURELY SACRIFICED IN WEEKS 1-3																

Gastrointestinal:

Severe mucosal necrosis was seen in the stomach and jejunum, together with necrotizing ileitis and submucosal edema, in one HD female which showed abnormalities of the gastro-intestinal tract at necropsy. Also mononuclear-cell infiltrate in the pyloric region of the stomach was seen in one HD male.

Mild inflammatory cell infiltration in the colon was seen in two MD animals which may relate to macroscopic findings.

Kidney:

In a number of animals in all groups (Groups 2-5), but not those receiving sterile water only (Group 1), renal tubule cells in the medullary rays had a mild or very mild granular appearance at histopathological evaluation. This was confirmed as an expected finding of intravenous HP $\beta$ CD administration by an external pathologist experienced in the evaluation of tissues from HP $\beta$ CD treated animals. The finding was absent after a 3 month recovery period. As described previously this renal toxicity is an expected finding in nonclinical toxicology studies.

Heart:

Very mild focal inflammatory cell infiltration was found in heart in all groups but the incidence increased at HD.

Thymus:

Moderate thymic atrophy was found in one HD and one MD animals. Mild or very mild thymic atrophy was also observed in 5 other HD animals. The Sponsor stated that the incidence of thymic atrophy at MD is of equivocal significance as thymic atrophy is an occasional background finding in Cynomolgus monkeys (b) (4). The increased incidence of thymic atrophy at HD was a probably consequence of the stress of the animals clinical condition and was considered to be indirectly related to DIC075U administration. *Note: this reviewer believes that this finding is drug related since it is dose dependent in terms of severity and incidence. Unfortunately there is no HD recovery group to evaluate the reversibility of the finding.*

Tail skin lesions

Tail lesions were observed in a number of animals from all groups, including the controls. Some had been observed in animals pre-trial and even before delivery from the supplier. However there was a tendency towards multiplicity and increased severity of lesions in animals treated at MD and HD.

**Special Evaluation:** None

**Toxicokinetics**

Toxicokinetic samples were obtained pre-dose and at 2, 10, (5, 15 for Group 2), 30, 60, 120, and 240 minutes post-dose from study animals at all DIC075U dose levels on Day 1 and at all DIC075U dose levels except 60 mg/kg/day on Day 28. No toxicokinetic samples were collected from the diclofenac 60 mg/kg/day animals on Day 28 because

all of these animals had been sacrificed prior to that time due to poor clinical conditions, although pre-terminal samples were collected prior to termination when possible.

Average diclofenac AUC determined on Day 1 ranged from 6.1 to 399.0  $\mu\text{g}\cdot\text{h}/\text{mL}$  in male, and from 7.4 to 413.3  $\mu\text{g}\cdot\text{h}/\text{mL}$  in female monkeys.  $C_{\text{max}}$  and AUC values of diclofenac increased with an increase in dose. AUC values showed a more than dose-proportional increase. The steady state volume of distribution was 128.2 mL/kg in males and 129.2 mL/kg in females. There were only marginal gender differences in AUC values. The half-life of elimination of diclofenac averaged between 0.6 and 1 hour and appeared to increase slightly after repeated dosing (see below the Sponsor's table 12). HP $\beta$ CD showed an average AUC value of 3918 and 3844  $\mu\text{g h}/\text{mL}$  in male and female monkeys, respectively, after a single IV dose of 533 mg/kg (see below the Sponsor's table 13).

**Table 12 Mean ( $\pm$  SD) Toxicokinetic Data of Diclofenac from Monkeys Treated 4 Weeks Intravenously with DIC075U**

Treatment	$T_{\text{max}}$ (h)	$C_{\text{max}}$ (ng/mL)	$T_{1/2}$ (h)	$V_{\text{ss}}$ (mL/kg)	$\text{AUC}_{0-t}$ (ng.h/mL)	$\text{AUC}_{0-\infty}$ (ng.h/mL)
<b>3 mg/kg/day</b>						
Males-Day 1	0.033 $\pm$ 0	29324 $\pm$ 2510	1.03 $\pm$ 0.19	180.9 $\pm$ 39.8	6059 $\pm$ 622	6151 $\pm$ 703
Females-Day 1	0.033 $\pm$ 0	32524 $\pm$ 1818	0.92 $\pm$ 0.22	146.2 $\pm$ 26.2	7292 $\pm$ 1306	7359 $\pm$ 1303
Males-Day 28	0.033 $\pm$ 0	32500 $\pm$ 1688	1.30 $\pm$ 0.37	205.8 $\pm$ 75.8	6054 $\pm$ 564	6200 $\pm$ 616
Females-Day 28	0.033 $\pm$ 0	33151 $\pm$ 6309	1.17 $\pm$ 0.28	182.7 $\pm$ 87.7	6493 $\pm$ 1206	6591 $\pm$ 1153
<b>15 mg/kg/day</b>						
Males-Day 1	0.033 $\pm$ 0	142514 $\pm$ 23383	1.00 $\pm$ 0.25	175.8 $\pm$ 29.8	54039 $\pm$ 18214	56002 $\pm$ 20482
Females-Day 1	0.033 $\pm$ 0	157456 $\pm$ 5857	0.85 $\pm$ 0.16	138.2 $\pm$ 20.1	58398 $\pm$ 6475	59291 $\pm$ 6529
Males-Day 28	0.033 $\pm$ 0	146482 $\pm$ 18002	1.07 $\pm$ 0.18	166.6 $\pm$ 35.5	49762 $\pm$ 8301	51129 $\pm$ 9384
Females-Day 28	0.033 $\pm$ 0	154524 $\pm$ 7518	0.95 $\pm$ 0.16	144.5 $\pm$ 21.7	50304 $\pm$ 8056	51090 $\pm$ 8036
<b>60 mg/kg/day</b>						
Males-Day 1	0.033 $\pm$ 0	528580 $\pm$ 42855	0.64 $\pm$ 0.07	124.3 $\pm$ 9.4	391474 $\pm$ 51691	398999 $\pm$ 55196
Females-Day 1	0.033 $\pm$ 0	552777 $\pm$ 39835	0.56 $\pm$ 0.02	107.8 $\pm$ 10.8	409790 $\pm$ 46234	413282 $\pm$ 46486

Source: NC-DFC-010, Appendix 37 (CTD 4.2.3.2.4)

**Table 13 Mean ( $\pm$  SD) Toxicokinetic Data of HP $\beta$ CD from Monkeys Dosed 4 Weeks Intravenously with 533 mg/kg HP $\beta$ CD**

Treatment	$\text{AUC}_{0-t}$ ( $\mu\text{g}\cdot\text{h}/\text{mL}$ )	$\text{AUC}_{0-\infty}$ ( $\mu\text{g}\cdot\text{h}/\text{mL}$ )	Cl (mL/kg.h)	$T_{1/2}$ (h)	$V_{\text{ss}}$ (mL/kg)
<b>Males-Day 1</b>	3810 $\pm$ 742.4	3918 $\pm$ 818.8	0.141 $\pm$ 0.030	0.7868 $\pm$ 16.06	128.2 $\pm$ 9.0
<b>Females-Day 1</b>	3769 $\pm$ 793.8	3844 $\pm$ 824.5	0.144 $\pm$ 0.031	0.7296 $\pm$ 0.061	129.2 $\pm$ 25.0
<b>Males-Day 28</b>	3203 $\pm$ 287.4	3263 $\pm$ 307.0	0.1644 $\pm$ 0.014	0.737 $\pm$ 0.040	137.6 $\pm$ 5.0
<b>Females-Day 28</b>	3411 $\pm$ 603.7	3463 $\pm$ 622.4	0.1576 $\pm$ 0.025	0.6864 $\pm$ 0.041	132.6 $\pm$ 17.0

Source: NC-DFC-010, Appendix 37 (CTD 4.2.3.2.4)

**6.2.2.2.2 Monkey****Study title: HPβCD, 4 Week Intravenous Toxicity Study in Cynomolgus Monkeys with a 3 Month Recovery Period**

Study no.: NC-DFC-011 (CTD 4.2.3.2.1)

Study report location: (b) (4)

Conducting laboratory and location: (b) (4)

Date of study initiation: February 6, 1997

GLP compliance: Yes

QA statement: Yes

Drug, lot # HPβCD; Lot # R &amp; D225

This study of the vehicle versus water control animals was conducted (separate from study NC-DFC-010) in conjunction with the safety evaluation of DIC075U.

5 male and 5 female monkeys (Group 2) received 533 mg/kg/day HPβCD at a dose volume of 2.4 mg/kg/day. This dose is approximately 50 times the expected intravenous dosage of HPβCD in humans when administered DIC075V as a single dose. Another group also containing 5 male and 5 female monkeys (Group 1) received sterile water at the same dose volume (water control). Three animals of each sex in each group were sacrificed at the end of the 4 week treatment period and the remaining animals were sacrificed after a further 13 week treatment-free recovery period.

Group	Treatment	Dose Level (HPβCD) (mg.kg <sup>-1</sup> .day <sup>-1</sup> )	Dose Volume (ml.kg <sup>-1</sup> .day <sup>-1</sup> )	Animal Numbers			
				Main Study		Recovery Study	
				Males	Females	Males	Females
1	Vehicle Control (Sterile water)	0	2.40	1-3	22-24	4-5	25-26
2	(b) (4) Control* (HPβCD)	533	2.40	6-8	27-29	9-10	30-31

Body weight, food consumption, ophthalmoscopy, electrocardiography, hematology, clinical chemistry, urinalysis, fecal analysis and organ weights were all unaffected by treatment for 4 weeks with HPβCD.

There were no clinical changes and no findings at necropsy considered to be associated with HPβCD treatment. However, at histopathological examination, renal tubular cells in the medullary rays had a granular appearance (mild or very mild) in most animals treated with HPβCD and examined at Week 4. The Sponsor stated that a pathologist (b) (4) experienced in examining tissues from animals treated with HPβCD an expected finding of intravenous

HP $\beta$ CD administration. This reviewer agrees with the Sponsor that these effects in kidneys are related to HP $\beta$ CD since these effects were not seen in water controls.

It has been shown that human subjects given a single dose of DIC075V receive approximately 66.4  $\mu\text{g}\cdot\text{h}/\text{mL}$  of HP $\beta$ CD. This exposure is approximately 50 times lower than that causing in monkeys very minimal light microscopic changes with no evidence for functional renal deficits.

Following the 3 month recovery period there were no differences in renal medullary rays in animals from the control or HP $\beta$ CD treated groups.

Toxicokinetic analysis showed a relatively short half-life for HP $\beta$ CD. There were no gender differences in HP $\beta$ CD kinetics and no accumulation in plasma in 4 weeks dosing period (see below TK data from NDA submission).

Male - Day 28

Parameter	N	Mean	SD	CV%	90% CI	
					(Lower)	(Upper)
Cl	5	0.1644	0.014	8.54	0.150	0.178
T $\frac{1}{2}$ el	5	0.737	0.040	5.37	0.697	0.777
AUC <sub>(0-t)</sub>	5	3203	287.4	8.97	2915	3490
AUC <sub>(0-<math>\infty</math>)</sub>	5	3263	307.0	9.41	2956	3570
V <sub>ss</sub>	5	137.6	5.0	3.27	133.0	142.0

Female - Day 28

Parameter	N	Mean	SD	CV%	90% CI	
					(Lower)	(Upper)
Cl	5	0.1576	0.025	15.63	0.133	0.182
T $\frac{1}{2}$ el	5	0.6864	0.041	6.01	0.645	0.728
AUC <sub>(0-t)</sub>	5	3411	603.7	17.70	2807	4014
AUC <sub>(0-<math>\infty</math>)</sub>	5	3463	622.4	17.98	2840	4085
V <sub>ss</sub>	5	132.6	17.0	12.84	116.0	150.0

## 7 Genetic Toxicology

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

Study title: **DF-HP $\beta$ CD Testing for Mutagenic Activity with Salmonella Typhimurium TA 1535, TA1537, TA 98 and TA 100, and Escherichia coli WP2uvrA**

Study no.: NC-DFC-007 (eCTD 4.2.3.3.1)

Study report location: [REDACTED] (b) (4)

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

Date of study initiation: May 21, 1997

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: DF-HP $\beta$ CD (DIC075U), # R&Q 227, 96.4%

#### Key Study Findings

- DIC075U was tested in the Ames Reverse Mutation Assays at concentrations of 17 to 5000  $\mu$ g/plate.
- The results of both direct plate and preincubation methods were similar. DIC075U did not induce mutagenic activity in any of the 5 bacterial strains used, in either metabolic activation condition.
- In the second mutation assay (pre-incubation method) toxicity was observed, in the presence of S9 mix only. Slightly thin background lawns of microcolonies were observed with S.typhimurium TA 96 and TA 100 at 2500  $\mu$ g per plate. Thin lawns with S.typhimurium TA 98 and TA 100 and slightly thin lawns with S.typhimurium TA 1537 were noted at 5000  $\mu$ g per plate.
- A reduction in the number of mutant colonies was observed in both assays in the presence and absence of S9 mix, most notably with TA 100 and at 5000  $\mu$ g/plate.
- No precipitation of the test material was observed.
- There was no difference in mutant colony count between the carrier solution (HF $\beta$ CD) and vehicle control.
- Under the conditions of the study, DIC075U was concluded to be negative in the bacterial reverse mutation assay when tested up to maximum limit of 5000 $\mu$ g/plate.

## Methods

Strains:	S. typhimurium TA98, TA100, TA1535, TA1537, and E. coli WP2 uvrA.
Concentrations in definitive study:	156.25, 312.5, 625, 1250, 2500 and 5000 µg/plate
Basis of concentration selection:	The dose levels used in the first mutation assay were chosen on the basis of the results of the toxicity test (in TA 100 only)
Negative control:	1) Vehicle control: water 2) A control sample of the (b) (4) (HPβCD) was included with the second mutation experiment to distinguish between the effects of DIC075U and the (b) (4)
Positive control:	2-aminoanthracene (2-AAN), Sodium azide (NaN <sub>3</sub> ), 9-aminoacridine (9-AA), 2-nitrofluorene (2-NF) and N-ethyl-N-nitro- N-nitrosoguanidine(ENNG).
Formulation/Vehicle:	<b>DIC075U</b> ( (b) (4) Diclofenac Sodium + (b) (4) HPβCD) (b) (4) Sterile Water for Injection USP
Incubation & sampling time:	<ul style="list-style-type: none"> <li>• Direct plate method: Incubation was for 2 days at 37°C.</li> <li>• Preincubation method: Pre-incubation was for 20 minutes at 37°C in the test tube prior to the addition of soft agar and placement on an agar plate. Incubation was for 2 days at 37°C.</li> </ul> <p>At the end of incubation period the colonies were counted using a Biotran III automated counter. The plates were examined for precipitation and microcolony growth.</p>

## Study Validity

All positive and vehicle control values were within acceptable ranges, and all criteria for a valid study were met. e.g. 1) the appropriate controls were used, the positive controls had mean reversion frequency 3-times or more greater than the mean reversion frequency of vehicle control 2) the highest concentration of DIC075U tested reached the maximum recommended concentration that is 5,000 µg/plate, 3) Two independent experiments were conducted to evaluate the mutagenicity of the test article.

## Results

- The results of the toxicity test for DIC075U are shown in below table:

### Test 1

DF-HPBCD - revertant colony numbers obtained per plate using bacterial strains :- *S. typhimurium* TA 100

Strain	Dose level µg/plate	Liver S-9	Individual revertant colony counts
TA 100	Solvent	-	150
	8	-	110
	25	-	114
	83	-	122
	250	-	126
	833	-	117
	2500	-	96
	5000	-	64
	Solvent	+	150
	8	+	132
	25	+	118
	83	+	100
	250	+	122
	833	+	112
	2500	+	115
	5000	+	57

- : Absence  
+ : Presence

A reduction in the number of colonies was observed in 5000 µg/plate. No precipitation of the test material occurred.

The dose levels used in the first mutation assay, chosen on the basis of the results of the toxicity test (in TA 100 only), were: 17, 50, 167, 500, 1667 and 5000 µg/plate. The dose levels were narrowed for the second assay to extend parameters: 156.25, 312.5, 625, 1250, 2500 and 5000 µg per plate.

- There was no apparent difference in mutant colony count between the carrier solution (DF-HPβCD) and vehicle control with pre-incubation method

DF-HPBCD (b)(4) revertant colony numbers per plate:-

Strain	Compound	Liver S-9	Mean revertant colony counts	SD	Individual revertant colony counts
TA 1535	(b)(4)	-	8	1	8, 8, 9
TA 1537		-	7	2	8, 7, 5
TA 98		-	21	5	16, 21, 26
TA 100		-	131	5	136, 126, 130
WP2uvrA		-	6	2	7, 6, 4
TA 1535		+	13	4	10, 17, 12
TA 1537		+	7	3	9, 7, 4
TA 98		+	11	2	9, 13, 11
TA 100		+	122	8	129, 114, 122
WP2uvrA		+	4	2	6, 2, 5

SD : Standard Deviation

- : Absence

+ : Presence

- DIC075U did not induce mutagenic activity in any of mutagenic assays (direct plate [test 2] or pre-incubation method [test 3]), in either activation condition ( see below the Sponsors tables, test 2 and test 3)

Test 2  
 Mean Number of *his*<sup>+</sup> and *trp*<sup>+</sup> Revertant Colonies Obtained when 4 Strains of  
*S. typhimurium* and one Strain of *E. coli* were Treated with DF-HPBCD  
 in the Presence of a Post-mitochondrial Fraction (S9 Mix) from the Livers  
 of Male Rats Treated with Aroclor 1254 (FLI 084)

Substance	Dose Level $\mu\text{g}$ per plate	TA 1535	TA 1537	TA 98	TA 100	WP2uvrA
		Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.
WATER	200 $\mu\text{l}$	10 $\pm$ 4	12 $\pm$ 3	20 $\pm$ 4	95 $\pm$ 12	5 $\pm$ 1
DF-HPBCD	17	14 $\pm$ 1	15 $\pm$ 4	24 $\pm$ 5	103 $\pm$ 6	10 $\pm$ 3
	50	12 $\pm$ 4	12 $\pm$ 1	20 $\pm$ 5	104 $\pm$ 11	9 $\pm$ 2
	167	13 $\pm$ 2	9 $\pm$ 3	21 $\pm$ 3	108 $\pm$ 14	11 $\pm$ 2
	500	11 $\pm$ 4	11 $\pm$ 3	19 $\pm$ 3	92 $\pm$ 9	8 $\pm$ 1
	1667	13 $\pm$ 3	9 $\pm$ 3	19 $\pm$ 3	80 $\pm$ 7	8 $\pm$ 3
	5000	12 $\pm$ 5	5 $\pm$ 2	13 $\pm$ 3	46 $\pm$ 6	11 $\pm$ 2
Positive Controls	Compound	2AAN	2AAN	2AAN	2AAN	2AAN
	Dose Level	2 $\mu\text{g}$	2 $\mu\text{g}$	0.5 $\mu\text{g}$	0.5 $\mu\text{g}$	20 $\mu\text{g}$
	Mean $\pm$ S.D.	375 $\pm$ 5	205 $\pm$ 17	827 $\pm$ 60	743 $\pm$ 71	332 $\pm$ 25

S.D. Standard Deviation

2AAN 2-Aminoanthracene

N.B. The mean values were generally calculated from triplicate plate counts.

Test 2  
 Mean Number of *his+* and *trp+* Revertant Colonies Obtained when 4 Strains of *S. typhimurium* and one Strain of *E. coli* were Treated with DF-HPBCD in the Absence of S9 Mix

Substance	Dose Level $\mu\text{g}$ per plate	TA 1535	TA 1537	TA 98	TA 100	WP2uvrA
		Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.
WATER	200 $\mu\text{l}$	11 $\pm$ 3	12 $\pm$ 1	18 $\pm$ 6	88 $\pm$ 11	9 $\pm$ 4
DF-HPBCD	17	12 $\pm$ 3	8 $\pm$ 1	24 $\pm$ 7	90 $\pm$ 4	9 $\pm$ 6
	50	8 $\pm$ 4	10 $\pm$ 3	23 $\pm$ 5	92 $\pm$ 10	9 $\pm$ 3
	167	12 $\pm$ 5	11 $\pm$ 5	21 $\pm$ 2	91 $\pm$ 11	8 $\pm$ 3
	500	12 $\pm$ 3	7 $\pm$ 3	19 $\pm$ 9	78 $\pm$ 13	7 $\pm$ 5
	1667	12 $\pm$ 3	11 $\pm$ 2	22 $\pm$ 4	76 $\pm$ 9	7 $\pm$ 2
	5000	15 $\pm$ 3	6 $\pm$ 3	17 $\pm$ 6	50 $\pm$ 11	4 $\pm$ 1
Positive Controls	Compound	NaN3	9AA	2NF	NaN3	ENNG
	Dose Level	1 $\mu\text{g}$	80 $\mu\text{g}$	1 $\mu\text{g}$	1 $\mu\text{g}$	2 $\mu\text{g}$
	Mean $\pm$ S.D.	182 $\pm$ 9	528 $\pm$ 161	200 $\pm$ 10	384 $\pm$ 4	143 $\pm$ 6

S.D. Standard Deviation

NaN3 Sodium azide  
 2NF 2-Nitrofluorene

9AA 9-Aminoacridine  
 ENNG N-Ethyl-N-nitro-N-nitrosoguanidine

N.B. The mean values were generally calculated from triplicate plate counts.

Test 3  
 Mean Number of *his+* and *trp+* Revertant Colonies Obtained when 4 Strains of  
*S. typhimurium* and one Strain of *E. coli* were Treated with DF-HPBCD  
 in the Presence of a Post-mitochondrial Fraction (S9 Mix) from the Livers  
 of Male Rats Treated with Aroclor 1254 (FLI 084)

Substance	Dose Level $\mu\text{g}$ per plate	TA 1535	TA 1537	TA 98	TA 100	WP2uvrA
		Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.
WATER	200 $\mu\text{l}$	10 $\pm$ 1	12 $\pm$ 1	23 $\pm$ 1	120 $\pm$ 14	6 $\pm$ 1
DF-HPBCD	156.25	8 $\pm$ 6	11 $\pm$ 2	17 $\pm$ 6	130 $\pm$ 13	4 $\pm$ 1
	312.5	9 $\pm$ 0	12 $\pm$ 2	20 $\pm$ 5	152 $\pm$ 28	6 $\pm$ 1
	625	12 $\pm$ 2	7 $\pm$ 2	15 $\pm$ 6	117 $\pm$ 14	3 $\pm$ 2
	1250	11 $\pm$ 2	7 $\pm$ 1	19 $\pm$ 5	105 $\pm$ 17	4 $\pm$ 3
	2500	12 $\pm$ 1	8 $\pm$ 1	13 $\pm$ 3 (STL)	70 $\pm$ 7 (STL)	6 $\pm$ 3
	5000	9 $\pm$ 4	4 $\pm$ 2 (STL)	6 $\pm$ 2 (TL)	16 $\pm$ 4 (TL)	7 $\pm$ 0
Positive Controls	Compound	2AAN	2AAN	2AAN	2AAN	2AAN
	Dose Level	2 $\mu\text{g}$	2 $\mu\text{g}$	0.5 $\mu\text{g}$	0.5 $\mu\text{g}$	20 $\mu\text{g}$
	Mean $\pm$ S.D.	80 $\pm$ 5	60 $\pm$ 3	124 $\pm$ 25	210 $\pm$ 4	348 $\pm$ 19

S.D. Standard Deviation

2AAN 2-Aminoanthracene

STL : SLIGHTLY THIN LAWN TL : THIN LAWN

N.B. The mean values were generally calculated from triplicate plate counts.

Test 3  
 Mean Number of *his+* and *trp+* Revertant Colonies Obtained when 4 Strains of  
*S. typhimurium* and one Strain of *E. coli* were Treated with DF-HPBCD  
 in the Absence of S9 Mix

Substance	Dose Level $\mu\text{g}$ per plate	TA 1535	TA 1537	TA 98	TA 100	WP2uvrA
		Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.
WATER	200 $\mu\text{l}$	12 $\pm$ 1	6 $\pm$ 3	16 $\pm$ 4	116 $\pm$ 5	7 $\pm$ 2
DF-HPBCD	156.25	9 $\pm$ 2	10 $\pm$ 2	12 $\pm$ 3	132 $\pm$ 4	7 $\pm$ 2
	312.5	11 $\pm$ 1	11 $\pm$ 3	14 $\pm$ 2	140 $\pm$ 8	7 $\pm$ 3
	625	15 $\pm$ 2	8 $\pm$ 1	17 $\pm$ 1	121 $\pm$ 6	6 $\pm$ 2
	1250	10 $\pm$ 1	7 $\pm$ 2	17 $\pm$ 3	134 $\pm$ 31	6 $\pm$ 3
	2500	9 $\pm$ 0	7 $\pm$ 3	13 $\pm$ 2	90 $\pm$ 36	6 $\pm$ 3
	5000	8 $\pm$ 3	6 $\pm$ 3	17 $\pm$ 1	81 $\pm$ 7	7 $\pm$ 2
Positive Controls	Compound	NaN3	9AA	2NF	NaN3	ENNG
	Dose Level	1 $\mu\text{g}$	80 $\mu\text{g}$	1 $\mu\text{g}$	1 $\mu\text{g}$	2 $\mu\text{g}$
	Mean $\pm$ S.D.	157 $\pm$ 6	406 $\pm$ 20	199 $\pm$ 28	349 $\pm$ 24	226 $\pm$ 5

S.D. Standard Deviation

NaN3 Sodium azide  
 2NF 2-Nitrofluorene

9AA 9-Aminoacridine  
 ENNG N-Ethyl-N-nitro-N-nitrosoguanidine

N.B. The mean values were generally calculated from triplicate plate counts.

## 7.2 *In Vitro* Mouse Lymphoma Mutation Assay

Study title: **DF-HP $\beta$ CD Mouse Lymphoma Mutation Assay**

Study no.: NC-DFC-009 (eCTD 4.2.3.3.1)

Study report location: (b) (4)

Conducting laboratory and location: (b) (4)

Date of study initiation: April 30, 1997

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: DF-HP $\beta$ CD (**DIC075U**), # R&Q 227, 96.4%

### Key Study Findings

- DIC075U was assayed for mutagenic potential in the mouse lymphoma L5178Y cell line, clone 3.7.2.C, scoring for forward mutations at the thymidine kinase locus: TK<sup>+/-</sup> to TK<sup>-/-</sup>.
- A cytotoxicity test showed that DF-HP $\beta$ CD was toxic at 312.5  $\mu$ g/ml in the absence and presence of S9 mix.
- Four independent mutation assays (2 in the absence and 2 in the presence of S9 mix), were conducted. The final concentrations of DIC075U in the treatment medium ranged between 10 and 250  $\mu$ g/ml. No significant evidence of mutagenic activity was obtained from cultures treated with DF-HP $\beta$ CD in any of these 4 assays
- DIC075U is not mutagenic in mouse lymphoma L5178Y cells, when tested at concentrations extending into the toxic range.

## Methods

**Strains:** Mouse lymphoma L5178Y cell line  
**Concentrations in definitive study ( $\mu\text{g/ml}$ ):** Assay 1 (in the absence of S9 mix): 10, 50, 90, 130, 170, 210 and 250  
 Assay 2 (in the presence of S9 mix): 10, 50, 90, 130, 170, 210 and 250  
 Assay 3 (in the absence of S9 mix): 10, 70, 130, 190 and 250  
 Assay 4 (in the presence of S9 mix): 50, 100, 150, 200 and 250

**Basis of concentration selection:** The dose levels chosen on the basis of the results of the toxicity test  
**Negative control:** water  
**Positive control:** ethyl methanesulphonate (EMS), methyl methanesulphonate (MMS), 3-methylcholanthrene

**Formulation/Vehicle:** **DIC075U** ( (b) (4) Diclofenac Sodium + (b) (4) HP $\beta$ CD) / (b) (4) Sterile Water for Injection USP

**Incubation & sampling time:** On the day of the test, samples of cell culture were dispensed to sterile tubes, each containing 3.9 ml media. Freshly prepared S9 mix or media was added to each tube followed by the test solution, vehicle or positive control.

All tubes were incubated on rotating at 37 °C, 10 r.p.m. for 4 h. After this, the cells were gently regimented by centrifuge. The cells were returned to the rotating drum and allowed to express their genetic lesions at 37°C for 2 days.

On Day 2, cell counts were determined. All cultures were selected for expression of genetic damage. This was determined by performing 2 parallel cloning assays: the viability assay and the mutant selection assay.

For the mutant selection assay, trifluorothymidine (TFT) was added to cloning medium. All plates were gelled at room temperature until the agar had set, then incubated at 37 °C in an atmosphere of 5% CO<sub>2</sub> :95% air (v/v) until the colonies were fully developed (usually 14 days). The colonies were then counted using a "Domino" image analyzer.

## Study Validity

The criteria for a valid assay were met: e.g. 1) cloning efficiency of solvent control of 56-129% (soft agar), 2) spontaneous mutant frequencies in solvent control (between 35-140 mutant/10<sup>+6</sup> clonable above solvent control value). 3) Positive control showed at least 100 mutants/10<sup>+6</sup> clonable cells above solvent control.

**Results**

- The preliminary toxicity test showed that a concentration of 156.25µg/ml reduced the relative suspension growth to 27% and 19% in the absence and presence of S9 mix, respectively (see below the Sponsor’s tables). Higher concentrations were lethal.

TABLE 1 Mouse Lymphoma Toxicity Test in the Absence of S9 Mix					
Chemical	Dose Level (µg.ml <sup>-1</sup> )	*Daily Suspension Count x 10 <sup>5</sup> .ml <sup>-1</sup> (ml Cells Kept )		Total	RSG %
		Day 1	Day 2		
		Water	(1 ml added)		
DF-HPβCD	19.5	13.8 (4.3)	13.0	19.9	68
	39.1	8.0 (7.5)	11.7	10.4	36
	78.125	7.0 (8.6)	11.9	9.3	32
	156.25	4.9 (12.2)	14.7	8.0	27
	312.5	0 -	-	0	0
	625	0 -	-	0	0
	1250	0 -	-	0	0
	2500	0 -	-	0	0

\* = Adjusted to 20 ml of 3 x 10<sup>5</sup>.ml<sup>-1</sup> after counting on Day 1  
RSG % = Relative suspension growth

TABLE 2 Mouse Lymphoma Toxicity Test in the Presence of S9 Mix						
Chemical	Dose Level (µg.ml <sup>-1</sup> )	*Daily Suspension Count x 10 <sup>5</sup> .ml <sup>-1</sup> (ml Cells Kept )			Total	RSG %
		Day 1		Day 2		
		Water	(1 ml added)	11.8 (5.1)		
DF-HPβCD	19.5	11.2 (5.4)	17.0	21.2	82	
	39.1	12.8 (4.7)	13.5	19.2	75	
	78.125	8.6 (7.0)	14.9	14.2	55	
	156.25	2.2 (20)	14.9	5.0	19	
	312.5	0 -	-	0	0	
	625	0 -	-	0	0	
	1250	0 -	-	0	0	
	2500	0 -	-	0	0	

\* = Adjusted to 20 ml of 3 x 10<sup>5</sup>.ml<sup>-1</sup> after counting on Day 1  
RSG % = Relative suspension growth

- Mutant assay 1 (with S9 mix) and assay 2 (without S9 mix): No evidence of mutagenic activity was obtained with DIC075U -treated cultures, in either the absence or the presence of S9 mix. The mean relative total growth (RTG) at the highest concentration was 16% and 15% in the absence and presence of S9 mix, respectively.

Mouse Lymphoma in the Absence of S9 Mix  
Data Summary  
(Assay 1)

Chemical	Dose Level ( $\mu\text{g}\cdot\text{ml}^{-1}$ )	Total Viable Count (VC)	Cloning Efficiency %	Suspension Growth	Total Cell Growth	Relative Total Growth %	Total Mutant Count (MC)	Mutant Fraction $\times 10^4$ $\frac{200 \text{ (MC)}}{\text{VC}}$	Fold Increase Over Control	
					Vehicle Mean = 15.0			Vehicle Mean = 42.0		
Water	(100 $\mu\text{l}$ added)	453	76	19.6	14.9	99	87	38	-	
		437	73	17.8	13.0	87	76	35		
		459	77	20.5	15.8	105	107	47		
		458	76	21.5	16.3	109	109	48		
Ethyl methanesulphonate	250	281	47	16.5	7.8	52†	731	520	12.4	
		376	63	16.0	10.1	67	644	343	8.2	
Methyl methanesulphonate	10	254	42	12.7	5.3	35	344	271	6.5	
		242	40	13.2	5.3	35	432	357	8.5	
DF-HP $\beta$ CD	10	542	90	18.3	16.5	110	135	50	1.2	
		486	81	23.9	19.4	129	102	42	1.0	
	50	NPS	-	-	-	-	-	NPS	-	-
		NPS	-	-	-	-	-	NPS	-	-

DF-HP $\beta$ CD	90	569	95	7.4	7.0	47	102	36	0.9	
		452	75	7.8	5.9	39	76	34	0.8	
	130	NPS	-	-	-	-	-	NPS	-	-
		NPS	-	-	-	-	-	NPS	-	-
	170	460	77	5.3	4.1	27	72	31	0.7	
		396	66	6.8	4.5	30	52	26	0.6	
	210	NPS	-	-	-	-	-	NPS	-	-
		NPS	-	-	-	-	-	NPS	-	-
	250	528	88	3.1	2.7	18	64	24	0.6	
		564	94	2.2	2.1	14	77	27	0.6	

Mouse Lymphoma in the Presence of S9 Mix (FLI 083)  
Data Summary  
(Assay 2)

Chemical	Dose Level (µg.ml <sup>-1</sup> )	Total Viable Count (VC)	Cloning Efficiency %	Suspension Growth	*Total Cell Growth	Relative Total Growth %	Total Mutant Count (MC)	Mutant Fraction x 10 <sup>-6</sup> $\frac{200 (MC)}{(VC)}$	Fold Increase Over Control
					Vehicle Mean = 17.4			Vehicle Mean = 34.3	
Water	(100 µl added)	524	87	18.4	16.0	92	66	25	-
		515	86	18.7	16.1	92	112	43	
		589	98	18.6	18.2	104	101	34	
		588	98	19.8	19.4	111	104	35	
3-Methylcholanthrene	2.5	321	54	14.4	7.8	45	452	282	8.2
		344	57	16.0	9.1	52	533	310	9.1
DF-HPβCD	10	NPS	-	-	-	-	NPS	-	-
		NPS	-	-	-	-	NPS	-	-
	50	NPS	-	-	-	-	NPS	-	-
		NPS	-	-	-	-	NPS	-	-

DF-HPβCD	90	432	72	13.7	9.9	57	61	28	0.8	
		436	73	12.3	9.0	52	72	33	1.0	
	130	642	107	7.6	8.1	46	87	27	0.8	
		558	93	10.2	9.5	55	86	31	0.9	
	170	567	95	6.9	6.6	38	122	43	1.3	
		531	89	7.0	6.2	36	113	43	1.3	
	210	647	108	2.5	2.7	15	96	30	0.9	
		623	104	2.5	2.6	15	96	31	0.9	
	250	NPT	-	-	-	-	-	NPT	-	-
		NPT	-	-	-	-	-	NPT	-	-

\* = Cloning efficiency x growth in suspension  
NPT = Not plated - toxic

In Assay 2, in the presence of S9 mix, DIC075U was assessed for mutagenic activity at concentrations of 90, 130, 170 and 210 µg/ml. Lower concentrations were surplus to requirement, while the highest concentration (250µg/ml) was too toxic for assessment.

- Mutant assay 3 (with S9 mix) and assay 4 (without S9 mix): No evidence of mutagenic activity was obtained with DIC075U treated cultures, in either the absence or the presence of S9 mix. The mean RTG at the highest concentration (250µg/ml) was 35% and 13% in the absence and presence of S9 mix, respectively.

Mouse Lymphoma in the Absence of S9 Mix  
Data Summary  
(Assay 3)

Chemical	Dose Level (µg.ml <sup>-1</sup> )	Total Viable Count (VC)	Cloning Efficiency %	Suspension Growth	*Total Cell Growth	Relative Total Growth %	Total Mutant Count (MC)	Mutant Fraction x 10 <sup>-4</sup> 200 (MC) (VC)	Fold Increase Over Control
					Vehicle Mean = 12.9			Vehicle Mean = 38.5	
Water	(100 µl added)	436	73	16.0	11.7	91	103	47	
		515	86	15.3	13.2	102	112	43	
		602	100	13.3	13.3	103	90	30	
		560	93	14.4	13.4	104	94	34	
Ethyl methanesulphonate	250	494	82	12.0	9.8	76	482	195	5.1
		440	73	13.4	9.8	76	464	211	5.5
Methyl methanesulphonate	10	256	43	7.9	3.4	26	206	163	4.2
		276	46	7.8	3.6	28	227	164	4.3
DF-HPβCD	10	NPS	-	-	-	-	NPS	-	-
		NPS	-	-	-	-	NPS	-	-
	70	522	87	5.8	5.0	39	110	42	1.1
		533	89	6.1	5.4	42	132	50	1.3

Chemical	Dose Level (µg.ml <sup>-1</sup> )	Total Viable Count (VC)	Cloning Efficiency %	Suspension Growth	*Total Cell Growth	Relative Total Growth %	Total Mutant Count (MC)	Mutant Fraction x 10 <sup>-4</sup> 200 (MC) (VC)	Fold Increase Over Control
					Vehicle Mean = 12.9			Vehicle Mean = 38.5	
DF-HPβCD	130	424	71	7.5	5.3	41	65	31	0.8
		527	88	6.5	5.7	44	75	28	0.7
	190	509	85	5.0	4.3	33	93	37	1.0
		493	82	4.7	3.9	30	80	32	0.8
	250	498	83	5.3	4.4	34	80	32	0.8
		486	81	5.7	4.6	36	73	30	0.8

\* = Cloning efficiency x growth in suspension

Mouse Lymphoma in the Presence of S9 Mix (FLI 084)  
Data Summary  
(Assay 4)

Chemical	Dose Level (µg.ml <sup>-1</sup> )	Total Viable Count (VC)	Cloning Efficiency %	Suspension Growth	*Total Cell Growth	Relative Total Growth %	Total Mutant Count (MC)	Mutant Fraction x 10 <sup>-6</sup> 200 (MC) (VC)	Fold Increase Over Control
					Vehicle Mean = 15.3			Vehicle Mean = 31.0	
Water	(100 µl added)	660	110	14.1	15.5	101	99	30	
		600	100	13.7	13.7	90	96	32	
		654	109	14.4	15.7	103	77	24	
		543	91	17.8	16.2	106	104	38	
3-Methylcholanthrene	2.5	360	60	8.5	5.1	33	526	292	9.4
		331	55	9.4	5.2	34	429	259	8.4
DF-HPβCD	50	NPS	-	-	-	-	NPS	-	-
		NPS	-	-	-	-	NPS	-	-
	100	538	90	8.6	7.7	50	102	38	1.2
		600	100	6.4	6.4	42	81	27	0.9

Chemical	Dose Level (µg.ml <sup>-1</sup> )	Total Viable Count (VC)	Cloning Efficiency %	Suspension Growth	*Total Cell Growth	Relative Total Growth %	Total Mutant Count (MC)	Mutant Fraction x 10 <sup>-6</sup> 200 (MC) (VC)	Fold Increase Over Control
					Vehicle Mean = 15.3			Vehicle Mean = 31.0	
DF-HPβCD	150	677	113	3.4	3.8	25	104	31	1.0
		668	111	5.2	5.8	38	110	33	1.1
	200	654	109	4.6	5.0	33	75	23	0.7
		694	116	2.6	3.0	20	112	32	1.0
	250	667	111	1.8	2.0	13	100	30	1.0
		672	112	1.8	2.0	13	103	31	1.0

\* = Cloning efficiency x growth in suspension

### 7.3 *In Vitro* Mouse Lymphoma Mutation Assay (Voltarol)

Study title: **Voltarol Mouse Lymphoma Mutation Assay**

Study no.: NC-DFC-008 (eCTD 4.2.3.3.1)

Study report location: [REDACTED] (b) (4)

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

Date of study initiation: April 30, 1997

GLP compliance: Yes

QA statement: Yes

Drug, lot #: Commercially available Voltarol (Batch No. 285800), manufactured by Geigy

#### Key Study Findings

- Voltarol was assayed for mutagenic potential in the mouse lymphoma L5178Y cell line, clone 3.7.2.C, scoring for forward mutations at the thymidine kinase locus: TK<sup>+/-</sup> to TK<sup>-/-</sup>.
- A cytotoxicity test showed that Voltarol was toxic at 156.25 µg/ml in the absence and presence of S9 mix.
- Four independent mutation assay (2 in the absence and 2 in the presence of S9 mix), were conducted. The final concentrations of test article in the treatment medium ranged between 130 and 250µg/ml.
- Dose-related mutagenic responses were obtained with Voltarol-treated cultures in all 4 assays. The responses were larger in the presence of S9 mix. The lowest effective concentration was 170 µg/ml.
- Voltarol is mutagenic in mouse lymphoma L5178Y cells when tested at concentration extending into the toxic range.

## Methods

Strains: Mouse lymphoma L5178Y cell line  
 Concentrations in definitive study ( $\mu\text{g/ml}$ ): All 4 assay: 130, 170, 210 and 250  
 Basis of concentration selection: The dose levels chosen on the basis of the results of the toxicity test  
 Negative control: water  
 Positive control: ethyl methanesulphonate (EMS), methyl methanesulphonate (MMS), 3-methylcholanthrene  
 Formulation/Vehicle: **Volrrol**  
 Incubation & sampling time: On the day of the test, samples of cell culture were dispensed to sterile tubes, each containing 3.9 ml media. Freshly prepared S9 mix or media was added to each tube followed by the test solution, vehicle or positive control.  
 All tubes were incubated on rotating at 37 °C, 10 r.p.m. for 4 h. After this, the cells were gently regimented by centrifuge. The cells were returned to the rotating drum and allowed to express their genetic lesions at 37°C for 2 days.  
 On Day 2, cell counts were determined. All cultures were selected for expression of genetic damage. This was determined by performing 2 parallel cloning assays: the viability assay and the mutant selection assay.  
 For the mutant selection assay, trifluorothymidine (TFT) was added to cloning medium. All plates were gelled at room temperature until the agar had set, then incubated at 37 °C in an atmosphere of 5% CO<sub>2</sub> :95% air (v/v) until the colonies were fully developed (usually 14 days). The colonies were then counted using a "Domino" image analyzer.

## Study Validity

The criteria for a valid assay were met: e.g. 1) cloning efficiency of solvent control of 56-129% (soft agar), 2) spontaneous mutant frequencies in solvent control (between 35-140 mutant/10<sup>+6</sup> clonable above solvent control value. 3) Positive control showed at least 100 mutants/10<sup>+6</sup> clonable cells above solvent control. 4) Relative growth between 10-20%.

**Results**

- The preliminary toxicity test showed that a concentration of 156.25 µg/ml reduced the relative suspension growth to 40% and 14% in the absence and presence of S9 mix, respectively (see below the Sponsor’s tables). Higher concentrations were lethal.

Mouse Lymphoma Toxicity Test in the Absence of S9 Mix						
Chemical	Dose Level (µg.ml <sup>-1</sup> )	*Daily Suspension Count x 10 <sup>5</sup> .ml <sup>-1</sup> (ml Cells Kept )		Total	RSG %	
		Day 1	Day 2			
Water	(1 ml added)	12.4	(4.8)	17.2	23.7	100
Voltarol	19.5	11.4	(5.3)	14.9	18.9	80
	39.1	12.6	(4.8)	15.0	21.0	89
	78.125	9.2	(6.5)	14.5	14.8	62
	156.25	6.0	(10.0)	14.2	9.5	40
	312.5	0.2	(20)	0.6	0.2	1
	625	0	(-)	-	-	-
	1250	0	(-)	-	-	-
	2500	0	(-)	-	-	-

Mouse Lymphoma Toxicity Test in the Presence of S9 Mix						
Chemical	Dose Level (µg.ml <sup>-1</sup> )	*Daily Suspension Count x 10 <sup>5</sup> .ml <sup>-1</sup> (ml Cells Kept)		Total Suspension Growth	RSG %	
		Day 1	Day 2			
Water	(1 ml added)	12.4	(4.8)	18.2	25.1	100
Voltarol	19.5	11.0	(5.4)	18.2	22.2	88
	39.1	10.4	(5.8)	14.8	17.1	68
	78.125	9.0	(6.7)	15.7	15.7	63
	156.25	3.4	(17.6)	9.2	3.5	14
	312.5	0	(-)	-	-	-
	625	0	(-)	-	-	-
	1250	0	(-)	-	-	-
	2500	0	(-)	-	-	-

\* = Adjusted to 20 ml of 3 x 10<sup>5</sup>.ml<sup>-1</sup> after counting on Day 1  
RSG % = Relative suspension growth

- Mutant assay 1 (with S9 mix) and assay 2 (without S9 mix): Both assays were classed positive.

In the absence of S9 mix, no response was obtained at the lowest concentration of 130 µg/ml , while slight increases (1.5-1.9-fold) in mutant fraction were obtained compared to control values at the next 2 concentrations. The highest concentration of 250 µg/ml resulted in a mean increase of 3.55-fold. The mean RTG at this concentration was 21%.

In the presence of S9 mix, no increase was obtained at the lowest concentration of 130 µg/ml. At 170 µg/ml, a mean increase of 3.4-fold over control was obtained in mutant fraction (mean RTG = 33%). This increased to 13.3-fold at 210 µg/ml, where mean RTG was 16.5%. At the highest concentration of 250 µg/ml the RTG of both cultures was below the minimum acceptable 10%.

Mouse Lymphoma in the Absence of S9 Mix  
Data Summary  
(Assay 1)

Chemical	Dose Level (µg.ml <sup>-1</sup> )	Total Viable Count (VC)	Cloning Efficiency %	Suspension Growth	*Total Cell Growth	Relative Total Growth %	Total Mutant Count (MC)	Mutant Fraction x 10 <sup>6</sup> 200 (MC) (VC)	Fold Increase Over Control
					Vehicle Mean = 16.8			Vehicle Mean = 61.5	
Water	(100 µl added)	417	70	23.7	16.6	99	152	73	-
		543	91	20.4	18.6	111	175	64	
		473	79	20.2	16.0	96	105	44	
		446	74	21.4	15.8	94	144	65	
Ethyl methanesulphonate	250	329	55	17.3	9.5	57†	704	428	7.0
		351	59	18.0	10.6	63	520	296	4.8
Methyl methanesulphonate	10	226	38	14.7	5.6	33	334	296	4.8
		181	30	15.7	4.7	28	281	310	5.0
Voltarol	10	NPS	-	-	-	-	NPS	-	-
		NPS	-	-	-	-	NPS	-	-
	50	NPS	-	-	-	-	NPS	-	-
		NPS	-	-	-	-	NPS	-	-

Voltarol	90	NPS	-	-	-	-	NPS	-	-
		NPS	-	-	-	-	NPS	-	-
	130	402	67	17.8	11.9	77	71	35	0.7
		396	66	15.3	10.1	65	148	75	1.4
	170	348	58	6.4	4.9	32	284	163	3.0
		440	73	7.3	5.3	34	448	204	3.8
	210	365	61	4.7	2.9	19	828	454	8.4
		406	68	3.3	2.2	14	1981	976	18.2
	250	294	49	2.1	1.0	6	1198	815	15.2
		318	53	1.4	0.7	5	1652	1039	19.3

\* = Cloning efficiency x growth in suspension  
NPS = Not plated - surplus

Mouse Lymphoma in the Presence of S9 Mix (FLI 083)  
Data Summary  
(Assay 2)

Chemical	Dose Level (µg.ml <sup>-1</sup> )	Total Viable Count (VC)	Cloning Efficiency %	Suspension Growth	*Total Cell Growth	Relative Total Growth %	Total Mutant Count (MC)	Mutant Fraction x 10 <sup>6</sup> $\frac{200 (MC)}{(VC)}$	Fold Increase Over Control
					Vehicle Mean = 15.6			Vehicle Mean = 53.8	
Water	(100 µl added)	414	69	20.2	13.9	89	97	47	-
		440	73	19.8	14.5	93	160	73	
		491	82	22.0	18.0	116	91	37	
		441	74	21.3	15.8	102	128	58	
3-Methylcholanthrene	2.5	328	55	13.1	7.2	46	660	402	7.5
		267	45	15.0	6.8	44	593	444	8.3
Voltarol	10	NPS	-	-	-	-	NPS	-	-
		NPS	-	-	-	-	NPS	-	-
	50	NPS	-	-	-	-	NPS	-	-
		NPS	-	-	-	-	NPS	-	-

Voltarol	90	NPS	-	-	-	-	NPS	-	-
		NPS	-	-	-	-	NPS	-	-
	130	402	67	17.8	11.9	77	71	35	0.7
		396	66	15.3	10.1	65	148	75	1.4
	170	348	58	8.4	4.9	32	284	163	3.0
		440	73	7.3	5.3	34	448	204	3.8
	210	365	61	4.7	2.9	19	828	454	8.4
		406	68	3.3	2.2	14	1981	976	18.2
	250	294	49	2.1	1.0	6	1198	815	15.2
		318	53	1.4	0.7	5	1652	1039	19.3

\* = Cloning efficiency x growth in suspension  
NPS = Not plated - surplus

- Mutant assay 3 (with S9 mix) and assay 4 (without S9 mix): Both assays were classed positive.

In the absence of S9 mix, a single dose increase was obtained at the highest concentration of 250 µg/ml, giving a mean increase in mutant fraction of 2.25-fold compared to control (mean RTG = 18.5%).

In the presence of S9 mix, no increase was obtained at the lowest concentration of 130 µg/ml (mean RTG = 65.5%). At 170 µg/ml, a slight increase (1.65-fold) was obtained (mean RTG = 32%). At 210 µg/ml, a mean increase of 4.2-fold over

control was obtained in mutant fraction (mean RTG = 14%). At the highest concentration of 250 µg/ml the RTG of both cultures was below the minimum acceptable 10%.

Mouse Lymphoma in the Absence of S9 Mix  
Data Summary  
(Assay 3)

Chemical	Dose Level (µg.ml <sup>-1</sup> )	Total Viable Count (VC)	Cloning Efficiency %	Suspension Growth	*Total Cell Growth	Relative Total Growth %	Total Mutant Count (MC)	Mutant Fraction x 10 <sup>6</sup> / 200 (MC) (VC)	Fold Increase Over Control
					Vehicle Mean = 17.6			Vehicle Mean = 38.5	
Water	(100 µl added)	616	103	16.5	17.0	97	110	36	-
		534	89	16.7	14.9	85	107	40	
		625	104	18.5	19.2	109	148	47	
		540	90	21.3	19.2	109	85	31	
Ethyl methanesulphonate	250	430	72	12.3	8.9	51	595	277	7.2
		413	69	15.2	10.5	60	616	298	7.7
Methyl methanesulphonate	10	295	49	12.2	6.0	34	435	295	7.7
		285	48	11.0	5.3	30	394	276	7.2
Voltarol	10	NPS	-	-	-	-	NPS	-	-
		NPS	-	-	-	-	NPS	-	-
	50	NPS	-	-	-	-	NPS	-	-
		NPS	-	-	-	-	NPS	-	-
Voltarol	90	NPS	-	-	-	-	NPS	-	-
		NPS	-	-	-	-	NPS	-	-
	130	529	88	12.6	11.1	63	112	42	1.1
		615	103	11.8	12.2	69	127	41	1.1
	170	544	91	8.6	7.8	44	115	42	1.1
		553	92	9.8	9.0	51	93	34	0.9
	210	527	88	6.1	5.4	31	129	49	1.3
		525	88	5.7	5.0	28	117	45	1.2
	250	541	90	3.8	3.4	19	241	89	2.3
		533	89	3.6	3.2	18	229	86	2.2

\* = Cloning efficiency x growth in suspension  
NPS = Not plated - surplus

Mouse Lymphoma in the Presence of S9 Mix (FLI 084)  
Data Summary  
(Assay 4)

Chemical	Dose Level (µg.ml <sup>-1</sup> )	Total Viable Count (VC)	Cloning Efficiency %	Suspension Growth	*Total Cell Growth	Relative Total Growth %	Total Mutant Count (MC)	Mutant Fraction x 10 <sup>4</sup> (VC)	Fold Increase Over Control
					Vehicle Mean = 14.8			Vehicle Mean = 40.5	
Water	(100 µl added)	547	91	15.1	13.7	93	105	38	-
		578	96	15.6	15.0	102	118	41	
		552	92	16.6	15.3	104	90	33	
		614	102	14.7	15.0	102	153	50	
3-Methylcholanthrene	2.5	315	53	5.3	2.8	19	668	424	10.5
		322	54	4.9	2.6	18	607	377	9.3
Voltarol	10	NPS	-	-	-	-	NPS	-	-
		NPS	-	-	-	-	NPS	-	-
	50	NPS	-	-	-	-	NPS	-	-
		NPS	-	-	-	-	NPS	-	-

Voltarol	90	NPS	-	-	-	-	NPS	-	-
		NPS	-	-	-	-	NPS	-	-
	130	579	97	9.8	9.5	64	94	32	0.8
		609	102	9.7	9.9	67	100	33	0.8
	170	423	71	5.8	4.1	28	169	80	2.0
		547	91	5.8	5.3	36	141	52	1.3
	210	347	58	3.7	2.1	14	239	138	3.4
		380	63	3.3	2.1	14	365	203	5.0
	250	318	53	2.0	1.1	7	441	277	6.8
		329	55	1.5	0.8	5	382	232	5.7

\* = Cloning efficiency x growth in suspension  
NPS = Not plated - surplus

#### 7.4 *In Vivo* mouse micronucleus test

Study title: **DF-HPβCD Micronucleus Test by Intravenous Administration in Bone Marrow of CD-1 Mice**

Study no.: NC-DFC-012 (eCTD 4.2.3.3.2.1)

Study report location: (b) (4)

Conducting laboratory and location: (b) (4)

Date of study initiation: July 22, 1997

GLP compliance: Yes

QA statement: Yes

Drug, lot #, purity: DF-HPβCD (**DIC075U**), # R&Q 227, 96.4%

#### Key Study Findings

- The *in-vivo* genotoxic potential of DIC075U was evaluated in micronuclei in bone marrow erythrocytes of male and female CD-1 mice using a 0h IV dosing and 24h and 48h sampling regimen.
- Based on the preliminary toxicity study, the maximum tolerated dose of DIC075U by IV route was 120 mg/kg
- Three groups were dosed at concentrations of 30, 60 and 120 mg/kg. A control group receiving vehicle (water) and a positive control receiving cyclophosphamide (CPH).
- DIC075U did not induce micronuclei in bone marrow cells when tested to the maximum tolerated dose of 120 mg/kg.

#### Methods

Strains: CD-1 mice

Number/sex/group: See below

Concentrations in definitive study (μg/ml): 30, 60 and 120 mg/kg

Basis of concentration selection: Base on maximum tolerated dose (MTD)

Negative control: water

Positive control: CPH (cyclophosphamide), 50mg/kg

Formulation/Vehicle: **DIC075U** ( (b) (4) Diclofenac Sodium + (b) (4) HPβCD) / (b) (4) Sterile Water for Injection USP

The mice were observed for clinical signs or mortality at frequent intervals post dosing, then twice daily prior to the scheduled kills for bone marrow (femur) preparation. Bone

marrow samples were taken 24h after dosing from all dose groups and at 48h from the vehicle and high dose groups.

Dose Group	Test Dose	Treatment and Number of Mice		
		Dosing (h)	24 h Sample	48 h Sample
Vehicle Control	10 ml water for injection.kg <sup>-1</sup>	0	5♂ + 5♀	5♂ + 5♀
Low Dose	30 mg DF-HPβCD.kg <sup>-1</sup>	0	5♂	
Mid Dose	60 mg DF-HPβCD.kg <sup>-1</sup>	0	5♂	
High Dose	120 mg DF-HPβCD.kg <sup>-1</sup>	0	5♂ + 5♀	5♂ + 5♀
Positive Control	50 mg Cyclophosphamide.kg <sup>-1</sup>	0	5♂	

Femur was removed for marrow extraction from five surviving animals in each treatment and control group. The bone marrow was flushed and smears were taken on slides. 2000 polychromatic erythrocytes (PCE) per control animal were analyzed for the frequency of micronuclei. The PCE/NCE ratio, a measure of any induced systemic toxicity, was determined by counting a minimum total of 1000 erythrocytes (PCE+ NCE) per marrow preparation.

### Study Validity

The criteria for a valid assay were met: e.g. 1) Dosing appeared to be adequate based upon the results of the dose range finding, 2) Positive controls exhibited appropriate responses, 3) The proportion of immature erythrocytes among total erythrocytes was not less than 20% of the control value (no bone marrow suppression), 4) % Micronucleated PCEs and the PCE/NCE ratio in the negative control and the positive control groups were within the historical data range for the testing laboratory.

### Results

In dose range finding, mice were dosed intravenously at 0h as follows:

Treatment Group	No. of Mice	Dose Level (mg.kg <sup>-1</sup> )
1	1♂ + 1♀	5
2	1♂ + 1♀	12.5
3	1♂ + 1♀	35
4	1♂ + 1♀	80
5	1♂ + 1♀	200

At 200 mg/kg clinical signs were laboured breathing, subdued behavior, prostration, and convulsions, rolling gait, hunched appearance and cold. One animal was killed in extremis and one died on day1. At 80mg/kg the animals were subdued with rolling gait and hunched appearance on day 1. No clinical sign were observed at lower doses.

- In the main toxicity test, findings were summarized as followed :

DF-HP $\beta$ CD  
 Micronucleus Test By Intravenous Administration in Bone Marrow of CD-1 Mice  
 Toxicity Study - Main Toxicity Test  
 Clinical Signs and Deaths

Dose Level (mg.kg <sup>-1</sup> )	Animal Nos.	Clinical Signs Observed (Day 1 Dosing Day)		No. of Deaths
80	11-13♂	Day 1	NAD.	0
	20-22♀	Days 2-4	NAD.	
120	14-16♂	Day 1	Subdued behaviour, rolling gait, hunched appearance, piloerection.	0
	23-25♀	Days 2-4	Subdued behaviour, tremors, hunched appearance, discharge (eyes), piloerection, cold, KIE.	
160	17-19♂	Day 1	Subdued behaviour, rolling gait, hunched appearance, FDC.	1
	26-28♀	Days 2-4	Piloerection, swollen abdomen, subdued behaviour, tremors, rolling gait, hunched appearance, cold, KIE.	

NAD = No abnormalities detected

KIE = Killed in *extremis*

FDC = Found dead in cage.

At 160 mg/kg 3 deaths and at 120 mg/kg 1 death occurred. At 120 and 160 mg/kg, subdued behavior, hunched appearance, piloerection, rolling gait, tremors, agitated, cold, discharge (eyes) and swollen abdomen were observed.

- In micronucleus test, two males died in 120 mg. Clinical signs in this group included subdued behavior, rolling gait, hunched appearance, discharge (eyes), piloerection and pale appearance.

There was no indication that DIC075U induced bone marrow micronuclei in the treated mice. The highest micronucleated PCE (MN-PCD) frequency recorded for the test article was in the HD males samples at 24h (incidence of 0.08%). The positive control (CPH) induced large increases in bone marrow micronuclei (incidence of 1.15%). See below the Sponsor's table.

**TABLE 3**  
**DF-HP $\beta$ CD**  
**Micronucleus Test By Intravenous Administration in Bone Marrow of CD-1 Mice**  
**Summary of Assessment Data**

Treatment	Time of Sample (h)	Sex	No. of Mice Assessed	Erythrocytes				PCE/NCE Mean $\pm$ S.D.
				Normochromatic Cells (NCE)	Polychromatic Cells (PCE)		PCE/NCE	
				No. of MN-NCE	PCE Analysed	No. of MN-PCE		
10 ml water for injection kg <sup>-1</sup>	24	♂	5 (5)	0	10000	4	0.04	1.09 $\pm$ 0.38
		♀	5 (5)	2	10000	6	0.06	
		♂♀	10 (10)	2	20000	10	0.05	
10 ml water for injection kg <sup>-1</sup>	48	♂	5 (5)	0	10000	1	0.01	1.76 $\pm$ 0.38
		♀	5 (5)	0	10000	5	0.05	
		♂♀	10 (10)	0	20000	6	0.03	
30 mg DF-HP $\beta$ CD kg <sup>-1</sup>	24	♂	5 (5)	0	10000	6	0.06	1.10 $\pm$ 0.45
60 mg DF-HP $\beta$ CD kg <sup>-1</sup>	24	♂	5 (5)	1	10000	3	0.03	0.94 $\pm$ 0.35
120 mg DF-HP $\beta$ CD kg <sup>-1</sup>	24	♂	5 (4)	3	8000	6	0.08	0.86 $\pm$ 0.28
		♀	5 (5)	3	10000	3	0.03	
		♂♀	10 (9)	6	18000	9	0.05	
120 mg DF-HP $\beta$ CD kg <sup>-1</sup>	48	♂	5 (4)	1	8000	4	0.05	1.69 $\pm$ 0.63
		♀	5 (5)	1	10000	4	0.04	
		♂♀	10 (9)	2	18000	8	0.04	
50 mg Cyclophosphamide. kg <sup>-1</sup>	24	♂	5 (5)	3	10000	115 $\Phi$	1.15	1.10 $\pm$ 0.38

PCE = Polychromatic erythrocytes  
 MN-PCE = Micronucleated PCE  
 NCE = Normochromatic erythrocytes  
 MN-NCE = Micronucleated NCE  
 $\Phi$  = Positive response in PCE

## 7.5 Other Genetic Toxicity Studies (for an impurity)

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

Study title: **Bacterial Reverse Mutation Assay with a Confirmatory Assay**

Study no.: NC-DFC-016 (eCTD 4.2.3.7.6.1)

Study report location: [REDACTED] (b) (4)

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

Date of study initiation: July 07, 2009

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: [REDACTED] (b) (4)

lot # 71206B, purity: 99.6%

**Key Study Findings**

- [REDACTED] (b) (4) was tested in the Ames Reverse Mutation Assays at concentrations of 1.6 to 5000µg/plate.
- In the initial mutagenicity assay and the confirmatory mutagenicity assay (plate incorporation method) with and without S9, revertant frequencies for all doses of the test article were less than control values.
- Normal growth was observed in all five tester strains in both assays.
- Precipitation of the test material was observed at 500, 1600 and 5000µg/plate.
- Under the conditions of the study, [REDACTED] (b) (4) was concluded to be negative in the bacterial reverse mutation assay when tested up to maximum limit of 5000µg/plate.

**Methods**

Strains: *S. typhimurium* TA98, TA100, TA1535, TA1537, and *E. coli* WP2 *uvrA*.  
 Concentrations in initial mutagenicity study: 1.60, 5.00, 16.0, 50.0, 160, 500, 1600, and 5000 µg/plate  
 Concentrations in definitive study (confirmatory assay): 5, 50, 50, 500, 500, 2500 and 5000 µg/plate (see protocol deviation)  
 Basis of concentration selection: Maximum recommended concentrations of test article  
 Negative control: Dimethylsulfoxide (DMSO)

## Positive control:

Tester Strain(s)	S9	Positive Control	Dose (µg/plate)
TA98	-	2-nitrofluorene	1.0
TA100, TA1535	-	sodium azide	2.0
TA1537	-	ICR-191	2.0
WP2 <i>uvrA</i>	-	4-nitroquinoline-N-oxide	1.0
TA98	+	benzo[a]pyrene	2.5
TA100, TA1535, TA1537	+	2-aminoanthracene	2.5
WP2 <i>uvrA</i>	+	2-aminoanthracene	25.0

Formulation/Vehicle: (b) (4) / DMSO

Incubation & sampling time:
 

- The plate incorporation method: Incubation was for 52 ± 4 hours at 37 ± 2°C.

Protocol Deviation: In the confirmatory mutagenicity assay (Trial C1), the second stock was dosed at 500 µg/plate instead of 1600 µg/plate, the fourth stock was dosed at 50.0 µg/plate instead of 160 µg/plate, and the sixth stock was dosed at 5.00 µg/plate instead of 16.0 µg/plate. The top dose of 5000 µg/plate was accurate, and the second dose in the initial trial (1600 µg/plate) showed no toxicity or increased revertant counts. The Sponsor stated that since this trial is the confirmatory mutagenicity assay, and it is confirming the results of the initial mutagenicity assay, the fact that the 1600, 160, and 16.0 µg/plate (doses used in the initial mutagenicity assay) doses were not accurate has no impact on study integrity.

**Study Validity**

All positive and vehicle control values were within acceptable ranges, and all criteria for a valid study were met.

**Results**

The test article did not produce any increases in the number of revertants in any tester strain under the conditions tested (see the Sponsor's tables). This is in concurrence with the Sponsor's conclusions.

**Initial Mutagenicity Assay Results with S9**

Study No.: 8213159  
 Trial No.: 8213159-B1  
 Plating Method: Plate incorporation assay

Date Plated: 7/16/2009  
 Date Counted: 7/21/2009 to 7/23/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/vehicle	Individual revertant colony counts
TA98	(b) (4)	5000	12.0	0.0	0.9	12 PM N, 12 PM N
		1600	18.0	2.8	1.3	20 PM N, 16 PM N
		500	19.5	0.7	1.4	19 N P, 20 MN P
		160	12.5	0.7	0.9	13 N, 12 N
		50.0	15.5	4.9	1.1	19 N, 12 N
		16.0	19.5	0.7	1.4	19 N, 20 MN
		5.00	15.0	2.8	1.1	13 N, 17 N
		1.60	13.5	3.5	1.0	16 N, 11 MN
		Dimethyl Sulfoxide	13.5	3.5		11 N, 16 MN
		TA100	(b) (4)	5000	56.5	6.4
1600	26.0			5.7	0.3	22 PM N, 30 PM N
500	73.0			22.6	0.8	89 N P, 57 MN P
160	100.5			9.2	1.1	94 MN, 107 N
50.0	86.5			9.2	1.0	93 N, 80 N
16.0	94.5			6.4	1.1	90 N, 99 N
5.00	106.0			9.9	1.2	113 N, 99 N
1.60	116.5			3.5	1.3	114 N, 119 N
Dimethyl Sulfoxide	89.5			10.6		97 N, 82 N
TA1535	(b) (4)			5000	8.5	0.7
		1600	6.0	2.8	0.5	4 PM N, 8 PM N
		500	10.5	0.7	0.8	10 MN P, 11 MN P
		160	12.0	1.4	1.0	11 N, 13 N
		50.0	12.0	0.0	1.0	12 N, 12 MN
		16.0	7.0	0.0	0.6	7 N, 7 MN
		5.00	11.5	4.9	0.9	8 MN, 15 N
		1.60	12.0	9.9	1.0	19 MN, 5 MN
		Dimethyl Sulfoxide	12.5	0.7		12 N, 13 N
		TA1537	(b) (4)	5000	5.0	1.4
1600	4.0			2.8	0.9	2 PM N, 6 PM N
500	8.5			2.1	1.9	10 N P, 7 N P
160	8.0			2.8	1.8	10 MN, 6 N
50.0	4.5			0.7	1.0	4 N, 5 MN
16.0	5.5			2.1	1.2	7 N, 4 N
5.00	8.5			0.7	1.9	9 MN, 8 N
1.60	6.0			0.0	1.3	6 MN, 6 MN
Dimethyl Sulfoxide	4.5			0.7		5 N, 4 N
WP2avrA	(b) (4)			5000	12.0	1.4
		1600	9.5	3.5	0.5	12 PM N, 7 PM N
		500	12.0	1.4	0.6	13 N P, 11 MN P
		160	11.5	0.7	0.6	12 MN, 11 N
		50.0	14.0	1.4	0.7	15 N, 13 N
		16.0	14.0	2.8	0.7	16 N, 12 N
		5.00	13.0	2.8	0.7	11 N, 15 MN
		1.60	17.0	1.4	0.9	16 N, 18 MN
		Dimethyl Sulfoxide	19.5	3.5		22 N, 17 N
		TA98	BP	2.5	452.0	76.4
TA100	2AA	2.5	2171.5	126.6	24.3	2261 N, 2082 N
TA1535	2AA	2.5	212.0	32.5	17.0	235 N, 189 N
TA1537	2AA	2.5	123.0	5.7	27.3	119 N, 127 N
WP2avrA	2AA	25.0	686.0	86.3	35.2	747 N, 625 N

Key to Positive Controls

BP Benzo[a]pyrene  
 2AA 2-aminoanthracene

Key to Plate Postfix Codes

P Precipitation of test article observed  
 M Plate counted manually  
 N Normal background bacterial lawn

**Initial Mutagenicity Assay Results without S9**

Study No.: 8213159

Trial No.: 8213159-B1

Plating Method: Plate incorporation assay

Date Plated: 7/16/2009

Date Counted: 7/21/2009 to 7/23/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/vehicle	Individual revertant colony counts	
TA98	(b) (4)	5000	9.0	2.8	0.7	7 P M N, 11 P M N	
		1600	9.0	2.8	0.7	11 P M N, 7 P M N	
		500	13.0	4.2	1.0	16 N P, 10 N P	
		160	11.0	9.9	0.8	18 N, 4 M N	
		50.0	9.5	2.1	0.7	8 M N, 11 N	
		16.0	7.0	1.4	0.5	6 M N, 8 M N	
		5.00	15.0	2.8	1.2	17 N, 13 N	
		1.60	16.5	2.1	1.3	15 N, 18 N	
			Dimethyl Sulfoxide		13.0	1.4	14 M N, 12 M N
		TA100	(b) (4)	5000	42.0	17.0	0.6
1600	41.0			7.1	0.6	36 P M N, 46 P M N	
500	64.5			3.5	0.9	67 N P, 62 N P	
160	100.5			13.4	1.4	91 N, 110 N	
50.0	62.0			4.2	0.9	59 N, 65 N	
16.0	49.5			3.5	0.7	47 N, 52 M N	
5.00	69.0			7.1	1.0	74 N, 64 N	
1.60	67.5			6.4	0.9	72 N, 63 N	
	Dimethyl Sulfoxide				71.5	3.5	74 N, 69 N
TA1535	(b) (4)			5000	11.5	6.4	1.4
		1600	7.0	0.0	0.9	7 P M N, 7 P M N	
		500	10.5	0.7	1.3	11 N P, 10 N P	
		160	16.0	0.0	2.0	16 M N, 16 N	
		50.0	10.0	1.4	1.3	11 N, 9 M N	
		16.0	12.5	0.7	1.6	12 N, 13 N	
		5.00	6.5	2.1	0.8	5 M N, 8 N	
		1.60	11.0	1.4	1.4	10 N, 12 M N	
			Dimethyl Sulfoxide		8.0	1.4	9 M N, 7 N
		TA1537	(b) (4)	5000	1.0	0.0	0.2
1600	0.5			0.7	0.1	0 P M N, 1 P M N	
500	4.0			2.8	0.8	6 M N P, 2 M N P	
160	5.5			2.1	1.1	7 N, 4 N	
50.0	3.0			1.4	0.6	2 M N, 4 M N	
16.0	6.5			3.5	1.3	4 N, 9 M N	
5.00	4.0			0.0	0.8	4 N, 4 M N	
1.60	3.5			2.1	0.7	5 N, 2 M N	
	Dimethyl Sulfoxide				5.0	1.4	4 M N, 6 N
WP2uvrA	(b) (4)			5000	11.5	0.7	0.5
		1600	8.5	3.5	0.4	11 P M N, 6 P M N	
		500	13.0	2.8	0.6	11 N P, 15 N P	
		160	16.5	3.5	0.8	19 N, 14 N	
		50.0	18.0	1.4	0.9	19 M N, 17 M N	
		16.0	17.5	2.1	0.8	16 N, 19 N	
		5.00	13.0	7.1	0.6	8 M N, 18 N	
		1.60	14.0	1.4	0.7	13 N, 15 N	
			Dimethyl Sulfoxide		21.0	4.2	18 N, 24 N
		TA98	2NF	1.0	278.0	1.4	21.4
TA100	SA	2.0	1110.5	17.7	15.5	1098 N, 1123 N	
TA1535	SA	2.0	910.5	9.2	113.8	904 N, 917 N	
TA1537	ICR	2.0	374.5	53.0	74.9	337 N, 412 N	
WP2uvrA	4NQO	1.0	202.5	3.5	9.6	205 N, 200 N	

Key to Positive Controls		Key to Plate Postfix Codes	
2NF	2-nitrofluorene	P	Precipitation of test article observed
SA	Sodium azide	M	Plate counted manually
ICR	ICR-191	N	Normal background bacterial lawn
4NQO	4-nitro-quinoline-oxide		

**Confirmatory Mutagenicity Assay Results with S9**

Study No.: 8213159  
 Trial No.: 8213159-C1

Plating Method: Plate incorporation assay

Date Plated: 7/28/2009  
 Date Counted: 7/30/2009 to 7/31/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/vehicle	Individual revertant colony counts
TA98	(b) (4)	5000	7.0	1.0	0.3	7 P M N, 8 P M N, 6 P M N
		500	16.0	5.6	0.7	22 P N, 15 P N, 11 P N
		500	18.0	3.0	0.8	15 P N, 18 P N, 21 P N
		50.0	25.0	6.9	1.1	17 N, 29 N, 29 N
		50.0	20.0	1.7	0.9	18 N, 21 N, 21 N
		5.00	15.7	5.5	0.7	21 N, 10 N, 16 N
	Dimethyl Sulfoxide		23.3	5.0		18 M N, 24 N, 28 N
TA100	(b) (4)	5000	51.0	14.5	0.5	37 P M N, 50 P M N, 66 P M N
		500	60.0	16.4	0.5	64 P N, 74 P N, 42 P N
		500	70.0	10.8	0.6	79 P N, 58 P N, 73 P N
		50.0	97.0	9.5	0.9	103 N, 102 N, 86 N
		50.0	103.3	16.7	0.9	113 N, 113 N, 84 N
		5.00	89.0	14.7	0.8	80 N, 81 N, 106 N
	Dimethyl Sulfoxide		109.7	7.0		103 N, 109 N, 117 N
TA1535	(b) (4)	5000	7.0	3.0	0.9	7 P M N, 10 P M N, 4 P M N
		500	10.0	7.0	1.3	7 P N, 18 P N, 5 P N
		500	4.3	0.6	0.6	4 P N, 5 P N, 4 P N
		50.0	8.0	2.0	1.0	10 N, 6 N, 8 N
		50.0	8.3	4.2	1.1	13 N, 5 N, 7 N
		5.00	9.3	5.9	1.2	16 N, 5 N, 7 N
	Dimethyl Sulfoxide		7.7	2.1		10 N, 6 N, 7 N
TA1537	(b) (4)	5000	2.7	0.6	0.4	2 P M N, 3 P M N, 3 P M N
		500	4.7	2.3	0.6	6 P N, 2 P N, 6 P N
		500	8.0	4.4	1.1	13 P N, 6 P N, 5 P N
		50.0	6.0	1.0	0.8	7 N, 6 N, 5 N
		50.0	9.7	2.9	1.3	13 N, 8 N, 8 N
		5.00	6.0	2.0	0.8	6 N, 8 N, 4 N
	Dimethyl Sulfoxide		7.3	3.5		4 N, 7 N, 11 N
WP2 <sup>uvrA</sup>	(b) (4)	5000	8.7	5.5	0.7	14 P M N, 3 P M N, 9 P M N
		500	11.0	6.0	0.9	17 P N, 11 P N, 5 P N
		500	12.3	1.2	1.0	11 P N, 13 P N, 13 P N
		50.0	15.7	4.2	1.2	17 N, 11 N, 19 N
		50.0	14.3	2.1	1.1	16 N, 12 N, 15 N
		5.00	15.0	4.4	1.2	17 N, 10 N, 18 N
	Dimethyl Sulfoxide		12.7	5.5		7 N, 13 N, 18 N
TA98	BP	2.5	297.0	96.7	12.7	186 N, 363 N, 342 N
TA100	2AA	2.5	3371.0	125.9	30.7	3277 N, 3322 N, 3514 N
TA1535	2AA	2.5	362.3	29.2	47.3	347 N, 344 N, 396 N
TA1537	2AA	2.5	222.3	16.3	30.3	228 N, 235 N, 204 N
WP2 <sup>uvrA</sup>	2AA	25.0	540.3	68.6	42.7	464 N, 560 N, 597 N

Key to Positive Controls

BP Benzo[a]pyrene  
 2AA 2-aminoanthracene

Key to Plate Postfix Codes

P Precipitation of test article observed  
 M Plate counted manually  
 N Normal background bacterial lawn

**Confirmatory Mutagenicity Assay Results without S9**

Study No.: 8213159

Trial No.: 8213159-C1

Plating Method: Plate incorporation assay

Date Plated: 7/28/2009

Date Counted: 7/30/2009 to 7/31/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	(b) (4)	5000	6.0	1.0	0.5	5 P M N, 6 P M N, 7 P M N
		500	13.7	2.3	1.1	11 P N, 15 P N, 15 P N
		500	16.3	4.2	1.3	21 P N, 15 P N, 13 P N
		50.0	12.3	2.5	1.0	12 N, 10 N, 15 N
		50.0	11.3	2.1	0.9	12 N, 9 N, 13 N
		5.00	9.7	1.5	0.8	10 N, 8 N, 11 N
	Dimethyl Sulfoxide		12.3	1.2		13 N, 13 N, 11 N
TA100	(b) (4)	5000	65.7	5.0	0.5	71 P M N, 61 P M N, 65 P M N
		500	81.0	5.6	0.7	75 P N, 82 P N, 86 P N
		500	88.0	9.5	0.7	87 P N, 98 P N, 79 P N
		50.0	107.0	8.9	0.9	117 N, 104 N, 100 N
		50.0	88.3	10.8	0.7	96 N, 93 N, 76 N
		5.00	101.7	13.9	0.8	98 N, 90 N, 117 N
	Dimethyl Sulfoxide		120.7	2.1		123 N, 119 N, 120 N
TA1535	(b) (4)	5000	12.3	5.5	0.5	18 P M N, 7 P M N, 12 P M N
		500	19.3	3.5	0.7	16 P N, 19 P N, 23 P N
		500	40.0	14.1	1.5	27 P N, 38 P N, 55 P N
		50.0	41.0	3.0	1.5	41 N, 44 N, 38 N
		50.0	30.0	7.0	1.1	33 N, 35 N, 22 N
		5.00	20.3	13.6	0.8	36 N, 13 N, 12 N
	Dimethyl Sulfoxide		26.7	16.9		46 M N, 15 N, 19 N
TA1537	(b) (4)	5000	5.3	0.6	1.1	5 P M N, 5 P M N, 6 P M N
		500	3.3	3.2	0.7	1 P N, 2 P N, 7 P N
		500	4.0	3.0	0.8	7 P N, 4 P N, 1 P N
		50.0	4.7	1.2	0.9	6 N, 4 N, 4 N
		50.0	5.7	2.1	1.1	4 N, 8 N, 5 N
		5.00	2.7	1.2	0.5	4 N, 2 N, 2 N
	Dimethyl Sulfoxide		5.0	3.0		2 N, 5 N, 8 N
WP2uvrA	(b) (4)	5000	11.7	4.5	1.2	7 P M N, 12 P M N, 16 P M N
		500	16.0	3.0	1.6	19 P N, 13 P N, 16 P N
		500	11.7	4.0	1.2	11 P N, 16 P N, 8 P N
		50.0	11.3	5.5	1.1	17 N, 11 N, 6 N
		50.0	11.7	5.5	1.2	12 N, 6 N, 17 N
		5.00	19.7	3.8	2.0	17 N, 24 N, 18 N
	Dimethyl Sulfoxide		10.0	3.6		6 M N, 13 N, 11 N
TA98	2NF	1.0	249.7	43.2	20.2	245 N, 209 N, 295 N
TA100	SA	2.0	1024.3	113.8	8.5	906 N, 1034 N, 1133 N
TA1535	SA	2.0	818.3	107.6	30.7	856 N, 697 N, 902 N
TA1537	ICR	2.0	419.7	74.9	83.9	452 N, 473 N, 334 N
WP2uvrA	4NQO	1.0	140.7	30.7	14.1	126 N, 120 N, 176 N

Key to Positive Controls

Key to Plate Postfix Codes

2NF 2-nitrofluorene

P

Precipitation of test article observed

SA Sodium azide

M

Plate counted manually

ICR ICR-191

N

Normal background bacterial lawn

4NQO 4-nitro-quinoline-oxide

### 7.5.2 *In Vitro* Chromosomal aberration assay

Study title: **Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes**

Study no.: NC-DFC-017 (eCTD 4.2.3.7.6.2)

Study report location: (b) (4)

Conducting laboratory and location: (b) (4)

Date of study initiation: July 06, 2009

GLP compliance: Yes

QA statement: Yes

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

lot # 71206B, purity: 99.6%

#### Key Study Findings

- (b) (4) was tested in the Chromosomal Aberration assay in cultured human peripheral blood lymphocytes
- The treatment period was for 3 and ~ 22 hours without metabolic activation and 3 hours with metabolic activation
- The high doses selected for analysis in the assay had a precipitate at the end of the treatment or  $\geq 50\%$  reduction in mitotic index
- Cultures treated with concentrations of 58.8, 84.0, and 120  $\mu\text{g}/\text{mL}$  without metabolic activation (3-hour treatment), 120, 172, and 245  $\mu\text{g}/\text{mL}$  without metabolic activation (~ 22-hour treatment), and 120, 172, and 245  $\mu\text{g}/\text{mL}$  with metabolic activation were analyzed for chromosomal aberrations.
- No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed.
- The test article, (b) (4) was considered negative for inducing chromosomal aberrations in cultured human lymphocytes without and with metabolic activation.

**Methods**

Cell line: cultured human peripheral blood lymphocytes

Concentrations in definitive study:
 

- 58.8, 84.0, and 120 µg/mL without metabolic activation (3-hour treatment),
- 120, 172, and 245 µg/mL without metabolic activation (~ 22-hour treatment),
- 120, 172, and 245 µg/mL with metabolic activation

Basis of concentration selection: solubility and mitotic index

Negative control: DMSO

Positive control: Mitomycin C (MMC), Cyclophosphamide (CP)

Formulation/Vehicle: (b) (4) / DMSO

Incubation & sampling time:

S9Activation Mix	Test Article Added	Exposure Completed	Colcemid® Added	Harvest Started
Without	0	3	20	22
Without	0	22	20	22
With	0	3	20	22

**Study Validity**

The following criteria for a valid assay were met: 1) the dose selection based upon solubility and mitotic index was acceptable for both non-activated and activated system; 2) the percentage of cells with aberrations in the negative and vehicle control did not exceed 5%, 3) the positive control produced significant chromosomal aberrations of the cells; 4) a minimum of 200 metaphase spreads (100 per duplicate treatment condition) were examined and scored for chromatid-type and chromosome-type aberrations

**Results**

DMSO was the vehicle for this assay. The highest concentration tested in the assay, 500 µg/mL, was above the solubility limit of the formulated (b) (4) after dosing into culture medium.

- In the assay without metabolic activation with a 3-hour treatment, mitotic index data are presented in below table:

**Table 1: Assessment of Toxicity for Chromosomal Aberrations Assay - Without Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest**

Study No.: 8213158      Trial No.: B1      Date: 07/16/09  
 Test Article: (b) (4)

Treatment			% Mitotic Index A Culture	% Mitotic Index B Culture	Average % Mitotic Index	% Mitotic Index Reduction
Vehicle Control	DMSO	10.0 µL/mL	7.5	5.3	6.4	0
Test Article		58.8 µg/mL	8.5	8.8	8.7	0
		84.0 µg/mL <sup>b</sup>	6.5	7.1	6.8	0
		120 µg/mL <sup>b</sup>	3.1 <sup>a</sup>	2.7 <sup>a</sup>	2.9	55
		172 µg/mL <sup>c</sup>	2.6 <sup>a</sup>	2.8 <sup>a</sup>	2.7	58
		245 µg/mL <sup>c</sup>	4.7 <sup>a</sup>	4.8 <sup>a</sup>	4.8	25
		350 µg/mL <sup>c</sup>	7.2 <sup>a</sup>	5.5 <sup>a</sup>	6.4	0
		500 µg/mL <sup>c</sup>	5.5 <sup>a</sup>	6.0 <sup>a</sup>	5.8	9

<sup>a</sup> Sparse number of cells present on slide.

<sup>b</sup> Precipitate observed at dose.

<sup>c</sup> Precipitate observed at dose, wash, and harvest.

DMSO = dimethylsulfoxide

Chromosomal aberrations were analyzed from the cultures treated with 58.8, 84.0, and 120 µg/mL. The high dose selected for analysis, 120 µg/mL, had a 55% reduction in mitotic index as compared with the vehicle control cultures. No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed (see below table).

**Table 2: Chromosomal Aberrations in Human Lymphocytes - Without Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest**

Study No.: 8213158      Trial No.: B1      Date: 07/16/09      Test Article: (b) (4)

	# Cells Scored for Aberrations	% Mitotic Index Reduction <sup>a</sup>	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judge-ment (+/-) <sup>b</sup>	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations						Judge-ment (+/-) <sup>d</sup>	
							gaps	simple breaks	chte	chre	mab	Totals <sup>c</sup>		
												-g		+g
<b>Controls</b>														
Vehicle:	DMSO	10.0 µL/mL	A	100	0	0	2					0	2	
			B	100	0	0		1				1	1	
			Total	200			2	1				1	3	
	Average	%	0	0.0	0.0	-	1.0	0.5			0.5	1.5		
Positive:	MMC	1.00 µg/mL	A	50	0	0	7	19	7			21	24	
			B	50	0	0	6	19	9			21	23	
			Total	100			13	38	16			42	47	
			Average	%	--	0.0	0.0	-	13.0	38.0	16.0		42.0	47.0
Test Article	58.8 µg/mL		A	100	0	0	3					0	3	
			B	100	0	0	4	2				2	6	
			Total	200			7	2				2	9	
			Average	%	0	0.0	0.0	-	3.5	1.0			1.0	4.5
			84.0 µg/mL	A	100	0	0	5	1				1	6
				B	100	0	0	3					0	3
	Total	200			8	1				1	9			
	Average	%	0	0.0	0.0	-	4.0	0.5			0.5	4.5		
	120 µg/mL	A	100	0	0	6	1				1	7		
		B	100	0	0	6	1				1	7		
		Total	200			12	2				2	14		
		Average	%	55	0.0	0.0	-	6.0	1.0			1.0	7.0	

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

<sup>a</sup> % Mitotic index reduction as compared to the vehicle control.

<sup>b</sup> Significantly greater in % polyploidy and % endoreduplication than the vehicle control, p ≤ 0.01.

<sup>c</sup> -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

<sup>d</sup> Significantly greater in -g than the vehicle control, p ≤ 0.01.      DMSO = dimethylsulfoxide      MMC = Mitomycin C

- In the assay without metabolic activation with a ~22-hour treatment, mitotic index data are presented in below table.

**Table 3: Assessment of Toxicity for Chromosomal Aberrations Assay - Without Metabolic Activation - ~22-Hour Treatment, ~22-Hour Harvest**

Study No.: 8213158 Trial No.: B1 Date: 07/16/09  
 Test Article: (b) (4)

Treatment			% Mitotic Index A Culture	% Mitotic Index B Culture	Average % Mitotic Index	% Mitotic Index Reduction
Vehicle Control	DMSO	10.0 µL/mL	10.3	10.5	10.4	0
Test Article		84.0 µg/mL <sup>b</sup>	-- <sup>a</sup>	-- <sup>a</sup>	--	--
		120 µg/mL <sup>b</sup>	9.4	9.2	9.3	11
		172 µg/mL <sup>b</sup>	8.7	9.0	8.9	14
		245 µg/mL <sup>c</sup>	10.1	10.0	10.1	3
		350 µg/mL <sup>c</sup>	11.5	10.9	11.2	0
		500 µg/mL <sup>c</sup>	10.4	10.5	10.5	0

<sup>a</sup> Dose not evaluated.  
<sup>b</sup> Precipitate observed at dose.  
<sup>c</sup> Precipitate observed at dose and harvest.  
 DMSO = dimethylsulfoxide

Chromosomal aberrations were analyzed from the cultures treated with 120, 172, and 245 µg/mL. The high dose selected for analysis, 245 µg/mL, had a precipitate at the end of the treatment period. No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed. (see below table).

**Table 4: Chromosomal Aberrations in Human Lymphocytes - Without Metabolic Activation - ~22-Hour Treatment, ~22-Hour Harvest**

Study No.: 8213158 Trial No.: B1 Date: 07/16/09 Test Article: (b) (4)

			# Cells Scored for Aberrations	% Mitotic Index Reduction <sup>a</sup>	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judge-Ment (+/-) <sup>b</sup>	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations					Judge-ment (+/-) <sup>d</sup>		
									gaps	simple breaks	chte	chre	mab		Totals <sup>c</sup>	
															-g	+g
Controls																
Vehicle:	DMSO	10.0 µL/mL	A 100		100	0	0					0	0			
			B 100		100	0	0						0	0		
			Total 200		200	0	0						0	0		
			Average %	0		0.0	0.0				0.0	0.0				
Positive:	MMC	0.300 µg/mL	A 75		100	0	0		9	17	5	20	28			
			B 50		100	0	0		4	14	2	16	19			
			Total 125		200	0	0		13	31	7	36	47			
			Average %	--		0.0	0.0	-	10.4	24.8	5.6	28.8	37.6	+		
Test Article	120 µg/mL		A 100		100	0	0		2	1		1	3			
			B 100		100	1	0		2	2		2	4			
			Total 200		200	1	0		4	3		3	7			
				Average %	11		0.5	0.0	-	2.0	1.5		1.5	3.5	-	
	172 µg/mL			A 100		100	1	0		3	2		2	5		
				B 100		100	1	0		4	1		1	5		
				Total 200		200	2	0		7	3		3	10		
				Average %	14		1.0	0.0	-	3.5	1.5		1.5	5.0	-	
	245 µg/mL			A 100		100	0	1		3			0	3		
B 100					100	1	0		3	1		1	4			
Total 200					200	1	1		6	1		1	7			
			Average %	3		0.5	0.5	-	3.0	0.5		0.5	3.5	-		

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

<sup>a</sup> % Mitotic index reduction as compared to the vehicle control.

<sup>b</sup> Significantly greater in % polyploidy and % endoreduplication than the vehicle control, p ≤ 0.01.

<sup>c</sup> -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

<sup>d</sup> Significantly greater in -g than the vehicle control, p ≤ 0.01. DMSO = dimethylsulfoxide MMC = Mitomycin C

- In the assay with metabolic activation with a 3-hour treatment, mitotic index data are presented in below table.

**Table 5: Assessment of Toxicity for Chromosomal Aberrations Assay - With Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest**

Study No.: 8213158 Trial No.: B1 Date: 07/16/09  
 Test Article: (b) (4)

Treatment	Dose	% Mitotic Index	Average % Mitotic Index	% Mitotic Index	
				A Culture	B Culture
Vehicle Control	DMSO	14.5	13.8		0
Test Article	10.0 µL/mL	-- <sup>a</sup>	--		--
	84.0 µg/mL <sup>b</sup>	8.8	9.0		35
	120 µg/mL <sup>b</sup>	14.0	13.9		0
	172 µg/mL <sup>b</sup>	11.7	13.0		6
	245 µg/mL <sup>c</sup>	-- <sup>a</sup>	--		--
	350 µg/mL <sup>c</sup>	9.5	9.6		30

<sup>a</sup> Dose not evaluated.  
<sup>b</sup> Precipitate observed at dose.  
<sup>c</sup> Precipitate observed at dose, wash, and harvest.  
 DMSO = dimethylsulfoxide

Chromosomal aberrations were analyzed from the cultures treated with 120, 172, and 245 µg/mL. The high dose selected for analysis, 245 µg/mL, had a precipitate at the end of the treatment period. No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed (see below table).

**Table 6: Chromosomal Aberrations in Human Lymphocytes - With Metabolic Activation - 3-Hour Treatment, ~22-Hour Harvest**

Study No.: 8213158 Trial No.: B1 Date: 07/16/09 Test Article: (b) (4)

	Treatment	Dose	# Cells Scored for Aberrations	% Mitotic Index Reduction <sup>a</sup>	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judge-ment (+/-) <sup>b</sup>	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations						Judge-ment (+/-) <sup>f</sup>			
									simple		chte	chre	mab	Totals <sup>c</sup>				
									gaps	breaks				-g		+g		
Controls	DMSO	10.0 µL/mL	A 100 B 100 Total 200	0	100	0	0	-	0	0	0	0	0	0	0	0	0	
			Average %	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	
Positive:	CP	25.0 µg/mL	A 50 B 50 Total 100		100	0	0	-	11	15	6	1	15	25	32	40	54	
			Average %	--	0.0	0.0	0.0	-	28.0	34.0	6.0	1.0	40.0	54.0	54.0	54.0	54.0	
Test Article	120 µg/mL	A 100	100		100	0	0	-	4				0	4	4	4		
		B 100	100		100	0	0	-	4				0	0	0	0		
			Average %	35		0.0	0.0	-	2.0				0.0	2.0	2.0	2.0	2.0	-
	172 µg/mL	A 100	100		100	0	0	-	2	1			1	2	2	2	2	
		B 100	100		100	0	0	-	2	1			1	2	2	2	2	
			Average %	0		0.0	0.0	-	1.0	0.5			0.5	1.0	1.0	1.0	1.0	-
245 µg/mL	A 100	100		100	0	0	-	3	1			0	3	3	3	3		
	B 100	100		100	0	0	-	3	1			1	3	3	3	3		
		Average %	6		0.0	0.0	-	1.5	0.5			0.5	1.5	1.5	1.5	1.5	-	

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication  
<sup>a</sup> % Mitotic index reduction as compared to the vehicle control.  
<sup>b</sup> Significantly greater in % polyploidy and % endoreduplication than the vehicle control, p ≤ 0.01.  
<sup>c</sup> -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.  
<sup>d</sup> Significantly greater in -g than the vehicle control, p ≤ 0.01. DMSO = dimethylsulfoxide CP = Cyclophosphamide

## 8 Carcinogenicity

None

## **9 Reproductive and Developmental Toxicology**

None

9.1 Fertility and Early Embryonic Development: None

9.2 Embryonic Fetal Development: None

9.2 Prenatal and Postnatal Development: None

## **10 Special Toxicity Studies**

The Sponsor conducted non-clinical studies with HP $\beta$ CD alone. In the nonclinical studies with DIC075U, HP $\beta$ CD was administered separately or in combination with diclofenac. In the dose-finding part of an IV bone marrow micronucleus test with DIC075U in mice, no adverse effects were observed at a dose level of 710 mg/kg of HP $\beta$ CD, study NC-DFC-012.

In the 4-week IV toxicity studies in rat with DIC075U and in monkeys with DIC075U or HP $\beta$ CD only, renal tubular vacuolization in the rat and very mild to mild granular appearance of renal tubular cells in the medullary rays in the monkey were attributed to HP $\beta$ CD and occurred at a dose level of equal to or higher than 26.6 mg HP $\beta$ CD/kg/day. The histopathological findings were partly reversible in the rat after a 9-week treatment free period and completely reversible in the monkey after 13 weeks without treatment as reviewed by this reviewer (NC-DFC-004, NC-DFC-010 and NC-DFC-011).

## **11 Local Tolerance Studies**

IV and IM local tolerance studies were conducted with DIC075V (to be marketed product) in rats and rabbits, respectively

### 11.1 Intravenous (IV) Local Tolerance Study

#### Study title: An Intravenous Local Tolerance Study in the Rat with a 7-day recovery period (draft report)\*

Study no.: NC-DFC-013 (CTD 4.2.3.6.1)

Study report location: (b) (4)

Conducting laboratory and location: (b) (4)

Date of study initiation: August 29, 2000

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: DF-HP $\beta$ CD; coded DIC075V  
Lot # 801345, % purity: 100.1%

*\*The Sponsor noted that this study was never finalized although the data was audited and found to be valid. As communicated to FDA on February 24, 2002, the sponsor of the study (b) (4) decided (b) (4) Additionally, the laboratory performing the study (b) (4) returning all raw data to the sponsor. Those data have since been purchased along with the rights to this product by Javelin Pharmaceuticals, Inc. According to the letter provided (b) (4) dated February 24, 2002, the data was audited and found to correctly reflect the raw data. Thus, while this report was never officially signed by the Study Director, Javelin believes these data are accurate and valid.*

*According to the data provided by the Sponsor this reviewer believes that this study is valid although it is a draft report.*

#### Key finding

- An IV local tolerance toxicity study including a 7 day recovery period was performed in rats testing DIC075V doses of 1.25 and 2.5 mg/kg (with respective HP $\beta$ CD doses of 11.1 and 22.5 mg once (QD) or four times (QID) daily for 1 or 7 consecutive days.
- Seventy-eight male animals were assigned to treatment groups. There were two control groups including saline once or saline four times daily.
- In gross necropsy, a red focus in the lung in a Day 2, 2.5 mg/kg QD male, enlarged iliac lymph nodes in a Day 8, 2.5 mg/kg QD male, and reddened renal lymph nodes in another Day 8, 2.5 mg/kg QID male were observed.

- In histopathology, perivascular hemorrhage and inflammation at the injection sites were observed within most dose groups including control group.
  - There was a slight increase in severity grades of perivascular inflammation in those rats receiving 1.25 mg/kg QID and 2.5 mg/kg QD or QID combined with inflammation and necrosis/degeneration in adjacent skeletal muscle at 2.5 mg/kg QD and 1.25 mg/kg QID, and vascular thrombi at 1.25 mg/kg QID and 2.5 mg/kg QID.
  - Injection site observations of toxicity were relatively absent after the 7 day recovery period.
- Due to microscopic findings at the injection sites (tails) and macroscopic observations, a dose level of 1.25 mg/kg diclofenac (11.1 mg/kg HP $\beta$ CD) is considered to be acceptable and reversible LOAEL.

#### Methods

Doses:	Diclofenac: 0, 1.25 and 2.5 mg/kg HP $\beta$ CD: 0, 11.1 and 22.5 mg/kg
Frequency of dosing:	Once or four times daily (see below table)
Route of administration:	Intravenous (Slow bolus, 1 ml/min)
Dose volume:	See below the Sponsor's table
Formulation/Vehicle:	DIC075V (37.5 mg Diclofenac Sodium + 333 mg HP $\beta$ CD) /mL Water for Injection USP; 2 mL/vial
Species/Strain:	(Sprague-Dawley CrI:CD®)
Number/Sex/Group:	Main group: 10 male /Group Recovery group: 3 male/group (see below table)
Age:	8 weeks
Weight:	244.4 to 351.9 g
Satellite groups:	N/A
Unique study design:	N/A
Deviation from study protocol:	No deviations in the study protocol were described by the applicant.

Seventy-eight male animals were assigned to treatment groups. Each animal was dosed once or four times daily for 1 or 7 consecutive days by intravenous bolus injection. The first day of dosing the animals was designated Day 1. The animals were evaluated for changes in clinical signs, body weight, food consumption and other parameters, five animals/group were euthanized on Days 2 and 8. The remaining 3 animals/group remained on test, untreated for an additional 7 days and served as recovery animals to determine reversibility of adverse effects. A gross necropsy was performed on all animals, tissues were preserved and histopathological evaluation was conducted on the injection sites.

Group No.	Number of Males	Test Article	Dose Level (mg/kg/Dose)	Dosing Interval/Day	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	No. Sac. Day 2	No. Sac. Day 8	No Sac. Day 15
1	13	Saline Control	0	1	0	0.067	5	5	3
2	13	Saline Control	0	4	0	0.067	5	5	3
3	13	DF-HP $\beta$ CD	1.25	1	1.25	0.033	5	5	3
4	13	DF-HP $\beta$ CD	2.5	1	2.5	0.067	5	5	3
5	13	DF-HP $\beta$ CD	1.25	4	5	0.033	5	5	3
6	13	DF-HP $\beta$ CD	2.5	4	10	0.067	5	5	3

**Mortality and clinical signs:**

No deaths. Only one 2.5 mg/kg Q.I.D male shows slight chromorhinorrhea (colored discharge from nose) on days 13 and 14. There were no other clinical observations.

**Body Weight and food consumption:**

There were no treatment related effects on body weights and food consumption.

**Gross Pathology:**

There were no gross treatment related necropsy findings in the organs and in the tails (injection sites).

Reviewer's table

Group Dose mg/kg	1 saline	2 Saline QID	3 1.25	4 2.5	5 1.25 QID	6 2.5 QID
Finding						
<b>Tan mesenteric nodule</b> (presumptive fat necrosis)	<b>1</b> <b>(day 15)</b>					
<b>Dilated kidney pelvis</b>	<b>1</b> <b>(day 15)</b>					
<b>Mottled kidneys</b>		<b>1 (day 8)</b>				
<b>Red focus lung</b> (presumptive hemorrhage)				<b>1 (day 2)</b>		
<b>Enlarged iliac lymph nodes</b> (presumptive reactive hyperplasia)				<b>1 (day 8)</b>		
<b>Reddened renal lymph nodes</b> (presumptive congestion/or hemorrhage)						<b>1 (day 8)</b>

*Note: above tissues were not examined microscopically.*

**Histopathology findings:**

Only the injection sites were examined microscopically (tails). Microscopic findings were summarized below for day 2, 8 and 15 by the Sponsor.

Perivascular hemorrhage and inflammation (generally characterized by mixed neutrophilic and mononuclear inflammatory cell infiltrates) were seen in all dose groups (day 2 and 8).

**At day 2**, the rat receiving 1.25 mg/kg Q.I.D and 2.5 mg/kg S.I.D and Q.I.D had slightly higher incidences and severity grades of perivascular inflammation.

### Incidence and Severity of Injection Site Observations at Day 2

Group Dose in mg/kg	1 Saline	2 Saline QID	3 1.25	4 2.5	5 1.25 QID	6 2.5 QID
Finding						
Perivascular hemorrhage	+2,+1,+2	+2,+1	+1	+1	+2	+3,+2
Perivascular inflammation	+1	+2		+1,+3,+2	+2,+2	+1,+3,+2,+2

**At day 8**, there was the highest incidence (3/5) in those rats given 7 consecutive multiple daily doses (Q.I.D.) of 1.25 mg/kg test article.

Perivascular inflammation had the highest incidences in rats receiving four daily doses of saline (4/5) or multiple daily doses (5/5 and 5/5) of 1.25 and 2.5 mg/kg test article.

Moderate (+3) inflammation was observed in rats receiving multiple daily doses of 1.25 mg/kg and those receiving single or multiple daily doses of 2.5 mg/kg DF-HP $\beta$ CD. Inflammation in 2/5 of 1.25 mg/kg Q.I.D and 2.5 mg/kg S.I.D rats extended into the adjacent skeletal muscle.

Necrosis/degeneration of skeletal muscle was observed in one 1.25 mg/kg Q.I.D injection site.

A focal mild vascular thrombus was present in one 1.25 mg/kg Q.I.D rat.

### Incidence and Severity of Injection Site Observations at Day 8

Group Dose in mg/kg	1 Saline	2 Saline QID	3 1.25	4 2.5	5 1.25 QID	6 2.5 QID
Finding						
Perivascular hemorrhage	+2	+1	+2	+1,+1	+1,+3,+1	+2
Perivascular inflammation	+2,+1	+1,+1,+1,+1	+2,+2	+3,+1	+2,+3,+3,+1,+2	+3,+2,+2,+2,+2
Perivascular edema			+2			
Inflam skeletal muscle				+2,+2	+2,+2	
Necrosis/degen muscle					+2	
Vascular thrombus					+2	

**At day 15** (following a 7 day recovery period), no perivascular hemorrhage was present. Focal minimal perivascular inflammation was observed in one 1.25 mg/kg S.I.D and two 1.25 mg/kg Q.I.D rat injection sites which is not dose dependent. A recanalized vascular thrombus was present at one 2.5 mg/kg Q.I.D injection site.

### Incidence and Severity of Injection Site Observations at Day 15

Group Dose in mg/kg	1 Saline	2 Saline QID	3 1.25	4 2.5	5 1.25 QID	6 2.5 QID
Finding						
Perivascular pigment			+1			
Perivascular inflammation			+1		+1,+1	
Recanalized vascular thrombus						+2

*Note: this reviewer agreed with the Sponsor that these microscopic changes indicate that the test article may be mildly irritating when injected intravascularly or when extravasated into the surrounding perivascular tissue.*

## 11.2 Intramuscular (IM) Local Tolerance Study

### 11.2.1: DF-HP $\beta$ CD Intramuscular 7-Day Local Tolerance Study in the Rabbit

A 7-day IM local tolerance study NC-DFC-005 (CTD 4.2.3.6.2) was performed with DIC075U, HP $\beta$ CD and Voltarol Ampoules 75 mg/3 mL in male New Zealand White rabbits (body weight range 2.47-2.76 kg on the first day of dosing; n = 3 per group) See below table copied from NDA submission. Rabbits were observed approximately 1 hour postdose for adverse clinical signs during the treatment phase then daily until termination on Day 11. Body weights and food consumption were recorded daily. After euthanasia, local injection sites were sampled, preserved and processed for light microscopy.

Test Article	Dose <sup>a</sup> (mg/kg)		Dosing Route	No. of Doses/day <sup>c</sup>	Treatment Duration (days)	Day of Sacrifice
	Diclofenac	HP $\beta$ CD				
HP $\beta$ CD	-	22.2	Intramuscular <sup>b</sup>	2	7	11
DIC075U	2.5	22.2	Intramuscular <sup>b</sup>	2	7	11
Voltarol	2.5	-	Intramuscular <sup>b</sup>	2	7	11

<sup>a</sup> 0.1 mL/kg of solutions containing (b)(4) diclofenac and/or (b)(4) HP $\beta$ CD. HP $\beta$ CD and DIC075U were administered in Water for Injection USP; Voltarol Ampoules 75 mg/3 mL was the commercially available preparation in the UK.

<sup>b</sup> Injections were to the left thigh muscle; an untreated site of the right thigh muscle was sampled for control purposes.

<sup>c</sup> Doses were given 6 hours apart.

Source: NC-DFC-005, Section: Experimental Procedure: Treatment (CTD 4.2.3.6.2)

Very slight erythema at the injection site was observed in 2 of 3 rabbits in each group. During histopathological evaluation, it became clear that the injection sites had been sampled incompletely for 5 out of 9 rabbits. As a consequence, sufficient data could not be generated to draw valid conclusions about the local tolerance of IM administration of DIC075U.

### 11.2.2: DF-HP $\beta$ CD Intramuscular 7-Day Local Tolerance Study in the Rabbit

This study NC-DFC-006 is a repeat of above study NC-DFC-005 using the same experimental design but with larger group sizes (n = 6 per group; body weight range 2.61- 2.92 kg on the first day of dosing).

Body weight changes were variable but failed to attain statistical intergroup differences. Food intake was not altered by any treatment. Local effects such as bruising and hematoma and very slight to slight erythema were observed at the injection site of various animals in all dose groups. Reddening of the skin and/or muscle was noticed in some DIC075U and Voltarol Ampoules 75 mg/3 mL-treated rabbits. No edema was noted at treatment sites. The main treatment-related histopathological finding at the injection sites was myositis. Myositis was observed in all treatment groups, but the inflammation was more severe and incidence was higher in DIC075U and Voltarol Ampoules 75 mg/3 mL-treated animals compared to HP $\beta$ CD-treated rabbits. There was no evidence of a clear difference in local effects between DIC075U and Voltarol Ampoules 75 mg/3 mL-treated animals.

### 11.2.3: DF-HP $\beta$ CD 7-Day Intramuscular Local Tolerance Study in the Rabbit

#### Study title: A 7-Day Intramuscular Local Tolerance Study in the Rabbit with a 14-day recovery period (draft report)\*

Study no.: NC-DFC-014 (CTD 4.2.3.6)

Study report location: (b) (4)

Conducting laboratory and location: (b) (4)

Date of study initiation: August 22, 2000

GLP compliance: Yes

QA statement: N/A

Drug, lot #, and % purity: DF-HP $\beta$ CD; coded DIC075V  
Lot # 801345, % purity: 100.1%

\* NOTE: This study was never finalized although the data was audited and found to be valid. As communicated to FDA on March 15, 2004, the sponsor of the study (b) (4) decided (b) (4). Additionally, the laboratory performing the study (b) (4) returning all raw data to the sponsor. Those data have since been purchased along with the rights to this product by Javelin Pharmaceuticals, Inc. According to the letter provided (b) (4) dated March 15, 2004, the data was audited and found to correctly reflect the raw data. Thus, while this report was never officially signed by the Study Director, Javelin believes these data are accurate and valid.

**Key finding**

- A 7-days IM local tolerance toxicity study including a 14 days recovery period was performed in rabbits testing DIC075V doses of 1.25 and 2.5 mg/kg (with respective HPβCD doses of 11.1 and 22.5 mg) once (S.I.D) or twice (B.I.D) daily for 1 or 7 consecutive days.
- Forty males were assigned to 4 treatment groups. Saline injections were performed in the right leg as a negative control at the same dose volume as the test article
- In gross necropsy, all test article injection sites (including control sites) from all rabbits had intramuscular discolorations that were pale, pink or red and corresponded microscopically to skeletal muscle degeneration/necrosis and/or chronic active inflammation with variable hemorrhage. There were no gross necropsy observations at either control or test article injection sites on day 22, after 14 days of recovery.
- In histopathology on day 2, increased severity of degeneration /necrosis and inflammation with hemorrhage at the test article injection sites were observed compared to the control sites. On day 8, most control sites were normal or had some areas of hemorrhage or inflammation and test article injection sites were characterized by multiple observations of skeletal muscle degeneration/necrosis that were mild to marked, chronic active inflammation, hemorrhage, sarcolemmal nuclear proliferation with variable areas of mineralization and vasculitis. There were no macroscopic observations (evidence of injection site reaction) by day 22 following 14 days of recovery.

**METHODS**

Doses:	Diclofenac: 0, 1.25 and 2.5 mg/kg HPβCD: 0, 11.1 and 22.5 mg/kg
Frequency of dosing:	Once or Twice daily for 1 or 7 consecutive days (see below table)
Route of administration:	Intramuscular (IM)
Dose volume:	See below the Sponsor's table
Formulation/Vehicle:	(37.5 mg Diclofenac Sodium + 333 mg HPβCD) /mL Water for Injection USP; 2 mL/vial
Species/Strain:	New Zealand White rabbits
Number/Sex/Group:	Main group: 8 male /Group Recovery group: 2 male/group (see below table)
Age:	16 weeks
Weight:	2.3 to 2.8 kg
Satellite groups:	N/A
Unique study design:	N/A

The first day of dosing the animals was designated Day 1. The animals were evaluated for changes in clinical signs, body weight, food consumption and other parameters as described below. Four animals/group were euthanized on Days 2 and 8. The remaining

2 animals/group remained on test, untreated for an additional 14 days and served as recovery animals to determine reversibility of adverse effects. A gross necropsy was performed on all animals, tissues were preserved and histopathological evaluation was conducted on the injection sites.

Group No.	Number of Males*	Test Article	Dose Level (mg/kg/dose)	Dosing Interval/Day	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	No. Sac. Day 2	No. Sac. Day 8	No. Sac. Day 22
1	10	DF-HP $\beta$ CD	1.25	1	1.25	0.033	4	4	2
2	10	DF-HP $\beta$ CD	2.5	1	2.5	0.067	4	4	2
3	10	DF-HP $\beta$ CD	1.25	2	2.5	0.033	4	4	2
4	10	DF-HP $\beta$ CD	2.5	2	5	0.067	4	4	2

\* Saline injections were performed in the contralateral (right) leg as a negative control at the same dose volume as the test article.

### **Mortality and clinical sign:**

No deaths or adverse clinical observations associated with systemic toxicity were observed.

### **Body weight and food consumption:**

No adverse effects were observed

### **Gross necropsy:**

Pink and red discolorations were present in 1, 4, 4 and 4 of injection sites receiving 1.25 mg/kg QD 2.5 mg/kg QD, 1.25 mg/kg B.I.D. and 2.5 mg/kg B.I.D. test article. These findings corresponded primarily to degeneration/necrosis of skeletal muscle with variable amounts of hemorrhage and/or inflammation.

Gross necropsy observations recorded on Day 22 following 7 days of dosing and 14 days of recovery did not reveal any test article-related findings.

### **Histopathology:**

Microscopic findings on day 2 of hemorrhage, inflammation and skeletal muscle degeneration/necrosis were variably present in control as well as test article sites, but control site observations were minimal to mild and often focal, occurring with greater frequency in rabbits receiving B.I.D. injections. Animals in groups 2 and 3 had increased severity of degeneration/necrosis and inflammation with hemorrhage at the test article injection site. By Day 22, after 14 days of recovery, there was no evidence of injection site reaction at test article sites.

On day 8, most control sites were normal or contained only sporadic foci of hemorrhage or inflammation. But most of the test article sites contained observation of degeneration/necrosis, chronic active to chronic active granulomatous inflammation, hemorrhage, and sarcolemmal nuclear proliferation with variable foci of mineralization and necrotizing vasculitis. There were no macroscopic observations (evidence of injection site reaction) by day 22 following 14 days of recovery.

Table's below summarizes the microscopic findings on day 2, 8 and Day 22.

	Group 1			Group 2			Group 3			Group 4		
Dose mg/kg IM	1.25 QD			2.5 QD			1.25 B.I.D.			2.5 B.I.D.		
	D2	D8	D22	D2	D8	D22	D2	D8	D22	D2	D8	D22
<b>Injected site Right</b>												
hemorrhage												
minimal	1/4	1/4	1/2			1/2	1/4			1/4		
mild											1/4	
Subacute inflammation												
minimal				1/4					1/2		1/4	
mild							2/4	1/4		2/4		
Sarcolemma nuclear proliferation												
minimal							1/4					
Degeneration/necrosis												
minimal							1/4			2/4		
mild							2/4					
<b>Injected site Left</b>												
Degeneration/necrosis												
minimal			1/2							1/4		
mild	1/4	1/4								1/4		
moderate		3/4		4/4	3/4		3/4	4/4		2/4	2/4	
marked					1/4		1/4				2/4	
Subacute inflammation												
minimal									1/2	1/4		
mild	1/4			3/4			2/4					
moderate		1/4		1/4								
Chronic active granulomatous inflammation												
moderate		1/4						1/4			1/4	
marked					2/4			1/4			1/4	
hemorrhage												
minimal		1/4	1/2	1/4	3/4			1/4				
mild		3/4		1/4	1/4		3/4	3/4		1/4	2/4	
moderate										1/4	1/4	
mineralization												
minimal					1/4			1/4				1/2
mild		2/4			2/4						1/4	
Sarcolemma nuclear proliferation												
minimal		1/4	1/2									
mild		2/4						3/4			1/4	
moderate					3/4						2/4	
marked					1/4							
Chronic active inflammation												
minimal									1/2			
moderate		1/4						1/4			1/4	
marked		2/4						1/4			1/4	

Necrotizing vasculitis												
minimal							1/4				1/4	
mild				1/4		1/4						
Acute inflammation												
minimal										1/4		
mild						1/4				2/4		
moderate						1/4						

See below the Sponsor's tables for SD2 and SD8:

Histologically observed degeneration/necrosis and inflammation was subjectively graded as: +1 = minimal; +2 = mild; +3 = moderate; and +4 = marked. Average severity grades of degeneration/necrosis and inflammation at test article injection sites were as follows:

	1.25 mg/kg sid	2.5 mg/kg sid	1.25 mg/kg bid	2.5 mg/kg bid
Degeneration/necrosis	.50	3.00	3.25	2.25
Inflammation	.50	2.25	2.25	1.50

The average severity grades of degeneration/necrosis and inflammation at Day 8 test article injection sites were as follows:

	1.25 mg/kg sid	2.5 mg/kg sid	1.25 mg/kg bid	2.5 mg/kg bid
Degeneration/necrosis	2.75	3.25	3.00	3.50
Inflammation	3.50	3.75	3.50	3.50

## 12 Integrated Summary and Safety Evaluation

The Applicant has submitted Dyloject as a 505(b) (2) application with reference made to NDA 20-142 to rely on previous findings of safety and efficacy for the approved listed drug Cataflam® (diclofenac potassium) and for support of current product labeling of diclofenac. The Applicant has obtained a Letter of Authorization to NDA 20-966, Sporanox® (itraconazole) Injection, and the current Sporanox labeling for background information on the toxicology of the major excipient HPβCD. Note that Sporanox was withdrawn on December 4, 2009 due to commercial reasons without evidence of a safety concern. The toxicity of the excipient HPβCD has been characterized by Janssen Pharmaceutica and its safety profile is additionally supported by the literature (Coussement et al, 1990, Gould et al, 2005 and Stella et al, 2008).

Daily dosage and Duration of use of Dyloject is within the listed drug Cataflam but the rout of administration is different. Also human AUC and Cmax values were not covered

by Cataflam. Therefore the applicant has conducted the nonclinical studies with IV formulation to support both systemic exposure and local tolerance.

It is thought that the toxicological effects of  $\beta$ -cyclodextrins ( $\beta$ -CD) are associated mainly to inclusion complexation with cholesterol and membrane lipids. Different substituents on the  $\beta$ -CD ring can confer diverse actions and various toxicological profiles such as hemolysis-inducing activity. HP $\beta$ CD with having hydroxypropyl groups on the CD molecule showed much lower toxic effect when compared to the native and methylated  $\beta$ -CDs (Kiss et al., 2010).

The Applicant conducted only one non-clinical pharmacology study to investigate the effects of diclofenac, DIC075V, and HP $\beta$ CD on the human cardiac delayed rectifier potassium channel using HEK 293 cells transfected with the *hERG* gene. The inhibition of the hERG current by DIC075V correlated closely with the inhibition of the current measured following exposure to HP $\beta$ CD. It can be concluded that the effect observed for DIC075V is derived from the interaction of HP $\beta$ CD with the hERG channel because diclofenac (API), was shown to have no effect on the hERG current. Quantitatively, IC<sub>50</sub> values for HP $\beta$ CD alone (13.13  $\mu$ g/mL) and for DIC075V (containing 16.8  $\mu$ g/mL HP $\beta$ CD) are similar. Diclofenac causes no inhibition of the hERG current density for the concentrations tested (1.58  $\mu$ g/mL - 474  $\mu$ g/mL). Himmel, et al demonstrated significant inhibition of the hERG currents when HEK 293 cells were exposed to HP $\beta$ CD and concluded that HP $\beta$ CD had a direct effect on the hERG current that could lead to misleading predictions of arrhythmic risk. Similarly findings were reported by Mikhail, et al, who demonstrated a significant inhibition of hERG currents with HEK 293 cells in the presence of cyclodextrins. According to the review by the medical officer, the results from a thorough QTc interval prolongation phase 1 clinical study DFC-011 in 77 normal volunteers revealed no evidence of altered the electrocardiograms or QTc interval prolongation following administration of DIC075V, 75 mg, which contained 666 mg of HP $\beta$ CD.

The nonclinical toxicology of Dyloject Injection 37.5 mg/mL (along with HP $\beta$ CD) has been characterized to evaluate safety for human use in several nonclinical studies as listed in section 3.1 of this document. The studies include a single IV dose in mice (*in vivo* micronucleus assay), 4-week IV repeat-dose toxicity studies (including recovery) in the rat and monkey, the full battery of *in vitro* and *in vivo* genotoxicity studies, an IV local tolerance study in rat and IM local tolerance studies in rabbit. Most of these studies were performed with the DIC075U formulation; local tolerance studies were performed with the DIC075V formulation. However, the main components in the DIC075V formulation (diclofenac sodium and HP $\beta$ CD) were present in the DIC075U formulation, dose levels were of relevance, and outcomes reflected individual known toxicities of an NSAID such as diclofenac and the  $\beta$ -CD, HP $\beta$ CD. Although monothioglycerol was not tested for systemic toxicity or genotoxicity, it is considered an acceptable excipient at the proposed concentration and daily dose limit for the IV route due to its inclusion in the Inactive Ingredients Guide at higher levels.

A single-dose of DIC075U administered by IV injection to male and female CD-1 mice at diclofenac dose levels of 120 or 160 mg/kg was associated with mortality (1 male at 160 mg/kg), sacrifice for humane reasons (1 male at 120 mg/kg, 2 females at 160 mg/kg) and clinical signs (subdued behavior, hunched appearance, piloerection, rolling gait,

tremors, agitation, cold on touch, discharge [eyes] and swollen abdomen). No such effects were observed at a diclofenac dose level of 80 mg/kg.

In a 4-week IV repeat-dose study NC-DFC-004, with a 9-week recovery period, when DIC075U was administered to rats at diclofenac dose levels of 3, 7, and 15 mg/kg/day (corresponding HP $\beta$ CD dose levels of 26.6, 62, and 133 mg/kg/day, respectively), systemic exposure was demonstrated at all dose levels. Maximum plasma concentration (C<sub>max</sub>) and area under the plasma-concentration-versus-time-curve (AUC) values increased with an increase in dose, with a slightly more than dose-proportional increase in AUC. Consistent with gender differences in toxicity, AUC values were consistently higher in females than males. Diclofenac-related findings were restricted to the highest dose level and comprised a low incidence of mortality/premature sacrifice (due to peritonitis), gastrointestinal toxicity and regenerative anemia (females only). These findings were shown to be reversible during the recovery period. The very mild to mild renal tubular vacuolation observed in treated animals was considered an expected finding following IV administration of HP $\beta$ CD in this study. There was evidence of reversibility since only very mild renal tubular vacuolation was observed at the end of the recovery period. The No Adverse Effect Level (NOAEL) for diclofenac-related effects was 7 mg/kg/day in rats which corresponds to an exposure (AUC<sub>0-t</sub>) on day 28 of 9,496 and 13,408 ng.hr/mL in males and females, respectively.

These GI findings are possibly the explanation for all the above though the HP $\beta$ CD could be playing a role in destabilizing red cell membrane integrity (which would lead to reduced RBC parameters and extramedullary hematopoiesis). Unfortunately the Sponsor did not include a control group that had HP $\beta$ CD alone in water. However these general findings are expected for an NSAID in cyclodextrin.

In the 4-week IV repeat-dose study NC-DFC-010, with a 13-week recovery period in monkeys, animals received DIC075U at diclofenac dose levels of 3, 15, and 60 mg/kg/day (with corresponding HP $\beta$ CD dose levels of 26.6, 133, and 533 mg/kg/day, respectively). In a companion study NC-DFC-011 with the same design, water control or the HP $\beta$ CD vehicle was administered alone at a dose level of 533 mg/kg/day. Systemic exposure to diclofenac was demonstrated at all dose levels in NC-DFC-010. C<sub>max</sub> and AUC values increased with increasing dose, and the increase in AUC was more than dose-proportional. There were only marginal gender differences in AUC values. At the diclofenac 60 mg/kg/day level, all monkeys were sacrificed prematurely as a consequence of poor general condition. Findings at this dose level were attributed to diclofenac-included gastrointestinal toxicity, regenerative anemia, and aggravated lesions of the tail skin. These findings are expected for an NSAID. NSAIDs cause a loss of the protective integrity of the GI tract leading to ulcerations/perforations with secondary consequence of anemia and negatively affect wound healing, therefore treated animals may not be able to heal as quickly as untreated animals. At dose levels of 15 mg/kg/day, only slight changes indicative of gastrointestinal blood loss and regenerative anemia were observed in combination with aggravated tail skin lesions. Due to the premature sacrifice of the high-dose group and the lack of recovery animals in the low and mid-dose groups, evaluation of reversibility of diclofenac-associated findings was not possible. In studies NC-DFC-010 and NC-DFC-011, a very mild to mild

granular appearance of the renal tubular cells in the medullary rays, observed in all treatment groups in both studies, was considered due to HP $\beta$ CD and resolved after a 3-month treatment-free period. The NOAEL for diclofenac-related toxicity was 3 mg/kg/day in monkeys for which the exposure (AUC<sub>0-t</sub>) on day 28 was 6054 and 6493 ng.hr/mL in males and females, respectively.

To support local tolerability, intravenous administration of DIC075V to rats once or 4 times daily for up to 7 days at diclofenac dose levels of 1.25 or 2.5 mg/kg/dose was associated with minimal to moderate perivascular inflammation at the injection sites. Following a 7-day recovery period, the incidence and severity of perivascular inflammation had decreased considerably as described in study NC-DFC-013. The repeated insult of IV administration (up to 4 times daily) into the tail vein of the rodent model may contribute a non-specific local effect. Local tolerability was evaluated in the clinical development program and based on the medical officer review appears to support the MRHD concentration in the injection site and the rate of administration.

In rabbits, given Dyloject IM into the leg (study NC-DFC-014), injections of DIC075V once or twice daily for up to 7 days at dose levels of 1.25 or 2.5 mg/kg/dose, injection sites showed pale, pink, and/or red discolorations, corresponding microscopically to degeneration/necrosis of skeletal muscle with infiltrations of inflammatory cells and variable degrees of hemorrhage. There was a slight but apparent association of increased severity with dosage and time. After a 14-day period without treatment, minimal or no effects were observed at the injection site. In another local tolerance study NC-DFC-006, effects at the injection sites were compared in rabbits following IM treatment with HP $\beta$ CD, DIC075U or Voltarol Ampoules 75 mg/3 mL. Doses were administered either once or twice a day for 1 or 7 days by IM injection in the left leg. Local effects indicative of myositis were observed in all treatment groups but with higher incidence and of a more severe nature in the DIC075U and Voltarol Ampoules 75 mg/3 mL-treated animals than in HP $\beta$ CD -treated rabbits. These local effects indicate that the Dyloject may be mildly irritating when injected intravascularly or when extravasated into the surrounding perivascular tissue in clinical use.

DIC075U was evaluated for genotoxicity, and found not to be mutagenic in the Ames test when tested up to a maximum diclofenac dose level of 5000  $\mu$ g/plate in study Toxicity to the bacteria was observed in the second mutation assay, in the presence of S9-mix only at 2500 and 5000  $\mu$ g DF-HP $\beta$ CD per plate. In the mouse lymphoma mutation assay, DIC075U caused a dose related reduction in relative total growth but did not cause an increase in mutant fraction. No significant evidence of mutagenic activity was obtained from cultures treated with DF-HP $\beta$ CD in any of the 4 assays (2 in the absence and 2 in the presence of S9-mix) and therefore DF-HP $\beta$ CD is not mutagenic in mouse lymphoma L5178Y cells, when tested at concentrations extending into the toxic range. An *in vivo* micronucleus study indicated that DIC075U did not induce an increase in micronucleated polychromatic erythrocytes when administered by IV injection to mice up to the maximum tolerated diclofenac dose of 120 mg/kg.

The impurity/degradation product, (b) (4), in drug substance and drug product exceeds ICH (Q3A and Q3B) threshold level for qualification. Therefore two *in vitro* genotoxicity studies and a repeat dose toxicity study

(of 14 days or greater in a single species using isolated impurity) were required. Two *in vitro* genotoxicity were conducted on the impurity/degradant. These results indicate (b) (4) was negative in the Bacterial Reverse Mutation Assay with a confirmatory assay under the conditions of the test protocol. Also (b) (4) was considered negative for inducing chromosomal aberrations in cultured human lymphocytes without and with metabolic activation. The repeat dose toxicity study for the impurity/degradant has not been conducted and there is no detail information for this specific impurity in non-clinical batches (DIC075U, Lot # R&D227) which support the safety of maximal human intake of the impurity using the proposed specification. Therefore the Sponsor revised the (b) (4) specification to NMT (b) (4) % to comply with ICHQ3B limits and proposed 18-month expiration dating for the product to ensure meeting the NMT (b) (4) % (b) (4) specification.

Due to lack of genotoxic effects in *in vitro* and *in vivo* genotoxicity tests with DIC075U and in view of the acute clinical treatment duration intended for DIC075V, the sponsor did not conduct carcinogenicity studies with diclofenac in a HPβCD-containing formulation. Data from 2-year oral carcinogenicity studies with diclofenac in mice (maximum dose levels of 0.3 and 1 mg/kg/day in males and females, respectively) and rats (maximum dose levels of 2 mg/kg/day) have demonstrated no carcinogenic potential as described in the approved Cataflam label.

No reproductive and development studies were performed to support this application. Novartis has performed a comprehensive evaluation of the reproductive and developmental toxicity of diclofenac for Cataflam as reflected in the approved label. Fertility and teratogenicity studies with diclofenac sodium showed no effect on fertility in rats (oral dose levels up to 4 mg/kg/day) and no evidence of teratogenicity in mice (oral dose levels up to 20 mg/kg/day), rats or rabbits (oral dose levels up to 10 mg/kg/day in both species). In reproduction studies conducted by Janssen to support approval of Sporanox, IV administration of HPβCD up to 100 mg/kg body weight (bwt) did not result in maternal toxicity or in effects on the litter. At 400 mg/kg bwt, a toxic dose as shown by repeated dose toxicity studies, a slightly lowered survival rate was noted. In addition, it has been demonstrated in a repeat Segment III IV study involving HPβCD with second undosed generation phase, that there were no adverse effects up to 400 mg/kg bwt. Postnatal behavior and reproductive capabilities of the second generation were not affected (Janssen Report 105919/1).

Based on the proposed recommended dose of DIC075V (37mg/ml diclofenac, 333 mg/mL HPβCD), with 4 times daily dosing, the maximum daily exposure to diclofenac and HPβCD in patients is approximately 2.1 mg/kg/day (4 doses of 0.53 mg/kg/dose) and 19 mg/kg/day (4 doses of 4.7 mg/kg/dose), respectively. The Applicant notes the dosing limit is 150 mg/day. In study DFC-PK-009 a dose of 37.5 mg of DIC075V produces maximum plasma concentration (C<sub>max</sub>) of 7.2 µg/mL and maximum total systemic exposure (AUC) of 1.95 µg·h/mL of diclofenac. Human clinical exposure to HPβCD was also determined in the same clinical trial. Mean maximum plasma concentrations in healthy volunteers were 50.3 µg/mL, while mean total systemic exposure (AUC) was 66.4 µg·h/mL.

**Safety Margin Table (Diclofenac and HP $\beta$ CD)**

	Dose (mg/kg/day)		HED <sup>a</sup> (mg/kg)		AUC(0-t) <sup>b</sup> ( $\mu$ g.hr/mL)		C <sub>max</sub> ( $\mu$ g/mL)		Human SM Based on AUC	
	DF	HP $\beta$ CD	DF	HP $\beta$ CD	DF	HP $\beta$ CD	DF	HP $\beta$ CD	DF	HP $\beta$ CD
Human (IV)										
37.5mg/mL Diclofenac, 333mg/mL HP $\beta$ CD	2.1 (0.53x4)	19 (4.7x4)			7.6 (1.95x4)	265.6 (66.4x4)	7.2	50.3		
Rats 4-week										
<b>NOAEL</b>	M	7	(62)	1.1	9.92	9.5		49.0	<b>1.5X</b>	
	F					13.4		54.6		
Monkeys 4-week										
<b>NOAEL</b>	M	3	(27)	0.96	8.64	6.0		32.5	<b>0.8X</b>	
	F					6.5		33.1		
<b>LOAEL<sup>c</sup></b>	M	15	(133)	4.8	43	49.7		146.5	<b>6.6X</b>	
	F					50.3		154.5		
<b>NOAEL</b>	--	<b>533</b>	--	171		3363				<b>12.7X</b>

<sup>a)</sup> HED: Human Equivalent Dose (Assume 70 kg human), <sup>b)</sup> AUC measured on day 28 for rats and monkeys but single dose for human (DFC-PK-009, appendix 2). <sup>c)</sup> Acceptable toxicity (minimal, reversible, and/or monitorable).

With the AUC corresponding to the NOAEL taken from the nonclinical studies, the calculation for a subject receiving 37.5 mg (4 times a day) of DIC075V would provide a 1.5-fold (Rat, 11/7.6  $\mu$ g.hr/mL) and a 0.8-fold ( Monkey, 6.25/7.6  $\mu$ g.hr/mL) systemic exposure margins for diclofenac. However, the AUC corresponding to acceptable LOAEL in 4 week study in monkey provides a 6.6-fold (50/7.6  $\mu$ g.hr/mL) systemic exposure margin. The calculation for a subject receiving DIC075V (4 time a day) would provide a 12.7-fold (Monkey, 3363/265.6  $\mu$ g.hr/mL) systemic exposure margins for HP $\beta$ CD. See table above.

Moreover, the duration of nonclinical studies were 28 days which is over 5 times longer than the duration proposed in the product label (5 days).

In summary, Dyloject® (DIC075V) Injection has low and expected acute toxicity at diclofenac and HP $\beta$ CD exposures equivalent to the exposure at the maximum recommended human dose; diclofenac demonstrates no genotoxic or carcinogenic potential, does not affect fertility, and is not teratogenic. The results of the local tolerance study suggested a mild to moderate irritation in injection sites when injected IV and moderate to marked irritation in injection sites when injected IM or with inadvertent injection into perivascular tissues which could lead to local muscle irritation or necrosis. Nonclinical studies have indicated HP $\beta$ CD may be delayed in its clearance from urinary epithelium. However it has been shown in animals and humans to not

interfere with renal function, lead to progressive renal disease, and is cleared with full histological reversibility. Therefore from the non-clinical pharmacology toxicology perspective, this NDA may be approved.

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## 14 Appendix/Attachments

### Appendix 1,

The PK of diclofenac, following IV administration of DIC075V and oral doses of Cataflam, are compared in table below (DFC-PK-006, healthy volunteer).

Study DFC-PK-006

September 10, 2007

Summary of diclofenac pharmacokinetic parameters after intravenous administration of 18.75 mg and 37.5 mg of DIC075V (diclofenac sodium) and oral administration of 50 mg of Cataflam (diclofenac potassium) every 6 hours for four doses to healthy volunteers.

Parameter <sup>1,2</sup>	DIC075V		Cataflam
	18.75 mg	37.5 mg	50 mg
<b>First Dose</b>			
C <sub>max</sub> (ng/mL)	2,904 ± 661 (36)	6,031 ± 1178 (36)	1,246 ± 732 (36)
T <sub>max</sub> (h)	0.083 (36) [0.083 – 0.150]	0.083 (36) [0.083 – 0.150]	1.50 (36) [0.33 – 3.00]
AUC(0-t) (h•ng/mL)	866 ± 221 (36)	1,843 ± 394 (36)	1,473 ± 488 (36)
AUC(inf) (h•ng/mL)	898 ± 231 (33)	1,859 ± 376 (34)	1,562 ± 519 (34)
λ <sub>z</sub> (h <sup>-1</sup> )	0.5221 ± 0.1108 (33)	0.4964 ± 0.0788 (34)	0.5656 ± 0.1223 (34)
t <sub>1/2</sub> (h)	1.39 ± 0.29 (33)	1.44 ± 0.27 (34)	1.28 ± 0.27 (34)
CL (mL/min)	344 ± 87.1 (33)	324 ± 63.0 (34)	526 ± 179 (34)
V <sub>z</sub> (L)	40.4 ± 10.1 (33)	40.1 ± 09.8 (34)	57.3 ± 20.4 (34)
F (%)	— <sup>3</sup>	— <sup>3</sup>	64.1
<b>Fourth Dose</b>			
C <sub>max</sub> (ng/mL)	3,090 ± 1,029 (36)	5,617 ± 1,799 (36)	851 ± 462 (36)
T <sub>max</sub> (h)	0.083 (36) [0.000 – 0.133]	0.083 (36) [0.067 – 0.183]	1.49 (36) [0.00 – 6.00]
AUC(0-t) (h•ng/mL)	935 ± 203 (36)	1,839 ± 506 (36)	1,350 ± 601 (36)
λ <sub>z</sub> (h <sup>-1</sup> )	0.4059 ± 0.1056 (35)	0.3256 ± 0.0917 (36)	0.2597 ± 0.0531 (36)
t <sub>1/2</sub> (h)	1.82 ± 0.48 (35)	2.29 ± 0.63 (36)	2.80 ± 0.66 (36)
CL (mL/min)	325 ± 71.6 (36)	387 ± 394 (36)	894 ± 1,392 (36)
V <sub>z</sub> (L)	50.4 ± 14.9 (35)	83.4 ± 127 (36)	242 ± 486 (36)
F (%)	— <sup>3</sup>	— <sup>3</sup>	54.6

<sup>1</sup>Arithmetic mean ± standard deviation (N) except for T<sub>max</sub> for which the median (N) [Range] is reported.

<sup>2</sup>CL and V<sub>z</sub> are CL/F and V<sub>z</sub>/F for Cataflam.

<sup>3</sup>Not applicable.

The data above shows that there is no significant different in PK profile of the first and fourth of daily dose.

**Appendix 2:**

Javelin Pharmaceuticals, Inc.  
Study DFC-PK-009

Page 9  
June 23, 2009

Summary of pharmacokinetic parameters for diclofenac after IV administration of DIC075V 37.5 mg to subjects with mild or moderate renal impairment and to healthy volunteers.

Parameter <sup>1</sup>	Renal Impairment		Healthy Matches
	Mild	Moderate	
C <sub>max</sub> (ng/mL)	7,286 ± 1,430 ( 8)	5,332 ± 1,629 ( 5)	7,163 ± 950 ( 7)
T <sub>max</sub> (h)	0.083 ( 8)	0.083 ( 5)	0.083 ( 7)
AUC(0-t) (h×ng/mL)	1,927 ± 409 ( 8)	1,531 ± 418 ( 5)	1,947 ± 313 ( 7)
AUC(inf) (h×ng/mL)	1,943 ± 409 ( 8)	1,550 ± 422 ( 5)	1,968 ± 315 ( 7)
λ <sub>z</sub> (h <sup>-1</sup> )	0.3856 ± 0.088 ( 8)	0.3427 ± 0.080 ( 5)	0.3725 ± 0.055 ( 7)
t <sub>1/2</sub> (h)	1.89 ± 0.46 ( 8)	2.10 ± 0.44 ( 5)	1.90 ± 0.30 ( 7)
CL (mL/min)	312 ± 73.0 ( 8)	401 ± 126 ( 5)	303 ± 55.6 ( 7)
V <sub>z</sub> (L)	49.8 ± 12.1 ( 8)	69.7 ± 9.22 ( 5)	50.2 ± 14.1 ( 7)

<sup>1</sup>Arithmetic mean ± standard deviation (N) except for T<sub>max</sub> for which the median (N) is reported.

Javelin Pharmaceuticals, Inc.  
Study DFC-PK-009

Page 11  
June 23, 2009

Summary of pharmacokinetic parameters for HPβCD after IV administration of DIC075V 37.5 mg to subjects with mild or moderate renal impairment and to healthy volunteers.

Parameter <sup>1</sup>	Renal Impairment		Healthy Matches
	Mild	Moderate	
C <sub>max</sub> (ng/mL)	60,750 ± 16,275 ( 8)	52,700 ± 18,565 ( 5)	50,329 ± 7,731 ( 7)
T <sub>max</sub> (h)	0.083 ( 8)	0.083 ( 5)	0.083 ( 7)
AUC(0-t) (h×ng/mL)	127,141 ± 90,489 ( 8)	169,042 ± 52,722 ( 5)	66,449 ± 12,642 ( 7)
AUC(inf) (h×ng/mL)	128,349 ± 91,132 ( 8)	165,728 ± 60,386 ( 4)	67,316 ± 12,615 ( 7)
λ <sub>z</sub> (h <sup>-1</sup> )	0.2549 ± 0.068 ( 8)	0.1226 ± 0.033 ( 4)	0.2510 ± 0.095 ( 7)
t <sub>1/2</sub> (h)	2.87 ± 0.69 ( 8)	6.04 ± 1.94 ( 4)	3.29 ± 1.66 ( 7)
CL (mL/min)	59.0 ± 31.3 ( 8)	36.2 ± 10.0 ( 4)	85.2 ± 16.5 ( 7)
V <sub>z</sub> (L)	13.6 ± 5.38 ( 8)	17.7 ± 1.88 ( 4)	23.3 ± 9.84 ( 7)

<sup>1</sup>Arithmetic mean ± standard deviation (N) except for T<sub>max</sub> for which the median (N) is reported.

**Appendix 3:**

## Necropsy and Histological Findings: Males and Females

PROJECT NUMBER: 453311  
 TREATMENT: Group 4 (15 mg.kg<sup>-1</sup>.day<sup>-1</sup>) FEMALES

---

ANIMAL NO: FINDINGS:

---

119 Killed Prematurely  
 Period on Study: 3 Week(s)  
 Main Study

## NECROPSY FINDINGS:

ABDOMEN : Contains fluid  
 SKIN AND SUBCUTIS : Staining (mild, ventral abdomen)

## HISTOLOGICAL FINDINGS:

ABDOMEN : \* Peritonitis (relates to necropsy finding)  
 ADRENAL GLAND : Mild capsular adhesion  
 CAECUM : Very mild peritoneal inflammatory cell  
 infiltration  
 COLON : Very mild peritoneal inflammatory cell  
 infiltration  
 DUODENUM : Mild peritoneal inflammatory cell infiltration  
 ILEUM : Moderate peritoneal inflammatory cell  
 infiltration  
 JEJUNUM : Mild peritoneal inflammatory cell infiltration  
 KIDNEY : Very mild medullary mineral deposit(s)  
 Mild capsular adhesion  
 Mild unilateral papillary oedema  
 LIVER : Very mild focal inflammatory cell infiltration  
 LUNG : Mild interstitial inflammatory cell infiltration  
 MESENTERIC LYMPH NODE : Mild histiocytosis  
 Moderate capsular adhesion  
 PANCREAS : Mild peritoneal inflammatory cell infiltration  
 PARATHYROID GLAND : Only one examined  
 PITUITARY GLAND : Section inadequate. No more available  
 RECTUM : Very mild peritoneal inflammatory cell  
 infiltration  
 SPLEEN : Moderate increased extramedullary haemopoiesis  
 Very mild adhesion  
 STOMACH : Mild hyperkeratosis in non-glandular region  
 Mild peritoneal inflammatory cell infiltration  
 URINARY BLADDER : Mild serosal adhesion  
 VAGINA : Section inadequate. No more available

\* = PROBABLE CAUSE OF DEATH

## Necropsy and Histological Findings: Males and Females

PROJECT NUMBER: 453311  
 TREATMENT: Group 4 (15 mg.kg<sup>-1</sup>.day<sup>-1</sup>) MALES

---

ANIMAL NO: FINDINGS:

---

51 Found Dead  
 Period on Study: 1 Week(s)  
 Main Study

## NECROPSY FINDINGS:

SYSTEMIC CONDITION : Autolysed

EYE : Both opaque

## HISTOLOGICAL FINDINGS:

ABDOMEN : \* Peritonitis

ADRENAL GLAND : Autolysed

CAECUM : Autolysed

COLON : Autolysed

DUODENUM : Autolysed

Moderate peritoneal inflammatory cell  
infiltration

EPIDIDYMIS : Autolysed

EYE : Autolysed (accounts for macroscopic finding)

HEART : Very mild cardiomyopathy

ILEUM : Autolysed

Moderate peritoneal inflammatory cell  
infiltration

JEJUNUM : Autolysed

\* = PROBABLE CAUSE OF DEATH

JEJUNUM : Moderate peritoneal inflammatory cell  
infiltration

KIDNEY : Autolysed

LIVER : Mild adhesion

Autolysed

LUNG : Autolysed

MAMMARY GLAND : Tissue absent from section. No more available

OPTIC NERVE : Tissue absent from section. No more available

PANCREAS : Autolysed

Adhesion

PARATHYROID GLAND : Only one examined

RECTUM : Autolysed

SALIVARY GLAND : Autolysed

SEMINAL VESICLE : Autolysed

SPLEEN : Autolysed

Adhesion

STOMACH : Autolysed

TESTIS : Autolysed

THYMUS : Autolysed

THYROID GLAND : Autolysed

**Appendix 4:**

The group means incidence of clinical signs observed are presented below (from final report).

TABLE 1

DF-HP $\beta$ CD and HP $\beta$ CD alone  
4 Week Intravenous Toxicity Study in Cynomolgus Monkeys with a 3 Month Recovery Period  
Clinical Signs: Group Mean Incidence of Most Common Signs

Clinical Sign	Week Number	Group/Dose Level (mg.kg <sup>-1</sup> .day <sup>-1</sup> )									
		1♂ (0)	2♂ (0)	3♂ (3)	4♂ (15)	5♂ (60)	1♀ (0)	2♀ (0)	3♀ (3)	4♀ (15)	5♀ (60)
Dark Faeces	1	0	0	0	3	5	0	0	0	2	5
	2	0	0	0	7	1	0	0	0	0	2
	3	0	0	0	3	0	0	0	0	0	0
	4	0	0	0	0	-	0	0	0	0	-
	Rec 1-4	0	0	-	-	-	0	0	-	-	-
	Rec 5-8	0	0	-	-	-	0	0	-	-	-
	Rec 9-13	0	0	-	-	-	0	0	-	-	-
Soft/Liquid Faeces	1	0	0	0	2	10	0	0	0	0	5
	2	0	0	0	2	1	0	0	0	0	0
	3	0	0	0	1	0	0	0	0	0	0
	4	0	0	0	1	-	0	0	0	0	-
	Rec 1-4	0	0	-	-	-	0	0	-	-	-
	Rec 5-8	0	0	-	-	-	0	0	-	-	-
	Rec 9-13	0	0	-	-	-	0	0	-	-	-
Red Mucoidal Material in Faeces	1	0	0	0	0	9	0	0	0	0	6
	2	0	0	0	0	1	0	0	0	1	0
	3	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	-	0	0	0	0	-
	Rec 1-4	0	0	-	-	-	0	0	-	-	-
	Rec 5-8	0	0	-	-	-	0	0	-	-	-
	Rec 9-13	0	0	-	-	-	0	0	-	-	-
Hunched	1	0	0	0	0	0	0	0	0	0	1
	2	0	0	0	0	3	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	-	0	0	0	0	-
	Rec 1-4	0	0	-	-	-	0	0	-	-	-
	Rec 5-8	0	0	-	-	-	0	0	-	-	-
	Rec 9-13	0	0	-	-	-	0	0	-	-	-
Subdued	1	0	0	0	0	0	0	0	0	0	1
	2	0	0	0	0	9	0	0	0	0	3
	3	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	-	0	0	0	0	-
	Rec 1-4	0	0	-	-	-	0	0	-	-	-
	Rec 5-8	0	0	-	-	-	0	0	-	-	-
	Rec 9-13	0	0	-	-	-	0	0	-	-	-

Maximum incidence = 7 x number of animals (3 or 5)  
(Recovery period = 28 or 35 days x number of animals (n=2))

***Appendix 5:***

(b) (4)



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immediately following this page

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
----- NDA-22396	----- ORIG-1	----- HOSPIRA INC	----- diclofenac sodium injection

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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/

-----  
ARMAGHAN EMAMI  
08/23/2010

ADAM M WASSERMAN  
08/30/2010

I concur with Dr. Emami that this application may be approved from the nonclinical standpoint. See my Supervisory memo for further discussion.



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

## **Supervisory Pharmacologist Memorandum**

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NDA NUMBER: 22-396  
SERIAL NUMBER: 000  
DATE RECEIVED BY CENTER: 12/3/2009  
PRODUCT:  
    **(Proposed) Trade Name:** Dyloject  
    **Established Name:** Diclofenac sodium intravenous injection

INDICATION: Treatment of acute moderate to severe pain  
in adults

SPONSOR: Hospira  
REVIEW DIVISION: Division of Anesthesia and Analgesia  
Products (HFD-170)  
PHARM/TOX REVIEWER: Armaghan Emami, Ph.D.  
PHARM/TOX SUPERVISOR: Adam Wasserman, Ph.D.  
DIVISION DIRECTOR: Bob Rappaport, M.D.  
PROJECT MANAGER: Kathleen Davies

## EXECUTIVE SUMMARY

### I. BACKGROUND

#### Regulatory Summary (Pharmacology/Toxicology)

The present NDA pertains to Dyloject®, an injectable solution of diclofenac sodium (b) (4) using hydroxyl-propyl  $\beta$ -cyclodextrin at 37.5 mg/mL which was developed and submitted by Javelin Pharmaceuticals for the acute treatment of moderate to severe pain in adults as Q6h dosing. Subsequent to NDA submission Javelin was bought by Hospira who now have control over the NDA and rights to the marketing of this drug product. Primary nonclinical review was conducted by Dr. Armaghan Emami.

Diclofenac was initially approved by Novartis in July 1988 as Voltaren® as an oral tablet and has been approved as the sodium or potassium salt in many types of products including immediate release tablets, delayed release tablets, oral solution, ophthalmic drops, topical patch, topical gel, and topical solution as described in Dr. Emami's review. Indications given for use include relief of mild to moderate pain, primary dysmenorrhea, signs and symptoms of osteoarthritis and rheumatoid arthritis, topical treatment of acute pain due to minor strains, sprains and contusions, actinic keratosis, and most recently migraine attacks. This submission utilizes as a listed drug Cataflam® (diclofenac potassium; NDA 20-142) oral tablets for this 505(b)(2) submission and the Applicant has a Letter of Authorization to NDA 20-966 Sporanox (itraconazole) which allows utilization of proprietary data for support of the excipients hydroxy-propyl  $\beta$ -cyclodextrin (HP $\beta$ CD also called Hydroxypropyl betadex NF). The NDA for Sporanox was withdrawn however this does not appear to be due to reasons of safety.

Dyloject represents the first intravenous formulation of diclofenac and as an intravenous NSAID would represent the 2<sup>nd</sup> or potentially 3<sup>rd</sup> IV formulation of this class to be approved. Caldolor® (intravenous ibuprofen; *management of fever and treatment of mild to moderate pain, moderate to severe pain as an adjunct to opioid analgesia* Cumberland Pharmaceuticals, Inc., NDA 22-348) was approved in June 2009 while Ofirmev (intravenous acetaminophen; *analgesic for pain and anti-pyretic for fever in adults, adolescents and children* Cadence pharmaceuticals, NDA 22-450) was resubmitted after a Complete Response and pending resolution of issues is currently under consideration with a PDUFA date of November 2010. It is worth noting that Dyloject is currently approved abroad though a recent recall of drug product in the United Kingdom was executed apparently due to the presence of an unidentified particulate.

This NDA was originally submitted to obtain approval for Dyloject use through the (b) (4) IV route and nonclinical studies were conducted to support this route of administration (b) (4)

(b) (4)

IV 37.5 mg/Q6 hr dosing appears to be supported based on CMC, biopharmaceutical and clinical evaluation.

## II. MAJOR NONCLINICAL ISSUES IDENTIFIED IN PRIMARY REVIEW

### ***Support for formulation***

As described in Dr. Emami's review, the Applicant has provided sufficient information to support the proposed formulation of Dyloject. There are no novel excipients, all being represented in the FDA Inactive Ingredients Database. Additionally, the levels of HP $\beta$ CD are within that of the approved Sporanox on a daily intake basis (1,332 mg/day vs. 16,000 mg/day) though the administered concentration in Dyloject (333 mg/mL) is higher than the diluted formulation of Sporanox (b) (4) and the administration rate for the current product may be much greater (bolus) versus Sporanox (slow infusion over 60 minutes). Support for the levels of HP $\beta$ CD was derived from data from the NDA as allowed by the Letter of Authorization the Applicant obtained from Johnson & Johnson Pharmaceutical Research & Development on behalf of Ortho McNeil Janssen Pharmaceuticals, Inc. Finally, nonclinical toxicology studies evaluated the local and systemic safety of HP $\beta$ CD as part of a separate vehicle arm in a 28-day monkey IV (bolus) monkey toxicology study which provided a significant (~13X) safety margin based on the area under the plasma concentration-time curve (AUC) of the study No Observed Adverse Effect Level (NOAEL).

The acceptability of using a different formulation (DIC075U (b) (4) (b) (4) in the majority of the nonclinical toxicology program to support the to-be-marketed Dyloject formulation (DIC075V) was considered by Dr. Emami. I agree with Dr. Emami that the critical support needed is local tolerance data and is provided by the use of the DIC075V formulation in dedicated local tolerance studies conducted in rat and rabbit. Though mild local toxicity was observed characterized as reversible perivascular inflammation, this does not preclude nonclinical recommendation for approval though it did predict data from clinical studies which notes some adverse findings.

The applicant previously sought acceptance of specifications for a drug product degradant, (b) (4) but provided an incomplete supporting safety qualification package. When informed of this inadequacy the Applicant agreed (b) (4) to comply with ICHQ3B limits (b) (4) which I find acceptable.

During CMC review, the compound (b) (4) was determined to be a leachable. (b) (4)

(b) (4) The total daily intake with 4 vials/day maximum would represent (4) µg. The compound was reported to be negative in two published genetic toxicity studies (Ames assay and Mouse Lymphoma assay) and has a high oral LD50 in rat of 8000 mg/kg which suggests low (oral) toxicity. I note that the Agency currently has no official guidance on acceptable levels of leachables in drug products; however, a position paper jointly created by Industry, Academia and FDA representatives under the auspices of the Product Quality Research Institute (PQRI) on this subject recommended a qualification threshold (b) (4)

I believe the publically available genetic toxicology information and acute oral toxicity study of this leachable compound, combined with the acute use of the product and very low levels of leachable is sufficient to consider the compound toxicologically qualified as it relates to its presence in the drug product at the end of shelf life.

#### **Support for systemic safety**

As can be seen in the below table taken from the Applicant's Overview of Clinical Pharmacology (and with extrapolation of AUC to 24 hr exposure) Dyloject exceeds the exposure parameters of the listed drug Cataflam.

	Dyloject 37.5 mg (as QID)	Cataflam 50 mg (as QID)	Dyloject vs. Cataflam
C <sub>max</sub> (ng/mL)	5,617 ± 1799	851 ± 462	6.6X
AUC <sub>0-t</sub> (ng*h/mL)	1,839 ± 506	1,350 ± 601	1.4X
AUC <sub>0-24 hr</sub> (ng*h/mL) (extrapolated as QID)	7,356	5,400	

It was therefore necessary for the Applicant to provide sufficient support for these systemic exposures from a nonclinical toxicology evaluation in two species. Intravenous toxicology studies of 28-days duration were performed in the rat and monkey incorporating a subset of animals allowed a 9 or 13 weeks treatment-free recovery period, respectively. Toxicities observed in the rat were considered by Dr. Emami to represent expected NSAID-related pathology, principally GI lesions and secondary hematologic and pathologic disturbances from anemia due to blood loss and the local inflammatory response, though some measure of anemia could be due to destabilization of the red blood cell due to a non-specific action of HPβCD. Toxicities observed in the monkey were similar with GI lesions associated with secondary regenerative anemia which in some cases were sufficiently severe to cause peritonitis and moribundity leading to early sacrifice of the highest dose group. Additional toxicity noted was increased incidence in severity of tail wounds which likely reflects well known NSAID inhibitory effects on wound repair. Histopathologic evidence of effects on the renal tract was

observed in both rats (renal tubular vacuolation) and monkey (granular appearance of medullary rays). The Applicant conducted a separate study in monkey in conjunction with the 28-day study which only evaluated the HP $\beta$ CD vehicle vs. saline and conclusively demonstrated this was an effect of the (b) (4) HP $\beta$ CD. The findings in rat kidney are typical of cyclodextrins as reflected in a number of publications (see Dr. Emami's review) and the prior Sporanox NDA studies to which the Applicant has a Right of Reference. Dr. Emami identified NOAELs which generally (rat: 1.5X AUC; 7.1X C<sub>max</sub>; monkey: 0.8X AUC; 4.5X C<sub>max</sub>) supports the clinical exposure to diclofenac at the maximum recommended human dose (MRHD) and I am in agreement with her evaluation.

The Applicant also conducted genetic toxicology studies on the DIC075U formulation and the product was negative in Ames and Mouse Lymphoma assays, as well as in an *in vivo* Micronucleus assay in the mouse.

### **Support for local safety**

(b) (4) IV route support is necessary for the current application. Single- or repeated-injection of rats with the to-be-marketed DIC075V product produced minimal to moderate perivascular inflammation at the injection sites commensurate with the number of injections and days administered (up to 4 times/day up to 7 days) but this demonstrated general reversibility. This was seen to a more limited extent with the saline vehicle but clearly indicates that in addition to expected local trauma the drug product possesses irritative potential. As Dr. Emami notes this was sufficiently captured and evaluated in the clinical safety data.

## **III. RECOMMENDATIONS**

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

The Applicant has provided nonclinical toxicology evaluation of the drug product in 28-day intravenous rat and monkey toxicology studies using an earlier developmental formulation which adequately support the safety of diclofenac systemic exposures associated with the maximum recommended human dose. Target organ toxicities were expected and are common to NSAID drug products being principally associated with GI lesions and secondary regenerative anemia as well as evidence of impaired wound healing from skin lesions. Histologic evidence of kidney effects at high dose levels is considered non-adverse and related to the vehicle containing hydroxy-propyl  $\beta$ -cyclodextrin. Levels of these excipients and others in formulation are also supported based on prior use in approved drug products. Local tolerance of the to-be-marketed drug product formulation was supported by a single- and repeat-dose IV study in the rat. While mild to moderate irritation was observed in this local tolerance study, this appears reversible and local safety is further

supported by clinical safety data. Other aspects of the formulation, including impurity/degradant specifications and a leachable compound observed in stability are acceptable based on ICH guidelines or are considered toxicologically qualified based on publicly available data.

On this basis, I concur with Dr. Emami that NDA 22-396 for Dyloject may be approved based on the nonclinical data provided.

**B. Recommendation for nonclinical studies**

None

**C. Recommendations on labeling**

The applicant has utilized the approved listed Cataflam label and adjusted safety margins based on a body surface area comparison between the described studies and their Dyloject product. Although safety margins from a direct toxicokinetic bridge using animal exposures under identical administration conditions and comparison to human exposures would be ideal, the proposed label changes with the corrections to include Dr. Emami's recalculations are acceptable.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22396	ORIG-1	HOSPIRA INC	diclofenac sodium injection

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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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ADAM M WASSERMAN  
08/19/2010

**PHARMACOLOGY/TOXICOLOGY NDA FILEABILITY CHECKLIST**

NDA/BLA Number: **22,396**

Applicant: **JAVELIN**

Stamp Date: **December 3,2009**

Drug Name: **Dyloject**

NDA/BLA Type: **505(b2)**

DAARP/OND/CDER/FDA

On **initial** overview of the NDA application for Refuse to File (RTF):

	<b>Parameters</b>	<b>Yes</b>	<b>No</b>	<b>Comment</b>
1	On its face, is the pharmacology section of the NDA/BLA organized (in accord with 21 CFR 314 and current guidelines for format and content) in a manner to allow substantive review to begin?	+		
2	Is the pharmacology/toxicology section of the NDA/BLA indexed and paginated in a manner allowing substantive review to begin?	+		
3	On its face, is the pharmacology/toxicology section of the NDA/BLA legible so that substantive review can begin?	+		
4	Are all required (*) and requested BBIND studies (in accord with 505(b1) and (b2) including referenced literature) completed and submitted in this NDA/BLA (carcinogenicity*, mutagenicity*, teratogenicity*, effects on fertility*, juvenile studies, acute and repeat dose adult animal studies*, maximum tolerated dose determination, dermal irritancy, ocular irritancy, photo co-carcinogenicity, animal pharmacokinetic studies, safety pharmacology, etc)?	+		

5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies been conducted with the appropriate formulation?	+	<table border="1" data-bbox="927 191 1409 373"> <thead> <tr> <th data-bbox="927 191 1084 218">Ingredient</th> <th data-bbox="1084 191 1242 218">DIC075U</th> <th data-bbox="1242 191 1409 218">DIC075V</th> </tr> </thead> <tbody> <tr> <td data-bbox="927 218 1084 245">Diclofenac</td> <td data-bbox="1084 218 1242 245">75 mg</td> <td data-bbox="1242 218 1409 245">75 mg</td> </tr> <tr> <td data-bbox="927 245 1084 273">HPβCD</td> <td colspan="2" data-bbox="1084 245 1409 273">(b) (4)</td> </tr> <tr> <td data-bbox="927 273 1084 333">monothioglycerol</td> <td colspan="2" data-bbox="1084 273 1409 333">(b) (4)</td> </tr> <tr> <td data-bbox="927 333 1084 373">water</td> <td colspan="2" data-bbox="1084 333 1409 373">(b) (4)</td> </tr> </tbody> </table> <p data-bbox="971 380 1507 520">DIC074U was used in most of the pivotal studies. However DIC075V (which will be the marketed product) was used in the IV and IM local tolerance studies.</p> <p data-bbox="971 562 1507 703"><i>Note: IV injections of DIC075V were associated with perivascular hemorrhage and inflammation within most dose group.</i></p>	Ingredient	DIC075U	DIC075V	Diclofenac	75 mg	75 mg	HPβCD	(b) (4)		monothioglycerol	(b) (4)		water	(b) (4)	
Ingredient	DIC075U	DIC075V																
Diclofenac	75 mg	75 mg																
HPβCD	(b) (4)																	
monothioglycerol	(b) (4)																	
water	(b) (4)																	
6	Is (are) the excipient(s) appropriately qualified (including interaction between the excipients if applicable)?	+	<p data-bbox="971 743 1507 846">The total daily exposure to the excipients was less than the maximum potency limits as listed in the IIG.</p> <ul data-bbox="971 856 1425 926" style="list-style-type: none"> <li data-bbox="971 856 1425 926">• Hydroxypropyl-β-cyclodextran (HPβCD)</li> </ul> <p data-bbox="971 930 1507 1283">At the MRDHD, the total daily intake of (b) (4) HPβCD is (b) (4) for Dyloject as compared to Sporanox® (16 g; NDA 20-966); However, the administered concentration is (b) (4) (333 mg/mL vs. (b) (4)), route and rate is not identical (IV (b) (4) bolus vs. IV slow infusion). Supporting nonclinical data to come from Sporanox NDA and/or prior human use/clinical trial safety.</p>															
7	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 <u>submitted</u> a rationale to justify the alternative route?	+																
8	Has the sponsor <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	+																
9	Has the sponsor submitted all special studies/ data requested by the Division during pre-submission discussions with	+	<ol data-bbox="971 1772 1479 1877" style="list-style-type: none"> <li data-bbox="971 1772 1479 1877">1. In vitro Validation of an Experimental Drug- HPβCD Complexation Module following of</li> </ol>															

	the sponsor?			<p>administration of Diclofenac sodium injection (37.5 mg/mL)</p> <p><i>Note: In vivo non-clinical data to assess for drug-drug interaction between diclofenac sodium injection and commonly used drugs which were requested by Pharm/Tox reviewer in pre-NDA meeting on March 2008 was not conducted.</i></p> <p><i>However, since this drug has been in the market for many years we believe there is no need for drug-drug interaction study.</i></p>
10	Are the proposed labeling sections relative to pharmacology, reproductive toxicology, and carcinogenicity appropriate (including human dose multiples expressed in either mg/m <sup>2</sup> or comparative serum/plasma levels) and in accordance with 201.57?		+	Proposed label lacks nonclinical study data in section 8.1 and the entire section 13. Exposure margin adjustment based on a mg/day dosing does not appear supported by comparative BA studies provided; Applicant will need to provide a scientific justification for adjusting the label margins
11	Has the sponsor submitted any toxicity data to address impurities, new excipients, leachables, etc. issues.		+	The only identifiable impurity was <span style="background-color: #cccccc; padding: 2px;">(b) (4)</span> The sponsor has qualified this impurity.
12	Has the sponsor addressed any abuse potential issues in the submission?			N/A
13	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A
14	From a pharmacology/ toxicology perspective, is the NDA/BLA fileable? If ``no`` please state below why it is not.		+	

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

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

**Comments to Sponsor:**

1. We note the proposed label lacks nonclinical study data which may be due to an omission in the NSAID class labeling effort as it affected your RLD product; earlier labels may contain this information.

- You will need to submit a revised label in which you add nonclinical data to Section 8.1 and the entire Section 13.
- Provide a scientifically justified explanation for proposed margin adjustments. Exposure margin adjustment based on a mg/day dosing does not appear supported by comparative BA studies provided.

Reviewing Pharmacologist: Armaghan Emami 02-04-10

Date

Team Leader: \_\_\_\_\_

Date

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22396	ORIG-1	JAVELIN PHARMACEUTICA LS INC	diclofenac sodium injection

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/s/

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ARMAGHAN EMAMI  
02/09/2010

ADAM M WASSERMAN  
02/11/2010