

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**204767Orig1s000**

**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/BLA REVIEW AND EVALUATION**

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Product: Acetaminophen Injection  
Indication: Management of mild to moderate pain;  
management of moderate to severe pain with  
adjunctive opioid analgesics; reduction of fever  
Applicant: Fresenius Kabi USA, LLC  
Review Division: Division of Anesthesia, Analgesia, and Addiction  
Products (DAAAP)  
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# 1 Executive Summary

## 1.1 Introduction

Intravenous acetaminophen (10 mg/mL) formulated in a freeflex (b) (4) is a new drug product presentation developed by Fresenius Kabi USA, LLC for the management of mild to moderate pain, management of moderate to severe pain with adjunctive opioid analgesics, and reduction of fever. The Applicant is submitting an application via the 505(b)(2) pathway that relies upon the Agency's previous findings of safety and efficacy of Cadence Pharmaceuticals' OFIRMEV (NDA 22450). The proposed nonclinical portions of the label for the Fresenius Kabi IV APAP formulation are the same as the referenced OFIRMEV product labeling.

After review of their initial NDA submission (initial NDA submission date of September 28, 2012), the Applicant received a complete response (see complete response letter dated July 25, 2013) from the Agency. Due to the lack of nonclinical data that supports the safety of the container closure system, in particular three identified leachables, a complete response action was recommended.

## 1.2 Brief Discussion of Nonclinical Findings

In this second cycle NDA resubmission, the Applicant submitted nonclinical data to support the safety of several identified leachables associated with the container closure system that were deemed deficiencies in the first cycle review of the NDA. To address the complete response letter from the Division dated July 25, 2013, the Applicant submitted a 4-week IV toxicity study with (b) (4) in rats and a 4-week IV toxicity study with (b) (4) in rats in order to qualify the safety of (b) (4) which are identified leachables from the container closure system. They also submitted 18-month and 24-month timepoints for stability which included updated leachable data and revised toxicological risk assessments, as requested in the complete response letter.

In the 4-week IV toxicology studies, rats were dosed via intravenous administration daily with (b) (4). Adverse local and systemic findings were noted in both repeat-dose toxicity studies with a systemic NOAEL of 50 mg/kg/day and 7.5 mg/kg/day identified for (b) (4), respectively, which confer exposure margins of (b) (4) fold based on a body surface area comparison, respectively. The Applicant also submitted the results of an Ames assay with (b) (4) and with (b) (4). Both leachables tested negative in an Ames assay and therefore are not considered mutagenic under the conditions tested. Review of the revised toxicological risk assessments showed that there is adequate coverage of the leachables (b) (4) to support the proposed 24-month shelf-life.

Taken together, the Applicant has provided adequate nonclinical data to support the safety of the identified leachables from the container closure system and therefore we recommend approval from the nonclinical pharmacology toxicology perspective.

### 1.3 Recommendations

#### 1.3.1 Approvability

From the pharmacology toxicology perspective, the Acetaminophen Injection (10 mg/mL) in freeflex® (b) (4) may be approved.

#### 1.3.2 Additional Non Clinical Recommendations

None.

#### 1.3.3 Labeling

Proposed changes to the Applicant's proposed labeling have not been altered since the first cycle review. Labeling will eventually have to be revised to conform to the Final Pregnancy Labeling and Lactation Rule (which will remove the Pregnancy Category and insert a Risk Summary Statement).

## 2 Drug Information

### 2.1 Drug

CAS Registry Number  
103-90-2

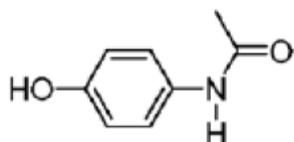
Generic Name  
Acetaminophen; paracetamol

Code Name  
N/A

Chemical Name  
N-acetyl-p-aminophenol;  
4'-hydroxyacetanilide;  
p-hydroxyacetanilide;  
p-acetamidophenol;  
p-acetaminophenol;  
p-acetylaminophenol

Molecular Formula/Molecular Weight  
C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub> / 151.16 g/mol

Structure



### Pharmacologic Class

There is no FDA-established pharmacological class for acetaminophen. Given the lack of clear understanding of the mechanism of action of acetaminophen, an established class cannot be recommended at this time.

## 2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA#	Drug Name	Div	Strength (route)	Marketing Status	AP Date	Indication	Company
22450	OFIRMEV (acetaminophen for injection)	DAAAP	1000 mg/100 mL (10 mg/mL) (IV infusion)	Prescription	November 2, 2010	Management of mild to moderate pain, management of moderate to severe pain with adjunctive opioid analgesics, reduction of fever	Cadence Pharmaceuticals, Inc.

The Applicant did not open an IND or preIND to support this NDA.

MF#	Subject of MF	Holder	Submit Date	Reviewer's Comment
		(b) (4)	October 14, 2003	Acceptable to support numerous NDAs and ANDAs.
26696	FREEFLEX® packaging system (b) (4) as manufactured in Halden, Norway for Fresenius Kabi Deutschland GmbH	Fresenius Kabi Deutschland GmbH	January 22, 2013	The MF contains inadequate safety justification for some identified leachable compounds (see nonclinical review dated June 20, 2013).

## 2.3 Drug Formulation

The drug formulation has not been amended since the time of the original nonclinical review. The reader is referred to the nonclinical review dated June 30, 2013 for information regarding the adequacy of the drug formulation.

## 2.4 Comments on Novel Excipients

The drug formulation has not been amended since the time of the original nonclinical review. It was previously determined that there are no novel excipients in the IV formulation of acetaminophen.

## 2.5 Comments on Impurities/Degradants of Concern

### Drug Substance and Drug Product

The drug substance specifications, drug product specifications, and residual solvents were deemed acceptable after the first cycle review and have not been amended since the time of the original nonclinical review. The reader is referred to the nonclinical review dated June 20, 2013 for information regarding the safety and adequacy of the specifications for drug substance impurities and drug product degradants.

### Container Closure System

To support the safety of the container closure system the Applicant submitted in the initial NDA submission, a LOA to DMF 26696, which contains several nonclinical migration studies (see nonclinical review of MF 26696). However after review of the DMF, several identified leachables were not adequately qualified for safety and were deemed deficiencies after the first NDA review cycle.

The Applicant has submitted updated migration studies to reflect 24 months of storage in various batches. The following table illustrates the storage scheme for the migration studies (from the Applicant's submission):

**Table 3.2.P.2- 16      Scheme for the storage conditions and testing frequency**

Storage Condition	Months							
	0	3	6	9	12	18	24	36
25 °C ± 2 °C / 40 % ± 5 % RH			X				X	
30 °C ± 2 °C / 35 % ± 5 % RH			X		X			
40 °C ± 2 °C / ≤ 25 % RH			X					

The following tables illustrate the migration studies done after 6, 12, and 24 months of storage (from the Applicant's submission):

Product: Acetaminophen Injection 10 mg/ mL Solution for Infusion  
 Batch: 12EGU94  
 Container: 100 mL **freeflex**® (b)(4)  
 Fill volume: 100 mL  
 Film: (b)(4)  
 Storage condition: 25 °C ± 2 °C/40 % RH ± 5 %

**Table 3.2.P.2- 17 Batch 12EGU94, 25 °C**

Target	Method	Unit	Months		
			0	6	24
(b)(4)	AP-829	µg/L	NT	NT	(b)(4)
(b)(4)	AP-829	µg/L	NT	NT	(b)(4)
(b)(4)	AP-829	µmol/L	NT	NT	(b)(4)
(b)(4)	AP-829	µg/L	NT	NT	(b)(4)
(b)(4)	AP-822	µg/L	NT	NT	(b)(4)
(b)(4)	AP-822	µg/L	NT	NT	(b)(4)
(b)(4)	AP-808	µg/L	NT	NT	(b)(4)

NT: not tested

Product: Acetaminophen Injection 10 mg/ mL Solution for Infusion  
 Batch: 12EGU95  
 Container: 100 mL **freeflex**® (b)(4)  
 Fill volume: (b)(4)  
 Film: (b)(4)  
 Storage condition: 25 °C ± 2 °C/40 % RH ± 5 %

**Table 3.2.P.2- 18 Batch 12EGU95, 25 °C**

Target	Method	Unit	Months		
			0	6	24
(b)(4)	AP-829	µg/L	NT	(b)(4)	(b)(4)
	AP-829	µg/L	NT		
	AP-829	µmol/L	NT		
	AP-829	µg/L	NT		
	AP-822	µg/L	NT		
	AP-822	µg/L	NT		
	AP-808	µg/L	NT		

NT: not tested

Product: Acetaminophen Injection 10 mg/ mL Solution for Infusion  
 Batch: 12EGU96  
 Container: 100 mL *freeflex*® (b)(4)  
 Fill volume: 100 mL  
 Film: (b)(4)  
 Storage condition: 25 °C ± 2 °C/40 % RH ± 5 %

**Table 3.2.P.2- 19 Batch 12EGU96, 25 °C**

Target	Method	Unit	Months		
			0	6	24
(b)(4)	AP-829	µg/L	NT	NT	(b)(4)
	AP-829	µg/L	NT	NT	
	AP-829	µmol/L	NT	NT	
	AP-829	µg/L	NT	NT	
	AP-822	µg/L	NT	NT	
	AP-822	µg/L	NT	NT	
	AP-808	µg/L	NT	NT	

NT: not tested

Product: Acetaminophen Injection 10 mg/ mL Solution for Infusion  
 Batch: 12EGU97  
 Container: 100 mL **freeflex**<sup>®</sup> (b) (4)  
 Fill volume: (b) (4)  
 Film: (b) (4)  
 Storage condition: 25 °C ± 2 °C/40 % RH ± 5 %

**Table 3.2.P.2- 20 Batch 12EGU97, 25 °C**

Target	Method	Unit	Months		
			0	6	24
(b) (4)	AP-829	µg/L	NT	NT	(b) (4)
	AP-829	µg/L	NT	NT	
	AP-829	µmol/L	NT	NT	
	AP-829	µg/L	NT	NT	
	AP-822	µg/L	NT	NT	
	AP-822	µg/L	NT	NT	
	AP-808	µg/L	NT	NT	

NT: not tested

Product: Acetaminophen Injection 10 mg/ mL Solution for Infusion  
 Batch: 12EGU95  
 Container: 100 mL *freeflex*® (b) (4)  
 Fill volume: (b) (4)  
 Film: (b) (4)  
 Storage condition: 30 °C ± 2 °C/35 % RH ± 5 %

**Table 3.2.P.2- 21 Batch 12EGU95, 30 °C**

Target	Method	Unit	Months		
			0	6	12
(b) (4)	AP-829	µg/L	NT	(b) (4)	(b) (4)
	AP-829	µg/L	NT		
	AP-829	µmol/L	NT		
	AP-829	µg/L	NT		
	AP-822	µg/L	NT		
	AP-822	µg/L	NT		
	AP-808	µg/L	NT		

NT: not tested

Product: Acetaminophen Injection 10 mg/ mL Solution for Infusion  
 Batch: 12EGU95  
 Container: 100 mL **freeflex**® (b)(4)  
 Fill volume: (b)(4)  
 Film: (b)(4)  
 Storage condition: 40 °C ± 2 °C/ NMT (b)(4) % RH

**Table 3.2.P.2- 22 Batch 12EGU95, 40 °C**

Target	Method	Unit	Months		
			0	3	6
(b)(4)	AP-829	µg/L	NT	NT	(b)(4)
	AP-829	µg/L	NT	NT	
	AP-829	µmol/L	NT	NT	
	AP-829	µg/L	NT	NT	
	AP-822	µg/L	NT	NT	
	AP-822	µg/L	NT	NT	
	AP-808	µg/L	NT	NT	

NT: not tested

The following table illustrates the highest levels of each identified leachable from these migration studies in the above three stability batches of this IV acetaminophen drug product formulation:

Leachable	Maximum Amount in Migration Studies*	Maximum TDI**
(b)(4)		

\* Maximum levels identified from the migration studies using this IV APAP formulation.

\*\* Maximum TDI (total daily intake) calculation is based on the MDD of APAP (4 g/day), which is obtained from (b)(4) via this IV APAP formulation.

Based on the information above, several of the leachables do appear to be increasing over time; however, a few appear to decrease suggesting degradation of the leachable with time.

For the purposes of the risk assessment of these leachables with the information given at the first and second review cycle, the maximum total daily intake values in the table above were calculated from the highest concentration of a specific leachable detected in the 4 batches to date multiplied by the maximum volume of (b) (4) using the proposed IV APAP formulation.

(b) (4)

As per the nonclinical review for MF 26696 (see nonclinical review), the maximum TDI of the remaining leachables from the migration studies using this IV APAP formulation yields an adequate safety margin except for (b) (4).

The Applicant has submitted an updated toxicology risk assessment for (b) (4),

(b) (4)  
(b) (4) product of the antioxidant (b) (4) (see figure below, from the Applicant's submission):

**Figure 1**                      **Structural Formula of** (b) (4)



Toxicological studies with (b) (4) that are used to determine the permitted daily exposure (PDE) value include an Ames test and two 90-day repeat dose study in rats and are illustrated in the following tables (from the Applicant's submission):

**Table 1**                      **Summary of Genotoxicity Information for** (b) (4)

Type of Assay	Design	Results	Reference
Ames test	5 Strains ± S9 mix	No mutagenicity	(b) (4) HKQ0015

**Table 2** Summary of NOAEL Values for (b) (4)

Study Type (Species)	Route of Exposure	NOAEL mg/kg-day	Critical Effects	Reference
90 day repeated dose study in rats (SD)	Oral by gavage	50 (LOAEL)	At (b) (4) mg/kg: thyroid hyperplasia	(b) (4)
90 day repeated dose study in rats (SD)	Oral by gavage	3	At (b) (4) mg/kg gamma globuline and cholesterol increase	(b) (4)

The calculation of the PDE for (b) (4) was then performed according to ICH Q3C(R5) by the Applicant (from the Applicant's submission):

$$PDE = \frac{NOEL(mg / kg / day) \times Weight\_Adjustment(kg)}{F1 \times F2 \times F3 \times F4 \times F5}$$

F1 is a factor for extrapolation between species

F2 is a factor of 10 for variability between individuals

F3 is a factor for short-term toxicity studies

F4 is a factor for severe toxicity (e.g. fetotoxicity and teratogenicity)

F5 is a variable factor if the no-effect level was not established.

The following table illustrates the calculation of the PDE for (b) (4) using the 90-day repeat dose studies (from the Applicant's submission):

**Table 3** Calculation of PDE for (b) (4)

Reference	Study Type	Species	NOAEL mg/kg/day	Modifying Factors	PDE mg
(b) (4)	90 days repeated dose oral toxicity study	Rat	50 (LOAEL)	F1= F2= F3= F4= F5=	(b) (4)
	90 days repeated dose oral toxicity study	Rat	3	F1= F2= F3= F4= F5=	

The following table illustrates the determination of the PDE for (b) (4) (from the Applicant's submission):

**Table 4** Estimated Daily Exposure to (b) (4) and its Calculated PDE

Maximum concentration of (b) (4) in Acetaminophen (µg/L)	Acetaminophen maximum daily dose volume (mL/day)	Maximum daily exposition with (b) (4) (mg/day)	PDE (mg)	
			enteral	iv
(b) (4)	400	(b) (4)	(b) (4)	(b) (4)

As shown in the table above, the maximum daily exposure to (b) (4) is (b) (4) mg/day, which represents an (b) (4)-fold safety margin when using the PDE IV value of (b) (4) mg.

Moreover, the leachable (b) (4) has been adequately qualified for safety as per the original nonclinical review during the first cycle (see nonclinical review dated June 20, 2013). It was noted that the Applicant planned to submit an Ames assay using (b) (4). The Ames assay with (b) (4) has been submitted in this second cycle submission and was reviewed and the compound was not mutagenic under the conditions of the study. Thus, (b) (4) does not represent a safety concern.

(b) (4)  
The potential leachable (b) (4) was not detected in any of the migration studies with the proposed IV APAP formulation that were submitted in this second cycle submission. Thus, (b) (4) does not represent a safety concern.

(b) (4)  
product of the (b) (4). The following (b) (4) figure illustrates the structures of (b) (4) (from the Applicant's submission):

**Figure 1**                      **Structural Formula of** (b) (4)  
(b) (4)



Toxicological studies with (b) (4) that are used to determine the permitted daily exposure (PDE) value include an Ames test and a 4-week repeat dose IV study in rats and are illustrated in the following tables (from the Applicant's submission):

**Table 1** Summary of Acute Toxicity and Genotoxicity Information for (b) (4)

Type of assay	Design	Results	Reference
Ames test	5 Strains ± S9 mix	No mutagenicity	(b) (4) HKQ0016

**Table 2** Summary of NOAEL Values for (b) (4)

Study Type (Species)	Route of Exposure	NOAEL mg/kg-day	Critical Effects	Reference
2 weeks repeated dose preliminary study in rats (SD)	IV bolus injection	50	At (b) (4) mg/kg local irritation, low body weight gain, swaying/unsteady gait	(b) (4) HKQ0011, 2014
4 weeks repeated dose toxicity study in rats (SD)	IV bolus injection	50	No critical effects	(b) (4) HKQ0012, 2014

This reviewer concurs with the Applicant's NOAEL of the 4-week IV toxicity study with (b) (4) (see review of the study below).

The calculation of the PDE for (b) (4) was then performed according to ICH Q3C(R5) by the Applicant (from the Applicant's submission):

$$PDE = \frac{NOEL(mg / kg / day) \times Weight\_Adjustment(kg)}{F1 \times F2 \times F3 \times F4 \times F5}$$

F1 is a factor for extrapolation between species

F2 is a factor of 10 for variability between individuals

F3 is a factor for short-term toxicity studies

F4 is a factor for severe toxicity (e.g. fetotoxicity and teratogenicity)

F5 is a variable factor if the no-effect level was not established.

The following table illustrates the calculation of the PDE for (b) (4) using the 4-weeks repeat dose IV study (from the Applicant's submission):

**Table 3** Calculation of PDE for (b) (4)

Reference	Study type	Species	NOAEL mg/kg/day	Modifying Factors	PDE mg
(b) (4) HKQ0012, 2012	4 weeks repeated dose toxicity study in rats (SD)	Rat	50	F1= F2= F3= F4= F5=	(b) (4)

The following table illustrates the determination of the PDE for (b) (4) (from the Applicant's submission):

**Table 4** Estimated Daily Exposure to (b) (4) and its Calculated PDE

Maximum concentration of (b) (4) in Acetaminophen (µg/L)	Acetaminophen maximum daily dose volume (mL/day)	Maximum daily exposition with (b) (4) (mg/day)	PDE (mg)
(b) (4)	400	(b) (4)	(b) (4)

As shown in the table above, the maximum daily exposure to (b) (4) mg/day, which represents a (b) (4) fold safety margin when using the PDE value of 5 mg.

Thus, the Applicant has adequately addressed the safety of (b) (4)

(b) (4) is the (b) (4) of the two components (b) (4) (see the following figure, from the Applicant's submission):

**Figure 1****Structural Formula of**

(b) (4)

(b) (4)

Toxicological studies with (b) (4) that are used to determine the permitted daily exposure (PDE) value include an LD<sub>50</sub> test, Ames test, and a 4-week repeat dose IV study in rats and are illustrated in the following tables (from the Applicant's submission):

**Table 1 Summary of Acute Toxicity and Genotoxicity Information for**

(b) (4)

Type of assay	Design	Results	Reference
LD50 test	Single dose oral toxicity in rats	LD50: > (b) (4) kg	ECHA 2012
Ames test	5 Strains ± S9 mix	No mutagenicity	ECHA 2012

**Table 2 Summary of NOAEL Values for**

(b) (4)

Study Type (Species)	Route of Exposure	NOAEL mg/kg-day	Critical Effects	Reference
2 weeks repeated dose preliminary study in rats (SD)	IV bolus injection	25	At (b) (4) mg/kg irregular respiration, underactivity, piloerection, impaired locomotion, low food consumption, treatment terminated on day 8	(b) (4) HKQ0013, 2014
4 weeks repeated dose toxicity study in rats (SD)	IV bolus injection	15	At (b) (4) mg/kg irritation at the injection site. Treatment terminated on day 15	(b) (4) HKQ0014, 2014

The calculation of the PDE for (b) (4) was then performed according to ICH Q3C(R5) by the Applicant (from the Applicant's submission) based on the 4-week toxicology study:

$$PDE = \frac{NOEL(mg / kg / day) \times Weight\_Adjustment(kg)}{F1 \times F2 \times F3 \times F4 \times F5}$$

F1 is a factor for extrapolation between species

F2 is a factor of 10 for variability between individuals

F3 is a factor for short-term toxicity studies

F4 is a factor for severe toxicity (e.g. fetotoxicity and teratogenicity)

F5 is a variable factor if the no-effect level was not established.

The following table illustrates the calculation of the PDE for (b) (4) using the 4-weeks repeat dose IV study (from the Applicant's submission):

**Table 3** Calculation of PDE for (b) (4)

Reference	Study type	Species	NOAEL mg/kg/day	Modifying Factors	PDE mg
(b) (4) HKQ0014, 2014	4 weeks repeated dose toxicity study in rats (SD)	Rat	15	F1= (b) (4) F2= F3= F4= F5=	(b) (4)

This reviewer concluded a lower NOAEL for the 4-week IV toxicology study with (b) (4) at 7.5 mg/kg/day (see review of the study below). This would make the PDE (b) (4).

The following table illustrates the determination of the PDE for (b) (4) (from the Applicant's submission):

**Table 4** Estimated Daily Exposure to (b) (4) and its Calculated PDE

Maximum concentration of (b) (4) in Acetaminophen ( $\mu\text{g/L}$ )	Acetaminophen maximum daily dose volume (mL/day)	Maximum daily exposition with (b) (4) (mg/day)	PDE (mg)
(b) (4)	400	(b) (4)	(b) (4)

As shown in the table above, the maximum daily exposure to (b) (4) is (b) (4) mg/day with the new PDE of (b) (4), which represents a (b) (4)-fold exposure margin.

Thus, the Applicant has adequately addressed the safety of (b) (4)

## 2.6 Proposed Clinical Population and Dosing Regimen

The indication is the management of mild to moderate pain, management of moderate to severe pain with adjunctive opioid analgesics, and reduction of fever. Due to the route of administration, this is not considered a chronic indication. As this product is administered in a hospital setting, the typical duration of use would be less than 14 days.

## 2.7 Regulatory Background

This is a 505(b)(2) application referencing the Agency's previous findings of safety to OFIRMEV (NDA 22450). The Applicant received a complete response (see complete response letter dated July 25, 2013) from the Agency at the conclusion of their initial NDA submission (Initial NDA submission date of September 28, 2012). The following nonclinical deficiencies were noted in the complete response letter:

### NONCLINICAL

1. You have not provided adequate safety justification for the levels of (b) (4) and (b) (4) leachables from the container closure system. To resolve this deficiency:
  - a. Submit the results of the proposed 4-week IV toxicology study of (b) (4) and a revised toxicological risk assessment.
  - b. Conduct and submit the results of a 4-week IV toxicology study of (b) (4) and a revised toxicological risk assessment for this compound. Alternatively, provide adequate data to support your conclusion that (b) (4) is virtually instantaneous in vivo such that exposure to the parent compound, when the product is used as directed, does not occur.

The following additional comment was in the Complete Response letter:

- You have proposed to complete in vitro bacterial reverse mutation studies (Ames tests) for both (b) (4) and (b) (4). The final reports for these studies are not required for approval. However, when the studies have been completed, the results should be submitted to the NDA.

### 3 Studies Submitted

#### 3.1 Studies Reviewed

The following table illustrates the nonclinical studies that were submitted and reviewed:

Study Number	Title	Location
HKQ0015	(b) (4) Bacterial Reverse Mutation Test	SDN 17; 4.2.3.7.7
HKQ0016	(b) (4) Bacterial Reverse Mutation Test	SDN 17; 4.2.3.7.7
HKQ0012	(b) (4) Toxicity Study by Intravenous Infusion to CD Rats for 4 Weeks	SDN 17; 4.2.3.7.7
HKQ0014	(b) (4) Toxicity Study by Intravenous (Bolus) Administration to CD Rat for 4 Weeks	SDN 17; 4.2.3.7.7

#### 3.2 Studies Not Reviewed

None. All studies in the table above were reviewed.

#### 3.3 Previous Reviews Referenced

Type of nonclinical Review	Application	Author	DARRTS Submission Date
First cycle primary review	NDA 204767	Dr. Carlic Huynh	June 20, 2013
MF review	DMF 26696	Dr. Carlic Huynh	June 20, 2013
First cycle secondary review	NDA 204767	Dr. R. Daniel Mellon	June 21, 2013

### 4 Pharmacology

#### 4.1 Primary Pharmacology

There were no primary pharmacology studies with acetaminophen submitted in this NDA.

## 4.2 Secondary Pharmacology

There were no secondary pharmacology studies with acetaminophen submitted in this NDA.

## 4.3 Safety Pharmacology

There were no safety pharmacology studies with acetaminophen submitted in this NDA.

# 5 Pharmacokinetics/ADME/Toxicokinetics

## 5.1 PK/ADME

There were no PK/ADME studies with acetaminophen submitted in this NDA.

## 5.2 Toxicokinetics

There were no toxicokinetics studies with acetaminophen submitted in this NDA.

# 6 General Toxicology

There were no general toxicology studies with acetaminophen submitted in this NDA.

The Applicant submitted two final reports for repeat-dose IV toxicity studies with either (b) (4) conducted in rats. These studies were submitted to the NDA to justify the safety of these two leachables present in the Freeflex® (b) (4) and are reviewed below.

## 6.2 Repeat-Dose Toxicity

**Study title:** (b) (4) **Toxicity Study by Intravenous Infusion to CD Rats for 4 Weeks**

Study no.: HKQ0012

Study report location: Module 4 of the SDN 17 electronic submission

Conducting laboratory and location:

(b) (4)

Date of study initiation: March 5, 2014

GLP compliance: Yes. Signature provided on August 18, 2014.

QA statement: Yes. Signature provided on August 18, 2014.

Drug, lot #, and % purity: (b) (4) Batch 047S-052S-EH is (b) (4) % pure, Batch 053054S-EH is (b) (4) % pure, and Batch 055/4-6S-A is (b) (4) % pure

### Key Study Findings

- Rats were dosed via intravenous administration with 0, 12.5, 25, or 50 mg/kg/day of (b) (4) for 4 weeks.
- The concentration of (b) (4) tested was (b) (4) fold greater than the concentration of (b) (4) found in migration studies; therefore, the local tissue toxicity effects noted are not clinically relevant.
- There were no deaths and no treatment-related changes in food consumption, ophthalmoscopy, hematology, and urinalysis.
- Injection site observations (clinical signs) include bruising, erosion and or ulceration, erythema, eschar, loss of flexibility, and reddening at the 25 and 50 mg/kg/day dose groups.
- There were no treatment-related changes in body weight; however, there was a significant decrease of 22.6% in the body weight gains in the female 50 mg/kg/day dose group.
- There was a significant increase in the triglyceride and phosphorous levels of 45.5 and 23.4%, respectively, in the male 50 mg/kg/day dose group with macroscopic and microscopic correlates in the liver and kidney.
- Macroscopically, hernia in the diaphragm, small epididymis, depression in the kidney, liver changes (irregular surfaces, masses, and small size), small testes, unilaterally absent thyroid glands, and depression and scab at the venous injection site were observed at the 50 mg/kg/day dose group.
- The histopathological findings at the injection site including perivascular hemorrhage, recanalized thrombus, vascular intimal proliferation, epidermal ulceration, and scabs at the 25 and 50 mg/kg/day dose groups are indicative of mild localized vascular irritation as opposed to systemic vascular irritation.
- Other histopathological changes at the injection site include perivascular inflammatory cell infiltration in the male 25 and 50 mg/kg/day dose groups, granuloma of the hair shaft in the female 50 mg/kg/day dose group, perivascular necrosis in male 50 mg/kg/day dose group, and abscess and epidermal hyperplasia in the female 50 mg/kg/day dose group.
- Histopathological changes in other sites include aggregates at the corticomedullary junction in the liver as well as perivascular infiltration of inflammatory cells and granuloma on the lung at the 50 mg/kg/day dose group.
- The pathologist's report concludes these systemic findings as incidental.
- The Applicant defines a NOAEL of 50 mg/kg/day and NOEL of 25 mg/kg/day for systemic toxicity. This reviewer concurs with this assessment.
- At the MTDD of 2 g/day of APAP, the total daily intake of (b) (4) is (b) (4) mcg/day. The rat NOAEL of 50 mg/kg/day confers an exposure -margin of (b) (4) fold based on a body surface area comparison.

## Methods

Doses: 0, 12.5, 25, and 50 mg/kg/day  
 Frequency of dosing: Once daily  
 Route of administration: Intravenous infusion into the left and right caudal vein at an infusion rate of 2.5 mL/kg/minute  
 Dose volume: Varies with dose (between 1.25 to 5 mL/kg), see table below  
 Formulation/Vehicle: 0.9% saline  
 Species/Strain: Rat/Crl:CD(SD)  
 Number/Sex/Group: 10/sex/group  
 Age: 43 to 50 days  
 Weight: 206 to 257 g (males); 167 to 199 g (females)  
 Satellite groups: None  
 Unique study design: None  
 Deviation from study protocol: The Applicant noted that for 1 day (April 7-8, 2014), the rats received 24 hours of light as opposed to a cycle of 12 hours light and 12 hours dark.

The following table illustrates the study design (from the Applicant's submission):

Group	Treatment	Dose level (mg/kg/day)#	Dose con. (mg/mL)	Dose volume (mL/kg)	Number of animals	
					Male	Female
1	Control	0	0	5	10	10
2	(b) (4)	12.5	(b) (4)	(b) (4)	10	10
3	(b) (4)	25	(b) (4)	(b) (4)	10	10
4	(b) (4)	50	(b) (4)	(b) (4)	10	10

# As supplied.

Saline flush was administered as 5 mL/kg; up to a maximum of 1 mL saline.

As shown in the table above, the concentration of (b) (4) tested is 10 mg/mL. In the original nonclinical review (dated June 20, 2013), the maximum amount of (b) (4) present in the migration studies was (b) (4) mcg/mL. This study tests concentrations of (b) (4) that are over (b) (4) fold the concentrations found in the migration studies. Thus, this study tests an adequate concentration of (b) (4)

## Observations and Results

### Mortality

Rats were observed visually twice daily for evidence of ill-health or reaction to treatment. There were no deaths or early sacrifice of rats in any of the dose groups.

### Clinical Signs

A detailed weekly physical examination was performed for each rat to monitor general health. Injection site observations were observed daily, prior to dosing. The signs associated with dosing were also examined once weekly during the first week of treatment and more detailed observations thereafter during Weeks 2 to 4, which included pre-dose and 1 to 2 hour post-dose observations.

The following table illustrates the clinical signs observed at the injection site (data from the Applicant's submission):

Observation	Male Dose Groups Affected	Female Dose Groups Affected	Days (Number of male rats/dose/day)	Days (Number of female rats/dose/day)
<b>N = 10</b>				
Bruising of the tail	25 and 50 mg/kg/day	12.5, 25, and 50 mg/kg/day	5 to 26 (up to 2 rats/dose/day)	2 to 30 (up to 3 rats/dose/day)
Erosion and or ulceration	50 mg/kg/day	None	26 to 29 (1 rat/day)	None
Erythema of the tail	25 and 50 mg/kg/day	25 and 50 mg/kg/day	6 to 29 (up to 3 rats/dose/day)	8 to 30 (up to 3 rats/dose/day)
Eschar of the tail	25 and 50 mg/kg/day	25 and 50 mg/kg/day	5 to 29 (1 rat/dose/day)	4 to 30 (up to 3 rats/dose/day)
Loss of flexibility	50 mg/kg/day	None	26 to 29 (1 rat/day)	None
Pale area on tail	50 mg/kg/day	25 and 50 mg/kg/day	8 to 18 (1 rat/day)	8 to 11 (1 rat/day from the 25 mg/kg/day dose group); 28 to 30 (1 rat/day from the 50 mg/kg/day dose group)
Reddening of the tail	50 mg/kg/day	None	26 to 29 (1 rat/day)	None

As shown in the table above, these effects seen at the injection site were observed mainly in the 25 and 50 mg/kg/day dose groups. The bruising of the tail in the female 12.5 mg/kg/day dose group was observed in 1 rat/day on Days 2, 3, 10, and 11. There were none of the above findings in the control group.

The remaining clinical signs were sporadic in nature and are common with this species of rat.

The Applicant noted that there were rats that did not receive the daily scheduled dose of (b) (4) as illustrated in the following table (from the Applicant's submission):

Text Table 1 - animals that did not receive their daily scheduled dose

Treatment	Day No.	Group/Sex/ Animal No.	Other information
0 mg/kg/day	25	1M 59	Had no flush as the needle flicked out at the end of dosing as the flush was about to be administered
12.5 mg/kg/day	2	2M 20	No flush given animal flicked needle out
	2	2F 63	No flush given animal flicked needle out
	2	2F 64	No flush given animal flicked needle out
	21	2F 63	Not dosed; unable to locate vein
	21	2F 68	No flush given animal flicked needle out
	26	2M 13	No flush given animal flicked needle out
25 mg/kg/day	4	3F 79	Not dosed due to signs seen on tail and unable to locate vein
	15	3F 77	No flush given animal flicked needle out
	19	3F 79	Not dosed; unable to locate vein
	21	3F 79	Not dosed; unable to locate vein
	22	3F 72	Not dosed; unable to locate vein
	26	3M 24	No flush given as cannula removed in error
50 mg/kg/day	7	4M 33	Small amount of dose appeared to be administered subcut
	7	4M 39	Small amount of dose appeared to be administered subcut
	8	4F 81	Not dosed; unable to locate vein
	14	4F 81	Not dosed; unable to locate vein
	18	4F 81	Not dosed; unable to locate vein
	19	4F 84	Not dosed; unable to locate vein
	21	4F 84	Not dosed; unable to locate vein
	21	4F 89	Not dosed; unable to locate vein
	22	4F 84	Not dosed; unable to locate vein
	24	4M 36	Not dosed; unable to locate vein
	25	4M 40	Had no flush given at the end of dosing as some dose noted to have gone subcut
	25	4F 82	Had no flush as the needle flicked out at the end of dosing as the flush was about to be administered
	27	4F 84	Not dosed; unable to locate vein
	28	4F 90	No flush given animal flicked needle out

As shown the table above, there were 6 occasions each from the 12.5 and 25 mg/kg/day dose groups and 14 occasions from the 50 mg/kg/day dose group where rats did not receive the daily scheduled dose of (b) (4). For each missed dose, the reason appears to be a technical error and not due to treatment-related adverse effects or moribundity. Moreover, the days of missing dosing as well as the number of animals missing the dose appear to be sporadic with any given animal not missing more than 4 doses out of a total of 28 dose administrations.

## Body Weights

The body weights were recorded for each rat weekly throughout the study and before the scheduled necropsy. There were no treatment-related changes in body weight or body weight gain over the 4 weeks of treatment in the males. Although there were no treatment-related changes in body weight in the females, the body weight gain over the 4 weeks of treatment was decreased by 22.6% in the 50 mg/kg/day dose group (see table below).

TABLE 3 (cont) Body weight - group mean values (g)

Dose Group Dose (mg/kg/day)		Control	(b) (4)				Change 0-4	
		1 0	2 12.5	3 25	4 50			
Group /Sex		Week P1	Week 0	1	2	3	4	Change 0-4
Statistics test								
1F	Mean	146	180	199	220	228	232	53
	SD	5.2	8.0	10.6	9.5	12.6	14.4	11.1
	N	10	10	10	10	10	10	10
2F	Mean	146	186	203	220	224	233	47
	SD	7.0	11.2	12.0	15.4	20.4	20.9	14.5
	N	10	10	10	10	10	10	10
X of 1F								0.90
3F	Mean	144	183	202	222	230	234	51
	SD	7.2	8.4	9.9	14.7	15.7	14.7	13.6
	N	10	10	10	10	10	10	10
X of 1F								0.97
4F	Mean	148	182	198	213	219	222	41*
	SD	4.4	7.3	11.2	13.5	16.0	13.9	10.2
	N	10	10	10	10	10	10	10
X of 1F								0.77

### Food Consumption

Food consumption was measured weekly throughout the study. There were no treatment-related changes in food consumption.

### Ophthalmoscopy

An eye examination was performed using a binocular indirect ophthalmoscope during pretreatment and at Week 4. The adnexae, conjunctiva, cornea, sclera, anterior chamber, iris (pupil dilated), lens, vitreous, and fundus were examined. There were no treatment-related changes in ophthalmoscopy.

### ECG

An ECG was not performed during this study.

### Hematology

Blood samples for hematology, coagulation, and clinical chemistry were collected after an overnight fast prior to dosing during Week 4 on all rats. The following hematology parameters were examined (from the Applicant's submission):

- Haematocrit (Hct)
- Haemoglobin concentration (Hb)
- Erythrocyte count (RBC)
- Absolute reticulocyte count (Retic)
- Mean cell haemoglobin (MCH)
- Mean cell haemoglobin concentration (MCHC)
- Mean cell volume (MCV)
- Red cell distribution width (RDW)
- Total leucocyte count (WBC)
- Differential leucocyte count:
  - Neutrophils (N)
  - Lymphocytes (L)
  - Eosinophils (E)
  - Basophils (B)
  - Monocytes (M)
  - Large unstained cells (LUC)
- Platelet count (Plt)
- Morphology:
  - Anisocytosis
  - Macrocytosis
  - Microcytosis
  - Hypochromasia
  - Hyperchromasia

The following coagulation parameters were examined (from the Applicant's submission):

Prothrombin time (PT) - using IL PT-Fibrinogen reagent.

Activated partial thromboplastin time (APTT) - using IL APTT reagent.

In males, there were decreases in the MCHC by 2.2, 1.2, and 1.5% in the 12.5, 25, and 50 mg/kg/day dose groups, respectively, compared to control. This finding is below 10%, is within the normal control range ( (b) (4) ), and is not dose-dependent. Thus, the decrease in MCHC does not represent a safety concern. There was an increase in monocytes by 63.2% in the 50 mg/kg/day dose group compared to control. Increases in monocytes in the blood may be caused by chronic infections and are not necessarily treatment-related. Moreover, there were no macroscopic (gross pathology) for microscopic (histopathology) correlates for this finding. Thus, the increase in monocytes does not represent a safety concern. There were no other treatment-related changes in hematology and coagulation in the males or females.

### Clinical Chemistry

The following clinical chemistry parameters were examined (from the Applicant's submission):

- Alkaline phosphatase (ALP)
- Alanine aminotransferase (ALT)
- Aspartate aminotransferase (AST)
- Total bilirubin (Bili)
- Urea
- Creatinine (Creat)
- Glucose (Gluc)
- Total cholesterol (Chol)
- Triglycerides (Trig)
- Sodium (Na)
- Potassium (K)
- Chloride (Cl)
- Calcium (Ca)
- Inorganic phosphorus (Phos)
- Total protein (Total Prot)
- Albumin (Alb)

In addition, the albumin/globulin ratio (A/G ratio) was calculated from total protein concentration and analyzed albumin concentration.

In males, there was an increase of 7.4 and 12.5% in glucose levels in the 25 and 50 mg/kg/day dose groups, respectively, an increase of 45.5% in triglyceride levels in the 50 mg/kg/day dose group, and an increase of 12.5, 6.3, and 23.4% in phosphorous levels in the 12.5, 25, and 50 mg/kg/day dose groups, respectively. In contrast, there was a decrease of 2.6, 5.3, and 2.6% in albumin in the 12.5, 25, and 50 mg/kg/day dose groups, respectively, and a decrease of 7.0% in the albumin/globulin ratio, respectively, compared to control. The changes in glucose, albumin, and the albumin/globulin ratio, however, are within the normal range ( (b) (4) ). Thus, the changes in glucose, albumin, and the albumin/globulin ratio do not represent a safety concern. The increase in triglycerides and phosphorous levels may be caused by liver and kidney damage. There were histopathological changes in the liver and kidney at the 50 mg/kg/day dose group including infiltration of inflammatory cells in the kidney as well as aggregates at the cortico-medullary junction and vacuolation in the liver at the male 50 mg/kg/day dose group; however, there were no liver and kidney findings in the 12.5 and 25 mg/kg/day dose groups.

In females, there was an increase of 2.0% in chloride levels in the 50 mg/kg/day dose group. This increase in the chloride levels is within the normal range ( (b) (4) ). Thus, the increase in chloride levels does not represent a safety concern.

There were no further treatment-related changes in clinical chemistry.

## Urinalysis

Rats were placed in individual metabolism cages overnight (fasted) to collect urine samples over a period of approximately 16 hours during Week 4. The following urinalysis parameters were examined (from the Applicant's submission):

### Using manual methods:

- Clarity and Colour (App) - by visual assessment
- Volume (Vol) - using a measuring cylinder
- pH - using a pH meter
- Specific gravity (SG) - by direct refractometry using a SG meter

### Using Multistix reagent strips, interpreted using a Clinitex<sup>®</sup> 500 instrument:

- Ketones (Keto)
- Bilirubin/bile pigments (Bili)
- Blood pigments (UBld)

### Using a Roche P Modular analyser:

- Protein (Prot and T-Prot)
- Creatinine (U-Creat and T-Creat)
- Glucose (U-Gluc And T-Gluc)
- Sodium (U-Na and T-Na)
- Potassium (U-K and T-K)
- Chloride (U-Cl and T-Cl)

There were no treatment-related changes in urinalysis in the males. There were 23.9 and 42.1% increases in sodium and chloride levels, respectively, in the female 50 mg/kg/day dose group. These increases in the sodium and chloride levels are within the normal range ( (b) (4) ). Thus, the increases in the sodium and chloride levels do not represent a safety concern. There were no further treatment-related changes in urinalysis.

### Gross Pathology

At the scheduled sacrificed, all rats were subjected to a detailed necropsy including a full macroscopic examination of the tissues. All external features and orifices were examined visually. Abnormalities in appearance or size of any organ and tissue (external and cut surface) were recorded. Organs and tissue samples were preserved.

The following table illustrates the gross pathology observations (data from the Applicant's submission):

Findings	Number of Rats Affected (N = 10 rats/dose examined)							
	Males (mg/kg/day)				Females (mg/kg/day)			
	Control	12.5	25	50	Control	12.5	25	50
Diaphragm Hernia	0	0	0	1	0	0	0	0
Epididymis Small	0	0	0	1	N/A	N/A	N/A	N/A
Kidneys Depression	0	0	0	1	0	0	0	0
Liver Irregular Surface Masses Small	0 0 0	0 0 0	0 0 0	1 1 1	0 0 0	0 0 0	0 0 0	0 0 0
Testes Small	0	0	0	1				
Thyroids Unilaterally Absent	0	0	0	0	0	0	0	1
Venous IS* (Lateral and Caudal) Depression Scab	0 0	0 0	0 0	2 0	0 0	0 0	0 0	1 1

\* Injection Site

As shown in the table above, macroscopic changes were observed in the diaphragm, epididymis, kidneys, liver, and testes in the male 50 mg/kg/day dose group as well as in the thyroids in the female 50 mg/kg/day dose group. The incidences of these findings were only in 1 rat/dose group. Additionally, injection site (IS) observations include depressions that were observed in 2 males from the 50 mg/kg/dose group. Depressions and scabs were also observed in 1 female from the 50 mg/kg/dose group. Depression and scab were correlated microscopically with perivascular necrosis, epidermal ulceration, and scabs (see histopathology below).

### **Organ Weights**

Each organ was weighed and recorded from all rats at the scheduled necropsy (end of Week 4). For bilateral organs, left and right organs were weighed together. In the males, there was a decrease of 15.8 and 17.5% in the absolute weight of the adrenals in the 25 and 50 mg/kg/day dose groups compared to control. There were no macroscopic (gross pathology) or microscopic correlates with the decrease in the weight of the adrenals and thus, the increase in the weight of the adrenals can be dismissed. There were no other treatment-related changes in organ weights in the males or females.

### **Histopathology**

#### **Adequate Battery**

The following table illustrates the organs and tissues that were weighed and examined microscopically (from the Applicant's submission):

Tissue and regions to be examined	Necropsy		Histology	Pathology
	Weigh	Fix		Light microscopy
Abnormalities		*	*	*
Adrenals	*	*	*	*
Aorta - thoracic		*	*	*
Bone marrow smear		*		c)
Brain (cerebellum, cerebrum, midbrain)	*	*	*	*
Caecum		*	*	*
Colon		*	*	*
Duodenum		*	*	*
Epididymides	*	*	*	*
Eyes		*	*	*
Femur (femorotibial joint)		b)	*	*
Harderian glands		*	*	*
Head		*	#	#
Heart (including auricular and ventricular regions)	*	*	*	*
Ileum		*	*	*
Jejunum		*	*	*
Kidneys	*	*	*	*
Liver (section from two lobes)	*	*	*	*
Lungs (section from two major lobes including bronchi)		*	*	*
Lymph nodes - mesenteric		*	*	*
- left axillary		*	*	*
- inguinal		*	*	*
Oesophagus		*	*	*
Ovaries	*	*	*	*
Pancreas		*	*	*
Parenteral sites (intravenous injection sites)		*	*	*
Pituitary	*	*	*	*
Prostate	*	*	*	*
Salivary glands - submandibular		*	†	†
- parotid		*	†	†
- sublingual		*	†	†
Sciatic nerves		*	†	†
Seminal vesicles	*	*	*	*
Skeletal muscle		*	†	†
Skin with mammary glands (inguinal area)		*	*	*
Spinal cord (transverse and longitudinal sections at the cervical level)		*	*	*
Spleen	*	*	*	*
Sternum		*	*	*
Stomach		*	*	*
Testes	*	*	*	*
Thymus	*	*	*	*
Thyroid with parathyroids	a)	*	*	*
Trachea		*	*	*
Urinary bladder		*	*	*
Uterus with cervix	*	*	*	*
Vagina		*	*	*

a) Weighed after partial fixation.

b) Both hindlimbs retained, one sectioned where appropriate.

c) Will be examined by the (b) (4) (if required).

\* Organs weighed, samples fixed or sections examined microscopically.

# Examined if effects suspected during the study.

† Only one examined.

This appears to be an adequate battery of tissues for histopathology.

Peer Review

A signed pathology report was provided on August 18, 2014. There appears to be one pathologist for the study and as such, a peer review was not evident.

### Histological Findings

The following table illustrates the microscopic findings at the injection site (data from the Applicant's submission):

Venous Injection Site (Lateral, Caudal) Observations (Histopathology)								
Findings	Number of Rats Affected							
	Males (mg/kg/day)				Females (mg/kg/day)			
	Control	12.5	25	50	Control	12.5	25	50
Number of rats examined	10	10	10	10	10	10	10	10
Hemorrhage, Perivascular								
Minimal	6	0	1	5	3	4	1	1
Slight	0	0	1	1	2	0	2	2
Moderate	0	0	0	0	0	0	1	0
<b>Total</b>	<b>6</b>	<b>0</b>	<b>2</b>	<b>6</b>	<b>5</b>	<b>4</b>	<b>4</b>	<b>3</b>
Infiltration, Inflammatory Cells, Perivascular								
Minimal								
Slight	0	2	2	2	3	1	0	3
Moderate	1	1	1	1	1	1	5	1
<b>Total</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>1</b>
<b>Total</b>	<b>1</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>4</b>	<b>2</b>	<b>6</b>	<b>5</b>
Fibrosis, Perivascular								
Minimal	3	1	1	0	2	0	0	4
Slight	0	2	4	0	3	2	4	2
Moderate	1	4	2	3	0	2	4	2
Marked	0	0	0	0	1	1	0	0
<b>Total</b>	<b>4</b>	<b>7</b>	<b>7</b>	<b>3</b>	<b>6</b>	<b>5</b>	<b>8</b>	<b>8</b>
Granuloma, Hair Shaft								
Minimal	1	1	0	0	2	0	0	0
Slight	1	0	0	0	0	0	0	0
Moderate	0	0	0	0	0	0	0	1
<b>Total</b>	<b>2</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>2</b>	<b>0</b>	<b>0</b>	<b>1</b>
Proliferation, Vascular Intimal								
Minimal	1	2	1	1	0	3	1	1
Slight	1	1	3	0	2	2	2	2
Moderate	0	1	0	0	0	0	2	1
Marked	0	0	0	0	0	0	0	1
<b>Total</b>	<b>2</b>	<b>4</b>	<b>4</b>	<b>1</b>	<b>2</b>	<b>5</b>	<b>5</b>	<b>5</b>
Scab(s)	0	0	0	2	0	0	0	1
Ulceration, Epidermal								
Slight	0	0	0	1	0	0	0	0
Moderate	0	0	0	1	0	0	0	1
<b>Total</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>2</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1</b>
Necrosis,								

Perivascular								
Minimal	0	0	1	1	3	1	0	1
Slight	0	1	0	0	0	1	3	1
Moderate	0	0	0	1	0	0	0	0
<b>Total</b>	<b>0</b>	<b>1</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>2</b>	<b>3</b>	<b>2</b>
Abscess								
Moderate	0	0	0	0	0	0	0	1
Hyperplasia, Epidermal								
Marked	0	0	0	0	0	0	0	1
Thrombus, Recanalizing	0	1	5	3	1	0	6	4

As shown in the tables above, there were a number of findings observed at the injection site microscopically. There was no clear dose-dependency on the incidence and severity of perivascular hemorrhage. Although the incidence of recanalized thrombus was greater than control, there does not appear to be a dose-dependency. The recanalizing thrombus observation reflects the traumatic character of repeated IV dosing as the observation was also noted in the female control group. Although the incidence of vascular intimal proliferation was greater than control, there does not appear to be dose-dependency in the incidence of this finding; however, the severity of the finding is dose dependent with marked severity observed in the female 50 mg/kg/dose group only. The increase in the incidence and severity of epidermal ulceration as well as the incidence of scabs were dose-dependent as these findings were observed in the 50 mg/kg/dose groups (male and female) only. These histopathological findings at the injection site are indicative of mild localized vascular irritation.

Other histopathological changes at the injection site include perivascular inflammatory cell infiltration, the severity of which appears dose-dependent for the male 25 and 50 mg/kg/day dose groups. Perivascular fibrosis does not appear to be clearly dose-dependent for incidence and severity. Granuloma of the hair shaft, abscess, and epidermal hyperplasia appears to be dose dependent in terms of severity as the finding was highest in the female 50 mg/kg/day dose group. The severity of perivascular necrosis appears to be dose dependent as the moderate severity of this finding was observed in male 50 mg/kg/day dose group.

Moreover, the following table illustrates additional histopathological changes that were observed (data from the Applicant's submission):

Findings	Number of Rats Affected							
	Males (mg/kg/day)				Females (mg/kg/day)			
	Control	12.5	25	50	Control	12.5	25	50
Number of rats examined	10			10	10			10
Epididymes Cell debris (intraluminal) Slight Sperm	0			1	N/A			N/A

(reduced, luminal) Moderate	0			1	N/A			N/A
Eyes Rosettes/ Folds (retina) Minimal	0			1	0			0
Kidneys Infiltration of Inflammatory Cells (interstitial) Minimal	0			2	1			1
Liver Aggregates (Cortico- Medullary Junction) Minimal Slight <b>Total</b>	4 0 4			4 1 5	8 0 8			8 0 8
Glycogen (decreased, periportal) Slight	0			1	0			0
Nodule (hepato- diaphragmatic)	0			1	0			0
Vacuolation (hepato- cellular, centrilobular) Minimal	0			1	0			0
Lungs Infiltration of Inflammatory Cells (perivascular) Minimal Hemorrhage (Aveoli)  Minimal  Granuloma Minimal Slight <b>Total</b>	1  0  0 0 0			4  1  0 0 0	1  1  2 0 0 2	Note: 1 rat exam.  1 Note: 1 rat exam.  1 Note: no rats exam.  0 0 0 0	0  0  0 0 0	1  0  4 1 5
Lymph Node (left axillary) Plasmacytosis Slight	0			0	0			1
Lymph Node (inguinal) Plasmocytosis								

Minimal	0			1	0			0
Pancreas Atrophy (Acinar Cells)								
Minimal	0			0	1			0
Slight	0			0	0			1
<b>Total</b>	<b>0</b>			<b>0</b>	<b>1</b>			<b>1</b>
Prostate Infiltration of Inflammatory Cells								
Minimal	0			1	N/A			N/A
Testes Degeneration, Tubular								
Moderate	0			1	N/A			N/A
Thyroids Aggregates, Lymphoid								
Minimal	0			0	0			1

It is noted that only the control and 50 mg/kg/day dose group were examined microscopically unless otherwise indicated. As shown in the table above, several findings were observed in the 50 mg/kg/day dose group. These findings include intraluminal cell debris and reduced luminal sperm in the epididymis, rosettes/folds in the retina, glycogen as well as hepatodiaphragmatic nodules and hepatocellular vacuolation in the liver, hemorrhage in the aveoli of the lungs, plasmacytosis in the left axillary and inguinal lymph nodes, atrophy of the acinar cells in the pancreas, infiltration of inflammatory cells in the prostate, degeneration of the tubules in the testes, and lymphoid aggregates in the thyroid. These findings were minimal to slight, only occurred in 1 rat from the 50 mg/kg/day dose group and did not occur in both sexes. Histopathological examination of the 12.5 and 25 mg/kg/day dose groups would most likely render these findings incidental or not-dose dependent and unlikely treatment related.

Other histopathological findings in the 50 mg/kg/day dose group were observed in more than 1 rat or demonstrated increased severity. These findings include aggregates at the corticomedullary junction in the liver as well as perivascular infiltration of inflammatory cells and granuloma in the lungs.

There were no further treatment-related histopathological changes.

### Special Evaluation

There was no special evaluation performed in this study.

### Toxicokinetics

Toxicokinetics was not performed for this study.

## Dosing Solution Analysis

A representative sample was taken from each batch of test substance and samples of formulations prepared for administration in Weeks 1, 3 (Day 15 preparation), and 4 (Day 22-23 and Day 24-29 preparations) were analyzed for concentration. In addition, stability and homogeneity were parameters examined during dosing solution analysis.

The following table illustrates the concentration and stability of (b) (4) in each sample taken (from the Applicant's submission):

### Group 1MF, Control-0.9% saline pH 4.5 - 8

Table: Sample occasion, sample date and measured concentration of the target substance of group 1MF:

Sample occasion / Sample date	Substance [mg/mL]	
	(b) (4)	
Week 1 / 12-Mar-14	n.d.	n.d.
Week 4 / 02-Apr-14	n.d.	n.d.

n.d. = not detectable

### Group 2, 3, 4 MF, 10mg/mL in 0.9% saline pH 4.5 - 8

Table: Sample occasion, sample date and measured concentration of the target substance group 2, 3, 4MF:

Sample occasion / Sample date	Substance [mg/mL]	
	(b) (4)	
Week 1 / 12-Mar-14	(b) (4)	n.d.
Week 3 / 27-Mar-14, filtered	(b) (4)	n.d.
Week 3 / 27-Mar-14, unfiltered	(b) (4)	n.d.
Week 4 / 03-Apr-14	(b) (4)	n.d.
Week 4 / 04-Apr-14, new batch	(b) (4)	n.d.

n.d. = not detectable

As shown in the table above, the (b) (4) did not break down to (b) (4) in any of the samples analyzed. Moreover, samples were analyzed and the concentrations were reported in the table above (from the Sponsor's submission). Stability testing, (b) (4) was relatively stable for up to 35 days at room temperature or at 2-8°C. Week 3 samples had concentrations that were approximately (b) (4)% lower than the target concentration of 10 mg/mL; however, it appears only Week 3 samples were lower while Week 1 and Week 4 samples were at the target concentration of 10 mg/mL. Dosing with this lower concentration does not in the opinion of this Reviewer negate the results and conclusions of this study.

**Study title:** (b) (4): **Toxicity Study by Intravenous (Bolus) Administration to CD Rat for 4 Weeks**

Study no.: HKQ0014

Study report location: Module 4 of the SDN 17 electronic submission

Conducting laboratory and location: (b) (4)

Date of study initiation: February 11, 2014

GLP compliance: Yes. Signature provided on August 12, 2014.

QA statement: Yes. Signature provided on August 12, 2014.

Drug, lot #, and % purity: (b) (4)  
batch 9-EQJ-134-2, (b) (4) % purity

### Key Study Findings

- Rats were dosed via intravenous administration with 0, 7.5, 15, or 25 mg/kg/day of (b) (4) for 4 weeks.
- This study tests concentrations of (b) (4) that are at least (b) (4) fold greater than the concentration of (b) (4) found in the migration studies; therefore, the local tissue toxicity effects noted are not clinically relevant.
- There were no treatment-related changes in food consumption, ophthalmoscopy, bone marrow smear parameters, and urinalysis.
- There 3 males and 2 females that were humanely sacrificed from the 25 mg/kg/day dose group. There was 1 male found dead in the 15 mg/kg/day dose group. The clinical signs in the death in the 15 mg/kg/day dose group (including rapid respiration, dull eyes, hunched posture, piloerection, and lachrymation) were reported to be different from the 25 mg/kg/day dose group (including scab, flaccid, rigid, and swollen tails as well as enlarged lymph nodes). The lack of any dose-dependent impact on mortality supports the conclusion that this was not likely treatment-related and most likely procedural.
- In the surviving rats, injection site clinical signs include reddening and bruising of the tail in the female 15 mg/kg/day dose group and discolored tail in the male and female 25 mg/kg/day dose groups.
- There were no treatment-related changes in body weight; however, body weight gains were lower during Weeks 0 to 1 but were no different than control by Weeks 1 to 2 in the 25 mg/kg/day dose group. In addition, body weight gains were lowered in Week 1 to 2 in the female 7.5 and 15 mg/kg/day dose groups; however, these levels were no different than control by Week 3 to 4.
- There were increases in lymphocytes, eosinophils, and neutrophils in the 15 mg/kg/day dose group that was correlated with decreased spleen weight (by 30.4%) and histopathological changes in the spleen (extramedullary hemopoiesis) in the 15 mg/kg/day dose group.

- There was an increase in triglycerides (by 75.9%) in the males and phosphorous (by 13.8%) in the females in the 15 mg/kg/day dose groups that was correlated with histopathological changes in the liver in the 15 mg/kg/day dose group (infiltration of inflammatory cells with and without hepatocellular necrosis and extramedullary hemopoiesis).
- Macroscopically (gross pathology), there were depression in the kidney and pale areas in the lung in the male 15 mg/kg/day.
- The spleen weight decrease by 30.4% in the 15 mg/kg/day dose group was the only treatment-related change in organ weight.
- Histopathological changes at the injection site include chronic thrombus, acute thrombus, perivascular inflammation, perivascular hemorrhage, and perivascular fibrosis at the 15 mg/kg/day dose group.
- Histopathological changes in other sites include extramedullary hemopoiesis in the spleen, perivascular inflammation in the lungs, and liver observations (infiltration of inflammatory cells with or without hepatocellular necrosis and extramedullary hemopoiesis) at the 15 mg/kg/day dose group.
- Taken together, the systemic NOAEL appears to be 7.5 mg/kg/day. This is in contrast to the Applicant's NOAEL of (b) (4) mg/kg/day for systemic toxicity.
- At the MTDD of 4 g/day of APAP, the total daily intake of (b) (4) is (b) (4) mcg/day. The NOAEL of 7.5 mg/kg/day confers an exposure margin of (b) (4) fold based on a body surface area comparison.

## Methods

Doses:	0 (saline), 0 (vehicle), 7.5, 15, and 25 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Intravenous injection into the left and right caudal veins at an infusion rate of 1 mL/minute
Dose volume:	5 mL/kg
Formulation/Vehicle:	10% DMSO in 1% hydroxypropyl-beta-cyclodextrin (there was also a saline control used)
Species/Strain:	Rat/Crl-CD(SD)
Number/Sex/Group:	10/sex/group with the exception of the saline control which was 5/sex/group.
Age:	48 to 55 days old
Weight:	244 to 313 g (males); 158 to 210 g (females)
Satellite groups:	None
Unique study design:	In addition to bone marrow smears being examined histopathologically, bone marrow parameters were examined (total myeloid cells, total erythroid cells, and myeloid/erythroid ratio).
Deviation from study protocol:	The Applicant notes that the optic nerves were retained in error.

The following table illustrates the study design (from the Applicant's submission):

Group	Treatment	Dose (mg/kg/day)	Formulated concentration (mg/mL)	Volume dose (mL/kg)
1	Saline Control	0	0	5
2	Vehicle Control	0	0	5
3	(b) (4)	7.5	(b) (4)	(b) (4)
4	(b) (4)	15.0	(b) (4)	(b) (4)
5	(b) (4)	25.0	(b) (4)	(b) (4)

As shown in the table above, the concentrations of (b) (4) tested in this study ranged from (b) (4) mg/mL. From the original nonclinical review (dated June 20, 2013), the maximum amount of (b) (4) in the migration studies was (b) (4) mcg/mL. Thus, this study tests between (b) (4) fold higher concentrations of (b) (4) and therefore evaluates an adequate concentration of (b) (4).

## Observations and Results

### Mortality

Rats were observed visually twice daily for evidence of ill-health or reaction to treatment. There were 3 males and 2 females from the 25 mg/kg/day dose group that were humanely killed due to the condition of their tails (dark/black color at the distal end, thickening, loss of flexibility, pale/white areas/patches, reddening, bruising, swollen areas, and eschar formation) on Day 14 (see table below from the Sponsor's submission). Macroscopic examination of the unscheduled deaths of rats on Day 14 and the early termination of rats on Day 15 from the 25 mg/kg/day dose group was performed and included findings in the tail and lymph nodes (lumbar, axillary, and inguinal) and are illustrated in the following tables (from the Sponsor's submission):

### Tail

Group/sex	5M	5F
Dose (mg/kg/day)	25.0	25.0
Dark area(s)	7	7
Pale area(s)	6	2
Scab(s)	4	6
Flaccid	1	1
Rigid	1	1
Swollen	1	0
Number of animals examined	<sup>a</sup> 10	<sup>a</sup> 10

<sup>a</sup> includes unscheduled decedents and animals terminated early, on Day 15

**Lumbar lymph nodes**

Group/sex	5M	5F
Dose (mg/kg/day)	25.0	25.0
Enlarged	2	2
Number of animals examined	<sup>a</sup> 10	<sup>a</sup> 10

<sup>a</sup> includes unscheduled decedents and animals terminated early, on Day 15

**Axillary lymph nodes**

Group/sex	5M	5F
Dose (mg/kg/day)	25.0	25.0
Enlarged	0	1
Number of animals examined	<sup>a</sup> 10	<sup>a</sup> 10

<sup>a</sup> includes unscheduled decedents and animals terminated early, on Day 15

**Inguinal lymph nodes**

Group/sex	5M	5F
Dose (mg/kg/day)	25.0	25.0
Enlarged	0	1
Number of animals examined	<sup>a</sup> 10	<sup>a</sup> 10

<sup>a</sup> includes unscheduled decedents and animals terminated early, on Day 15

As shown in the tables above, there were a number of findings at the injection site (tail) including dark areas, pale areas, scab, as well as flaccid, rigid, and swollen properties in the 25 mg/kg/day dose group. Other findings include axillary lymph node (enlarged) and inguinal lymph nodes (enlarged) in the 25 mg/kg/day dose group.

One male from the 15 mg/kg/day dose group was found dead on Day 7 approximately 1 hour after dosing. Clinical signs of this animal included rapid respiration and dull eyes on Day 6 as well as dull eyes, elevated gait, hunched posture, piloerection, and lachrymation immediately after dosing on Day 7. The Sponsor believes that the clinical signs following dosing were procedural rather than test article-related. The tail lesions observed in the deaths at the 25 mg/kg/day males was not observed in this animal. Although, there were no macroscopic or microscopic changes indicative of a cause of death, the relationship of this unscheduled death to the administration of (b) (4) is uncertain. The lack of any dose-dependent impact on mortality supports the conclusion that this was not likely treatment-related and most likely procedural.

**Clinical Signs**

A detailed weekly physical examination was performed for each rat to monitor general health. Injection site observations were observed daily, prior to dosing. The signs associated with dosing were also examined once weekly during the first week of treatment and more detailed observations thereafter during Weeks 2 to 4, which included pre-dose and 1 to 2 hour post-dose observations.

Regarding injection site observations, bruising of the tail on Days 8 and 9 were observed in the female 15 mg/kg/day dose group as well as reddening of the tails at the dose administration site on Day 6 was observed in the female 15 mg/kg/day dose group. In the 25 mg/kg/day dose group for both males and females, the tails of some animals became discolored (reddening/whitening) during or immediately after administration from Day 5 and an increasing number of rats from this dose group were not dosed because the tail vein could not be located as illustrated in the following table (from the Sponsor's submission):

Text Table 1 - Animals that did not receive their daily scheduled dose at 25.0 mg/kg/day

Treatment (mg/kg/day)	Day Numbers	Sex	Animal No.	Comment	
(b) (4) 25.0*	11, 14	M	36	Not dosed; unable to locate vein	
	9, 11, 14	M	37		
	5, 6, 9, 14	M	38		
	14	M	39		
	8, 9, 11, 14 <del>0</del>	M	40		
	9, 11, 14 <del>0</del>	M	41		
	9, 11, 14	M	42		
	6+7, 14 <del>0</del>	M	43		
	2, 6, 10, 11	M	44		
	5+8+9+10	M	45		
	7, 8, 9, 10, 11, 14 <del>0</del>	F	86		
	6, 10, 11, 14	F	87		
	8, 9, 14	F	88		
	10, 11, 14 <del>0</del>	F	89		
	5, 6, 8, 9, 11, 14	F	90		
	9, 10, 11, 14	F	91		Partial dose; dosing stopped; subcutaneous administration
	9, 11, 14	F	92		Not dosed; unable to locate vein
	5, 11, 14	F	93		
	9, 11	F	94		
	10, 11, 14	F	95		

\* Group 5 (25 mg/kg/day) were not dosed on Days 12 or 13 (Amendment 3)  
 0 Animals killed for welfare reasons before dosing

There was no dosing of any 25 mg/kg/day rat on Days 12 and 13. Dosing was scheduled to recommence on Day 14; however, the humane killing of 3 males and 2 females from this dose group occurred on Day 14 or 15. It was not possible to dose the remaining rats from this dose group and the 25 mg/kg/day dose group was terminated on Day 15.

There were also isolated incidences of individual animals from the 7.5 and 15.0 mg/kg/day group not receiving a dose because the tail veins could not be located (see Sponsor's table below). However it was noted by the Sponsor that treatment was successfully completed in all males and 7 of 10 females on at least 26 daily occasions. In the 7.5 mg/kg/day group, 17 of 20 animals were dosed on every occasion.

**Text Table 2 - Animals that did not receive their daily scheduled dose**

Treatment (mg/kg/day)	Day Numbers	Sex	Animal No.	Comment
Saline Control	2	M	4	Partial dose; subcutaneous administration
(b) (4) 7.5	18, 19	F	66	Not dosed; unable to locate vein
	9, 10, 21	F	67	
	20, 21, 26	F	74	
(b) (4) 15.0	20	M	27	Not dosed; unable to locate vein
	26	M	28	
	9, 12	M	31	
	13, 23	M	32	
	9	M	33	
	26	M	34	
	26	M	35	
	6, 14, 17, 19, 20, 23, 27	F	76	
	9, 13, 17, 26	F	77	
	2, 8, 26	F	78	
	10, 12, 26	F	80	
	8, 23, 25, 26	F	81	
	5, 26	F	82	

Clinical signs were observed in the other dose groups that were sporadic, transient in nature, or observed in all dose groups and as such, were not considered treatment-related. These observations include unsteady gait, decreased activity, breathing abnormalities (irregular, rapid, shallow, or slow), piloerection, red color in the urine, dull eyes, partially closed right eyelid, reduced body tone, posture abnormalities (flattened or prostrate), abnormal skin color (dark tail, white tail, or pallor of the whole body), hair loss in the forelimbs and head, brown stain at dorsal surface and nose, and injection site observations (bruising of the tail and reddening of the tail).

### Body Weights

The body weights were recorded for each rat weekly throughout the study and before the scheduled necropsy. There were no treatment-related changes in the absolute body weights in the males and females.

The following table illustrates the changes in the body weight gains in the males (adapted from the Applicant's submission):

TABLE 4 Body weight - group mean values (g)

Dose Group Dose (mg/kg/day)	Saline Control		Vehicle Control		(b) (4)		
	1	2	3	4	5		
	0	0	7.5	15.0	25.0		
Group /Sex	Change 0-1	Change 1-2	Change 2-3	Change 3-4	Change 0-4	4	
Statistics test	Wi	Wi	Wi	Wi	Wi		
1M	Mean	29	19	19	9	76	368
	SD	5.2	8.3	8.9	6.3	22.4	32.7
	N	5	5	5	5	5	5
	X of 2M	1.02	0.80	1.00	0.80	0.92	
2M	Mean	29	24	19	11	83	373
	SD	4.7	5.7	5.3	5.5	14.0	17.0
	N	10	10	10	10	10	10
3M	Mean	26	20	17	12	75	355
	SD	7.9	6.7	4.6	5.0	19.6	27.5
	N	10	10	10	10	10	10
	X of 1M	0.88	1.05	0.89	1.44	0.99	
	X of 2M	0.89	0.84	0.89	1.15	0.91	
4M	Mean	28	20	23	14	84	375
	SD	5.4	4.8	6.4	4.6	14.5	20.0
	N	9	9	9	9	9	9
	X of 1M	0.98	1.25	1.00	1.25	1.08	
	X of 2M	0.97	0.83	1.17	1.27	1.01	
5M	Mean	17**	26				
	SD	7.3	8.0				
	N	10	7				
	X of 2M	0.58	1.08				

Body weight gains in the males were decreased by 41.4% during Week 0 to 1 in the 25 mg/kg/day dose group compared to control. However, during Week 1 to 2, there were no treatment-related changes in body weight gains from this dose group compared to control before early termination of the 25 mg/kg/day dose group. There were no further treatment-related changes in body weight gains in the males.

The following table illustrates the changes in the body weight gains in the females (adapted from the Applicant's submission):

TABLE 4 (cont) Body weight - group mean values (g)

		Saline Control	Vehicle Control	(b) (4)			
Dose Group		1	2	3	4	5	
Dose (mg/kg/day)		0	0	7.5	15.0	25.0	
Group /Sex		Change 0-1	Change 1-2	Change 2-3	Change 3-4	Change 0-4	4
Statistics test		Wi	Wi	Wi	Wi	Wi	
1F	Mean	14	16	8	5	44	237
	SD	6.4	7.5	4.2	4.2	8.6	20.1
	N	5	5	5	5	5	5
	X of 2F	1.02	1.40	0.66	1.17	1.04	
2F	Mean	14	12	12	5	42	236
	SD	5.6	5.8	3.7	5.2	10.1	15.9
	N	10	10	10	10	10	10
3F	Mean	12	7 <sup>^^</sup>	11	6	36	224
	SD	4.0	4.7	2.7	4.6	4.4	14.9
	N	10	10	10	10	10	10
	X of 1F	0.81	0.46	1.42	1.11	0.82	
	X of 2F	0.82	0.64	0.94	1.30	0.85	
4F	Mean	11	8 <sup>^^</sup>	13 <sup>^</sup>	4	35	229
	SD	2.6	3.7	5.0	6.1	8.7	14.2
	N	10	10	10	10	10	10
	X of 1F	0.75	0.49	1.65	0.67	0.81	
	X of 2F	0.76	0.69	1.08	0.78	0.84	
5F	Mean	6 <sup>**</sup>	17 <sup>*</sup>				
	SD	5.8	5.9				
	N	10	8				
	X of 2F	0.43	1.50				

Body weight gains in the females were decreased by 57.1% during Week 0 to 1 in the 25 mg/kg/day dose group compared to control. However, there was an increase of 6.3% in body weight gains in the 25 mg/kg/day dose group compared to control before early termination of the 25 mg/kg/day dose group. During Week 1 to 2, there were decreases of 56.3 and 50.0% in in the 7.5 and 15 mg/kg/dose groups, respectively, compared to control. During Week 2 to 3, there was an increase of 62.5% in body weight gains in the 15 mg/kg/day dose group. By Week 2 to 3 in the 7.5 mg/kg/day dose group and by Week 3 to 4 in the 15 mg/kg/dose groups, there were no differences in the body weight gains compared to control until the end of the study. There were no further treatment-related changes in body weight gains in the females.

## Food Consumption

Food consumption was measured weekly throughout the study. There were no treatment-related changes in food consumption.

## Ophthalmoscopy

An eye examination was performed using a binocular indirect ophthalmoscope during pretreatment and at Week 4. The adnexae, conjunctiva, cornea, sclera, anterior chamber, iris (pupil dilated), lens, vitreous, and fundus were examined. There were no treatment-related changes in ophthalmoscopy.

## ECG

An ECG was not performed during this study.

## Hematology

Blood samples for hematology, coagulation, and clinical chemistry were collected after an overnight fast prior to dosing during Week 4 on all rats as well as for the rats terminated early from the 25 mg/kg/day dose group. The following hematology parameters were examined (from the Applicant's submission):

- Haematocrit (Hct)
- Haemoglobin concentration (Hb)
- Erythrocyte count (RBC)
- Absolute reticulocyte count (Retic)
- Mean cell haemoglobin (MCH)
- Mean cell haemoglobin concentration (MCHC)
- Mean cell volume (MCV)
- Red cell distribution width (RDW)
- Total leucocyte count (WBC)
- Differential leucocyte count:
  - Neutrophils (N)
  - Lymphocytes (L)
  - Eosinophils (E)
  - Basophils (B)
  - Monocytes (M)
  - Large unstained cells (LUC)
- Platelet count (Plt)
- Morphology:
  - Anisocytosis
  - Macrocytosis
  - Microcytosis
  - Hypochromasia
  - Hyperchromasia

The following coagulation parameters were examined (from the Applicant's submission):

Prothrombin time (PT) - using IL PT-Fibrinogen reagent.

Activated partial thromboplastin time (APTT) - using IL APTT reagent.

The following tables illustrate the red cell parameters and hematology parameters that are treatment related after Week 4 (from the Applicant's submission):

**Text Table 3: Fold changes in selected red cell parameters in Week 4, compared with Vehicle control**

Parameter	Dose (mg/kg/day)			
	Males		Females	
	7.5	15.0	7.5	15.0
Hct	0.98X	0.97X	1.02X	0.93X
Hb	0.96X	0.93X	1.03X	0.90X
RBC	0.95X	0.86X	1.03X	0.86X
Retic	1.31X	1.71X	1.00X	1.63X
MCH	1.02X	1.08X	1.00X	1.05X
MCHC	0.99X	0.96X	1.00X	0.97X
MCV	1.03X	1.12X	0.99X	1.09X
RDW	1.07X	1.12X	1.00X	1.11X

All values X Saline control

**Text Table 4: Fold changes in further haematology parameters in Week 4, compared with Vehicle control**

Parameter	Dose (mg/kg/day)			
	Males		Females	
	7.5	15.0	7.5	15.0
WBC	0.95X	1.31X	1.01X	1.38X
N	0.74X	1.47X	0.70X	2.20X
L	1.01X	1.29X	1.05X	1.26X
E	0.75X	1.00X	1.09X	1.64X
LUC	1.00X	1.83X	1.25X	1.50X
Plt	1.04X	1.30X	1.00X	1.11X

All values X Saline control

As shown in the tables above, there were significant decreases in red blood cells in the 15 mg/kg/day dose group in both males and females compared to control. The loss of red blood cells may be due to blood vessel injury as the test compound is injected into the caudal veins. There were significant increases in reticulocytes in the male 7.5 and 15 mg/kg/day dose groups as well as the female 15 mg/kg/day dose group compared to control. However, the reticulocytes are within the normal range ( (b) (4) ) and do not represent a safety concern. All other changes in the red cell parameters (Hct, Hb, MCH, MCHC, MCV, and RDW) at the 7.5 mg/kg/day dose groups were dismissed because they did not deviate significantly from the control; however, the changes at the 15 mg/kg/day dose groups were more significant. Although the change in hemoglobin in the MD females may have clinical significance, the LD was not significantly different than controls. There were significant increases in white blood cells (WBC) and lymphocytes in the both the male and female 15 mg/kg/day dose groups as well as in the eosinophils in the female 15 mg/kg/day dose group and large unstained cells (LUC) and the platelets in the male (with a smaller increase in females) 15 mg/kg/day dose group compared to control. The increases in WBC and platelets in both males and females are within normal range ( (b) (4) ). Thus, the increase in platelets does not represent a safety concern. There were significant decreases in neutrophils in the male and female 7.5 mg/kg/day dose groups followed by significant increases in neutrophils in the male and female 15 mg/kg/day dose groups compared to control. In males, the decrease in eosinophils in the 7.5 mg/kg/day dose group was not observed in the 15 mg/kg/day dose group compared to control. In females, there was a dose-dependent increase in the LUC in the 7.5 and 15 mg/kg/day dose groups compared to control. There were histopathological changes in the spleen (extramedullary hemopoiesis) that showed a dose-dependent increase in incidence and severity that may account for the increases in lymphocytes, WBC, eosinophils, neutrophils, and LUC (see histopathology below)

In addition, there were increases of 8.1% in the prothrombin time (PT) in the female 15 mg/kg/day dose group as well as in the APTT by 26.8 and 16.8% in the female 7.5 and 15 mg/kg/day dose groups, respectively, compared to control. The increase in PT was below 10%, which does not represent a biologically significant change and as such, does not represent a safety concern. The increases in the APTT are within the normal control range ( (b) (4) ) and do not represent a safety concern.

There were no further treatment-related changes in hematology and coagulation in the males and females.

### **Clinical Chemistry**

The following clinical chemistry parameters were examined (from the Applicant's submission):

Alkaline phosphatase (ALP)  
Alanine aminotransferase (ALT)  
Aspartate aminotransferase (AST)  
Total bilirubin (Bili)  
Urea  
Creatinine (Creat)  
Glucose (Gluc)  
Total cholesterol (Chol)  
Triglycerides (Trig)  
Sodium (Na)  
Potassium (K)  
Chloride (Cl)  
Calcium (Ca)  
Inorganic phosphorus (Phos)  
Total protein (Total Prot)  
Albumin (Alb)

In addition, the albumin/globulin ratio (A/G ratio) was calculated from total protein concentration and analyzed albumin concentration.

In males, there was an increase in triglycerides by 75.9% and an increase in phosphorous by 9.7% in the 15 mg/kg/day dose group. The increase in triglycerides may be caused by damage to the liver or kidney. There were histopathological changes in the liver at the 15 mg/kg/day dose group (see histopathology below), mainly infiltration of inflammatory cells with and without hepatocellular necrosis and extramedullary hemopoiesis observed in the 15 mg/kg/day dose group, that may account for the increases in the levels of triglycerides and phosphorous. However, the increase in phosphorous is below 10%, and as such, is not biologically significant.

There were no further treatment-related changes in clinical chemistry in the males. In females, there were decreases in the ALT, AST, total protein, and albumin in both the 7.5 and 15 mg/kg/day dose groups as well as decreases in the glucose level in the 15 mg/kg/day dose group that were within the normal range ( [REDACTED] <sup>(b) (4)</sup> ) and as such, do not represent a safety concern. There were increases in urea levels by 26.8 and 23.3% in the 7.5 and 15 mg/kg/day dose groups, respectively, compared to control that did not show a clear dose dependency. The increase in urea is not dose-dependent but was only observed in the 7.5 and 15 mg/kg/day dose groups. Increased urea was only observed in the female 15 mg/kg/day dose group. Thus, the increase in urea may not be treatment-related. There were decreases in the calcium levels by 3.7 and 4.1% in the 7.5 and 15 mg/kg/day dose group, respectively, compared to control do not appear to be a cause for concern. Thus, the decrease in calcium levels does not represent a safety concern. There was an increase in the phosphorous levels by 13.8% in the 15 mg/kg/day dose group. Increased phosphorous may be caused by kidney failure or liver disease. Infiltration of inflammatory cells with and without hepatocellular necrosis and extramedullary hemopoiesis in the liver were observed in the 15 mg/kg/day dose group in the histopathological examination that may account for the increase in phosphorous. There were no further treatment-related changes in clinical chemistry in the females.

## Urinalysis

Rats were placed in individual metabolism cages overnight (fasted) to collect urine samples over a period of approximately 16 hours during Week 4. The following urinalysis parameters were examined (from the Applicant's submission):

### Using manual methods:

- Appearance (App) - by visual assessment
- Volume (Vol) - using a measuring cylinder
- pH - using a pH meter
- Specific gravity (SG) - by direct refractometry using a SG meter

### Using Multistix reagent strips, interpreted using a Clinitek<sup>®</sup>500 instrument:

- Ketones (Keto)
- Bilirubin (bile pigments) (Bili)
- Blood pigments (UBld)

### Using a Roche P Modular analyser:

- Protein (Prot)
- Creatinine (U-Creat)
- Glucose (U-Gluc)
- Sodium (U-Na)
- Potassium (U-K)
- Chloride (U-Cl)

There was an increase of 1.5% in specific gravity in the male 7.5 mg/kg/day dose group. The increase in specific gravity is within the normal range ( (b) (4) ) and therefore is not considered biologically relevant. There were no further treatment-related changes in urinalysis in the males. There were no treatment-related changes in urinalysis in the females.

### Gross Pathology

At the scheduled sacrificed after Week 4 as well as during Week 2 (Day 15) following early termination of the 25 mg/kg/day dose group, all rats were subjected to a detailed necropsy including a full macroscopic examination of the tissues. All external features and orifices were examined visually. Abnormalities in appearance or size of any organ and tissue (external and cut surface) were recorded. Organs and tissue samples were preserved.

The following table illustrates the macroscopic findings after 4-weeks of treatment with (b) (4) (data from the Applicant's submission):

Findings	Number of Rats Affected							
	Males (mg/kg/day)				Females (mg/kg/day)			
	Saline Control	Vehicle Control	7.5	15	Saline Control	Vehicle Control	7.5	15
Number of rats examined	5	10	10	9	5	10	10	10
Kidneys Depression(s)	0	0	0	1	0	0	0	0
Lungs and Bronchi Pale area(s)	0	0	0	1	0	1	2	0
Oviducts Cyst(s)	N/A	N/A	N/A	N/A	0	0	0	1

As shown in the table above, there are a number of macroscopic findings in the kidneys, lungs, and oviducts. Kidney depression was observed in only 1/9 males from the 15 mg/kg/day dose group compared to control. There was 1/9 males from the 15 mg/kg/day dose group observed with pale areas of the lungs and bronchi compared to control. There were 1/10 females in the vehicle control and 2/10 females in the 7.5 mg/kg/day dose groups observed with this finding and as such, this finding does not appear to be dose-dependent. However, there are microscopic findings in the lungs (see histopathology below). There was 1/10 females observed with cyst(s) in the oviducts from the 15 mg/kg/day dose group compared to control. There were no further treatment-related changes in gross pathology (macroscopically) in both males and females.

### Organ Weights

Each organ was weighed and recorded from all rats at the scheduled necropsy (end of Week 4) as well as during Week 2 (Day 15) following early termination of the 25 mg/kg/day dose group. For bilateral organs, left and right organs were weighed together.

In males, there were decreases of 6.8% in the adrenal weight in the 15 mg/kg/day dose group as well as decreases of 5.2 and 5.5% in the brain weight in the 7.5 and 15 mg/kg/day dose groups, respectively, compared to control. The changes in the adrenal and brain are below 10% and as such, are not likely biologically relevant. Thus, the decreases in the adrenal and brain weights do not represent a safety concern. An increase of 30.4% in mean spleen weight in the 15 mg/kg/day dose group was also noted.

In females, there were increases of 6.8 and 4.5% in the kidneys and liver weights, respectively, in the 15 mg/kg/day dose group compared to control. The increases in the kidneys and liver weights are below 10% and as such, are not biologically relevant. Thus, the decreases in the kidneys and liver weights do not represent a safety concern.

There were no further treatment-related changes in organ weights in both males and females.

## **Histopathology**

### **Adequate Battery**

The following table illustrates the organs and tissues that were weighed and examined microscopically for the rats terminated early from the 25 mg/kg/day dose group during Week 2 (on Day 15) and the remaining rats at the end of Week 4 (from the Applicant's submission):

Tissue and regions examined	Necropsy		Histology	Pathology Light microscopy
	Weigh	Fix		
Abnormalities		*	*	*
Adrenals	*	*	*	*
Aorta - thoracic		*	*	*
Bone marrow smear		*		c)
Brain (cerebellum, cerebrum, midbrain)	*	*	*	*
Caecum		*	*	*
Colon		*	*	*
Duodenum		*	*	*
Epididymides	*	*	*	*
Eyes		*	*	*
Femur (femorotibial joint)		b)	*	*
Harderian glands		*	*	*
Head		*	#	#
Heart (including auricular and ventricular regions)	*	*	*	*
Ileum		*	*	*
Jejunum		*	*	*
Kidneys	*	*	*	*
Liver (section from two lobes)	*	*	*	*
Lungs (section from two major lobes including bronchi)		*	*	*
Lymph nodes - mesenteric		*	*	*
- mandibular		*	*	*
- inguinal		*	*	*
Oesophagus		*	*	*
Ovaries	*	*	*	*
Pancreas		*	*	*
Parenteral sites (intravenous injection sites)		*	*	*
Pituitary	*	*	*	*
Prostate	*	*	*	*
Salivary glands - submandibular		*	†	†
- parotid		*	†	†
- sublingual		*	†	†
Sciatic nerves		*	†	†
Seminal vesicles	*	*	*	*
Skeletal muscle		*	†	†
Skin with mammary glands (inguinal area)		*	*	*
Spinal cord (transverse and longitudinal sections at the cervical level)		*	*	*
Spleen	*	*	*	*
Sternum		*	*	*
Stomach		*	*	*
Testes	*	*	*	*
Thymus	*	*	*	*
Thyroid with parathyroids	a)	*	*	*
Trachea		*	*	*
Urinary bladder		*	*	*
Uterus with cervix	*	*	*	*
Vagina		*	*	*

a) Weighed after partial fixation.

b) Both hindlimbs retained, one sectioned where appropriate.

c) Examined by the (b) (4) (Groups 1-4 only).

\* Organs weighed, samples fixed or sections examined microscopically.

# Examined if effects suspected during the study.

† Only one examined.

This appears to be an adequate battery of tissues for histopathology.

#### Peer Review

A signed Pathology Report was provided and dated July 1, 2014. There appears to be one pathologist for the study and as such, a peer review was not evident.

#### Histological Findings

There were a number of changes noted in the spleen, injection site, and lungs. The following table illustrates the histopathological changes noted in the spleen after 4 weeks of treatment with (b)(4) (from the Applicant's submission):

**Text Table 5. Summary of treatment-related findings in the spleen for animals killed after 4 weeks of treatment**

Group/sex Dose (mg/kg/day)	1M	2M	3M	4M	1F	2F	3F	4F
	0	0	7.5	15.0	0	0	7.5	15.0
Extramedullary haemopoiesis								
minimal	1	3	7	3	2	1	2	2
slight	0	0	2	4	3	5	3	4
moderate	0	0	0	0	0	0	0	2
Total	1	3	9	7	5	6	5	8
Number of tissues examined	5	10	10	9*	5	10	10	10

\* Does not include unscheduled decedent

As shown in the table above, the incidence of extramedullary hemopoiesis is not clearly dose-dependent; however, the moderate severity of the finding was only observed in the 15 mg/kg/day dose group.

The following table illustrates the histopathological changes noted in the injection site after 4 weeks of treatment with (b)(4) (from the Applicant's submission):

**Text Table 6. Summary of treatment-related findings at the venous injection site for animals killed after 4 weeks of treatment**

Group/sex	1M	2M	3M	4M	1F	2F	3F	4F
Dose (mg/kg/day)	0	0	7.5	15.0	0	0	7.5	15.0
<b>Thrombus, chronic</b>								
minimal	0	0	0	1	0	2	1	0
slight	0	0	0	0	0	0	0	0
moderate	0	2	1	0	0	2	1	1
marked	0	0	0	5	0	3	4	0
severe	0	0	0	3	0	0	0	7
Total	0	2	1	9	0	7	6	8
<b>Thrombus, acute</b>								
minimal	0	1	0	2	0	1	0	0
slight	0	0	1	0	0	0	0	0
moderate	0	0	0	1	0	0	0	0
marked	0	0	0	1	0	0	0	0
Total	0	1	1	4	0	1	0	0
<b>Perivascular inflammation</b>								
minimal	0	1	2	1	0	4	1	2
slight	0	2	0	5	0	1	3	5
Total	0	3	2	6	0	5	4	7
<b>Perivascular haemorrhage</b>								
minimal	0	0	1	3	1	0	2	2
slight	1	1	1	1	0	2	1	2
moderate	0	0	0	0	0	0	0	1
Total	1	1	2	4	1	2	3	5
Number of tissues examined	5	10	10	9*	5	10	10	9

\* Does not include unscheduled decedent

As shown in the table above, chronic thrombus at the injection site was observed with dose-dependent incidence and severity with significant increases in both incidence and severity at the 15 mg/kg/day group compared to the controls. Acute thrombus at the injection site was observed with dose-dependent increases in incidence and severity with significant increases in both incidence and severity at the 15 mg/kg/day dose group compared to the controls. Perivascular inflammation at the injection site was observed with dose-dependent incidence and severity with significant increases in both incidence and severity at the 15 mg/kg/day dose group compared to the controls. According to the pathology report, this inflammation is thought to be chronic. Perivascular hemorrhage was observed with dose-dependent severity with significant increases in severity at the 15 mg/kg/day dose group compared to the controls.

In addition, these findings were also noted at the injection site (data from the Applicant's submission):

Additional Injection Site Findings								
Findings	Number of Rats Affected							
	Males (mg/kg/day)				Females (mg/kg/day)			
	Saline Control	Vehicle Control	7.5	15	Saline Control	Vehicle Control	7.5	15
Number of rats examined	5	10	10	9	5	10	10	9
Fibrosis, Perivascular								
Minimal	0	0	2	0	0	0	1	0
Slight	0	2	0	2	1	6	0	3
Moderate	0	0	2	0	0	1	1	3
Marked	0	0	0	0	0	0	2	0
<b>Total</b>	<b>0</b>	<b>2</b>	<b>4</b>	<b>2</b>	<b>1</b>	<b>7</b>	<b>4</b>	<b>6</b>

As shown in the table above, there were more females observed with perivascular fibrosis with moderate severity in the 15 mg/kg/day dose group compared to the controls.

The following table illustrates the histopathological changes noted in the lungs after 4 weeks of treatment with (b) (4) (from the Applicant's submission):

**Text Table 7. Summary of other findings in the lungs after 4 weeks of treatment**

Group/sex	1M	2M	3M	4M	1F	2F	3F	4F
Dose (mg/kg/day)	0	0	7.5	15.0	0	0	7.5	15.0
Thickening pulmonary arterioles								
minimal	0	5	1	3	0	6	0	5
slight	0	0	2	1	0	0	0	2
Total	0	5	3	4	0	6	0	7
Alveolar macrophages foamy								
minimal	0	1	4	3	0	3	4	4
slight	0	0	0	0	0	0	2	1
Total	0	1	4	3	0	3	6	5
Perivascular inflammation								
minimal	2	6	6	7	3	5	4	6
slight	0	0	1	1	1	5	5	3
Total	2	6	7	8	4	10	9	9
Granuloma								
minimal	1	5	4	3	1	4	3	5
slight	0	0	1	0	0	0	1	0
Total	1	5	5	3	1	4	4	5
Number of tissues examined	5	10	10	9*	5	10	10	10

\* Does not include unscheduled decedent

As shown in the table above, thickening pulmonary arterioles was observed in the 7.5 and 15 mg/kg/day dose group with increased severity when compared to the vehicle control group. However, the incidence of this finding is not dose dependent. The incidence and severity of foamy alveolar macrophages are not clearly dose dependent. The severity of perivascular inflammatory is not clearly dose dependent; however, the incidence is significantly increased in the 15 mg/kg/day dose group compared to the controls. The incidence and severity of granuloma is not dose dependent. These lung findings were dismissed in the pathology report as being not treatment-related. This is a reasonable conclusion.

The following table illustrates other noted histopathological changes (data from the Applicant's submission):

Findings	Number of Rats Affected							
	Males (mg/kg/day)				Females (mg/kg/day)			
	Saline Control	Vehicle Control	7.5	15	Saline Control	Vehicle Control	7.5	15
Number of rats examined	5	10	0	9	5	10	0	10
Bone, Femur including Joint Hypoplasia, Synovium Slight	0	0	0	1	0	0	0	0
Heart Infiltration, Inflammatory Cells, Myocardial Minimal	0	0	0	1	0	0	0	0
Slight	0	0	0	1	0	0	0	0
<b>Total</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>2</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
Liver (rats examined) Infiltration, Inflammatory Cells with or without Hepatocellular Necrosis Minimal	(5)	(10)	(10)	(9)	(5)	(10)	(10)	(10)
Slight	1	0	6	4	2	4	4	3
<b>Total</b>	<b>1</b>	<b>0</b>	<b>6</b>	<b>5</b>	<b>2</b>	<b>4</b>	<b>4</b>	<b>3</b>
Extramedullary Hemopoiesis Minimal	2	4	2	5	4	5	2	7
Slight	0	1	0	0	0	1	0	1
<b>Total</b>	<b>2</b>	<b>5</b>	<b>2</b>	<b>5</b>	<b>4</b>	<b>6</b>	<b>2</b>	<b>8</b>

Lymph Node, Inguinal Erythrocytosis/ Erythro- phagocytosis, Sinuses Minimal	0	0	0	0	0	0	0	1
Oviducts (rats exam.) Dilatation Moderate Infiltration, Inflammatory Cells Slight					(0)	(0)	(0)	(1)
	N/A	N/A	N/A	N/A	0	0	0	1
Prostate Aggregates, Lymphoid Minimal Inflammation Slight	0	0	0	1	N/A	N/A	N/A	N/A
	0	0	0	1	N/A	N/A	N/A	N/A
Skeletal Muscle Degeneration, Myofiber Minimal Slight Total	0	1	0	0	0	0	0	0
	0	0	0	1	0	0	0	0
	0	1	0	1	0	0	0	0

It is noted that the controls (saline and vehicle) and 15 mg/kg/day dose group were examined microscopically unless otherwise indicated. Hypoplasia of the synovium of the bone, myocardial infiltration of inflammatory cells of minimal and slight severity, lymphoid aggregates and inflammation in the prostate, and myofiber degeneration of skeletal muscle was observed in the male 15 mg/kg/day dose group. However, the incidence of these findings was in only 1 male rat. Erythrocytosis/erythrophagocytosis, sinuses in the inguinal lymph node as well as dilatation and infiltration of inflammatory cells in the oviducts were observed in the 15 mg/kg/day dose group. However, the incidence of these findings was in only 1 female rat. As these findings only occurred in only 1 rat from the 15 mg/kg/day dose group, histopathological examination of the 7.5 mg/kg/day dose group would most likely render these incidental findings unlikely to be dose dependent or treatment related.

The incidence of infiltration of inflammatory cells in the liver (with or without hepatocellular necrosis) was not clearly dose-dependent; however, the severity is dose-dependent with significant increases in severity at the 15 mg/kg/day dose group compared to the controls. The severity of extramedullary hemopoiesis in the liver was not dose-dependent; however, the incidence is dose-dependent with significant increases in incidence at the 15 mg/kg/day dose group.

### Special Evaluation

Although there was a histopathological examination of the bone marrow smears, several bone marrow parameters were measured as part of hematology on the smears (total myeloid cells in %, total erythroid cells in %, myeloid/erythroid ratio, and other). It is not clear from the protocol what “other” cell types were analyzed in the bone marrow. There were no treatment-related changes in the bone marrow parameters in the males and females except there were statistically significant increases in “other” from the female 7.5 and 15 mg/kg/day dose groups. As there was no explanation of the “other” category of cell types evaluated in the bone marrow smears in the protocol and this could represent multiple cell types, the reported change, if even real, is not interpretable.

### Toxicokinetics

Toxicokinetics were not performed in this study.

### Dosing Solution Analysis

A representative sample was taken from the batch of test substance and was analyzed for concentration. The following table illustrates the analyzed concentrations of (b) (4) from samples taken from batches of the test substance (from the Applicant's submission):

**Table 1**      **Analysed concentrations for (b) (4) in 10% DMSO in 1% HPβCD**

Occasion	Group	Nominal inclusion (mg/mL)	Analysed concentration (mg/mL)			RME (%)
			Analysis 1	Analysis 2	Mean	
Week 1	1	0	ND	ND	-	-
	2	0	ND	ND	-	-
	3					(b) (4)
	4					
	5					
Week 4	1	0	ND	ND	-	-
	2	0	ND	ND	-	-
	3					(b) (4)
	4					

RME    Relative mean error, representing the deviation from nominal

ND      Not detected

As shown in the table above, the analyzed concentration from each dose group from Week 1 and Week 4 were consistent and were within (b) (4) % of the expected concentration.

## 7 Genetic Toxicology

There were no new genetic toxicology studies with acetaminophen submitted in this second cycle submission to the NDA. The following information on the genetic toxicology (mutagenesis) of acetaminophen is from the OFIRMEV label (Cadence Pharma, November 2011):

### Mutagenesis

Acetaminophen was not mutagenic in the bacterial reverse mutation assay (Ames test). In contrast, acetaminophen tested positive in the in vitro mouse lymphoma assay and the in vitro chromosomal aberration assay using human lymphocytes. In the published literature, acetaminophen has been reported to be clastogenic when administered a dose of 1500 mg/kg/day to the rat model (3.6-times the MHDD, based on a body surface area comparison). In contrast, no clastogenicity was noted at a dose of 750 mg/kg/day (1.8- times the MHDD, based on a body surface area comparison), suggesting a threshold effect.

Results from two Ames assays that evaluated either [REDACTED] (b) (4) were submitted in this NDA to support the safety of the container closure system and are reviewed below.

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

**Study title:** [REDACTED] (b) (4) **Bacterial Reverse Mutation Test**

Study no.: HKQ0015

Study report location: Module 4 of the SDN 17 electronic submission

Conducting laboratory and location:

[REDACTED] (b) (4)

Date of study initiation: February 24, 2014

GLP compliance: Yes. Signature provided on April 17, 2014.

QA statement: Yes. Signature provided on April 17, 2014.

Drug, lot #, and % purity: [REDACTED] (b) (4) Batch Number I12012, [REDACTED] (b) (4) % pure

### Key Study Findings

- In the definitive test, concentrations of 0, 50, 150, 500, 1500, and 5000 mcg/plate of [REDACTED] (b) (4) were tested in *S. typhimurium* strains TA1535, TA1537, TA98, and TA100 as well as the *E. coli* strain WP2 uvrA.
- This study is considered valid.

- (b) (4) was not mutagenic under the conditions of this assay.

## Methods

Strains:	<i>S. typhimurium</i> strains TA1535, TA1537, TA98, and TA100; <i>E. coli</i> strain WP2 uvrA
Concentrations in definitive study:	0, 50, 150, 500, 1500, and 5000 mcg/plate
Basis of concentration selection:	High dose was 5000 mcg/plate, which is the standard limit concentration recommended in regulatory guidelines
Negative control:	DMSO
Positive control:	Without S9 activation, sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), 2-nitrofluorene (TA98), and 4-nitroquinoline-1-oxide (WP2 uvrA); With S9 activation, 2-aminoanthracene (TA100, TA1535, and WP2 uvrA) and benzo[a]pyrene (TA98 and TA1537)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	(b) (4) in vehicle (DMSO), vehicle alone (negative control), or positive control was pre-incubated with the bacteria with and without S9 mix in the top agar. The solution was mixed and overlaid onto a minimal bottom agar. After the overlay solidified, the plates were inverted and incubated at 37°C for 48 hrs. Afterwards, the plates were scored. All concentrations and controls (both positive and negative) in the initial toxicity-mutation assay and in the confirmatory mutagenicity assay were plated in triplicate.

## Study Validity

The study is considered valid for the following reasons: 1) the appropriate controls were used; 2) the appropriate strains were tested; 3) the positive control substances produced reliable positive results; 4) the highest concentration of (b) (4) tested reached the maximum recommended concentration of 5000 mcg/plate.

The study report states that the criteria for a positive is when a reproducible increase in revertant colony numbers of at least twice (three times in the case of TA1535 and TA1537) that of the concurrent vehicle controls, with some evidence of a positive concentration-response relationship.

## Results

Two sets of tests were performed (Test 1 and Test 2). The second test (Test 2) is considered a confirmatory test for the first test (Test 1).

The following table illustrates the number of revertants in Test 1 without metabolic activation (from the Applicant's submission):

Without metabolic activation

Strain	Addition	Concentration per plate	Mean revertants per plate	Standard Deviation	Fold increase relative to vehicle	Individual revertant colony counts (Sorcerer)
TA98	DMSO (b) (4)	5 µg	49.7	15.1		43, 39, 67
		15 µg				
		50 µg				
		150 µg				
		500 µg				
		1500 µg				
		5000 µg				
TA100	DMSO (b) (4)	5 µg	193.3	17.0		192, 177, 211
		15 µg				
		50 µg				
		150 µg				
		500 µg				
		1500 µg				
		5000 µg				
TA1535	DMSO (b) (4)	5 µg	38.3	8.1		42, 44, 29
		15 µg				
		50 µg				
		150 µg				
		500 µg				
		1500 µg				
		5000 µg				
TA1537	DMSO (b) (4)	5 µg	22.7	3.1		20, 22, 26
		15 µg				
		50 µg				
		150 µg				
		500 µg				
		1500 µg				
		5000 µg				
WP2 <i>uvrA</i> (pKMI01)	DMSO (b) (4)	5 µg	171.3	12.7		163, 165, 186
		15 µg				
		50 µg				
		150 µg				
		500 µg				
		1500 µg				
		5000 µg				
TA98	2NF	2 µg	236.7	30.0	4.8	255, 202, 253
TA100	NaN3	2 µg	756.7	97.0	3.9	678, 865, 727
TA1535	NaN3	2 µg	804.3	100.7	21.0	693, 831, 889
TA1537	AAC	50 µg	447.0	123.9	19.7	590, 373, 378
WP2 <i>uvrA</i> (pKMI01)	NQO	2 µg	2359.7	173.3	13.8	2197, 2542, 2340
Key to Positive Controls				Key to Plate Postfix Codes		
2NF	2-Nitrofluorene		P	Precipitate		
NaN3	Sodium azide					
AAC	9-Aminoacridine					
NQO	4-Nitroquinoline-1-oxide					

As shown in the table above, the number of revertants in the TA98 dose groups was not different than control. The number of revertants in 500 mcg/plate dose of TA100 was slightly greater than control; however, there was no clear dose dependency in the number of revertants in this strain and the number of revertants in all other dose groups was lower than control. The number of revertants in all dose groups in the remaining strains was no greater than their respective controls. The number of revertants in these strains in Test 1 without metabolic activation was (b) (4) than the historical control (see historical control table below).

The following table illustrates the number of revertants in Test 1 with metabolic activation (from the Applicant's submission):

With metabolic activation

Strain	Addition	Concentration per plate	Mean revertants per plate	Standard Deviation	Fold increase relative to vehicle	Individual revertant colony counts (Sorcerer)	
TA98	DMSO		81.3	6.5		88, 81, 75	
	(b) (4)	5 µg	(b) (4)				(b) (4)
		15 µg					
		50 µg					
		150 µg					
		500 µg					
		5000 µg					
TA100	DMSO		179.7	27.8		158, 170, 211	
	(b) (4)	5 µg	(b) (4)				(b) (4)
		15 µg					
		50 µg					
		150 µg					
		500 µg					
		5000 µg					
TA1535	DMSO		25.7	4.2		27, 21, 29	
	(b) (4)	5 µg	(b) (4)				(b) (4)
		15 µg					
		50 µg					
		150 µg					
		500 µg					
		5000 µg					
TA1537	DMSO		34.3	6.4		27, 37, 39	
	(b) (4)	5 µg	(b) (4)				(b) (4)
		15 µg					
		50 µg					
		150 µg					
		500 µg					
		5000 µg					
WP2 <i>uvrA</i> (pKMI01)	DMSO		223.7	2.5		221, 224, 226	
	(b) (4)	5 µg	(b) (4)				(b) (4)
		15 µg					
		50 µg					
		150 µg					
		500 µg					
		5000 µg					
TA98	B[a]P	5 µg	291.0	46.1	3.6	243, 335, 295	
TA100	AAN	5 µg	1322.7	717.6	7.4	496, 1785, 1687	
TA1535	AAN	5 µg	487.7	143.1	19.0	324, 589, 550	
TA1537	B[a]P	5 µg	141.0	19.2	4.1	154, 119, 150	
WP2 <i>uvrA</i> (pKMI01)	AAN	10 µg	1091.3	131.2	4.9	942, 1144, 1188	
Key to Positive Controls			Key to Plate Postfix Codes				
B[a]P	Benzo[a]pyrene		P		Precipitate		
AAN	2-Aminoanthracene						

As shown in the table above, there were no clear dose-dependent increases in the number of revertants in any of the strains. The number of revertants in these strains in Test 1 with metabolic activation was [REDACTED]<sup>(b) (4)</sup> than the historical control (see historical control table below).

The following table illustrates the number of revertants in Test 2 without metabolic activation (from the Applicant's submission):

Without metabolic activation

Strain	Addition	Concentration per plate	Mean revertants per plate	Standard Deviation	Fold increase relative to vehicle	Individual revertant colony counts (Sorcerer)
TA98	DMSO		38.7	3.5		35, 39, 42
	(b) (4)	50 µg	(b) (4)			
		150 µg				
		500 µg				
		1500 µg				
		5000 µg				
TA100	DMSO		146.7	18.8		167, 130, 143
	(b) (4)	50 µg	(b) (4)			
		150 µg				
		500 µg				
		1500 µg				
		5000 µg				
TA1535	DMSO		29.7	2.1		32, 29, 28
	(b) (4)	50 µg	(b) (4)			
		150 µg				
		500 µg				
		1500 µg				
		5000 µg				
TA1537	DMSO		14.7	3.5		18, 11, 15
	(b) (4)	50 µg	(b) (4)			
		150 µg				
		500 µg				
		1500 µg				
		5000 µg				
WP2 <i>uvrA</i> (pKM101)	DMSO		147.0	14.7		131, 160, 150
	(b) (4)	50 µg	(b) (4)			
		150 µg				
		500 µg				
		1500 µg				
		5000 µg				
TA98	2NF	2 µg	242.0	10.1	6.3	253, 233, 240
TA100	NaN3	2 µg	1021.7	32.8	7.0	1037, 1044, 984
TA1535	NaN3	2 µg	933.7	55.2	31.5	968, 963, 870
TA1537	AAC	50 µg	659.3	181.0	45.0	832, 471, 675
WP2 <i>uvrA</i> (pKM101)	NQO	2 µg	2142.7	197.6	14.6	2363, 1981, 2084

Key to Positive Controls

2NF	2-Nitrofluorene
NaN3	Sodium azide
AAC	9-Aminoacridine
NQO	4-Nitroquinoline-1-oxide

Key to Plate Postfix Codes

P	Precipitate
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As shown in the table above, in general, there were no clear dose-dependent increases in the number of revertants in any of the strains tested; however, in strains TA98 and TA100, the number of revertants were slightly increased in treated groups. Precipitate was noted at the 5000 mcg/plate. The number of revertants in these strains in Test 2 without metabolic activation was [REDACTED] <sup>(b) (4)</sup> than the historical control (see historical control table below).

The following table illustrates the number of revertants in Test 2 with metabolic activation (from the Applicant's submission):

With metabolic activation

Strain	Addition	Concentration per plate	Mean revertants per plate	Standard Deviation	Fold increase relative to vehicle	Individual revertant colony counts (Sorcerer)
TA98	DMSO		64.0	5.2		61, 61, 70
	(b) (4)	50 µg	(b) (4)			
		150 µg				
		500 µg				
		1500 µg				
		5000 µg				
TA100	DMSO		177.0	12.2		191, 171, 169
	(b) (4)	50 µg	(b) (4)			
		150 µg				
		500 µg				
		1500 µg				
		5000 µg				
TA1535	DMSO		25.0	3.5		29, 23, 23
	(b) (4)	50 µg	(b) (4)			
		150 µg				
		500 µg				
		1500 µg				
		5000 µg				
TA1537	DMSO		37.0	2.0		35, 39, 37
	(b) (4)	50 µg	(b) (4)			
		150 µg				
		500 µg				
		1500 µg				
		5000 µg				
WP2 <i>uvrA</i> (pKM101)	DMSO		192.0	20.1		215, 183, 178
	(b) (4)	50 µg	(b) (4)			
		150 µg				
		500 µg				
		1500 µg				
		5000 µg				
TA98	B[a]P	5 µg	264.7	12.1	4.1	266, 276, 252
TA100	AAN	5 µg	1178.3	488.2	6.7	1125, 1691, 719
TA1535	AAN	5 µg	265.0	57.3	10.6	279, 314, 202
TA1537	B[a]P	5 µg	206.0	20.1	5.6	225, 208, 185
WP2 <i>uvrA</i> (pKM101)	AAN	10 µg	1197.3	101.0	6.2	1138, 1140, 1314
Key to Positive Controls				Key to Plate Postfix Codes		
B[a]P	Benzo[a]pyrene		P		Precipitate	
AAN	2-Aminoanthracene					

As shown in the table above, in general, there were no increases in the number of revertants in any of the strains tested. The number of revertants in all other doses in these strains was lower than control. The number of revertants in these strains in Test 2 with metabolic activation was (b) (4) than the historical control (see historical control table below).

The following table illustrates the historical control for these strains in the DMSO vehicle (from the Applicant's submission):

#### HISTORICAL CONTROL DATA

Presented below are the historical control data (mean revertant colony counts) from the period 1 January 2009 to 31 December 2013.

##### DMSO

	TA100		TA1535		WP2 <i>uvrA</i> (pKM101)		TA98		TA1537	
	-	+	-	+	-	+	-	+	-	+
S9 Mix	-	+	-	+	-	+	-	+	-	+
Maximum	199	217	34	35	252	257	51	73	27	50
Minimum	98	108	10	12	90	108	24	32	5	11
Mean	141	160	22	23	134	152	37	50	13	28
No. of values	415	414	385	385	345	345	415	415	385	385
Standard deviation	16	20	4	3	22	23	4	6	3	5

##### Positive Controls

	TA100		TA1535		WP2 <i>uvrA</i> (pKM101)		TA98		TA1537	
	-	+	-	+	-	+	-	+	-	+
S9 Mix	-	+	-	+	-	+	-	+	-	+
	NaN <sub>3</sub> (2 µg)	AAN (5 µg)	NaN <sub>3</sub> (2 µg)	AAN (5 µg)	NQO (2 µg)	AAN (10 µg)	2NF (2 µg)	B[a]P (5 µg)	AAC (50 µg)	B[a]P (5 µg)
Maximum	2246	4438	2338	1457	4494	2806	920	727	1917	626
Minimum	390	522	145	119	524	294	125	127	133	68
Mean	970	1974	882	324	1774	908	321	292	663	178
No. of values	634	634	585	586	536	537	634	635	585	585
Standard deviation	283	671	313	142	587	462	120	91	280	55

NaN <sub>3</sub>	Sodium azide
2NF	2-Nitrofluorene
AAC	9-Aminoacridine
B[a]P	Benzo[a]pyrene
AAN	2-Aminoanthracene
NQO	4-Nitroquinoline-1-oxide

The number of revertants in the positive control groups was within the range of the historical control. Thus, taken together, (b) (4) was not mutagenic under the conditions of this assay.

**Study title:** (b) (4) **Bacterial Reverse Mutation Test**

Study no.: HKQ0016  
 Study report location: Module 4 of the SDN 17 electronic submission  
 Conducting laboratory and location: (b) (4)

Date of study initiation: February 24, 2014  
 GLP compliance: Yes. Signature provided on April 17, 2014.  
 QA statement: Yes. Signature provided on April 17, 2014.  
 Drug, lot #, and % purity: (b) (4) batch number 047S-052S-EH, (b) (4) % pure

**Key Study Findings**

- In the definitive test, concentrations of 0, 50, 150, 500, 1500, and 5000 mcg/plate of (b) (4) were tested in *S. typhimurium* strains TA1535, TA1537, TA98, and TA100 as well as the *E. coli* strain WP2 uvrA.
- This study is considered valid.
- (b) (4) was not mutagenic under the conditions of this assay.

**Methods**

Strains: *S. typhimurium* strains TA1535, TA1537, TA98, and TA100; *E. coli* strain WP2 uvrA

Concentrations in definitive study: 0, 50, 150, 500, 1500, and 5000 mcg/plate

Basis of concentration selection: High dose was 5000 mcg/plate, which is the standard limit concentration recommended in regulatory guidelines

Negative control: DMSO

Positive control: Without S9 activation, sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), 2-nitrofluorene (TA98), and 4-nitroquinoline-1-oxide (WP2 uvrA);  
 With S9 activation, 2-aminoanthracene (TA100, TA1535, and WP2 uvrA) and benzo[a]pyrene (TA98 and TA1537)

Formulation/Vehicle: DMSO

Incubation & sampling time: (b) (4) in vehicle (DMSO), vehicle alone (negative control), or positive control was pre-incubated with the bacteria with and without S9 mix in the top agar. The solution was mixed and overlaid onto a

minimal bottom agar. After the overlay solidified, the plates were inverted and incubated at 37°C for 48 hrs. Afterwards, the plates were scored. All concentrations and controls (both positive and negative) in the initial toxicity-mutation assay and in the confirmatory mutagenicity assay were plated in triplicate.

### **Study Validity**

The study is considered valid for the following reasons: 1) the appropriate controls were used; 2) the appropriate strains were tested; 3) the positive control substances produced reliable positive results; and 4) the highest concentration of [REDACTED] (b) (4) tested reached the maximum recommended concentration of 5000 mcg/plate

The study report states that the criteria for a positive is when a reproducible increase in revertant colony numbers of at least twice (three times in the case of TA1535 and TA1537) that of the concurrent vehicle controls, with some evidence of a positive concentration-response relationship.

### **Results**

The following table illustrates the number of revertants in Test 1 without metabolic activation (from the Applicant's submission):

Without metabolic activation

Strain	Addition	Concentration per plate	Mean revertants per plate	Standard Deviation	Fold increase relative to vehicle	Individual revertant colony counts (Sorcerer)
TA98	DMSO		55.3	10.7		53, 67, 46
	(b) (4)	5 µg				(b) (4)
		15 µg				
		50 µg				
		150 µg				
		500 µg				
		5000 µg				
TA100	DMSO		203.7	38.0		222, 160, 229
	(b) (4)	5 µg				(b) (4)
		15 µg				
		50 µg				
		150 µg				
		500 µg				
		5000 µg				
TA1535	DMSO		35.3	6.4		40, 28, 38
	(b) (4)	5 µg				(b) (4)
		15 µg				
		50 µg				
		150 µg				
		500 µg				
		5000 µg				
TA1537	DMSO		28.7	2.1		27, 28, 31
	(b) (4)	5 µg				(b) (4)
		15 µg				
		50 µg				
		150 µg				
		500 µg				
		5000 µg				
WP2 <i>uvrA</i> (pKM101)	DMSO		186.3	8.5		183, 196, 180
	(b) (4)	5 µg				(b) (4)
		15 µg				
		50 µg				
		150 µg				
		500 µg				
		5000 µg				
TA98	2NF	2 µg	398.7	30.0	7.2	400, 428, 368
TA100	NaN3	2 µg	659.7	87.0	3.2	565, 678, 736
TA1535	NaN3	2 µg	877.3	122.7	24.8	763, 1007, 862
TA1537	AAC	50 µg	421.0	115.2	14.7	554, 352, 357
WP2 <i>uvrA</i> (pKM101)	NQO	2 µg	2124.0	165.2	11.4	1934, 2204, 2234
Key to Positive Controls			Key to Plate Postfix Codes			
2NF	2-Nitrofluorene		T	Thinning of background lawn		
NaN3	Sodium azide		S	Slight thinning of background lawn		
AAC	9-Aminoacridine					
NQO	4-Nitroquinoline-1-oxide					

As shown in the table above, there were no dose-dependent increases in the number of revertants in all strains.

The following table illustrates the number of revertants in Test 1 with metabolic activation (from the Applicant's submission):

With metabolic activation

Strain	Addition	Concentration per plate	Mean revertants per plate	Standard Deviation	Fold increase relative to vehicle	Individual revertant colony counts (Sorcerer)	
TA98	DMSO		69.0	8.7		79, 64, 64	
	(b) (4)	5 µg					(b) (4)
		15 µg					
		50 µg					
		150 µg					
		500 µg					
		1500 µg					
	5000 µg						
TA100	DMSO		200.0	11.1		188, 202, 210	
	(b) (4)	5 µg					(b) (4)
		15 µg					
		50 µg					
		150 µg					
		500 µg					
		1500 µg					
	5000 µg						
TA1535	DMSO		30.3	3.8		33, 32, 26	
	(b) (4)	5 µg					(b) (4)
		15 µg					
		50 µg					
		150 µg					
		500 µg					
		1500 µg					
	5000 µg						
TA1537	DMSO		38.7	4.7		44, 35, 37	
	(b) (4)	5 µg					(b) (4)
		15 µg					
		50 µg					
		150 µg					
		500 µg					
		1500 µg					
	5000 µg						
WP2 <i>uvrA</i> (pKM101)	DMSO		233.3	2.5		231, 233, 236	
	(b) (4)	5 µg					(b) (4)
		15 µg					
		50 µg					
		150 µg					
		500 µg					
		1500 µg					
	5000 µg						
TA98	B[a]P	5 µg	338.3	89.5	4.9	248, 427, 340	
TA100	AAN	5 µg	1440.0	928.4	7.2	368, 1974, 1978	
TA1535	AAN	5 µg	432.0	10.6	14.2	428, 424, 444	
TA1537	B[a]P	5 µg	151.0	13.2	3.9	161, 136, 156	
WP2 <i>uvrA</i> (pKM101)	AAN	10 µg	780.3	128.3	3.3	633, 841, 867	
Key to Positive Controls							
B[a]P	Benzo[a]pyrene						
AAN	2-Aminoanthracene						

As shown in the table above, there were no dose-dependent increases in the number of revertants. The number of revertants in the TA100, TA1535, TA1537, and WP2 uvrA strains at all doses tested were within the historical control range for those strains with metabolic activation in DMSO.

It is noted that despite the thinning of colonies in the 5000 mcg/plate dose of (b) (4) is the TA98, TA100, TA1535, and TA1537 strains without metabolic activation in Test 1, these bacterial strains are viable and the results may be considered valid as shown in the table below (from the Applicant's submission):

**Viability plate summary**

Strain		Mean counts per plate	Standard Deviation	Individual colony counts (100 $\mu$ L aliquots of $10^{-6}$ dilution of 10-hour culture)
<b>TA98</b>	Viability	109.3	7.4	115, 101, 112
<b>TA100</b>	Viability	205.3	68.1	251, 238, 127
<b>TA1535</b>	Viability	264.3	47.9	209, 293, 291
<b>TA1537</b>	Viability	167.0	43.9	214, 127, 160
<b>WP2 uvrA (pKM101)</b>	Viability	141.0	57.7	186, 76, 161

The following table illustrates the number of revertants in Test 2 without metabolic activation (from the Applicant's submission):

Without metabolic activation

Strain	Addition	Concentration per plate	Mean revertants per plate	Standard Deviation	Fold increase relative to vehicle	Individual revertant colony counts (Sorcerer)
TA98	DMSO		40.7	7.4		49, 35, 38
	(b) (4)	50 µg	(b) (4)			
		150 µg				
		500 µg				
		1500 µg				
		5000 µg				
TA100	DMSO		128.7	11.2		137, 116, 133
	(b) (4)	50 µg	(b) (4)			
		150 µg				
		500 µg				
		1500 µg				
		5000 µg				
TA1535	DMSO		25.0	3.5		21, 27, 27
	(b) (4)	50 µg	(b) (4)			
		150 µg				
		500 µg				
		1500 µg				
		5000 µg				
TA1537	DMSO		15.3	3.1		18, 16, 12
	(b) (4)	50 µg	(b) (4)			
		150 µg				
		500 µg				
		1500 µg				
		5000 µg				
WP2 <i>uvrA</i> (pKM101)	DMSO		158.3	17.8		171, 138, 166
	(b) (4)	50 µg	(b) (4)			
		150 µg				
		500 µg				
		1500 µg				
		5000 µg				
TA98	2NF	2 µg	283.0	26.2	7.0	287, 307, 255
TA100	NaN3	2 µg	992.7	58.3	7.7	1060, 960, 958
TA1535	NaN3	2 µg	547.7	260.6	21.9	812, 540, 291
TA1537	AAC	50 µg	839.3	394.4	54.7	780, 478, 1260
WP2 <i>uvrA</i> (pKM101)	NQO	2 µg	1782.7	244.9	11.3	2019, 1530, 1799

Key to Positive Controls

2NF	2-Nitrofluorene
NaN3	Sodium azide
AAC	9-Aminoacridine
NQO	4-Nitroquinoline-1-oxide

Key to Plate Postfix Codes

T	Thinning of background lawn
V	Severe thinning of background lawn

As shown in the table above, the number of revertants from the TA100 strain shows slight increases; however, the number of revertants from all remaining strains does not show a clear dose-dependency. It is noted that with the exception of the 150 mcg/plate dose group in the TA98 strain, the number of revertants in all dose groups in all strains are within the historical control range for those strains without metabolic activation in DMSO (see historical control table below).

The following table illustrates the number of revertants in Test 2 with metabolic activation (from the Applicant's submission):

With metabolic activation

Strain	Addition	Concentration per plate	Mean revertants per plate	Standard Deviation	Fold increase relative to vehicle	Individual revertant colony counts (Sorcerer)
TA98	DMSO		61.7	6.0		61, 68, 56
	(b) (4)	50 µg	(b) (4)			
		150 µg				
		500 µg				
		1500 µg				
		5000 µg				
TA100	DMSO		153.7	10.2		161, 142, 158
	(b) (4)	50 µg	(b) (4)			
		150 µg				
		500 µg				
		1500 µg				
		5000 µg				
TA1535	DMSO		28.7	2.5		29, 26, 31
	(b) (4)	50 µg	(b) (4)			
		150 µg				
		500 µg				
		1500 µg				
		5000 µg				
TA1537	DMSO		30.0	3.5		34, 28, 28
	(b) (4)	50 µg	(b) (4)			
		150 µg				
		500 µg				
		1500 µg				
		5000 µg				
WP2 uvrA (pKM101)	DMSO		185.3	11.9		177, 199, 180
	(b) (4)	50 µg	(b) (4)			
		150 µg				
		500 µg				
		1500 µg				
		5000 µg				
TA98	B[a]P	5 µg	234.3	14.2	3.8	219, 247, 237
TA100	AAN	5 µg	826.7	444.6	5.4	1340, 573, 567
TA1535	AAN	5 µg	302.0	9.5	10.5	308, 307, 291
TA1537	B[a]P	5 µg	142.7	14.6	4.8	159, 138, 131
WP2 uvrA (pKM101)	AAN	10 µg	1261.7	76.0	6.8	1321, 1176, 1288

Key to Positive Controls

B[a]P Benzo[a]pyrene  
 AAN 2-Aminoanthracene

Key to Plate Postfix Codes

T Thinning of background lawn  
 V Severe thinning of background lawn  
 S Slight thinning of background lawn

As shown in the table above, there were incidences of slight increases in the number of revertants in the TA98 and TA100 strains. However, there was no dose-dependency in the number of revertants in all strains tested. The number of revertants in all dose groups in all strains were within the historical control range for each strain with metabolic activation in DMSO (see historical control table below).

It is noted that despite the thinning of colonies in the 5000 mcg/plate dose of (b) (4) in all strains with and without metabolic activation in Test 2, these bacterial strains are viable and may be considered valid as shown in the table below (from the Applicant's submission):

Viability plate summary

Strain		Mean counts per plate	Standard Deviation	Individual colony counts (100 $\mu$ L aliquots of $10^{-6}$ dilution of 10-hour culture)
TA98	Viability	179.0	6.9	175, 187, 175
TA100	Viability	259.0	16.5	276, 243, 258
TA1535	Viability	277.0	27.2	307, 254, 270
TA1537	Viability	166.3	20.2	188, 148, 163
WP2 uvrA (pKM101)	Viability	208.7	13.3	200, 224, 202

The historical control is illustrated in the following table (from the Applicant's submission):

**HISTORICAL CONTROL DATA**

Presented below are the historical control data (mean revertant colony counts) from the period 1 January 2009 to 31 December 2013.

**DMSO**

	TA100		TA1535		WP2 <i>uvrA</i> (pKM101)		TA98		TA1537	
	-	+	-	+	-	+	-	+	-	+
S9 Mix	-	+	-	+	-	+	-	+	-	+
Maximum	199	217	34	35	252	257	51	73	27	50
Minimum	98	108	10	12	90	108	24	32	5	11
Mean	141	160	22	23	134	152	37	50	13	28
No. of values	415	414	385	385	345	345	415	415	385	385
Standard deviation	16	20	4	3	22	23	4	6	3	5

**Positive Controls**

	TA100		TA1535		WP2 <i>uvrA</i> (pKM101)		TA98		TA1537	
	-	+	-	+	-	+	-	+	-	+
S9 Mix	NaN <sub>3</sub> (2 µg)	AAN (5 µg)	NaN <sub>3</sub> (2 µg)	AAN (5 µg)	NQO (2 µg)	AAN (10 µg)	2NF (2 µg)	B[a]P (5 µg)	AAC (50 µg)	B[a]P (5 µg)
Maximum	2246	4438	2338	1457	4494	2806	920	727	1917	626
Minimum	390	522	145	119	524	294	125	127	133	68
Mean	970	1974	882	324	1774	908	321	292	663	178
No. of values	634	634	585	586	536	537	634	635	585	585
Standard deviation	283	671	313	142	587	462	120	91	280	55

NaN <sub>3</sub>	Sodium azide
2NF	2-Nitrofluorene
AAC	9-Aminoacridine
B[a]P	Benzo[a]pyrene
AAN	2-Aminoanthracene
NQO	4-Nitroquinoline-1-oxide

The number of revertants in the positive control groups was within the range of the historical control. Thus, taken together, (b) (4) was not mutagenic under the conditions of this assay.

**8 Carcinogenicity**

There were no carcinogenicity studies with acetaminophen submitted in this NDA. The following information on the carcinogenicity of acetaminophen is from the OFIRMEV label (Cadence Pharma, November 2011):

Carcinogenesis

Long-term studies in mice and rats have been completed by the National Toxicology Program to evaluate the carcinogenic potential of acetaminophen. In 2-year feeding studies, F344/N rats and B6C3F1 mice were fed a diet containing acetaminophen up to 6000 ppm. Female rats demonstrated equivocal evidence of carcinogenic activity based on increased incidences of mononuclear cell leukemia at 0.8 times the maximum human daily dose (MHDD) of 4 grams/day, based on a body surface area comparison. In contrast, there was no evidence of carcinogenic activity in male rats (0.7 times) or mice (1.2-1.4 times the MHDD, based on a body surface area comparison).

## 9 Reproductive and Developmental Toxicology

There were no reproductive and developmental toxicology studies with acetaminophen submitted in this NDA. The following information on the impairment of fertility of acetaminophen is from the OFIRMEV label (Cadence Pharma, November 2011):

### Impairment of fertility

In studies conducted by the National Toxicology Program, fertility assessments have been completed in Swiss mice via a continuous breeding study. There were no effects on fertility parameters in mice consuming up to 1.7 times the MHDD of acetaminophen, based on a body surface area comparison. Although there was no effect on sperm motility or sperm density in the epididymis, there was a significant increase in the percentage of abnormal sperm in mice consuming 1.7 times the MHDD (based on a body surface area comparison) and there was a reduction in the number of mating pairs producing a fifth litter at this dose, suggesting the potential for cumulative toxicity with chronic administration of acetaminophen near the upper limit of daily dosing.

Published studies in rodents report that oral acetaminophen treatment of male animals at doses that are 1.2 times the MHDD and greater (based on a body surface area comparison) result in decreased testicular weights, reduced spermatogenesis, reduced fertility, and reduced implantation sites in females given the same doses. These effects appear to increase with the duration of treatment. The clinical significance of these findings is not known.

There were no further reproductive and developmental toxicology studies with IV acetaminophen submitted in this NDA. The following information on the reproductive and developmental toxicology of acetaminophen is from the pregnancy section of the OFIRMEV label (Cadence Pharma, November 2011):

Pregnancy Category C. There are no studies of intravenous acetaminophen in pregnant women; however, epidemiological data on oral acetaminophen use in pregnant women show no increased risk of major congenital malformations. Animal reproduction studies have not been conducted with IV acetaminophen, and it is not known whether OFIRMEV can cause fetal harm when administered to a pregnant woman. OFIRMEV should be given to a pregnant woman only if clearly needed.

The results from a large population-based prospective cohort, including data from 26,424 women with live born singletons who were exposed to oral acetaminophen during the first trimester, indicate no increased risk for congenital malformations, compared to a control group of unexposed children. The rate of congenital malformations (4.3%) was similar to the rate in the general population. A population-based, case-control study from the National Birth Defects Prevention Study showed that 11,610 children with prenatal exposure to acetaminophen during the first trimester had no increased risk of major birth defects compared to 4,500 children in the control group. Other epidemiological data showed similar results.

While animal reproduction studies have not been conducted with intravenous acetaminophen, studies in pregnant rats that received oral acetaminophen during organogenesis at doses up to 0.85 times the maximum human daily dose (MHDD = 4 grams/day, based on a body surface area comparison) showed evidence of fetotoxicity (reduced fetal weight and length) and a dose-related increase in bone variations (reduced ossification and rudimentary rib changes). Offspring had no evidence of external, visceral, or skeletal malformations. When pregnant rats received oral acetaminophen throughout gestation at doses of 1.2-times the MHDD (based on a body surface area comparison), areas of necrosis occurred in both the liver and kidney of pregnant rats and fetuses. These effects did not occur in animals that received oral acetaminophen at doses 0.3-times the MHDD, based on a body surface area comparison.

In a continuous breeding study, pregnant mice received 0.25, 0.5, or 1.0% acetaminophen via the diet (357, 715, or 1430 mg/kg/day). These doses are approximately 0.43, 0.87, and 1.7 times the MHDD, respectively, based on a body surface area comparison. A dose-related reduction in body weights of fourth and fifth litter offspring of the treated mating pair occurred during lactation and post-weaning at all doses. Animals in the high dose group had a reduced number of litters per mating pair, male offspring with an increased percentage of abnormal sperm, and reduced birth weights in the next generation pups.

## 10 Special Toxicology Studies

There were no special toxicology studies with acetaminophen submitted in this NDA.

## 11 Integrated Summary and Safety Evaluation

Fresenius Kabi USA, LLC has submitted a 505(b)(2) application for an intravenous formulation of acetaminophen (10 mg/mL) in a freeflex® (b)(4) for the management of mild to moderate pain, management of moderate to severe pain with adjunctive opioid analgesics, and reduction of fever. In the first cycle NDA review, the safety of the drug substance impurities, drug product degradants and excipients were reviewed and deemed adequate. However, the safety of several leachables was not adequately qualified for safety and a complete response was recommended from a nonclinical pharmacology toxicology perspective.

To address the complete response letter from the Division dated July 25, 2013, the Applicant submitted a 4-week IV toxicity study with (b)(4) in rats and a 4-week IV toxicity study with (b)(4) in rats in order to qualify the safety of (b)(4) two identified leachables from the container closure system. The Applicant also submitted the results of an Ames assay with (b)(4) and an Ames assay with (b)(4)

(b)(4) is not mutagenic in a valid Ames assay. (b)(4) is not mutagenic in a valid Ames assay. Rats were dosed via intravenous administration with 0, 12.5, 25, or 50 mg/kg/day of (b)(4) for 4 weeks. Injection site observations (clinical signs) include bruising, erosion and or ulceration, erythema, eschar, loss of flexibility, and reddening at the 25 and 50 mg/kg/day dose groups. There was a significant increase in the triglyceride and phosphorous levels of 45.5 and 23.4%, respectively, in the male 50 mg/kg/day dose group with macroscopic and microscopic correlates in the liver and kidney. Macroscopically, hernia in the diaphragm, small epididymis, depression in the kidney, liver changes (irregular surfaces, masses, and small size), small testes, unilaterally absent thyroid glands, and depression and scab at the venous injection site were observed at the 50 mg/kg/day dose group. The histopathological findings at the injection site including perivascular hemorrhage, recanalized thrombus, vascular intimal proliferation, epidermal ulceration, and scabs at the 25 and 50 mg/kg/day dose groups are indicative of mild localized vascular irritation as opposed to systemic vascular irritation. Other histopathological changes at the injection site include perivascular inflammatory cell infiltration in the male 25 and 50 mg/kg/day dose groups, granuloma of the hair shaft in the female 50 mg/kg/day dose group, perivascular necrosis in male 50 mg/kg/day dose group, and abscess and epidermal hyperplasia in the female 50 mg/kg/day dose group. Histopathological changes in other sites include aggregates at the corticomedullary junction in the liver as well as perivascular infiltration of inflammatory cells and granuloma on the lung at the 50 mg/kg/day dose group. The pathologist's report concludes these systemic findings as incidental. The Applicant's NOAEL of 50 mg/kg/day and NOEL of 25 mg/kg/day. This reviewer concurs with this assessment. At the MTDD of 2 g/day of APAP, the total daily intake of (b)(4) is (b)(4) mcg/day. The rat NOAEL of 12.5 mg/kg/day confers an exposure margin of (b)(4) fold based on a body surface area comparison.

Rats were dosed via intravenous administration with 0, 7.5, 15, or 25 mg/kg/day of (b) (4) for 4 weeks. There were 3 males and 2 females that were humanely sacrificed from the 25 mg/kg/day dose group. There was 1 male found dead in the 15 mg/kg/day dose group. In the surviving rats, injection site clinical signs include reddening and bruising of the tail in the female 15 mg/kg/day dose group and discolored tail in the male and female 25 mg/kg/day dose groups. There were increases in lymphocytes, eosinophils, and neutrophils in the 15 mg/kg/day dose group that was correlated with decreased spleen weight (by 30.4%) and histopathological changes in the spleen (extramedullary hemopoiesis) in the 15 mg/kg/day dose group. There was an increase in triglycerides (by 75.9%) in the males and phosphorous (by 13.8%) in the females in the 15 mg/kg/day dose groups that was correlated with histopathological changes in the liver in the 15 mg/kg/day dose group (infiltration of inflammatory cells with and without hepatocellular necrosis and extramedullary hemopoiesis). Macroscopically (gross pathology), there were depression in the kidney and pale areas in the lung in the male 15 mg/kg/day. Histopathological changes at the injection site include chronic thrombus, acute thrombus, perivascular inflammation, perivascular hemorrhage, and perivascular fibrosis at the 15 mg/kg/day dose group. Histopathological changes in other sites include extramedullary hemopoiesis in the spleen, perivascular inflammation in the lungs, and liver observations (infiltration of inflammatory cells with or without hepatocellular necrosis and extramedullary hemopoiesis) at the 15 mg/kg/day dose group. Taken together, the NOAEL appears to be 7.5 mg/kg/day. This is in contrast to the Applicant's NOAEL of (b) (4) mg/kg/day. At the MTDD of 4 g/day of APAP, the total daily intake of (b) (4) is (b) (4) mcg/day. The NOAEL of 7.5 mg/kg/day confers an exposure margin of (b) (4) fold based on a body surface area comparison.

Review of the revised toxicological risk assessments showed that there is adequate coverage of the leachables (b) (4).

From the pharmacology toxicology perspective, the Applicant has addressed all the deficiencies outlined in the complete response letter and therefore the NDA for the drug product, Acetaminophen Injection in freeflex® (b) (4) may be approved.

## 12 Appendix/Attachments

### References

(b) (4)

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/s/  
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CARLIC K HUYNH  
10/13/2015

NEWTON H WOO  
10/13/2015

RICHARD D MELLON  
10/13/2015

I concur that NDA 204767 may be approved from a nonclinical pharmacology toxicology perspective.



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

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

**Date:** June 20, 2013

**To:** **NDA 204767** (Acetaminophen Injection)

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

**Subject:** **Complete Response Recommendation**

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Dr. Carlic Huynh has recommended that a complete response action be taken for NDA 204767 (Acetaminophen Injection). I concur with the deficiencies noted in Dr. Huynh's review and the information he recommends to address these deficiencies.

There were no communications with the Sponsor of this NDA application prior to submission of this 505(b)(2) NDA that relies upon the Agency's previous finding of safety for Ofirmev (NDA 22450). There are outstanding patents listed in the Orange Book for Ofirmev. The focus of the nonclinical pharmacology toxicology review was on the potential need for an IV toxicology study, local tissue toxicology study, the safety of the drug substance and drug product specifications, the excipients in the drug product formulation, and the FreeFlex® container closure system (extractables/leachables).

As noted in his review, Dr. Huynh has concluded that there are no safety concerns with the proposed drug substance and drug product specifications. The formulation is comparable to the referenced product and is isotonic, therefore, we did not feel that an IV toxicology study or a local tissue toxicity study was necessary to support approval of this product. However, at the time of NDA submission, adequate information on the safety of the container closure system was not readily available as supporting data was referenced via a CBER Master File and long-term stability data were not yet completed. Nonetheless, based on the information available to us at the time of filing, adequate safety information for several identified leachable compounds did not appear to be available to support the NDA. Following discussion with the Sponsor they agreed to submit a Master File to CDER for the FreeFlex® (b) (4) (MF 26696). As noted in the 74-day letter, we also informed them that additional data

would likely be required to support the safety of several identified leachables from the container closure system.

Dr. Huynh reviewed the levels of leachables detected to date in the existing stability studies that were submitted during the review cycle. In addition, he reviewed the risk assessments submitted for the leachables. Based on the highest levels of the compounds detected to date, Dr. Huynh concluded that there are inadequate data to support the safety of at least two compounds identified as leachables from the container closure system, (b) (4)

The Sponsor proposed to complete a 4-week IV toxicology study for (b) (4) to address this concern. The study has not yet been completed and will be required to support the safety of the container closure system. The Sponsor's toxicology risk assessment for (b) (4) is based on the safety of the two main metabolites (b) (4). Although Dr. Huynh notes that there are no safety concerns with the levels of these metabolites detected to date, the rate of (b) (4) in vivo is not known and therefore exposure to the parent compound is likely. Dr. Huynh recommends that the Sponsor should either conduct a 28-day IV toxicology study for (b) (4) or provide convincing evidence that (b) (4) is virtually instantaneously (b) (4).

It must be noted that the risk assessments on the leachables completed to date were based on the highest levels detected to date and the assumption that they will not increase upon longer stability. The stability data we have to date is limited, and must be confirmed by adequate data, as recommended by the CMC review team. As the CMC review team has concluded, data from at least three batches over the entire course of stability should be provided in order to fully characterize the potential leachables via this product. As the Sponsor has not yet provided adequate data to that effect to date, we will have to revisit the toxicological risk assessments based on definitive stability data in order to recommend approval of this product.

I concur with Dr. Huynh's recommendations on the nonclinical sections of the drug product labeling, which are identical to the referenced product. I also concur with his deficiencies and recommendations to resolve the deficiencies, as reproduced below:

### **Deficiencies**

You have not provided adequate safety justification for the levels of (b) (4) two of the identified leachables from the container closure system.

### **Information needed to resolve the deficiency**

1. Submit the results of the proposed 4-week IV toxicology study of (b) (4) and a revised toxicological risk assessment.
2. Conduct and submit the results of a 4-week IV toxicology study of (b) (4) and a revised toxicological risk assessment for this compound. Alternatively, you may be able to provide adequate data to support your conclusion that (b) (4) is virtually instantaneous in vivo such that exposure to the parent compound, when the product is used as directed, would not occur and your risk assessment based on the major metabolites alone is adequate to address the safety of the parent compound.

### **Additional Nonclinical Recommendations**

The following comments are not deficiencies, but should still be relayed to the Sponsor nonetheless.

1. You have proposed to complete in vitro bacterial reverse mutation studies (Ames tests) for both (b) (4). The final reports for these studies are not required for approval; however, should the studies be completed, the results should be submitted to the NDA.
2. Once you have evaluated the levels of leachables in the drug product over the course of the entire intended shelf-life, you must submit revised risk assessments based on the worst-case exposures. Final determination of the adequacy of your leachables safety assessment can only be provided upon review of the definitive stability data.

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/s/  
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RICHARD D MELLON  
06/21/2013

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

**PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION**

Application number: 204767

Supporting document/s: SDNs 1, 3, 4, 5, 6, 8, and 9

Applicant's letter date: September 28, 2012 (SDN 1); December 27, 2012 (SDN 3); February 4, 2013 (SDN 4); February 22, 2013 (SDN 5); March 13, 2013 (SDN 6); March 29, 2013 (SDN 8); and April 17, 2013 (SDN 9)

CDER stamp date: September 28, 2012 (SDN 1); December 27, 2012 (SDN 3); February 4, 2013 (SDN 4); February 22, 2013 (SDN 5); March 13, 2013 (SDN 6); March 29, 2013 (SDN 8); and April 17, 2013 (SDN 9)

Product: Acetaminophen Injection

Indication: Management of mild to moderate pain; management of moderate to severe pain with adjunctive opioid analgesics; reduction of fever

Applicant: Fresenius Kabi USA, LLC

Review Division: Division of Anesthesia, Analgesia, and Addiction Products (DAAAP)

Reviewer: Carlic K. Huynh, Ph.D.

Supervisor/Team Leader: R. Daniel Mellon, Ph.D.

Division Director: Bob A. Rappaport, M.D.

Project Manager: Diana L. Walker, Ph.D.

*Template Version: September 1, 2010*

**Disclaimer**

Except as specifically identified, all data and information discussed below and necessary for approval of 204767 are owned by Fresenius Kabi USA, LLC or are data for which Fresenius Kabi USA, LLC has obtained a written right of reference. Any information or data necessary for approval of 204767 that Fresenius Kabi USA, LLC does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of 204767.

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# 1 Executive Summary

## 1.1 Introduction

Fresenius Kabi USA, LLC is developing an intravenous formulation of acetaminophen (10 mg/mL). The Applicant is submitting an application via the 505(b)(2) pathway, proposing to rely upon the Agency's previous findings of safety and efficacy of Cadence Pharmaceuticals' OFIRMEV (NDA 22450). The proposed nonclinical portions of the label for the Fresenius Kabi IV APAP formulation are the same as the referenced OFIRMEV product labeling.

## 1.2 Brief Discussion of Nonclinical Findings

No new toxicology studies for acetaminophen were submitted. Although there was no communication with the Applicant prior to submission, based on the drug product formulation, no new toxicology studies on acetaminophen are required. The focus of this review is on the safety of the drug substance impurities, drug product degradants, the excipients in the formulation, and the container closure system.

The drug substance and drug product specifications are acceptable. There are no novel excipients in this IV APAP formulation.

To justify why a local tissue toxicity study is not necessary, the Applicant submitted data to show that the osmolality of their IV APAP formulation is comparable to the referenced product OFIRMEV. Given the similarity in the formulation to the reference product and the osmolality data, this justification is adequate.

In order to characterize the container closure system, the NDA references data in the Master File (MF) and contains leachable data with their IV APAP formulation.

Ongoing assessment of leachables during stability submitted during the review cycle suggests the presence of multiple leachable compounds in the drug product. Preliminary results of migration studies from limited batches this IV APAP drug product formulation under various storage conditions for up to 12 months have been submitted in this NDA to date. Identified leachables include (b) (4)

Migration studies have also been reported with various aqueous solutions under various storage conditions and are located in MF 26696 (see nonclinical review). The MF also contains toxicological risk assessments. Based on the data submitted both to the MF and the NDA, most of the identified leachables in migration studies with this IV APAP formulation are qualified for safety except for (b) (4) (and related substances), (b) (4). It is noted that (b) (4) (and related substances) were not detected in the migration studies with this IV APAP formulation to date and thus does not represent a safety concern. The lack of adequate data was discussed with the Applicant during the course of this review cycle. In response to these discussions, the Applicant submitted additional data and proposals to

conduct additional studies to support the safety of the (b) (4)

(b) (4) is presumably a degradation product of (b) (4) the plastic materials comprising the freeflex® (b) (4). A 3-month repeat-dose oral toxicology study in rats with (b) (4) was submitted during the review cycle. Rats were dosed 0, 3, 10, 30, and 100 mg/kg of (b) (4) for 3 months via oral gavage. A NOAEL of 10 mg/kg was defined by a slight increase in liver weights and histopathologic findings of hypertrophy of the hepatocytes in the 30 and 100 mg/kg dose groups. The rat NOAEL of 10 mg/kg supports a human equivalent dose (HED) of (b) (4). Since this toxicity study used oral administration and this IV APAP formulation is for intravenous administration, an additional safety factor of 10 was applied. The maximum level of (b) (4) found to date upon stability using this IV acetaminophen formulation was (b) (4) mcg at the MDD of 4 g/day of APAP, which suggests a safety margin of 363. Thus, the levels of (b) (4) that could leach into this product do not represent a safety concern from the general toxicity perspective. The Applicant plans to submit an Ames test for (b) (4). A QSAR analysis of (b) (4) does not suggest the potential for mutagenicity. Given the low levels detected and lack of concerns regarding mutagenicity, no further studies are required to support the safety of (b) (4).

The second leachable that was not deemed adequately qualified via the data in the MF is (b) (4). As per the MF toxicological risk assessment, (b) (4)

(b) (4) There are no toxicology data on this compound. The toxicological risk assessment based on the parent compound was not deemed adequate. The Sponsor has proposed to conduct a 4-week IV toxicity study in rats and an Ames test for (b) (4). As the final reports for these studies have not been received by the Agency, approval of this NDA cannot be recommended from the nonclinical pharmacology toxicology perspective at this time. As there are no structural alerts for genotoxicity, the proposed Ames test will not be required for approval.

Additionally, (b) (4) has been identified as a leachable in migration studies with this IV APAP formulation. This compound appears to be coming from the secondary container closure system upon stability. There are no data on (b) (4). The Applicant proposed a risk assessment based on the primary metabolites only. The weight of evidence approach for (b) (4) that was presented in MF 26696 is not adequate to justify the safety of the levels of (b) (4) detected in migration studies with this IV APAP formulation at the MDD of APAP of 4 g/day as the rate of (b) (4) to the metabolite is not known. Thus, a 28-day IV toxicity study with (b) (4) is needed to justify the safety of this leachable unless the Applicant can demonstrate virtually instantaneous (b) (4) of the parent. As these data are necessary for approval, we cannot recommend approval from the nonclinical pharmacology toxicology perspective at this time.

The label for the Fresenius Kabi IV APAP formulation is the same as the referenced OFIRMEV for the mutagenesis, carcinogenesis, impairment of fertility, and pregnancy sections. The labeling will eventually have to be revised to conform to the proposed Pregnancy Labeling and Lactation Rule (which will remove the Pregnancy Category and insert a Risk Summary Statement).

### 1.3 Recommendations

#### 1.3.1 Approvability

From a nonclinical pharmacology toxicology perspective, we cannot recommend approval of this product at this time. We recommend a complete response.

#### Deficiency

You have not provided adequate safety justification for the levels of (b) (4) leachables from the container closure system.

#### Information needed to resolve the deficiency

1. Submit the results of the proposed 4-week IV toxicology study of (b) (4) and a revised toxicological risk assessment.
2. Conduct and submit the results of a 4-week IV toxicology study of (b) (4) and a revised toxicological risk assessment. Alternatively, you may be able to provide adequate data to support your conclusion that (b) (4) is virtually instantaneous in vivo such that exposure to the parent compound, when the product is used as directed, would not occur and your risk assessment based on the major metabolites alone is adequate to address the safety of the parent compound.

#### 1.3.2 Additional Nonclinical Recommendations

The Applicant proposed to submit results from in vitro bacterial reverse mutation studies (Ames tests) for both (b) (4). The final reports for these studies are not required for approval; however, should the studies be completed, the results should be submitted to the NDA.

#### 1.3.3 Labeling

The following changes to the Applicant’s proposed labeling are illustrated in the table below:

<i>Applicant’s proposed labeling</i>	<i>Reviewer’s proposed changes</i>	<i>Rationale for changes</i>

<p><b>INDICATIONS AND USAGE</b> Acetaminophen injection is indicated for the:</p> <ul style="list-style-type: none"> <li>• Management of mild to moderate pain. (1)</li> <li>• Management of moderate to severe pain with adjunctive opioid analgesics. (1)</li> <li>• Reduction of fever. (1)</li> </ul>	<p><b>INDICATIONS AND USAGE</b> Acetaminophen injection is indicated for the:</p> <ul style="list-style-type: none"> <li>• Management of mild to moderate pain. (1)</li> <li>• Management of moderate to severe pain with adjunctive opioid analgesics. (1)</li> <li>• Reduction of fever. (1)</li> </ul>	<p>No changes were necessary.</p>
<p><b>USE IN SPECIFIC POPULATIONS</b></p> <ul style="list-style-type: none"> <li>• Pregnancy: Category C. There are no studies of intravenous acetaminophen in pregnant women. Use only if clearly needed. (8.1)</li> </ul>	<p><b>USE IN SPECIFIC POPULATIONS</b></p> <ul style="list-style-type: none"> <li>• Pregnancy: Category C. There are no studies of intravenous acetaminophen in pregnant women. Use only if clearly needed. (8.1)</li> </ul>	<p>No changes were necessary. As per the Maternal Health Team labeling initiative, nonclinical pregnancy information with a reference to Section 8.1 was placed in the Highlights section.</p>
<p><b>8.1 Pregnancy</b> (b) (4)</p> <p>Pregnancy Category C.</p> <p>There are no studies of intravenous acetaminophen in pregnant women; however, epidemiological data on oral acetaminophen use in pregnant women show no increased risk of major congenital malformations. Animal reproduction studies have not been conducted with IV acetaminophen, and it is not known whether acetaminophen can cause fetal harm when administered to a pregnant woman. Acetaminophen should be given to a pregnant woman only if clearly needed.</p> <p>The results from a large population-based prospective cohort, including data from 26,424 women with live born singletons who were exposed to oral acetaminophen during the</p>	<p><b>8.1 Pregnancy</b> (b) (4)</p> <p>Pregnancy Category C.</p> <p>There are no studies of intravenous acetaminophen in pregnant women; however, epidemiological data on oral acetaminophen use in pregnant women show no increased risk of major congenital malformations. Animal reproduction studies have not been conducted with IV acetaminophen, and it is not known whether acetaminophen can cause fetal harm when administered to a pregnant woman. Acetaminophen should be given to a pregnant woman only if clearly needed.</p> <p>The results from a large population-based prospective cohort, including data from 26,424 women with live born singletons who were exposed to oral acetaminophen during the first trimester, indicate no</p>	<p>No changes were necessary to the content. The header is being removed as per the draft PLLR. Ultimately, the pregnancy category will be removed and a risk summary statement will be inserted.</p> <p>The Applicant’s proposed labeling is identical to the current Cadence’s OFIRMEV (acetaminophen) injection label (version approved on November 2, 2010) including an introductory paragraph as per the CFR for Category C drugs and human data to be placed first as per the Maternal Health Team initiative.</p> <p>Like the referenced product labeling, this entire section will have to be revised to remove the pregnancy category and include a risk summary statement once</p>

<p>first trimester, indicate no increased risk for congenital malformations, compared to a control group of unexposed children. The rate of congenital malformations (4.3%) was similar to the rate in the general population. A population-based, case-control study from the National Birth Defects Prevention Study showed that 11,610 children with prenatal exposure to acetaminophen during the first trimester had no increased risk of major birth defects compared to 4,500 children in the control group. Other epidemiological data showed similar results.</p> <p>While animal reproduction studies have not been conducted with intravenous acetaminophen, studies in pregnant rats that received oral acetaminophen during organogenesis at doses up to 0.85 times the maximum human daily dose (MHDD = 4 grams/day, based on a body surface area comparison) showed evidence of fetotoxicity (reduced fetal weight and length) and a dose-related increase in bone variations (reduced ossification and rudimentary rib changes). Offspring had no evidence of external, visceral, or skeletal malformations. When pregnant rats received oral acetaminophen throughout gestation at doses of 1.2 times the MHDD (based on a body surface area comparison), areas of necrosis occurred in both the liver and kidney of pregnant rats</p>	<p>increased risk for congenital malformations, compared to a control group of unexposed children. The rate of congenital malformations (4.3%) was similar to the rate in the general population. A population-based, case-control study from the National Birth Defects Prevention Study showed that 11,610 children with prenatal exposure to acetaminophen during the first trimester had no increased risk of major birth defects compared to 4,500 children in the control group. Other epidemiological data showed similar results.</p> <p>While animal reproduction studies have not been conducted with intravenous acetaminophen, studies in pregnant rats that received oral acetaminophen during organogenesis at doses up to 0.85 times the maximum human daily dose (MHDD = 4 grams/day, based on a body surface area comparison) showed evidence of fetotoxicity (reduced fetal weight and length) and a dose-related increase in bone variations (reduced ossification and rudimentary rib changes). Offspring had no evidence of external, visceral, or skeletal malformations. When pregnant rats received oral acetaminophen throughout gestation at doses of 1.2 times the MHDD (based on a body surface area comparison), areas of necrosis occurred in both the liver and kidney of pregnant rats and fetuses. These effects did not occur in animals that received oral acetaminophen at</p>	<p>the pregnancy labeling and lactation rule is finalized. As this product cannot be approved as of the date of this review, no further action is necessary at this time.</p>
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<p>and fetuses. These effects did not occur in animals that received oral acetaminophen at doses 0.3 times the MHDD, based on a body surface area comparison.</p> <p>In a continuous breeding study, pregnant mice received 0.25, 0.5, or 1% acetaminophen via the diet (357, 715, or 1430 mg/kg/day). These doses are approximately 0.43, 0.87, and 1.7 times the MHDD, respectively, based on a body surface area comparison. A dose-related reduction in body weights of fourth and fifth litter offspring of the treated mating pair occurred during lactation and post-weaning at all doses. Animals in the high dose group had a reduced number of litters per mating pair, male offspring with an increased percentage of abnormal sperm, and reduced birth weights in the next generation pups.</p>	<p>doses 0.3 times the MHDD, based on a body surface area comparison.</p> <p>In a continuous breeding study, pregnant mice received 0.25, 0.5, or 1% acetaminophen via the diet (357, 715, or 1430 mg/kg/day). These doses are approximately 0.43, 0.87, and 1.7 times the MHDD, respectively, based on a body surface area comparison. A dose-related reduction in body weights of fourth and fifth litter offspring of the treated mating pair occurred during lactation and post-weaning at all doses. Animals in the high dose group had a reduced number of litters per mating pair, male offspring with an increased percentage of abnormal sperm, and reduced birth weights in the next generation pups.</p>	
<p><b>13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility</b>  <u>Carcinogenesis</u>                  Long-term studies in mice and rats have been completed by the National Toxicology Program to evaluate the carcinogenic potential of acetaminophen. In 2-year feeding studies, F344/N rats and B6C3F1 mice were fed a diet containing acetaminophen up to 6000 ppm. Female rats demonstrated equivocal evidence of carcinogenic activity</p>	<p><b>13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility</b>  <u>Carcinogenesis</u>                  Long-term studies in mice and rats have been completed by the National Toxicology Program to evaluate the carcinogenic potential of acetaminophen. In 2-year feeding studies, F344/N rats and B6C3F1 mice were fed a diet containing acetaminophen up to 6000 ppm. Female rats demonstrated equivocal evidence of carcinogenic activity</p>	<p>No changes were necessary to the content.</p> <p>The Applicant’s proposed labeling is identical to the current Cadence’s OFIRMEV (acetaminophen) injection label (version approved on November 2, 2010).</p>

<p>based on increased incidences of mononuclear cell leukemia at 0.8 times the maximum human daily dose (MHDD) of 4 grams/day, based on a body surface area comparison. In contrast, there was no evidence of carcinogenic activity in male rats (0.7 times) or mice (1.2 to 1.4 times the MHDD, based on a body surface area comparison).</p> <p><u>Mutagenesis</u> Acetaminophen was not mutagenic in the bacterial reverse mutation assay (Ames test). In contrast, acetaminophen tested positive in the <i>in vitro</i> mouse lymphoma assay and the <i>in vitro</i> chromosomal aberration assay using human lymphocytes. In the published literature, acetaminophen has been reported to be clastogenic when administered a dose of 1500 mg/kg/day to the rat model (3.6 times the MHDD, based on a body surface area comparison). In contrast, no clastogenicity was noted at a dose of 750 mg/kg/day (1.8 times the MHDD, based on a body surface area comparison), suggesting a threshold effect.</p> <p><u>Impairment of Fertility</u> In studies conducted by the National Toxicology Program, fertility assessments have been completed in Swiss mice via a continuous breeding study. There were no effects on fertility parameters in mice consuming up to 1.7 times the MHDD of acetaminophen, based on a</p>	<p>based on increased incidences of mononuclear cell leukemia at 0.8 times the maximum human daily dose (MHDD) of 4 grams/day, based on a body surface area comparison. In contrast, there was no evidence of carcinogenic activity in male rats (0.7 times) or mice (1.2 to 1.4 times the MHDD, based on a body surface area comparison).</p> <p><u>Mutagenesis</u> Acetaminophen was not mutagenic in the bacterial reverse mutation assay (Ames test). In contrast, acetaminophen tested positive in the <i>in vitro</i> mouse lymphoma assay and the <i>in vitro</i> chromosomal aberration assay using human lymphocytes. In the published literature, acetaminophen has been reported to be clastogenic when administered a dose of 1500 mg/kg/day to the rat model (3.6 times the MHDD, based on a body surface area comparison). In contrast, no clastogenicity was noted at a dose of 750 mg/kg/day (1.8 times the MHDD, based on a body surface area comparison), suggesting a threshold effect.</p> <p><u>Impairment of Fertility</u> In studies conducted by the National Toxicology Program, fertility assessments have been completed in Swiss mice via a continuous breeding study. There were no effects on fertility parameters in mice consuming up to 1.7 times the MHDD of acetaminophen, based on a body</p>	
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<p>body surface area comparison. Although there was no effect on sperm motility or sperm density in the epididymis, there was a significant increase in the percentage of abnormal sperm in mice consuming 1.7 times the MHDD (based on a body surface area comparison) and there was a reduction in the number of mating pairs producing a fifth litter at this dose, suggesting the potential for cumulative toxicity with chronic administration of acetaminophen near the upper limit of daily dosing.</p>	<p>surface area comparison. Although there was no effect on sperm motility or sperm density in the epididymis, there was a significant increase in the percentage of abnormal sperm in mice consuming 1.7 times the MHDD (based on a body surface area comparison) and there was a reduction in the number of mating pairs producing a fifth litter at this dose, suggesting the potential for cumulative toxicity with chronic administration of acetaminophen near the upper limit of daily dosing.</p>	
<p>Published studies in rodents report that oral acetaminophen treatment of male animals at doses that are 1.2 times the MHDD and greater (based on a body surface area comparison) result in decreased testicular weights, reduced spermatogenesis, reduced fertility, and reduced implantation sites in females given the same doses. These effects appear to increase with the duration of treatment. The clinical significance of these findings is not known.</p>	<p>Published studies in rodents report that oral acetaminophen treatment of male animals at doses that are 1.2 times the MHDD and greater (based on a body surface area comparison) result in decreased testicular weights, reduced spermatogenesis, reduced fertility, and reduced implantation sites in females given the same doses. These effects appear to increase with the duration of treatment. The clinical significance of these findings is not known.</p>	

## 2 Drug Information

### 2.1 Drug

CAS Registry Number  
103-90-2

Generic Name  
Acetaminophen; paracetamol

Code Name

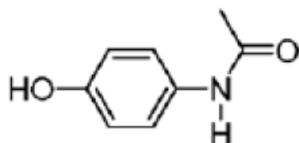
## Chemical Name

N-acetyl-p-aminophenol;  
4'-hydroxyacetanilide;  
p-hydroxyacetanilide;  
p-acetamidophenol;  
p-acetaminophenol;  
p-acetylaminophenol

## Molecular Formula/Molecular Weight

C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub> / 151.16 g/mol

## Structure



## Pharmacologic Class

There is no FDA-established pharmacological class for acetaminophen. We recommend not including an established class given the lack of clear understanding of the mechanism of action of acetaminophen.

## 2.2 Relevant INDs, NDAs, BLAs and MFs

NDA#	Drug Name	Div	Strength (route)	Marketing Status	AP Date	Indication	Company
22450	OFIRMEV (acetaminophen for injection)	DAAAP	1000 mg/100 mL (10 mg/mL) (IV infusion)	Prescription	November 2, 2010	Management of mild to moderate pain, management of moderate to severe pain with adjunctive opioid analgesics, reduction of fever	Cadence Pharmaceuticals, Inc.

The Applicant did not open an IND or preIND to support this NDA.

MF#	Subject of MF	Holder	Submit Date	Reviewer's Comment
		(b) (4)	October 14, 2003	Acceptable to support numerous NDAs and ANDAs
26696	FREEFLEX® packaging system (b) (4) as manufactured in Halden, Norway for Fresenius Kabi Deutschland GmbH	Fresenius Kabi Deutschland GmbH	January 22, 2013	The MF contains inadequate safety justification for some identified leachable compounds.

### 2.3 Drug Formulation

The following table represents the drug formulation for IV acetaminophen (from the Applicant's submission):

Ingredient	Function	Content (per mL)	Content (at MDD of 4 g/day)
Acetaminophen	therapeutic agent	10.0 mg	4000 mg
Mannitol	(b) (4)	36.7 mg	14,680 mg
Cysteine	(b) (4)	0.10 mg	40 mg

It is noted that the levels of mannitol and cysteine in this formulation would result in (b) (4) than in the reference OFIRMEV at the maximum daily dose of 4 g/day of acetaminophen. The following table illustrates the differences between this formulation and OFIRMEV (from the Applicant's submission):

Excipients	mg per vial/bag		Functionality
	RLD (Ofirmev™)	Fresenius Kabi's Acetaminophen Injection <sup>1</sup>	
Mannitol USP	3850	3670	(b) (4)
Cysteine (b) (4)	-	10	(b) (4)
Cysteine Hydrochloride (monohydrate), USP	25	-	(b) (4)
Dibasic Sodium Phosphate (b) (4) USP	10.4	-	(b) (4)

### 2.4 Comments on Novel Excipients

As shown in the table above, there are no novel excipients in this IV formulation of acetaminophen. The excipients in this formulation are mannitol, cysteine (b) (4). The amounts of mannitol and cysteine in this IV APAP formulation (b) (4) reference product OFIRMEV.

## 2.5 Comments on Impurities/Degradants of Concern

### Drug Substance

Information related to the drug substance information is contained in MF (b) (4) (b) (4). The following table represents the specification for the drug substance (from the Applicant's submission):

Impurities	Acceptance Criteria
(b) (4)	

The drug substance specifications for (b) (4) are the same as the FDA-approved reference product OFIRMEV. The drug substance specifications are acceptable.

### Residual Solvents

(b) (4) of acetaminophen production. Residual solvents, including (b) (4) are not listed in the drug substance specification from (b) (4) as none were present at the limit of detection (LOD) of 0.5%. Thus, there are no safety issues with the residual solvents in the drug substance.

### Drug Product

The following table represents the specification for the drug product (from the Applicant's submission):

Degradant	Acceptance Criteria
(b) (4)	(b) (4) % release (b) (4) % shelf life
Any Other Individual Impurity	(b) (4) %
Total Impurities	(b) (4) % release (b) (4) % shelf life

The drug product specifications for (b) (4) are lower than or equivalent to the FDA-approved reference product OFIRMEV. The specifications are acceptable.





(b) (4)

**Container Closure System**

Additional information related to the container closure system, including the Applicant's toxicological risk assessments for actual and potential leachables, can be found in MF 26696 for the freeflex® (b) (4). The Applicant will use the 100 mL (b) (4) filled with (b) (4) 100 mL IV acetaminophen formulation as illustrated in the table below (from the Applicant's submission):

<b>Packaging Configuration</b>	(b) (4)
	100 mL in 100-mL freeflex® (b) (4) code 434100)
(b) (4)	

The Applicant has submitted migration studies using various solutions, time points, and storage conditions, to identify leachables in the MF submission (see nonclinical review of MF 26696 for the safety evaluation of the identified leachables). The MF does not contain leachable studies for this specific drug product. In the NDA, the Applicant has submitted results of ongoing migration studies under various storage conditions and time points using this IV acetaminophen drug product formulation as illustrated in the table below in this NDA submission (from the Applicant's submission):

**Table 3.2.P.2- 16 Scheme for the storage conditions and testing frequency**

Storage Condition	Months							
	0	3	6	9	12	18	24	36
25 °C ± 2 °C / 40 % ± 5 % RH		/	X	/	/	/	X	/
30 °C ± 2 °C / 35 % ± 5 % RH		/	X	/	X	/	/	/
40 °C ± 2 °C / ≤ 25 % RH		/	X	/	/	/	/	/

The Applicant has submitted 6-month and 12-month results of these migration studies in the tables below (from the Applicant's submission):

Product: Acetaminophen Injection 10 mg/ mL Solution for Infusion  
 Batch: 12EGU95  
 Container: 100 mL free/flex<sup>®</sup> (b) (4)  
 Fill volume: (b) (4)  
 Film: (b) (4)  
 Storage condition: 40 °C ± 2 °C/ NMT (b) (4) % RH

**Table 3.2.P.2- 17 Batch 12EGU95, 40 °C**

Target	Method	Unit	Months		
			0	3	6
(b) (4)	AP-829	µg/L	NT	NT	(b) (4)
	AP-829	µg/L	NT	NT	
	AP-829	µmol/L	NT	NT	
	AP-829	µg/L	NT	NT	
	AP-822	µg/L	NT	NT	
	AP-822	µg/L	NT	NT	
	AP-808	µg/L	NT	NT	

NT: not tested

Storage condition: 25 °C ± 2 °C/40 % RH ± 5 %

**Table 3.2.P.2- 18 Batch 12EGU95, 25 °C**

Target	Method	Unit	Months		
			0	6	24
(b) (4)	AP-829	µg/L	NT	(b) (4)	P
	AP-829	µg/L	NT		P
	AP-829	µmol/L	NT		P
	AP-829	µg/L	NT		P
	AP-822	µg/L	NT		P
	AP-822	µg/L	NT		P
	AP-808	µg/L	NT		P

NT: not tested

P: Planned

Storage condition: 30 °C ± 2 °C/35 % RH ± 5 %

**Table 3.2.P.2- 19 Batch 12EGU95, 30 °C**

Target	Method	Unit	Months		
			0	6	12
(b) (4)	AP-829	µg/L	NT	(b) (4)	(b) (4)
	AP-829	µg/L	NT		
	AP-829	µmol/L	NT		
	AP-829	µg/L	NT		
	AP-822	µg/L	NT		
	AP-822	µg/L	NT		
	AP-808	µg/L	NT		

NT: not tested

The following table illustrates the highest levels of each identified leachable from these migration studies in the above three stability batches of this IV acetaminophen drug product formulation:

Leachable	Maximum Amount in Migration Studies*	Maximum TDI**
(b) (4)		

\* Maximum levels identified from the migration studies using this IV APAP formulation.

\*\* Maximum TDI (total daily intake) calculation is based on the MDD of APAP (4 g/day), which is obtained from (b) (4) via this IV APAP formulation.

Only one of the three batches contains both 6-month and 12-month data. Based on the data submitted to date, there does not appear to be an appreciable increase of these leachables over time. However, final determination will require additional stability data from multiple batches and longer storage periods (see CMC review for further discussion).

For the purposes of the risk assessment of these leachables based on the limited data obtained to date, the maximum total daily intake values in the table above were calculated from the highest concentration of a specific leachable detected in the three batches to date multiplied by the maximum volume of (b) (4)

mL) using either formulation. This risk assessment will have to be revised upon submission of adequate stability data as per the CMC review team.

(b) (4)

As per the nonclinical review for MF 26696 (see nonclinical review), the maximum TDI of all identified leachables from the migration studies using this IV APAP formulation yields an adequate safety margin except for (b) (4). The adequacy of the safety justification of (b) (4) will be addressed below as part of a review for a 3-month oral toxicity study in rats using (b) (4).

The weight of evidence approach for (b) (4) that was presented in MF 26696 is not adequate to justify the safety of the levels of (b) (4) detected in migration studies with this IV APAP formulation at the MDD of APAP of 4 g/day. Thus, a 28-day IV toxicity study with (b) (4) is needed to justify the safety of this leachable or the Applicant must provide adequate data to conclude that the (b) (4) virtually instantly such that the risk assessment based exclusively on the metabolites could be deemed adequate.

## 2.6 Proposed Clinical Population and Dosing Regimen

The indication is the management of mild to moderate pain, management of moderate to severe pain with adjunctive opioid analgesics, and reduction of fever. Due to the route of administration, this is not considered a chronic indication. As this product is administered in a hospital setting, the typical duration of use would be < 14 days.

## 2.7 Regulatory Background

This is a 505(b)(2) application referencing the Agency's previous findings of safety to OFIRMEV (NDA 22450).

The Agency communicated with the Applicant to address potential deficiencies identified in the filing communication (see filing communication dated December 4, 2012). According to the filing communication, the following potential approval issues were noted:

1. Provide osmolalities of your proposed product and the referenced drug to ensure that the change in mannitol composition in the proposed product formulation with respect to the referenced drug has no significant impact on relative bioavailability. If the solution is not isotonic, provide justification for why any differences in osmolality between your drug product and the referenced drug product do not represent a safety concern.

2. The degradant (b) (4), which is also called (b) (4) in the drug product specification should be reduced to as low as technically feasible based on manufacturing capability.
3. Based on preliminary review of the NDA, it appears as though there are inadequate safety justifications for the systemic levels of all leachables from the container closure system for the intravenous route of administration. Specifically, there does not appear to be adequate safety justification for the following three identified leachables: (b) (4). Unless adequately justified otherwise, the IV safety of these three identified leachables from the container closure system should be qualified via an IV toxicology study that provides an adequate safety margin for the levels of these identified leachables that a person would be exposed to when treated with up to 4 grams of acetaminophen per day via this drug product formulation. The duration of such a toxicology study should be comparable to the predicted maximum duration proposed in your drug product labeling (i.e., up to 14 days duration).

The osmolalities of this IV acetaminophen drug product compared to the reference OFIRMEV were submitted and are reviewed in the "Special Toxicology Studies" section below.

The adequacy the level of (b) (4) in the drug product specification has been addressed in the "Comments on Impurities/Degradants of Concern" section above.

The Applicant has submitted a 3-month oral toxicity study of (b) (4) in rats in support of the level of (b) (4) found in the freeflex® (b) (4) and is reviewed in the "Repeat-Dose Toxicity" section below. The Applicant will also perform the Ames test using (b) (4). Based on QSAR analysis, there are no structural alerts for genotoxicity. The proposed Ames test will not be required for approval.

To address the safety of (b) (4) found in the freeflex® (b) (4) the Applicant has submitted a protocol for a preliminary toxicity study in rats and a 4-week intravenous toxicity study in rats, both using (b) (4). The preliminary toxicity study in rats appears to be a standard dose-range finding study to determine doses for a definitive toxicity study. The 4-week intravenous toxicity study in rats appears to be a standard toxicity study with appropriate endpoints which includes general histopathology of an adequate battery of tissues and histopathology at the injection site. The Applicant will also perform the Ames test using (b) (4). In the absence of final study reports for both the 4-week IV toxicity study in rats and Ames test, the safety of (b) (4) still needs to be justified in this drug product. Based on QSAR analysis, there do not appear to be any structural alerts for genotoxicity. Given the maximum daily dose of this leachable of (b) (4) mcg, an Ames assay is not required for approval.

To address the safety of (b) (4) (and related substances) found in the freeflex® (b) (4) the Applicant provided data from the ongoing migration (leachables)

studies using this IV acetaminophen drug product of up to 12 months that indicates that (b) (4) (and related substances) has not been detected as a leachable compound to date. The detection limit for (b) (4) (and related substances) is (b) (4). At the MDD of 4 g/day of acetaminophen, the maximum level of (b) (4) (and related substances) would be below (b) (4) mcg for the 100 mL fill (b) (4) below the toxicological limit for an unqualified genotoxic impurity of NMT 1.5 mcg/day. Thus, the level of (b) (4) (and related substances) found in the freeflex® (b) (4) do not represent a safety concern as long as this leachable is not detected in ongoing migration studies.

### 3 Studies Submitted

#### 3.1 Studies Reviewed

Study Number 8E CAP-0024: (b) (4): 3 month oral toxicity study in rats (b) (4)

#### 3.2 Studies Not Reviewed

There were no other studies submitted in this NDA.

#### 3.3 Previous Reviews Referenced

There were no previous reviews referenced.

### 4 Pharmacology

#### 4.1 Primary Pharmacology

There were no primary pharmacology studies with IV acetaminophen submitted in this NDA.

#### 4.2 Secondary Pharmacology

There were no secondary pharmacology studies with IV acetaminophen submitted in this NDA.

#### 4.3 Safety Pharmacology

There were no safety pharmacology studies with IV acetaminophen submitted in this NDA.

## 5 Pharmacokinetics/ADME/Toxicokinetics

### 5.1 PK/ADME

There were no PK/ADME studies with IV acetaminophen submitted in this NDA.

### 5.2 Toxicokinetics

There were no general toxicology studies with IV acetaminophen submitted in this NDA. There were no separate toxicokinetics studies done with IV acetaminophen submitted in this NDA.

## 6 General Toxicology

There were no general toxicology studies with IV acetaminophen submitted in this NDA.

The Applicant submitted a final report for a 3 month oral toxicity study with the leachable (b) (4) that was conducted by (b) (4). This study was submitted to justify the safety of the levels of (b) (4) found in the Freeflex® (b) (4)

### 6.2 Repeat-Dose Toxicity

**Study title:** (b) (4): **3 month oral toxicity study in rats**

Study no.: 8E CAP-0024  
(GU project # 810895)

Study report location: SDN 9

Conducting laboratory and location: (b) (4)

Date of study initiation: May 12, 1982

GLP compliance: This is an audited final study report that states that it was but conducted in accordance with OECD GLPs.

QA statement: Yes. Signature provided on July 22, 1983.

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

### Key Study Findings

- SPF rats were dosed 0, 3, 10, 30, and 100 mg/kg of (b) (4) for 3 months via oral gavage.
- There were no clear treatment related deaths in the study. One female from the 100 mg/kg dose group died prematurely; however, there was no clear cause of death.
- Minor changes in clinical chemistry were noted; however, none of these changes were deemed to be of biological significance.

- An increase in liver weight, hepatocyte hypertrophy and relatively minor elevations in liver transaminases were noted in the 30 and 100 mg dose groups.
- Necrosis was noted in the liver of one male 100 mg/kg dose group. Cholangiofibrosis of the intrahepatic bile duct was observed in one male each treated with 30 and 100 mg/kg dose group male and one female treated with 100 mg/kg.
- Histopathologic changes in the lung were noted; however, these were likely due to the gavage process.
- The Applicant had determined a no observed effects level (NOEL) of 3 mg/kg. We concur with this assessment.
- The NOAEL is 10 mg/kg based on changes in the liver at 30 and 100 mg/kg and foamy cell accumulation in the alveoli of the lungs at 100 mg/kg.
- The rat NOAEL of 10 mg/kg supports a human equivalent dose (HED) of (b) (4) mg of (b) (4). Since this toxicity study used oral administration and this IV APAP formulation is for intravenous administration, an additional safety factor of 10 may be used. The HED is then reduced to (b) (4) mg of (b) (4). The levels (b) (4) found in migration studies using this IV acetaminophen drug product formulation were (b) (4) mcg at the MDD of 4 g/day of APAP, which represents a safety margin of 363.
- Based on the levels of (b) (4) that were noted in MF 26696 (see nonclinical review) and on migration studies using this IV acetaminophen drug product formulation, there is an adequate safety margin based on the NOAEL from this rat study to support the levels of (b) (4) in the Freeflex® (b) (4) to be used with this IV acetaminophen drug product.

## Methods

Doses: 0, 3.0, 10, 30, and 100 mg/kg  
Frequency of dosing: Once daily, 7 times a week, for 3 months  
Route of administration: Oral gavage  
Dose volume: 10 mL/kg  
Formulation/Vehicle: Carboxymethyl-cellulose (0.5%) and Tween 80 (0.1%)  
Species/Strain: Rat, Tif; RAIf (SPF)  
Number/Sex/Group: 20/sex/group  
Age: Approximately 6 weeks  
Weight: 165-169 g males; 135-141 g females  
Satellite groups: None  
Unique study design: Addition parameters were measured including hearing test, water consumption, and food conversion. Urinalysis was not specifically measured. There were no toxicokinetics performed in this study. There was 20/sex/group.  
Deviations from study protocol: The animals were transferred to a new pathogen free facility during the course of the study. The transfer took place under close supervision of the quality assurance unit on July 8, 1982 (Week 7 of the study).  
Necropsy was performed between Day 92 to Day 96 during Week 14 of the study. Dosing continued in all study animals until the day prior to sacrifice.  
There were slightly higher temperatures than specified in the protocol. Temperatures of 23 to 24°C on Days 52, 55-62, 64-74, 76-77, 80-82, 86, 90, 92, and 94-95 were reported during the study.  
The relative humidity was above the specified range in the protocol. The relative humidity was 74% during Day 60 of the study.  
These deviations were considered to have no impact on the validity of the study.

## Observations and Results

### Mortality

Mortality was observed daily. A total of three animals died during the test period. A male from the 100 mg/kg dose group died on Day 62 of treatment due to gavage error (lung edema). A female from the 100 mg/kg dose group died on Day 82 with no macroscopic findings. A male from the 10 mg/kg dose group died on Day 88 due to gavage error (perforation of the esophagus). All mortalities were considered accidental;

however, the 1 female death from the 100 mg/kg dose group was not stated as caused by gavage error as there were no macroscopic findings.

### **Clinical Signs**

Clinical signs were observed daily. No clinical symptoms and no signs of local and/or systemic toxicity were observed.

### **Body Weights**

Body weights were measured and recorded weekly. There were no treatment-related changes in body weights.

### **Food Consumption**

Food consumption, water consumption, and food conversion were measured and recorded weekly. There were no treatment-related changes in food consumption, water consumption, and food conversion.

### **Ophthalmoscopy**

Eye examination was performed at the beginning and towards the end of the treatment period. There were no treatment-related changes noted during the eye examination.

### **ECG**

None.

### **Hematology**

Blood was collected for hematology, coagulation, and clinical chemistry at the end of the treatment period. Food was withheld for 20 hours prior to blood collection. Out of the 20/sex/group, only 10 animals were used for hematology, coagulation, and clinical chemistry.

The following parameters were measured for hematology and coagulation (from the Sponsor's submission):

## PARAMETERS USED IN HAEMATOLOGY

PARAMETER	METHOD	SI-UNITS
ERYTHROCYTES (RBC)	Estimated by Coulter Counter S-Plus	T/l
MEAN CORPUSCULAR VOLUME (MCV)	Estimated by Coulter Counter S-Plus	f/l
HAEMATOCRIT (PCV)	Estimated by Coulter Counter S-Plus	l
HAEMOGLOBIN (Hb)	Cyanmethaemoglobin Method estimated by Coulter Counter S-Plus	mmol/l
MEAN CORPUSCULAR HAEMOGLOBIN (MCH)	Estimated by Coulter Counter S-Plus	fmol
THROMBOCYTES (PLT) (Platelet count)	Estimated by Coulter Counter S-Plus	G/l
LEUCOCYTE COUNT (WBC)		
TOTAL COUNT	Estimated by Coulter Counter S-Plus	G/l
DIFFERENTIAL COUNT	Blood smear stained with a modified polychrome methylene blue (Ames, Hema-Tek Stain-Pak) using the Ames Hema-Tek Slide Stainer  Counting by means of the Honeywell ACS 1000  Meta-Myel. = Metamyelocytes Band = Band Neutrophils Seg = Segmented Neutrophils	Rel.: l Abs.: G/l

	<b>Eo</b>	= Eosinophils	
	<b>Ba</b>	= Basophils	
	<b>Mo</b>	= Monocytes	
	<b>Ly</b>	= Lymphocytes	
	<b>Pl</b>	= Plasma Cells	
	<b>NEN</b>	= Nucl. RBC-Normo- blasts	& WBC
	<b>NEE</b>	= Nucl. RBC-Erythro- blasts	& WBC
<b>RETICULOCYTES</b>		Supravital staining with brilliant cresyl blue	1
<b>PROTHROMBIN TIME (PT)</b>		Quick's one-stage method using Microcoagulometer Greiner Electronics, Plasma and Activated Thromboplastin (Dade)	sec

Changes in hematology and coagulation are noted in the clinical chemistry section below.

### Clinical Chemistry

The following parameters were measured for clinical chemistry (from the Sponsor's submission):

**PARAMETERS USED IN BLOOD CHEMISTRY**

<b>PARAMETER</b>	<b>METHOD</b>	<b>SI-UNITS</b>
Glucose	(b) (4)	mmol/l
Urea (Urea-N)	(b) (4)	mmol/l
Total Bilirubin	(b) (4)	umol/l
Creatinine	(b) (4)	umol/l
Total Protein	(b) (4)	g/l
Albumin	(b) (4)	g/l
Globulin	Calculated value: (Total Protein-Albumin)	g/l

<b>A/G Ratio</b>	<b>Calculated Value: Albumin/Globulin</b>	<b>1</b>
<b>Sodium (Na)</b>		<sup>(b) (4)</sup> mmol/l
<b>Potassium (K)</b>		mmol/l
<b>Chloride (Cl)</b>		mmol/l
<b>Calcium (Ca)</b>		mmol/l
<b>Phosphorus Inorganic</b>		mmol/l
<b>Asp. Aminotransferase (GOT) EC 2.6.1.1.</b>		U/l
<b>Ala. Aminotransferase (GPT) EC 2.6.1.2.</b>		U/l
<b>Alkaline Phosphatase (AP) EC 3.1.3.1.</b>		U/l
<b>Gamma-Glutamyl Transpeptidase (GGT) EC 2.3.2.2.</b>		U/l

(b) (4)

Total cholesterol

mmol/l

The following table illustrates the changes in hematology, coagulation, and clinical chemistry at Week 14 in males and females (from the Sponsor's submission):

SEX : MALE COMPOUND

PARAMETER	TEST WEEK 14										TREND										
	DOSE IN MG/KG BW/DAY																				
	0.000		3.000		10.000		30.000		100.000												
	NO	MEAN	NO	MEAN	NO	MEAN	NO	MEAN	NO	MEAN											
-- HAEMATOLOGY --																					
ERYTHROCYTES	T/L	10	9.0	10	9.1	10	8.9	10	9.1	10	8.5										
HAEMOGLOBIN	MMOL/L	10	10.0	10	10.2	10	10.0	10	10.0	10	9.7	*									
HAEMATOCRIT	1	10	0.46	10	0.46	10	0.45	10	0.45	10	0.44										
RETICULOCYTES	1	10	3.720	10	3.600	10	3.260	9	3.600	9	3.820										
MEAN CORP. VOLUME (MCV)	FL	10	51	10	51	10	51	10	50	10	52										
MEAN CORP. HB (MCH)	FMOL	10	1.12	10	1.12	10	1.12	10	1.11	10	1.14										
LEUKOCYTES	G/L	10	7.4	10	9.5	10	9.2	10	9.5	10	8.3										
DIFFERENTIAL BLOOD COUNT - REL.																					
METAMYELOCYTES	1	10	0.00	10	0.00	10	0.00	10	0.00	10	0.00										
BAND NEUTROPHILS	1	10	0.00	10	0.00	10	0.00	10	0.00	10	0.00										
SEGMENTED NEUTROPHILS	1	10	0.13	10	0.15	10	0.16	10	0.12	10	0.12										
EOSINOPHILS	1	10	0.01	10	0.01	10	0.02	10	0.02	10	0.02										
BAZOPHILS	1	10	0.00	10	0.00	10	0.00	10	0.00	10	0.00										
MONOCYTES	1	10	0.01	10	0.01	10	0.02	10	0.01	10	0.01										
LYMPHOCYTES	1	10	0.85	10	0.83	10	0.81	10	0.85	10	0.85										
PLASMA CELLS	1	10	0.00	10	0.00	10	0.00	10	0.00	10	0.00										
OTHER CELLS	1	10	0.00	10	0.00	10	0.00	10	0.00	10	0.00										
NUCL. RBC-NORMOBLASTS	X MBC	10	0.00	10	0.00	10	0.10	10	0.10	10	0.00										
THROMBOCYTES	G/L	10	639	10	715	10	744	10	950	10	821	*									
COAGULATION																					
PROTHROMBIN TIME	SEC	10	15.3	9	15.0	10	15.1	10	15.6	10	14.7										
-- BLOOD CHEMISTRY																					
ALK. PHOSPHATASE	U/L	10	214.3	10	222.9	10	244.2	10	239.6	10	268.3										
ALA. AMINOTRANSF.(GPT)	U/L	10	35.4	10	34.4	10	38.3	10	40.2	10	46.7	* -->									
ASP. AMINOTRANSF.(GOT)	U/L	10	56.2	10	53.2	10	54.6	10	59.1	10	50.5										
G-GLUTAMYL TRANSPEPT.	U/L	10	0.9	10	1.7	10	1.3	10	1.6	10	1.5	* -->									
SODIUM	MMOL/L	10	140	10	141	10	142	10	143	10	142	* -->									
POTASSIUM	MMOL/L	10	3.80	10	3.98	10	4.02	10	4.07	10	4.03										
CHLORIDE	MMOL/L	10	100.9	10	100.6	10	98.9	9	98.2	10	99.9										
CALCIUM	MMOL/L	10	2.44	10	2.57	10	2.50	9	2.62	10	2.39										

NO = NO. OF VALUES / GROUP  
 \* = SIGN. DIFFERENCE (LOCATION AND/OR DISPERSION) BETWEEN CONTROL (GROUP 1) AND GROUP X (SIGN.L.= 0.050)  
 --> = SIGN. POS. TREND FROM CONTROL TO HIGHEST DOSAGE-GROUP (SIGN.L.= 0.010)

SEX : MALE

COMPOUND

TEST WEEK 14

PARAMETER		DOSE IN MG/KG BW/DAY										TREND
		0.000		3.000		10.000		30.000		100.000		
		NO	MEAN	NO	MEAN	NO	MEAN	NO	MEAN	NO	MEAN	
PHOSPHATE INORG.	MMOL/L	10	1.67	10	1.94 #	10	1.90	10	2.11 #	10	1.58	
GLUCOSE	MMOL/L	10	6.6	10	6.4	10	6.2	10	6.2 #	10	6.0	
UREA-N	MMOL/L	10	6.0	10	5.0	10	6.0	10	5.4	10	5.9	
CREATININE	MMOL/L	10	44	10	45	10	45	9	48	10	49 #	-->
BILIRUBIN	MMOL/L	10	1.4	10	1.6 #	10	1.7	10	1.2	10	0.0	
CHOLESTEROL	MMOL/L	10	2.0	10	2.1	10	2.3	0	2.5 #	10	2.5 #	-->
TOTAL PROTEINS	G/L	10	62.1	10	63.0	10	63.6	10	66.5 #	10	67.5 #	-->
ALBUMIN	G/L	10	33.7	10	34.6	10	33.9	10	34.0	10	34.6	
TOTAL GLOBULIN	G/L	10	28.4	10	29.2	10	29.7	10	31.8 #	10	32.0 #	-->
A/G RATIO		10	1.10	10	1.19	10	1.15	10	1.09 #	10	1.05 #	<--

NO = NO. OF VALUES / GROUP  
 # = SIGN. DIFFERENCE (LOCATION AND/OR DISPERSION) BETWEEN CONTROL (GROUP 1) AND GROUP X (SIGN.L. = 0.050)  
 --> = SIGN. POS. TREND FROM CONTROL TO HIGHEST DOSAGE-GROUP (SIGN.L. = 0.010)  
 <-- = SIGN. NEG. TREND FROM CONTROL TO HIGHEST DOSAGE-GROUP (SIGN.L. = 0.010)

SEX : FEMALE

COMPOUND

TEST WEEK 14

PARAMETER		DOSE IN MG/KG BW/DAY										TREND
		0.000		3.000		10.000		30.000		100.000		
		NO	MEAN	NO	MEAN	NO	MEAN	NO	MEAN	NO	MEAN	
-- HAEMATOLOGY --												
ERYTHROCYTES	T/L	10	0.0	10	0.3	10	0.3	10	0.3	10	7.9	
HAEMOGLOBIN	MMOL/L	10	9.2	10	9.6	10	9.7 #	10	9.6 #	10	9.2	
HAEMATOCRIT	1	10	0.42	10	0.43	10	0.43	10	0.43	10	0.41	
RETICULOCYTES	1	10	3.620	10	3.450 #	10	3.500	10	3.620	10	3.640	
MEAN CORP. VOLUME (MCV)	FL	10	52	10	52	10	52	10	52	10	52	
MEAN CORP. WB (MCH)	MMOL	10	1.15	10	1.15	10	1.10	10	1.15	10	1.17	
LEUKOCYTES	G/L	10	5.5	10	7.2 #	10	6.6	10	6.7	10	4.0	
DIFFERENTIAL BLOOD COUNT - REL.												
RETAYELOCYTES	1	10	0.00	10	0.00	10	0.00	10	0.00	10	0.00	
BAND NEUTROPHILS	1	10	0.00	10	0.00 #	10	0.00 #	10	0.00	10	0.00 #	
SEGMENTED NEUTROPHILS	1	10	0.22	10	0.20	10	0.15	10	0.19	10	0.16	
EOSINOPHILS	1	10	0.01	10	0.01	10	0.01	10	0.01	10	0.01	
BASOPHILS	1	10	0.00	10	0.00	10	0.00	10	0.00	10	0.00	
MONOCYTES	1	10	0.01	10	0.01	10	0.01	10	0.01	10	0.01	
LYMPHOCTES	1	10	0.75	10	0.77	10	0.03	10	0.79	10	0.02	
PLASMA CELLS	1	10	0.00	10	0.00	10	0.00	10	0.00	10	0.00	
OTHER CELLS	1	10	0.00	10	0.00	10	0.00	10	0.00	10	0.00	
NUCL. RBC-NORMOBLASTS % WBC		10	0.00	10	0.00	10	0.20	10	0.20	10	0.10	
THROMBOCYTES	G/L	10	766	10	917	10	1059 #	10	896	10	070	
COAGULATION PROTHROMBIN TIME	SEC	10	14.4	10	15.9 #	10	15.5 #	10	15.5 #	10	15.1	
-- BLOOD CHEMISTRY												
ALK. PHOSPHATASE	U/L	10	157.1	10	129.7	10	159.3	10	151.0	10	220.5 #	-->
ALA. AMINOTRANSF.(GPT)	U/L	10	37.3	10	41.7	10	47.3	10	03.3	10	47.7	
ASP. AMINOTRANSF.(GOT)	U/L	10	50.5	10	55.6	10	59.3	10	100.9	10	40.7	
G-GLUTAMYL TRANSPEPT.	U/L	10	1.5	10	1.2	10	1.9 #	10	1.0	10	1.7	
SODIUM	MMOL/L	10	140	10	143 #	10	144 #	10	142 #	10	141	
POTASSIUM	MMOL/L	10	3.62	10	3.04	10	3.98	10	3.93	10	3.36	
CHLORIDE	MMOL/L	10	100.0	10	98.0 #	10	100.0	10	100.2	10	99.3	
CALCIUM	MMOL/L	10	2.45	10	2.50	10	2.62 #	10	2.54 #	10	2.57 #	-->

NO = NO. OF VALUES / GROUP  
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 --> = SIGN. POS. TREND FROM CONTROL TO HIGHEST DOSAGE-GROUP (SIGN.L. = 0.010)

SEX : FEMALE

COMPOUND

		TEST WEEK 14										
PARAMETER		DOSE IN MG/KG BW/DAY										TREND
		0.000		3.000		10.000		30.000		100.000		
		NO	MEAN	NO	MEAN	NO	MEAN	NO	MEAN	NO	MEAN	
PHOSPHATE INORG.	MMOL/L	10	1.46	10	1.62 *	10	1.65 *	10	1.55	10	1.54	
GLUCOSE	MMOL/L	10	7.6	10	6.3 *	10	6.2 *	10	6.0 *	10	6.1 *	<--
UREA-N	MMOL/L	10	5.6	10	4.9 *	10	4.2 *	10	4.5 *	10	4.7 *	<--
CREATININE	MMOL/L	10	52	10	47 *	10	50	10	51	10	51	
BILIRUBIN	MMOL/L	10	1.3	10	1.0	10	0.9	10	1.1	10	1.7	-->
CHOLESTEROL	MMOL/L	10	1.7	10	1.7	10	1.0	10	2.0	10	1.9	
TOTAL PROTEINS	G/L	10	65.6	10	66.0	10	67.3	10	67.4	10	68.8 *	-->
ALBUMIN	G/L	10	39.0	10	38.3	10	38.5	10	38.5	10	38.3	
TOTAL GLOBULIN	G/L	10	26.7	10	27.8	10	28.9 *	10	28.9 *	10	30.5 *	-->
A/G RATIO		10	1.46	10	1.38	10	1.34 *	10	1.34 *	10	1.26 *	<--

NO = NO. OF VALUES / GROUP  
 \* = SIGN. DIFFERENCE (LOCATION AND/OR DISPERSION) BETWEEN CONTROL (GROUP 1) AND GROUP X (SIGN.L. = 0.050)  
 --> = SIGN. POS. TREND FROM CONTROL TO HIGHEST DOSAGE-GROUP (SIGN.L. = 0.010)  
 <-- = SIGN. NEG. TREND FROM CONTROL TO HIGHEST DOSAGE-GROUP (SIGN.L. = 0.010)

As shown in the tables above, several changes were observed in hematology, coagulation, and clinical chemistry in males and females at Week 14.

In males, hemoglobin was decreased by 3% in 100 mg/kg dose group compared to control. Thrombocytes were increased by 11.9, 48.7, and 28.5% in the 3, 30, and 10 mg/kg male dose groups, respectively, compared to control. The changes observed in thrombocytes did not occur in a dose-dependent manner and thus do not represent a safety concern. The levels of alanine aminotransferase were increased by 31.9% in the 100 mg/kg male dose group compared to control. The levels of gamma-glutamyl transpeptidase were increased by 88.9, 44.4, 11.1, and 66.7% in the 3, 10, 30, and 300 mg/kg male dose groups, respectively, compared to control. Although the changes observed in the levels of gamma-glutamyl transpeptidase did not occur in a dose-dependent manner, they may be indicative of changes in liver weight and histopathology at higher doses (hepatocellular hypertrophy; see organ weight and histopathology sections). Creatinine was increased by 11.4% in the 100 mg/kg male dose group compared to control. Cholesterol was increased by 25% in both the 30 and 100 mg/kg male dose groups compared to control. There is no clear dose-dependency to the increase in cholesterol and thus does not represent a safety concern. Total proteins were increased by 7.1 and 8.7% in the 30 and 100 mg/kg male dose groups, respectively, compared to control. Total globulin was increased by 12.0 and 15.5% in the 30 and 100 mg/kg male dose groups, respectively, compared to control. The albumin to globulin ratio (A/G ratio) was decreased by 7.6 and 10.2% in the 30 and 100 mg/kg male dose groups. There were no other treatment-related changes in hematology, coagulation, and clinical chemistry in the males. None of these changes are believed to have biological significance.

In females, band neutrophils were noted as having a statistically significant change in the 3, 10, and 100 mg/kg dose groups; however, the mean values in each of those groups were the same as control. As such, the finding in band neutrophils is dismissed.

The levels of alkaline phosphatase were increased by 45.4% in the 100 mg/kg female dose group compared to control. Calcium was increased by 6.9, 3.7, and 4.8% in the 10, 30, and 100 mg/kg female dose groups, respectively, compared to control. The changes observed in calcium did not occur in a dose-dependent manner and thus do not represent a safety concern. Glucose was decreased by 17.1, 18.4, 21.1, and 19.7% in the 3, 10, 30, and 100 mg/kg female dose groups, respectively, compared to control. The changes observed in glucose did not occur in an overt dose-dependent manner and thus do not represent a safety concern. Urea was decreased by 12.5, 25.0, 19.6, and 16.1% in the 3, 10, 30, and 100 mg/kg female dose groups, respectively, compared to control. The changes observed in urea did not occur in a dose-dependent manner and thus do not represent a safety concern. Total globulin was increased by 8.2, 8.2, and 14.2% in the 10, 30, and 100 mg/kg female dose groups, respectively, compared to control. The changes observed in total globulin did not occur in an overt dose-dependent manner and thus do not represent a safety concern. The albumin to globulin ratio (A/G ratio) was decreased by 12.0, 12.0, and 13.7% in the 10, 30, and 100 mg/kg female dose groups, respectively, compared to control. The changes observed in the albumin to globulin ratio (A/G ratio) did not occur in an overt dose-dependent manner and thus do not represent a safety concern. There were no other treatment related changes in hematology, coagulation, and clinical chemistry in the females.

All changes in hematology, coagulation, and clinical chemistry are considered monitorable in a clinical setting and, based on the magnitude of the changes, do not appear to have biological significance.

### **Urinalysis**

Urine samples were not collected. It is noted that urea was measured in the blood.

### **Gross Pathology**

At the end of the treatment period, all treatment and control animals were sacrificed and necropsied.

The following table illustrates the macroscopic findings in the male and female rats after 3-months of exposure to (b) (4) (from the Sponsor's submission):

DATE: 19/07/83

TOX DISPO NO: 010895    COMPOUND: (b) (4)    DURATION: 3 MONTHS    PAGE: 1

SUMMARY OF MACROSCOPICAL FINDINGS

SPECIES: RAT

	0 MG/KG		3 MG/KG		10 MG/KG		30 MG/KG		100 MG/KG		H	F
	M	F	M	F	M	F	M	F	M	F		
ANIMALS EXAMINED MACROSCOPICALLY	20	20	20	20	20	20	20	20	20	20		
ABDOMINAL CAVITY SMALL MASS	---	1	---	---	---	---	---	---	---	1	---	---
AORTA NOT TAKEN	---	---	---	1	---	---	---	---	---	---	---	---
BACK INJURY DURING LIFE	---	---	---	---	---	---	1	---	---	---	---	---
BODY AS A WHOLE NO CHANGES OBSERVED	19	19	18	19	19	19	18	18	17	19	---	---
EPIDIDYMIS DAMAGED DURING AUTOPSY NODULE	1	---	---	---	---	---	---	---	1	---	---	---
EYE SMALL MASS	---	---	1	---	---	---	---	---	---	---	---	---
LUNG OEDEMA	---	---	---	---	---	---	---	---	1	---	---	---
ESOPHAGUS PERFORATION	---	---	---	1	---	---	---	---	---	---	---	---
TESTIS SMALL	1	---	1	---	---	---	2	---	1	---	---	---
THORACIC CAVITY CONTENTS PURULENT	---	---	---	---	1	---	---	---	---	---	---	---
THORACIC CAVITY FIBRINOUS ADHESION	---	---	---	---	1	---	---	---	---	---	---	---
URINARY BLADDER NOT TAKEN	---	---	---	---	---	1	---	---	---	---	---	---
UTERUS DAMAGED DURING AUTOPSY	---	---	---	---	---	---	---	1	---	---	---	---

As shown in the table above, there are a number macroscopic changes. There was a small mass in the abdominal cavity observed in 1/20 control females in 1/20 females in the 100 mg/kg dose group. Because the small mass in the abdominal cavity occurred with the same incidence in the 100 mg/kg female dose group as in the female control, this finding does not represent a safety concern. A nodule in the epididymis was observed in 1/20 males from the 100 mg/kg dose group. Oedema in the lung was observed in 1/20 males from the 100 mg/kg dose group. Oedema in the lung was attributed to error in the oral gavage method and thus dismissed. Small testis was observed in 1/20, 1/20, 2/20, and 1/20 males from the control, 3, 30, and 100 mg/kg dose group. There was no clear dose-dependency in this finding and thus does not represent a safety concern. There were no other treatment related changes in gross pathology.

### Organ Weights

Brain, heart, liver, kidneys, adrenals, thymus, and gonads were weighed. The following table illustrates the changes in organ weights, organ to body weight ratios, and the organ to brain weight ratios in males and females at Week 14 (from the Sponsor's submission):

SEX : MALE

TEST WEEK 14

COMPOUND : T

O R G A N S	DOSE IN MG/KG BW/DAY										TREND
	0.000		3.000		10.000		30.000		100.000		
	NO	MEAN	NO	MEAN	NO	MEAN	NO	MEAN	NO	MEAN	
+ BODY	20	455.544	20	448.604	19	463.772	20	474.484	19	451.415	
+ BRAIN	20	2.448	20	2.458	19	2.502	20	2.463	19	2.443	
BRAIN / BODY	20	0.544	20	0.551	19	0.546	20	0.521*	19	0.543	
+ HEART	20	1.344	20	1.303	19	1.323	20	1.349	19	1.378	
HEART / BODY	20	0.298	20	0.291	19	0.287	20	0.284	19	0.305	
HEART / BRAIN	20	54.952	20	52.953	19	52.861	20	54.772	19	56.507	
+ LIVER	20	15.091	20	14.895*	19	16.216*	20	19.170*	19	21.301*	-->
LIVER / BODY	20	3.303	20	3.317*	19	3.496*	20	4.047*	19	4.725*	-->
LIVER / BRAIN	20	616.824	20	605.978*	19	647.437*	20	778.738*	19	875.511*	-->
+ KIDNEYS	20	2.940	20	3.038	19	3.060	20	3.039	19	3.081	
KIDNEYS / BODY	20	0.650	20	0.678	19	0.662	20	0.642	19	0.684	
KIDNEYS / BRAIN	20	120.163	20	123.602	19	122.118	20	123.486	19	126.272	

NO = NO. OF VALUES / GROUP

\* = SIGN. DIFFERENCE (LOCATION AND/OR DISPERSION) BETWEEN CONTROL (GROUP 1) AND GROUP X (SIGN.L. = 0.050)

--> = SIGN. POS. TREND FROM CONTROL TO HIGHEST DOSAGE-GROUP (SIGN.L. = 0.010)

SEX : MALE

TEST WEEK 14

COMPOUND : T

O R G A N S	DOSE IN MG/KG BW/DAY										TREND
	0.000		3.000		10.000		30.000		100.000		
	NO	MEAN	NO	MEAN	NO	MEAN	NO	MEAN	NO	MEAN	
+ ADRENALS	20	0.071	20	0.073	19	0.071	20	0.066	19	0.071	
ADRENALS / BODY	20	0.0157	20	0.0161	19	0.0155	20	0.0140	19	0.0157	
ADRENALS / BRAIN	20	2.915	20	2.954	19	2.840	20	2.677	19	2.908	
+ THYMUS	20	0.382	20	0.384	19	0.434	20	0.417	19	0.402	
THYMUS / BODY	20	0.083	20	0.085	19	0.094	20	0.088	19	0.089	
THYMUS / BRAIN	20	15.566	20	15.625	19	17.291	20	16.953	19	16.500	
+ GONADES	20	3.952	20	3.773	19	4.121	20	3.919	19	3.737	
GONADES / BODY	20	0.880	20	0.844	19	0.899	20	0.828*	19	0.834	
GONADES / BRAIN	20	161.622	20	153.208	19	164.783	20	159.141	19	153.070	

NO = NO. OF VALUES / GROUP

\* = SIGN. DIFFERENCE (LOCATION AND/OR DISPERSION) BETWEEN CONTROL (GROUP 1) AND GROUP X (SIGN.L. = 0.050)

\*\*\* NO STATISTICALLY SIGN. TRENDS (SIGN.L. = 0.010)

SEX : FEMALE TEST WEEK 14 COMPOUND : T

ORGANS	DOSE IN MG/KG BW/DAY										TREND
	0.000		3.000		10.000		30.000		100.000		
	NO	MEAN	NO	MEAN	NO	MEAN	NO	MEAN	NO	MEAN	
+ BODY	20	279.060	20	274.810	20	273.859	20	270.020	19	276.078	
+ BRAIN	20	2.132	20	2.156	20	2.193*	20	2.175	19	2.183*	
BRAIN / BODY	20	0.769	20	0.790	20	0.808	20	0.811	19	0.794	
+ HEART	20	0.857	20	0.820	20	0.833	20	0.809	19	0.830	
HEART / BODY	20	0.307	20	0.300	20	0.306	20	0.300	19	0.302	
HEART / BRAIN	20	40.217	20	38.174	20	38.021	20	37.215	19	38.101	
+ LIVER	20	9.959	20	10.229	20	11.125*	20	11.707*	19	13.453*	-->
LIVER / BODY	20	3.581	20	3.730	20	4.065*	20	4.341*	19	4.875*	-->
LIVER / BRAIN	20	467.044	20	474.914	20	507.369	20	538.877*	19	616.009*	-->
+ KIDNEYS	20	1.905	20	1.805	20	1.868	20	1.803*	19	1.864	
KIDNEYS / BODY	20	0.686	20	0.660	20	0.686	20	0.669	19	0.676	
KIDNEYS / BRAIN	20	89.447	20	83.873*	20	85.191	20	83.002*	19	85.470*	

NO = NO. OF VALUES / GROUP  
 \* = SIGN. DIFFERENCE (LOCATION AND/OR DISPERSION) BETWEEN CONTROL (GROUP 1) AND GROUP X (SIGN.L. = 0.050)  
 --> = SIGN. POS. TREND FROM CONTROL TO HIGHEST DOSAGE-GROUP (SIGN.L. = 0.010)

SEX : FEMALE TEST WEEK 14 COMPOUND : T

ORGANS	DOSE IN MG/KG BW/DAY										TREND
	0.000		3.000		10.000		30.000		100.000		
	NO	MEAN	NO	MEAN	NO	MEAN	NO	MEAN	NO	MEAN	
+ ADRENALS	20	0.104	20	0.095	20	0.097	20	0.086*	19	0.089*	<--
ADRENALS / BODY	20	0.0376	20	0.0349	20	0.0357	20	0.0320*	19	0.0324*	<--
ADRENALS / BRAIN	20	4.897	20	4.408	20	4.418	20	3.961*	19	4.078*	<--
+ THYMUS	20	0.391	20	0.342	20	0.365	20	0.381	19	0.401	
THYMUS / BODY	20	0.140	20	0.125	20	0.133	20	0.141	19	0.145	
THYMUS / BRAIN	20	18.357	20	16.014*	20	16.704	20	17.518	19	18.370	
+ GONADES	20	0.155	20	0.153	20	0.153	20	0.152	19	0.151	
GONADES / BODY	20	0.056	20	0.056	20	0.057	20	0.057	19	0.055	
GONADES / BRAIN	20	7.251	20	7.124	20	6.986	20	6.988	19	6.927	

NO = NO. OF VALUES / GROUP  
 \* = SIGN. DIFFERENCE (LOCATION AND/OR DISPERSION) BETWEEN CONTROL (GROUP 1) AND GROUP X (SIGN.L. = 0.050)  
 <-- = SIGN. NEG. TREND FROM CONTROL TO HIGHEST DOSAGE-GROUP (SIGN.L. = 0.010)

As shown in the tables above, there are a number changes in the organ weights, the organ to body weight ratios, and the organ to brain weight ratios.

In the 30 mg/kg male dose group, there was a decrease in the brain to body weight ratio by 4.2% compared to control. This finding was not observed in the 100 mg/kg male dose group as there was no dose-dependency and thus does not represent a safety concern. There was an initial decrease in the liver weights by 1.3% in the 3 mg/kg male dose group followed by an increase of 7.5, 27.0, and 41.2% in the 10, 30, and 100 mg/kg male dose groups, respectively, compared to control. There was an increase in the liver to body weight ratio by 0.4, 5.8, 22.5, and 43.1% in the male 3, 10, 30, and 100 mg/kg dose groups, respectively, compared to control. There was an initial decrease in

the liver to brain weight ratio by 1.8% in the male 3 mg/kg dose group followed by an increase of 5.0, 26.2, 41.9% in the male 10, 30, and 100 mg/kg dose groups, respectively, compared to control. These liver weight changes occurred with changes in liver enzymes (e.g. gamma-glutamyl transpeptidase, alanine aminotransferase, and alkaline phosphatase) beginning at 3 mg/kg. Moreover, the changes in liver weight at the lower doses of 3 and 10 mg/kg were not considered because there were no treatment related changes in liver histopathology at those doses (see histological findings below). There was decrease in the gonads to body weight ratio by 5.9% in the male 30 mg/kg dose group compared to control. This finding was not observed in the male 100 mg/kg dose as there was no dose-dependency and thus does not represent a safety concern. There were no other treatment related changes in organ weight, organ to body weight ratio, and organ to brain weight ratio in the males.

In females, the brain weight increased by 2.9 and 2.4% in the 10 and 100 mg/kg dose group, respectively, compared to control. As there is no clear dose-dependency in this finding, brain weight changes do not represent a safety concern. There was an increase in the liver weight by 11.7, 17.6, and 35.1% in the female 10, 30, and 100 mg/kg dose groups, respectively, compared to control. There was an increase in the liver to body weight ratio by 13.5, 21.2, and 36.1% in the female 10, 30, and 100 mg/kg dose groups, respectively, compared to control. There was an increase in the liver to brain weight ratio by 8.6, 15.4, and 31.9% in the female 10, 30, and 100 mg/kg dose groups, respectively, compared to control. These liver weight changes occurred with changes in liver enzymes (e.g. gamma-glutamyl transpeptidase, alanine aminotransferase, and alkaline phosphatase) beginning at 3 mg/kg. Moreover, the changes in liver weight at the lower dose of 10 mg/kg were not considered because there were no treatment-related changes in liver histopathology at this dose (see histological findings below). There was a decrease in the weight of the kidneys by 5.4% in the 30 mg/kg dose group compared to control. This finding was not observed in the female 100 mg/kg dose group as there was no dose-dependency and thus does not represent a safety concern. There was a decrease in the kidney to brain weight ratio by 6.2, 7.2, and 4.4% in the female 3, 30, and 100 mg/kg dose groups, respectively, compared to control. This finding was not observed in the female 10 mg/kg dose group as there was no dose-dependency and thus does not represent a safety concern. There was a decrease in the weight of the adrenal glands by 17.3 and 14.4% in the female 30 and 100 mg/kg dose groups, respectively, compared to control. This finding was not dose dependent and thus does not represent a safety concern. There was a decrease in the adrenals to body weight ratio by 14.9 and 13.8% in the female 30 and 100 mg/kg dose groups, respectively, compared to control. This finding was not dose dependent and thus does not represent a safety concern. There was a decrease in the adrenals to brain weight ratio by 19.1 and 16.7% in the female 30 and 100 mg/kg dose groups, respectively, compared to control. This finding was not dose dependent and thus does not represent a safety concern.

## Histopathology

### Adequate Battery

The following tissues and organs were collected, preserved, and examined microscopically (from the Sponsor's submission):

brain  
spinal cord  
eye with optic nerve  
orbital gland  
pituitary gland  
salivary gland  
heart  
aorta  
thymus  
thyroid with parathyroid gland  
lungs with mainstem bronchi  
trachea  
spleen  
sternum  
lymph nodes  
sciatic nerve  
oesophagus  
stomach  
small and large intestine  
adrenal gland  
pancreas  
liver  
kidneys  
urinary bladder  
ovaries  
testis  
seminal vesicle  
epididymis  
prostate  
uterus  
skin  
mammary area  
femur  
skeletal muscle  
organs and tissues showing macroscopic changes

This appears to be an adequate histopathology battery.

### Peer Review

There was no dedicated Pathology Report but the signature of scientist responsible for the Pathology section was provided on September 14, 1983. Peer review was not clear but an additional signature from the Pathology staff was provided on September 14, 1983.

### Histological Findings

The histopathological findings are illustrated in the following table (adapted from the Sponsor's submission):

TOX DISPO NR: 010895    COMPOUND: (b) (4)    DURATION: 3 MONTHS    PAGE: 1

SUMMARY OF MICROSCOPICAL FINDINGS

SPECIES: RAT

	0 MG/KG		3 MG/KG		10 MG/KG		30 MG/KG		100 MG/KG		H	F
	M	F	M	F	M	F	M	F	M	F		
ANIMALS EXAMINED MICROSCOPICALLY	20	20	20	20	20	20	20	20	20	20		
LYMPH NODE CONGESTION	---	---	---	---	---	---	---	---	---	---	1	---
BRONCHUS FOREIGN BODY	---	---	---	---	---	---	---	---	1	1	---	---
LUNG CONGESTION	---	---	---	---	1	---	---	---	1	1	---	---
HAEMORRHAGE	---	---	---	---	---	---	---	1	2	1	---	---
METASTATIC CALCIFICATION	---	---	---	---	---	---	---	---	---	---	---	---
LUNG ALVEOLUS FOREIGN BODY	---	---	---	---	---	---	---	---	13	7	---	---
FOAM CELL	2	1	2	1	2	2	1	2	---	---	---	---
LIVER CONGESTION	---	---	---	---	1	---	---	---	1	1	---	---
LYMPHOHISTIOCYTIC INFILTRATION	1	---	---	1	1	2	---	---	---	1	---	---
FATTY CHANGE	---	---	---	---	---	2	---	1	---	---	---	---
RECENT NECROSIS	---	---	---	---	---	---	---	---	1	---	---	---
ORGANIZING NECROSIS	---	---	1	---	---	---	---	---	---	---	---	---
INTRAHEPATIC BILE DUCT CHOLANGIOFIBROSIS	---	---	---	---	---	---	1	---	1	1	---	---
LIVER HEPATOCYTE HYPERTROPHY	---	---	---	---	---	---	6	5	20	14	---	---
RENAL TUBULE DILATATION	---	---	---	---	---	---	---	---	1	---	---	---
CAST	---	---	---	---	---	---	---	---	1	---	---	---
EPIDIDYMHIS IMPACTION	---	---	---	---	---	---	---	---	1	---	---	---
CELLULAR DEBRIS	---	---	1	---	---	---	---	---	---	---	---	---
SPERMATIC GIANT CELL	---	---	1	---	1	---	2	---	1	---	---	---
ABSENCE OF SPERMATOZOA	1	---	---	---	---	---	---	---	---	---	---	---
UTERUS CONGESTION	---	---	---	---	---	---	---	---	---	1	---	---
ADRENAL GLAND CONGESTION	---	---	---	---	---	---	---	---	---	1	---	---
THYROID GLAND CONGESTION	---	---	---	---	---	---	---	---	---	1	---	---
THYMUS CONGESTION	---	---	---	---	1	---	---	---	1	1	---	---
HAEMORRHAGE	---	---	---	---	---	---	---	---	1	---	---	---

As shown in the table above, there are a number of histopathological findings. There was congestion in the lymph node in 1/20 females from the 100 mg/kg dose group. In the renal tubule, there was 1/20 males observed with dilatation and 1/20 males observed with cast from the 100 mg/kg dose group. Impaction of the epididymis was observed in 1/20 males from the 100 mg/kg dose group. Congestion of the uterus was observed in 1/20 females from the 100 mg/kg dose group. Congestion of the adrenal gland was observed in 1/20 females from the 100 mg/kg dose group. Congestion of the thyroid gland was observed in 1/20 females from the 100 mg/kg dose group. Hemorrhage of the thymus was observed in 1/20 males from the 100 mg/kg dose group.

There was a foreign body in the bronchus of 1/20 males and females each, from the 100 mg/kg dose group. Foreign body in the bronchus could be the result of error in the oral gavage technique. Congestion in the lung was observed in 1/20 males and

females each, from the 10 mg/kg dose groups as well as 1/20 females from the 100 mg/kg dose group. There was no dose-dependency in this finding and thus, congestion in the lung does not represent a safety concern. Hemorrhage in the lung was observed in 1/20 males and 2/20 males from the 10 and 100 mg/kg dose groups as well as in 1/20 females from the 100 mg/kg dose group. Hemorrhage in the lung was not dose-dependent and thus, does not represent a safety concern. There was a foreign body observed in the lung alveolus in 1/20 males and females each, from the 100 mg/kg dose group. Foreign body in the alveolus could be the result of error in the oral gavage technique. Foamy cells in the lung alveolus was observed in 2/20, 2/20, 2/20, 1/20, and 13/20 males as well as 1/20, 1/20, 2/20, 2/20, and 7/20 females from the 0, 3, 10, 30, and 100 mg/kg dose groups, respectively.

In the liver, recent necrosis was noted in 1/20 males from the 100 mg/kg dose group. Cholangiofibrosis of the intrahepatic bile duct was observed in 1/20 males each from the 30 and 100 mg/kg dose groups as well as 1/20 females from the 100 mg/kg dose group. Hepatocyte hypertrophy in the liver was observed in 6/20 and 20/20 males as well as 5/20 and 14/20 females from the 30 and 100 mg/kg dose groups. Changes in the liver at 30 and 100 mg/kg dose groups were preceded by changes in liver enzymes (e.g. gamma-glutamyl transpeptidase, alanine aminotransferase, and alkaline phosphatase) that were observed beginning at 3 mg/kg.

The Pathology Report notes the slight hypertrophy of the hepatocytes in the liver in the 30 and 100 mg/kg dose groups as well as the slight focal accumulation of foamy cells in the alveoli of the lungs in the 100 mg/kg dose group as treatment-related changes due to the test article. Foamy cells in the alveoli of the lungs are a part of the recycling process of surfactant production in type II alveolar cells which accounts for the finding in the control animals and the 3, 10, and 30 mg/kg dose groups ( (b) (4) ); however, due to the high incidence of the finding in the 100 mg/kg dose group, this finding is considered treatment-related, although still likely a byproduct of the gavage process. All other lung changes were due to gavage error. All other histopathological changes were spontaneous in nature and common in this strain of rats. This reviewer concurs with the assessment of the Pathology Report. However, the Pathology Report does not determine a NOAEL.

### **Special Evaluation**

In addition, a hearing test was performed at the beginning and towards the end of the treatment period. There were no treatment related effects on hearing.

### **Toxicokinetics**

Toxicokinetics were not assessed in this study.

### **Dosing Solution Analysis**

No dedicated dosing solution analysis was performed. There was chemical analysis of the stability performed. Pretest samples of the suspension were analyzed for stability

prior to the initiation of the study for 4 hours. A concentration of 96-100% was obtained under the conditions of the analysis, whereas the initial concentration was 85-107%.

## Conclusions

In conclusion, the NOAEL is 10 mg/kg based on histopathological findings such as hepatocyte hypertrophy in the liver at 30 and 100 mg/kg and foamy cell accumulation in the alveoli of the lungs at 100 mg/kg. Other histopathological findings include congestion in the lymph node, dilatation and cast in the renal tubule, impaction of the epididymis, congestion of the uterus, congestion of the adrenal gland, congestion of the thyroid gland, and hemorrhage of the thymus in the 100 mg/kg dose group, as well as cholangiofibrosis of the intrahepatic bile duct in the 30 and 100 mg/kg dose groups.

No NOAEL was determined in the Pathology Report; however, the Applicant had determined a no observed effects level (NOEL) of 3 mg/kg.

The rat NOAEL of 10 mg/kg would support a human equivalent dose (HED) of (b) (4) mg of (b) (4). Since this toxicity study used oral administration and this IV APAP formulation is for intravenous administration, an additional safety factor of 10 may be used. The HED is then reduced to (b) (4) mg of (b) (4). The maximum level of (b) (4) found in migration studies using this IV acetaminophen formulation was (b) (4) mcg at the MDD of 4 g/day of APAP, which represents a safety margin of 363.

Based on the levels of (b) (4) that were noted in MF 26696 (see nonclinical review) and on migration studies using this IV acetaminophen drug product formulation, there is an adequate safety margin based on the NOAEL from this rat study to support the levels of (b) (4) in the free/ex® (b) (4) to be used with this IV acetaminophen formulation. Thus, (b) (4) is adequately qualified for systemic safety.

## 7 Genetic Toxicology

There were no genetic toxicology studies with IV acetaminophen submitted in this NDA. The following information on the genetic toxicology (mutagenesis) of acetaminophen is from the OFIRMEV label (Cadence Pharma, November 2011):

### Mutagenesis

Acetaminophen was not mutagenic in the bacterial reverse mutation assay (Ames test). In contrast, acetaminophen tested positive in the in vitro mouse lymphoma assay and the in vitro chromosomal aberration assay using human lymphocytes. In the published literature, acetaminophen has been reported to be clastogenic when administered a dose of 1500 mg/kg/day to the rat model (3.6-times the MHDD, based on a body surface area comparison). In contrast, no clastogenicity was noted at a dose of 750 mg/kg/day (1.8-times the MHDD, based on a body surface area comparison), suggesting a threshold effect.

## 8 Carcinogenicity

There were no studies on the carcinogenicity of IV acetaminophen submitted in this NDA. The following information on the carcinogenicity of acetaminophen is from the OFIRMEV label (Cadence Pharma, November 2011):

### Carcinogenesis

Long-term studies in mice and rats have been completed by the National Toxicology Program to evaluate the carcinogenic potential of acetaminophen. In 2-year feeding studies, F344/N rats and B6C3F1 mice were fed a diet containing acetaminophen up to 6000 ppm. Female rats demonstrated equivocal evidence of carcinogenic activity based on increased incidences of mononuclear cell leukemia at 0.8 times the maximum human daily dose (MHDD) of 4 grams/day, based on a body surface area comparison. In contrast, there was no evidence of carcinogenic activity in male rats (0.7 times) or mice (1.2-1.4 times the MHDD, based on a body surface area comparison).

## 9 Reproductive and Developmental Toxicology

There were no fertility studies with IV acetaminophen submitted in this NDA. The following information on the impairment of fertility of acetaminophen is from the OFIRMEV label (Cadence Pharma, November 2011):

### Impairment of fertility

In studies conducted by the National Toxicology Program, fertility assessments have been completed in Swiss mice via a continuous breeding study. There were no effects on fertility parameters in mice consuming up to 1.7 times the MHDD of acetaminophen, based on a body surface area comparison. Although there was no effect on sperm motility or sperm density in the epididymis, there was a significant increase in the percentage of abnormal sperm in mice consuming 1.7 times the MHDD (based on a body surface area comparison) and there was a reduction in the number of mating pairs producing a fifth litter at this dose, suggesting the potential for cumulative toxicity with chronic administration of acetaminophen near the upper limit of daily dosing.

Published studies in rodents report that oral acetaminophen treatment of male animals at doses that are 1.2 times the MHDD and greater (based on a body surface area comparison) result in decreased testicular weights, reduced spermatogenesis, reduced fertility, and reduced implantation sites in females given the same doses. These effects appear to increase with the duration of treatment. The clinical significance of these findings is not known.

There were no further reproductive and developmental toxicology studies with IV acetaminophen submitted in this NDA. The following information on the reproductive and developmental toxicology of acetaminophen is from the pregnancy section of the OFIRMEV label (Cadence Pharma, November 2011):

Pregnancy Category C. There are no studies of intravenous acetaminophen in pregnant women; however, epidemiological data on oral acetaminophen use in pregnant women show no increased risk of major congenital malformations. Animal reproduction studies have not been conducted with IV acetaminophen, and it is not known whether OFIRMEV can cause fetal harm when administered to a pregnant woman. OFIRMEV should be given to a pregnant woman only if clearly needed.

The results from a large population-based prospective cohort, including data from 26,424 women with live born singletons who were exposed to oral acetaminophen during the first trimester, indicate no increased risk for congenital malformations, compared to a control group of unexposed children. The rate of congenital malformations (4.3%) was similar to the rate in the general population. A population-based, case-control study from the National Birth Defects Prevention Study showed that 11,610 children with prenatal exposure to acetaminophen during the first trimester had no increased risk of major birth defects compared to 4,500 children in the control group. Other epidemiological data showed similar results.

While animal reproduction studies have not been conducted with intravenous acetaminophen, studies in pregnant rats that received oral acetaminophen during organogenesis at doses up to 0.85 times the maximum human daily dose (MHDD = 4 grams/day, based on a body surface area comparison) showed evidence of fetotoxicity (reduced fetal weight and length) and a dose-related increase in bone variations (reduced ossification and rudimentary rib changes). Offspring had no evidence of external, visceral, or skeletal malformations. When pregnant rats received oral acetaminophen throughout gestation at doses of 1.2-times the MHDD (based on a body surface area comparison), areas of necrosis occurred in both the liver and kidney of pregnant rats and fetuses. These effects did not occur in animals that received oral acetaminophen at doses 0.3-times the MHDD, based on a body surface area comparison.

In a continuous breeding study, pregnant mice received 0.25, 0.5, or 1.0% acetaminophen via the diet (357, 715, or 1430 mg/kg/day). These doses are approximately 0.43, 0.87, and 1.7 times the MHDD, respectively, based on a body surface area comparison. A dose-related reduction in body weights of fourth and fifth litter offspring of the treated mating pair occurred during lactation and post-weaning at all doses. Animals in the high dose group had a reduced number of litters per mating pair, male offspring with an increased percentage of abnormal sperm, and reduced birth weights in the next generation pups.

## 10 Special Toxicology Studies

The Applicant has submitted information regarding the osmolality of their drug product and the referenced product (OFIRMEV). The following table illustrates the osmolality between their drug product and OFIRMEV (from the Applicant's submission):

**Table 1.11.1- 1 Osmolality Results for FK Finished Product**

Lot no.	Manufacturing Date	Osmolality (mOsm/kg)
12EGU94	July 2011	275
12EGU95	July 2011	276
12EGU96	July 2011	275
12EGU97	July 2011	275

**Table 1.11.1- 2 Osmolality Results for Ofirmev<sup>®</sup> Finished Product (RLD)**

Lot no.	Expiration Date	Osmolality (mOsm/kg)
V005710	May 2012	286
2B70898	February 2014	281
2G71267	July 2014	282
2H60284	August 2014	282

As shown in the table above, the Applicant's drug product and OFIRMEV have similar osmolality and both are considered isotonic. As such, blood compatibility testing on the Applicant's IV acetaminophen drug product will not be necessary. Thus, there are no issues with the osmolality of this IV acetaminophen drug product.

## 11 Integrated Summary and Safety Evaluation

The Applicant has submitted an osmolality comparison between their IV APAP formulation and OFIRMEV, migration studies with their IV APAP formulation, and a 3-month oral toxicity study in rats to justify the levels of the (b) (4) leachable from the container closure system. There were no other nonclinical studies submitted in this NDA.

There are no novel excipients in this IV APAP formulation that preclude approval. There are no safety concerns with the drug substance specifications, including the specifications for (b) (4) as well as the residual solvents of the drug substance. There are no additional safety concerns with the drug product specifications, including the specifications for (b) (4) compared to the FDA-approved OFIRMEV. There are no safety concerns with the residual solvents (b) (4) of the drug product. The label for the Fresenius Kabi IV APAP formulation is the same as the referenced OFIRMEV for the mutagenesis, carcinogenesis, impairment of fertility, pregnancy sections. The osmolality of this IV APAP formulation indicates that the solution is isotonic, and similar to OFIRMEV. As such, blood compatibility testing with this IV APAP formulation will not be necessary.

Migration studies (Leachables) with this IV APAP formulation were done under various storage conditions for up to 24 months. Results up to 12 months have been submitted in this NDA to date. Identified leachables include (b) (4)

(b) (4). Migration studies were also done with various aqueous solutions under various storage conditions and were submitted to MF 26696 (see nonclinical review). Based on the safety evaluations of the identified leachables in the migration studies submitted to MF 26696, the identified leachables in migration studies with this IV APAP formulation and in migration studies submitted to MF 26696 are qualified for safety except for (b) (4) (and related substances), (b) (4). It is noted that (b) (4) (and related substances) were not detected in the migration studies with this IV APAP formulation to date and thus does not appear to represent a safety concern. It is also noted that (b) (4) has been qualified as a Class 3 residual solvent and the levels of (b) (4) are acceptable as per ICH Q3C. The existing leachable data for the limited stability batches is not deemed adequate by the CMC review team to be able to conclude that no further leachables will be found on stability. Therefore, the risk assessment of these leachables must be deemed preliminary pending further data.

A 3-month oral toxicology study in rats with (b) (4) has been submitted in this NDA. SPF rats were dosed 0, 3.0, 10, 30, and 100 mg/kg of (b) (4) for 3 months via oral gavage. A NOAEL of 10 mg/kg was determined based on histopathology, namely the slight hypertrophy of the hepatocytes in the liver in the 30 and 100 mg/kg dose groups as well as the slight focal accumulation of foamy cells in the alveoli of the lungs in the 100 mg/kg dose group were regarded as treatment-related changes due to the test article. Other histopathological findings include congestion in the lymph node, dilatation and cast in the renal tubule, impaction of the epididymis, congestion of the uterus, congestion of the adrenal gland, congestion of the thyroid gland, and hemorrhage of the thymus in the 100 mg/kg dose group, as well as cholangiofibrosis of the intrahepatic bile duct in the 30 and 100 mg/kg dose groups. A NOAEL was not determined in the Pathology Report; however, the Applicant had determined a no observed effects level (NOEL) of 3 mg/kg. The rat NOAEL of 10 mg/kg supports a human equivalent dose (HED) of (b) (4) mg of (b) (4). Since this toxicity study used oral administration and this IV APAP formulation is for intravenous administration, an additional safety factor of 10 may be used. The HED is then reduced to (b) (4) mg of (b) (4). The maximum level of (b) (4) found in migration studies using this IV acetaminophen formulation was (b) (4) mcg at the MDD of 4 g/day of APAP, which represents an adequate safety margin of 363. Thus, (b) (4) does not represent a safety concern.

The weight of evidence approach for (b) (4) that was presented in MF 26696 is not adequate to justify the safety of the levels of (b) (4) detected in migration studies with this IV APAP formulation at the MDD of APAP of 4 g/day. As there are no data for the parent compound, the Applicant completed their risk assessment on the primary metabolites alone. No data were provided to characterize the rate of (b) (4) or determine if the parent compound was detected systemically. Thus, a 28-day IV toxicity study with (b) (4) is needed to justify the safety of this leachable. It may be possible to provide data to show that the (b) (4) is virtually instantly (b) (4) to the metabolites

to support the conclusion that the risk assessment on the metabolites alone is adequate. However, definitive data will be required to support such a conclusion.

The Applicant also plans to submit an Ames test for (b) (4) and a 4-week IV toxicity study in rats and an Ames test for (b) (4). As the final report for the repeat-dose toxicology study for (b) (4) as well as the 4-week IV toxicity study with (b) (4) (or data to show such a study is not needed) have not been received and deemed adequate by the Agency, from a nonclinical pharmacology toxicology perspective, we cannot recommend approval of this NDA at this time.

## 12 Appendix/Attachments

(b) (4)

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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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CARLIC K HUYNH  
06/20/2013

RICHARD D MELLON  
06/20/2013

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

**NDA/BLA Number: 204-767    Applicant: Fresenius Kabi USA, LLC    Stamp Date: September 28, 2012**

**Drug Name: Acetaminophen Injection    NDA/BLA Type: 505(b)(2)    DAAAP/ODEII/OND/CDER/OMPT/FDA**

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

	<b>Content Parameter</b>	<b>Yes</b>	<b>No</b>	<b>Comment</b>
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?			Not applicable. The Sponsor did not conduct any new nonclinical studies. The submitted 505(b)(2) New Drug Application (NDA) included referenced nonclinical studies. The Sponsor relies upon the literature for 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 by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).			Not applicable. The Sponsor did not conduct any new nonclinical studies. The submitted 505(b)(2) New Drug Application (NDA) included a reference to Ofirmev™.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?			Not applicable. The Sponsor did not conduct any new nonclinical studies. The submitted 505(b)(2) New Drug Application (NDA) included a reference to Ofirmev™.

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

	<b>Content Parameter</b>	<b>Yes</b>	<b>No</b>	<b>Comment</b>
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?			Not applicable. The Sponsor did not conduct any new nonclinical studies. The submitted 505(b)(2) New Drug Application (NDA) included a reference to Ofirmev™.
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?			Not applicable. The Agency did not have any previous discussions with the Applicant.
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		The Applicant's proposed labeling is the same as the referenced product Ofirmev™ for the pharmacology/toxicology sections (carcinogenesis, mutagenesis, teratogenic effects, nonteratogenic effects, and impairment of fertility).
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)		X	DS specifications appear acceptable. The DP specification for (b)(4) should be reduced to as low as reasonably possible ((b)(4)) based on information suggesting the impurity is genotoxic.  In terms of leachables and extractables from the container closure system, based on other reviews of the data in the referenced BB-DMF ((b)(4)), migration studies performed by the DMF holder show leachables that have not been deemed adequately qualified for systemic safety. However, there are other FDA-approved IV drug products that employ this container closure system; therefore, this will require further review.
11	Has the applicant addressed any abuse potential issues in the submission?	X		The submitted 505(b)(2) New Drug Application (NDA) included a reference to Ofirmev™.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable. This is a 505(b)(2) New Drug Application (NDA) submitted to support a Rx.

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

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

File name: 5\_Pharmacology\_Toxicology Filing Checklist for NDA\_BLA or Supplement 010908

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

Upon preliminary review, there were several potential filing issues identified by the nonclinical review team. These are discussed below:

1. The NDA references a DMF that has been submitted to CBER ( (b) (4) ). This document is not readily available to CDER reviewers and we cannot submit formal reviews of the DMF via our archival system (DARRTs). The Division discussed this challenge with the Applicant prior to the filing date and the Applicant indicated that they will submit the information to a CDER DMF as soon as possible. No time line has yet been provided for this submission; however, as the CBER DMF is something we could access, the Division cannot make this a filing issue.
2. Based on information available to the review team from another Fresenius Kabi product that references the same CBER DMF (owned by Fresenius Kabi), the DMF does not include adequate justification for the systemic exposure to 3 leachables identified in the migration studies with the *freeflex*® container closure ( (b) (4) ). The 3 leachables were (b) (4) . The Applicant should provide justification of these leachables up to the MDD of 4 g/day of APAP via your drug product. Justification may be obtain via either literature references or a general toxicology study using the intravenous route in a single species evaluating these 3 leachables. However, as the *freeflex*® (b) (4) appears to be used in other FDA-approved IV drug products, the safety of the systemic exposure to these 3 leachables will have to be deemed a review issue at this time. Nonetheless, a comment on this potential review issue should be included in the 74-day letter.
3. The submission does not appear to include information regarding the osmolality of the solution. As the formulation is not identical to the referenced product and would appear to be of lower osmolality, this information should be submitted to the application. As the formulations differ in what appears to be minor ways, this is not deemed a filing issue and should be readily corrected and a comment should be included in the 74-day letter.
4. The DP specification for (b) (4) appears to be able to be tightened. The referenced drug product specification is lower than that proposed and therefore, further justification would be necessary if the spec can not be tightened. This is not deemed a filing issue but a comment should be included in the 74-day letter.

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

1. Submit osmolality data for the final drug product formulation. If the solution is not isotonic, provide justification why any differences in osmolality between your drug product and the referenced drug product do not represent a safety concern.
2. The degradant (b) (4) which is also called (b) (4) , in the drug product specification should be reduced to as low as technically feasible based on manufacturing capability.
3. Based on preliminary review of the NDA, it appears as though there are inadequate safety justifications for the systemic levels of all leachables from the container closure system for the intravenous route of administration. Specifically, there does not appear to be adequate safety justification for the following three identified leachables: (b) (4) ,

File name: 5\_Pharmacology\_Toxicology Filing Checklist for NDA\_BLA or Supplement  
010908

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

(b) (4). Unless adequately justified otherwise, the IV safety of these three identified leachables from the container closure system should be qualified via an IV toxicology study that provides an adequate safety margin for the levels of these identified leachables that a person would be exposed to when treated with up to 4 grams of acetaminophen per day via this drug product formulation. The duration of such a toxicology study should be comparable to the predicted maximum duration proposed in your drug product labeling (i.e., up to 14 days duration).

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Reviewing Pharmacologist

Date

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Team Leader/Supervisor

Date

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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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CARLIC K HUYNH  
11/27/2012

RICHARD D MELLON  
11/27/2012