

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**204200Orig1s000**

**204200Orig2s000**

**PHARMACOLOGY REVIEW(S)**

## Memo-To-File

TO: NDA 204-200  
JHP Pharmaceuticals LLC; Adrenalin<sup>®</sup> (Epinephrine injection, USP 1:1000)

FROM: Timothy W. Robison, Ph.D., D.A.B.T.  
Pharmacology and Toxicology Team Leader  
Division of Pulmonary, Allergy, and Rheumatology Products

DATE: November 15, 2012

I concur with Dr. Jane Sohn's review dated October 30, 2012 that recommends approval of NDA 204-200 as well as changes to the product label. JHP Pharmaceuticals LLC submitted this 505(b)(2) NDA for Adrenalin<sup>®</sup> (epinephrine), which is already marketed, but without an approved NDA. The active pharmaceutical ingredient (API) is epinephrine. The indication is treatment of anaphylaxis. The sponsor reference the approved Listed Drug EpiPen<sup>®</sup>, marketed under NDA 19-430, which also utilizes the same API and is approved for the treatment of anaphylaxis by the IM and SC routes. The safety of epinephrine is based on extensive previous clinical experience, which is supported in the literature. Nonclinical studies were conducted to support the safety of an impurity; these studies were reviewed by Dr. Sohn under a Chemistry Consult dated June 1, 2012.

Changes to the proposed product label were recommended by Dr. Sohn in Sections 8.1, 12.1, and 13. Potential reproductive toxicity is conveyed in Section 8.1. Clinical experience has identified concerns for fetal anoxia, spontaneous abortion, or both. Adverse developmental effects were also identified in reproductive toxicology studies with animals. Results of genetic toxicology tests are described in Section 13.1. Epinephrine was positive in the *in vitro* *Salmonella* bacterial reverse mutation assay and *in vitro* mouse lymphoma assay, but negative in the *in vivo* micronucleus assay. Epinephrine is an oxidative mutagen based on the *E. Coli* WP2 Mutoxitest bacterial reverse mutation assay. It is possible that a degradant of epinephrine might be responsible for the positive results observed in the two *in vitro* assays. These positive results have little or no safety implications for the acute use of this product to treat anaphylaxis.

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

/s/  
-----

TIMOTHY W ROBISON

11/16/2012

See Dr. Sohn's reviews dated June 1, 2012 and October 30, 2012

**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: 204-200  
Supporting document/s: SD 1 (EDR)  
Applicant's letter date: 3/7/12  
CDER stamp date: 3/7/12  
Product: Adrenalin® (epinephrine injection, USP 1:1000)  
Indication: Treatment of anaphylaxis  
Applicant: JHP Pharmaceuticals LLC  
Review Division: Division of Pulmonary, Allergy and  
Rheumatology Drug Products (DPARP)  
Reviewer: Jane J. Sohn, Ph.D.  
Supervisor/Team Leader: Timothy Robison, Ph.D., DABT  
Division Director: Badrul Chowdhury, M.D., Ph.D.  
Project Manager: Carol Hill

*Template Version: September 1, 2010*

**Disclaimer**

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 204200 are owned by JHP Pharmaceuticals LLC or are data for which JHP Pharmaceuticals LLC has obtained a written right of reference. Any information or data necessary for approval of NDA 204200 that JHP Pharmaceuticals 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 NDA 204200.

## TABLE OF CONTENTS

<b>1</b>	<b>EXECUTIVE SUMMARY .....</b>	<b>4</b>
1.1	INTRODUCTION .....	4
1.3	RECOMMENDATIONS .....	4
<b>2</b>	<b>DRUG INFORMATION .....</b>	<b>7</b>
2.1	DRUG .....	7
2.2	RELEVANT INDS, NDAs, BLAs AND DMFs .....	8
2.3	DRUG FORMULATION .....	8
2.4	COMMENTS ON NOVEL EXCIPIENTS .....	8
2.5	COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN .....	8
2.6	PROPOSED CLINICAL POPULATION AND DOSING REGIMEN .....	8
2.7	REGULATORY BACKGROUND .....	9
<b>3</b>	<b>STUDIES SUBMITTED.....</b>	<b>10</b>
3.1	STUDIES REVIEWED.....	10
3.2	STUDIES NOT REVIEWED .....	10
3.3	PREVIOUS REVIEWS REFERENCED.....	10
<b>4</b>	<b>INTEGRATED SUMMARY AND SAFETY EVALUATION .....</b>	<b>10</b>
<b>5</b>	<b>APPENDIX/ATTACHMENTS.....</b>	<b>12</b>

## Table of Tables

Table 1: Maximum dose of epinephrine by age group ..... 9

# 1 Executive Summary

## 1.1 Introduction

JHP Pharmaceuticals LLC (JHP) has submitted this 505 (b) (2) NDA for Adrenalin® (epinephrine), which is already marketed, but without an approved NDA. The active pharmaceutical ingredient (API) is epinephrine. The indication for this review is treatment of anaphylaxis. The additional indication of maintenance of mydriasis in cataract surgery is under review within the Division of Transplant and Ophthalmic Drug Products (DTOP). The review for the anaphylaxis indication is being conducted under a standard review clock (10 months), while the review for the mydriasis indication is under a priority review clock (6 months) that was extended due to the submission of a major chemistry, manufacturing, and controls (CMC) during the NDA review cycle extending the review cycle to 9 months.

For treatment of anaphylaxis, the sponsor proposes their product for the intramuscular (IM), subcutaneous (SC), <sup>(b) (4)</sup> routes. <sup>(b) (4)</sup>

The sponsor refers to the approved Listed Drug EpiPen®, marketed under NDA 19-430, which also utilizes the same API and is approved for the treatment of anaphylaxis via the IM and SC routes. Differences between the proposed product and EpiPen® include: 1) the sponsor proposes a vial of epinephrine with no device, 2) increased doses of the API, and 3) a different impurity profile. The safety of epinephrine is based on extensive previous clinical experience, which is supported in the literature. Nonclinical studies were conducted to support the safety of an impurity. These studies were reviewed under a Chemistry Consult (submitted June 1, 2012). The Agency accepts reference to nonclinical information in the approved labeling for EpiPen® and the public literature to support NDA 204200.

## 1.3 Recommendations

### 1.3.1 Approvability

NDA 204200 is recommended for approval from the nonclinical perspective, pending the suggested revisions to the proposed label (see below).

### 1.3.2 Additional Recommendations:

None.

### 1.3.3 Labeling:

The following changes to the proposed labeling for sections 8.1, 12.1, and 13 are presented below with changes presented as strikethroughs for deletions or in red font for additions. Sections 8.3 and 10 were reviewed to ensure that they do not contain any nonclinical information and are shown below as a verification of their review. Changes in Sections 8.3 and 10 reflect changes proposed by other disciplines:

2 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

(b) (4)



## 2 Drug Information

### 2.1 Drug

CAS Registry Number (Optional): 51-43-4

Generic Name: epinephrine injection, adrenaline injection

Code Name: None.

Chemical Name:

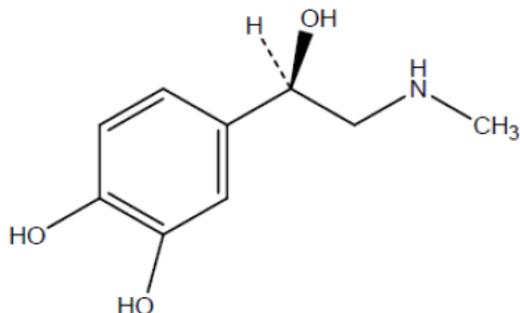
1,2-Benzenediol, 4-[1-hydroxy-2-(methylamino)ethyl]-, (R) (USP)

(-)-3,4,-Dihydroxy-(-(methylamino)methyl]benzyl alcohol (CAS)

R-1-(3,4-dihydroxyphenyl)-2-methylaminoethanol (BP)

Molecular Formula/Molecular Weight: C<sub>9</sub>H<sub>13</sub>NO<sub>3</sub>/183.20442

Structure or Biochemical Description:



Pharmacologic Class: Sympathomimetic catecholamine

## 2.2 Relevant INDs, NDAs, BLAs and DMFs

pIND 111712, Adrenalin® (JHP Pharmaceuticals), Division of Pulmonary, Allergy and Rheumatology Products (DPARP).

## 2.3 Drug Formulation

A 1 mL (b) (4) drug product is proposed:

Ingredient	Function	mM	mg/mL
(b) (4)	(b) (4)	(b) (4)	(b) (4)
NaCl	Tonicity agent		9.00
Sodium Metabisulfite (as Sodium Bisulfite)	Anti-oxidant		1.00
HCl (b) (4)	(b) (4)		(b) (4)
Epinephrine USP Synthetic	Active Ingredient		

- indicates that the sponsor did not provide the information

## 2.4 Comments on Novel Excipients

There are no novel excipients.

## 2.5 Comments on Impurities/Degradants of Concern

Degradants were reviewed in a Chemistry Consult submitted into DARRTS on June 1, 2012, based on the specifications provided in the original NDA submission (March 7, 2012).

## 2.6 Proposed Clinical Population and Dosing Regimen

The following doses are proposed by Medical Officer Dr. Peter Starke, and reflect an update to the sponsor's proposed doses.

Adrenalin® (epinephrine) is proposed for adults and pediatric patients (all ages) with anaphylaxis. In adults and children weighing greater than 30 kg, Adrenalin® is recommended via the intramuscular (IM) or subcutaneous (SC) routes at 0.3 to 0.5 mg (0.3 to 0.5 mL of 1 mg/mL [1:1000] solution) up to a maximum of 0.5 mg per injection. Injections may be repeated every 5 to 10 minutes as necessary. No maximum dosage is proposed by the sponsor for the IM and SC routes for adults. Medical Officer, Dr. Peter Starke recommends up to 3 IM/SC doses in adults and adolescents weighing greater than 30 kg, which translates into a maximum IM/SC exposure of 1.5 mg.

In children weighing equal to or less than 30 kg, Adrenalin® is recommended via the intramuscular (IM) or subcutaneous (SC) routes at up to 0.3 mg per injection. Injections may be repeated every 5 to 10 minutes as necessary, and no maximum number of injects is recommended by the sponsor. Medical Officer, Dr. Peter Starke recommends up to 3 IM/SC doses in children weighing equal to or less than 30 kg, which translates into a maximum IM/SC exposure of 0.9 mg.

The maximum total dose is the sum of the exposure the maximum 3 doses via the IM/SC route:

**Table 1: Maximum dose of epinephrine by age group**

Adult and children greater than 30 kg (mg)			
IM/SC			Maximum dose
1 dose	2 dose	3 dose	
0.5	1.0	1.5	<b>1.5</b>
Children equal to or less than 30 kg (mg)			
IM/SC			Maximum dose
1 dose	2 dose	3 dose	
0.3	0.6	0.9	<b>0.9</b>

## 2.7 Regulatory Background

On March 10, 2011, JHP Pharmaceuticals (JHP) submitted a type B meeting request regarding the submission for a 505(b)(2) application for epinephrine injection, USP.

The pre-IND meeting package was submitted on June 3, 2011 under pre-IND 111712 in the Division of Pulmonary, Allergy and Rheumatology Products (DPARP). Meeting minutes of the pre-IND teleconference were sent to JHP on August 4, 2011.

JHP stated in their pre-IND Meeting Package their intent to submit a 505(b)(2) NDA for epinephrine injection, USP based on FDA's prior review and approval of the NDAs for EpiPen® Auto-Injector (Meridian Medical Technologies, Inc.) and Twinject® Auto-Injector (Shionogi Pharma, Inc.) for the treatment of anaphylaxis. JHP intended to seek approval (b)(4) of epinephrine supported by consensus reports found in the literature.

On March 7, 2012, JHP submitted a New Drug Application (NDA) for Adrenalin® (epinephrine injection, USP) pursuant to Section 505(b)(2) of the Federal Food, Drug and Cosmetic Act. The NDA referred to the Listed Drug, EpiPen, marketed under Meridian Medical Technology's NDA 19-430 (b) (4)

Indications included anaphylaxis (reviewed in DPARP), and maintenance of mydriasis during cataract surgery (reviewed in DTOP).

On August 21, 2012, an Information Request was sent to address issues with high levels of impurities identified by the CMC reviewer. A teleconference was held with JHP on June 21, 2012 to discuss CMC issues with impurities. On September 6, 2012, two major amendments were submitted to address issues with impurity levels, and the review timeline was extended for NDA 204200 Original 2 by 3 months.

Based on the Memorandum to File from the RPM Ms. Carol Hill (August 23, 2012), this application was split for administrative purposes into Original 1 under DPARP (for severe acute anaphylactic reaction) and Original 2 under DTOP (for maintenance of mydriasis in cataract surgery). DTOP instituted a priority review with a due date of September 7, 2012 and DPARP's review timeline was standard with a due date of January 7, 2013. (b) (4)

### **3 Studies Submitted**

#### **3.1 Studies Reviewed**

No nonclinical studies were submitted or required for epinephrine. Studies were submitted to support the safety of degradants, and were reviewed under a chemistry consult.

#### **3.2 Studies Not Reviewed**

None.

#### **3.3 Previous Reviews Referenced**

None.

### **4 Integrated Summary and Safety Evaluation**

The sponsor JHP Pharmaceuticals has submitted this 505 (b) (2) NDA for the approval Adrenalin® (epinephrine), an already marketed product with an approved NDA. The active pharmaceutical ingredient (API) is epinephrine. Adrenalin® is proposed for the treatment of anaphylaxis via the intramuscular (IM), subcutaneous (SC) (b) (4) routes. The sponsor refers to the approved Listed Drug EpiPen®,

marketed under NDA 19-430, which utilizes the same API and is approved for the treatment of anaphylaxis via the IM and SC routes. (b) (4)

Thus, the relevant clinical doses under this review are through the IM and SC routes only. The Medical Officer supports 0.3 to 0.5 mg per injection for adults and children weighing greater than 30 kg, and 0.3 mg per injection for children weighing equal to or less than 30 kg, with a maximum of 3 injections for all patients. Based on the provided data, the safety of epinephrine at these levels is supported from the nonclinical perspective.

The degradants of concern were identified by the CMC reviewer. The safety from the nonclinical perspective was reviewed under a Chemistry Consult (submitted June 1, 2012).

The Agency agrees that referencing the nonclinical information in the approved labeling for EpiPen® (NDA 19-430) is sufficient to support filing of NDA 204200. The sponsor also submitted references in Annotated Draft Labeling. The nonclinical sections of the Sponsor's originally proposed labeling were updated to reflect the most currently available epinephrine information contained in the Auvi-Q approved label (August 2012) and to align with 21 CFR Part 201.57 labeling recommendations. No leachables and extractables requiring nonclinical evaluation were identified by the chemistry reviewer.

The reviewer recommends approval of this NDA from the nonclinical perspective.

## 5 Appendix/Attachments

The following references were used in conjunction with the label for the approved Listed Drug EpiPen® (marketed under NDA 19-430) to support the statements in the labeling review for NDA 204200.

Auletta FJ. Effect of epinephrine on implantation and foetal survival in the rabbit. *J Reprod Fertil.* 1971 Nov;27(2):281-2.

Bruce WR, Heddle JA. The mutagenic activity of 61 agents as determined by the micronucleus, *Salmonella*, and sperm abnormality assays. *Can J Genet Cytol.* 1979 Sep;21(3):319-34.

Daston GP, Rogers JM, Versteeg DJ, Sabourin TD, Baines D, Marsh SS. Interspecies comparisons of A/D ratios: A/D ratios are not constant across species. *Fundam Appl Toxicol.* 1991 Nov;17(4):696-722.

Hirsch KS, Fritz HI. Teratogenic effects of mescaline, epinephrine, and norepinephrine in the hamster. *Teratology.* 1981 Jun;23(3):287-91.

Martínez A, Urios A, Blanco M. Mutagenicity of 80 chemicals in *Escherichia coli* tester strains IC203, deficient in OxyR, and its *oxyR(+)* parent WP2 *uvrA/pKM101*: detection of 31 oxidative mutagens. *Mutat Res.* 2000 Apr 13;467(1):41-53.

McGregor DB, Riach CG, Brown A, Edwards I, Reynolds D, West K, Willington S. Reactivity of catecholamines and related substances in the mouse lymphoma L5178Y cell assay for mutagens. *Environ Mol Mutagen.* 1988;11(4):523-44.

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

/s/  
-----

JANE J SOHN  
10/30/2012

TIMOTHY W ROBISON  
10/30/2012  
I concur

**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: NDA 204200

Supporting document/s: Electronic submission

Applicant's letter date: March 7, 2012

CDER stamp date: March 7, 2012

Product: Adrenalin<sup>®</sup> (epinephrine injection, USP)

Indication: For induction and maintenance of mydriasis during intraocular surgery; and for treatment of anaphylaxis. (The ophthalmic use will be reviewed by DTOP here; the anaphylactic use will be reviewed by Division of Pulmonary, Allergy and Rheumatology Products (DPARP), separately).

Applicant: JHP Pharmaceuticals

Review Division: Division of Transplant and Ophthalmology Products (DTOP)

Reviewer: Conrad H. Chen, Ph.D. (DTOP)

Supervisor/Team Leader: Lori E. Kotch, Ph.D. (acting Team Leader)

Division Director: Renata Albrecht, MD (DTOP)

Project Manager: Judit Milstein (DTOP)

<b>1</b>	<b>EXECUTIVE SUMMARY .....</b>	<b>3</b>
1.1	RECOMMENDATIONS .....	3
1.1.1	APPROVABILITY .....	3
1.1.2	<i>Additional Non Clinical Recommendations</i> .....	3
1.1.3	<i>Labeling</i> .....	3
1.2	BRIEF DISCUSSION OF NONCLINICAL FINDINGS .....	5
<b>2</b>	<b>DRUG INFORMATION .....</b>	<b>5</b>
2.1.1	<i>CAS Registry Number (Optional): 51-43-4</i> .....	5
2.1.2	<i>Generic Name: Epinephrine</i> .....	5
2.1.3	<i>Code Name: None</i> .....	5
2.1.5	<i>Molecular Formula/Molecular Weight:</i> .....	6
2.1.6	<i>Structure:</i> .....	6
2.1.7	<i>Pharmacologic class: Epinephrine is an endogenous catecholamine</i> .....	6
2.2	<i>Relevant IND/s, NDA/s, and DMF/s</i> .....	6
2.3	<i>Clinical Formulation</i> .....	6
2.3.1	<i>Drug Formulation</i> .....	6
2.3.1.1	<i>Comments on Novel Excipients:</i> .....	7
2.3.1.2	<i>Comments on Impurities/Degradants of Concern:</i> .....	7
<b>3</b>	<b>STUDIES SUBMITTED .....</b>	<b>8</b>
3.1	STUDIES REVIEWED .....	9
3.2	STUDIES NOT REVIEWED .....	15
<b>11</b>	<b>INTEGRATED SUMMARY AND SAFETY EVALUATION.....</b>	<b>16</b>

# 1 Executive Summary

Epinephrine injection (EP), USP (currently manufactured by JHP as Adrenalin®), has been available on the market for over 100 years. However, FDA has only approved two single-entity epinephrine drug products for the emergency treatment of severe allergic reactions. JHP has made the decision to file an NDA for approval of Adrenalin® for the treatment of anaphylaxis and induction of mydriasis during cataract surgery. General nonclinical safety evaluation of Adrenalin® for subcutaneous/intramuscular use in anaphylaxis will be reviewed in a separate review by DPARP. The current review will only cover the ocular use of Adrenalin® for this NDA.

The nonclinical safety assessment of Adrenalin® for ocular use relied on reports obtained from the literature. All of the effects seen in animals were due to the expected pharmacologic and supra-pharmacologic actions of epinephrine. No unexpected non-clinical effects of epinephrine have been reported. Ocular studies with commercially available epinephrine formulations have shown adverse effects on the cornea, including increased corneal thickness, increased corneal epithelial cell density and morphological changes. Published nonclinical studies have shown that these effects are due to a combination of the dose of Na sulfite (antioxidant in the formulation) to the eyes and the low pH of buffered epinephrine formulations. However, this will not be an issue in the ocular use of Adrenalin® since the 1:1,000 formulation will be diluted by at least 100-fold before use and the concentration of Na sulfite after dilution will not cause significant effects on cornea. The use of Adrenalin® for the induction of mydriasis during cataract surgery, as described in the labeling, is recommended from the nonclinical perspective.

## 1.1 Recommendations

### 1.1.1 Approvability

The approval of Adrenalin® for induction and maintenance of mydriasis during intraocular surgery is recommended.

### 1.1.2 Additional Non Clinical Recommendations

None

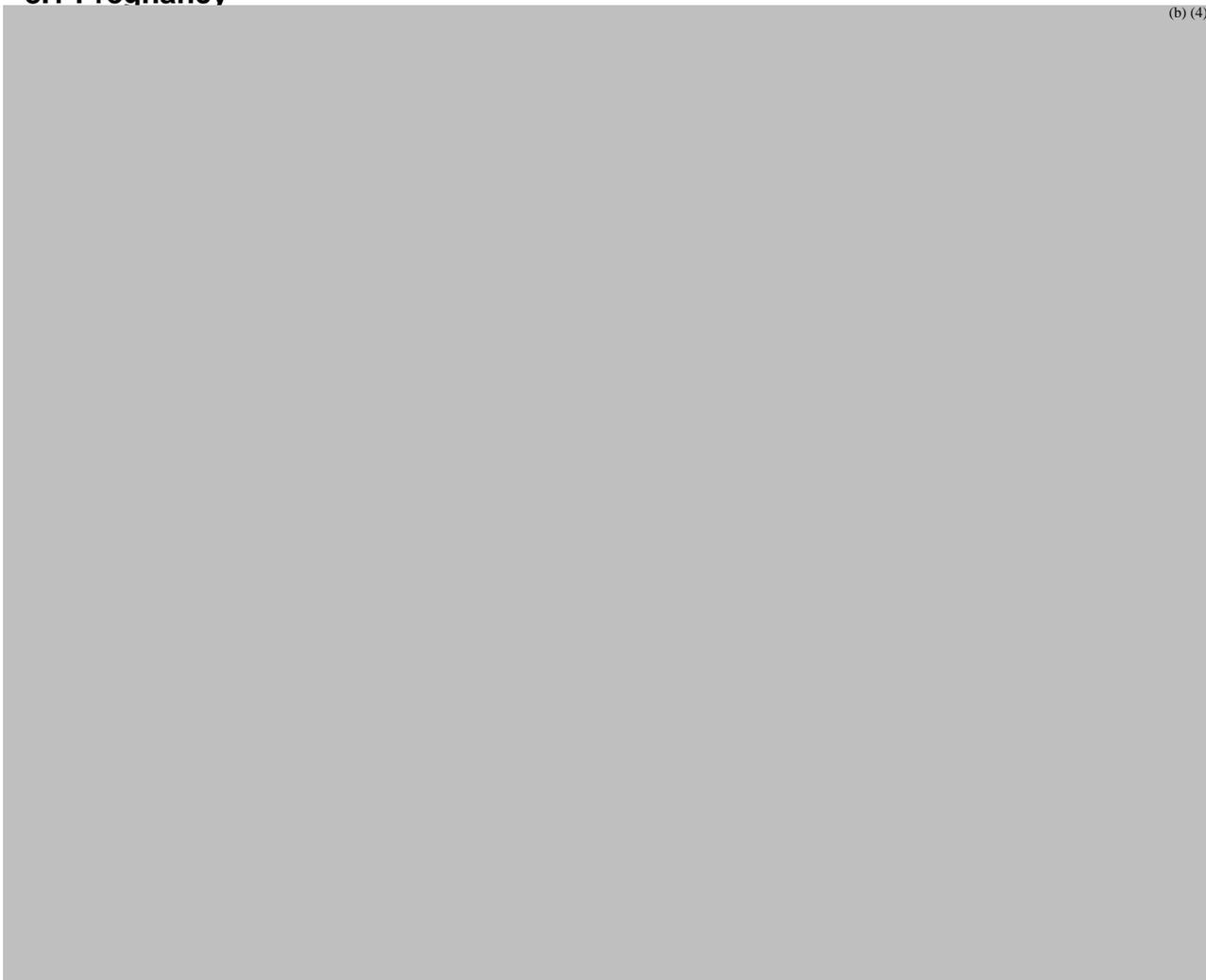
### 1.1.3 Labeling

The recommended label for sections 8.1 and 13.1 are as follows:

**Strikethrough represents deleted text, and italicized font represents inserted text.**

**8.1 Pregnancy**

(b) (4)



**13 NONCLINICAL TOXICOLOGY**

**13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility**

(b) (4)



## 1.2 Brief Discussion of Nonclinical Findings

The nonclinical safety assessment of Adrenalin® for ocular use relied on reports obtained from the literature. All of the effects seen in animals were due to the expected pharmacologic and supra-pharmacologic actions of epinephrine. No unexpected non-clinical effects of epinephrine have been reported.

Ocular studies with commercially available epinephrine formulations have shown adverse effects on the cornea, including increased corneal thickness, increased corneal epithelial cell density and morphological changes. Published nonclinical studies have shown that these effects are due to a combination of the dose of Na sulfite to the eyes and the low pH of buffered epinephrine formulations. However, this will not be an issue in the ocular use of Adrenalin® since the 1:1,000 formulation will be diluted by at least 100-fold before use and the concentration of Na sulfite after dilution is not expected to cause significant effects on cornea.

## 2 Drug Information

2.1 Drug: Adrenaline®

2.1.1 CAS Registry Number (Optional): 51-43-4

2.1.2 Generic Name: Epinephrine

2.1.3 Code Name: None

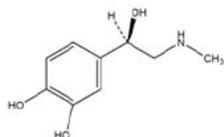
2.1.4 Chemical Name:

(-)-3,4-Dihydroxy- $\alpha$ -[(methylamino)methyl]benzyl alcohol

2.1.5 Molecular Formula/Molecular Weight:

C<sub>9</sub>H<sub>13</sub>NO<sub>3</sub>/ 183.20 (free base) (b) (4)

2.1.6 Structure:



2.1.7 Pharmacologic class: Epinephrine is an endogenous catecholamine that is a nonselective agonist of adrenergic receptors.

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

IND 111,712

2.3 Clinical Formulation

2.3.1 Drug Formulation

Table 2.3.P.1.1 Quantitative Composition (b) (4)

Ingredient	Grade	Function	Batch Quantity	Unit Formula
Epinephrine	USP	Active	(b) (4)	(b) (4)
Sodium Chloride	USP	Tonicity adjustor	(b) (4)	9.0 mg
Sodium Metabisulfite	NF	Antioxidant	(b) (4)	1.0 mg
(b) (4) Hydrochloric Acid	USP	(b) (4)	(b) (4)	(b) (4)
Water for Injection	USP	(b) (4)	(b) (4)	(b) (4)



**c) Comparison of the proposed formulation to approved epinephrine injection NDA's**

Based on the current formulation from the package insert for EpiPen<sup>®</sup> (NDA 19-430) , JHP's Adrenalin<sup>®</sup> (epinephrine injection, USP) is similar to the currently approved NDA's as indicated by the formulations presented in the table below.

Ingredient	Adrenalin <sup>®</sup>	EpiPen <sup>®</sup> Auto-injector (mg/mL)
	(mg/mL)	
Epinephrine	1:1000	1:1000
Sodium Metabisulfite	1	(b) (4)
Sodium Chloride	9	(b) (4)
Hydrochloric Acid	(b) (4)	(b) (4)
Water for Injection	(b) (4)	(b) (4)

**2.3.1.1 Comments on Novel Excipients:**

Sodium metabisulfite is used as an antioxidant (b) (4)

**2.3.1.2 Comments on Impurities/Degradants of Concern:**

(b) (4)

## 2.4 Proposed Clinical Population and Dosing Regimen

The use of Adrenaline® in hypersensitivity reactions (severe acute anaphylactic reactions (b) (4)) will be reviewed separately by DPARP (Dr. Jane Sohn).

Only the ophthalmic use will be reviewed here.

The Ophthalmic use: Induction of Mydriasis during Cataract Surgery

Adults and Pediatric: to maintain mydriasis, Adrenalin® may be added to the irrigation fluid at very low doses (1:100,000 to 1:1,000,000 [ $10^1$  mcg to 1 mcg/mL]). (b) (4)

Adrenalin® may also be injected intraocularly as a bolus dose in 0.1 mL at a dilution of 1:100,000 to 1:400,000 ( $10^1$  mcg/mL to 2.5 mcg/mL).

<sup>1</sup>Actual value is (b) (4) based on API/mL listed in the clinical drug product.

## 2.5 Regulatory Background

Epinephrine injection, USP (currently manufactured by JHP as Adrenalin®), has been available on the market for over 100 years and in addition to the emergent treatment of severe allergic reactions, has been well accepted in the medical community as a safe and effective drug for such unapproved uses as hemostasis, decongestion, bronchial asthmatic paroxysms, inhibiting uterine contractions, and glaucoma (AHFS, American Society of Health-System Pharmacists Drug Information manual). Epinephrine injection, USP is also the main drug used during resuscitation from cardiac arrest as outlined in the advanced cardiovascular life support (ACLS) algorithm for management of cardiac arrest from the American Heart Association. While FDA has only approved two single-entity epinephrine drug products for the emergency treatment of severe allergic reactions (EpiPen® Auto-injector, NDA 19-430, approved 12-22-1987 and Twinject® Auto-injector, NDA 20-800 approved 5-30-2003), there are many unapproved epinephrine drug products available on the market.

Although unapproved epinephrine drug products have been available on the market for over 100 years, JHP has made the decision to file an NDA for approval of Adrenalin® (epinephrine injection, USP) for the treatment of anaphylaxis (b) (4) supported by consensus reports found in the literature. JHP submitted background materials for Adrenaline® on June 3, 2011 to the FDA to discuss the filing of NDA. The meeting date was July 5, 2011. A memorandum of meeting minutes dated August 8, 2011 was sent to JHP by the FDA.

## 3 Studies Submitted

(b) (4)  
The sponsor-conducted 14-day IV studies and genotoxicity studies

for epinephrine also evaluated the toxicity of [REDACTED] (b) (4). These studies will be reviewed by DPARP in a separate review.

The nonclinical safety assessment of Adrenalin® for ocular use relied on reports obtained from the literature. Assessment included literature data on general toxicity when epinephrine is applied topically to the eye and by intracameral injections. The potential toxicity of Na bisulfite, the anti-oxidant included in the Adrenalin® drug product, was also assessed using the literature.

### 3.1 Studies Reviewed

Table 2.6.6.8.1 lists representative published studies where animals were exposed to EP by the ocular route at doses many times over the dose proposed for the ocular use of Adrenalin®.

There are 6 studies using the topical route, 5 studies using the intracameral route, 2 *ex vivo* studies, and 2 studies with Na bisulfite alone using a subconjunctival injection. Just a few pertinent studies will be discussed here.

Besides pupillary dilation, other effects of EP on the eye include decreases in IOP associated with effects on circulation of aqueous humor. There is also the potential effect on vasoconstriction. Formal GLP ocular toxicity studies have not been performed with Adrenalin® specifically or EP in general. Many published studies have been looking at the pharmacological and potential adverse effects of EP when applied to the eye. Potential adverse effects would be due to the supra-pharmacological activity of EP.

The doses proposed for maintenance of mydriasis during cataract surgery are: 1:1,000,000 to 1:100,000 dilutions (1 to 10 µg/mL) for topical irrigation, and a 0.1 mL intracameral injection of 2.5 to 10 µg/mL (0.25 to 1 µg total injected) [REDACTED] (b) (4).

The topical exposures in all the animal studies were by bolus application. To provide an estimate of the ratio of the animal dose to the proposed human irrigation dose, a human dose of 10 µg given topically was used in the calculation. For intracameral injections, the top dose of 1 µg was used to estimate the ratio of animal exposure over human exposure.

For topical application, doses tested ranged from 0.2 to 2000 µg/eye, corresponding to 0.02x to 200x ratios compared to the highest human topical dose. No unexpected pharmacology or toxicity was reported in any of these studies using topical EP. Intracameral doses ranged from 0.2 to 500 µg/eye, corresponding to 0.2x to 500x ratios compared to the highest human intracameral injection. As with topical application, intracameral injections did not result in unexpected pharmacological effects.

Ocular studies with commercially available epinephrine formulations have shown adverse effects on the cornea, including increased corneal thickness, increased corneal epithelial cell density and morphological changes. Published nonclinical studies have shown that these effects are due to a combination of the dose of Na sulfite to the eyes and the low pH of buffered epinephrine formulations.

The corneal epithelial effects of Na sulfite in EP formulations used for mydriasis in cataract surgery were studied by Hull et al (1975 [245-250]) and Hull (1979 [1380-1381]). These studies were done with *ex vivo* tissue, using eyes collected from rabbit and owl monkeys. Corneal thickness was measured, and tissues examined by SEM (scanning electron microscopy) and TEM (transmission electron microscopy). After a 5 minute perfusion with commercial EP at 1,000 µg/mL (Group 1) significant increases in corneal thickness were observed. This dose of EP has 1,000 µg/mL Na bisulfite; 100x the concentration of the recommended human dose with Adrenalin®. Scanning electron microscopy showed loss of endothelial cell margins with apparent rupture of the plasma membrane in some endothelial cells. The TEM examination revealed severe damage to the corneal epithelium with swelling of the nucleus, mitochondria and endoplasmic reticulum; along with irregularity of the posterior cell membrane. When the 1,000 µg/mL EP formulation was diluted 5-fold (to 200 µg/mL Na bisulfite) (Group 2) no swelling was seen and there were no SEM or TEM morphological changes. The concentration of 200 µg/mL is 20x the human dose for Adrenalin®. No swelling or morphological changes were seen when EP bitartrate (1,000 µg/mL EP without Na bisulfite) (Group 3) was perfused. When corneas were perfused with 1,000 µg/mL of Na bisulfite alone (Group 4), the same adverse effects were seen as with 1,000 µg/mL EP with 1,000 µg/mL Na bisulfite. Na bisulfite alone was tested at two lower concentrations. At 800 µg/mL the swelling was about half as that at 1,000 µg/mL. At the low concentration of 500 µg/mL, no swelling was observed. The concentration of 500 µg/mL Na bisulfite is 50x the maximum concentration to be used with Adrenalin®.

Olson et al (1980 [2224]) looked at Na bisulfite containing EP formulations injected intracamerally in cats. At the highest doses tested (500 µg/eye EP; 500 µg/eye Na bisulfite) there were no effects on corneal epithelial cell density. These doses are both 500x the maximum dose in humans.

Edelhauser et al (1982 [327-333]) tested four commercial formulations of EP, all containing Na bisulfite. (See Table 2.6.6.8.1 for the EP and Na bisulfite content of each formulation.) Injections of the EP formulations resulted in marked corneal edema occurring 3 hr after injection, and lasting 10 days. Loss of corneal epithelial cells was also observed. The corneas developed pannus and vessel invasion within the corneal stroma. Importantly, the buffered salt solution control, when acidified to pH 4.0, resulted in the same morphological lesions as the EP formulations. The authors examined the buffering capacity of each of the formulations and found the three products that are provided at 1:10,000 (100 µg/mL) all have very high buffering capacity compared to the Parke-Davis product which is provided at 1:1,000 (1,000 µg/mL) and needs to be diluted 1:10 to be used, thus lowering its buffering capacity further.

The conclusion from the authors was the final pH change in the anterior chamber was a very significant contributor to the morphological changes seen with intracamerally injected EP formulations. Adrenalin® will be provided at 1:1,000 (1,000 µg/mL) which will be diluted, at the least, 100-fold to 10 µg/mL for both irrigation and bolus intracameral use. This will significantly reduce the acidity of the 1:1,000 formulation. (b) (4)

Therefore,

the ocular use of Adrenalin® will not damage the corneal epithelium from a pH stand point. And providing a high concentration of EP (1:1,000), which requires dilution for use, is a benefit. In addition, 100-fold dilution will also provide a significant dilution of the Na bisulfite.

Slack et al (1990 [77-82]) looked at corneal edema with Na bisulfite-free EP injected intracamerally at a dose 200x that of the maximum Adrenalin® dose. Some non-statistically significant corneal edema was observed, compared to controls, but the authors concluded the edema was markedly less than the edema reported with Na sulfite-containing EP formulations (reported in Edelhauser et al, 1982). The same EP formulation was tested *ex vivo* in excised human eyes, and no corneal edema or SEM changes were seen, when the eyes were perfused for 3 hours at 1:250,000 and 1:500,000 dilutions (2 and 4 µg/mL). This provides further support that diluting out 1:000 EP decreases the buffering capacity, and decreases effects on the cornea.

Two studies assessed the toxicity of Na bisulfite alone in rabbits given by the subconjunctival route. Doses of 45, 180, and 360 µg/eye of Na bisulfite resulted in intracellular vacuolization and thickening of the corneal endothelial cell layer, as seen by light microscopy (Weinreb et al 1986 [525-531]). The doses are 45x, 180x, and 360x that which will be injected intracamerally with Adrenalin®. The low dose of 45 µg/eye resulted in only slightly increases of intracellular vacuoles compared to controls.

Treatment with 180 and 360 µg/eye resulted in more significant vacuolization. In another study, Na bisulfite was given to rabbits by subconjunctival injection at a dose of 3200 µg/eye/day for 3 days (Chapman et al 1992 [189-196]). In this study, no pathology was seen using light microscopy.

In conclusion, ocular studies with commercially available epinephrine formulations have shown adverse effects on the cornea are due to Na sulfite. However, this will not be an issue in the ocular use of Adrenalin® since the 1:1,000 formulation will be diluted by at least 100-fold before use and the concentration of Na sulfite after dilution is not expected to cause significant effects on cornea.

Table 2.6.6.8.1 Ocular Studies with Epinephrine

Species Route	Treatment and Regimen Sample Size	Summary of Results <sup>a</sup> Source of EP	Ratio Over Human Dose <sup>b</sup>	References	Sulfite Content <sup>c</sup>
<b>Topical</b>					
Rabbits	L-EP 500 and 1100 µg/eye  n = 3 or 4	Decreased IOP.  Source = Epitrate and Epifrin	EP: 50x to 110x	Birss et al, 1978 [1049]	yes, unknown
Rabbits	L-EP and D-EP 2000 µg/eye  n= 6	Initial increase in IOP, then decrease in IOP. Increase in pupillary diameter. D-EP elicited the same effects to similar degrees. Small, but measurable, effects of L-EP and D-EP on systemic arterial blood pressure.  Source = Sigma (L-EP = Sterling Winthrop)	EP: 200x	Rowland and Potter, 1981 [30]	none
Rhesus monkey	L-EP 330 µg/eye, 5000 µg/mL  2 drops, 15 min between each drop, assume 0.033 mL/drop  n=12	No effect on iris vasculature, decreased IOP.  Source = Epifrin	EP: 33x	Virdi and Hayreh, 1984 [6]	yes, unknown
Rabbits	L-EP and D-EP 7.8 to 1000 µg/eye (in 50 µL)  n=6	Decreased IOP, and increased pupillary diameter. L-EP 4-5x more potent than D-EP on pupillary diameter and IOP.  Source = NR	EP: 0.8x to 100x	Gherezghiher and Koss, 1985 [22-25]	NR
Rabbits	L-EP 250 µg/eye  n=10	Decreased IOP, increased aqueous humor outflow.  Eppy (Smith and Nephew Pharmaceuticals)	EP: 25x	Anderson and Wilson, 1990 [121-124]	NR

Species Route	Treatment and Regimen Sample Size	Summary of Results <sup>a</sup> Source of EP	Ratio Over Human Dose <sup>b</sup>	References	Sulfite Content <sup>c</sup>			
Rabbit	L-EP 500 µg/eye	Decreased IOP, increased aqueous humor outflow, increased pupillary diameter.  Source = Research Biochemical	EP: 50x	Crosson and Petrovich, 1999 [2056-2059]	NR			
Rabbit	EP 0.2 to 360 µg/eye  n=6	Expected effects on IOP.  Source = NR (EP bitartrate)	EP: 0.02x to 36x	Rom et al, 1997 [315-318]; Schwartz et al, 2002 [135-136]	NR			
<b>Intracamerally</b>								
Cat	EP 20, 100, 500 µg/eye, in 1 mL  n = 7 to 10	No effects observed on corneal endothelial cell density.		Olson et al, 1980 [2224]	yes, 200-1000 µg/mL			
		<b>Group</b>	<b>EP (µg/eye)</b>			<b>Na bisulfite (µg/eye)</b>	<b>EP</b>	<b>Sulfite</b>
		1	20			20	20x	20x
		2	100			100	100x	100x
3	500	500	500x	500x				
Rabbit	EP 20 to 200 µg/eye (0.2 mL injected)  n = NR	Injections of the EP formulations resulted in marked corneal edema occurring 3 hr after injection, and lasting 10 days. Loss of corneal epithelial cells was also observed. The corneas developed pannus and vessel invasion within the corneal stroma. The effects on the cornea were associated with Na bisulfite content and the pH of the formulations. See text for more detail.		Edelhauser et al, 1982 [327-333]	yes, 500-2000 µg/mL			
		<b>EP (µg/eye)</b>	<b>Na bisulfite (µg/eye)</b>			<b>EP</b>	<b>Sulfite</b>	
		IMS Min-I-Jet	20			400	20x	400x
		Bristoject	20			100	20x	100x
		Abboject	20			100	20x	100x
Parke-Davis 1 mL	200	200	200x	200x				

Species Route	Treatment and Regimen Sample Size	Summary of Results <sup>a</sup> Source of EP	Ratio Over Human Dose <sup>b</sup>	References	Sulfite Content <sup>c</sup>				
Rabbit	EP 200 µg/eye  n=6	Na bisulfite-free EP resulted in a small amount of non-statistically significant corneal edema. The edema was markedly less compared to previously reported studies with Na bisulfite-containing EP formulations. See text for more detail.  Source = American Regent Laboratories - EP, preservative - and Na bisulfite-free	EP: 200x	Slack et al, 1990 [77-82]	none				
Rabbit	EP 2, 4 and 20 µg/eye  n=7	No morphological effects on corneal epithelium or corneal thickness, using in vivo specular microscopy, and light and scanning EM.  Source = Bosmin Inj, Daiichi Seiyaku Co, Japan	EP: 2x, 4x, 20x	Liou et al, 2002 [469]	NR				
Rabbit	EP 0.2 µg/eye, 0.02 mL of 10 µg/mL  n=10	Expected pupillary dilation. No difference in corneal thickness or endothelial cell density, compared to controls. Increased endothelial cytoplasmic vacuolization as seen with transmission EM, and decreased microprojections as seen with scanning EM. Using the TUNEL assay, EP-treated eyes had an apoptosis index of 5%.  Source = Dan Han Pharm	EP: 0.2x	Kim et al, 2010 [563-570]	NR				
Species Route	Treatment and Regimen Sample Size	Summary of Results <sup>a</sup> Source of EP	Ratio Over Human Dose <sup>b</sup>	References	Sulfite Content <sup>c</sup>				
<b>Ex vivo perfusion</b>									
Rabbits, Owl monkeys ( <i>ex vivo</i> )	EP 200 to 1000 µg/mL perfused  n = NR	See text for full summary of these studies.			Hull et al, 1975 [245-250]; Hull, 1979 [1380-1381]	yes, 1000 µg/mL			
		Group	Time of perfusion (min)	EP µg/mL			Na bisulfite µg/mL	EP	Sulfite
		1	5	1000			1000	100x	100x
		2	360	200			200	20x	20x
		3	360	1000			0	100x	
		4	5	0			500		50x
	5	0	800		80x				
	5	0	1000		100x				
Source = EP with 1000 µg/mL Na bisulfite; EP bitartrate without Na bisulfite									
Human <i>ex vivo</i>	EP 3 hour perfusion at 1:250,000 and 1:500,000 dilutions from a 1:1,000 formulation, resulting in 2 and 4 µg/mL perfusion  n=10	Scanning EM revealed no morphological changes.  Source = American Regent Laboratories - EP, preservative- and Na bisulfite-free	EP: 0.2x to 0.4x	Slack et al, 1990 [79]	none				
<b>Subconjunctival injection</b>									
Rabbit	Na bisulfite 45, 180, 360 µg/eye  n=3	Dose-responsive increased intercellular vacuolization as seen by light microscopy and thickening of the corneal endothelial cell layer.  Source = n.a.	sulfite: 45x, 180x, 360x	Weinreb et al, 1986 [525-531]	n.a.				

Species Route	Treatment and Regimen Sample Size	Summary of Results <sup>a</sup> Source of EP	Ratio Over Human Dose <sup>b</sup>	References	Sulfite Content <sup>c</sup>
Rabbit	Na bisulfite 3200 µg/eye/day, for 3 days  n=3	No pathology seen with hematoxylin-eosin staining, and light microscopic examination.  Source = n.a.	sulfite: 3200x	Chapman et al, 1992 [189-196]	n.a.

<sup>a</sup> No non-pharmacologically expected effects reported.

<sup>b</sup> Maximum human doses: 10 µg/mL topical irrigation; 1 µg injected intracamerally.

For the purposes of calculating a safety margin, 10 µg per eye (or 10 µg/mL) was used for topical studies and perfusion studies, and 1 µg/eye for studies where EP was injected. For calculation of Na bisulfite ration, for the Adrenalin<sup>®</sup> 1 mL vials, a 1 µg EP injection contains 1 µg Na bisulfite.

EP = ratio for epinephrine

sulfite = ratio for Na bisulfite

<sup>c</sup> Amount of Na bisulfite in the material used:

NR = not reported

None = no Na bisulfite in the material used

Yes, unknown = Na bisulfite in the material, but concentration unknown

Yes, XX µg/mL = Na bisulfite in the material at XX µg/mL.

D-EP = D-epinephrine

EM = electron microscopy

EP = epinephrine, but D and/or L content unknown

L-EP = L-epinephrine

n.a. = not applicable

intracamerally = injected into anterior chamber of the eye

IOP = intraocular pressure

NR = not reported

## Developmental and Reproductive Toxicity

**Key Findings:** The sponsor provided four published articles that highlighted the potential for epinephrine to produce reproductive toxicity. The developmental and reproductive toxicity-related articles submitted provided an overview of the potential effect of epinephrine administration during the human 1<sup>st</sup> trimester equivalent in mice, rabbits, hamsters and rats. In animal models epinephrine was shown to affect implantation, produce structural teratogenicity, decrease embryofetal survival and development, and alter postnatal behavior when administered early in gestation (human 1<sup>st</sup> and 2<sup>nd</sup> month equivalent). These studies provided the basis for data included in sections 8.1 and 13.1 of the proposed label, and are summarized as follows.

Subcutaneous administration of epinephrine to rabbits at a dose of 1.2 mg/kg/day (~19,000-fold the human highest intraocular dose) on gestational days 3 to 9 produced an increased incidence of arrested fetal development and structural teratogenicity (gastroschisis). Subcutaneous administration of epinephrine to mice at a dose of 1 mg/kg/day (~4,000-fold the human highest intraocular daily dose) on gestational days 6 to 15 produced delayed skeletal ossification. These effects were not observed in mice at a dose of 0.5 mg/kg/day (2,000-fold the human highest intraocular daily dose). Subcutaneous administration of epinephrine to hamsters at a dose of 0.5 mg/kg/day (~3,000-fold the highest intraocular human dose) on gestational days 7 to 10 resulted in decreased litter size and delayed skeletal ossification. Behavioral effects were reported in the offspring of rats receiving subcutaneous administration of 0.4mg/kg epinephrine (~3,000-fold the human

highest intraocular daily dose) on gestational days 7-12. (The above dose human dose multiples are based on an intraocular human dose of 1 µg / 50 kg individual).

Epinephrine has also been shown to affect fertility. Decreased implantation was shown in female rats administered epinephrine subcutaneously at 0.4mg/kg/day (~3,000-fold the human highest intraocular daily dose) on gestational days 1 to 6; female rabbits administered epinephrine subcutaneously at 1.2 mg/kg/day (~19,000-fold the human highest intraocular daily dose) on gestational days 3 to 9; and female hamsters administered epinephrine subcutaneously at 0.5mg/kg/day (3,000-fold the human highest intraocular daily dose) on gestational days 7 to 10.

*Reviewer Comments;*

- It should be noted that bioavailability data for epinephrine are not available. The extent of human systemic exposure at the recommended human intraocular dose is unknown. The above safety margin/human dose multiples are likely overestimated given that bioavailability following ocular administration is expected to be considerably less than 100%. As such the collective nonclinical data indicate that potential reproductive and teratogenic effects at or near the clinical dose are highly unlikely, but cannot be entirely excluded.
- While the literature indicates that the administration of epinephrine produces its predominant effects early in gestation (reduced implantation and teratogenesis), no data was submitted to address the effects of epinephrine administration during the human 2<sup>nd</sup> and 3<sup>rd</sup> trimester equivalent. It should be noted that epinephrine causes vasoconstriction of placental vessels, and the resulting fetal anoxia/hypoxia would be expected to affect development throughout pregnancy, the extent of which would be determined by dose and the temporal window of exposure. Exposure during the 2<sup>nd</sup> or 3<sup>rd</sup> trimester is often associated with developmental delay and behavioral effects, as growth and neural development peak during these developmental stages. Accordingly, behavioral/neurologic effects (as well as fetal deaths) have been reported in the fetuses/offspring of both animals and humans as a result of epinephrine administration during late gestation (e.g. for anaphylaxis).

### 3.2 Studies Not Reviewed

General nonclinical safety evaluation of Adrenalin® for subcutaneous/intramuscular use in anaphylaxis is supported by the EpiPen® and Twinject® approvals for subcutaneous and intramuscular treatment for anaphylaxis. General nonclinical safety evaluation (b) (4) is supported by (b) (4) the GLP rat intravenous 14-day toxicity studies conducted by the Sponsor. The Sponsor also conducted a GLP bacterial reverse mutation study and an *in vitro* CHO cell chromosomal aberration study with epinephrine (study #s 246549 and 246548) and

provided data showing that epinephrine has mutagenic potential under the conditions of these assays. In conjunction with data contained in submitted literature, the sponsor concluded that epinephrine has mutagenic potential.

No original studies were submitted to assess *in vivo* mutagenicity or carcinogenicity potential. Given the acute nature of the proposed epinephrine dosing regimen for ocular use, the lack of *in vivo* data does not pose a significant safety concern. These genotoxicity studies will be reviewed by DPARP in support of the anaphylaxis indication.

### 3.3 Previous Reviews Referenced

None.

## 11 Integrated Summary and Safety Evaluation

Epinephrine injection, USP (currently manufactured by JHP as Adrenalin®) has been available on the market for over 100 years and in addition to the emergent treatment of severe allergic reactions, has been well accepted in the medical community as a safe and effective drug for such unapproved uses as hemostasis, decongestion, bronchial asthmatic paroxysms, inhibiting uterine contractions, and glaucoma. Epinephrine injection, USP is also the main drug used during resuscitation from cardiac arrest.

While FDA has only approved two single-entity epinephrine drug products for the emergency treatment of severe allergic reactions (EpiPen® Auto-injector NDA 19-430 approved on 12-22-1987 and Twinject® Auto-injector NDA 20-800 approved on 5-30-2003), there are many unapproved epinephrine drug products available on the market. JHP has made the decision to file an NDA for approval of Adrenalin® for the treatment of anaphylaxis and induction of mydriasis during cataract surgery. General nonclinical safety evaluation of Adrenalin® for anaphylaxis will be reviewed in a separate review by DPARP.

The nonclinical safety assessment of Adrenalin® for ocular use relied on reports obtained from the literature. Assessment included literature data on general toxicity when epinephrine is applied topically to the eye and by intracameral injections. For ocular use of Adrenalin®, no unexpected non-pharmacological effects of epinephrine have been reported. The potential toxicity of Na bisulfite, the anti-oxidant included in the Adrenalin® drug product, was also assessed using the literature. Ocular studies with commercially available epinephrine formulations have shown adverse effects on the cornea, including increased corneal thickness, increased corneal epithelial cell density and morphological changes. The data have shown that these effects are due to a combination of the dose of Na sulfite to the eyes and the low pH of buffered epinephrine formulations. However, this will not be a safety issue for ocular use of Adrenalin® since the 1:1,000 formulation will be diluted by at least 100-fold before the use and the concentration of Na sulfite after dilution is not expected to cause significant effects on cornea. The use of Adrenalin® for induction and maintenance of mydriasis during

intraocular surgery, as described in the labeling, is recommended from the nonclinical perspective. The recommendations for changes to sections 8.1 and 13.1 of the label are contained in the executive summary of this review.

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

/s/  
-----

LORI E KOTCH  
08/17/2012

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

**PHARMACOLOGY/TOXICOLOGY NDA CHEMISTRY CONSULTATION**

Application number: 204-200  
Supporting document/s: SD 1 (EDR)  
Applicant's letter date: 3/7/12  
CDER stamp date: 3/7/12  
Product: Adrenalin® (epinephrine injection)  
Indication: Treatment of anaphylaxis  
Applicant: JHP Pharmaceuticals LLC  
Review Division: Division of Pulmonary, Allergy and  
Rheumatology Drug Products (DPARP)  
Reviewer: Jane J. Sohn, Ph.D.  
Supervisor/Team Leader: Molly E. Shea, Ph.D.  
Division Director: Badrul Chowdhury, M.D., Ph.D.  
Project Manager: Carol Hill

*Template Version: September 1, 2010*

**Disclaimer**

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 204200 are owned by JHP Pharmaceuticals LLC or are data for which JHP Pharmaceuticals LLC has obtained a written right of reference. Any information or data necessary for approval of NDA 204200 that JHP Pharmaceuticals 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 NDA 204200.

## TABLE OF CONTENTS

<b>1</b>	<b>EXECUTIVE SUMMARY .....</b>	<b>4</b>
1.1	INTRODUCTION .....	4
1.2	BRIEF DISCUSSION OF NONCLINICAL FINDINGS .....	4
1.3	RECOMMENDATIONS .....	8
<b>2</b>	<b>DRUG INFORMATION .....</b>	<b>8</b>
2.1	DRUG .....	8
2.2	RELEVANT INDS, NDAs, BLAs AND DMFs .....	9
2.3	DRUG FORMULATION .....	9
2.4	COMMENTS ON NOVEL EXCIPIENTS .....	11
2.5	COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN .....	11
2.6	PROPOSED CLINICAL POPULATION AND DOSING REGIMEN .....	13
2.7	REGULATORY BACKGROUND .....	14
<b>3</b>	<b>STUDIES SUBMITTED.....</b>	<b>14</b>
3.1	STUDIES REVIEWED.....	15
	ALL STUDIES WERE REVIEWED.....	15
3.2	STUDIES NOT REVIEWED .....	15
3.3	PREVIOUS REVIEWS REFERENCED.....	15
<b>3</b>	<b>PHARMACOKINETICS .....</b>	<b>15</b>
<b>4</b>	<b>GENERAL TOXICOLOGY.....</b>	<b>18</b>
4.1	REPEAT-DOSE TOXICITY .....	18
<b>5</b>	<b>GENETIC TOXICOLOGY .....</b>	<b>35</b>
5.1	<i>IN VITRO</i> REVERSE MUTATION ASSAY IN BACTERIAL CELLS (AMES).....	35
5.2	<i>IN VITRO</i> ASSAYS IN MAMMALIAN CELLS.....	41
5.3	OTHER GENETIC TOXICITY STUDIES: COMPUTATIONAL TOXICOLOGY .....	46
<b>6</b>	<b>INTEGRATED SUMMARY AND SAFETY EVALUATION .....</b>	<b>52</b>

## Table of Tables

Table 1: Safety Margins for proposed (b) (4) specifications.....	5
Table 2: Components and quantitative composition.....	9
Table 3: Drug product specifications .....	10
Table 4: Proposed specifications for impurities .....	11
Table 5 Impurities/degradants.....	12
Table 6: Maximum exposure for each impurity.....	13
Table 7: Maximum dose by age group .....	14
Table 8: 2 week rat dose ranging study: Mortalities .....	20
Table 9: 2 week rat dose ranging study: Body weight gain .....	21
Table 10: 2 week rat dose ranging study: Feed consumption .....	22
Table 11: 2 week IV rat study: Clinical Signs .....	26
Table 12: 2 week IV rat study: Body weight gain.....	26
Table 13: 2 week IV rat study: Food consumption .....	27
Table 14: 2 week IV rat study: Ophthalmic findings .....	28
Table 15: 2 week IV rat study: Percent change in hematology parameters.....	29
Table 16: 2 week IV rat study: Percent change in clinical chemistry parameters. ....	29
Table 17: 2 week IV rat study: Gross pathology lesions.....	30
Table 18: 2 week IV rat study: Organ weights (absolute and normalized).....	31
Table 19: 2 week IV rat study: Histological findings in animals that survived to Day 14	33
Table 20: 2 week IV rat study: (b) (4) dosage formulation concentrations .....	34
Table 21: Bacterial mutation assay: Dose preparation analysis .....	37
Table 22: (b) (4) bacterial mutation assay results in TA98 strain (+S9) .....	38
Table 23: (b) (4) bacterial mutation assay results in TA1537 strain (+S9) .....	38
Table 24: Epinephrine bacterial mutation assay results in TA100 strain (-S9) .....	39
Table 25: Relative cell growth as an indication of cytotoxicity for dose selection.....	44
Table 26: Chromosomal aberrations: (b) (4) .....	45
Table 27: Chromosomal aberrations: epinephrine .....	46
Table 28: Safety Margins for proposed (b) (4) specifications.....	53
Table 29: Impurity specifications and exposures.....	57

# 1 Executive Summary

## 1.1 Introduction

The sponsor JHP Pharmaceuticals LLC (JHP) is proposing [REDACTED] (b) (4) Adrenalin® with the API epinephrine for the treatment of anaphylaxis in adults and pediatric patients (all ages by the intramuscular (IM), subcutaneous (SC) [REDACTED] (b) (4) routes). Toxicology studies were conducted to support the safety for proposed specifications [REDACTED] (b) (4). The safety of four additional epinephrine-related impurities [REDACTED] (b) (4) is addressed.

## 1.2 Brief Discussion of Nonclinical Findings

Adrenalin is an unapproved product marketed by JHP. Impurities were discussed with the sponsor on July 9, 2011 at the Pre-IND Meeting. The sponsor conducted toxicology studies to support the safety of [REDACTED] (b) (4) and submitted literature and levels of [REDACTED] (b) (4) to support the safety of [REDACTED] (b) (4). Safety evaluations [REDACTED] (b) (4) were requested by CMC for this consult, and are summarized below.

[REDACTED] (b) (4) are epinephrine-related impurities that are considered degradants for this review.

[REDACTED] (b) (4)

(b) (4)

### Other impurities

(b) (4)

A comprehensive computational genetic toxicology assessment from CDER predicted that all three impurities are negative for mutagenicity for the Ames assay and *E.coli*.

### **1.3 Recommendations**

The proposed levels for the impurity (b) (4) are not supported by adequate safety margins in the pivotal toxicology study, or the levels in approved products. Additionally, the levels (b) (4) in the drug product are not supported by the levels in currently approved products. Although (b) (4) does not contribute to additional safety risk compared to the API (b) (4) its presence in the drug product offers no benefit to the patient population.

This nonclinical review recommends that the sponsor reduce the level (b) (4) and recommends further evaluation (b) (4) from Quality. It may be appropriate for the sponsor to reduce levels (b) (4) to those found in currently approved products per Guidance for Industry: ANDAs: Impurities in Drug Products (November 2010). It is difficult for this nonclinical review to recommend a decrease in levels (b) (4) without a defined adverse effect due to (b) (4) alone.

A comment from nonclinical was forwarded on May 24, 2012 to CMC to include in a joint Information Request. The comment is as follows:

“5. Your proposed levels for the impurity (b) (4) are not supported by adequate safety margins in your toxicology study, or the levels in approved products. Reduce the level (b) (4) and include your plan with revised acceptance criterion (see comment 1.)”

## **2 Drug Information**

### **2.1 Drug**

CAS Registry Number (Optional): 51-43-4

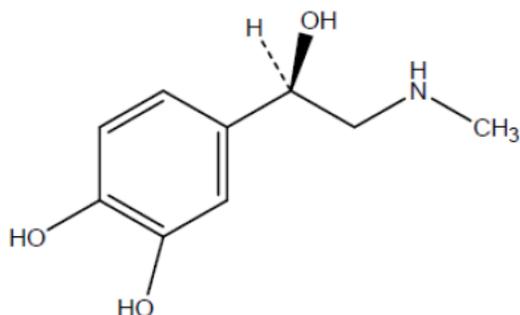
Generic Name: epinephrine injection, adrenaline injection

Code Name: Adrenalin®

Chemical Name: epinephrine

Molecular Formula/Molecular Weight: C9-H13-N-O3/183.20442

Structure or Biochemical Description:



Pharmacologic Class: Sympathomimetic catecholamine

## 2.2 Relevant INDs, NDAs, BLAs and DMFs

pIND 111712, Adrenalin® (JHP Pharmaceuticals), Division of Pulmonary, Allergy and Rheumatology Products (DPARP).

## 2.3 Drug Formulation

The sponsor is proposing (b) (4) for the indication of anaphylaxis in a single-use vial (b) (4). The active pharmaceutical ingredient epinephrine is proposed at a (b) (4) overage (Section 3.2.P.3.2.).

(b) (4)  
Sodium metabisulfite is present (b) (4)

**Table 2: Components and quantitative composition**

Ingredient	Function	mg/mL
(b) (4)	(b) (4)	(b) (4)
NaCl	Tonicity agent	9.00
Sodium Metabisulfite (as Sodium Bisulfite)	Anti-oxidant	1.00 (b) (4)
HCl (b) (4)	(b) (4)	

Epinephrine USP Synthetic	Active Ingredient	(b) (4)
		(b) (4)

**Table 3: Drug product specifications**

JHP proposes to utilize the following Test and Acceptance Criteria for release and end of shelf life for the drug product, Adrenalin® Injection 1 mg/mL, as indicated below.

Test	Specification	Specification
	Release	Stability
Description	(b) (4)	
Assay	(b) (4)	
Individual Unidentified Impurity	(b) (4)	
Total Impurities*	(b) (4)	
Identification	(b) (4)	
pH	(b) (4)	
Sodium Bisulfite	(b) (4)	
Total Acidity	(b) (4)	
Color & Clarity	(b) (4)	
Sterility	(b) (4)	
Particulate Matter	(b) (4)	
Bacterial Endotoxin	(b) (4)	
AME	(b) (4)	

\* Total Impurities

Sponsor's table (Section 3.2.P.5.1)

[Redacted] (b) (4)

**Table 4: Proposed specifications for impurities**

[Redacted] (b) (4)

**2.4 Comments on Novel Excipients**

None for this consult. Excipients will be reviewed in the NDA review.

**2.5 Comments on Impurities/Degradants of Concern**

Toxicology studies were conducted to support the safety for proposed specifications (b) (4)  
[Redacted] The safety of four additional epinephrine-related (b) (4)  
impurities (b) (4) is addressed  
using literature and levels in approved products. (b) (4)  
[Redacted]

**Table 5 Impurities/degradants**

Impurity (IUPAC; Common Name)	Origin	Structure
(b) (4)		

The method of analysis for related substances in Adrenalin<sup>®</sup> Injection 1 mg/mL is stability indicating and capable of detecting and quantifying all the known and unknown impurities.

Sponsor's Table (Section 3.2.P.5.5)

An additional impurity shown in Table 3 is listed as an "individual unidentified impurity" and is present at a (b) (4) specification release. CMC reviewer Dr. Ying Wang is requesting that the sponsor identify the impurity (draft IR sent to this reviewer via email on May 24, 2012.)

The maximum exposure to each impurity, (b) (4) is shown below. (b) (4)

--	--

**Table 6: Maximum exposure for each impurity**

(b) (4)

**2.6 Proposed Clinical Population and Dosing Regimen**

Adrenalin® is proposed for adults and pediatric patients (all ages) with anaphylaxis. In adults, Adrenalin is recommended via the intramuscular (IM) or subcutaneous (SC) routes at (b) (4) to 0.5 mg ((b) (4) to 0.5 mL of 1 mg/mL [1:1000] solution) up to a maximum of 0.5 mg per injection. Injections may be repeated every 5 to 10 minutes as necessary, (b) (4) No maximum dosage is proposed by the sponsor for the IM and SC routes for adults. Medical Officer Dr. Peter Starke recommends up to 3 IM/SC doses in adults and adolescents >12 years old, which translates into a maximum IM/SC exposure of 1.5 mg.

(b) (4)

For pediatric subjects, Adrenalin® is recommended at 0.01 mg/kg via the IM and SC routes, with additional injections every 5 to 10 minutes as necessary (b) (4) No maximum dosage or number of injections is given for IM and SC administration in pediatric patients. Dr. Starke recommends a maximum single dose of 0.3 mg for children 6-12 years old, and 0.15 mg for children <6 years old, with up to 3 doses for both populations. This translates into a maximum IM/SC exposure of 0.9 mg for children 6-12 years, and 0.45 mg for children <6 years old.

(b) (4)

[Redacted] (b) (4)

[Redacted] (b) (4)

[Redacted] (b) (4)

**2.7 Regulatory Background**

The FDA has had the following communications with JHP regarding Adrenalin®:

Date	Communication
March 11, 2011	Type B Meeting request regarding 505 (b) (2) application
June 3, 2011	Pre-IND 111712 Meeting Package
June 30, July 1, 2011	Pre-IND 111712 Preliminary Meeting Comments
July 5, 2011	Pre-IND 111712 Teleconference
August 4, 2011	Pre-IND 111712 FDA Meeting minutes sent to sponsor
March 7, 2012	NDA Submitted

**3 Studies Submitted**

The following studies were submitted to NDA 204200, and are reviewed under this consult:

Pharmacokinetics	Study #
------------------	---------

Dev. and validation of an LC/MS/MS method for the measurement of epinephrine (b) (4) in dosing solutions and its use in support of toxicity studies.	246977
<b>Toxicology</b>	<b>Study #</b>
A 14-day repeated IV dose range finding toxicity study in SD rats given epinephrine and (b) (4)	246111
A 14-day IV toxicity study in SD rats given epinephrine and (b) (4)	246269
In vitro bacterial reverse mutation assay of epinephrine (b) (4)	246549
In vitro chromosome aberration test of epinephrine (b) (4) in CHO cells	246548
Computational toxicity assessment using the Leadscope FDA model applier (b) (4)	2010.10.1
Computational toxicity assessment using the Leadscope FDA model applier for epinephrine	2011.04.07

### 3.1 Studies Reviewed

All studies were reviewed.

### 3.2 Studies Not Reviewed

None.

### 3.3 Previous Reviews Referenced

None.

## 3 Pharmacokinetics

### Development and validation of an LC/MS/MS method for the measurement of epinephrine and (b) (4) in dosing solutions and its use in support of toxicity studies (study no. 246977) (GLP)

The sponsor developed a method for measuring (b) (4) and epinephrine in dosing solutions. This review assesses if requirements for full validation were met, per Guidance for Industry: Bioanalytical Method Validation, May 2001.

The study includes statements for GLP compliance and Quality Assurance.

Experiments were performed (b) (4)

#### **Methods and Validity:**

An Agilent 6410 LC/MS/MS system was operated in the MS/MS mode with an Agilent Model 1200 liquid chromatograph for the duration of this validation.

The reference standards were epinephrine (lot # 1047153) and (b) (4) (lot # WH-83-52-29), supplied by JHP Pharmaceuticals LLC. The purities of Epinephrine and (b) (4) were 99.6% and 96.3%, respectively as

reported on the Certificates of Analysis (b) (4) These were supplied by reputable commercial sources, and are considered authenticated analytical reference standards. The reference standards were used as the analyte in spiked calibration (reference) standards. The internal standard was USP Diphenhydramine hydrochloride reference standard supplied by (b) (4)

The matrix was 0.9% sodium chloride solution (saline) lot # J1L 406 (expiry March 2014). This LC/MS/MS method is proposed, however, to measure test article in dosing solutions made with 0.9% sodium chloride solution (saline) acidified with HCl. Although the use of saline as the matrix is not optimal, further partial validation using saline acidified with HCl as the matrix was conducted in other study reports by using calibration standards made with saline acidified with HCl (see **“In Vitro Reverse Mutation Assay in Bacterial Cells” study #246549** under Section 5.1 of this review.)

Calibration standards were prepared by spiking saline with known amounts of epinephrine and (b) (4) to create one set of 9 non-zero calibration standards ranging from 2.00 to 1500 µg/mL (2.00, 4.00, 10.0, 50.0, 100, 500, 1000, 1300 and 1500 µg/mL). This meets the recommendation for spiking of matrix with analyte, number of non-zero standards, and a non-zero standard included at the LLOQ. The set of standards was stored at  $-80^{\circ}\text{C} \pm 10^{\circ}\text{C}$ .

The quality control samples for epinephrine and (b) (4) were designated QC A (6.00 µg/mL), QC B (750 µg/mL), QC C (1200 µg/mL) and QC D (2400 µg/mL). Eighteen QC samples, 6 at each concentration level (QC A, QC B and QC C), were included with each validation batch. The QC samples were stored at  $-80^{\circ}\text{C} \pm 10^{\circ}\text{C}$ . This exceeds the recommended number of replicates at each concentration, and meets the minimum 3 concentrations in the expected range (reported as 2.00 µg/mL to 1500 µg/mL.)

The stability of (b) (4) and epinephrine was assessed using the following conditions, by comparison to freshly thawed and diluted samples:

- Freeze-thaw stability was assessed using 6 replicates of the QC A and QC C samples frozen at  $-80^{\circ}\text{C} \pm 10^{\circ}\text{C}$  for three (3) consecutive cycles (for a minimum of 24 hours per cycle) and thawed for three (3) consecutive cycles by keeping the samples at ambient room temperature for less than one hour each cycle.
- Short-term stability was assessed using 6 replicates of the QC A and QC C samples removed from the freezer ( $-80^{\circ}\text{C} \pm 10^{\circ}\text{C}$ ) and kept on the bench at ambient room temperature for 7.1 hours.
- Long-term stability was assessed two ways.
  - “Storage stability”: Six (6) replicates of each of the QC A, QC B, QC C samples and the calibration standards from one batch were stored at  $2^{\circ}\text{C}$  to  $8^{\circ}\text{C}$  for 72.8 hours after their initial injections. At the end of the storage period these samples were re-injected.
  - “Fridge stability”: Fridge stability was assessed using six (6) replicates of each of the QC A and QC C samples. The undiluted stability samples were removed from the freezer ( $-80^{\circ}\text{C} \pm 10^{\circ}\text{C}$ ) and kept at  $2^{\circ}\text{C}$  to  $8^{\circ}\text{C}$  for 169.3 hours.

- Post-preparative stability (autosampler stability) was assessed by storing 6 replicates of each of the QC A and QC C samples in the autosampler at 2°C to 8°C for 47.2 hours after their initial injections. At the end of the storage period these samples were reinjected and compared to freshly thawed and diluted QC samples.

Notably, long-term stability sampling was not repeated on three separate occasions, after storage exceeding the time between the date of first sample collection and date of last sample analysis, as recommended by the Guidance for Industry: Bioanalytical Method Validation, May 2001. However, repeated assessment on 5 days (November 16, 17, 18, 19, 22 of 2011) was performed for QC A (6.00 µg/mL), QC B (750 µg/mL), and QC C (1200 µg/mL) for determining precision and accuracy for the method as a whole.

Stock solution stability does not appear to have been performed by evaluating the stability of stock solutions of test article and the internal standard at room temperature for at least 6 hours. The short-term stability assay (described above), however, is adequate to assess the affect on room temperature for 6 hours on both test articles. Thus, the stability testing methods overall are adequate.

### Results:

For sensitivity, the sponsor identified the lower limit of quantitation (LLOQ) to be 2.00 µg/mL. The LLOQ was determined based on 6 replicate injections of 2.00 µg/mL, with a precision of 4.2% and an accuracy of 95.5% (b)(4) and precision (% CV) of 2.1% and accuracy (% nominal) of 96.5% for epinephrine. The precision and accuracy at the LLOQ are acceptable. For selectivity, a representative chromatogram shows that at the LLOQ, discrete peaks are present for each (b)(4) and epinephrine, which have a different retention time than diphenhydramine.

Precision and accuracy were acceptable, based on (b)(4) and epinephrine samples QC LLOQ (2.00 µg/mL), QC A (6.00 µg/mL), QC B (750 µg/mL), and QC C (1200 µg/mL). For both test articles, analysis was performed for within batch analysis on 5 separate days (November 16, 17, 18, 19, 22 of 2011) for QC A/B/C. QC LLOQ was included in 3 separate experiments). For both (b)(4) and epinephrine QC A/B/C, the within and between batch mean %CV was ≤15%, and mean % nominal were between 85 to 115%. For epinephrine QC LLOQ, the within and between batch mean %CV was ≤20%, and mean % nominal were between 80 to 120%.

The calibration curves showed acceptable linearity. The correlation coefficients were ≥0.9996 and ≥0.9952 for (b)(4) and epinephrine, respectively. For both (b)(4) and epinephrine, Standard A (2.00 µg/mL) had a mean %CV ≤20%, and mean % nominal between 80 to 120%. Additional standards had mean %CV ≤15%, and mean % nominal between 85 to 115%.

The stability of (b)(4) and epinephrine were assessed, and all samples, upon extraction, were compared to freshly prepared samples, and were within ±15%.

**Conclusion:** This method is validated for the measurement of (b) (4) and epinephrine in saline. For further validation of saline of acidified with HCl as the matrix, see the review of “**In Vitro Reverse Mutation Assay in Bacterial Cells (Ames) (study #246549)**” in Section 5.1 of this review.

## 4 General Toxicology

### 4.1 Repeat-Dose Toxicity

**Study title:** A 14-day repeated IV dose range finding toxicity study in SD rats

(b) (4)

Study no.:	246111
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 29, 2011
GLP compliance:	Non-GLP
QA statement:	No.
Drug, lot #, and % purity:	Epinephrine, lot 1047153, 99.6% purity; (b) (4) lot WH-83-52-29, 96.3% purity

The goal of this dose range finding study is to determine the maximum tolerated dose (MTD) of epinephrine and the combination of epinephrine and (b) (4) in Sprague-Dawley rats via the intravenous (IV) route of exposure based on once daily injections for 14 days. Animals that survived until the end of the study were sacrificed without further examination.

### Key Study Findings

- Within 5 minutes of dosing,  $\geq 50$  mcg/kg of epinephrine led to passivity, respiratory distress, tachypnoea, convulsions and death. Animals dosed with 25-50 mcg/kg epinephrine displayed passivity and tachypnoea. The MTD was determined to be 30 mcg/kg based on daily dosing for 2 weeks.

## Methods

## Doses &amp; Frequency:

Group	Dose (mcg/kg)
Single dose	
1) Epinephrine	1000
2) Epinephrine	300
Exploratory group: epinephrine	100
	50
	25
Repeat dose (daily for 14 days)	
3) Epinephrine	30
4) Epinephrine	50
5/6) Epinephrine + (b) (4)	9.3 + 20.7 (day 1), 30 + 67 (days 2-14)

*Adaptation of Table 4 on page 15 of the sponsor's study report*

The sponsor originally planned on dosing animals with epinephrine at 30, 50, 300, and 1000 mcg/kg IV daily, for 14 days. When toxicity and mortality was observed after Day 1 of dosing, unused rats were utilized for single dose exploratory studies at 25, 50, and 100 mcg/kg (see page 12 of study report for this explanation, and the original plan that was changed.) Groups dosed with 300 and 1000 mcg/kg died immediately after treatment, and therefore they only received a single dose.

For the combination of epinephrine and (b) (4), the conducting laboratory stated that treatment was initiated with 9.3 mcg/kg epinephrine, and 20.7 mcg/kg (b) (4) in Group 5. On Day 2, the dose was adjusted to 30 mcg/kg epinephrine and 67 mcg/kg (b) (4) at the request of the sponsor. This group was renamed Group 6, and animals continued to receive the updated dose daily from Day 2 to Day 14. This group is referred to as "Group 5/6" in this review.

Dose volume: 1 mL/kg  
 Formulation/Vehicle: Sodium chloride (0.9% w/v), pH 5-6  
 (adjusted with 2 N HCl)  
 Species/Strain: *Rattus norvegicus*/CD® [CrI:CD®(SD)BR] (Sprague-Dawley rats)  
 Number/Sex/Group: 3 males/group  
 Age: 7 - 8 weeks  
 Weight: 273 - 335 g  
 Satellite groups: Exploratory group.  
 Unique study design: After initiation of the study, animals that received 100 and 300 mcg/kg of epinephrine died. As a result, a single dose "exploratory" group was injected with 25, 50, 100 mcg/kg of epinephrine to test multiple doses. For further explanation, see

“Dose & Frequency” above. There was no vehicle control group. Deviation from study protocol: Group 5 animals were dosed with 9.3 mcg/kg epinephrine and 20.7 mcg/kg (b) (4) on day 1, and adjusted to 30 mcg/kg epinephrine and 67 mcg/kg (b) (4) thereafter. The name of this group was changed to Group 6. This group is referred to as “Group 5/6” in this review.

Group 1 and 2 animals were originally planned to receive 100 and 300 mcg/kg epinephrine IV daily for 14 days. As a result of mortality in all animals receiving these doses on Day 1, the animals only received a single dose.

Other protocol deviations appeared to not affect the substance or validity of the study.

## Observations and Results

### Mortality

Mortality checks were performed twice per day throughout the study.

There were mortalities observed in animals dosed with greater than 30 mcg/kg of epinephrine alone, as shown in the table below. The sponsor reported that deaths occurred within 5 min. of dosing, “preceded by respiratory distress, tachypnoea, convulsions and in some cases, bloody nasal discharge.” An exception was an animal dosed with 50 mcg/kg epinephrine that was found dead on day 10.

**Table 8: 2 week rat dose ranging study: Mortalities**

Group	Dose (mcg/kg)	Mortalities per number dosed	Percent mortality
Single dose			
1) Epinephrine	1000	2/2	100%
2) Epinephrine	300	2/2	100%
Exploratory group: epinephrine	100	2/3	67%
	50	1/1	100%
	25	0/1	0%
Repeat dose			
3) Epinephrine	30	0/3	0%
4) Epinephrine	50	1/3	33%
5/6*) Epinephrine + (b) (4)	9.3 + 20.7 (day 1), 30 + 67 (days 2-14)	0/3	0%

Note: There was no vehicle control group

\*This group was initially designated “group 5”, and received 9.3 mcg/kg epinephrine + 20.7 mcg/kg (b) (4) on Day 1. On Day 2, the dosing was changed to 30 mcg/kg epinephrine + 60 mcg/kg (b) (4), at the request of the sponsor. Upon updating the dosing, the sponsor designated this group as “group 6”.

## Clinical Signs

Animals were inspected twice daily. Clinical signs were recorded once a day during the morning observation period. If the afternoon observations differed they were also recorded. Detailed clinical examinations were performed weekly, including observations for general appearance, respiration, abnormalities for behavior and movement, and appearance of external organs, skin and any lesions.

Animals were also monitored closely for about 2 hours after each dosing. Observations included: reaction to treatment such as changes in skin, fur, eyes and mucous membranes. Respiratory, circulatory, autonomic, central nervous system, somatomotor activity and behavior patterns were also monitored along with any other signs of ill-health.

Within 5 minutes of dosing,  $\geq 50$  mcg/kg of epinephrine led to passivity, respiratory distress, tachypnoea, convulsions and death (see "Mortality"). Animals dosed with 25-50 mcg/kg epinephrine displayed passivity and tachypnoea.

## Body Weights

The unfasted body weight of each rat was recorded during the acclimatization period. Each rat was weighed again (unfasted) before dosing on Days 1, 7 and 14.

Body weights increased in animals dosed with epinephrine (30 and 50 mcg/kg) and epinephrine in combination with (b)(4). Body weight gain is calculated as the difference in mean weight on day 14 versus day 1, in the same dosage group (no vehicle controls available). Percent change was calculated with respect to time, within each dosage group. There was no difference in body weight gain between epinephrine alone (30 mcg/kg) and epinephrine plus (b)(4) (30 mcg/kg + 67 mcg/kg).

**Table 9: 2 week rat dose ranging study: Body weight gain**

Group	Dose (mcg/kg)	Percent gain	Weight gain (g)
3) Epinephrine	30	12%	38.5
4) Epinephrine	50	23%	69.7
5/6) Epinephrine + (b)(4)	9.3 + 20.7 (day 1), 30 + 67 (days 2-14)	13%	40.8

\*This group was initially designated "group 5", and received 9.3 mcg/kg epinephrine + 20.7 mcg/kg (b)(4) on Day 1. On Day 2, the dosing was changed to 30 mcg/kg epinephrine + 60 mcg/kg (b)(4), at the request of the sponsor. Upon updating the dosing, the sponsor designated this group as "group 6".

## Feed Consumption

The food intake of each rat was recorded during the 1-week pretreatment period and weekly during the study period.

Food consumption increased in animals dosed with epinephrine (30 and 50 mcg/kg) and epinephrine in combination with (b) (4). Change in food consumption is calculated as the difference in mean food consumption measured on days 7-14 versus days 1-7, in the same dosage group. Percent change was calculated with respect to time, within each dosage group (no vehicle controls available). There were no notable differences in feed in epinephrine alone (30 mcg/kg) and epinephrine plus (b) (4) (30 mcg/kg + 67 mcg/kg).

**Table 10: 2 week rat dose ranging study: Feed consumption**

Group	Dose (mcg/kg)	Percent change	Change in food consumption (g)
3) Epinephrine	30	15%	25.5
4) Epinephrine	50	30%	45.8
5/6)* Epinephrine + (b) (4)	9.3 + 20.7 (day 1), 30 + 67 (days 2-14)	14%	22.2

\*This group was initially designated "group 5", and received 9.3 mcg/kg epinephrine + 20.7 mcg/kg (b) (4) on Day 1. On Day 2, the dosing was changed to 30 mcg/kg epinephrine + 60 mcg/kg (b) (4), at the request of the sponsor. Upon updating the dosing, the sponsor designated this group as "group 6".

### Dosing Solution Analysis

Not done.

**Study title:** A 14-day repeated IV dose range finding toxicity study in SD rats given epinephrine and (b) (4)

Study no.: 246269  
 Study report location: EDR  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: October 31, 2011  
 GLP compliance: GLP  
 QA statement: Yes  
 Drug, lot #, and % purity: Epinephrine, lot 1047153, 99.6% pure;  
 (b) (4) lot WH-83-52-29, 96.3% pure.

### Key Study Findings

- There were 4 unscheduled deaths on Days 1 and 2 that led the sponsor to lower levels of epinephrine, and also (b) (4) (proportional to epinephrine.) On Day 1, one animal died that received epinephrine alone (30 mcg/kg, #019), one died that received epinephrine and (b) (4) (30 mcg/kg + 26 mcg/kg, #040), and one died that received epinephrine and (b) (4) (30 mcg/kg + 67 mcg/kg, #043). On Day 2, an additional animal that received epinephrine and (b) (4) (30 mcg/kg + 67 mcg/kg, #045) died. The sponsor reported mild pulmonary congestion (Rat #040), moderate pulmonary congestion (Rats #019 and #043), and moderate

hemorrhage (Rat #045). These animals are excluded from the analysis below as the dose of epinephrine was lowered from 30 to 20 mcg/kg epinephrine on Day 2.

- A low incidence of incipient anterior cortical cataracts is associated with epinephrine exposure (2/10 epinephrine only, 1/10 epinephrine and (b) (4) LD, 1/10 epinephrine and (b) (4) HD). Incidence did not increase in the presence of (b) (4).
- There was a moderate dose-dependent increase in monocytes (9% epinephrine and (b) (4) MD, 18% epinephrine and (b) (4) HD), with respect to levels of (b) (4). These moderate changes are not dose-limiting.
- There were no gross findings attributable to (b) (4). Gross unilateral hydronephrosis was observed in 1/10 control and 1/9 animals dosed with epinephrine and HD (b) (4). This was determined to be a sporadic finding. Enlarged mandibular lymph nodes were observed in 1/10 epinephrine only, 1/10 combination LD, and 1/9 combination MD animals. These findings are not attributable to (b) (4) alone.
- Microscopic unilateral hydronephrosis was observed in 2 animals that received combination HD treatment (#049, #050). Importantly, gross unilateral hydronephrosis was also observed in a control animal (#010) that developed microscopic kidney papillary cysts, and gross unilateral hydronephrosis was observed in a combination HD animal (#049). Based on the presence of gross unilateral hydronephrosis in one control animal, and the unilateral nature of this finding, the unilateral hydronephrosis is considered a background finding.
- One combination HD animal developed acute/focal inflammation in the heart (1/9). The incidence in SD male rats in the literature is reported as 13.8%. Thus, this is determined to be an incidental finding.
- The dosage solution for (b) (4) was not within 10% of the intended concentration.
- (b) (4) plus epinephrine did not result in greater and/or different toxicities than epinephrine alone.

## Methods

Doses:

Group	Dose (mcg/kg)		n#
	Epinephrine	(b) (4)	
1) Control	0	0	10
2) Epinephrine	20*	0	10
3) Epinephrine + (b) (4)	20*	6	10
4) Epinephrine + (b) (4)	20*	17	9
5) Epinephrine + (b) (4)	20*	45	9

\*Due to four unscheduled deaths by Day 2, the dose of epinephrine was lowered to the levels shown above (30 to 20 mcg/kg, and the dose concentrations of epinephrine/ (b) (4) were also adjusted.) The original doses on Day 1 were 30 mcg/kg of epinephrine with 9, 26 or 67 mcg/kg of (b) (4)

#Numbers of animals after Day 2

Frequency of dosing: Daily for 14 days  
Route of administration: Intravenous (IV)  
Dose volume: 1 mL/kg  
Formulation/Vehicle: Sodium chloride (0.9% w/v), pH 5-7 (adjusted with 2 N HCl)  
Species/Strain: *Rattus norvegicus*/CD® [CrI:CD®(SD)BR] (Sprague-Dawley rats)  
Number/Sex/Group: 10-11 males/group initially. 9-10 males/group after deaths on day 1.  
Age: 7 - 8 weeks  
Weight: 269-323 g  
Satellite groups: None  
Unique study design: Dosage solution containing epinephrine was spiked (b) (4)

Deviation from study protocol: The dosage solution (b) (4) was not within 10% of the intended concentration for all doses, and was in fact outside of 15% of the target dose for 5 out of 15 samples. Although the HD was within 10% of the target concentration, the lack of accuracy in 5 of 15 samples (which all showed lower measured doses compared to target doses) supports the use of the actual quantified doses for all dosage calculations. (See "dosage solution analysis" below for more information.)

## Observations and Results

## Mortality

Mortality checks were performed twice per day.

There were 4 unscheduled deaths on Days 1 and 2 that led the sponsor to lower levels of epinephrine and (b) (4) (proportionally to epinephrine.) On Day 1, one animal died that received epinephrine alone (30 mcg/kg, #019), one died that received epinephrine and (b) (4) (30 mcg/kg + 26 mcg/kg, #040), and one died that received epinephrine and (b) (4) (30 mcg/kg + 67 mcg/kg, #043). On Day 2, an additional animal that received epinephrine and (b) (4) (30 mcg/kg + 67 mcg/kg, #045) died. The sponsor reported mild pulmonary congestion (Rat #040), moderate pulmonary congestion (Rats #019 and #043), and moderate hemorrhage (Rat #045). These rats exhibited moderate passivity (1 rat) or marked convulsions (3 rats) prior to death. These deaths are consistent with epinephrine overdose.

**Table: 2 week IV rat study: Unscheduled deaths**

Group	Dose (mcg/kg)		unscheduled mortality
	Epinephrine	(b) (4)	
2) Epinephrine	20*	0	N=1, Day 1
4) Epinephrine	20*	17	N=2, Day 1
5) Epinephrine + (b) (4)	20*	45	N=1, Day 2

\*Due to four unscheduled deaths by Day 2, the dose of epinephrine was lowered from 30 to 20 mcg/kg and the dose concentrations of epinephrine/(b) (4) were also adjusted to the doses shown.

## Clinical Signs

Animals were inspected twice daily. Clinical signs were recorded once a day during the morning observation period. If the afternoon observations differed, they were also recorded. Detailed clinical examinations were performed weekly, including observations for general appearance, respiration, abnormalities for behavior and movement, and appearance of external organs, skin and any lesions.

Animals were also monitored closely for about 2 hours after each dosing. Observations included: reaction to treatment such as changes in skin, fur, eyes and mucous membranes. Respiratory, circulatory, autonomic, central nervous system, somatomotor activity and behavior patterns were also monitored along with any other signs of ill-health.

On Day 1, all rats dosed with 30 mcg/kg epinephrine exhibited mild tachypnoea and mild to moderate passivity within 5 minutes of dosing. With the exception of 4 rats that died, all animals recovered within 60 minutes of dosing.

Starting on Day 2, the dose of epinephrine was lowered from 30 mcg/kg to 20 mcg/kg. Several animals showed slight tachypnoea and/or passivity from Day 2 to Day 14 of the study, indicated in the table below. There was no increase in clinical signs based on

exposure to (b) (4) compared to epinephrine alone. Thus, the observed clinical signs appear to be attributable to epinephrine.

**Table 11: 2 week IV rat study: Clinical Signs**

Group	Dose (mcg/kg)		Number of animals exhibit clinical signs Day 2-14
	Epinephrine	(b) (4)	
1) Control	0	0	0/10
2) Epinephrine	20*	0	3/10
3) Epinephrine + (b) (4)	20*	6	0/10
4) Epinephrine + (b) (4)	20*	17	3/10
5) Epinephrine + (b) (4)	20*	45	3/10

\*Due to four unscheduled deaths by Day 2, the dose of epinephrine was lowered from 30 to 20 mcg/kg and the dose concentrations of epinephrine/ (b) (4) were also adjusted to the doses shown.

### Body Weights

The unfasted body weight of each rat was recorded three times during the acclimatization period: 2 weeks prior to study start; 1 week prior to study start for randomization; and the day prior to dosing to ensure mean body weights of the groups were similar. Each rat was weighed again (unfasted) before dosing on Days 1, 8 and 14. Finally, following an overnight period (approximately 12 to 18 hours) of food deprivation, each rat was weighed terminally prior to necropsy.

Body weight gain was calculated by determining the difference in weight on Day 14 versus Day 1 (predose). Percent change was calculated based on the percent of body weight gain in treated versus untreated groups. Percent change in body weight gain does not appear to be dependent on either test article as animals in groups 2 and 5 had the greatest percent change, but those in groups 2 and 3 showed little change. Therefore, the addition (b) (4) had no effect on body weight gain compared to epinephrine alone.

**Table 12: 2 week IV rat study: Body weight gain**

Group	Epinephrine	(b) (4)	Percent change	Body Weight gain (g)
1) Control	0	0	-	46.4
2) Epinephrine	20*	0	-21%	36.6
3) Epinephrine + (b) (4)	20*	6	0%	46.2
4) Epinephrine + (b) (4)	20*	17	-3%	45.1
5) Epinephrine + (b) (4)	20*	45	-28%	33.4

\*Due to four unscheduled deaths by Day 2, the dose of epinephrine was lowered from 30 to 20 mcg/kg and the dose concentrations of epinephrine/ (b) (4) were also adjusted to the doses shown.

### Feed Consumption

The food intake of each rat was recorded during the 1-week pretreatment period and weekly during the study period.

Food consumption was calculated based on total feed consumed from Day 1 to Day 14. Percent change was calculated based on the percent of food consumed in treated versus untreated groups. The percent change with respect to control animals does not appear to be dependent on either test article as animals in groups 2 and 5 had the greatest percent change, but those in groups 2 and 3 showed less change. This is reflected in body weight gain. Therefore, the addition (b) (4) had no effect on feed consumption compared to epinephrine alone.

**Table 13: 2 week IV rat study: Food consumption**

Group	Dose (mcg/kg)		Percent change	Food consumption (g)
	Epinephrine	(b) (4)		
1) Control	0	0	-	335.7
2) Epinephrine	20*	0	-6%	314.8
3) Epinephrine + (b) (4)	20*	6	-1%	333.6
4) Epinephrine + (b) (4)	20*	17	-4%	321.4
5) Epinephrine + (b) (4)	20*	45	-7%	312.9

\*Due to four unscheduled deaths by Day 2, the dose of epinephrine was lowered from 30 to 20 mcg/kg and the dose concentrations of epinephrine/ (b) (4) were also adjusted to the doses shown.

### Ophthalmoscopy

Funduscopy (indirect ophthalmoscopy) and biomicroscopic (slit lamp) examinations were performed on all study animals once during the pretreatment period and again prior to necropsy.

A low incidence of incipient anterior cortical cataracts is associated with epinephrine exposure (2/10 epinephrine only, 1/10 epinephrine and (b) (4) LD, 1/10 epinephrine and (b) (4) HD), with no increase in incidence due to exposure to (b) (4). Background findings include crystalline deposits, lens cataracts, and hyperreflective lesions in the posterior segment. Therefore, the addition (b) (4) had no effect on ophthalmic lesions compared to epinephrine alone.

**Table 14: 2 week IV rat study: Ophthalmic findings**

	Epinephrine/ (b) (4)									
	0	20 / 0	20 / 6	20 / 17	20 / 45	0	20 / 0	20 / 6	20 / 17	20 / 45
N	10	11	10	10	11	10	10	10	9	9
Lesion	Before first dose*					After last dose				
Cornea - Subepithelial Crystalline Deposits	5	7	3	4	2	5	5	5	6	3
Lens - Cataracts	0	1	0	0	0	0	1	0	0	0
Lens - Incipient Anterior Cortical Cataract	0	0	0	0	0	0	2	1	0	1
Posterior segment -hyperreflective lesion	0	0	0	0	1	0	0	0	0	2

### Hematology

Blood was collected on Day 14 prior to necropsy, and an adequate battery for hematology and coagulation was performed: red blood cell count (RBC), hemoglobin (Hb), Hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin volume (MCHV), mean platelet volume (MPV), platelets, white blood cell count (WBC), neutrophils, lymphocytes, monocytes, eosinophils, basophils, leucocytes (LUC), reticulocytes, prothrombin time, activated partial thromboplastin time (APTT). Animals were fasted overnight, with water *ad libitum*, and blood was collected (~6 mL) via the abdominal aorta following anesthesia induced by isoflurane. For coagulation parameters, citrate was used as an anticoagulant. The following parameters were analyzed:

There was a moderate dose-dependent increase in monocytes (9% MD, 18% HD), with respect to levels of (b) (4) dosing. There were increases in eosinophils (20%) and basophils (50%) in the epinephrine dosed group only, which were not observed in animals dosed with both epinephrine and (b) (4). It is difficult to determine if this is due to epinephrine alone because there is only one group dosed with epinephrine only. Most likely, changes in eosinophils and basophils are sporadic, and not due to epinephrine. The increase in monocytes due to (b) (4) is slight, and not considered dose-limiting. Therefore, the (b) (4) has no dose-limiting effects on hematology compared to epinephrine alone.

**Table 15: 2 week IV rat study: Percent change in hematology parameters**

Parameter	Unit	Epinephrine/ (b) (4)			
		20 / 0	20 / 6	20 / 17	20 / 45
Monocytes	x10 <sup>9</sup> /L	0%	0%	9%	18%
Eosinophils	x10 <sup>9</sup> /L	20%	0%	0%	-20%
Basophils	x10 <sup>9</sup> /L	-50%	0%	0%	0%

### Clinical Chemistry

Blood was collected on Day 14 prior to necropsy, and an adequate battery for Clinical chemistry was performed: albumin/globulin ratio (A/G), albumin (ALB), globulin (GLOB), alkaline phosphatase (ALP), bilirubin total (Bil(T)), urea nitrogen (BUN), calcium (Ca), chloride (Cl), creatinine, glucose, lactate dehydrogenase (LDH), phosphorus (P), potassium (K), total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), sodium (Na), triglycerides, creatine kinase (CK), cholesterol, gamma glutamyl transferase (GGT). Animals were fasted overnight, with water *ad libitum*, and blood was collected (~6 mL) via the abdominal aorta following anesthesia induced by isoflurane.

There were no changes that were clearly attributable to epinephrine or (b) (4). The sponsor reported a statistically significant change ( $P < 0.05$ ) in chloride in MD and HD groups dosed with both test articles, but these percent changes were very slight (2%) compared to control. There were dose-dependent decreases in lactate dehydrogenase (LDH) and creatine kinase (CK) that were observed in the MD and HD combination groups, but similar changes were also observed with epinephrine alone and not in the LD combination group. Therefore, the addition (b) (4) had no notable effect on clinical chemistry parameters compared to epinephrine alone.

**Table 16: 2 week IV rat study: Percent change in clinical chemistry parameters.**

Parameter	Unit	Epinephrine/ (b) (4)			
		20 / 0	20 / 6	20 / 17	20 / 45
Cl	mmol / L	1%	1%	<b>2%</b>	<b>2%</b>
LDH	u / L	-31%	4%	-41%	-45%
CK	u / L	-31%	9%	-31%	-42%

Note: Bold indicates  $P < 0.05$

### Urinalysis

Urine was collected (over approximately a 12 to 18 hour period) by placing rats in metabolic cages during the week prior to necropsy. Animals were fasted overnight, with water *ad libitum*, prior to urine collection. In the event that urine was not obtained for any animal using the conventional collection technique, a sample was recovered by cystocentesis during the necropsy procedure.

There were no notable findings based on urinalysis.

## Gross Pathology

Gross necropsy observations included an examination of the external surfaces of the body; all orifices; cranial cavity; external surfaces of the brain and spinal cord; nasal cavity and paranasal sinuses; joints; thoracic, abdominal, and pelvic cavities and viscera.

The four animals that died before scheduled sacrifice were found with diffuse hemorrhaging and fluid/edema in the lungs and trachea, consistent with epinephrine overdose. One animal also displayed autolysis in the abdominal cavity, resulting from the beginning stages of decomposition. The addition (b) (4) had no effect on death rates compared to epinephrine alone.

There were no clear findings attributable to (b) (4). Unilateral hydronephrosis was observed in 1/10 control and 1/9 animals dosed with epinephrine and HD (b) (4). Enlarged mandibular lymph nodes were observed in 1/10 epinephrine only, 1/10 combination LD, and 1/9 combination MD animals. No similar findings were seen in animals dosed with epinephrine and HD (b) (4); thus, this was determined to be a sporadic finding. Overall, the addition (b) (4) had no effect on gross pathology compared to epinephrine alone.

**Table 17: 2 week IV rat study: Gross pathology lesions**

Lesion	Control n = 10	Epinephrine/ (b) (4)			
		20 / 0 n = 10	20 / 6 n = 10	20 / 17 n = 9	20 / 45 n = 9
Kidney - unilateral, hydronephrosis	1	0	0	0	1
Mandibular lymph nodes -- enlarged	0	1	1	1	0

## Organ Weights

Organ weights were determined for each animal euthanized as per schedule at the end of the study. Paired organs were weighed together. The following organs were dissected, trimmed free of fat and weighed: adrenals, brain, heart, kidneys, liver, lungs, pituitary gland, prostate, spleen, thymus, and testes. The epididymides were not weighed, nor were any female sex organs available. Weights are reported as absolute, normalized to body weight (BW), and normalized to brain weight (BrW)

For epinephrine alone, percent changes >10% (compared to control) were observed in the adrenal glands (11% BW), prostate gland (-19% BW), lungs (17% BW), and thymus (-10% BrW). For the lung, there was one animal that received only epinephrine, and survived until the end of the study, which showed hemorrhage. There were no microscopic findings for the other organs that showed percent change in weight >10%, and these changes were not consistently seen in animals dosed with both epinephrine and (b) (4)

For epinephrine and (b) (4) in combination, there were no organs that showed changes that were dose-dependent on (b) (4), with greater severity than observed with epinephrine alone. Percent weight changes consistently >10% were observed in pituitary glands of animals dosed with both test articles, but they were not dose dependent with (b) (4). Therefore, the addition (b) (4) had no effect on organ weights compared to epinephrine alone.

**Table 18: 2 week IV rat study: Organ weights (absolute and normalized)**

Organ	Absolute			
	Epinephrine/ (b) (4)			
	20 / 0	20 / 6	20 / 17	20 / 45
	n =10	n =10	n = 9	n = 9
Adrenal Glands	8%	2%	12%	12%
Prostate Gland	-21%	-9%	-4%	-15%
Lungs	14%	12%	14%	7%
Thymus	-11%	-17%	-11%	-23%
Pituitary gland	-8%	-14%	-17%	-15%

Organ	Normalized by body weight				Normalized by brain weight			
	Epinephrine/ (b) (4)				Epinephrine/ (b) (4)			
	20 / 0	20 / 6	20 / 17	20 / 45	20 / 0	20 / 6	20 / 17	20 / 45
	n =10	n =10	n = 9	n = 9	n =10	n =10	n = 9	n = 9
Adrenal Glands	11%	1%	13%	18%	10%	2%	10%	13%
Prostate Gland	-19%	-9%	-4%	-11%	-19%	-9%	-5%	-14%
Lungs	17%	11%	15%	13%	16%	12%	13%	8%
Thymus	-9%	-17%	-10%	-19%	-10%	-17%	-12%	-22%
Pituitary gland	-5%	-14%	-16%	-11%	-6%	-14%	-18%	-14%

## Histopathology

Adequate Battery: Yes.

The following organs were preserved and evaluated:

Adrenals; Aorta (thoracic); Brain (3 levels); Cecum; Colon; Duodenum; Epididymides; Esophagus; Eyes; Heart; Ileum; Jejunum; Kidneys; Lacrimal Glands; Liver (sample of central and left lobes); Lungs (left & right diaphragmatic lobes); Lymph Node (Mandibular); Lymph Node (Mesenteric); Optic Nerves; Pancreas; Pituitary; Prostate; Salivary Gland (mandibular); Sciatic Nerve; Skeletal Muscle (quadriceps); Skin (inguinal); Spinal Cord (cervical); Spleen; Sternum & Marrow; Stomach; Testes; Thymus; Thyroid / Parathyroids; Tongue; Trachea; Urinary Bladder; and Injection site.

(Note: The following tissues were not examined: bone marrow smear, bone (femur), gall bladder, Harderian gland, larynx, nasal cavity, peripheral nerve, pharynx, rectum, seminal vesicles, zymbal gland, and female-only tissues.)

Peer Review: No.

### Histological Findings

Changes observed in tissues were graded on a scale of: None = 0; Minimal = 1; Mild = 2; Moderate = 3; Marked =4; Severe = 5.

On Day 1, one animal died that received epinephrine alone (30 mcg/kg, #019), one died that received epinephrine and (b) (4) (30 mcg/kg + 26 mcg/kg, #040), and one died that received epinephrine and (b) (4) (30 mcg/kg + 67 mcg/kg, #043). On Day 2, an additional animal that received epinephrine and (b) (4) (30 mcg/kg + 67 mcg/kg, #045) died. The sponsor reported mild pulmonary congestion (Rat #040), moderate pulmonary congestion (Rats #019 and #043), and moderate hemorrhage (Rat #045). **These animals are excluded from the analysis below.**

For animals that survived the duration of the study, lung hemorrhage and focal liver inflammation in 1/10 animals was observed in animals that received epinephrine alone. With respect to (b) (4), unilateral hydronephrosis was observed in 2 animals that received combination HD treatment (#049, #050). Interestingly, gross unilateral hydronephrosis was observed in a control animal (#010) that developed microscopic kidney papillary cysts, and gross unilateral hydronephrosis was observed in a combination HD animal (#049). Based on the presence of gross unilateral hydronephrosis in one control animal, and the unilateral nature of this finding, the unilateral hydronephrosis is considered a background finding.

One combination HD animal developed acute/focal inflammation in the heart (1/9). The incidence in SD male rats is reported as 13.8%<sup>9</sup>. Thus, this is determined to be an incidental finding.

---

<sup>9</sup> Peckham, JC (2002). Animal Histopathology. In MJ Derelanko and MA Hollinger (Eds.) Handbook of Toxicology (649-740). Washington DC: CRC Press.

**Table 19: 2 week IV rat study: Histological findings in animals that survived to Day 14**

Organ	Lesion	ID #	Epinephrine/ <sup>(b) (4)</sup>				
			Control	20 / 0	20 / 6	20 / 17	20 / 45
		N	1-10	11-20	21-30	31-40	41-50
			10	10	0/10	0/9	9
Kidneys:	inflammation, focal, subchronic, cortical	Grade 2	1	1	ND	ND	0
	cyst papillary	Grade 2	1	0	ND	ND	0
	hydronephrosis, unilateral	Grade 2	0	0	ND	ND	1
		Grade 3	0	0	ND	ND	1
Liver	inflammation, subacute, focal	Grade 2	0	1	ND	ND	0
Lungs	hemorrhage, focal	Grade 2	0	1	ND	ND	0
Heart	inflammation, acute, focal	Grade 2	0	0	ND	ND	1
Eyes	retinal thinning, bilateral	Grade 2	1	0	ND	ND	0
Injection site	inflammation, subchronic, perivascular	Grade 2	5	0	ND	ND	2
		Grade 3	0	0	ND	ND	1
	foreign body (hair) in perivascular tissue	Grade 2	0	0	ND	ND	1

ND = scoring not done

### Toxicokinetics

Note done.

### Dosing Solution Analysis

The concentration of epinephrine and <sup>(b) (4)</sup> were measured in aliquots collected from dosing solution preparations on Days 1, 2, and 13 (stored at  $-80 \pm 10^\circ\text{C}$ ), for a total of 15 samples per test article. Concentrations were determined using an Agilent 6400 series LC/MS/MS system. The lower limit of quantitation (LLOQ) was established to be 2.00 mcg/mL, with a calibration range from 2.00 mcg/mL to 1500 mcg/mL for epinephrine and <sup>(b) (4)</sup> (based on spiked standards). The internal standard was USP diphenhydramine hydrochloride, and the matrix was saline solution. The use of saline solution is not optimal, considering that the test article was diluted in acidified saline solution first, then further diluted in saline solution. This, however, does not constitute a need to repeat this study.

Fifteen dose formulation samples were assayed in duplicate, for a total of 30 samples for each test article, assayed on November 24, 2011. For epinephrine, dose formulation samples from Days 1, 2 and 13 ranged from 93.1% to 105.3%, which are within an acceptable range.

Samples (b) (4), however, ranged from 83.3% to 99.1% (b) (4) on initial analysis (November 24, 2011). In addition, all samples on Day 1 were higher than measured in Day 2 and Day 13 because of the decrease in dosage on Day 2. For the purpose of this review, the lower concentration used on Days 2 and 14 will be utilized for safety margin calculations.

The sponsor stated that a “total of five (5) samples were selected for analytical repeats (b) (4). All five (5) samples were repeated due to RCV (reassay to obtain confirming value, sample did not meet specification for percent targeted claim of ±15%)” (page 209 of the study report). For the purpose of this review, initial (November 24, 2011) and repeat assay results (November 25, 2011, December 14, 2011) from Day 2 and Day 13 are shown in the table below. Although the HD was within 10% of the target concentration, the lack of accuracy in 5 of 15 samples (which all showed lower measured doses compared to target doses) supports the use of the actual quantified doses for all dosage calculations.

**Table 20: 2 week IV rat study: (b) (4) dosage formulation concentrations**

Target concentration (mcg/mL)	(b) (4) Concentration (mcg/mL)								Overall concentration
	Day2				Day 13				
	Assay 1	Assay 2	Assay 3	Average	Assay 1	Assay 2	Assay 3	Average	
0	0.00	-	-	0.00	0.00	-	-	0.00	0.00
	0.00	-	-	0.00	0.00	-	-	0.00	
0	0.00	-	-	0.00	0.00	-	-	0.00	0.00
	0.00	-	-	0.00	0.00	-	-	0.00	
6.000	5.13	-	-	5.13	4.68	5.89	5.34	5.30	5.26
	5.04	5.58	5.29	5.30	5.05	5.66	5.25	5.32	
17.000	16.00	-	-	16.00	16.85	-	-	16.85	16.31
	16.31	-	-	16.31	14.10	16.85	17.32	16.09	
45.000	43.36	-	-	43.36	41.16	-	-	41.16	41.83
	41.83	-	-	41.83	40.98	-	-	40.98	

“-“ indicates that samples that were within 15% of the target concentration and were not assayed again

APPEARS THIS WAY ON ORIGINAL

## 5 Genetic Toxicology

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

**Study title:**

Study no.:	246549
Study report location:	SD 1 (EDR)
Conducting laboratory and location:	(b) (4)
	M1H 2W4
Date of study initiation:	October 4, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) Batch No. WH-83-52-29, 96.3%; Epinephrine, Batch No. 1047153, 99.6%

**Key Study Findings**

- (b) (4) was negative for genotoxic responses in the *in vitro* bacterial reverse mutation assay using both the plate incorporation and preincubation methods, in the presence and absence of metabolic activation.
- This study was found to be valid.

## Methods

- Strains: *Salmonella typhimurium* strains, TA98, TA100, TA1535, TA1537 and *Escherichia coli* strain WP2 *uvrA*.
- Concentrations in definitive study: For both the plate incorporation and preincubation tests, plates were treated with 12, 37, 110, 330 and 1000 mcg/plate (b) (4). For epinephrine, plates were treated with 62, 190, 560, 1700, and 5000 mcg/plate. Testing was conducted with and without S9 (10%; rat liver homogenate from male Sprague-Dawley rats treated with Aroclor 1254). Concentrations of positive controls are stated under "Positive controls."
- Basis of concentration selection: (b) (4) concentration was based on limit of solubility; precipitation (b) (4) was observed at 1000 mcg/plate with S9. Epinephrine concentration was based on the maximum recommendation by ICH S2 (R1). The sponsor's dose selection appears acceptable.
- Negative control: 0.9% Sodium Chloride Injection, USP (Saline) acidified to 10.5 to 14% v/v of 2N HCl in saline.
- Positive control: Positive controls used are shown below:

Strain	S9	Positive Control	Concentration/plate
TA98	-	2-nitrofluorene	5 mcg
	+	benzo[a]pyrene	5 mcg
TA100	-	2-nitrofluorene	5 mcg
	+	benzo[a]pyrene	5 mcg
TA1535	-	sodium azide	5 mcg
	+	cyclophosphamide	100 mcg
TA1537	-	9-aminoacridine	100 mcg
	+	benzo[a]pyrene	5 mcg
WP2 <i>uvrA</i>	-	methyl methanesulfonate	1 mL
	+	2-aminoanthracene	100 mcg

- Formulation/Vehicle: Test article was solubilized in saline acidified with HCl, then added to saline.
- Incubation & sampling time: The plate incorporation and preincubation (20 minute preincubation at 37 °C) methods were used in these studies. Plates were incubated for approximately 48-72 hours at 37 ± 2°C, which is acceptable.

## Study Validity

The study is considered valid for the following reasons:

- 1) The appropriate controls were tested and positive controls produced significant increases ( $P < 0.01$ ) in the frequency of revertant colony counts in the presence and absence of S9.
- 2) The appropriate strains were tested.

- 3) (b) (4) concentration was based on limit of solubility; precipitation (b) (4) was observed at 1000 mcg/plate with S9. Epinephrine was dosed up to 5000 mcg/plate, based on the maximum recommendation by ICH S2 (R1). The sponsor's dose selection is acceptable.

The method for quantification of (b) (4) and epinephrine was validated using saline as the matrix in the study “**Dev. and validation of an LC/MS/MS method for the measurement of epinephrine and (b) (4) in dosing solutions and its use in support of toxicity studies**” (study #246977). The matrix used in the dosage formulations for all four toxicology studies in this review is saline acidified with HCl. A partial validation was performed in each toxicology study, and is reviewed for this study. To address the change in matrix from saline to acidified saline, the latter was used for spiked calibration samples. The lower limit of quantitation (LLOQ) was determined to be 2.00 mcg/mL, with a calibration range of 2.00 to 1500 mcg/mL. The internal standard was USP diphenhydramine hydrochloride. Calibration standards were made with epinephrine (lot #1047153, 99.6% purity, 0.9994 correlation coefficient) and (b) (4) (lot #WH-83-52-29, 96.3% purity, 0.9991 correlation coefficient).

The concentration of test article preparations was validated by LC/MS/MS method in duplicate. The final concentrations of epinephrine in the dosing formulations ranged from 87.8% to 106.1% of the targeted concentrations. (b) (4) in the dosing formulations ranged from 79.4% to 151.2%. The percent of the targeted claim is shown below for the HD for each test article within 15% of the targeted claim (shown below). Thus, the dose preparations are acceptable.

**Table 21: Bacterial mutation assay: Dose preparation analysis**

	Purity of HD (% of targeted claim)	
	S9	
	-	+
Epinephrine		
Plate incorporation	95.0	88.5
Preincubation	104.8	

(b) (4)	Purity of HD (% of targeted claim)	
	S9	
	-	+
Plate incorporation	126.2	148.1
Preincubation	91.6	

## Results

(b) (4) was tested up to doses of 1000 mcg/plate, with precipitation observed at 1000 mcg/plate with S9.

(b) (4) did not induce notable increases in the frequency of revertant colonies in TA100, TA1535, and WP *uvrA*, by the plate incorporation or preincubation methods, with or without metabolic activation. In the TA98 (+S9) by plate incorporation, the 1000 mcg/plate induced 61 revertants, compared to 52 in the untreated group (not statistically significant). Data from this experiment was higher than historical control data provided by the sponsor. Data from TA98 (+S9) treated with (b) (4) by pre-incubation, however, was unremarkable. Therefore, (b) (4) was considered negative for genotoxicity in TA98.

**Table 22:** (b) (4) bacterial mutation assay results in TA98 strain (+S9)

(b) (4) (mcg/plate)	Plate			
Plate incorporation	1	2	3	Mean
0	51	55	50	52
12	61	49	55	55
37	64	50	50	55
110	56	49	58	54
330	50	54	51	52
1000	58	59	66	61
benzo[a]pyrene	457	465	400	441
Preincubation	1	2	3	Mean
0	52	55	60	56
12	67	60	47	58
37	68	61	61	63
110	70	64	52	62
330	63	68	67	66
1000	60	71	56	62
benzo[a]pyrene	533	495	588	539

In TA1537 (+S9) with preincubation, 330 mcg/mL induced a significant increase ( $P < 0.01$ ) of 21 revertants, versus 14 revertants in control, but there was no dose response at 1000 mcg/mL (20 revertants). Data from TA1537 by plate incorporation did not show genotoxicity by (b) (4) (see data below). Therefore, (b) (4) is negative in the *in vitro* bacterial reverse mutation assay.

**Table 23:** (b) (4) bacterial mutation assay results in TA1537 strain (+S9)

(b) (4) (mcg/plate)	Plate			
Plate incorporation	1	2	3	Mean
0	17	19	23	20
12	18	19	20	19
37	19	22	20	20
110	21	26	27	25
330	20	28	23	24
1000	28	17	26	24
benzo[a]pyrene	231	226	190	216
Preincubation	1	2	3	Mean
0	12	14	16	14

12	19	20	16	18
37	20	22	17	20
110	20	19	14	18
330	20	21	23	<b>21*</b>
1000	19	20	21	20
benzo[a]pyrene	82	190	208	160

\*Bold indicates P<0.01

### Epinephrine

Although epinephrine is not the focus of this study, the data are evaluated below. Epinephrine induced moderate but statistically significant ( $P < 0.01$ ) increases in revertant colonies counts in TA100 without S9 by plate incorporation (5000 mcg/plate) and by preincubation (1700, 5000 mcg/plate). The latter results do not appear dose-dependent, which makes it difficult to determine if this is a true effect. Epinephrine was negative for all other test strains. Based on the data with TA100 without metabolic activation, the data with epinephrine is equivocal for genotoxicity.

**Table 24: Epinephrine bacterial mutation assay results in TA100 strain (-S9)**

Epinephrine (mcg/plate)	Plate			Mean
	1	2	3	
Plate incorporation				
0	163	165	154	161
62	149	163	155	156
190	137	153	155	148
560	164	171	166	167
1700	171	165	160	165
5000	200	175	219	<b>198*</b>
sodium azide	1819	1714	1939	1824
Preincubation	1	2	3	Mean
0	145	149	149	148
62	144	152	151	149
190	151	170	150	157
560	162	132	172	155
1700	211	196	197	<b>201*</b>
5000	231	195	195	<b>207*</b>
sodium azide	1857	1997	1877	1910

\*Bold indicates  $P < 0.01$

Epinephrine has been published previously to be negative by the Ames *Salmonella* assay<sup>10</sup>, but 500 mcg/plate was the highest concentration tested and a limited number

<sup>10</sup> Bruce WR and JA Heddle. The Mutagenic activity of 61 agents as determined by the micronucleus, *Salmonella*, and sperm abnormality assays. Canadian Journal of Genetics and Cytology 1979 Sep; 21(3): 319-34

of strains were tested. *S. typhimurium* strains TA1537, TA98, and TA100 were tested with 0.05, 0.5, 5, 50 and 500 mcg/plate with and without S9. The vehicle was water. A positive response was considered a 50% increase about the spontaneous mutation frequency.

[REDACTED] (b) (4)

Despite the lack of clear positive mutagenicity in the Ames assay (see additional information under the Computational Toxicology in Section 5.3 and a further description of the genotoxic potential of epinephrine in the Integrated Summary in Section 6), labels for the approved epinephrine products (for anaphylaxis) list epinephrine as genotoxic in bacteria. [REDACTED] (b) (4)

[REDACTED] (b) (4)

**Based on results [REDACTED] (b) (4) in the literature, epinephrine is positive for bacterial mutagenicity.**

[REDACTED] (b) (4)

## 5.2 *In Vitro* Assays in Mammalian Cells

### Study title:

Study no.: 246548  
 Study report location: SD-1 (EDR)  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: October 29, 2011  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: (b) (4) Batch No. WH-83-52-29, 96.3%; Epinephrine, Batch No. 1047153, 99.6%

### Key Study Findings

- (b) (4) induced chromosomal aberrations at the HD 1.0 mg/mL with 3 hr exposure and metabolic activation. The percentage of cells with structural aberrations was 17%, significantly higher ( $P < 0.01$ ) than 2.5% in negative controls. (b) (4) without metabolic activation, with 3 hr and 19 hr exposure, did not induce an increase in cells with structural aberrations, but dosing was not appropriate as the sponsor did not dose to cytotoxic levels.

### Methods

Cell line: Chinese hamster ovary (CHO) cells  
 Concentrations in (b) (4) was tested at 0.25, 0.50 and 1.0 mg/mL  $\pm$ S9 for 3 hrs, and at 0.13, 0.25, and 0.50 without S9 for 19 hrs. Epinephrine was tested at 0.28, 0.83 and 2.5 mg/mL  $\pm$ S9 for 3 hours. A longer-term incubation with epinephrine was omitted due to a positive result at the 3 hr incubation. S9 was used at 25 mL/mL  
 Basis of concentration selection: Concentrations for (b) (4) and epinephrine did not meet the 1997 OECD guidelines for testing up to 5 mcg/mL or to 50% inhibition of confluency, cell count, or mitotic index. Mitotic index is appropriate for suspension cells according to OECD, which does not apply to CHO cells. (b) (4) and epinephrine concentrations are acceptable due to the positive signal for chromosomal aberrations. The sponsor stated that greater cytotoxicity was observed with epinephrine in dose-ranging studies not included in the study report, but the data submitted does not support the high dose (HD) selected.

The sponsor reported cell count as percent relative cell growth (RCG): ((viable cell count in test flask/viable cell

count in negative control flask) x 100). (b) (4) induced a maximum cytotoxicity at the HD 1.0 mg/mL of 73% RCG (3 hr +S9), and 59% RCG (19 hr - S9). Epinephrine induced a maximum cytotoxicity at the HD 2.5 mg/mL of 56% RCG (3 hr incubation -S9), with greater cytotoxicity at 5.0 mg/mL in a dose-ranging study (data not reported).

Concentration (mg/mL)	Relative cell growth (%)		
	-S9, 3 hr	+S9, 3 hr	-S9, 19 hr
	(b) (4)		
0	100	100	100
0.13	-	-	90
0.25	107	118	78
0.5	104	113	85
1	103	73	59
Epinephrine			
0	100	100	100
0.093	108	-	ND
0.28	115	116	ND
0.83	66	95	ND
2.5	56	71	ND
0.015	ND	ND	101
0.03	ND	ND	97
0.06	ND	ND	100
0.12	ND	ND	90

"- " indicates not read because no toxicity at higher concentration.

"ND" indicates not done

Relative mitotic index (RMI) was also reported, but RCG is the preferred indicator of toxicity in CHO cells.

Negative control: 0.9% Sodium Chloride Injection, USP (Saline) acidified to 8.6 to 10% v/v of 2N HCl in saline. This stock was diluted 2-fold as the test solutions were also diluted to make the formulations equal in pH.

Positive control: Mitomycin C (MMC) was used for cultures not treated with S9 at 0.2 and 0.5 µg/mL for the 3 and 19-hour exposure periods. Cyclophosphamide (CP) was used at 3.75 and 7.5 µg/mL for treatment with S9 for the 3-hour exposure period. Results of only one concentration of each positive control were reported.

Formulation/Vehicle: Test article was solubilized in saline acidified with HCl,

then added to saline.

Incubation & sampling time: Cells were incubated with and without metabolic activation in humidified incubator at  $37 \pm 2^\circ\text{C}$  and  $5 \pm 2\%$   $\text{CO}_2$ .

Treatment time (hr)	Treatment condition	Recovery Time (hr)	Concentration (mg/mL)
(b) (4)			
3	Non-activated (-S9)	20.5	0.25, 0.50, 1.0
	Activated (+S9)	20.5	0.25, 0.50, 1.0
19	Non-activated (-S9)	19	0.13, 0.25, 0.50, 1.0
Epinephrine			
3	Non-activated (-S9)	20.5	0.093*, 0.28, 0.83, 2.5
	Activated (+S9)	20.5	0.093*, 0.28, 0.83, 2.5
19	Non-activated (-S9)	19	0.015, 0.030, 0.060, 0.12

\* 0.093 mg/mL was used for epinephrine but not evaluated.

### Study Validity

The study is considered valid based on the following parameters:

- The appropriate positive controls were used based on OECD 1997 guidelines.
- An acceptable number of cells were evaluated. Duplicate cultures were prepared for each exposure concentration. One hundred cells from each negative control and test article treated culture, or a minimum of 50 cells when an obvious positive result was observed, were scored. A minimum of 50 cells from a positive control culture were examined. Chromatid gaps and chromosome gaps were recorded when encountered, but not included in the calculations. The 1997 OECD guidelines recommend that 200 well-spread metaphases be scored per concentration (100/duplicate), but allow that this number can be reduced when high numbers of aberrations are observed. Therefore, the number of cells evaluated is acceptable as it did not appear to affect the integrity of this evaluation.
- Well-spread metaphase cells with 19 - 23 chromosomes were analyzed for chromosome aberrations. Numerical aberrations (polyploidy, endoreduplication) were enumerated, but analyzed separately from structural aberrations. Chromatid gaps (tg) and chromosome gaps were noted but not included in the analyses.
- The exposure time and recovery time are acceptable. Exposure was performed for 3 hours  $\pm$ S9, and 19 hours without S9, with 20.5 and 19 hour recovery times, respectively.

- The dose selection is acceptable, based on the positive evaluation for induction of chromosomal aberrations. Valid concentrations are based on 50% or more cytotoxicity at the HD under each condition (incubation time and metabolic status), which was not shown here. However, (b) (4) and epinephrine were determined in this study to be positive for induction of chromosomal aberrations, thus higher concentrations are not required.
- Protocol deviations do not appear to have negatively impacted the validity of the study. Of note, epinephrine dosing preparations were stored with nitrogen gas due to the observation in preliminary testing that epinephrine-treated media changed from orange/red to red/brown.

## Results

The sponsor did not dose up to required levels of cytotoxicity for (b) (4) or epinephrine. For (b) (4), the concentrations analyzed were 0, 0.25, 0.50, and 1.0 mg/mL for 3 hr exposure ±S9, and 0, 0.13, 0.25, 0.50, and 1.0 mg/mL for 19 hr exposure without S9. The highest (b) (4) concentration (1 mg/mL) did not inhibit cell growth greater than 50% under any of the tested conditions. The dosage for epinephrine was also below acceptable cytotoxic levels; therefore the conditions may underestimate the clastogenic effects of epinephrine.

**Table 25: Relative cell growth as an indication of cytotoxicity for dose selection**

Concentration (mg/mL)	Relative cell growth (%)		
	-S9, 3 hr	+S9, 3 hr	-S9, 19 hr
(b) (4)			
0	100	100	100
0.13	-	-	90
0.25	107	118	78
0.5	104	113	85
1	103*	73*	59*
Epinephrine			
0	100	100	100
0.093	108	-	ND
0.28	115	116	ND
0.83	66	95	ND
2.5	56*	71*	ND
0.015	ND	ND	101
0.03	ND	ND	97
0.06	ND	ND	100
0.12	ND	ND	90*

\* Values are >50%, therefore doses are insufficient

(b) (4) induced an increase in structural aberrations. The percentage of cells with structural aberrations with 3 hr exposure +S9 was 17%, significantly higher ( $P < 0.01$ ) than 2.5% in negative controls. The positive control cyclophosphamide (7.5 mcg/mL, +S9) also increased the percentage of cells with structural aberrations. Lower concentrations of (b) (4) in the 3 hr +S9 group do not appear to induce structural aberrations based on comparison to control, and were generally within the historical control data (0-3.1%). (b) (4) without metabolic activation, with 3 hr and 19 hr exposure, did not induce an increase in cells with structural aberrations, but dosing was not appropriate as the sponsor did not dose to proper cytotoxic levels. The positive control mitomycin C (0.5 mcg/mL) induced an increase in cells with chromosomal aberration.

**Table 26: Chromosomal aberrations:** (b) (4)

(b) (4) (mg/mL)	% cells with structural aberrations			% cells with endoreduplication		
	3 hr -S9	3 hr +S9	19 hr - S9	3 hr - S9	3 hr +S9	19 hr - S9
0	2	2.5	4.5	0	2.9	0
0.13	-	-	3	-	-	0
0.25	1	2	2	0	1.0	0
0.50	2	3.5	3	0	1.0	0
1.0	1.5	17	-	0.5	8.3	-
Positive Control	53	37	31	0	3.8	0

“-“indicates not done

Although epinephrine is not the focus of this review, epinephrine was evaluated for induction of chromosomal aberrations. Epinephrine induced an increase in chromosomal aberrations without metabolic activation with 3 hr exposure at both the MD (8.3 mg/mL;  $P < 0.01$ ) and HD (2.5 mg/mL;  $P < 0.03$ ). The reason for the lack of dose response is not clear, but may be related to the increased cytotoxicity at the HD, based on RCG, or a decrease in mitosis. Results for the MD and HD were higher than historical controls (0-5%) using DMSO and water. There was also an increase in the total number of endoreduplicated cells. The positive controls mitomycin C (0.5 mcg/mL, -S9) and cyclophosphamide ((7.5 mcg/mL, +S9) induced an increase in cells with structural aberrations. Therefore, epinephrine is considered mutagenic under these experimental conditions. The sponsor also concluded epinephrine to be a clastogen based on the 3 hr exposure without S9, and did not perform a confirmatory test with longer-term exposure (19 hr; study report p. 15).

**Table 27: Chromosomal aberrations: epinephrine**

Epinephrine (mg/mL)	% cells with structural aberrations		% cells with endoreduplication	
	3 hr -S9	3 hr +S9	3 hr -S9	3 hr +S9
0	2	2.5	0	3.0
0.28	2	4	0	2
0.83	15	3.9	3	15.0
2.5	7.3	5.8	6.0	31.0
Positive Control	53	37	0	4

Note: 19 hr incubation without S9 was not reported

Addition of metabolizing enzymes (S9) diminished the clastogenic effect of epinephrine. Treatment with 2.5 mg/mL epinephrine induced 5.8% cells with structural aberrations, compared to control (2.5%) and historical control (0-3.1%). Although epinephrine induced an increase in the percent of cells with structural aberration, the effect was marginal, and the results are considered to be inconclusive for induction of structural aberration with S9. Importantly, epinephrine induced an increase in endoreduplicated cells with metabolic activation (see table above).

The sponsor noted that the “positive result for mutagenicity of EP [epinephrine] in absence of metabolizing enzymes is in accordance with the study by McGregor and colleagues (McGregor, D.B. et al, 1988)<sup>13</sup>. These authors demonstrated an increase in the colony formation on mouse lymphoma L5178Y cells treated with EP.”

### 5.3 Other Genetic Toxicity Studies: Computational Toxicology

Computational toxicology assessments were submitted by the sponsor for (b) (4) (study 2010.10.1) and epinephrine (study 2011.04.07) using Leadscope for multiple endpoints for genetic toxicology and carcinogenesis. (b) (4) and epinephrine were positive by multiple endpoints.

The FDA currently recognizes data derived using multiple computational toxicology programs for the bacterial mutagenesis assay. To further assess the genotoxic potential of (b) (4) and epinephrine, the FDA CDER Computational Toxicology Group performed a comprehensive computational toxicology test using three software programs: Derek Nexus 2.0.2 (DX), Leadscope Model Applier 1.3.3-3 (LMA), and MC4PC 2.4.0.7 (MC). (b) (4) was predicted as positive for the *Salmonella* mutagenicity, and no prediction was made for *E. coli* mutagenicity based on the lack of representation in the model training data sets. (b) (4) was predicted as positive for the *Salmonella* mutagenicity based on published literature. Further details of these results

<sup>13</sup> McGregor DB et al. Reactivity of catecholamines and related substances in the mouse lymphoma L5178Y cell assay for mutagens. Environ Mol Mutagen. 1988;11(4):523-44.

are discussed in the Integrated Summary below. No prediction was made for *E. coli* mutagenicity based on the lack of representation in the model training data sets.

 (b) (4) were also analyzed by the FDA CDER Computational Toxicology Group, and all 3 structures were predicted to be negative for the Ames test and *E. coli* mutagenicity. The computational toxicology reports are shown below.

4 Page(s) has been Withheld in Full as B4 (CCI/TS)  
immediately following this page

## 6 Integrated Summary and Safety Evaluation

Adrenalin is an unapproved product marketed by JHP. Impurities were discussed with the sponsor on July 9, 2011 at the Pre-IND Meeting. The sponsor conducted toxicology studies to support the safety of (b) (4), and submitted literature to support the safety of (b) (4) based on Agency recommendations. Safety evaluations (b) (4) were requested by CMC for this consult, and are summarized below.

(b) (4) are epinephrine-related impurities that are considered degradants for this review.



(b) (4)

An additional impurity shown in Table 2 is listed as an “individual unidentified impurity” and is present at a (b) (4) specification release. CMC reviewer Dr. Ying Wang is requesting that the sponsor identify the impurity (draft IR sent to this reviewer via email on May 24, 2012), and the safety of this impurity is not reviewed in this consult.

Overall, the levels of the three impurities (b) (4) are reasonably safe from the nonclinical perspective.

(b) (4)

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

/s/  
-----

JANE J SOHN  
06/01/2012

MOLLY E SHEA  
06/01/2012  
I concur.

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

**NDA/BLA Number: NDA  
204200**

**Applicant: JHP Pharmaceuticals, Stamp Date: March 7, 2012  
LLC**

**Drug Name: Adrenalin®**

**NDA/BLA Type: NDA**

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

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

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

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

	<b>Content Parameter</b>	<b>Yes</b>	<b>No</b>	<b>Comment</b>
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		See p.50 (Section 12.221) in Information Package for P-IND 111712 (Meeting on July 5, 2011).
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		See 5 in above.
11	Has the applicant addressed any abuse potential issues in the submission?			NA
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			NA

**IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? \_\_\_Yes\_\_\_**

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

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

Conrad H. Chen, Ph.D.	April 6, 2012
_____ Reviewing Pharmacologist	_____ Date
Lori E. Kotch, Ph.D.	April 9, 2012
_____ Team Leader/Supervisor	_____ Date

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

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

/s/  
-----

CONRAD H CHEN  
04/17/2012

LORI E KOTCH  
04/17/2012

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

NDA/BLA Number: 204200    Applicant: JHP Pharmaceuticals    Stamp Date: 3/7/12  
LLC

Drug Name: Adrenalin®    NDA/BLA Type: 505 (b)(2)  
(epinephrine)

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	x		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	x		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	x		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	x		The sufficiency of the provided literature review to support the safety (b)(4) will be assessed.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	x		The formulation used in the toxicology studies are different than those used in the to be marketed products. See comment for item 6.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	x		The IV route of exposure is used to qualify the degradant (b)(4)
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	x		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?			Not applicable.

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

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

	<b>Content Parameter</b>	<b>Yes</b>	<b>No</b>	<b>Comment</b>
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	x		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	x		The provided literature review to support the safety (b) (4) will be assessed.
11	Has the applicant addressed any abuse potential issues in the submission?	x		The sponsor states: “No reports of addiction to Adrenalin have been found in the literature” (Clinical overview, p. 43).
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable.

**IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? \_\_Yes\_\_\_\_\_**

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

Not applicable.

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

No comments from nonclinical.

See electronic signatures

\_\_\_\_\_  
Reviewing Pharmacologist

\_\_\_\_\_  
Date

\_\_\_\_\_  
Team Leader/Supervisor

\_\_\_\_\_  
Date

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

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

/s/  
-----

JANE J SOHN  
04/12/2012

MOLLY E TOPPER  
04/12/2012  
I concur.