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APPLICATION NUMBER:

209575Orig1s000

NON-CLINICAL REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	209575
Supporting document/s:	0025; 0028
Applicant's letter date:	June 21, 2019; August 21, 2019
CDER stamp date:	June 21, 2019; August 21, 2019
Product:	NUMBRINO (cocaine hydrochloride) nasal solution
Indication:	Induction of local anesthesia of the mucous membranes when performing diagnostic procedures and surgeries on or through the nasal cavities in adults
Applicant:	Cody Laboratories, Inc
Review Division:	Division of Anesthesiology, Addiction Medicine, and Pain Medicine (DAAP)
Reviewer:	BeLinda A. Hayes, PhD
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Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

Cody Laboratories Inc resubmitted NDA 209575 for marketing approval of Numbrino (cocaine hydrochloride, 4% and 10% topical solution formulations), for the indication of induction of local anesthesia when performing diagnostic procedures and surgeries on or through the mucous membranes of the nasal cavities in adults via 505(b)(2) application. The Applicant relied upon studies conducted by themselves and published literature to support the nonclinical requirements for this 505(b)(2) application.

This NDA was orginially submitted on September 21, 2017 and was not approved due to deficiencies from numerous disciplines. The compete response letter dated July 20, 2018 included two nonclinical deficiencies that related to the leachables evaluation and genotoxicity evaluation. The nonclinical deficiencies were:

1. You have not provided adequate leachables evaluation to justify the safety of the proposed container closure system. Specifically, your leachables evaluation did not evaluate at least three batches of your to-be-marketed drug product for leachables and include assessments at multiple timepoints over the course of your stability studies as we advised at the Pre-NDA meeting and in accordance with best practices per USP <1664>: Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems. Further, you have not provided an adequate extractables-leachables correlation to ensure that leachable compound levels can be extrapolated from data collected from simulation studies under accelerated conditions.

   To resolve this deficiency:
   Conduct a new leachables study under standard storage conditions that evaluates at least three batches of the to-be-marketed topical cocaine solution products for leachables and include assessments at multiple timepoints over the course of your stability studies (beginning, middle, and end of proposed shelf-life) in order to identify trends in leachable levels over time. Evaluate all container closure systems you intend to market. Clearly delineate how you leveraged the existing extraction studies to inform your leachables assessment. Submit a toxicological assessment justifying the safety of the maximum level achieved over the course of stability fo any leachable that exceeds 5 mcg/day, taking into consideration the maximum daily dose of the drug product. Submit a discussion of the extractables leachables correlation of the findings.

2. Several of your final study reports did not report the purity of the test articles.

   To resolve this deficiency:

   Revise the final study reports for Study 16-01138-G2, 16-01139-G2, and 16-01140- G2 to include purity information of the test articles evaluated.

1.2 Brief Discussion of Nonclinical Findings

In the NDA resubmission, the Applicant submitted new leachables data from nine different lots of Cocaine Hydrochloride topical solution (4%) and two lots of 10%

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Cocaine Hydrochloride Hydrochloride topical solution in the proposed container closure system intended for marketing. It is noted that in the resubmission, the Applicant states they will only market (b) (4) originally proposed container closure systems, which is the (b) (4) container closure configuration and therefore new leachables data was only submitted for this container closure configuration. Three timepoints (beginning of stability, mid, and end of stability) were evaluated for the 4% Cocaine Hydrochloride topical solution. (b) (4) .

As per the CMC review team the extractable/leachable data were considered acceptable for the (b) (4) container closure system. All leachables detected in the leachables assessment were lower than the 5 mcg/mL qualification threshold. Thus, there are no nonclinical safety concerns with the container closure. To address the second nonclinical deficiency, the Applicant submitted purity information and certificate of analyses for the test articles evaluated in the genotoxicity evaluation and has adequately addressed this deficiency. Taken together, the Applicant has addressed all nonclinical deficiencies. However, there are several outstanding issues with regard to the potential effects of cocaine on reproductive and developmental endpoints and its potential effects on the CNS and reproductive organs in the adolescent pediatric population and therefore postmarketing requirements will be recommended to address these outstanding issues.

1.3 Recommendations

1.3.1 Approvability

From a nonclinical pharmacology toxicology perspective, NDA 209575 may be approved. Adequate data were provided to address the nonclinical deficiencies outlined in the complete response letter dated July 20, 2018 for the (b) (4) container closure configuration and therefore NDA 209575 may be approved for the (b) (4) container closure configuration with the following postmarketing requirements (see 1.3.2 below).

1.3.2 Additional Non Clinical Recommendations

The following postmarketing requirements (PMRs) are recommended:

1. Conduct a female fertility and early embryonic development study in the rat model to adequately characterize the effect of cocaine on female fertility and early embryonic development.
2. Conduct an embryo-fetal development study in the rat model to characterize the teratogenic potential of cocaine.

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3. Conduct an embryo-fetal development study in the rabbit model to characterize the teratogenic potential of cocaine.

4. Conduct a pre- and post-natal development study in the rat model to characterize the impact of cocaine on development, including exposure during lactation to weaning, growth and development, functional assessments, and reproductive capacity of the offspring.

5. Conduct a juvenile animal study to characterize the impact of cocaine on brain development and male reproductive tissue and development to support pediatric dosing in children 12 to 17 years of age.

(b) (4)

1.3.3 Labeling

The following changes to the Applicant's proposed labeling are recommended in the table below (deletions are highlighted in ~~red strikethrough~~ and additions are highlighted in purple). Refer to the action letter for final drug product labeling.

Applicant's proposed labeling	Reviewer's proposed changes	Rationale for changes
HIGHLIGHTS OF PRESCRIBING INFORMATION (b) (4)	**HIGHLIGHTS OF PRESCRIBING INFORMATION** INDICATIONS AND USAGE NUMBRINO is a liquid formulation of cocaine hydrochloride, an ester local anesthetic, indicated for the introduction of local (topical) anesthesia for diagnostic procedures and surgeries on or through the accessible mucous membranes of the nasal cavities. (1)	Established pharmacological class was added to highlights as per regulations. For final indication, see the final labeling and clinical review.
8 USE IN SPECIFIC POPULATIONS	**8 USE IN SPECIFIC POPULATIONS**	
8.1 Pregnancy (b) (4)	**8.1 Pregnancy** Risk Summary There are no adequate and well-controlled studies of NUMBRINO in pregnant women. Limited available data from published literature on use of cocaine in pregnant women are not sufficient to inform a drug-associated risk for major birth defects and miscarriage. Adverse pregnancy outcomes,	The Applicant did not propose a nonclinical risk summary statement. Refer to the DPMH Review and final labeling for evaluation of the proposed human risk summary statement.

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(b) (4)	including premature delivery and low birth weight, have been seen in infants born to mothers dependent on cocaine [see Clinical Considerations].	
	In published animal reproduction studies, cocaine administered to pregnant females during the gestational period produced hydronephrosis (0.5 times the human reference dose (HRD) of 37.5 mg via the 4% solution), developmental delays in the offspring (1.5 times the HRD), cerebral hemorrhage and fetal edema (2.2 times the HRD), reduced fetal body weights and brain weights (2.6 times the HRD), and reduced fetal survival (3.7 times the HRD).	(Mahalik et al., 1980)
	Single dose administration of cocaine intravenously during organogenesis in mice produced cryptochidism, anophthalmia, exencephaly, and delayed ossification at 7.8 times the HRD. In rats, a single dose of cocaine administered by intraperitoneal injection produced edematous fetuses, hemorrhages and limb defects at 13 times the HRD [*see Animal Data*].	(Webster and Brown-Woodman, 1990)
(b) (4)	(b) (4)	Older CFR language removed.
(b) (4)	The estimated background risk of major birth defects and miscarriage for the indicated population is unknown. All pregnancies have a background risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.	Generic boilerplate labeling added.
Data (b) (4) (b) (4)	Data Animal Data (b) (4)	

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(b) (4)	(b) (4)	Unclear from submission what they cited. We believe the mouse study is Mahalik.
(b) (4)	(b) (4)	Unclear from submission what they cited. We believe the rat study is El-Bizri. Statement on Benzoylecgonine from Spear 1989a
	Formal animal reproduction and development studies have not been conducted with intranasal cocaine hydrochloride. However, reproduction and development studies with cocaine have been reported in the published literature. Exposure margins below are based on body surface area comparison to the human reference dose (HRD) of 37.5	Introduction statement to cover how exposure margins were characterized.

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	mg (estimated amount absorbed from the 160 mg (4%) cocaine-soaked pledgets).	
	Hydronephrosis was noted in fetuses from pregnant rats treated wtih cocaine 2.1 mg/kg (0.5 times the HRD) and higher from Gestation Days 0-19. Cerebral hemorrhage and endematous fetuses were noted at 2.2 times the HRD and above).	Data source: (el-Bizri et al., 1991)
	Developmental delays and altered spontaneous exploratory behavior in response to cocaine were reported in rat pups born to dams treated intravenously with 6 mg/kg cocaine (1.5 times the HRD) from Gestation Days 8-20 in the absense of maternal toxicity.	Data Source: (Kunko et al., 1993)
	Reduced fetal body and brain weights and alterations in fetal central neurotransmitter levels were noted following treatment of pregnant mice with 20 mg/kg cocaine (2.6 times the HRD) from Gestation Days 8-12 or 12-18.	Data source: (Middaugh et al., 1996; Song et al., 2002)
	Reduced fetal survival was noted when pregnant nonhuman primates were dosed with 0.3 mg/kg/h cocaine (3.7 times the HRD on per day basis) via a subcutaneous minipump from Gestation Day 24 to birth.	Data Source: (Howell et al., 2001)
	Exencephaly, cryptochidism, hydronephrosis, anophthalmia, delayed ossification, limb anomalies, and cerebral and intra-abdominal hemorrhage were reported following a single subcutaneous injection of 60 mg/kg cocaine (7.8 times the HRD) to pregant mice between Gestation Days 7 to 12. No significant maternal toxicity was reported at this dose.	Data source: (Mahalik et al., 1980)
	Deficits in associational learning were reported when pregnant rats were treated with cocaine during gestation (10 times the HRD) in the absence of maternal toxicity.	Data Source: (Spear et al., 1989)
	Hemorrhage, fetal edema, and	Data source: (Webster and

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	limb defects were reported when pregmant rats were administered a single injection of cocaine at a dose of 50 mg/kg/day or higher (13 times the HRD) during Gestation Days 9 to 19. Increased resorptions were noted at doses higher than 70 mg/kg/day (18 times the HRD) when administered on Gestation Day 16. No adverse effects were reported at a dose of 40 mg/kg (10 times the HRD).	Brown-Woodman, 1990)
	Fetal deaths, decreased fetal body weights, edematous fetuses and single incidences of cleft palate and hypertrophic ventricle were observed when pregnant rats were treated with intraperitoneal cocaine at 60 mg/kg (16 times the HRD) from Gestation Days 8 to 12. Maternal toxicity was noted at this dose (mortality). No adverse effect level for fetal and maternal toxicity was noted at 50 mg/kg/day (13 times the HRD).	Data source: (Fantel and Macphail, 1982)
	Decreased body weights, overall body length and crown circumference of offspring were reported when pregnant Rhesus monkeys were treated with escalating doses up to 7.5 mg/kg cocaine TID intramuscularly per day for 5 days per week from prior to conception to term (12 times the HRD).	Data Source: (Morris et al., 1997)

2 Drug Information

2.1 Drug

CAS Registry Number: 53-21-4

Generic Name: Cocaine hydrochloride

Code Name:

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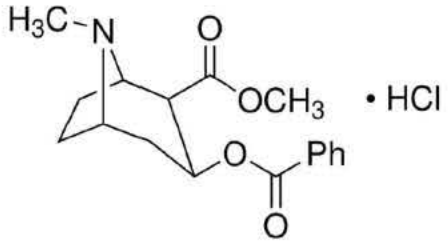
Chemical Name: Ecgonine methyl ester benzoate hydrochloride 3β-hydroxy-1αH, 5αH-tropane-2β-carboxylic acid methyl ester benzoate hydrochloride

Molecular Formula/Molecular Weight: $C_{17}N_{21}NO_4 \cdot HCl$/339.1

Structure or Biochemical Description:



Pharmacologic Class: Ester local anesthetic (Established Pharmacologic Class)

2.2 Relevant INDs, NDAs, BLAs and DMFs

INDs	Status	Division	Indication	Stamp Date	Applicant
106499	Active	DAAAP	Local anesthesia for diagnostic procedures and surgeries on or through the accessible mucous membranes of the nasal cavities	04/06/2013	Cody Laboratories Inc

DMF	Status	Subject of DMF	Stamp Date	Holder
(b) (4)	Closed	(b) (4)	02/27/2018	(b) (4)
	Active		05/06/2005	
	Active		09/03/1970	
	Active		03/14/1975	
	Active		12/03/1997	
	Active		03/10/2015	
	Active		3/9/1998	
29120	Active		08/07/2017	Cody Laboratories Inc

2.3 Drug Formulation

There were no changes with the formulation, drug substance specifications, and drug product specfications from the first review cycle. For more details refer to the nonclinical review in DARRTS dated June 21, 2018.

2.4 Comments on Novel Excipients

There are no novel excipients in the drug product.

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2.5 Comments on Impurities/Degradants of Concern

For more detail refer to the nonclinical review in DARRTS dated June 21, 2018.

Drug Substance Impurities

All impurity specifications are within the ICH Q3A(R2) qualification levels for the drug substance, except for (b) (4) [redacted]. However, (b) (4) [redacted] was adequately qualified by nonclinical studies. For more details refer to the nonclinical review in DARRTS dated June 21, 2018.

Residual Solvents in the Drug Substance

All residual solvents in the drug drug substance meets ICH Q3C acceptable exposure limits. For more details refer to the nonclinical review in DARRTS dated June 21, 2018.

Drug Product Degradants

Several drug product degradants exceed ICH Q3B qualification thresholds; however, all were adequately qualififed by nonclinical studies. For more details refer to the nonclinical review in DARRTS dated June 21, 2018.

2.6 Container Closure System

In the resubmission, as depicted in the table below, the Applicant selected (b) (4) [redacted] container closure system configurations that was proposed in the initial NDA submission. The proposed container closure system consists of a (b) (4) [redacted] (b) (4) [redacted] glass bottle and a (b) (4) [redacted] cap closure.

Table 1. Proposed Container Closure System for Cocaine Hydrochloride Topical Solution

Configuration	Container	Closure
1	(b) (4) [redacted] (b) (4) [redacted] (b) (4) [redacted] Type III [redacted] Glass Bottle	(b) (4) [redacted] (b) (4) [redacted] cap (b) (4) [redacted]

To address the nonclinical deficiency, the Applicant submitted new leachables data from several batches at several timepoints. The leachables studies included all components of the container closure system. It is noted that no new extraction study was conducted for the resubmission. Results from the extraction study described in the original submission was used to inform what potential compounds may be present during storage of the drug product for the leachable study. As noted in the first review cycle, the analytical evaluation threshold (AET) for the extractables studies was (b) (4) [redacted], which was acceptable. Results from this study are described in the original review (DARRTs June 21, 2018).

Analytical Evaluation Threshold (AET)

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(b) (4)

An Information Request was sent to the Applicant on August 9, 2019:

> Clarify whether your leachables study tested the to be marketed container closure system containing all the components of the (b) (4) cap listed in your 3.2.P.7 Table 1 ((b) (4) Cap, (b) (4) (b) (4)

The Applicant response to the Information request was as follows:

> The leachables studies included all components of the (b) (4) cap.

Reviewer Comments: The response is adequate.

Leachables screening evaluation – initial and additional screening

Study 2. The leachables screening evaluation (RPT63682.0) was submitted during the first review cycle (for more detail refer to the nonclinical review in DARRTS dated June 21, 2018) but will be presented and summarized along with the new data (Study 3).

Study 3. Different batches at different timepoints were analyzed for volatile, semi-volatile, non-volatile, and metal leachables, which are summarized in the Table below.

Volatile and Semi-volatile Analysis by Headspace GC/MS. The Applicant reported that no compounds were detected in the proposed container closure at beginning timepoint and the aged retained timepoints (middle and end).

Non-volatile Analysis by LC/UV/MS. The Applicant reported that no compounds were detected in any of the all bottle/cap combination.

Reviewer's comment. This analysis differs from the one employed for Study 2, which used HPLC/MS to measure non-volatile in the orginial NDA submission. The CMC review team noted that the methods were acceptable. Refer to the CMC review for additional details.

Metal Analysis by ICP/MS. The Applicant's table (From Applicant submission, pages 11 and 12 of study Summary Report 834RD2) below illustrates the leachables identified in the propose container closures analysis. (b) (4) and (b) (4) were identified in all Cocaine HCL topical solution 4% samples. (b) (4) was identified in several cocaine HCL Topical solution 4% samples, and Al was identified in one lot.

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(b) (4)

Although various elements were identified as leachables from the proposed container closure, no additional controls are required for elemental leachables as the levels were consistently less than 30% of the PDE.

2.6 Proposed Clinical Population and Dosing Regimen

There were no changes in the proposed clinical population. The proposed indication is for use as a local anesthetic for diagnostic and surgical procedures on or through the mucous membranes of the nasal cavities in adults. It is available as a 4% or 10% topical solution (marketed unapproved product). It is administered at the time of the surgical procedure as a single, 160 mg (4%) or 400 mg (10%) intranasal dose as a solution using specific sized pledgets (1/2″ x 3″). One or two pledgets will be applied topically per nares, with a maximum of two pledgets per nare or a maximum of four pledgets per procedure. One mL of the cocaine topical solution is applied to each pledget. Following soaking, two pledgets are to be placed against the septum in each nasal cavity for up to twenty minutes and then are to be removed and discarded. The total theoretical dose for any one procedure is 160 mg or 400 mg cocaine hydrochloride if the total amount of cocaine in the pledget is absorbed. However, in their document titled "Summary of Biopharmaceutics studies" the Applicant has indicated that a mean of 23.44% and 33.34% of the total administered 160 mg dose (4% cocaine solution) and 400 mg dose (10% cocaine solution was absorbed), respectively. Therefore, some of the drug product is retained within the cottonoid pledgets after delivery. Therefore, a dose of 37.5 mg/day and (b) (4) mg/day was the calculated maximum recommended human dose (MRHD) for the 4% and 10% topical solution, respectively; and was used as a reference human dose for all subsequent margin calculations.

2.7 Regulatory Background

A preIND meeting (IND 106499) was held on December 15, 2009 to discuss the drug development program for this marketed unapproved product. The Division informed the Applicant that the NDA should include a comprehensive genotoxicity assessment of cocaine, comprehensive review of the literature and specifically address what studies identified provide the most relevant data with respect to current ICH M3(R2) requirements. The NDA must also review and analyze the existing toxicology data for cocaine, including general and local toxicology, genetic toxicology and reproductive and developmental toxicology (fertility and early embryonic development, embryofetal development, and pre- and postnatal development). We also advised that any impurity or degradant that exceeded ICH Q3A(R2)/Q3B(R2) thresholds would have to be qualified for safety in accordance with these guidance documents. In addition,

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potentially genotoxic impurities should be adequately controlled and adequate information on extractables and leachables must be provided to support the safety of the container closure system. Key regulatory milestones are noted below:

- The orginial IND was opended on April 6, 2013.
- On December 22, 2014, an email correspondence was conveyed in respond to the Sponsor advice/information request on December 22, 2014 to the Sponsor regarding toxicology testing.
- A pre-NDA meeting was held in April 2017.
- NDA 209575 was originally submitted September 27, 2017. The Applicant submitted a 505(b)(2) application for cocaine hydrochloride topical solution 4% and 10% that relies upon published scientific literature.
- The original NDA submission was not approved. The complete response letter dated July 20, 2018 included a nonclinical deficiency related to their leachables evaluation and genotoxicity study.

3 Studies Submitted

3.1 Studies Reviewed

- Extractable/Leachable assessment for Cocaine Hydrochloride Topical Solution, 4% and 10% (Study Report 834RD2, Revision: 00)
- Chromosomal aberration assay – OECD 473 (Study Report № 16-01139-G2, Amended)
- Chromosomal aberration assay – OECD 473 (Study Report № 16-01138-G2, Second Amended)
- Chromosomal aberration assay – OECD 473 (Study Report № 16-01140-G2, Second Amended)

3.2 Studies Not Reviewed

N/A

3.3 Previous Reviews Referenced

During the first review cycle, the following nonclinical studies were reviewed (DARRTS dated, June 21, 2018)

Report №	Study Title	Module/CTD Description
Study Report	**General Toxicology**	
№ 16-01711-G1	A 14-day repeat dose intranasal instillation toxicity study in Sprague-Dawley rats with a 14-day recovery period.	4.2.3.2.1
	Genetic Toxicology	
№ 11-4138-G1	*Salmonella typhimurium* and *Escherichia coli* reverse mutation assay (OECD 471)	

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№ 16-01138-G3 Amended	*Salmonella typhimurium* and *Escherichia coli* reverse mutation assay (OECD 471)	4.2.3.3.1
№ 16-01139-G3 Amended	*Salmonella typhimurium* and *Escherichia coli* reverse mutation assay (OECD 471)	
№ 16-01140-G3	*Salmonella typhimurium* and *Escherichia coli* reverse mutation assay (OECD 471)	
№ 11-4138-G2	Chromosomal Aberration Assay – OECD 473	
№ 16-01138-G2	Chromosomal Aberration Assay – OECD 473	
№ 16-01139-G2	Chromosomal Aberration Assay – OECD 473	
№ 16-01140-G2	Chromosomal Aberration Assay – OECD 473	
№ 11-4138-G3	Rodent bone marrow micronucleus assay- OECD 474 cocaine.	
№ 54873.0001	Evaluation of micronuclei in B6C3F1in mice and DNA damage In Sprague Dawley rats administered ecgonine hydrochloride.	

7 Genetic Toxicology

In the initial NDA submission, a nonclinical deficiency was the omission of test article purity in several final study reports. To address this nonclinical deficiency the Applicant amended the final study reports with a copy of the certificate of analysis for each test article as summarized in the table below.

Test Article	Certificate of Analysis	Chemical Purity (HPLC)	Related Study Report
(b) (4) [redacted]	Lot # 265-32	(b) (4) [redacted]	Study 16-01138-G2
	Lot # 246-96		Study 16-01139-G2
	Lot # 2015507989		Study 16-01140-G2

Reviewer Comment: The Applicant has submitted the purity of the test article for each study report that was identified in the deficiency and therefore the response is deemed adequate.

9 Reproductive and Developmental Toxicology

(b) (4) [redacted] the submitted nonclinical information provided from the publish literature did not adequately address the endpoints for fertility and early development, embryo-fetal development and pre- and post-natal development but does provide some data and information to inform labeling. Therefore, the standard battery of reproductive and developmental toxicology studies will be recommended as a postmarketing requirement.

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9.1 Fertility and Early Embryonic Development

Standard fertility and early embryonic development studies have not been conducted in animals with cocaine to understand the effects of cocaine on male and female reproductive functions (i.e., development and maturation of gametes, mating behavior and fertilization). Male reproductive toxicity studies should evaluate the potential of a drug to damage reproductive organs, gamete maturation and release, reduction in sperm count, alteration in sperm motility or morphology, but also evaluate functional effects such as libido/mating behavior. Female reproductive toxicity studies should evaluate the impact of a drug on the estrus cycle, tubal transport, implantation, development of preimplantation states of the embryo.

The Applicant summarized the existing literature to address these endpoints of male and female fertility. This Reviewer grouped the literature references into fertility and reproduction effects, hormonal effects, and testicular effects.

Fertility and Reproduction

Several published reports in the literature have studied the effects of cocaine on some endpoints typically covered by standard fertility studies. The table below summarizes the literature references supplied by the Applicant. Comments on their utility with respect to typical fertility and early embryonic development study endpoints are provided.

Table 2: Effects of Cocaine on Female Fertility Endpoints

Effect Reported	Species	Treatment Period	Dose	HED (per 60 kg person)	Estimated Exposure Margin for % Cocaine Topical Solution Based on BSA‡		Citation	Comment
					4%	10%		
Cocaine delayed first estrus but did not impact fertility rate	Female Mice	PND 22 – 35 prior to mating	20 mg/kg BID SC (40 mg/kg/day) NOAEL not defined	195 mg/day	5.2x	1.5x	(Chen and Vandenbergh, 1994)	Really a juvenile animal study. Only includes fertility endpoint, not early embryonic development
Disrupted cyclicity and reduced ovulation and serum LH. Effect statistically	Female Rat	Several weeks; no mating	1 5 10 20 mg/kg SC (4 weeks)	9.7 mg 48 mg 97 mg 194 mg	0.26x 1.3x 2.6x 5.2x	0.073x 0.36x 0.73x 1.5x	(King et al., 1993)	No mating, not a fertility and early embryonic development study

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significant at 10 and 20 mg/kg; trending at 5 mg/kg.								

‡: Referenced MDD is 37.5 mg/day for the 4% solution; mean of 23.44% of the total administered 160 mg dose is absorbed.
Referenced MDD is (b) (4) for the 10% solution; mean of 33.34% of the total administered 400 mg dose is absorbed.

As noted in the table above, none of the submitted studies included appropriate dosing intervals or endpoints to be considered adequate to address the fertility and early embryonic development requirements for cocaine.

Hormonal Effects

In terms of effects on female reproductive hormones, published studies suggest that cocaine can result in transient changes in progesterone and alterations in menstrual cyclicity.

Table 3: Effects on Cocaine on Female Reproductive Hormones

Effect Reported	Species	Dose	HED (per 60 kg person)	Estimated Exposure Margin for % Cocaine Topical Solution Based on BSA‡		Citation
				4%	10%	
Reduced serum progesterone though not entirely dose dependent, follicular fluid progesterone decreased dose dependently at all doses.	Rabbits	0 10 20 40 80 mg/kg SC (5 days)	 194 mg 387 mg 774 mg 1548 mg	 5.2x 10.3x 20.6x 41.3x	 1.5x 2.9x 5.8x 11.6x	(Kaufmann et al., 1990)
Reduced estradiol levels and altered menstrual cyclicity and folliculogenesis	Monkey	0 or 4 mg/kg IV (13 days)	77 mg	2.1x	0.58x	(Potter et al., 1998)

‡: Referenced MDD is 37.5 mg/day for the 4% solution; mean of 23.44% of the total administered 160 mg dose is absorbed.
Referenced MDD is (b) (4) for the 10% solution; mean of 33.34% of the total administered 400 mg dose is absorbed.

Testicular Effects

Reference ID: 4580670

The Applicant's literature search identified numerous articles examining the impact of cocaine administration on testes. These are summarized in the table below with more detailed analysis of several key studies presented thereafter.

Table 4: Effects of Cocaine on Male Fertility Endpoints

Effect Reported	Species	Dosing Regimen	HED (per 60 kg person)	Estimated Exposure Margin for % Cocaine Topical Solution Based on BSA‡		Citation	Comment
				4%	10%		
No change in sexual behavior (e.g., latency to first intromission, number of intromissions until ejaculation, latency to ejaculation) or fertility. No histopathological changes to testes. Increased abnormal sperm morphology at 30 mg/kg. Increased hyperactivity of offspring.	Long Evans Rats (114-117 days old = ~ 16 weeks old) Allowed to mate with untreated females	15 or 30 mg/kg, SC (72 days)	145 mg 290 mg	3.9x 7.8x	1.1x 2.2x	(Abel et al., 1989)	Duration of treatment is appropriate; included fertility, sperm motility studies, testes histopathology data. Even have assessment of offspring for potential paternally mediated effects. A reasonable male fertility study. Rats older than typically used for fertility studies.
Necrosis of interstitial cells and seminiferous tubules at Day 15, progressed to Day 90. No histopathology at Day 7 reported.	Wistar Rats ("adult") (based on body weight reported, estimated to be ~6 weeks old)	30 mg/kg, IP (sacrificed on Days 7, 15, 30, 45, 60, 75, and 90)	290 mg	7.8x	2.2x	(Barroso-Moguel et al., 1994)	No NOAEL identified, suggests repeated exposure necessary to histopathological changes.

Reference ID: 4580670

Effect Reported	Species	Dosing Regimen	HED (per 60 kg person)	Estimated Exposure Margin for % Cocaine Topical Solution Based on BSA‡		Citation	Comment
				4%	10%		
Reduced pregnancy rates; reduced birth weights of pups; reduced diameter of seminiferous tubules, reduced thickness of germinal epithelial cells, reduced Step VII spermatids	Sprague Dawley rats (30 days old)	15 mg/kg SC (Daily for 100 days & twice weekly for 150 days prior to mating)	145 mg	3.9x	1.1x	(George et al., 1996)	Duration appropriate, endpoints for male fertility reasonable. See below. This study seems adequate to substitute for a male fertility study. No NOAEL identified.
Reduced seminiferous tubule diameter and thickness of germinal epithelium, increased degeneration of germ cells, failure to release mature spermatids	Rats (25 days old)	15 mg/kg SC (100 days)	145 mg	3.9x	1.1x	(Li et al., 1997)	Assessed morphology of testes, appropriate duration of study. No NOAEL identified.
Cocaine reduced testicular blood flow within 15 minutes and the effect lasted up to 60 minutes.	Sprague Dawley Rats (25 days old)	30 mg/kg, SC (single dose)	290 mg	7.8x	2.2x	(Li et al., 1999)	Not a fertility study. Measured testicular blood flow. Perhaps suggesting mechanism contributing to cocaine induced testicular toxicity. No NOAEL.

‡: Referenced MDD is 37.5 mg/day for the 4% solution; mean of 23.44% of the total administered 160 mg dose is absorbed.
Referenced MDD is (b) (4) for the 10% solution; mean of 33.34% of the total administered 400 mg dose is absorbed.

Collectively, the published literature on the effects of cocaine in adult male rats is not entirely consistent. Abel et al. report no adverse effects of adult males treated for 72 days via the SC route at doses up to 7.8 times the maximum daily dose based on body surface area. In contrast, Barroso-Moguel et al. (1994) report necrosis of male

Reference ID: 4580670

reproductive tissues after 7 days of this dose given IP. In theory, it is possible that IP could result in higher C_{max} values compared to SC, there could be strain differences, or the Wistar rats tested by Barroso-Moguel may have been younger than those tested by Abel.

The literature does report consistent adverse effects in peripubertal rats (George et al., 1996, Li et al., 1997). Specifically, in peripubertal male rats treated with cocaine, morphometric analysis of the testes showed a decrease in mean diameter of seminiferous tubules in peripubertal male rats administered cocaine daily for 100 days and twice weekly for 150 days. Similarly, the thickness of the germinal epithelium was decreased in peripubertal males administered cocaine daily for 100 days and twice weekly for 150 days. Cocaine also had significant effects on spermatogenesis in peripubertal rats; the number of spermatids was reduced (Step VII).

Abel et al., 1989

Abel and colleagues (1989) examined the effects of cocaine hydrochloride on reproductive function and sexual behavior of adult male rats and their offspring. Three groups of Long-Evan male rats were subcutaneously administered cocaine at doses of 0, 15, or 30 mg/kg for a minimum of 72 days. Rats in the 0 and 15 mg/kg groups were pair-fed with animals receiving the 30 mg/kg dose to account for reduced food intake. A fourth group served as a non-treated ad libitum-fed control. Male endpoints evaluations included: hormones (estradiol, LH, FSH, and T level), sexual behavior, fertility, sperm test (conducted 6 hours after the sexual behavior test), testis weight and histology, and effects on offspring. Offspring endpoint evaluation included: birth weight, litter size, postnatal mortality, weight at age Day 21, behavior (activity at 16 days of age, passive avoidance at 17 day of age, spontaneous alternation (T-maze test at 33 to 35 days of age, and sperm morphology at 58 days of age. Body weight and estradiol level and T/estradiol ratio were decreased in a dose-dependent manner by the subcutaneous administration of cocaine to male rats. Cocaine administration increased locomotor activity in a dose-dependent manner. Results from this study suggested that cocaine had no effects on male reproductive function, sexual behavior, or fertility. As depicted in the tables below reproduced from the publication, cocaine did affect sexual behavior or fertility as measured by the number of days to impregnate 2 females. Relative to control, a slight decrease in seminal vesicle (7.1%) weight was observed in the HD males (Table 2 below, page 8). An increase in the percentage of spermatozoa with heads separated from the tail was associated with the high dose of cocaine. Cocaine also did not affect weights of the testis or accessory organs, histology of the testis, number of implantations, resorptions, fetal or newborn weight of offspring, or offspring weight at 21 days of age. Cocaine had no effect on the behavior of the offspring as measured in the passive avoidance and T-maze models.

Reference ID: 4520620

TABLE 1. Effects of Cocaine on Sexual Behavior of Male Rats*

	Dose			
	30 mg/kg N = 24	15 mg/kg N = 26	0 mg/kg N = 15	*Ad Libitum* N = 15
Latency to Intromission (sec)	90 ± 22	99 ± 23	119 ± 30	150 ± 32
Number of mounts	16 ± 3	11 ± 1	13 ± 1	15 ± 2
Latency to Ejaculate (sec)	895 ± 148	855 ± 97	849 ± 179	1206 ± 195
Post Ejaculation (sec)	419 ± 24	436 ± 24	472 ± 34	453 ± 46
Period				
Days to first plug	3.0 ± 0.6	3.3 ± 0.4	3.8 ± 0.6	†
Days to second plug	3.9 ± 0.8	3.5 ± 0.6	5.2 ± 1.3	†

*Mean ± SEM.
†Not recorded.

TABLE 2. Effects of Cocaine on the Testis and Seminal Vesicles of Rats*

	Dose			
	30 mg/kg N = 5	15 mg/kg N = 6	0 mg/kg N = 5	*Ad Libitum* N = 6
Body wt (g)	465 ± 8	524 ± 10	538 ± 21	603 ± 9
Testes (g)	3.8 ± 2.2	3.5 ± 0.1	4.1 ± 0.2	3.8 ± 0.2
Seminal vesicles (g)	1.5 ± 0.2	2.0 ± 0.1	2.1 ± 0.1	1.9 ± 0.1
Relative testis wt	8 ± 0	7 ± 0	8 ± 1	6 ± 0
Relative seminal vesicle wt†	3 ± 0	4 ± 0	4 ± 0	3 ± 0

*Mean ± SEM.
†(Organ wt/body wt) × 1000.

George et al., 1996

The effects of chronic administration of cocaine on spermatogenesis and fertility were evaluated in prepubertal male Sprague-Dawley rats (George et al., 1996). Four groups of male rats (25 day of age) were subcutaneously administered a single dose of cocaine (0 or 15 mg/kg) either daily or twice weekly (Saturday and Sunday) for 100 days. Male's endpoints evaluation included: sexual behavior, fertility, sperm test (conducted 6 hours after the sexual behavior test), testis and epididymides weight and histology, hormonal analysis (follicle stimulating hormone, luteinizing hormone, and serum testosterone levels), histological examination of the testes (quantitative and qualitative morphologic assessment of the seminiferous tubules to assess the effect on the various stages of spermatogenesis), Sertoli cell count, and spermatid count. Pregnancy weight and birth weights were evaluated. Offspring endpoint evaluation included: birth weight and weight at 10 days of age.

Results from the study suggested that cocaine adversely affected male fertility after chronic administration. Compared to the control (86%) group ($p<0.05$), only 33% (3/9) of the female in the daily cocaine group became pregnant after 10 days of mating. After 20 days of mating, the pregnancy rate in the daily cocaine group was 50% compared to a pregnancy rate of 100% in the control group ($p<0.05$). Cocaine treatment on weekends only had no effect on fertility; pregnancy rate in the weekend cocaine group was not significantly different from the control group after 10 days (100% vs 100%) of dosing and 20 days of dosing (100% vs 86%). The birth weights of offspring from the group receiving daily cocaine were 12% less than that of controls ($p < 0.05$), but by 10

Reference ID: 4500620

days there was no difference between groups. The weight of the testis and epididymis was not affected by cocaine exposure. There was no significant difference in the levels of testosterone, FSH, or LH among the cocaine treated groups and the controls. Morphologic analysis suggests that cocaine produced significant effects on spermatogenesis. Compared to the control group, the mean diameter of seminiferous tubules in the daily and twice weekly cocaine groups was significantly ($p<0.05$) reduced. The mean diameter of the tubules in the daily cocaine group was 213.16 ± 2.66 mcm and in the control group it was 272.95 ± 5.35 mcm. In the weekend-treated group the mean diameter was 230.36 ± 5.63 mcm, and controls showed 250.19 ± 4.26 mcm. Similarly, compared to the control, the thickness (height) of the germinal epithelium was also significantly ($p<0.05$) reduced in the cocaine-treated groups. The thickness of the germinal epithelium in the daily treated group was 70.80 ± 1.34 mcm. (control 89.71 ± 2.0 mcm.), and in the weekend treated group was 78.98 ± 1.53 mcm. (control 86.92 ± 1.76 mcm). However, there was no evidence of sloughed Sertoli cells or germ cells in the tubular lumen or epididymis. Cocaine treatment of prepubertal rats adversely affected spermatids. The number of step VII spermatids was reduced in both daily and twice weekly cocaine groups, a difference that was statistically significant ($p < 0.05$). Corrected true germ cell count (as reflected in counts of Step VII spermatids) in the daily cocaine group was 89.33 ± 3.92 compared with 110.83 ± 5.69 in the control ($p < 0.05$). In the weekend cocaine group the number was 80.57 ± 2.14, and in the controls it was 95.8 ± 4.63 ($p <0.05$).

Li et al., 1997

The histological effects of chronic administration of cocaine on the testes were evaluated in prepubertal male Sprague-Dawley rats (Li et al., 1997). Four groups of male rats (25 day of age) were subcutaneously administered a single dose of cocaine (0 or 15 mg/kg) either daily or twice weekly (Saturday and Sunday) for 100 days. Male endpoint evaluations included: histological examination of the testes (quantitative and qualitative morphologic assessment of the seminiferous tubules to assess the effect on the various stages of spermatogenesis), Sertoli cell count, and spermatid count. Compared to the control group, the mean diameter of seminiferous tubules in the daily and twice weekly cocaine groups was significantly ($p<0.05$) reduced. The mean diameter of the tubules in the daily cocaine group was 70.80 ± 1.34 mcm compared to 89.71 ± 2.00 mcm from the control animals. In the weekend-treated group the mean diameter was 78.98 ± 2.54 mcm, and tubule thickness in control animals was 86.92. ± 1.76 mcm. Compared to the control, the thickness (height) of the germinal epithelium was also significantly ($p<0.05$) reduced in the cocaine-treated groups. The thickness of the germinal epithelium in the daily treated group was 70.80 ± 1.34 mcm. (control 89.71 ± 2.0 mcm.), and in the weekend treated group was 78.98 ± 1.53 mcm. (control 86.92 ± 1.76 μm). There was evidence that the number of degenerating germ cells was significantly greater in the cocaine-treated animals compared to the control animals. However, there was no evidence of sloughed Sertoli cells or germ cells in the tubular lumen or in the epididymis. The cocaine-treated animals also failed to release mature spermatids.

Li et al., 1999

Reference ID: 4500620

The effects of subcutaneously administered cocaine on blood flow to the testes were evaluated in prepubertal male Sprague-Dawley rats (Li et al., 1999). Two groups of male rats (25 day of age) were subcutaneously administered a single dose of cocaine (0 or 30 mg/kg). Testicular blood blow was measured via Xenon clearance. Xenon-133 wash out experiments were carried out at 5, 10, 15, 20, 30, 45, 60, 90, and 270 minutes after cocaine or saline administration; individual animals were used for each time point. Compared to the control, subcutaneous injections of cocaine (30 mg/kg) to rats resulted in significantly ($p<0.05$) reduced testicular blood flow 15 to 60 minutes post-injection.

Summary

As per ICH S5(R2), fertility and early embryonic development studies are intended to test for toxic effects of a compound resulting from treatment prior to mating (males and females) through mating and implantation. For females, the studies should characterize effects on the estrus cycle, tubal transport, implantation, and development of preimplantation states of the embryo as well as mating behavior and fertility assessments. The acceptable dosing regimen is to dose females at least 2 weeks prior to mating through implantation (for rats, implantation is generally by Gestation Day 6-7 or pregnancy). For males, the studies should evaluate the impact of a drug on functional effects (e.g., libido, epididymal sperm maturation) that may not be detected via histopathological examination of the male reproductive tissues. Although spermatogenesis in the rat lasts 63 days, acceptable dosing regiments require that the rats be tested for at least 28 days prior to mating. Male rodents are considered sexually mature at approximately 50 days of age (7 weeks). However, male rodents do not have sperm in the cauda epididymis until Day 70 (Week 7). As such most standard study protocols test males between 10 and 12 weeks of age. As noted in the table above, most of the published studies actually tested juvenile animals and as such, these findings are relevant to pediatric indications and should be included in Section 8.4.

Evaluation of the dosing regimens and endpoints examined in the literature cited above indicates that a GLP female fertility and early embryonic development study with cocaine is warranted because the appropriate endpoints have not been fully characterized. Nonetheless, some of the information in the literature is appropriate for labeling. In terms of male fertility, histopathological evaluation of the testes, as well as fertility assessment and sperm morphology data do appear in the published literature. These studies do not clearly define a NOAEL but do characterize potential toxicity for peripubertal rats. These data clearly suggest a greater risk for younger male rats than older male rats.

9.2 Embryonic Fetal Development

Applicant did not conduct standard embryonic fetal development studies. However, the Applicant selected several embryo-fetal development studies that characterize cocaine induced toxicity, which included studies conducted in mice, rats, sheep, and primates.

Reference ID: 4520620

As per ICH S5(R2), embryo-fetal development studies are intended to detect adverse effects on the pregnant female and development of the embryo and fetus consequent to test article exposure of the female implantation to closure of the hard palate. The studies should characterize the toxicity profile of the test article compared to nonpregnant females as well as assess embryofetal death, altered growth and structural changes (including soft tissue and skeletal examination). The acceptable dosing regimen is to dose pregnant females from implantation to the closure of the hard palate (GD 6 to 18 for mice and rats; GD 6-20 for rabbits) and then perform Cesarean section on females one day prior to parturition (GD 18 mice; GD 20-21 rats; GD 29 rabbits). At terminal examination, the following endpoints are recorded: macroscopic examination of all adults (histological evaluation), corpora lutea number, number of live and dead implantations, individual fetal body weight, fetal abnormalities (soft tissue and skeletal changes), and gross evaluation of the placenta.

Several studies summarized by the Applicant for this section were not deemed relevant by this Reviewer given that several citations examined nonclinical animal models that do not conform to ICH S5 study designs (e.g. in vitro assays, chick embryo, sheep studies), did not examine standard embryofetal endpoints, or were not peer-reviewed journal articles but rather were conference abstracts that lacked sufficient detail. Only those published nonclinical literature submitted by the Applicant that contained sufficient details and data to permit substantive review and evaluated standard embryofetal study endpoints are summarized below.

Mice

The developmental toxicity of cocaine administration was evaluated in several strains of mice using subcutaneous and intraperitoneal routes during the early period of organogenesis. These studies show that cocaine at low multiples of clinically relevant doses produced skeletal and visceral malformations in mice. The primary malformations observed included urogenital defects (i.e. enlarged renal pelvis, hydronephrosis and cryptorchidism), ocular defects (i.e. anophthalmia and microphthalmia), and various defects in skeletal ossification.

Mahalik et al., 1980

Mahalik et al. (1980) evaluated the teratogenic effects of cocaine once daily by subcutaneous injection in pregnant CF-1 mice that were divided into one of thirteen groups of eight mice (Mahalik et al., 1980). Six groups were subcutaneously administered a single dose of 60 mg/kg cocaine on Day 7, 8, 9, 10, 11, or 12. Dose selection was based on dose range-finding studies that demonstrated that doses of 70 mg/kg and higher resulted in convulsions and doses of 100 mg and higher resulted in mortality. Six groups received a single administration of saline on Day 7, 8, 9, 10, 11, or 12. One group of mice was untreated and served as a control group. Pregnant mice were sacrificed on Day 18 prior to the termination of gestation. After Cesarean section, uterine horns were examined grossly for fetal swellings and resorption sites. Fetal endpoint evaluation included body weight, sex, gross examination for external fetal

Reference ID: 4520620

malformations, visceral examination in every other fetus. Relative to vehicle control, no significant difference in maternal body weight was observed. There were no maternal mortalities or convulsions. The only clinical signs reported at this dose were described as mild excitatory responses and a Straub tail. Significant differences were noted in resorption on Day 10 and sex ratio on Day 9 when compared to vehicle control. Results from the study are presented in the Applicant's tables below. Compared to controls, soft tissue abnormalities were observed on each day of cocaine treatment. On Days 7 to 9, cocaine showed a significant increase in skeletal abnormalities compared with the vehicle control. Following subcutaneous administration of cocaine hydrochloride (2%) at 60 mg/kg/day, a significant incidence of fetuses with exencephaly, cryptorchidism, hydronephrosis, anophthalmia, malformed or missing lenses, delayed ossification of the skull, paws or centrum, extra ribs, malformed or crankshaft sternebrae and split or butterfly xiphoid processes were reported. Cocaine alone group showed a significant increase in visceral malformations as compared to control group, such as cerebral hemorrhage, hydrocephalus, intra-abdominal hemorrhage, and anomalies of the limb.

Table I—Mean Values of the Test Groups

Treatment[a]	Day	Maternal Weight Ratio, S/T[b]	Fetal Ratio, Right Horn/ Left Horn	Resorption Ratio, Right Horn/ Left Horn	Mean Fetal Weight, g	Sex Ratio, M/F	Soft Tissue Abnormalities	Skeletal Abnormalities
Untreated	—	28.2/50.9	5.6/5.3	0.3/0.2	1.13	4.7/6.2	0.0	1.3
Saline	7	30.2/56.3	6.1/5.6	0.0/0.2	1.16	8.0/3.7	0.1	3.5
	8	28.1/54.2	6.3/5.3	0.2/0.2	1.14	7.0/4.7	0.2	1.7
	9	28.4/53.3	6.3/5.1	0.3/0.0	1.17	8.3/3.1	0.1	1.6
	10	30.7/55.6	5.7/5.7	0.0/0.6	1.15	6.8/4.6	0.0	4.0
	11	30.6/53.0	5.8/5.5	0.0/0.5	1.11	6.1/5.2	0.0	2.6
	12	27.4/52.1	6.5/4.6	0.5/0.0	1.15	7.1/4.0	0.0	3.8
Cocaine	7	29.5/54.8	5.5/6.2	0.2/0.3	1.14	5.6/6.1	6.0[c]	7.7[c]
	8	28.6/50.9	5.7/5.0	0.5/0.2	1.10	6.2/4.5	2.1[c]	7.3[c]
	9	29.8/54.4	6.7/4.7	0.7/0.2	1.12	6.3[c]/5.1[c]	2.2[c]	8.6[c]
	10	30.1/52.0	5.7/4.3	1.1[c]/1.0	1.13	6.2/3.8	2.1[c]	5.2
	11	30.4/53.6	6.5/4.3	0.6/0.7	1.13	6.6/4.2	2.6[c]	6.0
	12	28.6/53.3	5.8/5.2	0.1/0.2	1.19	6.0/5.0	1.0[c]	8.1

[a] The dose of saline was 0.3 ml sc, and the dose of cocaine was 60 mg/kg sc. [b] S = starting weight, and T = terminal weight. [c] Statistically significant in comparison with respective saline controls, $p < 0.05$.

Table II—Occurrence of Exencephaly, Cryptorchidism, and Hydronephrosis on Day of Treatment (Compared with Saline)

		Exencephaly				Cryptorchidism				Hydronephrosis			
		Litters		Fetuses		Litters		Fetuses		Litters		Fetuses	
Treatment[a]	Day	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Untreated	—	8	0	43	0	8	0	43	0	8	0	43	0
Saline	7	8	0	45	0	7	1	44	1	8	0	45	0
	8	8	0	46	0	7	1	45	1	8	0	46	0
	9	8	0	44	0	7	1	43	1	8	0	44	0
	10	8	0	44	0	8	0	44	0	8	0	44	0
	11	8	0	44	0	8	0	44	0	8	0	44	0
	12	8	0	43	0	8	0	43	0	8	0	43	0
Cocaine	7	7	1	44	1	6	2	42	3	4	4[b]	37	8[b]
	8	7	1	39	2	4	4	35	6[b]	7	1	40	1
	9	4	4[b]	41	5[b]	5	3	42	4	4	4[b]	41	5[b]
	10	7	1	39	1	4	4[b]	33	7[b]	6	2	37	3
	11	8	0	44	0	5	3	39	5[b]	6	2	41	3
	12	8	0	44	0	6	2	42	2	7	1	43	1

[a] The dose of saline was 0.3 ml sc, and the dose of cocaine was 60 mg/kg sc. [b] Statistically significant in comparison with respective saline controls, $p < 0.05$.

Reference ID: 4500620

Table III—Occurrence of Anophthalmia, Malformed Lenses, and Missing Lenses on Day of Treatment (Compared with Saline)

		Anophthalmia				Malformed Lenses				Missing Lenses			
		Litters		Fetuses		Litters		Fetuses		Litters		Fetuses	
Treatment[a]	Day	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Untreated Saline	—	8	0	43	0	8	0	43	0	8	0	43	0
	7	8	0	45	0	8	0	45	0	8	0	45	0
	8	8	0	46	0	8	0	46	0	8	0	46	0
	9	8	0	44	0	8	0	44	0	8	0	44	0
	10	8	0	44	0	8	0	44	0	8	0	44	0
	11	8	0	44	0	8	0	44	0	8	0	44	0
	12	8	0	43	0	8	0	43	0	8	0	43	0
Cocaine	7	5	3	41	4[b]	3	5[b]	31	14[b]	4	4[b]	39	6[b]
	8	6	2	39	2	5	3	38	3	6	2	39	2
	9	6	2	44	2	6	2	44	2	8	0	46	0
	10	5	3	37	3	7	1	39	1	7	1	39	1
	11	5	3	40	4[b]	6	2	42	2	5	3	41	3
	12	6	2	42	2	8	0	44	0	5	3	41	3

[a] The dose of saline was 0.3 ml sc, and the dose of cocaine was 60 mg/kg sc. [b] Statistically significant in comparison with respective saline controls, $p < 0.05$.

Table IV—Occurrence of Delayed Ossification of the Skull, Paws, and Centrum on Day of Treatment (Compared with Saline)

		Delayed Ossification Skull				Delayed Ossification Paws				Delayed Ossification Centrum			
		Litters		Fetuses		Litters		Fetuses		Litters		Fetuses	
Treatment[a]	Day	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Untreated Saline	—	8	0	45	0	3	5	36	9	8	0	45	0
	7	8	0	50	0	3	5	34	16	8	0	50	0
	8	8	0	48	0	4	4	41	7	8	0	48	0
	9	8	0	48	0	7	1	43	5	8	0	48	0
	10	8	0	48	0	2	6	32	16	8	0	48	0
	11	8	0	47	0	4	4	33	14	8	0	47	0
	12	7	1	45	1	2	6	32	14	8	0	46	0
Cocaine	7	8	0	49	0	2	6	26	23	8	0	49	0
	8	7	1	42	3	2	6	22	23[b]	8	0	46	0
	9	7	1	45	1	1	7[b]	16	30[b]	7	1	45	1
	10	7	1	40	1	1	7	20	21	8	0	41	0
	11	6	2	38	5[b]	2	6	24	19	8	0	43	0
	12	2	5[b]	34	10[b]	0	7	19	25[b]	6	1	40	4[b]

[a] The dose of saline was 0.3 ml sc, and the dose of cocaine was 60 mg/kg sc. [b] Statistically significant in comparison with respective saline controls, $p < 0.05$.

Table V—Occurrence of Split Sternebrae, Malformed Sternebrae, and Crankshaft Sternebrae on Day of Treatment (Compared with Saline)

		Split Sternebrae				Malformed Sternebrae				Crankshaft Sternebrae			
		Litters		Fetuses		Litters		Fetuses		Litters		Fetuses	
Treatment[a]	Day	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Untreated Saline	—	7	1	44	1	8	0	45	0	7	1	44	1
	7	3	5	40	10	8	0	50	0	7	1	49	1
	8	5	3	43	5	8	0	48	0	7	1	47	1
	9	5	3	44	4	6	2	46	2	6	2	46	2
	10	3	5	41	7	4	4	43	5	7	1	47	1
	11	6	2	45	2	8	0	47	0	6	2	45	2
	12	4	4	41	5	4	4	41	5	6	2	43	3
Cocaine	7	2	6	39	10	2	6[b]	35	14[b]	6	2	46	3
	8	4	4	38	7	4	4[b]	37	8[b]	4	4	39	6[b]
	9	1	7[b]	33	13[b]	5	3	39	7	3	5	37	9[b]
	10	5	3	37	4	1	7	29	12[b]	7	1	40	1
	11	5	3	38	5	3	5[b]	35	8[b]	6	2	41	2
	12	3	4	34	10	2	5	38	6	6	1	43	1

[a] The dose of saline was 0.3 ml sc, and the dose of cocaine was 60 mg/kg sc. [b] Statistically significant in comparison with respective saline controls, $p < 0.05$.

A developmental NOAEL was not identified in this study as only one dose, which produced adverse embryofetal effects, was tested. This dose of 60 mg/kg/day is 7.8-times the human reference dose of 37.5 mg of cocaine via the 4% solution.

Ohnaka et al., 2001

Ohnaka et al evaluated the effects of cocaine alone, alcohol alone, and the combination of cocaine in combination with alcohol (Ohnaka et al., 2001). Pregnant ICR mice were

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divided into 4 groups as follows: Group A received saline solution IP on Gestational Days 7, 8, 9. Group B received sucrose solution diluted in distilled water with a caloric value similar to that of the 10% ethanol solution. Group C received 10% ethanol solution given ad libitum. Group D received 50 mg/kg cocaine IP on GD 7, 8, and 9. Tap water was given ad libitum. Group E received 50 mg/kg/day cocaine IP on GD 7, 8, 9 with 10% ethanol solution given ad libitum. Mean number of implants, mean fetal body weights, mean placental weights were not different between the cocaine group compared to the control group.

Group	Treatment	Fetal Mortality rate (%)	External Malformation rate (%)
A	Control	5.2	0
B	Sucrose	15.9	1.7
C	Ethanol p.o.	15.3	0
D	Cocaine i.p.	6.8	1.6
E	Ethanol p.o. +Cocaine i.p.	27.0*	1.6

*Significantly different from Group A and D (P<0.05)

Group	Treatment	No. of live fetuses	Cleft palate	polydactyly	Tail anomaly or anal atresia
A	Control	139	0	0	0
B	Sucrose p.o.	93	1	1	0
C	Ethanol p.o.	132	0	0	0
D	Cocaine i.p.	131	1	1	0
E	Ethanol p.o. +Cocaine i.p.	105	1	0	1

Cleft palate, polydactyl and tail anomaly were observed in fetuses exposed to cocaine but the rate of malformations were not significantly higher in any group. However, in dams that received both cocaine and alcohol, increased fetal mortality and fetal weight loss were noted. Results suggest that 50 mg/kg IP cocaine administered at early stages of organogenesis did not exhibit embryotoxicity.

Hunter et al., 1995

Hunter et al. evaluated the teratogenic effects of cocaine administrated to ICR mice during early organogenesis (Hunter et al., 1995). On Gestation Day 9, cocaine was intraperitoneally administered at doses ranging from 20 to 100 mg. Control animals were injected (IP) with saline. The developmental effects of 60 mg/kg cocaine on

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different days of gestation were evaluated following administration on Day 8, 9, or 10 or on all three days (8-10). Results from the study are presented in the tables below.

TABLE 1
Effects of Intraperitoneal Cocaine Administration on Day 9 of Gestation: Maternal

Treatment (mg/kg)	Maternal deaths % (*N*)	Maternal body weight change (g)	
		Days 9–10	Days 9–19
Control	0 (0/28)	1.0 ± 0.1 (11)	22.3 ± 1.1
20	0 (0/12)	0.8 ± 0.3 (7)	23.3 ± 1.2
30	0 (0/12)	1.6 ± 0.7 (9)	20.8 ± 2.3
40	0 (0/11)	−0.5 ± 0.2 (3)	19.2 ± 2.3
50	0 (0/10)	0.0 ± 0.1 (5)	22.6 ± 1.3
60	12 (2/16)	−0.9 ± 0.4* (4)	21.3 ± 0.8
70	48 (22/46)	−4.2 ± 0.7* (9)	18.8 ± 1.1
80	75 (9/12)	—	21.8 ± 0.4
100	100 (3/3)	—	—
Fasted	0 (0/11)	−4.4 ± 0.3* (9)	22.7 ± 1.3

* $p < 0.05$ compared to control.

TABLE 2
Effects of Cocaine Administration on Day 9 of Gestation: Developmental[a]

Treatment (mg/kg)	No. litters	Implants	Live pups	Nonlive conceptuses			Pup weights (g)
				Total	Early	Late	
Control	28	12.7 ± 0.5	11.7 ± 0.5	1.1 ± 0.2	0.9 ± 0.2	0.2 ± 0.1	1.24 ± 0.02
Intraperitoneal administration							
20	12	13.9 ± 0.7	12.5 ± 0.9	1.4 ± 0.5	1.2 ± 0.4	0.2 ± 0.1	1.28 ± 0.04
30	11	12.7 ± 1.5	12.0 ± 1.4	1.0 ± 0.3	0.8 ± 0.3	0.2 ± 0.2	1.24 ± 0.03
40	11	11.7 ± 1.5	10.3 ± 1.4	1.5 ± 0.7	1.3 ± 0.7	0.2 ± 0.1	1.28 ± 0.05
50	10	14.3 ± 1.0	12.4 ± 0.8	1.9 ± 0.7	1.6 ± 0.6	0.3 ± 0.2	1.24 ± 0.03
60	14	12.9 ± 0.3	12.5 ± 0.4	0.4 ± 0.3	0.2 ± 0.2	0.2 ± 0.2	1.17 ± 0.04
70	9	12.9 ± 0.6	11.3 ± 0.9	1.6 ± 0.5	0.8 ± 0.2	0.8 ± 0.5*	1.18 ± 0.03
Fasted	11	12.9 ± 0.9	12.3 ± 0.9	0.6 ± 0.2	0.5 ± 0.2	0.1 ± 0.1	1.34 ± 0.03*
Subcutaneous administration							
70	13	13.5 ± 0.7	12.7 ± 0.8	0.7 ± 0.2	0.4 ± 0.1	0.4 ± 0.2	1.29 ± 0.02

[a] The mean ± SEM are presented on a per litter basis.
* $p < 0.05$ compared to control.

Maternal lethality occurred at doses ≥ 60 mg/kg. One hundred percent lethality occurred at the 100 mg/kg dose of cocaine. Cocaine administration on Day 9, no significant effect was noted on the number of implantation or number of live pups/litter when evaluated on Day 19. However, a significant increase in dead conceptus during late gestation was observed at higher dose that also produced maternal deaths.

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TABLE 3
Effects of Intraperitoneal Cocaine Administration on Day 9 of Gestation: Pup Abnormalities[a]

Treatment (mg/kg)	External abnormalities		Visceral abnormalities	
	Fetuses	Litters	Fetuses	Litters
Control	0 (0/298)	0 (0/25)	7.4 (22/298)	32 (8/25)
20	0.7 (1/146)	8 (1/12)	15.8 (23/146)*	83 (10/12)*
30	0 (0/132)	0 (0/11)	11.4 (15/132)	55 (6/11)
40	0.9 (1/112)	9 (1/11)	15.2 (17/112)*	64 (7/11)
50	1.6 (2/123)	20 (2/10)	18.7 (23/123)*	100 (10/10)*
60	1.5 (2/132)	18 (2/11)	15.9 (21/132)*	64 (7/11)
70	1.3 (1/79)	14 (1/7)	43.0 (34/79)*	100 (7/7)*
Fasted	0 (0/119)	0 (0/10)	3.4 (4/119)	40 (4/10)

[a] Data are presented as percentage affected (*n* affected/*n* evaluated).
* $p < 0.05$ compared to control.

No cocaine-induced external malformations were noted. However, a statistically significant increase incidence of visceral abnormalities was noted, namely enlarged renal pelvis. Incidences of enlarged renal pelvis were comparable whether cocaine was administered on Days 8, 9, or 10 or on all 3 days.

TABLE 4
Effect of Cocaine Administration on Day 9 of Gestation: Enlarged Renal Pelvis

Treatment (mg/kg)	Pups[a]	Litters[a]	% pups/litter[b]	Kidney morphological score[b]
Control	5.4 (16/298)	32.0 (8/25)	11.4 ± 4.1	1.08 ± 0.02
Intraperitoneal administration				
20	11.0 (16/146)*	58.3 (7/12)	11.6 ± 4.0	1.15 ± 0.04
30	9.8 (13/132)	45.5 (5/11)	18.1 ± 9.2	1.14 ± 0.04
40	11.6 (13/112)*	45.5 (5/11)	20.9 ± 11.1	1.16 ± 0.05
50	13.8 (17/123)*	80.0 (8/12)*	14.2 ± 3.9	1.18 ± 0.05*
60	13.6 (18/132)*	63.6 (7/11)	13.6 ± 4.5	1.22 ± 0.06*
70	38.0 (30/79)*	100 (7/7)*	40.3 ± 7.3*	1.51 ± 0.09*
Fasted	2.5 (3/119)	30.0 (3/10)	2.8 ± 1.4	1.04 ± 0.03
Subcutaneous administration				
70	2.2 (3/139)	27.3 (3/11)	2.2 ± 1.1	1.05 ± 0.02

[a] Data are presented as percentage affected (*n* affected/*n* evaluated).
[b] Mean ± SEM.
* $p < 0.05$ compared to control.

Results suggest that cocaine exposure at early stages of organogenesis resulted in an increase in the percentage of litters presenting with an enlarged renal pelvis. However,

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when pups from cocaine-treated mothers were allowed to develop to PND 21 and then evaluated, the kidneys were normal, suggesting that morphological alterations observed on GD 19 represent a delay of normal development and not a malformation.

Al-Motabagani and Mohamed, 2005

Al-Motabagani and colleagues (2005) evaluated the teratogenic effects of cocaine, alcohol or the combination of alcohol and cocaine administration (Al-Motabagani and Mohamed, 2005). Pregnant female mice were administered either 50% ethanol alone (25 mg/kg; oral) twice daily, cocaine (20 mg/100 g body weight or 200 mg/kg; IP) daily, 50% ethanol and cocaine, or vehicle control from Gestational Days 6 to 15. Pregnant mice were sacrificed on Day 20 and uterine contents were evaluated and the number of dead and live fetuses were determined as well as the number of resorption. The fetuses were examined for soft tissue anomalies. No significant effect was noted on the number of implantation or number of live pups/litter. An increase in anomalies in the head and neck (i.e., partial cleft palate and hydrocephaly), limb, and kidneys (i.e., unilateral hydronephrosis) were observed as shown in the table below (Table 2 from the article). A significant increase in some anomalies of the head and neck was observed in both alcohol and cocaine groups alone.

Parameter/group	Control group	Alcohol group	Cocaine group	Combined group
No. of foetuses examined for anomalies	57	54	53	63
No. of foetuses with head and neck anomalies	3	18	16	35
Complete cleft palate	1	3	1	7
Partial cleft palate	0	8	8	1
Excencephaly	1	0	0	18
Hydrocephaly	1	7	7	9
No. of foetuses with limb anomalies	3	14	9	25
Phocomelia	0	0	0	1
Wrist drop	1	5	0	9
Clubbing of the foot	1	6	8	10
Syndactly	0	3	1	3
Webbing of toes	1	0	0	1
Ectrodactly of the hind limb	0	0	0	1
No. of fetuses with renal anomalies	4	11	10	25
Unilateral hydronephrosis	2	1	9	3
Bilateral hydronephrosis	1	1	0	11
Ectopic kidney	1	9	1	11
No. of foetuses with kink tail	1	1	1	12

These findings suggest the potential for teratogenicity in fetuses exposed to cocaine during critical periods of early gestation at high doses of cocaine. It is notable that the

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dose of 200 mg/kg appears to exceed the lethal IP dose of cocaine that has been reported in several other publications that utilize the mouse model.

Rats

Several embryo-fetal development studies were identified in the published literature that were conducted in Sprague-Dawley and Long-Evans rats evaluating subcutaneous, and intraperitoneal cocaine administration during organogenesis. These studies demonstrated that cocaine was teratogenic in rats and are described in more detail below.

Study title: The Teratogenicity of Cocaine (Alan G. Fantel and Bonita J. Macphail, 1982)

Key Study Findings

Female rats were treated with cocaine hydrochloride (0, 50, 60, and 75 mg/kg/day) on Gestation Days 8-12 with the following findings:

1. Treatment-related mortalities were observed in maternal rats intraperitoneally administered cocaine at 75 mg/kg/day.
2. Maternal and fetal body weight was significantly reduced at 60 mg/kg/day.
3. Fetal edema, increased resorption and single incidences of cleft palate and hypertrophic left ventricle, and severe autolysis of the fetus were observed at 60 mg/kg/day of cocaine.
4. Maternal and developmental NOAEL = 50 mg/kg/day (HED = 8.1 mg/kg or 484 mg/60 kg person based on body surface area) was defined (based on decreased fetal body weight, resorption and edema findings at the 60 mg/kg dose).
5. Based on a body surface area comparison, the dose of 50 mg/kg in a rat is 8.1-times the maximum recommended dose of 160 mg/60 kg person in which 23.4% of cocaine (37.5 mg) is completely absorbed from the nasopharynx following intranasal administration. For the 10% cocaine topical solution, the dose of 50 mg/kg is 3.6-times the maximum recommended dose of 400 mg/60 kg person in which 33.4% of cocaine is absorbed completely from the pledgets (133.6 mg)

Methods

Doses:	0, 50, 60, 75 mg/day (rats); 60 mg/kg/day (mice)
Frequency of dosing:	Daily on Gestation Days 8-12 (rats); Daily on Gestation Days 7-16 (mice)
Dose volume:	Unknown
Route of administration:	Intraperitoneal

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Formulation: Cocaine HCl was dissolved in Hank's Balanced Salt Solution
Species/Strain: Rats/Sprague-Dawley; Mice/Swiss Webster
Number/Sex/Group: Rats
0 mg – 8 litters
50 mg – 12 litters
60 mg – 9 litters
75 mg – 0 litters (lethal)

Mice
0 mg – 8 litters
60 mg – 8 litters

Observations and Results

Examination of Embryo and Fetuses	
Cesarean/Necropsy	Day 20 of gestation (rats); Day 18 of gestation (mice)
Cesarean Section	The number of corpora lutea, early and late resorptions, dead and live fetuses, placental weight of live fetuses
Fetal Observations	Each fetus was weighed, sexed and examined for external alterations
Skeletal Examination	Examined for external malformations. Fetuses were fixed in a 3:1 ratio in Bouin's solution and 95% ethanol and subsequently cleared with potassium hydroxide and stained with alizarin red for skeletal examination.

Mortality
Cocaine was lethal at the high dose (75 mg/kg/day). All maternal dams died on or before Gestation Day 14.

Body Weight

The mean body weight of dams intraperitoneally administered cocaine at 50 mg/kg/day during the gestation period was comparable to the vehicle-treated dams. Treatment-related effects on the mean body weight were observed in the dams administered the high dose (60 mg/kg/day). Relative to control, the mean body weight was statistically ($p<0.025$) significantly decreased by 18% on Gestation Day 20.

Gestation Day 20 Laparohysterectomy Data

In the low-dose group, a total of 136 fetuses were delivered in 12 litters. Body weight was comparable in the fetuses from the low dose group. Nine resorptions occurred. In the mid-dose group, a total of 94 fetuses were delivered in nine litters; 79 of these fetuses were normal. Compared to control fetuses, fetal weight was reduced; the

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growth retardation was correlated with the reduced maternal weight at this dose. Relative to control, a significant number (p<0.001) of resorptions was observed; 38 resorptions were observed in fetuses from the mid-dose dams. As depicted in the table below, mean fetal weight was reduced approximately 27% in fetus born in the 60 mg/kg/day group.

TABLE 1. Average maternal and fetal weights (in grams) from cocaine-treated and control Sprague-Dawley rats

		Maternal weights		Fetal weights
		Day 8	Day 20	(day 20)
50 mg/kg	N	12	8	136
	$\bar{X}$	278.25	340.88	3.66
	Sx	33.97	43.05	0.40
60 mg/kg	N	9	8	94
	$\bar{X}$	286.33	284.50*	2.67**
	Sx	36.30	57.32	0.99
Control group	N	8	8	100
	$\bar{X}$	256.88	347.00	3.67
	Sx	22.90	33.04	0.33

*P < 0.025 compared to controls.
**P < 0.005 compared to controls.

Offspring (malformations, variations, etc)

No treatment-related effects in the type or incidence of gross external, soft tissue or skeletal malformations were noted following intraperitoneal administration of cocaine in the low dose group that received 50 mg/kg/day. Treatment-related effects were observed in fetuses from the mid-dose group that included single incidences of cleft palate and hypertrophic left ventricle, and severe autolysis of a fetus. It was also noted that in the mid-dose group that two litters had 13 fetuses with edema.

Study title: Effects of Cocaine on Rat Embryo Development in Vivo and in Cultures (Hiba el-Bizri, Ian Guest, and Daya Varma, 1991)

Key Study Findings

Reference ID: 4500620

Pregnant SD rats were treated with cocaine hydrochloride (0, 6.25, 12.5, 25, 50, 100 mcmol/kg = 0, 2.1, 4.2, 8.5, 17, and 34 mg/kg, IP) on Gestation Days 0-19 with the following findings:

1. Treatment-related mortalities were observed in maternal rats intraperitoneally administered cocaine 50 mcmol/kg/day and above.

2. Mean maternal body weight was reduced 4.8% at ≥ 25 mcmol/kg/day.

3. Cocaine caused a dose-dependent increase in soft tissue genitourinary tract findings, which include hydronephrosis and enlarged bladder at all doses.

4. Maternal NOAEL = 12.5 mcmol/kg/day (based on mortality and decreased body weight).

5. A developmental NOAEL was not identified as hydronephrosis was observed at all doses tested.

Methods

Doses:	0, 6.25, 12.5, 25, 50, and 100 mcmol/kg = 0 (saline), 2.1, 4.2, 8.5, 17 and 34 mg/kg/day
Frequency of dosing:	Once daily on Gestation Day 0 to 19
Dose volume:	Unknown
Route of administration:	Intraperitoneal
Formulation:	Cocaine was dissolved in saline
Species/Strain:	Rats/Sprague-Dawley
Number/Sex/Group:	5 to 13 litters/group

Observations and Results

Examination of Embryo and Fetuses	
Cesarean/Necropsy	Day 20 of gestation (rats)
Cesarean Section	The number of corpora lutea, resorption sites, dead and live fetuses, number of runts or edematous fetuses, and fetal and placental weights were recorded. A number of pregnant rats treated with saline or 15 mg/kg cocaine were allowed to deliver spontaneously.
Fetal Observations	Each fetus was weighed, sexed and examined for external alterations.
Fetal Examination	Day 20 fetuses were either fixed in Bouin's fluid for internal organ examination or in ethanol for skeletal examination after staining by Alizarin Red.

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Overt cocaine-induced toxicity included excitation that occurred 15 to 30 minutes following treatment after dosing 5 to 6 days in the dose range of 6.25 to 50 mcmol/kg. In the 100 mcmol/kg group, the animals appeared lethargic, and 3 out of the 5 animals died within 15 to 30 minutes after dosing on Day 6 or 7 of treatment; convulsions preceded the death of these 3 animals. At 100 mcmol/kg, all implants were loss. Maternal and fetal results from the study report are presented in the author's table below. Relative to vehicle control, a small but significant decrease in food consumption and body weight was reported. Prenatal cocaine exposure did not affect gestational duration nor the growth pattern. At 100 mcmol/kg, three of the five dams died after six to seven injections.

Table 1. *Maternal and fetal effects of cocaine in rats**

	Cocaine (μmol/kg)					
Variables	0	6.25	12.5	25	50	100
n	13	6	6	6	17	5
Day 0 body wt (g)	231 ± 4	227 ± 5	235 ± 4	224 ± 6	234 ± 4	227 ± 4
Day 20 body wt (g)	375 ± 6	368 ± 8	374 ± 8	357 ± 10	357 ± 6†	
Food intake (g/d)	23.2 ± 0.2	23.7 ± 0.4	22.6 ± 0.4	22.1 ± 0.5	20.5 ± 0.4†	16 ± 0.4†
Maternal death (*n*)	0	0	0	0	2†	3†
Litter size (*n*)	13.1 ± 0.8	13.1 ± 0.8	14.8 ± 0.8	13.0 ± 0.9	13.3 ± 0.5	0†
Fetal body wt (g)	3.6 ± 0.1	3.4 ± 0.1	3.4 ± 0.1	3.6 ± 0.1	3.6 ± 0.1	
Placental wt (mg)	465 ± 14	440 ± 21	431 ± 17	476 ± 13	438 ± 7	
Edematous fetuses (*n*)	0/170	0/79	0/80	5/63	15/160	
Runts (*n*)	0/170	2/79	3/89†	2/63	12/160†	
Resorptions (*n*)	6	9†	1	5	12†	

* Cocaine was injected intraperitoneally once a day from d 0 to 19 of gestation inclusive and animals were killed on d 20; 0 dose refers to saline injections.

† Significantly ($p < 0.05$) different from the corresponding value for the saline-treated animals; data are either means ± SEM or total numbers.

Cocaine did not affect mean fetal and placental weights, although it increased the number of runts and edematous fetuses. Three pups born to one cocaine-treated animal died 20 days after birth. Brain catecholamine levels, which includes dopamine, epinephrine, and norepinephrine, were measured from Gestational Day 20 fetuses and newborn pups and were slightly elevated but were not significantly different compared to control animals. Cocaine caused a dose-dependent increase in soft tissue genitourinary tract findings, which include hydronephrosis and enlarged bladder as well as cerebral hemorrhage. Results from this study suggest that cocaine is a potential teratogen as evidenced by visceral malformations.

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Table 2. *Fetal abnormalities after daily intraperitoneal injections of cocaine in rats from d 0 to 19 of gestation*

	Cocaine (μmol/kg)				
Variables*	0	6.25	12.5	25	50
Litters examined (*n*)	13	6	6	5	12
Fetuses examined (*n*)	37	19	21	18	34
Enlarged bladder	0	3†	6†	5†	9†
Hydronephrosis	2	7†	8†	8†	16†
Cerebral hemorrhage	0	0	0	1	4
Total abnormal fetuses (*n*)	2	8†	8†	9†	20†

* Refer to fetuses on d 20 of gestation.

† Significantly ($p < 0.05$) different from corresponding values for saline-treated (0 dose of cocaine) animals.

Effects of cocaine (1 mcM – 1 mM) on the growth of embryos in culture were studied to find out if cocaine's fetal toxicity is correlated with the maternal toxicity. In the in vitro embryo culture study, rats were mated during a 2-hour period. Embryos (10-12 somite stage) was harvested from 10.5 day pregnant rats were cultured for 48 hours. Embryo size, mortality (absence of heart beat), and protein content were assessed at the end of the incubation period. Results are shown in the study report figure below. Cocaine elicited a concentration-dependent inhibition of embryo development in culture and was lethal to all embryos at a concentration of 300 mcM. Results from the in vitro embryo culture assay suggest that cocaine can exert direct fetal toxicity but may act independent of interactions with catecholamines as procaine a local anesthetic was also embryotoxic suggestive of a class effect.

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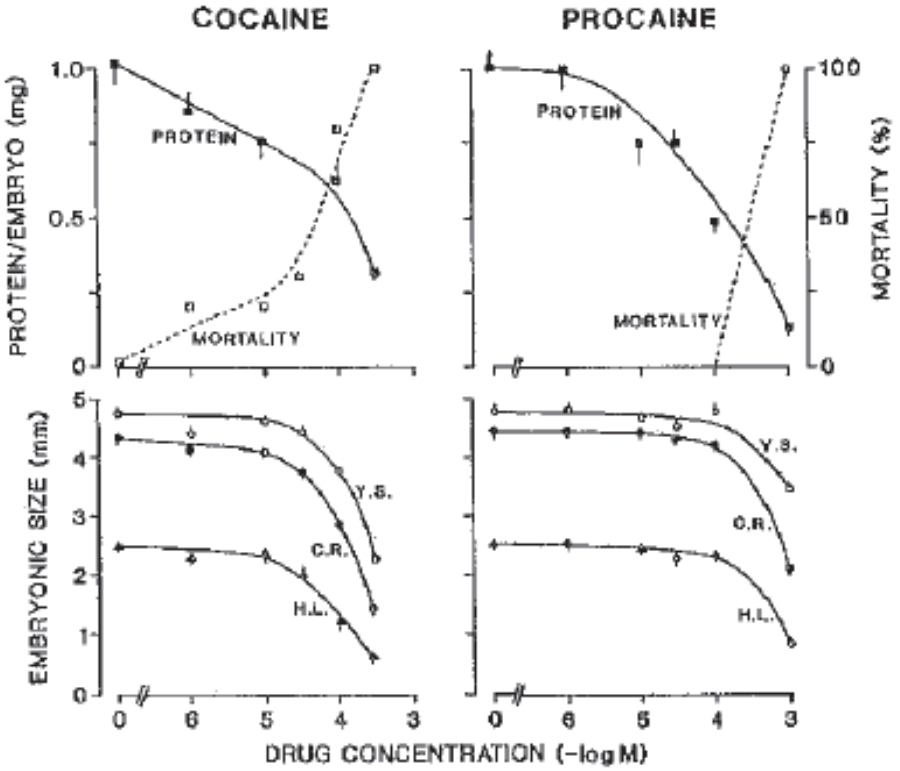

Fig. 1. Effects of cocaine and procaine on the development of rat embryos in culture. Each data point represents mean ± SEM of five to 10 embryos. *Y.S.*, yolk sac diameter; *C.R.*, crown rump; and *H.L.*, head length; absence of heart beats denoted mortality. Significant ($p < 0.05$) differences (not shown in the figure to avoid overcrowding): effects of cocaine on protein content and mortality were significant at all concentrations; procaine significantly inhibited protein content at $>10^{-5}$ M and was lethal to embryos only at 10^{-3} M; embryonic sizes at $>10^{-5}$ cocaine and $>10^{-4}$ procaine are significantly different from the corresponding values at 0 drug (saline) concentration.

Webster and Brown-Woodman, 1990

Webster and Brown-Woodman (1990) evaluated the teratogenic effects of cocaine in pregnant Sprague-Dawley rats as a single or multiple doses following intraperitoneal administration in three different study aims (Webster and Brown-Woodman, 1990). Subsequently, the fetuses were examined at various times post-dose for evidence of hemorrhage and external malformations.

Dose-Response Study. Pregnant Sprague-Dawley rats received a single dose (0, (distilled water) 40, 50, 60, 70, or 80 mg/kg) on Gestational Day 16. Forty hours post-dosing, the fetuses were examined with a dissecting microscope for external malformations and cleft palate. As suggested in the table below, cocaine-induced maternal lethality occurred at the highest dose (80 mg/kg). Percent of implants

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resorbed was significant increased by cocaine exposure to 70 mg/kg relative to vehicle control. Teratogenicity was observed in a dose-dependent manner over a narrow dose range. A single dose of cocaine on Gestation Day 16 induced severe hemorrhage in the fetuses which primarily affected the footplates of both forelimbs and hindlimbs, and distal part of the tail. Hemorrhage was visible in the fetuses 2 hours after dosing. The hemorrhages were also observed in the genital tubercle, and the upper lip and adjacent part of the nose. "Less severe damage was restricted to the distal parts of the middle digits of the limb and the distal part of the tail."

TABLE 1. Effect of dose on cocaine teratogenesis on embryonic day 16 in the rat (fetuses examined 48 hours later)

Treatment (mg/kg)	No. of rats	No. of litters	Mean litter size	Percent of implants resorbed	Percent of live fetuses abnormal	No. of affected litters	Mean fetal weight (g)
40	5	5	17.2	1.1	0.0	0/5	1.23 ± 0.06*
50	7	7	14.0	5.0	7.3*	3/7	1.29 ± 0.08
60	6	5	16.0	3.4	21.5*	4/5	1.27 ± 0.16*
70	5	2	11.0	18.7*	45.8*	2/2	1.24 ± 0.09
80	4	0					
Controls	5	5	12.8	0.0	0.0	0/5	1.41 ± 0.10

*Significantly different from the control; $P < 0.05$.

Treatment Interval Study. On Gestation Day 16, rats were given two injections of 50 mg/kg cocaine at intervals of 1, 2, 3, or 4 hours. Forty hours post-dosing, the fetuses were weighed and examined. Percent of implants resorbed was significant increased by cocaine exposure to 70 mg/kg relative to vehicle control. Findings suggested that when two doses of cocaine (50 mg/kg) were administered 1 to 4 hours apart, the incidence of affected fetuses increases as the time interval between the two doses decreased. "When two doses were given 1 hour apart, the number of affected fetuses increased to 29.5%, two doses 2 hours apart affected 23%, and two doses 3 hours apart affected 17%."

Treatment Interval Study. On Gestation Day 16, rats were given two injections of 50 mg/kg cocaine at intervals of 1, 2, 3, or 4 hours. Forty hours post-dosing, the fetuses were weighed and examined. Percent of implants resorbed was significant increased by cocaine exposure to 70 mg/kg relative to vehicle control. Findings suggested that when two doses of cocaine (50 mg/kg) were administered 1 to 4 hours apart, the incidence of affected fetuses increases as the time interval between the two doses decreased. "When two doses were given 1 hour apart, the number of affected fetuses increased to 29.5%, two doses 2 hours apart affected 23%, and two doses **3** hours apart affected 17%."

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TABLE 2. Effect of treatment interval on cocaine teratogenesis in the rat on embryonic day 16 (fetuses examined 48 hours later)

Dose (50 mg/kg)	No. of rats	No. of litters	Mean litter size	Percent of implants resorbed	Percent of live fetuses abnormal	No. of affected litters	Mean fetal weight (g)
1 injection	7	7	14.0	5.0	7.3	3/7	1.29 ± 0.08
2×1 hr	5	5	13.4	12.0*	29.5*	4/5	1.30 ± 0.09
2×2 hr	7	5	12.8	17.5*	23.1*	4/5	1.20 ± 0.19
2×3 hr	5	5	13.0	13.3	17.0	3/5	1.16 ± 0.12*
2×4 hr	5	4	11.0	13.1	1.8	1/4	1.30 ± 0.02
Controls	4	4	14.3	1.5	0.0	0/4	1.33 ± 0.02

*Significantly different from control; $P<0.05$.

Stage of Pregnancy Study. Pregnant rats were given two intraperitoneal injections of 50 mg/kg cocaine at 2 hour intervals on Gestation Day 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19. The pregnant rats were sacrificed on Gestational Day 21 and the fetuses were weighed and examined. Another group of pregnant rats were given two injections of 50 mg/kg cocaine at 1 hour apart on either Gestation Day 13, 14, 15, 16, 17, 18, or 19. Findings showed that this marginally teratogenic dose of cocaine (50 mg/kg) induced a low incidence of externally visible malformations, and was restricted to litters exposed to cocaine.

TABLE 3. Effect of stage of pregnancy on cocaine teratogenesis in the rat (2 i.p. doses 50 mg/kg 2 hours apart, killed on day 21)

Day of treatment	No. of rats	No. of litters	Mean litter size	Percent of implants resorbed	Percent of live fetuses abnormal	No. of affected litters	Mean fetal weight (g)
9	4	3	9.0	35.4	0.0	0/3	4.90 ± 0.79
10	6	1	18.0	0.0	0.0	0/1	4.34
11	4	1	15.0	0.0	0.0	0/1	4.58
12	3	3	14.3	0.0	0.0	0/3	4.53 ± 0.16
13	3	2	14.0	0.0	0.0	0/2	4.62 ± 0.16
14	3	3	10.7	6.3	0.0	0/3	5.20 ± 0.39*
15	4	4	15.0	0.0	7.8	1/4	4.31 ± 0.13
16	4	3	15.7	5.7	4.2	1/3	4.51 ± 0.41
17	5	4	11.8	17.3*	4.5	1/4	4.55 ± 0.16
18	5	4	12.3	9.9*	7.3	3/4	4.43 ± 0.66
19	5	2	3.0	12.5	16.7	1/2	5.13 ± 0.28
Controls	6	6	13.0	0.9	1.1	1/6	4.46 ± 0.79

*Significantly different from the control; $P<0.05$.

For this study, a NOAEL of 40 mg/kg was identified (HED = 6.5 mg/kg or 387 mg/60 kg person based on body surface area), which was based on hemorrhage, edema and external malformations at doses ≥ 50 mg/kg/day). Based on a body surface area comparison, the dose of 40 mg/kg in a rat is 10.4-times the maximum recommended dose of 160 mg/60 kg person in which 23.4% of cocaine (37.4 mg) is completely absorbed from the nasopharynx following intranasal administration. For the 10% cocaine topical solution, the dose of 40 mg/kg is 2.9-times the maximum recommended dose of 400 mg/60 kg person in which 33.4% of cocaine is absorbed completely from the pledgets (133.6 mg)

Reference ID: 4500620

Summary

The table below summarizes the literature references submitted by the Applicant. Reviewer's comments on the study design, adequacy with respect to typical embryofetal development study endpoints, and utility for labeling language are provided.

Table 5: Summary "Embryo-fetal Development" Studies Submitted by Applicant

Effect Reported	Species	Treatment Period	Doses*	HED (per 60 kg person) of NOAEL / LOAEL	Estimated Exposure Margin for % Cocaine Topical Solution Based on BSA‡		Citation	Comment
					4%	10%		
Exencephaly, cryptorchidism, hydronephrosis, anophthalmia, delayed ossification	CF-1 mice	Single administration on GD 7, 8, 9, 10, 11 or 12	60 mg/kg (SC)	293 mg	7.8x	2.2x	(Mahalik et al., 1980)	Sample size is small. Maternal toxicity was not observed. Dosing period does not span organogenesis (GD 6-17) but study is informative as adverse effects are observed after single dose. Only a single dosing group was evaluated. As adverse findings were observed, a NOAEL was not identified.
Cocaine reduced crown rump length, increased vasodilation and hemorrhage and increased incidence of neural tube defects.	ICR mice	GD 8	78 mg/kg, IP	380 mg	10x	2.8x	(Fisher et al., 1994; Zimmerman et al., 1994)	Not a typical study design. Cocaine given on GD 8, embryos removed GD 10, 12, or 14
Single incidences of cleft palate, polydactyl and tail anomaly were reported but incidences were not significantly different than control.	ICR mice	GD 7-9	50 mg/kg (IP)	244 mg	6.5x	1.8x	(Ohnaka et al., 2001)	Sample size is small. Maternal toxicity was not observed. Dosing period does not span organogenesis (GD 6-17).
Although	ICR	Single	0				(Hunter et	Sample size is small.

Reference ID: 4580670

external malformations were not noted, higher incidences of visceral abnormalities were observed in cocaine exposed pups. Enlarged renal pelvis, which may be indicative of hydronephrosis, was the most consistent abnormality reported.	mice	administration on GD 8, 9, or 10, or repeated administration from GD 8-10	20 30 40 50 60 70 80 100 mg/kg (IP)	97.6 mg 146 mg 194 mg 244 mg 293 mg 342 mg 390 mg 488 mg	2.6x 3.9x 5.2x 6.5x 7.8x 9.1x 10x 13x	0.73x 1.1x 1.5x 1.8x 2.2x 2.6x 2.9x 3.7x	al., 1995)	Maternal deaths were observed at ≥ 60 mg/kg. Dosing period does not span organogenesis (GD 6-17).
Increases in incidences of partial cleft palate, hydrocephaly, clubbing of the foot and hydronephrosis were reported in cocaine exposed pups.	Mice (strain not specified)	GD 6-15	20 mg/ 100 mg BW (or 200 mg/kg) (IP)	975.6 mg	26x	7.3x	(Al-Motabagani and Mohamed, 2005)	Sample size is small. A NOAEL was not identified. Maternal toxicity was not reported, which is surprising given the dose. Other studies have reported that cocaine IP administration is lethal at IP doses greater than 60 mg/kg.
Fetal edema, increased resorption, single incidences of cleft palate and hypertrophic ventricle. Maternal toxicity was observed at the 60 (decreased body weight) and 75 mg/kg/day dose (death) groups.	Sprague-Dawley rats	GD 8 - 12	0 **50** 60 75 mg/kg (IP)	484 mg 580 mg 726 mg	13x 15x 19x	3.6x 4.3x 5.4x	(Fantel and Macphail, 1982)	Sample size is small. Dosing period does not span organogenesis (GD 6-17). Maternal toxicity was observed at the 60 and 75 mg/kg/day dose groups.
Hemorrhage, fetal edema, limb defects	Sprague-Dawley rats	Single administration on GD 9, 10, 11, 12, 13,14, 15, 16, 17, 18 or 19	0 **40** 50 60 70 80 mg/kg (IP)	387 484 581 677 774	10x 13x 15x 18x 20x	2.9x 3.6x 4.3x 5.1x 5.6x	(Webster and Brown-Woodman, 1990)	Cesarean section as well as skeletal and visceral examination were not performed. Sample size is small. Dosing period does not span organogenesis (GD 6-17) but study is informative as adverse effects are observed after single

Reference ID: 4580670

								dose.
Hydronephrosis (all doses), cerebral hemorrhage, fetal edema (7.6, 15, and 30)	Sprague-Dawley rats	GD 0 - 19	2.1 4.2 8.5 17 34 mg/kg (IP)	20.3 mg 40.6 82.2 164.5 329	0.54 x 1.1x 2.2x 4.4x 8.8x	0.15x 0.30x 0.62x 1.2x 2.5x	(el-Bizri et al., 1991)	Sample size is small. Dosing started earlier than typical organogenesis period (GD 6-17). A developmental NOAEL was not identified as hydronephrosis was reported at all doses. Findings were observed at lower doses and may have been due to the initiation of dosing prior to implantation. Most EFD studies are initiated at GD 6.
Decreased litter weight and fetal weights, not entirely dose dependent.	Sprague-Dawley Rats	GD 7-19	20 30 40 50 mg/kg, BID, SC	194 290 387 484 mg BID	5.2x 7.7x 10x 13x Single dose	1.5x 2.2x 2.9x 3.6x Single dose	(Church et al., 1995)	Not an EFD study. Maternal lethality in HD, decreased BW in all doses. Unclear results to inform labeling.
Location in uterine horn impacts potential cocaine exposure and teratogenicity results.	Sprague Dawley Rat	GD 7-21	30 mg/kg, SC	146 mg	3.9x	1.1x	(Lipton et al., 1998)	Not an EFD study. Study examined impact of location in uterine horn on cocaine exposures.

‡: Referenced MDD is 37.5 mg/day for the 4% solution; mean of 23.44% of the total administered 160 mg dose is absorbed.
Referenced MDD is (b) (4) for the 10% solution; mean of 33.34% of the total administered 400 mg dose is absorbed.

Evaluation of the literature cited above indicates the dosing regimens and endpoints examined are not consistent with ICH S5A study designs and therefore GLP embryofetal development studies with cocaine are warranted. Nonetheless, some of the information in the literature are appropriate for labeling given the absence of definitive GLP studies. Collectively these studies demonstrate that cocaine can produce teratogenic effects at multiples of clinically relevant doses and it is also noted that many of the studies did not clearly define a developmental NOAEL. Taken together, these data suggest that cocaine administration during organogenesis as well as a single high exposure of cocaine can result in significant adverse effects to the fetus and mother. Therefore these data should be included into labeling until more definitive GLP studies are conducted.

Reference ID: 4580670

9.3 Prenatal and Postnatal Development

GLP standard prenatal and postnatal development (PPD) studies have not been conducted. The Applicant submitted several published reports in the literature to characterize the effects of cocaine on pre- and postnatal development in rats and primates, which are summarized in the table below. The reviewer has commented on the utility of these studies with respect to typical prenatal and postnatal development study endpoints. As per ICH S5, PPD studies are intended characterize the effects of a drug on the pregnant/lactating female and on development of the conceptus and the offspring after exposure of the female from implantation through weaning. Endpoints in the maternal animals should include clinical signs and mortality, food intake and body weight changes, duration of pregnancy, and partition. Endpoints in the offspring include macroscopic examinations at necropsy, abnormalities, number of live/dead offspring, body weight (at birth, pre- and post-weaning), survival and growth, maturation and fertility of the offspring, physical development, sensory function and reflexes, and behavior. Specific functional testing methods are not outlined by ICH S5; however, studies that assess sensory function, motor activity, and learning and memory are recommended.

Table 6: Pre- and Postnatal Development Studies Identified by the Applicant

Effect Reported	Species	Treatment Period	Doses/NOAEL	HED (mg/day per 60 kg person)	Estimated Exposure Margin for % Cocaine Based on BSA		Citation	Comment
					4%	10%		
No material toxicity Infant toxicity included: Reduced body weight, overall body length, and crown circumference No change in infant reflexes when examined at birth	Pregnant Rhesus Monkeys (n-10 per group)	Prior to conception throughout gestation	Escalating doses up to 7.5 mg/kg IM TID, 5 consecutive days per week (22.5 mg/kg/day)	145 mg TID 435 mg/day	3.9x 12x	1.1x 3.3x	(Morris et al., 1997)	Not a typical PPD study, dosing earlier than typical and no lactational exposures. Detailed assessments of offspring.
Reduced offspring survival Of surviving offspring, no change in heart rate or Apgar scores.	Pregnant Rhesus Monkeys	GD 24 to term	0.3 mg/kg/h cocaine SC via mini-osmotic pump (7.2 mg/kg/day)	5.8 mg/h 139 mg/day	0.15x 3.7x	0.043x 1.0x	(Howell et al., 2001)	Not a standard PPD study, no lactational exposure. Endpoints included fetal survival, heart rate, body weight,

Reference ID: 4520620

								biparietal diameter, body length and "Apgar scores"
No effect on offspring mortality, birthweights, postnatal growth and ontogeny of motor activity. HD pups demonstrated heightened motor activity on PND 20-23.	Female Wistar Rat	GD 8-22	30 mg/kg, PO 60 mg/kg, PO	290 mg 580 mg	7.7x 15x	2.2x 4.3x	(Hutchings et al., 1989)	Not a complete PPD study in terms of dosing or endpoints. Dams has reduced food and water intake and BW deficits. Controls were pair fed to control for decreased food consumption. Pups were fostered by untreated dams.
No effect on offspring body weights, physical maturation, live births. Deficits in learning of an odor/milk association Increased locomotor activity, attenuated response to footshock ‡without change in sensitivity to shock suggesting cognitive deficit	Female Sprague Dawley Rat	GD 8-20 No maternal toxicity reported.	40 mg/kg SC NOAEL for effects on pups was not identified.	387 mg/day	10x	2.9x	(Spear et al., 1989)	Not a complete PPD study No dosing during lactation period No maternal toxicity reported.
Offspring of cocaine treated and pair fed dams demonstrated higher growth rats but constant deficit in forebrain weights compared to offspring from untreated	Pregnant Wistar Rats	GD 8-22	60 mg/kg, SC	580 mg	15x	4.4x	(Silva et al., 1995)	Although not a PPD study, does suggest deficits in CNS development in offspring from cocaine treated dams like the results of food deprivation. A NOAEL was not determined.

Reference ID: 4520620

control or saline dams.								
Prenatal cocaine reduced striatal D2 receptor density in the first week of life but these returned to normal by the second week.	Pregnant Sprague-Dawley rats	GD 7-21	60 mg/kg, PO	580 mg	15x	4.4x	(Stadlin et al., 1995)	Not a PPD study, but does suggest potential for CNS changes during rapid brain growth following in utero exposure to cocaine.
Study suggests differential toxicity during vs. after pregnancy may be due to differential biodistribution of cocaine	Fischer Rat	GD 16, GD 20, post delivery	20 mg/kg, IP	194 mg	5.2x	1.5x	(Dwivedi et al., 1993)	Biodistribution study Clinical significance unclear Not appropriate for labeling at this time
Offspring challenged on PND 20 with cocaine. Prenatal cocaine exposure reduced cocaine induced c-fos in brain and attenuated cocaine-induced behavioral responses	Pregnant Dutch Belted Rabbits	GD 8-29	4 mg/kg, IV BID	77 mg BID (154 mg/day)	2.1x 4.1x	0.58x 1.2x	(Tilakaratne et al., 2001)	Study supports conclusion that cocaine in utero can alter brain development and likely through changes in neurotransmitter receptors. Functional endpoints and species are not standard for a PPD study.

‡: Referenced MDD is 37.5 mg/day for the 4% solution; mean of 23.44% of the total administered 160 mg dose is absorbed. Referenced MDD is (b) (4) for the 10% solution; mean of 33.34% of the total administered 400 mg dose is absorbed.

Below
are some of the published nonclinical studies submitted by the Applicant that evaluated some of the standard pre- and postnatal developmental endpoints, summarized in greater detail.

Study title: The effect of chronic exposure during pregnancy on maternal and infant outcomes in the rhesus monkey (P. Morris, Z. Bintenda, M.P Gilliam, J Klein, K. McMartin, G. Korean, H.M. Duhart, W. Slikker JR. Paule, 1997. Neurotoxicology and Teratogenicity 19(1): 47-57)

GLP compliance: Unknown
Drug, lot #, and % purity: Cocaine, unknown, and 100%

Reference ID: 4520620

Key Study Findings

Pregnant female monkeys were treated with escalating doses of cocaine hydrochloride up to 7.5 mg/kg three times per day, 5 consecutive days per week, prior to conception and throughout gestation with the following findings:
Cocaine and benzoylecgonine levels were detected in the maternal hair and infant hair at the end of the gestation period.

1. Cocaine prenatal exposure during pregnancy had no significant effects on maternal indices.

2. Significant effects at birth were observed in the following infant indices: body weight, overall length, and crown circumference.

3. There were no changes in infant reflexes at birth.

4. Based on observed effects on fetal body weight, length, and crown circumference, the fetal LOEL was defined at 15.6 mg/kg (average dose at term).

5. Based on a body surface area comparison, the dose of 15.6 mg/kg in primates (HED of 302 mg/day) is 8.1-times the maximum recommended dose of 160 mg/60 kg person in which 23.44% of cocaine (37.5 mg) is completely absorbed. For the 10% cocaine topical solution, the dose of 15.6 mg is 2.3-times the maximum recommended dose of 400 mg mg/kg person in which 33.34% of cocaine is (133.6 mg) is completely absorbed.

Methods

Doses:	0 (saline), escalating doses was initially started at 1.0 mg/kg (1-2 weeks), followed by 1.5 mg/kg (1 to 2 weeks), the dose was gradually increased to as much as 2.5-3.0 mg/kg/injection in some monkeys
Frequency of dosing:	Prior to conception and throughout gestation, three times per day, 5 consecutive days per week
Dose volume:	0.1 mL/kg
Route of administration:	Intramuscular
Formulation/Vehicle:	Saline
Species/Strain:	Primates/Rhesus monkey
Number/Sex/Group:	10/female/group

Observations and Results

Clinical Observation (Female)	Body weight at term Body weight gain at term Body weight gain during pregnancy

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Fetal Observations	Body weight Overall length Crown-to-rump length Rump-to-heel length Biparietal diameter Crown circumference
Infant's Behavioral Measures (at birth)	The following reflex measured once: vocalization (the presence of spontaneous vocalization by the infant), rooting (the infant directs its mouth toward an index finger placed on its cheek near the corner of the mouth), eye blink to air puff (the infant blinks in response to a puff of air directed at its face), eye blink to a glabellar tap (the infant blinks in response to the tap of an index finger on its forehead between the eyes), pupillary response (the infant's pupils contract in response to a bright examination light), nystagmus (the presence of uncoordinated or asymmetric movements of the infant's eyeballs), visual orientation (the infant directs its gaze at different objects in the visual field), visual following (the infant follows the movement of an index finger across the visual field), auditory orientation (the infant turns its head toward the sound of a lips-smack made at the side of the head), auditory startle (the infant exhibits startling to a loud shout), placing (the infant places its hand/foot on the surface of the examining table in response to the stroke of an index finger on the dorsum of its hand/foot), prone progression (the infant attempts to move forward/backward when placed prone on the surface of the examining table), plantar grasp (the infant grasps an index finger placed on the palmar surface of its hand), Hoffman sign (the infant flexes its thumb in response to the stretch of a finger) Moro reflex (the infant struggles to remain upright when held supine and its head is suddenly unsupported), and Babinski reflex (the infant flexes its toes in response to the stroke of an index finger across the plantar surface of its foot).
Toxicokinetic Analysis	Not performed. However, at end of gestation, hair sample was collected from the mothers and infants to measure cocaine and benzoylecgonine level.

Mortality

No treatment mortality was observed in the dams.

Maternal Indices

Reference ID: 4520620

EFFECT OF CHRONIC COCAINE EXPOSURE ON MATERNAL OUTCOME: PHYSICAL MEASURES

Index of Outcome	Experimental Group: Control ($N = 10$)	Experimental Group: Cocaine ($N = 10$)
Body weight at term (kg)	8.14 ± 0.40	7.52 ± 0.34
Body weight gain during pregnancy (kg)	1.36 ± 0.20	1.15 ± 0.25
Length of pregnancy (days)	165.10 ± 2.13	163.00 ± 1.21

Rhesus monkeys were assigned to either a Control group ($N = 10$) or a Cocaine group ($N = 10$) that was administered (IM) escalating doses of cocaine, three times per day, 5 consecutive days per week, prior to conception and throughout gestation. The physical measures shown here were obtained from the mothers throughout gestation. Data represent means ± SEMs.

As depicted in the author's table (Table 5, page 54) above, no significant treatment-related effects in maternal primate indices were reported. "There were no significant correlations between any of the indices of maternal outcome and any of the indices of infant outcome."

Infant Indices

EFFECT OF CHRONIC COCAINE EXPOSURE ON INFANT OUTCOME: PHYSICAL MEASURES

Index of Outcome	Experimental Group: Control ($N = 10$)	Experimental Group: Cocaine ($N = 10$)
Body weight (kg)	0.49 ± 0.01	0.43 ± 0.01*
Overall length (cm)	29.86 ± 0.53	27.75 ± 0.70*
Crown-to-rump length (cm)	18.96 ± 0.48	17.50 ± 0.54
Rump-to-heel length (cm)	10.90 ± 0.41	10.25 ± 0.21
Biparietal diameter (cm)	5.13 ± 0.08	5.09 ± 0.10
Crown circumference (cm)	19.25 ± 0.20	18.45 ± 0.24*

Rhesus monkeys were assigned to either a Control group ($N = 10$) or a Cocaine group ($N = 10$) that was administered (IM) escalating doses of cocaine, three times per day, 5 consecutive days per week, prior to conception and throughout gestation. The physical measures shown here were obtained from their infants at birth. Data represent means ± SEMs.

*Cocaine significantly different from Control ($p < 0.05$, unpaired Student's t-test).

Infant indices data is presented in the authors' table (Table 6, page 54) above. At birth, body weight (-12.2%), overall length (-7.1%), and crown circumference (-4.2%) were significantly ($p<0.05$) reduced compared to control infants. No significant treatment-related effects in infant indices at birth with respect to: crown-to-rump length, rump-to-heel length, and biparietal diameter. The infant reflexes in infants exposed to cocaine

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during gestation were comparable to control infants. No significant differences in the cumulative score were observed.

EFFECT OF CHRONIC COCAINE EXPOSURE ON INFANT OUTCOME: BEHAVIORAL MEASURES

	Experimental Group	
Reflex	Control (N = 10)	Cocaine (N = 10)
Vocalization	1.00 ± 0.00	1.00 ± 0.00
Rooting	1.00 ± 0.00	1.00 ± 0.00
Eye blink (puff)	1.00 ± 0.00	1.00 ± 0.00
Eye blink (tap)	1.00 ± 0.00	1.00 ± 0.00
Pupillary response	1.00 ± 0.00	1.00 ± 0.00
Nystagmus	0.00 ± 0.00	0.00 ± 0.00
Visual orientation	1.00 ± 0.00	0.80 ± 0.13
Visual following	0.10 ± 0.09	0.10 ± 0.10
Auditory orientation	0.80 ± 0.13	0.80 ± 0.13
Auditory startle	0.50 ± 0.17	0.10 ± 0.10
Placing	1.00 ± 0.00	1.00 ± 0.00
Prone progression	0.90 ± 0.09	1.00 ± 0.00
Plantar grasp	1.00 ± 0.00	1.00 ± 0.00
Moro reflex	1.00 ± 0.00	1.00 ± 0.00
Babinski reflex	1.00 ± 0.00	1.00 ± 0.00
Cumulative reflex score	11.50 ± 0.27	11.10 ± 0.38

Rhesus monkeys were assigned to either a Control group (N = 10) or a Cocaine group (N = 10) that was administered (IM) escalating doses of cocaine, three times per day, 5 consecutive days per week, prior to conception and throughout gestation. The reflexes shown here were tested in their infants at birth. Assigning a value of "1" for the presence of the reflex and a value of "0" for the absence of the reflex, the algebraic sum of these values was considered a cumulative reflex score. Data represent means ± SEMs.

Toxicokinetic Analysis

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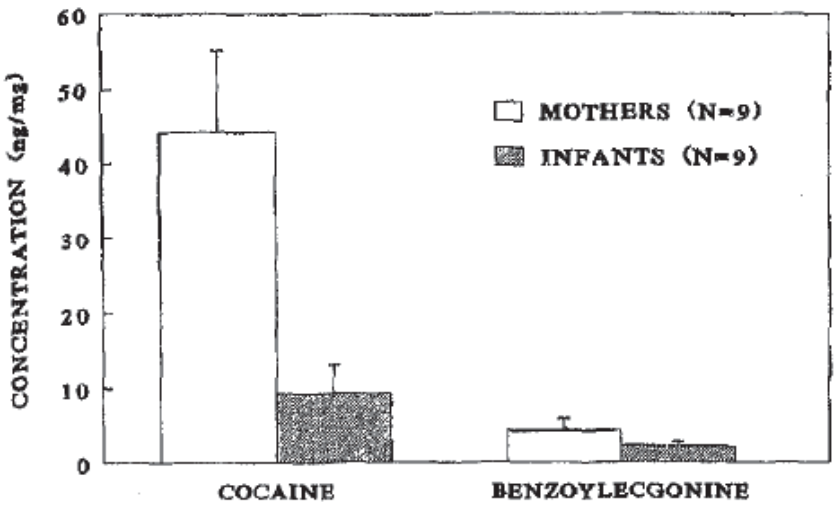

FIG. 2. Effect of chronic cocaine exposure on biochemical measures: maternal and infant hair cocaine and benzoylecgonine. Data represent the levels (ng/mg), as determined by RIA, of cocaine and benzoylecgonine in the first 1 cm (measured from the scalp) of maternal and infant hair samples taken at term, following chronic administration of cocaine to the mothers prior to conception and throughout gestation.

Hair analysis data is presented in the author's figure (Figure 1, page 2) above. Detectable levels of cocaine and benzoylecgonine were measured in all samples of maternal and infant hair from the cocaine treatment group. Hair cocaine levels were approximately 44 and 9 ng/mg for the mothers and the infants, respectively. Cocaine and benzoylecgonine were detected in maternal plasma. Plasma cocaine levels increased rapidly reaching C_{max} at approximately 175 ng/mL at 15 minutes following intramuscular injection; then steadily declined to approximately 30 ng/mL by 120 minutes after injection. The C_{max} level correspond to approximately 4 times the human plasma level of 43 ng/mL following use of this product. AUC data in the primate study were not provided.

Study title: Fetal development in rhesus monkeys exposed prenatally to cocaine (Howell LL, Schama KF, Ellis JE, Grimley PJ, Kitchens AJ, Byrd LD. 2001. Neurotoxicology and Teratology 23:133-140)

GLP compliance:	Unknown
Drug, lot #, and % purity:	Cocaine, unknown, and unknown

Key Study Findings

Pregnant female monkeys were treated with 0.3 mg/kg/h via SC osmotic minipump from postconception Day 24 to birth (Group 1) and conception through Day 42 of gestation (Group 2) with the following findings in the offspring delivered at term (Howell et al., 2001):

1. Cocaine exposure resulted in 22% reduced fetal survival

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2. No evidences that prenatal cocaine exposure were associated with abnormal physical growth in surviving fetuses.

3. No developmental toxicity was observed in surviving fetuses.

4. Survival rate of maternal animals was significantly affected in both early- and late-exposure to cocaine.

Methods

Doses:	Continuous SC infusion of Saline or cocaine 0.3 mg/kg/h via Alzet osmotic minipumps
Frequency of dosing:	Postconception Day 24 to gestation (Group 1); Conception through Day 42 of gestation (Group 2)
Dose volume:	Not specified
Route of administration:	Subcutaneous (osmotic minipump)
Formulation/Vehicle:	Saline
Species/Strain:	Primates/Rhesus monkey
Number/Sex/Group:	18/female/group 1; Group 2: n =12 (treatment group, n= 8 (vehicle control group)

Observations and Results

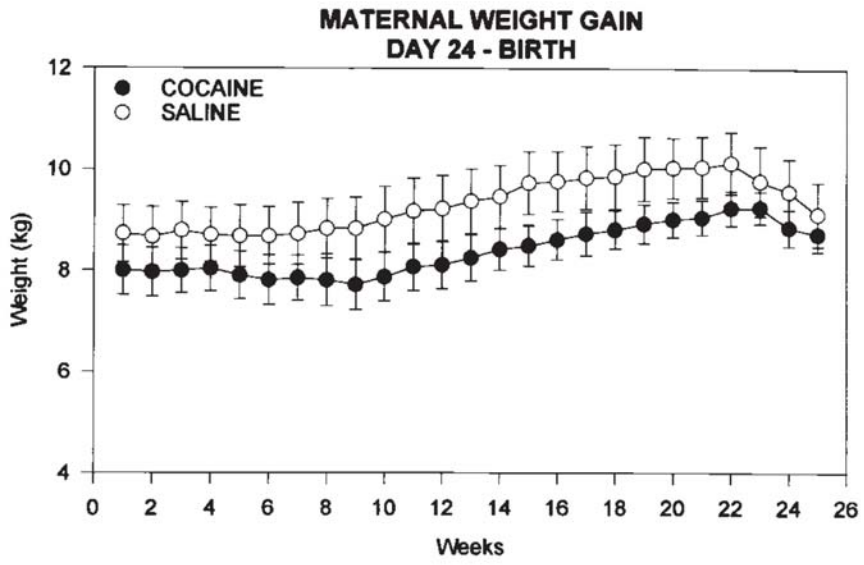
Clinical Observation (Female)	Food consumption (daily) Body weight (weekly) throughout gestation; Body weight at term
Fetal Observations	Diagnostic ultrasonography (at 28-day intervals) to visualize and confirm implantation of the fertilized ovum and to monitor utero development throughout gestation Implantation and development of the blastocyst 24 days after breeding, Development and positioning of the umbilical cord Visualization of the heart beat and cardiac activity Head circumference (biparietal diameter)
Postnatal Development at Birth (immediately after upon removal from their mothers and at two subsequent 30-min intervals)	Gestational age Body weight Biparietal diameter Body length (crown to rump) Apgar scale (heart rate, respiratory effect, muscle tone, behavioral state and color) was used to allow early assessment of neonate physical and behavioral states
Toxicokinetic Analysis	Maternal blood sample.

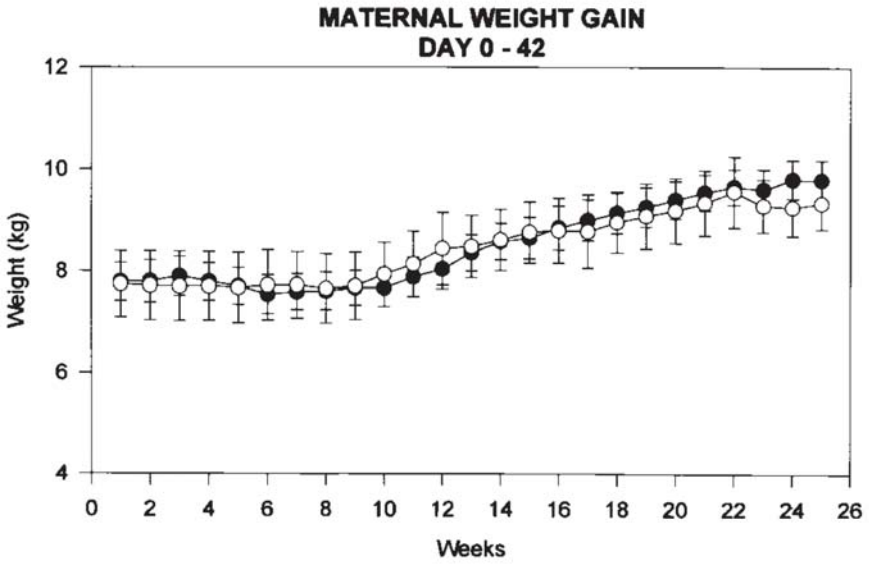
Mortality

No treatment mortality was observed in the dams.

Maternal Data

Reference ID: 4520620





As depicted in the author's figure (Figure 3, Page 137) above, body weight increased during pregnancy (Gestation Day 24 to birth, Gestation Day 0-42). There were no significant differences in weight gain of the cocaine groups and saline groups. The mean body weight in animals exposed to cocaine was 9.2 ± 0.3 kg from Day 24 postconception through gestation. The average body weight in animals exposed to cocaine from conception through Day 42 of gestation was 9.8 ± 0.2 kg.

Fetuses Data

Survival rates were significantly lower in cocaine-exposed fetuses when results were combined for the late- and early-exposure groups. In the late-exposure group, 61% resulted in live births compared to 78% of live births in the control group. Similarly, in

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the early-exposure group, 67% resulted in live births compared to 75% in the pair-fed control group. Spontaneous abortion occurred in four fetuses and two fetuses in the cocaine-exposed group and control group, respectively. In the cocaine-exposed group, one fetus was spontaneously aborted, one was resorbed and two were born dead. One fetus was spontaneously aborted and one was born dead in the control group.

Pregnancy outcomes

	Total pregnancies	Total survived	% Survival
Saline (day 24–birth)	18	14	78
Cocaine (day 24–birth)	18	11	61
Saline (days 0–42)	8	6	75
Cocaine (days 0–42)	12	8	67

Chi-squared analysis was used to determine the statistical significance of survival rates. When data were combined for the late- and early-exposure groups, survival rates were significantly ($P < .05$) lower in cocaine-exposed fetuses.

As depicted in the author's table (Table 6, page 138) below, fetal heart rate was in the normal range. Also, fetal biparietal diameter increased normally during gestation.

Table 6
Fetal heart rate and biparietal diameter

	Month				
	2	3	4	5	6
Heart rate (beats/min)					
Saline (day 24 birth)	188±4	192±2	184±5	190±5	180±4
Cocaine (day 24 birth)	186±4	196±4	194±4	200±4	190±3
Saline (days 0 42)	177±4	187±6	189±6	181±6	174±7
Cocaine (days 0 42)	182±4	186±3	192±9	184±5	179±8
Biparietal diameter (cm)					
Saline (day 24 birth)		2.5±0.1	3.7±0.1	4.3±0.1	4.6±0.0
Cocaine (day 24 birth)		2.7±0.1	3.6±0.1	4.5±0.1	4.6±0.1
Saline (days 0 42)		2.3±0.1	3.7±0.1	4.4±0.1	4.7±0.0
Cocaine (days 0 42)		2.5±0.0	3.5±0.1	4.3±0.1	4.7±0.1

Reference ID: 4500620

Table 7
Physical growth measures at birth

	Gestational age (days)	Weight (g)	BPD (cm)	Crown-rump (cm)
Saline (day 24 birth)	170.5±2.2	524.9±23.2	5.1±0.1	19.9±0.4
Cocaine (day 24 birth)	163.9±2.6	499.9±28.4	5.0±0.1	19.2±0.4
Saline (days 0 42)	160.7±6.1	464.3±42.9	5.0±0.2	18.7±0.5
Cocaine (days 0 42)	174.1±1.9	531.0±21.9	5.1±0.1	19.6±0.3

As depicted in the author's table above (Table 7, page 138), mean gestational length was within normal range for both cocaine and saline groups. No significant differences between control and cocaine groups were observed on body weight, biparietal diameter, and body length.

At birth, no significant differences in Apgar scores were observed between cocaine and control groups.

Table 8
Apgar scores

Time	1	2	3
Saline (day 24–birth)	8.5±0.8	9.1±0.5	9.1±0.5
Cocaine (day 24–birth)	9.4±0.3	9.5±0.4	9.7±0.2
Saline (days 0–42)	8.2±1.0	8.2±1.3	8.0±1.2
Cocaine (days 0–42)	8.9±0.6	8.6±0.6	9.3±0.3

Apgar test — modified for rhesus monkeys[a]

Sign	0	1	2
Respiration	no respiration	<60/min	>60/min
Heart rate	no heart rate	150 bpm	>150 bpm
Muscle tone	flaccid	some tone	flexed
Skin color	gray	gray and pink	pink
State (irritability)	unconscious	passive	active

[a] Apgar scores are assigned at birth and at two subsequent 20-min intervals.

Reference ID: 4500620

Toxicokinetic Analysis

Mean maternal plasma level was 151 ng/mL, approximately 3.5 times the reported human plasma levels. However, AUC data were not provided.

PPD Overall Conclusions

As noted in the data summarized above, although the primate study conducted by NCTR was not a traditional PPD study, they did dose over the entire duration of gestation typically covered by a traditional PPD study. The existing data also suggest adverse effects on the offspring in terms of survival and growth. The existing data fail to test reproductive capacity of the offspring, a standard endpoint in a modern PPD study. Although the existing literature suggest adverse effects are likely to occur in a PPD study, given the lack of reproductive testing, lack of exposure via lactation, evidence for drug accumulation in milk, and lack of adequate toxicokinetic data to fully inform these endpoints, a dedicated rat PPD study is recommended as a postmarketing requirement.

10 Special Toxicology Studies

Juvenile Animal Studies

Juvenile animal studies are intended to obtain safety data for pediatric clinical dosing. They are generally targeted toxicity studies focusing on the developing organ systems of concern for a specific drug and are recommended if the target organ of toxicity for a given drug is undergoing significant changes in the indicated patient population. Cocaine is a CNS active drug. The CNS undergoes dramatic changes over the course of development and is potentially uniquely vulnerable over the entire age range from prior to birth to 20 plus years of age. Given the known CNS target of the drug, juvenile animal studies are recommended to characterize the impact of cocaine during critical periods of brain development.

The Applicant cited three abstracts and one publication in their summary for the Juvenile Toxicity section of the Toxicology Written Summary. Because abstracts are considered preliminary data, may not be reproducible, and are not peer reviewed, these will not be reviewed here. The single publication (NIDA monograph) did not dose juvenile animals and will also not be reviewed here.

The Applicant did reference a few published studies in their discussion of pre- and postnatal development studies that are actually juvenile animal studies. Magalhaes et al. dosed male and female Wistar rat pups with 15 mg/kg cocaine hydrochloride via SC injection twice daily from PND 1 to 27 and examined forced swim behavior on PND 26 and 27 (Magalhaes et al., 2002). They report that cocaine treated animals were less

Reference ID: 4520620

active in the forced swim test and displayed clinical signs interpreted to be consistent with “behavioral despair” suggesting altered ability to cope with stressful situations.

Chen et al. (1993) doses Sprague Dawley rat pups with 40, 60, or 80 mg/kg cocaine from PND 4-9. Pups in the 60 and 80 mg/kg group demonstrated a dose-dependent reduction in survival. All doses produced reduced body weights. There were no reported effects on the mass of the whole brain, forebrain, cerebellum, or brainstem.

A summary of the reviewed literature cited by the Applicant is shown in the Table below. It is noted that a vast amount of literature is available for cocaine and brain development, and the number of publications submitted by the Applicant is not considered exhaustive. As the human pediatric dose is not yet determined and the size of the pledget will likely be smaller pending age of use, the total daily dose will likely be lower for some age groups. As such, exposure margin estimates are not provided at this time.

Table 7: Summary of "Juvenile Animal Studies" Submitted by the Applicant

Key Findings Reported	Species	Ages Tested	Dosing regimen and NOAEL if applicable	Citation	Comment
Dose-dependent reduction in survival rate was observed (60 and 80 mg/kg). All doses reduced body weight No effects on the mass of total brain or forebraln, cerebellum, or brainstem.	Sprague-Dawley Rats (Neonates)	PND 4 – PND 9/SC Corresponds to third trimester in humans	40, 60, 80 mg/kg SC No NOAEL based on body weight reductions	(Chen et al., 1993)	Dosed during brain growth spurt in rat. Measured survival rate, somatic growth, and brain development (brain weight only) Crude brain assessments, not appropriate for labeling.
Cocaine exposure during PND 1-10, but not PND 11-20, resulted in a small increased ASR amplitude on the second day of testing, a finding consistent with an alteration in long-term habituation.	Sprague–Dawley rats (female only)	PND 1–10 or PND 11–20 Corresponds to third trimester and first two years of life in terms of brain development	50 mg/kg, SC	(Dow-Edwards and Hughes, 1995)	Examined acoustic startle response (ASR)
Cocaine treated animals were less active in the forced swim test and displayed clinical signs interpreted to be consistent with “behavioral despair” suggesting altered	Wistar rat pups	PND 1-27	15 mg/kg SC	(Magalhaes et al., 2002)	Examined forced swim test

Reference ID: 4520620

Key Findings Reported	Species	Ages Tested	Dosing regimen and NOAEL if applicable	Citation	Comment
ability to cope with stressful situations					

Juvenile animal studies were not completed by the Applicant to support this application. Rather, the Applicant conducted a literature review to address the safety of the drug product for use in pediatric patients. However,the literature provided by the Applicant are inadequate to characterize the impact of cocaine on the developing brain.

As noted in the agreed Pediatric Study Plan (PSP), juvenile animal studies was required to support future clinical studies in pediatric patients. The Applicant agreed to conduct juvenile animal studies to characterize the impact of cocaine on the developing brain and assess the potential for long-term cognitive/behavioral changes in animals following exposure during the critical periods of brain development. The protocols for the juvenile toxicology studies were submitted to IND (b) (4) (sequence 0037). Cody has requested a waiver for pediatric patients under 12 years of age. On October 29, 2019, the Division met with PeRC to address the Applicant waiver request. The Applicant submitted a revised timeline for the pediatric development plan and requested a waiver for the Infant/Young Child cohort, ages 0 (birth) to < 8 years and the Child cohort, ages 8 to 11 years. PeRC granted the waiver request. Pediatric studies will only be required for children aged 12 to 17 years. During the meeting, it was discussed whether juvenile animal toxicology studies will have any utility given that cocaine has a long history of clinical use. PeRC members questioned the need for studies given the single dose administration proposed. However, the Division does not agree given the literature that single administration of cocaine has been demonstrated to produce effects on reproductive organs. Further other literature have demonstrated that single administration of other CNS active drugs may have profound effects on brain and based on the published literature suggested CNS alterations with no clear NOAEL to date. As such, we continue to recommend that the Applicant be required to conduct the juvenile studies to characterize the potential for long-term effects of cocaine on the developing brain during the critical periods of brain development and the potential for effects on reproductive organs during development.

11 Integrated Summary and Safety Evaluation

See Executive Summary

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/s/

NEWTON H WOO on behalf of BELINDA A HAYES
11/15/2019 02:52:54 PM

NEWTON H WOO
11/15/2019 02:53:23 PM

RICHARD D MELLON
11/15/2019 03:08:13 PM
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:	209575
Supporting document/s:	0001
Applicant's letter date:	September 21, 2017
CDER stamp date:	September 21, 2017
Product:	NUMBRINO (cocaine hydrochloride) nasal solution
Indication:	Induction of local anesthesia of the mucous membranes when performing diagnostic procedures and surgeries on or through the nasal cavities in adults
Applicant:	Cody Laboratories, Inc
Review Division:	Division of Anesthesia, Analgesia, and Addiction Products
Reviewer:	BeLinda A. Hayes, PhD
Supervisor:	R. Daniel Mellon, PhD
Team Leader:	Newton H. Woo, PhD
Division Director:	Sharon Hertz, MD
Project Manager:	Shelly Kapoor

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 209575 are owned by Cody Laboratories Inc or are data for which Cody Laboratories Inc has obtained a written right of reference.
Any information or data necessary for approval of NDA 209575 that Cody Laboratories Inc 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 209575.

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1 Executive Summary

1.1 Introduction

Cody Laboratories Inc submitted a 505(b)(2) NDA for cocaine hydrochloride, 4% and 10% topical solution formulations, for the indication of induction of local anesthesia when performing diagnostic procedures and surgeries on or through the mucous membranes of the nasal cavities in adults. The Applicant is relying upon studies conducted by themselves and published literature to support the nonclinical requirements for this 505(b)(2) application. The Applicant included a 14-day repeat-dose toxicology study in rats to support the local tolerance toxicity of the topical formulation of cocaine hydrochloride and a full battery of genetic toxicology studies to inform labeling. The Applicant was also required to complete nonclinical studies to support any drug substance and drug product specifications that exceeded ICH qualification thresholds and to support the safety of several excipients that have not been used in FDA-approved drug products indicated for intra-nasal use.

1.2 Brief Discussion of Nonclinical Findings

Given the long clinical history of use of cocaine hydrochloride via the intranasal route of administration, the Agency agreed that nonclinical general or reproductive and developmental toxicology studies with cocaine would not be required to support filing the NDA. However, the Applicant was required to submit a detailed literature review to address all requirements for the NDA and submit a comprehensive evaluation of the genotoxic potential of cocaine. The adequacy of the literature to address the standard requirements could only be determined upon review of the literature submitted and the adequacy of the scientific bridge to the referenced studies.

The proposed drug substance and drug product specifications are acceptable. The Applicant provided adequate qualification data for the systemic toxicity and local safety of the drug product formulation and any specifications that exceeded the ICH Q3A/B qualification thresholds. In addition, the Applicant submitted genetic toxicology assays to qualify several impurities/degradants, including (b) (4) was negative in these in vivo assays. Therefore, these impurities have been adequately qualified for safety at the proposed specifications.

The Applicant provided extractable and leachable studies to characterize and justify the safety of potential leachables arising from the container closure systems to be employed in the proposed drug products. However, the leachable studies were deficient because they were not performed using at least three batches of the drug product with testing at multiple timepoints over the course of stability studies. The

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Applicant should be required to submit adequate leachable data over the course of stability to support the safety of the container closure systems.

A standard battery of genotoxicity assays, including the Ames, in vitro chromosome aberration, in vivo bone marrow micronucleus assays were submitted to characterize the genotoxic potential of cocaine. Cocaine tested negative in these studies.

The Applicant submitted a literature search to address the reproductive and developmental toxicity study requirements for cocaine. The literature search was limited and included references to numerous abstracts, which were not reviewed because these data may be preliminary, not reproducible, not peer reviewed, and lack adequate detail to permit substantive review. Based on a preliminary search of the literature, the submitted published studies identified by the Applicant are not complete given the vast literature on cocaine. However, the studies could be used to inform labeling until definitive studies with pharmacokinetic data are completed.

As true for all acute use drug product, the interpretation of reproductive and developmental studies is challenging. To adequately characterize the effect of a drug on rapidly developing systems, dosing must be done over critical periods of development. This requires more prolonged exposure in order to minimize the use of animals and characterize risk. Although some of the adverse effects reported in such studies may not occur with single doses, we cannot dismiss the risks reported given that adverse findings have been reported after a single dose and have to assume they could have been the result of exposures during critical periods unless definitively demonstrated otherwise.

Adequate data appear to exist in the published literature to address male fertility consistent with the endpoints outlined in ICH S5A including fertility, sperm motility and morphology, male reproductive tissue histopathology, and sexual behavior. Although many of the published studies do not clearly define a NOAEL, at least one study suggests that doses of up to 3.9 times the human referenced dose of 37.5 mg via the use of the 4% product are not likely to result in adverse effects on adult male fertility. Younger males appear to be more susceptible to adverse testicular effects and higher doses can clearly result in adverse histopathology finding in the rat testes.

Adequate published studies to fully characterize the impact of cocaine on female fertility and early embryonic development were not identified by the Applicant. The published studies do suggest that cocaine can alter female reproductive hormones and cyclicity suggesting a potential impact on female fertility and early embryonic effects. These findings should be included in labeling. A definitive study should be completed.

The Applicant submitted many published studies that dosed pregnant animals during organogenesis which contain some of the standard embryofetal development (EFD) study endpoints outlined in ICH S5. Many of these identify clear adverse effects at relatively high doses and via routes that are likely to result in higher C_{max} values than the proposed drug product but fail to define a no adverse effect level (NOAEL). The

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Applicant maintains that "cocaine *is* a human and animal teratogen" and proposes to include labeling to that effect. These adverse effects should be included in labeling should the product be approved; however, dedicated embryo-fetal studies in the rat and rabbit are recommended as post-marketing requirements not only to provide valid GLP studies but also to obtain adequate toxicokinetic data to provide a comparison to the human exposures via this route of administration and further inform labeling.

Similar to EFD studies, the published literature submitted also contain some endpoints similar to modern pre- and postnatal development studies (PPD) described by ICH S5. These studies suggest the potential for adverse effects, particularly on the central nervous system and testes in the offspring of pups born to pregnant mothers treated with cocaine. None of the studies dosed the pregnant animals during gestation and through weaning as per standard protocols. Nonetheless, offspring birth weights and brain weights are reduced at higher doses of cocaine. Offspring survival is also impacted in some studies. Limited postnatal functional studies were submitted; however, the data does suggest the potential for adverse effects on the CNS. The significance of the findings are not clear given the lack of adequate PK data to compare to the human exposures via this drug product. A definitive GLP study with PK data is recommended to be completed as a PMR to fully characterize the effects of cocaine on PPD, including defining a NOAEL, to fully inform labeling and risk.

The Applicant submitted inadequate data to characterize the effects of cocaine on juvenile animals. Although their submitted references cited do suggest that cocaine can have an impact on the developing brain, many of the cited documents were primarily abstracts and are not considered adequate for the reasons cited above. Preliminary searches and review of selected publications by the review team noted that there are a considerable number of publications that address the developmental effects of cocaine that were not submitted or reviewed by the Applicant. For example, a PubMed search for "cocaine and 'brain development' and animal" alone conducted on June 12, 2018 resulted in 70 publications retrieved. Nonetheless, as most of these studies will not be via the intranasal route and will not likely identify a NOAEL or include all appropriate endpoints, they are not likely to be adequate to justify the safety of the product. An appropriate juvenile animal study would include a comprehensive evaluation that includes histopathology plus functional behavioral assessments to support pediatric studies is recommended. As agreed in the pediatric study plan (PSP) dedicated juvenile animal studies will be completed. The studies should be designed to test the effects of cocaine on brain development and should be completed prior to clinical studies in pediatric patients. Therefore, dedicated juvenile animal studies are recommended to be completed as post-marketing requirements should the product be approved. The design of the study will be dictated by the age range this product is believed to be useful for. PeRC concluded that the pediatric development plan should address safety in children aged 8 to >17 and were willing to consider a waiver for studies under the age of 8 if adequately justified by the Applicant.

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1.3 Recommendations

1.3.1 Approvability

From a nonclinical pharmacology toxicology perspective, inadequate nonclinical data have been provided to support an approval recommendation. Specifically, there are inadequate data to support the safety of the container closure system and inadequate study reports for several genetic toxicology studies were submitted (lacked purity information).

Deficiencies:

1. You have not provided adequate leachables evaluation to justify the safety of the proposed container closure system. Specifically, your leachables evaluation did not evaluate at least three batches of your to-be-marketed drug product for leachables and include assessments at multiple timepoints over the course of your stability studies as we advised at the Pre-NDA meeting and in accordance with best practices per USP <1664>: Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems. Further, you have not provided an adequate extractables-leachables correlation to ensure that leachable compound levels can be extrapolated from data collected from simulation studies under accelerated conditions.

To resolve this deficiency:

> Conduct a new leachables study under standard storage conditions that evaluates at least three batches of the to-be-marketed topical cocaine solution products for leachables and include assessments at multiple timepoints over the course of your stability studies (beginning, middle, and end of proposed shelf-life) in order to identify trends in leachable levels over time. Evaluate all container closure systems you intend to market. Clearly delineate how you leveraged the existing extraction studies to inform your leachables assessment. Submit a toxicological assessment justifying the safety of the maximum level achieved over the course of stability for any leachable that exceeds 5 mcg/day, taking into consideration the maximum daily dose of the drug product. Submit a discussion of the extractables leachables correlation of the findings.

2. Several of your final study reports did not report the purity of the test articles.

To resolve this deficiency:

> Revise the final study reports for Study 16-01138-G2, 16-01139-G2, and 16-01140-G2 to include purity information of the test articles evaluated.

1.3.2 Additional Nonclinical Recommendations

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As per the agreements made prior to submission of the NDA, should the NDA be approved, we recommend the following postmarketing requirements (PMRs):

1) You have not provided adequate literature references to characterize the potential adverse effects of cocaine hydrochloride on reproductive and developmental endpoints consistent with standard endpoints outlined in ICH S5(R2). Conduct the standard battery of reproductive and developmental toxicology studies outlined in ICH S5(R2), including pharmacokinetic data for the following:

   a) a female fertility and early embryonic development study in the rat model to adequately characterize the effect of cocaine on female fertility and early embryonic development.

   b) an embryo-fetal development study in the rat model to characterize the teratogenic potential of cocaine.

   c) an embryo-fetal development study in the rabbit model to characterize the teratogenic potential of cocaine.

   d) a pre- and post-natal development study in the rat model to characterize the impact of cocaine on development, including exposure during lactation to weaning, growth and development, functional and behavioral assessments, and reproductive capacity of the offspring.

2) Conduct a juvenile animal study to characterize the impact of cocaine on brain development and male reproductive tissue and development to support pediatric dosing.

1.3.3 Labeling

The following changes to the Applicant's proposed labeling are recommended in the table below (deletions are highlighted in ~~red strikethrough~~ and additions are highlighted in purple). Refer to the action letter for final drug product labeling.

Applicant's proposed labeling	Reviewer's proposed changes	Rationale for changes
(b) (4) [redacted]	**HIGHLIGHTS OF PRESCRIBING INFORMATION** INDICATIONS AND USAGE NUMBRINO is a liquid formulation of cocaine hydrochloride, an ester local anesthetic, indicated for the introduction of local (topical) anesthesia for diagnostic procedures and surgeries on or through the accessible mucous membranes of the nasal cavities. (1)	Established pharmacological class was added to highlights as per regulations. For final indication, see the final labeling and clinical review.

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Applicant's proposed labeling	Reviewer's proposed changes	Rationale for changes
8 USE IN SPECIFIC POPULATIONS	**8 USE IN SPECIFIC POPULATIONS**	
8.1 Pregnancy (b) (4) (b) (4)	**8.1 Pregnancy** Risk Summary There are no adequate and well-controlled studies of NUMBRINO in pregnant women. Limited available data from published literature on use of cocaine in pregnant women are not sufficient to inform a drug-associated risk for major birth defects and miscarriage. Adverse pregnancy outcomes, including premature delivery and low birth weight, have been seen in infants born to mothers dependent on cocaine [see Clinical Considerations].	The Applicant did not propose a nonclinical risk summary statement. Refer to the DPMH Review and final labeling for evaluation of the proposed human risk summary statement.
	In published animal reproduction studies, cocaine administered to pregnant females during the gestational period produced hydronephrosis (0.5 times the human reference dose (HRD) of 37.5 mg via the 4% solution), developmental delays in the offspring (1.5 times the HRD), cerebral hemorrhage and fetal edema (2.2 times the HRD), reduced fetal body weights and brain weights (2.6 times the HRD), and reduced fetal survival (3.7 times the HRD).	
	Single dose administration of cocaine intravenously during organogenesis in mice produced cryptochidism, anophthalmia, exencephaly, and delayed ossification at 7.8 times the HRD.	(Mahalik et al., 1980)
	In rats, a single dose of cocaine administered by intraperitoneal injection produced edematous fetuses, hemorrhages and limb defects at 13 times the HRD [*see Animal Data*].	(Webster and Brown-Woodman, 1990)
(b) (4)		Older CFR language removed.

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Applicant's proposed labeling	Reviewer's proposed changes	Rationale for changes
(b) (4) [redacted]	The estimated background risk of major birth defects and miscarriage for the indicated population is unknown. All pregnancies have a background risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.	Generic boilerplate labeling added.

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Applicant's proposed labeling	Reviewer's proposed changes	Rationale for changes
Data (b) (4)	Data Animal Data	
(b) (4)		Unclear from submission what they cited. We believe the mouse study is Mahalik.
(b) (4)		Unclear from submission what they cited. We believe the rat study is El-Bizri.
		Statement on Benzoylecgonine from Spear 1989a

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Applicant's proposed labeling	Reviewer's proposed changes	Rationale for changes
	Formal animal reproduction and development studies have not been conducted with intranasal cocaine hydrochloride. However, reproduction and development studies with cocaine have been reported in the published literature. Exposure margins below are based on body surface area comparison to the human reference dose (HRD) of 37.5 mg (estimated amount absorbed from the 160 mg (4%) cocaine-soaked pledgets).	Intro statement to cover how exposure margins were characterize.
	Hydronephrosis was noted in fetuses from pregnant rats treated wtih cocaine 2.1 mg/kg (0.5 times the HRD) and higher from Gestation Days 0-19. Cerebral hemorrhage and endematous fetuses were noted at 2.2 times the HRD and above).	Data source: (el-Bizri et al., 1991)
	Developmental delays and altered spontaneous exploratory behavior in response to cocaine were reported in rat pups born to dams treated intravenously with 6 mg/kg cocaine (1.5 times the HRD) from Gestation Days 8-20 in the absense of maternal toxicity.	Data Source: (Kunko et al., 1993)
	Reduced fetal body and brain weights and alterations in fetal central neurotransmitter levels were noted following treatment of pregnant mice with 20 mg/kg cocaine (2.6 times the HRD) from Gestation Days 8-12 or 12-18.	Data source: (Middaugh et al., 1996; Song et al., 2002)
	Reduced fetal survival was noted when pregnant nonhuman primates were dosed with 0.3 mg/kg/h cocaine (3.7 times the HRD on per day basis) via a subcutaneous minipump from Gestation Day 24 to birth.	Data Source: (Howell et al., 2001)

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Applicant's proposed labeling	Reviewer's proposed changes	Rationale for changes
	Exencephaly, cryptochidism, hydronephrosis, anophthalmia, delayed ossification, limb anomalies, and cerebral and intra-abdominal hemorrhage were reported following a single subcutaneous injection of 60 mg/kg cocaine (7.8 times the HRD) to pregant mice between Gestation Days 7 to 12. No significant maternal toxicity was reported at this dose.	Data source: (Mahalik et al., 1980)
	Deficits in associational learning were reported when pregnant rats were treated with cocaine during gestation (10 times the HRD) in the absence of maternal toxicity.	Data Source: (Spear et al., 1989)
	Hemorrhage, fetal edema, and limb defects were reported when pregmant rats were administered a single injection of cocaine at a dose of 50 mg/kg/day or higher (13 times the HRD) during Gestation Days 9 to 19. Increased resorptions were noted at doses higher than 70 mg/kg/day (18 times the HRD) when administered on Gestation Day 16. No adverse effects were reported at a dose of 40 mg/kg (10 times the HRD).	Data source: (Webster and Brown-Woodman, 1990)
	Fetal deaths, decreased fetal body weights, edematous fetuses and single incidences of cleft palate and hypertrophic ventricle were observed when pregnant rats were treated with intraperitoneal cocaine at 60 mg/kg (16 times the HRD) from Gestation Days 8 to 12. Maternal toxicity was noted at this dose (mortality). No adverse effect level for fetal and maternal toxicity was noted at 50 mg/kg/day (13 times the HRD).	Data source: (Fantel and Macphail, 1982)

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Applicant's proposed labeling	Reviewer's proposed changes	Rationale for changes
	Decreased body weights, overall body length and crown circumference of offspring were reported when pregnant Rhesus monkeys were treated with escalating doses up to 7.5 mg/kg cocaine TID intramuscularly per day for 5 days per week from prior to conception to term (12 times the HRD).	Data Source: (Morris et al., 1997)
	8.3 Females and Males of Reproductive Potential Published animal studies suggest that cocaine can alter female reproductive hormone levels, disrupt the estrous cycle, and reduce ovulation at doses 1-2 times the HRD based on body surface area (*See 13.1 Nonclinical Toxicology*).	Sponsor did not propose an 8.3. Changes were made as per the PLLR when adverse effects are noted in nonclinical studies.
(b) (4) [redacted]	**8.4 Pediatric Use** Safety and effectiveness of NUMBRINO in pediatric patients under the age of 18 has not been established. In juvenile male rats, 30 mg/kg subcutaneous cocaine administration for longer than 7 days (7.8 times the HRD) produced testicular necrosis. Treatment of juvenile male rats with 15 mg/kg (3.9 times the HRD) for 100 days resulted in abnormal sperm morphology and reduced pregnancy rates.	Data Source: (Barroso-Moguel et al., 1994; George et al., 1996, Li et al., 1997)
(b) (4) [redacted]	**11 DESCRIPTION** Each mL of NUMBRINO™ (cocaine hydrochloride topical solution) contains 40 mg (4%: 160 mg/4 mL or 400 mg/10 mL) or (b) (4) mg (10%: 400 mg/(b) (4) of cocaine hydrochloride, an ester local anesthetic, as aqueous solution. …	Established pharmacological class (ester local anesthetic) was updated as per regulations. Defer to CMC team regarding the rest of this section.
12 CLINICAL PHARMACOLOGY **12.1 Mechanism of Action**	**CLINICAL PHARMACOLOGY** **12.1 Mechanism of Action** Cocaine hydrochloride is a local	Mechanism of action

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	anesthetic of the ester type. Cocaine hydrochloride prevents conduction in nerve fibers by reversibly blocking voltage-gated sodium channels and preventing the transient rise in sodium conductance necessary for generation of an action potential.	should be focused on local anesthesia, as that is the indication. As the clinical evaluation fo the drug was not designed to characterize vasoconstriction, these statements are promotional.
(b) (4) [redacted] (b) (4)		
13 NONCLINICAL TOXICOLOGY **13.1 Carcinogenesis, Mutagenesis, and Impairment of fertility**		
(b) (4) [redacted] (b) (4) [redacted]	**CARCINOGENESIS** Long-term studies in animals have not been performed to evaluate the carcinogenic potential of cocaine (b) (4) [redacted]	Human data are not to be included in Section 13.
MUTAGENESIS (b) (4) [redacted]	**MUTAGENESIS** Cocaine hydrochloride was not mutagenic in an in vitro bacterial reverse mutation assay (Ames test) and was not clastogenic in an in vitro chromosomal aberrations assay or in the in vivo rat micronucleus test.	Genetic toxicity data for impurities/metabolites are not included in labeling unless there is a clear safety signal that warrants attention.
(b) (4) [redacted]		Proposed labeling is likely from the George et al. reference 1996. However, this study evaluated

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(b) (4)		juvenile rats (4-week old) rats and therefore these findings were moved to Section 8.4.
	Studies in animals to characterize the effects of cocaine on fertility have not been completed. There are published studies that provide some information on the potential impact of cocaine on fertility. Exposure margins below are based on body surface area comparison to the human reference dose (HRD) of 37.5 mg (estimated amount absorbed from the 160 mg cocaine-soaked pledgets).	General statement added to explain source of exposure margins.
	Suppression of estrous/menstrual cyclicity and ovulation was reported in rats at 1.3 to 2.6 times the HRD and in monkeys at 2.1 times the HRD.	Data Source: (King et al., 1993; Potter et al., 1998)
	In a published study in older male rats (16 weeks) 30 mg/kg cocaine SC (7.8 times the HRD) for 72 days prior to mating did not alter male fertility or alter male reproductive tissue histopathology but did increase the incidence of abnormal sperm and resulted in hyperactivity of next generation offspring.	Data Source: (Abel et al., 1989)

2 Drug Information

2.1 Drug

CAS Registry Number: 53-21-4

Generic Name: Cocaine hydrochloride

Code Name:

Chemical Name: Ecgonine methyl ester benzoate hydrochloride 3β-hydroxy-1αH, 5αH-tropane-2β-carboxylic acid methyl ester benzoate hydrochloride

Molecular Formula/Molecular Weight: $C_{17}N_{21}NO_4 \cdot HCl$/339.1

Structure or Biochemical Description:

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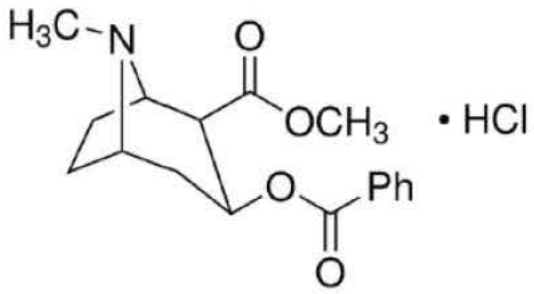

Pharmacologic Class:
Ester local anesthetic (Established Pharmacologic Class)

2.2 Relevant INDs, NDAs, BLAs and DMFs

	Status	Division	Indication	Stamp Date	Applicant
INDs					
106499	Active	DAAAP	Local anesthesia for diagnostic procedures and surgeries on or through the accessible mucous membranes of the nasal cavities	04/06/2013	Cody Laboratories Inc

DMF	Status	Subject of DMF	Stamp Date	Holder
(b) (4)	Closed	Glass (Type III) containers for parental preparation	02/27/2018	(b) (4)
	Active	(b) (4)	05/06/2005	
	Active		09/03/1970	
	Active		03/14/1975	
	Active		12/03/1997	
	Active		03/10/2015	
	Active		3/9/1998	
29120	Active		08/07/2017	Cody Laboratories Inc

2.3 Drug Formulation

Two strengths of cocaine hydrochloride topical solution are proposed: 4% (40 mg/mL) and 10% (b) (4) The following tables illustrate the composition of these cocaine hydrochloride topical solutions (from the Applicant's submission). As depicted in the table, the 4% strength will be available in a 4-mL single use bottles and 10-mL multi-dose bottles. (b) (4).

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Table 1. Unit Composition of Cocaine Hydrochloride Topical Solution, 4%

Ingredient Name	Quality Standard	mg per mL	% per w/w	Function
Cocaine Hydrochloride, USP	USP	40	4.0	Active Ingredient
Sodium Benzoate, NF	NF	(b) (4)	(b) (4)	(b) (4)
D & C Yellow #10	N/A			
FD & C Green #3	N/A			
Citric Acid Anhydrous, USP	USP			
Purified Water, USP	USP			
Theoretical Amount				

Table 2. Unit Composition of Cocaine Hydrochloride Topical Solution, 10%

Ingredient Name	Quality Standard	mg per mL	% per w/w	Function
				(b) (4)

The drug product is acidic with a pH specification of (b) (4) over the course of stability.

2.4 Comments on Novel Excipients

Prior to submission of the NDA, cocaine hydrochloride topical solution had three novel excipients for the intranasal route of administration: sodium benzoate, D & C Yellow #10 and FD & C Green #3. Subsequently; these excipients have been used in a FDA-approved intranasal drug product at comparable dose/concentration and for comparable use. As of the date of this review, there are no novel excipients in the drug product.

2.5 Comments on Impurities/Degradants of Concern

Qualification thresholds are based on the maximum daily dose (MDD). The MDD for this drug product is not straightforward due to the method of topical delivery proposed. As per the drug product labeling, the 4% and 10% cocaine solution vial contains 160 mg and 400 mg of cocaine hydrochloride, respectively. However the solution is exposed to

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the nasal passages via cotton or cottonoid pledgets inserted into the nasal passages for up to 20 minutes. The Applicant has indicated that a mean of 23.4% and 33.4% of the total administered 160 mg dose (4% cocaine solution) and 400 mg dose (10% cocaine solution) was absorbed, respectively, based on analysis of residual cocaine still in the cotton pledgets after application to the nasal passages of 31 subjects in a single center, randomized, double-blind, single-dose, 2-period crossover study (Study LNT-P6-733). The results are summarized in the table below, reproduced from the submission:

Table 3: Fraction (%) of Administered Dose of Cocaine HCl Absorbed From the Pledgets in Humans

Parameter (%F)	160 mg Cocaine HCl 4% (SD)	400 mg Cocaine HCl 10% (SD)
Mean (SD)	23.44% (8.876)	33.34% (10.710)
Median	23.91%	34.13%
Range (min, max)	6.9, 36.9	14.3, 67.5

Source: CSR Study LNT-P6-733, Table 14.3.10

As noted in the study results above, a significant portion of the administered cocaine is retained within the cottonoid pledgets after delivery. The variability after administration of the 10% solution does appear greater than after the 4% solution based on the range of reported fractions absorbed. However, for the purposes of this application, the MDD will be 37.5 mg/day (23.44% of 160 mg) for the 4% solution and (b) (4) [redacted] (33.34% of 400 mg)) for the 10% solution.

Drug Substance Impurities

The cocaine hydrochloride drug substance is manufactured synthetically by Cody Laboratories as described in the referenced DMF 29120. The drug substance impurity specifications are presented in the table below. According to ICH Q3A(R2), (b) (4) [redacted]

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Table 4: Drug Substance Specifications

Impurity	Structure	Proposed Specification	Acceptable? (Yes/No)	Reviewer's Comments
		(b) (4)	Yes	Within ICH Q3A(R2) qualification threshold
			Yes	Within ICH Q3A(R2) qualification threshold
			Yes	Within ICH Q3A(R2) qualification threshold
			Yes	Within ICH Q3A(R2) qualification threshold
			Yes	Exceeds ICH Q3A(R2) qualification threshold; however, see qualification data below
			Yes	Within ICH Q3A(R2) qualification threshold
			Yes	Within ICH Q3A(R2) qualification threshold
			Yes	Within ICH Q3A(R2) qualification threshold

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Impurity	Structure	Proposed Specification	Acceptable? (Yes/No)	Reviewer's Comments
		(b) (4)	Yes	Exceeds ICH Q3A(R2) qualification threshold; however, see qualification data below
(b) (4)			Yes	Within ICH Q3A(R2) qualification threshold
			Yes	Within ICH Q3A(R2) qualification threshold
Individual Unknown			Yes	Within ICH Q3A(R2) qualification threshold

Safety Justification for (b) (4) and (b) (4) (and (b) (4)

In several interactions with the Division, the Applicant was advised to qualify any impurity that was above qualification thresholds. Three compounds (two as drug substance impurities and two as drug product degradants) exceeded the qualification threshold and therefore the Applicant conducted a 14-day local tolerance study with a drug product solution spiked with the three impurities/degradants and conducted a standard genetic toxicology qualification battery with each compound that included an Ames assay and a chromosomal aberration assay (which are reviewed in Sections 6 and 7 of this review). In the 14-day instillation study, the impurities were not associated with any adverse local findings to the local nasal tissue or olfactory bulb. A local NOEL of < 23.7 mg/kg/day was identified based on the absence of adverse findings in the nasal cavity or olfactory bulb. (b) (4) and (b) (4) were not genotoxic; they were non-mutagenic in the Ames assay or non-clastogenic in the in vitro chromosomal assay. However, (b) (4) was clastogenic in the in vitro chromosomal assay in the presence of metabolic activation but tested negative in an in vivo Comet assay and was negative in the Ames assay.

Residual Solvents in the Drug Substance

Potential residual solvents in the drug substance are depicted in the table below. Drug substance specification includes (b) (4) residual solvents: (b) (4) All are

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within established Permissible Daily Exposure limits stated in ICH Q3C(R6) and are acceptable (see Table below).

Table 5: Residual Solvents Drug Substance

Residual Solvent	Class	Cody Laboratories Proposed Specifications (ppm)	ICH Q3C(R6) PDE (ppm)	Acceptable (Yes/No)
(b) (4) [redacted]				Yes; meets ICH Q3C threshold for a Class 3 residual
				Yes; meets ICH Q3C threshold for a Class 2 residual
				Ys; meets ICH Q3C threshold for a Class 2 residual solvent
				Yes: Acceptable, meets ICH Q3C threshold for a Class 2 residual solvent
				Yes; Acceptable, meets ICH Q3C threshold for a Class 3 residual solvent
				Yes; Acceptable, meets ICH Q3C threshold for a Class 3 residual solvent
				Yes; Acceptable, meets ICH Q3C threshold for a Class 3 residual solvent

Drug Product Degradants

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The drug product degradant specifications are presented in the table below. According to ICH Q3B(R2), the identification and qualification thresholds for a degradant with a maximum daily dose (MDD) of a drug product that is (b) (4)

Table 6: Drug Product Degradant Stability Specifications (b) (4)

Impurity	Structure	Proposed Specification	Acceptable? (Rationale)	Reviewer's Comments
(b) (4)		(b) (4)	Yes; exceeds ICH Q3B(R2) qualification threshold (b) (4)	Acceptable. Local safety of this impurity is qualified via a 14-day local tolerance study **Not mutagenic; negative in the Ames assay. Not clastogenic; negative chromosomal aberrations in vitro vivo assay.**
		(b) (4)	Yes; exceeds ICH Q3B(R2) qualification threshold	Acceptable. Local safety of this impurity is qualified via a 14-day local tolerance study **Not mutagenic; negative in the Ames assay. Not clastogenic; negative chromosomal aberrations in vitro assay.**
(b) (4)		(b) (4)	Yes; exceeds ICH Q3B(R2) qualification threshold	Acceptable. Systemic safety justified as this is a major metabolite of cocaine. Local safety of this impurity is qualified via a 14-day local tolerance study. **Mutagenic and clastogenic potential qualified in Ames assay, and chromosomal aberration assay**
Individual Unknown		(b) (4)	Yes	Does not exceed identification or qualification threshold

Container Closure System:

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The container closure system consists of an (b) (4) glass bottle with a (b) (4) cap closure. Both topical solutions (4% and 10%) are clear blue-green colored liquids that are packaged in a (b) (4) amber glass bottle with a (b) (4) cap closure. (b) (4) container/closure configurations are proposed (b) (4)

Container Closure Configuration	(b) (4)		
Bottle Size			
Bottle & Description			
Cap Size & Description			
Product Strength/Fill Size	**4%:** 4 mL and 10 mL **10%:** (b) (4)	**4%:** 4 mL and 10 mL **10%:** (b) (4)	**4%:** 4 mL and 10 mL **10%:** (b) (4)

(b) (4) will also be used (b) (4) and (b) (4), both manufactured by (b) (4) and covered by DMF (b) (4) According to the Application, (b) (4) container closure systems can be used for (b) (4) presentations.

Methodology of the Extraction Study:

Container closure components subject to extraction include primary components in contact with drug substance: amber glass vials and cap. The extraction studies were performed on the (b) (4)

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2.6 Proposed Clinical Population and Dosing Regimen

The drug product is an aqueous solution of cocaine hydrochloride that is indicated for use as a local anesthetic for diagnostic and surgical procedures on or through the mucous membranes of the nasal cavities in adults. It is available as a 4% or 10% topical solution. It is administered at the time of the surgical procedure as a single, 160 mg (4%) or 400 mg (10%) intranasal dose as a solution using specific sized pledgets (1/2″ x 3″). One or two pledgets will be applied topically per nares, with a maximum of two pledgets per nare or a maximum of four pledgets per procedure. One mL of the cocaine topical solution is applied to each pledget. Following soaking, two pledgets are to be placed against the septum in each nasal cavity for up to twenty minutes and then are to be removed and discarded. The total theoretical dose for any one procedure is 160 mg or 400 mg cocaine hydrochloride if the total amount of cocaine in the pledget is absorbed. However, in their document titled "Summary of Biopharmaceutics studies" the Applicant has indicated that a mean of 23.44% and 33.34% of the total administered 160 mg dose (4% cocaine solution) and 400 mg dose (10% cocaine solution was absorbed), respectively. Therefore, some of the drug product is retained within the cottonoid pledgets after delivery. Therefore, a dose of 37.5 mg/day and (b) (4) was the calculated maximum recommended human dose (MRHD) for the 4% and 10% topical solution, respectively; and was used as a reference human dose for all subsequent margin calculations.

2.7 Regulatory Background

The Applicant has submitted a 505(b)(2) application for cocaine hydrochloride topical solution 4% and 10% that relies upon published scientific literature.

A preIND meeting (IND 106499) was requested by Sponsor on September 9, 2009 and the preIND meeting was held on December 15, 2009 to discuss the drug development program for this marketed unapproved product. In those responses, the Division informed the Applicant that the NDA should include a comprehensive genotoxicity assessment of cocaine, comprehensive review of the literature and specifically address what studies identified provide the most relevant data with respect to current ICH M3(R2) requirements. The NDA must also review and analyze the existing toxicology data for cocaine, including general and local toxicology, genetic toxicology and reproductive and developmental toxicology (fertility and early embryonic development, embryofetal development, and pre- and postnatal development). We also advised that any impurity or degradant that exceeded ICH Q3A(R2)/Q3B(R2) thresholds would have to be qualified for safety in accordance with these guidance documents. In addition, potentially genotoxic impurities should be adequately controlled and adequate information on extractables and leachables must be provided to support the safety of the container closure system.

An IND was opened on April 6, 2013. An email correspondence was conveyed in respond to the Sponsor advice/ information request on December 22, 2014 to the Sponsor regarding toxicology testing.

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During development, the Applicant sought clarification if the required testing should be performed on the drug substance or the drug product. If the drug product should be tested, should the 4% and 10% strengths both be analyzed or is testing on only the 10% strength (highest strength) adequate for the NDA. The Division responded that the drug substance should be evaluated up to a limit dose as stated in the ICH S2(R1) Guidance. The Applicant inquired about whether a repeat-dose study was required since the drug product is intended for a single application for a surgery or diagnostic procedure. The Division responded that a 14-day repeat-dose toxicity study is recommended. On February 14, 2016, a telecon was held to discuss the design of the 14-day repeat-dose toxicity study. The Applicant and the Division agreed on the design of the repeat-dose toxicity study.

Pediatrics: PeRC discussed the pediatric study plan for this application on May 16, 2018. PeRC concluded that (b) (4) They also agreed with the request to defer studies of the 4% solution in pediatric studies in children aged 8 to 17 years. They noted that they would agree to waive studies of the 4% solution in children under 8 if the Applicant can provide adequate information to justify such a waiver. Based on the concern for the effects of cocaine on brain development, they agreed with the Division that juvenile animal studies are required to support clinical trials in pediatric patients. Final determination of the exact juvenile animal studies necessary to support pediatric use will have to be deferred until a final determination on the patient population to be studied is made.

3 Studies Submitted

3.1 Studies Reviewed

Report №	Study Title	Module/CTD Description
Study Report	**General Toxicology**	
№ 16-01711-G1	A 14-day repeat dose intranasal instillation toxicity study in Sprague-Dawley rats with a 14-day recovery period.	4.2.3.2.1
	Genetic Toxicology	
№ 11-4138-G1	*Salmonella typhimurium* and *Escherichia coli* reverse mutation assay (OECD 471)	4.2.3.3.1
№ 16-01138-G3 Amended	*Salmonella typhimurium* and *Escherichia coli* reverse mutation assay (OECD 471)	
№ 16-01139-G3 Amended	*Salmonella typhimurium* and *Escherichia coli* reverse mutation assay (OECD 471)	
№ 16-01140-G3	*Salmonella typhimurium* and *Escherichia coli* reverse mutation assay (OECD 471)	
№ 11-4138-G2	Chromosomal Aberration Assay – OECD 473	
№ 16-01138-G2	Chromosomal Aberration Assay – OECD 473	
№ 16-01139-G2	Chromosomal Aberration Assay – OECD 473	
№ 16-01140-G2	Chromosomal Aberration Assay – OECD 473	

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№ 11-4138-G3	Rodent bone marrow micronucleus assay- OECD 474 cocaine.	
№ 54873.0001	Evaluation of micronuclei in B6C3F1in mice and DNA damage In Sprague Dawley rats administered ecgonine hydrochloride.	

In addition to the new studies conducted by the Applicant listed above, the Applicant submitted published literature to address all other aspects of the NDA. See the reference list at the end of the document for the citations leveraged to support this application.

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

There were no new nonclinical pharmacology studies completed for this 505(b)(2) application. The Applicant conducted a review of the scientific literature between January 1965 and May 2017, and identified nine published literature that they reviewed as relevant for the nonclinical primary pharmacology of cocaine. The Applicant submitted a summary of these identified published reports; however, these reports did not discuss the primary pharmacology of cocaine. The pharmacology information from the published literature is summarized as follows:

Mechanism of action:

Local Anesthesia

Cocaine is a local anesthetic of the ester type. The Applicant submitted a review article summarizing the conclusion that local anesthetics, including cocaine, blocks voltage-gated sodium channels necessary for the propagation of electrical signals along the nerve axon, thereby preventing sensory messages to be received by the central nervous system (Scholz, 2002).

4.2 Secondary Pharmacology

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In addition to local anesthesia, cocaine blocks the reuptake of dopamine, serotonin, and norepinephrine. The positive reinforcement effects are likely primarily mediated by the increase in central dopamine levels (Johanson and Schuster, 2000).

4.3 Safety Pharmacology

Dedicated safety pharmacology studies were not completed nor was an integrated assessment of the impact of cocaine on the central nervous, cardiovascular, or respiratory system submitted. As per the Applicant, “safety pharmacology studies may not be needed for locally applied agents where the pharmacology of the test substance is well-characterized”. Cocaine has been used therapeutically and illicitly for decades and therefore, dedicated safety pharmacology studies to support the safety of clinical trials were not deemed necessary.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

No nonclinical absorption, distribution, and excretion studies using the intranasal route of administration were conducted by the Applicant. An integrated literature search review was also not completed. The Applicant submitted a waiver request for these studies. As the nonclinical ADME were not deemed necessary to support the proposed clinical studies and will not be used in labeling, no further studies are necessary.

6 General Toxicology

6.1 Single-Dose Toxicity

The Applicant did not conduct any single-dose toxicity studies. The Applicant requested a waiver for the single-dose toxicity studies because the Applicant deemed it unnecessary based on published literature, controlled clinical studies, nonclinical studies and toxicology studies performed and provided in other sections of the submission.

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6.2 Repeat-Dose Toxicity

Study title: A 14-Day Repeat-dose Intranasal Instillation Toxicity Study in Sprague-Dawley Rats with a 14 Day Recovery Period.

Study no.:	16-01711
Study report location:	\\cdsesub1\evsprod\nda209575\0001\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\16-01711-g1\16-01711-g1-pre-clinical-study-report.pdf
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	September 22, 2016
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	**Test Article 1:** Cocaine HCl 4% solution containing (b) (4) [redacted] w/v benzoate; Lot 276-54-A, 101% purity (COA dated 9/30/2016 was provided) **Test Article 2:** Cocaine HCl 10% solution containing (b) (4) [redacted] w/v benzoate; Lot 276-54-B, 100% (COA dated 9/30/2016 was provided)

Key Study Findings

Intranasal instillation of saline, vehicle, 4% (5.4 to 9.6 mg/kg) and 10% (13.5 to 23.7 mg/kg) cocaine solution spiked with (b) (4) [redacted] % benzoate in rats for 14 consecutive days produced the following results:

1. There were no test article-related effects on hematological and clinical chemistry parameters.

2. There were no test article-related macroscopic or microscopic changes observed in the nasal cavity or olfactory bulb after intranasal administration of the spiked cocaine solution.

3. A local NOEL of 23.7 mg/kg/day for cocaine (10%) containing (b) (4) [redacted] benzoate was based on the absence of adverse findings in the nasal cavity or olfactory bulb.

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Methods

Doses:

Group	Treatment	№ animals/sex	Dose Range (mg/kg)	Dose Volume (µL)
1	Sterile Saline Control	21/sex	0	50
2	Vehicle Control	21/sex	0	50
3	Cocaine HCl 4%	27/sex	5.4 to 9.6	50
4	Cocaine HCl 10%	27/sex	13.5 to 23.7	50

Frequency of dosing: Once a day
Route of administration: Intranasal instillation
Dose volume: 50 mcL (25 mcL to each nostril)
Formulation/Vehicle: **Control Article 1:** Solution/Sterile saline
Control Article 2: Solution/vehicle control
Species/Strain: Rats/Sprague Dawley
Number/Sex/Group: 10/sex/main study control group; 15/sex/main study test article group
Age: At least 5 weeks old at study initiation
Weight: 207.8-323.1 grams
Satellite groups: Recovery Group
5/sex/group
Toxicokinetic Group
6/sex/control group; 12/sex/test article group
Unique study design: None
Deviation from study protocol: None

Observations and Results

Endpoints	Time of Observation
Mortality/Moribundity	Daily
Clinical signs	**Daily:** Clinical examinations performed between 1 and 4 hours after dosing. "Clinical observations included, but were not limited to, changes in the skin, fur, eyes and mucous membranes, respiratory system, circulatory system, autonomic central system, somatomotor activity, and behavior pattern."
Body weights	Prior to dose administration on Day 1, all animals were weighed. During the dosing period, body weigh were recorded weekly. Terminal (fasted) body weights were recorded on the day prior to the scheduled necropsy on Day 15 and 29 (main study and recovery animals, respectively).
Food Consumption	"Food consumption was quantitatively assessed weekly during the in-life period for all main study and recovery animals."
Ophthalmoscopy	**Pre-Dosing:** Ophthalmic examinations were performed in all main study and recovery animals between Day -3 and -1. **Post-Dosing:** Ophthalmic examinations were performed in all main study and recovery animals during Week 2 (Days 8-14). Ophthalmic examinations were also performed in the recovery animals between Days 24 and 28.

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Endpoints	Time of Observation
Electrocardiography	Not performed
Hematology	Animals were fasted overnight prior to blood collection. The following parameters were evaluated at the end of the dosing period (Day 15) and on Day 29 at the end of the recovery period: RBC, WBC, hemoglobin (HGB), hematocrit (HCT), MCV, MCH, MCHC, platelet count (PLA), differential white blood cell count, reticulocytes, blood cell morphology (abnormal and immature cells).
Clinical Chemistry	The following parameters were evaluated on Day 15 at the end of the dosing period and on Day 29 at the end of the recovery period: blood urea nitrogen, glucose, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma glutamyltransferase, sodium, potassium, inorganic phosphorus, chloride, total protein, albumin, globulin, albumin/globulin ratio, cholesterol, creatinine, total bilirubin, calcium, and triglycerides
Coagulation	Coagulation parameters assessment include: Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT)
Urinalysis	The following parameters were evaluated on Day 15 at the end of the dosing period and on Day 29 at the end of the recovery period: appearance, (color and clarity) microscopic evaluation of sediments, specific gravity, total volume, pH, protein, glucose, bilirubin, blood, ketones, nitrites, and urobilinogen
Gross pathology	At sacrifice; on Day 15 at the end of dosing period and on Day 29 at the end of the recovery period
Organ weight	At sacrifice; on Day 15 at the end of dosing period and on Day 29 at the end of the recovery period
Histopathology	At sacrifice. Animals in all treatment groups were examined. Seven coronal cross sections of the brain were taken. Also, microscopic examination of four level of the nasal cavities were examined.
Toxicokinetics	Blood samples (approx. 0.5 mL/timepoint) were collected via the retro-orbital plexus for toxicokinetic evaluation at pre-dose (Day 1), 0.5, 1, 1.5, 2, and 8 hours after dosing on Day 14 and at 1 hour from the control animals (Groups 1 and 2).

Mortality

No treatment-related deaths occurred during the study; all animals survived the duration of the study.

Clinical Signs

There were no apparent test-article related changes. Soft stools were the only abnormal clinical signs observed in 5 males (control and test article groups).

Body Weights

There was no apparent test-article or vehicle-control related effect on bodyweight or body weight change.

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Food Consumption

There was no apparent test article-related or vehicle control article-related changes.

Ophthalmoscopy

No test article changes were observed.

ECG

Not performed.

Hematology

There were no remarkable changes in hematology parameters at Days 15 and 29 administration of test article or vehicle control.

Clinical Chemistry

There were no remarkable changes in serum chemistry parameters at Days 15 of placebo administration of test article or vehicle. There was one statistically significant difference at the end of the treatment period. Compared to vehicle control, mean phosphorous level was lower in males in the 10% cocaine group (13.63%, $p<0.05$). Per the Applicant, phosphorous value was within the historical control reference range (9.20 -18.20 mg/dL).

Clinical Chemistry Group Means with Statistically Significant Differences

Sex	Day	Parameter	Group 1	Group 2	Group 3	Group 4	Toxikon Historical Reference Interval
Male	15	Phosphorus (Phos)	17.63 mg/dL	16.40 mg/dL	16.77 mg/dL	15.23[1] mg/dL	9.20-18.20 mg/dL

[1]($p \leq 0.05$)

Urinalysis

There was no apparent test article-related or control groups-related changes at end of the treatment and recovery periods.

Gross Pathology

No test article-related, saline-, or vehicle-control alterations in organ weights were observed.

Organ Weights

No test article-related, saline-, or vehicle-control macroscopic changes were observed.

Histopathology

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Adequate Battery: Yes

Peer Review: Yes, Formal peer review was conducted by (b) (4), DVM, MS, DACVP ((b) (4), April 14, 2017).

Histological Findings
No treatment-related microscopic findings were observed in the test-article groups relative to the saline control or vehicle control groups at Days 15 and 29. Histological analysis of seven brain sections that included the olfactory region in level 4 showed no treat-related microscopic findings. Nasal analysis across four separate areas of the nasal cavity did not reveal treat-induced injury to the nasal cavity after repeated intranasal administration of cocaine at 4% (5.4 to 9.6 mg/kg) and 10% (13.4 to 23.7 mg)/kg) for 14 days.

Special Evaluation

None

Toxicokinetics

A dose related increase between 4% and 10% cocaine solutions was observed for C_{max} and AUC on Days 1 and 14 for cocaine, (b) (4). However, the plasma concentrations were very low for each analyte measured. Levels of benzoyl ecgonine showed the highest levels.

Dosing Solution Analysis

A certificate of analysis for the material tested was provided indicating that the drug solution prepared for study conformed with the intended concentration of compounds.

7 Genetic Toxicology

The Applicant submitted a full battery of genotoxicity assays to characterize the genotoxic potential of cocaine. In addition, the Applicant submitted genetic toxicology studies to qualify several impurities, including (b) (4) As (b) (4) tested positive in the chromosomal aberrations assay, two in vivo genotoxicity assays were also conducted.

Reference ID: 4263670

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

Study title: *Salmonella Typhimurium* and *Escherichia Coli* Reverse Mutation Assay (OECD 471)

Study no.:	11-4138-G1
Study report location:	(b) (4) [redacted]
Conducting laboratory and location:	
Date of study initiation:	September 2, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Cocaine HCl, Lot 11330001,100%

Key Study Findings

Cocaine hydrochloride at concentrations up to 5.0 mg/plate was not mutagenic in the in vitro reverse mutation assay using strains of *Salmonella typhimurium* and *Escherichia coli* with or without enhanced metabolic enzymes.

Methods

Strains:	*Salmonella typhimurium* TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2uvrA
Concentrations in definitive study:	0.02, 0.06, 0.18, 0.55, 1.66, and 5 mg/plate
Basis of concentration selection:	A preliminary dose-range finding toxicity mutation assay was conducted to establish the cocaine hydrochloride doses to be used for the definitive mutation tests. Using the test strains TA98, and TA100 in the presence and absence of S9 mix, cocaine at concentrations of 0.02, 0.06, 0.18, 0.55, 1.66 and 5 mg/plate) evaluated. Neither precipitation nor background toxicity was observed up to 5 mg/plate.
Negative control:	Vehicle = sterile water for injection (100 mcL/plate)
Positive control:	2-nitrofluorene (2-NF), sodium azide (NaAZ), 9-aminoacridine (9AA), 9-nitroquinoline 1-oxide (9-NQ 1-O) and 2-aminoanthracene (2AA) were selected for positive controls based on the bacterial strain as indicated in the following table:

Reference ID: 4263670

Test Strain	Positive Control Substance (mcg/mL)	
	-S9	+S9
TA100	NaAZ (100.0)	2AA (10.0)
TA1535	NaAZ (5.0)	2AA (20.0)
TA98	2-NF (10.0)	2AA (5.0)
TA1537	9AA (800.0)	2AA (30.0)
WP2*uvr*A	4-NQ 1-O (100.0)	2AA (200.0)

Formulation/Vehicle: Cocaine was dissolved in sterile water for injection.

Incubation & sampling time: The definitive mutagenicity test was performed using the plate-incorporation method. Triplicate plates for positive controls, negative controls, and cocaine hydrochloride concentrations were prepared for each bacterial strain in the presence and absence of S9 mix. The S9 metabolic activator, the tester strain, and the test article were combined in molten agar which is overlaid onto the minimal agar plate. When the agar had set, the plates were inverted and incubated for approximately 70 ± 2 hours at 37 ± 1°C, and observed for compound insolubility and cytotoxicity, and scored.

Study Validity

The study is valid for the following reasons: 1) the appropriate strains were tested, 2) the appropriate controls were used, and 3) the positive control substances produced positive and reliable results. The test article was considered to be positive if it caused a dose-related increase in the mean revertants per plate of at least one tester stain over a minimum of two increasing concentrations of the test article. The assay methods, positive and negative controls, and the concentrations of drug used for the definitive study were adequate.

Results

Under the conditions of the study, cocaine was negative in the bacterial reverse mutation assay.

Cocaine hydrochloride was evaluated at the following concentrations: 0.02, 0.06, 0.18, 0.056, 1.66, and 5 mg/plate in the mutagenicity assay with or without S9 metabolic enzyme activation. In the mutagenicity assay, the positive controls induced mutation frequencies as expected. Compared to the vehicle control group, cocaine did not result in an increase in revertant bacterial colonies. As depicted in the Applicant's tables (pages 17 and 18) below, compared to the vehicle control groups, cocaine

Reference ID: 4263670

hydrochloride did not result in an increase in revertant bacterial colonies in the *Salmonella typhimurium* strains (TA987, TA100, TA1535 or TA1537) or the *Escherichia coli* test strain (WP2*uvr*A) in the presence or absence of the metabolic activator which would be indicative of its mutagenic potential. No precipitation or cytotoxicity was observed at any concentration level.

Table 7. Confirmatory Bacterial Mutation Assay without Metabolic Activation

Revertants/Plate*

Strain	Controls		Test Substance					
	Positive**	Negative***	Dosing Concentrations (mg/plate)					
			5	1.66	0.55	0.18	0.06	0.02
TA98	198	17	7	15	17	14	17	17
	219	15	4	16	13	16	15	17
	279	16	4	15	17	16	16	16
Mean	**232**	**16**	**5**	**15**	**16**	**15**	**16**	**17**
SD	**42**	**1**	**2**	**1**	**2**	**1**	**1**	**1**
TA100	893	82	20	74	76	77	80	77
	972	83	28	70	77	78	73	78
	941	81	30	71	75	79	75	77
Mean	**935**	**82**	**26**	**72**	**76**	**78**	**76**	**77**
SD	**40**	**1**	**5**	**2**	**1**	**1**	**4**	**1**
TA1535	212	7	6	7	8	9	8	8
	231	7	5	6	7	6	7	9
	243	8	6	6	7	6	7	6
Mean	**229**	**7**	**6**	**6**	**7**	**7**	**7**	**8**
SD	**16**	**1**	**1**	**1**	**1**	**2**	**1**	**2**
TA1537	271	1	0	2	1	2	1	1
	239	1	1	1	1	1	2	1
	249	1	1	1	1	2	1	2
Mean	**253**	**1**	**1**	**1**	**1**	**2**	**1**	**1**
SD	**16**	**0**	**1**	**1**	**0**	**1**	**1**	**1**
WP2 *uvr*A	402	40	37	37	42	45	41	50
	341	45	35	35	50	49	45	41
	338	50	35	44	40	48	49	43
Mean	**360**	**45**	**36**	**39**	**44**	**47**	**45**	**45**
SD	**36**	**5**	**1**	**5**	**5**	**2**	**4**	**5**

* All plates were applied at 100 mL/plate.
** The positive control used was Sodium Azide for strains TA100 and TA1535, 2-Nitrofluorene for strain TA98, 9-Aminoacridine for strain TA1537, and 4-Nitroquinoline 1-oxide for strain WP2 *uvr*A.
*** The negative control used in the assay was Sterile Water For Injection (SWFI).
SD = Standard Deviation

Reference ID: 4263670

Table 8. Confirmatory Bacterial Mutation Assay with Metabolic Activation

Revertants/Plate*

Strain	Controls		Test Substance					
			Dosing Concentrations (mg/plate)					
	Positive**	Negative***	5	1.66	0.55	0.18	0.06	0.02
TA98	198	17	15	16	17	19	15	18
	262	18	15	19	19	19	18	18
	214	19	18	18	15	14	17	15
Mean	**225**	**18**	**16**	**18**	**17**	**17**	**17**	**17**
SD	**33**	**1**	**2**	**2**	**2**	**3**	**2**	**2**
TA100	982	84	70	71	69	70	67	73
	812	83	73	72	79	77	101	74
	706	79	75	70	73	71	70	71
Mean	**833**	**82**	**73**	**71**	**74**	**73**	**79**	**73**
SD	**139**	**3**	**3**	**1**	**5**	**4**	**19**	**2**
TA1535	196	7	5	6	5	5	6	5
	241	8	9	5	5	8	6	8
	292	8	7	6	7	7	9	5
Mean	**243**	**8**	**7**	**6**	**6**	**7**	**7**	**6**
SD	**48**	**1**	**2**	**1**	**1**	**2**	**2**	**2**
TA1537	197	2	2	2	2	1	2	1
	210	2	1	2	2	3	2	2
	102	2	2	2	2	2	2	2
Mean	**170**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
SD	**59**	**0**	**1**	**0**	**0**	**1**	**0**	**1**
WP2 *uvr*A	371	37	39	51	40	37	50	41
	382	51	51	35	52	49	41	34
	321	49	40	33	53	41	40	42
Mean	**358**	**46**	**43**	**40**	**48**	**42**	**44**	**39**
SD	**33**	**8**	**7**	**10**	**7**	**6**	**6**	**4**

* All plates were applied at 100 mL/plate.
** The positive control used was 2-Aminoanthacene for all strains.
*** The negative control used in the assay was Sterile Water For Injection (SWFI).
SD = Standard Deviation

Reference ID: 4263670

Study title: *Salmonella Typhimurium* and *Escherichia Coli* reverse mutation assay (OECD 471)

Study no.:	16-01138-G3 Amended
Study report location:	\\cdsesub1\evsprod\nda209575\0001\m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\16-01138-g3\16-01138-g3-pre-clinical-study-report.pdf
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	March 2, 2017
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) [redacted], 99.9%

Key Study Findings

(b) (4) [redacted] at concentration of 5358.87 mcg/plate was not mutagenic in the in vitro reverse mutation assay using strains of *Salmonella typhimurium* and *Escherichia coli* with or without enhanced metabolic enzymes.

Methods

Strains:	*Salmonella typhimurium* TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2uvrA
Concentrations in definitive study:	0.14, 1.93, 3.48, 11.71, 23.01, 66.92, 240.10, 628.11, 2842.42, 5358.87 mcg/plate
Basis of concentration selection:	A preliminary dose-range finding toxicity mutation assay was conducted to establish the cocaine hydrochloride doses to be used for the definitive mutation tests. Using the test strains TA98, and TA100 in the presence and absence of S9 mix, cocaine at concentrations of 0.02, 0.06, 0.18, 0.55, 1.66 and 5 mg/plate) evaluated. Neither precipitation nor background toxicity was observed up to 5 mg/plate.
Negative control:	Vehicle = 60% menthol in water (100 mcL/plate), and physiological saline
Positive control:	2-nitrofluorene (2-NF), sodium azide (NaAZ), 9-aminoacridine (9AA), 9-nitroquinoline 1-0xide (9-NQ 1-O) and 2-aminoanthracene (2AA) were selected for positive controls based on the bacterial strain as indicated in the following table:

Reference ID: 4263670

Test Strain	Positive Control Substance (mcg/plate)	
	-S9	+S9
TA100	NaAZ (10.0)	2AA (1.0)
TA1535	NaAZ (0.5)	2AA (2.0)
TA98	2-NF (1.0)	2AA (0.5)
TA1537	9AA (80.0)	2AA (3.0)
WP2uvrA	4-NQ 1-O (10.0)	2AA (20.0)

Formulation/Vehicle: [redacted] (b) (4) was dissolved in 60% menthol in water.

Incubation & sampling time: The definitive mutagenicity test was performed using the plate-incorporation method. Triplicate plates for positive controls, negative controls, and cocaine hydrochloride concentrations were prepared for each bacterial strain in the presence and absence of S9 mix. The S9 metabolic activator, the tester strain, and the test article were combined in molten agar which is overlaid onto the minimal agar plate. When the agar had set, the plates were inverted and incubated for 48 to 72 hours at 37 ± 1°C, and observed for compound insolubility and cytotoxicity, and scored.

Study Validity

The study is valid for the following reasons: 1) the appropriate strains were tested, 2) the appropriate controls were used, and 3) the positive control substances produced positive and reliable results. The test article was considered to be positive if at least concentration exhibits a reproducible and statistically significant increase ($p \leq 0.05$) and a two-fold increase in the number of mutants over its concurrent negative control. Also, the test article was considered positive if a concentration-dependent increase in the number of mutants was observed.

Results

Under the conditions of the study, [redacted] (b) (4) was negative in the bacterial reverse mutation assay.

[redacted] (b) (4) was evaluated at the following concentrations: 0.14, 1.93, 3.48, 11.71, 23.01, 66.92, 240.10, 628.11, 2842.42, 5358.87 mcg/plate in the mutagenicity assay with or without S9 metabolic enzyme activation. In the mutagenicity assay, the positive controls induced mutation frequencies as expected. Compared to the negative control group, [redacted] (b) (4) did not result in an increase in revertant bacterial colonies in the presence of metabolic activation. However, increase in the frequency of revertants was observed relative to the negative control in the TA1535 strain exposed to 628.11

Reference ID: 4263670

mcg/plate in the absence of metabolic activation. The induced mutation frequency was within the historical control data for the laboratory (range: 4 to 10). As depicted in the Applicant's tables (pages 16 and 18) below, compared to the vehicle control groups, (b) (4) did not result in an increase in revertant bacterial colonies in the *Salmonella typhimurium* strains (TA987, TA100, TA1535 or TA1537) or the *Escherichia coli* test strain (WP2*uvr*A) in the presence or absence of the metabolic activator which would be indicative of its mutagenic potential. No precipitation or cytotoxicity was observed at any concentration level.

Table 9. Definitive Bacterial Mutation Assay without Metabolic Activation

Revertants/Plate

Strain	Controls			Test Substance									
	Positive	Negative		Dosing Concentrations (µg/plate)									
		NaCl	MeOH	5,358.87	2,842.42	628.11	240.10	66.92	23.01	11.71	3.48	1.93	0.14
TA98	35	26	14	7	13	17	20	6	19	6	5	3	5
	38	16	5	12	6	10	7	9	13	6	5	7	8
	42	14	8	17	6	12	12	8	7	12	10	6	7
Mean	**38* (P = 0.003)**	**19**	**9**	**12**	**8**	**13**	**13**	**8**	**13**	**8**	**7**	**5**	**7**
SD	**4**	**6**	**5**	**5**	**4**	**4**	**7**	**2**	**6**	**3**	**3**	**2**	**2**
TA100	480	67	70	65	45	64	52	52	63	62	48	63	67
	488	74	45	69	43	73	60	55	51	62	65	47	57
	510	46	56	64	59	65	57	49	56	53	56	53	66
Mean	**493* (P = 0.000)**	**62**	**57**	**66**	**49**	**67**	**56**	**52**	**57**	**59**	**56**	**54**	**63**
SD	**16**	**15**	**13**	**3**	**9**	**5**	**4**	**3**	**6**	**5**	**9**	**8**	**6**
TA1535	68	1	4	3	6	6	2	4	5	4	4	4	3
	68	6	2	2	7	9	2	3	5	1	2	3	2
	63	1	2	3	4	8	4	4	3	8	3	2	2
Mean	**66* (P = 0.001)**	**3**	**3**	**3**	**6**	**8* (P = 0.020)**	**3**	**4**	**4**	**4**	**3**	**3**	**2**
SD	**3**	**3**	**1**	**1**	**2**	**2**	**1**	**1**	**1**	**4**	**1**	**1**	**1**
TA1537	80	2	1	3	1	5	1	1	2	2	2	2	2
	25	1	1	3	3	3	1	1	2	2	1	1	1
	92	2	1	1	3	2	1	1	1	1	1	1	1
Mean	**66** (P = 0.088)**	**2**	**1**	**2**	**2**	**3**	**1**	**1**	**2**	**2**	**1**	**1**	**1**
SD	**36**	**1**	**0**	**1**	**1**	**2**	**0**	**0**	**1**	**1**	**1**	**1**	**1**
WP2 *uvr*A	350	26	31	25	23	31	39	38	38	30	33	28	28
	442	19	39	34	21	31	19	30	30	52	26	27	25
	490	39	34	31	29	30	41	40	30	44	37	26	37
Mean	**427* (P = 0.011)**	**28**	**35**	**30**	**24**	**31**	**33**	**36**	**33**	**42**	**32**	**27**	**30**
SD	**71**	**10**	**4**	**5**	**4**	**1**	**12**	**5**	**5**	**11**	**6**	**1**	**6**

* = < 0.05 SD = Standard Deviation

** = > 0.05 compared to MeOH but more than two-fold increase.

Reference ID: 4263670

Table 10. Definitive Bacterial Mutation Assay with Metabolic Activation

Revertants/Plate

Strain	Controls			Test Substance									
	Positive	Negative		Dosing Concentrations (µg/plate)									
		NaCl	MeOH	5,358.87	2,842.42	628.11	240.10	66.92	23.01	11.71	3.48	1.93	0.14
TA98	50	30	24	20	18	27	22	19	22	23	23	18	21
	60	21	22	20	22	18	19	13	24	20	22	22	21
	49	19	25	20	20	20	19	25	24	25	23	27	25
Mean	**53* (P = 0.015)**	**23**	**24**	**20**	**20**	**22**	**20**	**19**	**23**	**23**	**23**	**22**	**22**
SD	**6**	**6**	**2**	**0**	**2**	**5**	**2**	**6**	**1**	**3**	**1**	**5**	**2**
TA100	344	109	118	101	105	97	87	79	96	81	94	84	93
	364	87	107	85	108	110	108	99	105	101	95	79	100
	388	91	108	115	101	102	98	83	79	104	92	98	100
Mean	**365* (P = 0.003)**	**96**	**111**	**100**	**105**	**103**	**98**	**87†**	**93**	**95**	**94†**	**87†**	**98**
SD	**22**	**12**	**6**	**15**	**4**	**7**	**11**	**11**	**13**	**13**	**2**	**10**	**4**
TA1535	72	11	11	8	9	3	13	8	10	7	6	7	6
	109	9	7	7	7	7	7	9	5	9	8	6	5
	101	9	9	6	4	10	10	3	14	9	8	8	10
Mean	**94* (P = 0.017)**	**10**	**9**	**7**	**7**	**7**	**10**	**7**	**10**	**8**	**7**	**7**	**7**
SD	**19**	**1**	**2**	**1**	**3**	**4**	**3**	**3**	**5**	**1**	**1**	**1**	**3**
TA1537	73	4	6	5	3	2	6	7	4	6	11	3	8
	93	6	7	5	3	4	3	8	5	9	6	7	7
	78	4	5	9	6	5	4	3	5	7	6	3	7
Mean	**81* (P = 0.006)**	**5**	**6**	**6**	**4**	**4**	**4**	**6**	**5**	**7**	**8**	**4**	**7**
SD	**10**	**1**	**1**	**2**	**2**	**2**	**2**	**3**	**1**	**2**	**3**	**2**	**1**
WP2 *uvr*A	320	52	51	53	51	55	70	52	67	60	47	48	50
	358	51	54	53	48	51	72	44	52	42	48	50	48
	394	45	54	69	51	50	47	36	64	50	52	47	69
Mean	**357* (P = 0.005)**	**49**	**53**	**58**	**50**	**52**	**63**	**44**	**61**	**51**	**49**	**48†**	**56**
SD	**37**	**4**	**2**	**9**	**2**	**3**	**14**	**8**	**8**	**9**	**3**	**2**	**12**

* = < 0.05 SD = Standard Deviation

† = < 0.05 compared to MeOH but less than two-fold increase.

Study title: *Salmonella Typhimurium* and *Escherichia Coli* reverse mutation assay (OECD 471)

Study no.: 16-01138-G3 Amended

Study report location: \\cdsesub1\evsprod\nda209575\0001\m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\16-01140-g3\16-01140-g3-pre-clinical-study-report.pdf

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

Date of study initiation: June 2, 2016

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: [redacted] (b) (4), 98.9%

Key Study Findings

[redacted] (b) (4) at concentration of 5,382.65 mcg/plate was not mutagenic in the in vitro reverse mutation assay using strains of *Salmonella typhimurium* and *Escherichia coli* with or without enhanced metabolic enzymes.

Reference ID: 4363670

Methods

Strains: *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2uvrA

Concentrations in definitive study: 0.25, 0.79, 2.29, 6.41, 23.27, 69.59, 206.14, 605.26, 2063.93, and 5382.65 mcg/plate

Basis of concentration selection: Basis for selection of the concentration to be used for the definitive mutation tests was not described.

Negative control: Vehicle = 60% menthol in water (100 mcL/plate), and physiological saline

Positive control: 2-nitrofluorene (2-NF), sodium azide (NaAZ), 9-aminoacridine (9AA), and 4-nitroquinoline 1-0xide (4-NQ 1-O) were selected for positive controls based on the bacterial strain as indicated in the following table:

TABLE 2:
Positive Control Articles

Strain Designation		Strain-Specific Positive Controls per Metabolic Activation (MA)			
		Without MA		Plus MA	
		Agent	µg/plate	Agent	µg/plate
S. typhimurium	TA98	2-Nitrofluorene	1.0	2-Aminoanthracene	0.5
	TA100	Sodium Azide	10.0	2-Aminoanthracene	1.0
	TA1535	Sodium Azide	0.5	2-Aminoanthracene	2.0
	TA1537	9-Aminoacridine	80.0	2-Aminoanthracene	3.0
E. coli	WP2	4-Nitroquinoline 1-Oxide	10.0	2-Aminoanthracene	20.0

Formulation/Vehicle: [redacted] (b) (4) was dissolved in 60% menthol in water.

Incubation & sampling time: The definitive mutagenicity test was performed using the plate-incorporation method. Triplicate plates for positive controls, negative controls, and cocaine hydrochloride concentrations were prepared for each bacterial strain in the presence and absence of S9 mix. The S9 metabolic activator, the tester strain, and the test article were combined in molten agar which is overlaid onto the minimal agar plate. When the agar had set, the plates were inverted and incubated for 48 to 72 hours at 37 ± 1°C, and observed for compound insolubility and cytotoxicity, and scored.

Study Validity

The study is valid for the following reasons: 1) the appropriate strains were tested, 2) the appropriate controls were used, and 3) the positive control substances produced positive and reliable results. The test article was considered to be positive if at least concentration exhibits a reproducible and statistically significant increase ($p \leq 0.05$) and a two-fold increase in the number of mutants over its concurrent negative control. Also,

Reference ID: 4263670

the test article was considered positive if a concentration-dependent increase in the number of mutants was observed.

Results

Under the conditions of the study, [redacted] (b) (4) was negative in the bacterial reverse mutation assay.

[redacted] (b) (4) was evaluated at the following concentrations: 0.25, 0.79, 2.29, 6.41, 23.27, 69.59, 206.14, 605.26, 2063.93, and 5382.65 mcg/plate in the mutagenicity assay with or without S9 metabolic enzyme activation. In the mutagenicity assay, the positive controls induced mutation frequencies as expected. Compared to the negative control group, [redacted] (b) (4) did not result in an increase in revertants bacterial colonies in the presence of metabolic activation. As depicted in the Applicant's tables (pages 15 and 16) below, compared to the vehicle control groups, [redacted] (b) (4) did not result in an increase in revertant bacterial colonies in the *Salmonella typhimurium* strains (TA987, TA100, TA1535 or TA1537) or the *Escherichia coli* test strain (WP2*uvr*A) in the presence However, a statistically significant increase in the frequency of revertants was observed relative to the negative control in the TA1535 and TA1537 strains exposed to 23.27 and 206.14 mcg/plate in the absence of metabolic activation. The induced mutation frequency was within the historical control data for the laboratory (Range: 5 to 95). No precipitation or cytotoxicity was reported at any concentration level.

TABLE 3:
Reverse Mutation Assay without Metabolic Activation

Revertants/Plate

Strain	Controls			Test Substance									
	Positive	Negative		Dosing Concentrations (µg/plate)									
		NaCl	MeOH	5,382.65	2,063.93	605.26	206.14	69.59	23.27	6.41	2.29	0.79	0.25**
TA98	35	26	14	9	13	8	10	8	8	12	5	4	6
	38	16	5	8	6	9	8	2	2	9	9	12	2
	42	14	8	12	8	8	9	8	11	7	10	10	13
Mean	**38* (P = 0.003)**	**19**	**9**	**10**	**9**	**8**	**9**	**6**	**7**	**9**	**8**	**9**	**7**
SD	**4**	**6**	**5**	**2**	**4**	**1**	**1**	**3**	**5**	**3**	**3**	**4**	**6**
TA100	480	67	70	52	51	39	64	58	59	63	55	52	55
	488	74	45	43	49	41	38	58	58	50	49	61	59
	510	46	56	59	66	55	34	58	58	63	58	77	54
Mean	**493* (P = 0.000)**	**62**	**57**	**51**	**55**	**45**	**45**	**58**	**58**	**59**	**54**	**63**	**56**
SD	**16**	**15**	**13**	**8**	**9**	**9**	**16**	**0**	**1**	**8**	**5**	**13**	**3**
TA1535	68	1	4	3	1	4	3	2	8	5	3	3	5
	68	6	2	4	4	3	4	4	8	4	4	6	4
	63	1	2	4	2	4	4	2	6	7	5	8	2
Mean	**66* (P = 0.001)**	**3**	**3**	**4**	**2**	**4**	**4**	**3**	**7* (P = 0.008)**	**5**	**4**	**6**	**4**
SD	**3**	**3**	**1**	**1**	**2**	**1**	**1**	**1**	**1**	**2**	**1**	**3**	**2**
TA1537	80	2	1	2	2	2	2	1	1	4	1	2	1
	25	1	1	1	2	2	2	1	1	1	3	3	1
	92	2	1	3	1	1	2	2	3	1	2	2	3
Mean	**66** (P = 0.088)**	**2**	**1**	**2**	**2**	**2**	**2* (P = 0.000)**	**1**	**2**	**2**	**2**	**2**	**2**
SD	**36**	**1**	**0**	**1**	**1**	**1**	**0**	**1**	**1**	**2**	**1**	**1**	**1**
WP2 *uvr*A	350	26	31	24	34	25	34	36	35	34	32	37	38
	442	19	39	32	27	36	25	28	33	38	24	44	41
	490	39	34	27	46	26	33	34	29	45	36	36	39
Mean	**427* (P = 0.011)**	**28**	**35**	**28**	**36**	**29**	**31**	**33**	**32**	**39**	**31**	**39**	**39**
SD	**71**	**10**	**4**	**4**	**10**	**6**	**5**	**4**	**3**	**6**	**6**	**4**	**2**

* = < 0.05 SD = Standard Deviation
** = Concentration was not measured.
† = Not statistically significant but greater than 30 fold higher than the negative controls.

Reference ID: 4263670

TABLE 4:
Reverse Mutation Assay with Metabolic Activation

Revertants/Plate

Strain	Controls			Test Substance									
	Positive	Negative		Dosing Concentrations (µg/plate)									
		NaCl	MeOH	5,382.65	2,063.93	605.26	206.14	69.59	23.27	6.41	2.29	0.79	0.25**
TA98	50	30	24	21	23	25	22	24	21	21	27	20	28
	60	21	22	7	18	31	23	29	24	23	34	29	28
	49	19	25	22	16	23	21	24	28	20	27	26	29
Mean	**53* (P = 0.015)**	**23**	**24**	**17**	**19**	**26**	**22**	**26**	**24**	**21**	**29**	**25**	**28* (P = 0.038)**
SD	**6**	**6**	**2**	**8**	**4**	**4**	**1**	**3**	**4**	**2**	**4**	**5**	**1**
TA100	344	109	118	86	92	113	97	110	96	75	97	87	105
	364	87	107	94	153	94	87	43	43	56	87	103	116
	388	91	108	73	119	107	97	69	90	80	83	115	111
Mean	**365* (P = 0.003)**	**96**	**111**	**84**	**121**	**105**	**94**	**74**	**76**	**70**	**89**	**102**	**111**
SD	**22**	**12**	**6**	**11**	**31**	**10**	**6**	**34**	**29**	**13**	**7**	**14**	**6**
TA1535	72	11	11	7	6	9	10	4	8	7	13	8	11
	109	9	7	12	10	11	5	8	8	7	15	8	13
	101	9	9	4	11	14	12	11	10	6	8	9	15
Mean	**94* (P = 0.017)**	**10**	**9**	**8**	**9**	**11**	**9**	**8**	**9**	**7**	**12**	**8**	**13**
SD	**19**	**1**	**2**	**4**	**3**	**3**	**4**	**4**	**1**	**1**	**4**	**1**	**2**
TA1537	73	4	6	9	4	5	6	7	8	6	5	10	8
	93	6	7	3	6	3	7	5	1	4	5	3	4
	78	4	5	3	1	7	6	6	3	12	8	5	8
Mean	**81* (P = 0.006)**	**5**	**6**	**5**	**4**	**5**	**6**	**6**	**4**	**7**	**6**	**6**	**7**
SD	**10**	**1**	**1**	**3**	**3**	**2**	**1**	**1**	**4**	**4**	**2**	**4**	**2**
WP2 *uvrA*	320	52	51	44	57	66	69	57	64	62	52	40	58
	358	51	54	47	50	64	57	40	56	53	66	70	44
	394	45	54	39	50	41	72	55	44	68	54	74	61
Mean	**357* (P = 0.005)**	**49**	**53**	**43**	**52**	**57**	**66**	**51**	**55**	**61**	**57**	**61**	**54**
SD	**37**	**4**	**2**	**4**	**4**	**14**	**8**	**9**	**10**	**8**	**8**	**19**	**9**

* = < 0.05
** = Concentration was not measured.
SD = Standard Deviation

Study title: *Salmonella Typhimurium* and *Escherichia Coli* reverse mutation assay (OECD 471)

Study no.: 16-01139-G3 Amended
Study report location: 4.2.3.3 \\cdsesub1\evsprod\nda209575\0001\m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\16-01139-g3\16-01139-g3-pre-clinical-study-report.pdf
Conducting laboratory and location: (b) (4) [redacted]
Date of study initiation: June 2, 2016
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: [redacted] (b) (4), 99.3%

Key Study Findings

[redacted] (b) (4) at concentration of 4926.29 mcg/plate was not mutagenic in the in vitro reverse mutation assay using strains of *Salmonella typhimurium* and *Escherichia coli* with or without enhanced metabolic enzymes.

Reference ID: 4263670

Methods

Strains: *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2uvrA

Concentrations in definitive study: 0.25, 1.04, 2.14, 8.02, 20.84, 60.23, 180.79, 556.54, 1799.10, and 4926.29 mcg/plate

Basis of concentration selection: Basis for selection of the concentration to be used for the definitive mutation tests was not described.

Negative control: Vehicle = 60% menthol in water (100 mcL/plate), and physiological saline

Positive control: 2-nitrofluorene (2-NF), sodium azide (NaAZ), 9-aminoacridine (9AA), and 4-nitroquinoline 1-0xide (4-NQ 1-O) were selected for positive controls based on the bacterial strain as indicated in the following table:

TABLE 2:
Positive Control Articles

Strain Designation		Strain-Specific Positive Controls per Metabolic Activation (MA)			
		Without MA		Plus MA	
		Agent	µg/plate	Agent	µg/plate
S. typhimurium	TA98	2-Nitrofluorene	1.0	2-Aminoanthracene	0.5
	TA100	Sodium Azide	10.0	2-Aminoanthracene	1.0
	TA1535	Sodium Azide	0.5	2-Aminoanthracene	2.0
	TA1537	9-Aminoacridine	80.0	2-Aminoanthracene	3.0
E. coli	WP2	4-Nitroquinoline 1-Oxide	10.0	2-Aminoanthracene	20.0

Formulation/Vehicle: [redacted] (b) (4) was dissolved in 60% menthol in water.

Incubation & sampling time: The definitive mutagenicity test was performed using the plate-incorporation method. Triplicate plates for positive controls, negative controls, and cocaine hydrochloride concentrations were prepared for each bacterial strain in the presence and absence of S9 mix. The S9 metabolic activator, the tester strain, and the test article were combined in molten agar which is overlaid onto the minimal agar plate. When the agar had set, the plates were inverted and incubated for 48 to 72 hours at 37 ± 1°C, and observed for compound insolubility and cytotoxicity, and scored.

Study Validity

The study is valid for the following reasons: 1) the appropriate strains were tested, 2) the appropriate controls were used, and 3) the positive control substances produced positive and reliable results. The test article was considered to be positive if at least concentration exhibits a reproducible and statistically significant increase ($p \leq 0.05$) and a two-fold increase in the number of mutants over its concurrent negative control. Also,

Reference ID: 4263670

the test article was considered positive if a concentration-dependent increase in the number of mutants was observed.

Results

Under the conditions of the study, (b) (4) was negative in the bacterial reverse mutation assay.

(b) (4) was evaluated at the following concentrations: .25, 1.04, 2.14, 8.02, 20.84, 60.23, 180.79, 556.54, 1799.10, and 4926.29 mcg/plate in the mutagenicity assay with or without S9 metabolic enzyme activation. In the mutagenicity assay, the positive controls induced mutation frequencies as expected. Compared to the negative control group, (b) (4) did not result in an increase in revertants bacterial colonies in the presence of metabolic activation. As depicted in the Applicant's tables (pages 15 and 16) below, compared to the vehicle control groups, (b) (4) did not result in an increase in revertant bacterial colonies in the *Salmonella typhimurium* strains (TA987, TA100, TA1535 or TA1537) or the *Escherichia coli* test strain (WP2*uvr*A) in the presence of metabolic activation. However, a statistically significant increase in the frequency of revertants was observed relative to the negative control in the TA1537 strain exposed to 1.04, 2.14 and 556.54 mcg/plate in the absence of metabolic activation. The induced mutation frequency was within the historical control data for the laboratory (Range: 2 to 7); thus is not considered to be biologically significant. No precipitation or cytotoxicity was reported at any concentration level.

TABLE 3:
Reverse Mutation Assay without Metabolic Activation

Revertants/Plate

Strain	Controls			Test Substance									
	Positive	Negative		Dosing Concentrations (µg/plate)									
		NaCl	MeOH	4,926.29	1,799.10	556.54	180.79	60.23	20.84	8.02	2.14	1.04	0.25++
TA98	35	26	14	11	14	8	13	8	7	16	8	11	8
	38	16	5	9	6	7	6	9	6	11	9	6	10
	42	14	8	10	11	6	7	7	8	5	8	8	4
Mean	**38* (P = 0.003)**	**19**	**9**	**10**	**10**	**7**	**9**	**8**	**7**	**11**	**8**	**8**	**7**
SD	**4**	**6**	**5**	**1**	**4**	**1**	**4**	**1**	**1**	**6**	**1**	**3**	**3**
TA100	480	67	70	57	59	57	38	52	68	60	65	51	61
	488	74	45	62	58	59	69	51	60	55	45	43	54
	510	46	56	64	53	71	57	41	48	41	40	53	61
Mean	**493* (P = 0.000)**	**62**	**57**	**61**	**57**	**62**	**55**	**48**	**59**	**52**	**50**	**49**	**59**
SD	**16**	**15**	**13**	**4**	**3**	**8**	**16**	**6**	**10**	**10**	**13**	**5**	**4**
TA1535	68	1	4	1	2	1	4	4	9	4	3	3	2
	68	6	2	3	5	4	6	5	7	5	5	4	4
	63	1	2	4	3	2	3	3	3	6	3	2	2
Mean	**66* (P = 0.001)**	**3**	**3**	**3**	**3**	**2**	**4**	**4**	**6**	**5**	**4**	**3**	**3**
SD	**3**	**3**	**1**	**2**	**2**	**2**	**2**	**1**	**3**	**1**	**1**	**1**	**1**
TA1537	80	2	1	1	1	2	4	1	3	3	2	3	1
	25	1	1	1	3	2	1	2	1	1	2	2	1
	92	2	1	2	2	2	1	3	2	2	2	3	2
Mean	**66** (P = 0.088)**	**2**	**1**	**1**	**2**	**2* (P = 0.000)**	**2**	**2**	**2**	**2**	**2* (P = 0.000)**	**3* (P = 0.038)**	**1**
SD	**36**	**1**	**0**	**1**	**1**	**0**	**2**	**1**	**1**	**1**	**0**	**1**	**1**
WP2 *uvr*A	350	26	31	27	28	32	52	30	33	24	32	33	47
	442	19	39	31	33	33	24	47	33	29	35	31	27
	490	39	34	40	30	32	33	40	34	47	26	27	38
Mean	**427* (P = 0.011)**	**28**	**35**	**33**	**30**	**32**	**36**	**39**	**33**	**33**	**31**	**30**	**37**
SD	**71**	**10**	**4**	**7**	**3**	**1**	**14**	**9**	**1**	**12**	**5**	**3**	**10**

* = < 0.05 SD = Standard Deviation
** = Not statistically significant but several folds higher than negative control.
++ = Theoretical concentration

Reference ID: 4263670

TABLE 4:
Reverse Mutation Assay with Metabolic Activation

Revertants/Plate

Strain	Controls			Test Substance									
	Positive	Negative		Dosing Concentrations (µg/plate)									
		NaCl	MeOH	4,926.29	1,799.10	556.54	180.79	60.23	20.84	8.02	2.14	1.04	0.25++
TA98	50	30	24	24	23	20	13	22	22	28	14	14	23
	60	21	22	23	23	25	23	22	26	28	18	26	17
	49	19	25	23	15	27	17	29	30	28	14	28	19
Mean	**53* (P = 0.015)**	**23**	**24**	**23**	**20**	**24**	**18**	**24**	**26**	**28+**	**15**	**23**	**20**
SD	**6**	**6**	**2**	**1**	**5**	**4**	**5**	**4**	**4**	**0**	**2**	**8**	**3**
TA100	344	109	118	90	92	75	99	98	87	130	119	116	93
	364	87	107	127	118	99	103	105	109	94	122	102	84
	388	91	108	88	103	102	78	102	120	102	97	73	94
Mean	**365* (P = 0.003)**	**96**	**111**	**102**	**104**	**92**	**93**	**102**	**105**	**109**	**113**	**97**	**90**
SD	**22**	**12**	**6**	**22**	**13**	**15**	**13**	**4**	**17**	**19**	**14**	**22**	**6**
TA1535	72	11	11	11	9	7	11	6	9	12	7	9	11
	109	9	7	11	15	8	10	6	9	9	10	15	8
	101	9	9	10	14	10	10	10	4	14	12	10	6
Mean	**94* (P = 0.017)**	**10**	**9**	**11**	**13**	**8**	**10**	**7**	**7**	**12**	**10**	**11**	**8**
SD	**19**	**1**	**2**	**1**	**3**	**2**	**1**	**2**	**3**	**3**	**3**	**3**	**3**
TA1537	73	4	6	8	10	5	5	6	11	5	4	10	7
	93	6	7	6	8	6	8	7	8	3	10	6	6
	78	4	5	8	4	6	6	9	6	8	3	12	9
Mean	**81* (P = 0.006)**	**5**	**6**	**7**	**7**	**6**	**6**	**7**	**8**	**5**	**6**	**9**	**7**
SD	**10**	**1**	**1**	**1**	**3**	**1**	**2**	**2**	**3**	**3**	**4**	**3**	**2**
WP2 *uvr*A	320	52	51	57	40	48	51	56	54	53	48	56	46
	358	51	54	39	57	41	58	51	78	47	56	52	38
	394	45	54	60	53	55	57	47	48	39	48	49	55
Mean	**357* (P = 0.005)**	**49**	**53**	**52**	**50**	**48**	**55**	**51**	**60**	**46**	**51**	**52**	**46**
SD	**37**	**4**	**2**	**11**	**9**	**7**	**4**	**5**	**16**	**7**	**5**	**4**	**9**

* = < 0.05 SD = Standard Deviation
+ = Statistically significant from negative control but less than two-fold increase.
++ = Theoretical concentration

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Chromosomal Aberration Assay – OECD 473

Study no.: 11-4138
Study report location: \\cdsesub1\evsprod\nda209575\0001\m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\11-4138-g2\11-4138-g2-pre-clinical-study-report.pdf
Conducting laboratory and location: (b) (4) [redacted]
Date of study initiation: September 2, 2011
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Cocaine HCl, Lot 11330001,100%

Key Study Findings

Cocaine at concentrations of 0.185, 0.555, and 1.666 mg/mL without metabolic activation and at 0.061, 0.185, and 0.555 mg/mL in the presence of metabolic activation was negative in the in vitro chromosomal aberration test using Chinese Hamster ovary.

Methods

Reference ID: 4263670

Cell line: Chinese Hamster Ovary (CHO)

Concentrations in definitive study: Without S9 mix:
0.185, 0.555, and 1.666 mg/mL

With S9 mix:
0.061, 0.185, and 0.555 mg/mL

Basis of concentration selection: Doses selection was based upon a dose range-finding assay in which cocaine hydrochloride was evaluated at 0.020, 0.061, 0.185, 0.555, 1.666, and 5 mg/mL. Cytotoxicity was measured based on reduction in cell confluences. Cocaine was cytotoxic. Significant (100%) cytotoxicity was observed at the 1.666 and 5 mg/mL concentration in the presence of metabolic activation. and partial toxicity was observed at 1.666 mg/mL in the absence of metabolic activation. Partial toxicity was noted at the lower concentration.

Negative control: Ham's F-12 medium

Positive control: Mitomycin C (MMC): 0.075, mcg/mL (3-hr treatment in the absence of S9 mix)

Cyclophosphamide (CP): 35 mcg/mL (in the presence of S9 mix)

Formulation/Vehicle: Solution/sterile water for injection

Incubation & sampling time: The CHO cells were incubated with or without metabolic activation in a cell culture incubator at 37 ±1°C, 5 ±1% CO_2 and saturated humidity.

Treatment Condition	Treatment Time	Recovery Time	Drug Concentration
Non-activated (-S9)	3 hr	21 hr	0.185, 0.555, 1.666 mg/mL
	20 hr		0.020, 0.061, 0.185 mg/mL
Activated (S9)	3 hr	4 hr	0.061, 0.185, 0.555 mg/mL

Study Validity

The study appears valid for the following reasons:

- The appropriate positive controls were employed according to OECD guidelines and produced expected results.
- An acceptable number of mitotic cells were evaluated (minimal of 200 cells per culture).
- The conditions of the assay are appropriate based upon OECD guidelines.
- The frequency of cells with structural chromosome aberrations in the vehicle controls was within the historical control range for the vehicle controls.
- The dose selection based upon cytotoxicity (i.e., reduction of mitotic index) was acceptable.

Reference ID: 4263670

Results

As depicted in the tables below, there was no evidence of chromosomal aberrations in cocaine treated CHO cells in the presence and absence of metabolic activation. For the 3-hour non-activated main group, the highest cocaine concentration tested (1.666 mg/mL) did not produce a significant increase in the number of structural chromosomal aberrations or polyploidy. Similar results were observed in the confirmatory assay performed using an extended period of exposure (20 hours); a significant increase in chromosomal aberration was not observed at the highest concentration (0.555 mg/mL) tested. The positive control, mitomycin C (0.075 mcg/mL) increased the number of CHO cells with structural aberrations (20).

Table 11. The Effect of Cocaine on the Induction of Chromosomal Aberrations in CHO Cells (Non-activated)

Treatment	# Cells Analyzed	Number and Types of Aberrations: Gaps		Breaks			ICRA					Other			# Cells with Aberrations*
		TG	SG	TB	SB	D	TR	QR	CR	R	DC	DM	PV	PP	
Negative Control: Ham's F–12 Medium	200	2	0	0	0	1	0	0	0	0	0	0	0	3	1
Positive Control: Mitomycin C (0.075 µg/mL)	100	1	0	10	9	4	2	1	0	0	0	0	0	12	20
Test Substance: 1.666 mg/mL	200	3	1	1	1	0	0	0	0	0	0	0	0	4	2
Test Substance: 0.555 mg/mL	200	1	1	3	0	0	0	0	0	0	0	0	0	1	3
Test Substance: 0.185 mg/mL	200	2	1	2	1	0	0	0	0	0	0	0	0	3	3

* Not including Gaps or Polyploid Cells.

CODE FOR IDENTIFYING CHROMOSOMAL ABERRATIONS

TG = Chromatid Gap
SB = Chromosome Break
ICRA = Inter Chromosomal
R = Ring
PV = Pulverized

SG = Chromosome Gap
DM = Double Minute
TR = Triradial
D = Deletion
PP = Polyploid Cells

TB = Chromatid Break
DC = Dicentric Chromosome
QR = Quadradial Rearrangement
CR = Complex Rearrangement

For the 3-hour activated group, the highest cocaine concentration tested (0.555 mg/mL) did not produce a significant increase in the number of structural chromosomal aberrations or polyploidy. The positive control, cyclophosphamide (35 mcg/mL) increased the number of CHO cells with structural aberrations (22 cells compared to 3 in the negative control).

Reference ID: 4263670

Number and Types of Aberrations

Treatment	# Cells Analyzed	Gaps		Breaks			ICRA					Other			# Cells with Aberrations*
		TG	SG	TB	SB	D	TR	QR	CR	R	DC	DM	PV	PP	
Negative Control: Ham's F–12 Medium	200	2	0	2	1	0	0	0	0	0	0	0	0	3	3
Positive Control: Cyclophosphamide (35 µg/mL)	100	3	0	15	12	01	5	4	0	0	0	0	0	12	22
Test Substance: 0.555 mg/mL	200	4	0	4	0	0	0	1	0	0	0	0	0	1	5
Test Substance: 0.185 mg/mL	200	2	0	0	0	1	3	0	0	0	0	0	0	2	3
Test Substance: 0.061 mg/mL	200	4	0	1	0	3	1	0	0	0	0	0	0	2	5

* Not including Gaps or Polyploid Cells.

CODE FOR IDENTIFYING CHROMOSOMAL ABERRATIONS

TG = Chromatid Gap
SB = Chromosome Break
ICRA = Inter Chromosomal
R = Ring
PV = Pulverized

SG = Chromosome Gap
DM = Double Minute
TR = Triradial
D = Deletion
PP = Polyploid Cells

TB = Chromatid Break
DC = Dicentric Chromosome
QR = Quadradial Rearrangement
CR = Complex Rearrangement

Study title: Chromosomal Aberration Assay – OECD 473

Study no.: 16-01138-G2

Study report location: \\cdsesub1\evsprod\nda209575\0001\m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\16-01138-g2\16-01138-g2-pre-clinical-study-report.pdf

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

Date of study initiation: February 15, 2017

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: (b) (4) [redacted], Lot 265-32, purity unreported

Key Study Findings

Under the conditions of this assay, (b) (4) [redacted] at concentrations of 0.48, 0.94, and 1.81 mg/mL was not clastogenic in cultured CHO cells cultures in vitro in the absence and presence of metabolic activation. However, purity information on the test article was not provided and therefore, the study is not acceptable in its current form.

Methods

Cell line: Chinese Hamster Ovary (CHO)

Concentrations in definitive study: Without S9 mix: 0.185, 0.555, and 1.666 mg/mL

With S9 mix: 0.061, 0.185, and 0.555 mg/mL

Basis of concentration selection: No preliminary dose range-finding study was performed to determine the concentration selection for the definitive studies.

Negative control: 60% methanol in water and 95% ethanol in saline

Reference ID: 4263670

Positive control: Mitomycin C (MMC): 0.075 mcg/mL (3-hr treatment in the absence of S9 mix), 0.5 mcg/mL (confirmation assay, 20-h treatment in the absence of S9 mix)

Cyclophosphamide (CP): 7 mcg/mL (in the presence of S9 mix)

Formulation/Vehicle: Solution/60 menthanol:40 ethanol

Incubation & sampling time: The CHO cells were incubated with or without metabolic activation in a cell culture incubator at 37 ±1°C, 5 ±1% CO_2 and in humidity atmosphere.

Treatment Condition	Treatment Time	Recovery Time	Drug Concentration
Non-activated (-S9)	3 to 6 hr	18 to 21 hr	0.48, 0.94, 1.81 mg/mL
	20 hr		0.48, 0.94, 1.81 mg/mL
Activated (S9)	3 hr	4 hr	0.48, 0.94, 1.81 mg/mL

Study Validity

The study appears valid for the following reasons:

- The appropriate positive controls were employed according to OECD guidelines and produced expected results.
- An acceptable number of mitotic cells were evaluated (minimal of 200 cells per culture).
- The conditions of the assay are appropriate based upon OECD guidelines.

However, purity information on the test article was not provided and therefore, the study is not acceptable in its current form.

Results

There was no evidence of chromosomal aberrations in (b) (4) treated (b) (4) cells. For the 3-hour non-activated group, the highest cocaine concentration tested (1.8 mg/mL) did not produce a significant increase in the number of structural chromosomal aberrations or polypoidy. The positive control, mitomycin C increased the percentage of CHO cells with structural aberrations in the main (35%) and confirmatory assay (20%) relative to negative control. Cytotoxicity was observed with a reduction in mitotic index to 2.0% compared to the negative control in the absence of S9 mix.

For the 3-hour activated group, the highest concentration tested (1.8 mg/mL) produced 34% mitotic inhibition relative to negative control. No significant increase in percentage of CHO cells with structural aberrations was observed.

Reference ID: 4263670

Table 12. Summary of results from chromosomal aberration assay with (b) (4)

Summary of Results

Assay	Article	Exposure Concentrations	Cytotoxicity (%)*	Chrom. Ab. (%)	χ^2 p-value
Non-Activated (Main)	Negative Control (MeOH)	0.6%	0	0.3	N/A
	Negative Control (EtOH)	1%	0	0.3	1.000
	Positive Control (MMC)	0.05 µg/mL	28	35	0.000
	Test Article	1.81 mg/mL	0	2.0	0.057
	Test Article	0.94 mg/mL	0	1.0	0.316
	Test Article	0.48 mg/mL	0	2.0	0.057
Activated (Main)	Negative Control (MeOH)	0.6%	0	1.3	N/A
	Negative Control (EtOH)	1%	24	1.3	1.000
	Positive Control (MMC)	0.05 µg/mL	31	34	0.000
	Test Article	1.81 mg/mL	0	2.3	0.361
	Test Article	0.94 mg/mL	0	2.7	0.243
	Test Article	0.48 mg/mL	15	2.7	0.243
Non-Activated (Conf.)	Negative Control (MeOH)	0.6%	0	0.7	N/A
	Negative Control (EtOH)	1%	7	1.3	0.412
	Positive Control (MMC)	0.075 µg/mL	17	20	0.000
	Test Article	1.81 mg/mL	55	1.3	0.412
	Test Article	0.94 mg/mL	0	1.3	0.412
	Test Article	0.48 mg/mL	10	2.0	0.155

Conf.: Confirmation Chrom. Ab.: Chromosomal Aberration
* Cytotoxicity = reduction in percentage Relative Population Doubling of respective negative control (MeOH)
N/A = Not Applicable

Study title: Chromosomal Aberration Assay – OECD 473

Study no.: 16-01139-G2
Study report location: \\cdsesub1\evsprod\nda209575\0001\m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\16-01139-g2\16-01139-g2-pre-clinical-study-report.pdf
Conducting laboratory and location: (b) (4)
Date of study initiation: February 14, 2017
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: (b) (4) purity unreported

Key Study Findings

(b) (4) was clastogenic in cultured CHO cells in vitro. (b) (4) at concentrations of 0.82, and 1.66 mg/mL, induced structural chromosomal aberration. In

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the absence of metabolic activation, (b) (4) induced structural chromosomal aberration at a concentration of 1.66 mg/mL. However, purity information on the test article was not provided and therefore, the study is not acceptable in its current form.

Methods

Cell line:	Chinese Hamster Ovary (CHO)
Concentrations in definitive study:	Without S9 mix: 0.44, 0.82, and 1.66 mg/mL With S9 mix: 0.44, 0.82, and 1.66 mg/mL
Basis of concentration selection:	No preliminary dose range finding study was performed to determine the concentration selection for the definitive studies.
Negative control:	60% methanol in water and 95% ethanol in saline
Positive control:	Mitomycin C (MMC): 0.075, mcg/mL (3-hr treatment in the absence of S9 mix), 0.5 mcg/mL (confirmation assay, 20-h treatment in the absence of S9 mix) Cyclophosphamide (CP): 7 mcg/mL (in the presence of S9 mix)
Formulation/Vehicle:	Solution/60 methanol:40 ethanol
Incubation & sampling time:	The CHO cells were incubated with or without metabolic activation in a cell culture incubator at 37 ±1°C, 5 ±1% CO_2 and in humidity atmosphere.

Treatment Condition	Treatment Time	Recovery Time	Drug Concentration
Non-activated (-S9)	3 to 6 hr	18 to 21 hr	0.44, 0.82, 1.66 mg/mL
	20 hr		0.44, 0.82, 1.66 mg/mL
Activated (S9)	3 hr	18 to 21 hr	0.44, 0.82, 1.66 mg/mL

Study Validity

The chromosomal aberration test performed was considered acceptable as it met the following criteria:

- The aberrations percentage for positive control is statistically higher than that of the negative control.
- The aberration percentage for the positive and negative controls was within the historical control range for the vehicle controls.

However, purity information on the test article was not provided and therefore, the study is not acceptable in its current form.

Results

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There was evidence of that (b) (4) induced chromosomal aberrations in vitro in CHO cells. Results are presented in the Sponsor's table below. (b) (4) induced a statistically significant increase in the percentage of cells with structural chromosomal aberrations in the presence of S9 metabolic activation. In the 3-hour test with metabolic activation, at 0.82 and 1.66 mg/mL, a statistically significant increase in the percentage of CHO cells with structural aberrations was observed when compared to the negative control.

For the 3-hour main activated assay, no statistically significant increase in the number of CHO cells with structural chromosomal aberrations was observed. In the confirmation assay (prolong exposure), a statistically significant increase in the percentage of CHO cells with structural aberrations was observed at the 1.66 mg/mL concentration in the absence of S9 metabolic activation relative to the negative control. The structural aberrations included: gaps, breaks, and inter chromosomal rearrangement.

Table 13. Summary of results from chromosomal aberration assay with Ecgonine.

Summary of Results

Assay	Article	Exposure Concentrations	Cytotoxicity (%)*	Chrom. Ab. (%)	χ^2 p-value
Non-Activated (Main)	Negative Control (MeOH)	0.6%	0	0.3	N/A
	Negative Control (EtOH)	1%	0	0.3	1.000
	Positive Control (MMC)	0.05 µg/mL	28	28	0.000
	Test Article	1.66 mg/mL	19	0.7	0.563
	Test Article	0.82 mg/mL	0	1.7	0.102
	Test Article	0.44 mg/mL	12	2.0	0.057
Activated (Main)	Negative Control (MeOH)	0.6%	0	1.3	N/A
	Negative Control (EtOH)	1%	24	1.3	1.000
	Positive Control (CP)	7 µg/mL	31	34	0.000
	Test Article	1.66 mg/mL	41	5.3	0.006
	Test Article	0.82 mg/mL	20	4.3	0.027
	Test Article	0.44 mg/mL	12	2.0	0.524
Non-Activated (Conf.)	Negative Control (MeOH)	0.6%	0	0.7	N/A
	Negative Control (EtOH)	1%	7	1.3	0.412
	Positive Control (MMC)	0.075 µg/mL	17	20	0.000
	Test Article	1.66 mg/mL	25	3.7	0.012
	Test Article	0.82 mg/mL	1	1.7	0.254
	Test Article	0.44 mg/mL	7	0.3	0.563

Conf.: Confirmation Chrom. Ab.: Chromosomal Aberration
* Cytotoxicity = reduction in percentage Relative Population Doubling of respective negative control (MeOH)
N/A = Not Applicable

Reference ID: 4263670

Table 14. Chromosomal aberration assay in the presence of S9 activation.

Chromosomal Aberrations Results (Activated Assay)

Treatment	Replicate	#Cells Analyzed	# Cells with Aberrations*	Specific Aberrations observed**														
				Gaps		Breaks			ICRA					Other				
				TG	SG	TB	SB	D	TR	QR	CR	R	DC	DM	PV	PP	ER	AN
Negative Control (MeOH)	A	150	2	1	0	2	1	0	0	0	0	0	1	0	0	1	0	0
	B	150	2	3	0	1	1	0	0	0	0	0	1	0	0	1	0	0
	Total	**300**	**4**	**4**	**0**	**3**	**2**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**2**	**0**	**0**
Negative Control (EtOH)	A	150	2	1	0	1	0	0	0	0	0	0	0	1	0	2	0	0
	B	150	2	2	0	0	0	0	0	1	1	0	1	0	0	3	0	1
	Total	**300**	**4**	**3**	**0**	**1**	**0**	**0**	**0**	**1**	**1**	**0**	**1**	**1**	**0**	**5**	**0**	**1**
Positive Control	A	50	16	2	0	6	4	1	6	1	0	0	0	0	1	1	0	1
	B	50	18	3	0	8	3	2	15	2	1	1	1	0	0	0	0	0
	Total	**100**	**34**	**5**	**0**	**14**	**7**	**3**	**21**	**3**	**1**	**1**	**1**	**0**	**1**	**1**	**0**	**1**

Treatment	Replicate	#Cells Analyzed	# Cells with Aberrations*	Specific Aberrations observed**														
				Gaps		Breaks			ICRA					Other				
				TG	SG	TB	SB	D	TR	QR	CR	R	DC	DM	PV	PP	ER	AN
Test Article (1.66 mg/mL)	A	150	12	2	1	3	5	5	4	2	0	0	1	0	0	5	0	2
	B	150	4	2	0	2	0	0	1	3	0	0	0	0	0	3	0	0
	Total	**300**	**16**	**4**	**1**	**5**	**5**	**5**	**5**	**5**	**0**	**0**	**1**	**0**	**0**	**8**	**0**	**2**
Test Article (0.82 mg/mL)	A	150	9	3	0	3	3	1	2	2	0	1	0	1	0	5	1	2
	B	150	4	1	0	4	3	0	2	0	0	0	1	1	0	4	0	2
	Total	**300**	**13**	**4**	**0**	**7**	**6**	**1**	**4**	**2**	**0**	**1**	**1**	**2**	**0**	**9**	**1**	**4**
Test Article (0.44 mg/mL)	A	150	4	3	0	6	0	1	3	1	0	0	0	0	0	3	0	2
	B	150	2	2	2	1	0	0	0	0	0	0	1	0	0	0	0	0
	Total	**300**	**6**	**5**	**2**	**7**	**0**	**1**	**3**	**1**	**0**	**0**	**1**	**0**	**0**	**3**	**0**	**2**

* Not including Gaps, PP, AN, or ER. ** Multiple aberrations can be observed within one cell N/A: Not Applicable

CODE FOR IDENTIFYING CHROMOSOMAL ABERRATIONS

TG = Chromatid Gap
SG = Chromosome Gap
CR = Complex Rearrangement
SB = Chromosome Break
ER = Endoreduplication
TR = Triradial
QR = Quadradial
TB = Chromatid Break
ICRA = Inter Chromosomal Rearrangement
AN = Aneuploidy
DM = Double Minute
DC = Dicentric Chromosome
PV = Pulverized
R = Ring
D = Deletion
PP = Polyploidy

Table 15. Chromosomal aberration confirmation assay.

Chromosomal Aberrations Results (Confirmation)

Treatment	Replicate	#Cells Analyzed	# Cells with Aberrations*	Specific Aberrations observed**														
				Gaps		Breaks			ICRA					Other				
				TG	SG	TB	SB	D	TR	QR	CR	R	DC	DM	PV	PP	ER	AN
Negative Control (MeOH)	A	150	1	1	0	0	2	0	0	0	0	0	0	0	0	2	0	0
	B	150	1	1	0	0	7	0	0	0	0	1	0	5	0	3	0	3
	Total	**300**	**2**	**2**	**0**	**0**	**9**	**0**	**0**	**0**	**0**	**1**	**0**	**5**	**0**	**5**	**0**	**3**
Negative Control (EtOH)	A	150	2	0	0	1	1	0	0	0	0	1	0	0	0	3	0	0
	B	150	2	0	0	0	0	0	0	0	0	1	1	0	0	2	0	2
	Total	**300**	**4**	**0**	**0**	**1**	**1**	**0**	**0**	**0**	**0**	**2**	**1**	**0**	**0**	**5**	**0**	**2**
Positive Control	A	100	10	2	1	6	0	2	3	2	0	0	0	0	0	1	0	0
	B	50	20	3	0	8	3	1	7	2	0	1	0	1	0	0	0	0
	Total	**150**	**30**	**5**	**1**	**14**	**3**	**3**	**10**	**4**	**0**	**1**	**0**	**1**	**0**	**1**	**0**	**0**

Reference ID: 4263670

Treatment	Replicate	# Cells Analyzed	# Cells with Aberrations*	Specific Aberrations observed**														
				Gaps		Breaks			ICRA					Other				
				TG	SG	TB	SB	D	TR	QR	CR	R	DC	DM	PV	PP	ER	AN
Test Article (1.66 mg/mL)	A	150	4	1	0	1	2	0	0	0	0	0	2	0	0	1	0	1
	B	150	7	3	0	3	1	0	1	0	0	0	3	1	0	2	0	1
	Total	**300**	**11**	**4**	**0**	**4**	**3**	**0**	**1**	**0**	**0**	**0**	**5**	**1**	**0**	**3**	**0**	**2**
Test Article (0.82 mg/mL)	A	150	3	1	0	0	3	0	0	0	0	0	1	0	0	1	0	1
	B	150	2	0	0	1	1	1	1	0	0	0	0	0	0	3	0	0
	Total	**300**	**5**	**1**	**0**	**1**	**4**	**1**	**1**	**0**	**0**	**0**	**1**	**0**	**0**	**4**	**0**	**1**
Test Article (0.44 mg/mL)	A	150	1	1	1	0	0	1	0	0	0	0	0	0	0	2	0	0
	B	150	0	1	1	0	0	0	0	0	0	0	0	0	0	2	0	0
	Total	**300**	**1**	**2**	**2**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**4**	**0**	**0**

* Not including Gaps, PP, AN, or ER ** Multiple aberrations can be observed within one cell N/A: Not Applicable

CODE FOR IDENTIFYING CHROMOSOMAL ABERRATIONS

TG = Chromatid Gap
SG = Chromosome Gap
CR = Complex Rearrangement
SB = Chromosome Break
ER = Endoreduplication
TR = Triradial
QR = Quadradial
TB = Chromatid Break
ICRA = Inter Chromosomal Rearrangement
AN = Aneuploidy
DM = Double Minute
DC = Dicentric Chromosome
PV = Pulverized
R = Ring
D = Deletion
PP = Polyploidy

A dose-dependent response in percentage of chromosomal aberrations was observed in the main activated assay. In the 3-hour test with metabolic activation, at 0.82 and 1.66 mg/mL, a statistically significant increase in the percentage of CHO cells with structural aberrations was observed when compared to the negative control.

Study title: Chromosomal Aberration Assay – OECD 473

Study no.: 16-01140-G2
Study report location: \\cdsesub1\evsprod\nda209575\0001\m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\16-01140-g2\16-01140-g2-pre-clinical-study-report.pdf
Conducting laboratory and location: (b) (4) [redacted]
Date of study initiation: February 15, 2017
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: [redacted] (b) (4), purity unreported

Key Study Findings

Although [redacted] (b) (4) appeared to increase the incidence of chromosomal aberrations over controls, the effects were not clearly concentration dependent and within the historical control range. As such, [redacted] (b) (4) was not clastogenic in cultured CHO cells in vitro. However, purity information on the test article was not provided and therefore, the study is not acceptable in its current form.

Reference ID: 4363670

Methods

Cell line:	Chinese Hamster Ovary (CHO)
Concentrations in definitive study:	Without S9 mix: 0.40, 0.83, and 1.7 mg/mL With S9 mix: 0.40, 0.83, and 1.7 mg/mL
Basis of concentration selection:	No preliminary dose range-finding study was performed to determine the concentration selection for the definitive studies.
Negative control:	60% methanol in water and 95% ethanol in saline
Positive control:	Mitomycin C (MMC): 0.075, mcg/mL (main assay, 3-h treatment in the absence of S9 mix); 0.5 mcg/mL (confirmation assay, 20-h treatment in the absence of S9 mix) Cyclophosphamide (CP): 7 mcg/mL (in the presence of S9 mix)
Formulation/Vehicle:	Solution/60 methanol:40 ethanol
Incubation & sampling time:	The CHO cells were incubated with or without metabolic activation in a cell culture incubator at 37 ±1°C, 5 ±1% CO_2 and in humidity atmosphere.

Treatment Condition	Treatment Time	Recovery Time	Drug Concentration
Non-activated (-S9)	3 to 6 hr	18 to 21 hr	0.40, 0.83, 1.70 mg/mL
	20 hr		0.40, 0.83, 1.70 mg/mL
Activated (S9)	3 hr to 6 hr	18 to 21 hr	0.40, 0.83, 1.70 mg/mL

Study Validity

The following abnormalities were recorded and considered for the calculation of the percentage of cell with aberrations: chromatid breaks, inter chromosomal rearrangement, deletion, triradial, quadradial, ring chromosome, double minute, pulverized chromosome, and dicentric chromosome. The chromosomal aberration test performed was considered acceptable as it met the following criteria:

- The aberrations percentage for positive control is statistically higher than that of the negative control.
- The aberration percentage for the positive and negative controls was within the historical control range for the vehicle controls.

However, purity information on the test article was not provided and therefore, the study is not acceptable in its current form.

Reference ID: 4263670

Results

Results are presented in the Sponsor's table below. There was evidence of (b) (4) r inducing a small increase in structural chromosomal aberrations in vitro in CHO cells. In the 3-hour main assay, (b) (4) induced, at 0.40, 0.83, and 1.7 mg/mL, a statistically significant increase in the percentage (2.7%) of cells with structural chromosomal aberrations in the absence of S9 metabolic activation compared to the negative control. However, the mean percentage of structural chromosomal aberration was judged to be negative because the percentage was within the historical negative control range (mean of 2.2; 95% confidence interval limits 1.7 – 2.7). Furthermore, the small changes were not dose-dependent and likely achieved statistical significance due to the low aberration % in the negative control groups, which were lower than historical control levels. (b) (4) did not induce a statistically significant increase in the percentage of CHO cells with structural chromosomal aberration in the presence of metabolic activation (main assay) nor in the confirmation assay after prolong exposure.

Table 16. Summary of results from chromosomal aberration assay with (b) (4)

Summary of Results

Assay	Article	Exposure Concentrations	Cytotoxicity (%)*	Chrom. Ab. (%)	X^2 p-value
Non-Activated (Main)	Negative Control (MeOH)	0.6%	0	0.3	N/A
	Negative Control (EtOH)	1%	0	0.3	1.000
	Positive Control (MMC)	0.05 µg/mL	28	28.0	0.000
	Test Article	1.70 mg/mL	0	2.7	0.019
	Test Article	0.83 mg/mL	0	2.7	0.019
	Test Article	0.40 mg/mL	26	2.7	0.019
Activated (Main)	Negative Control (MeOH)	0.6%	0	1.3	N/A
	Negative Control (EtOH)	1%	24	1.3	1.000
	Positive Control (CP)	7 µg/mL	30	34.0	0.000
	Test Article	1.70 mg/mL	24	3.3	0.105
	Test Article	0.83 mg/mL	13	3.3	0.105
	Test Article	0.40 mg/mL	0	2.3	0.361
Non-Activated (Conf.)	Negative Control (MeOH)	0.6%	0	0.7	N/A
	Negative Control (EtOH)	1%	7	1.3	0.412
	Positive Control (MMC)	0.075 µg/mL	17	20.0	0.000
	Test Article	1.70 mg/mL	30	1.0	0.653
	Test Article	0.83 mg/mL	21	2.0	0.155
	Test Article	0.40 mg/mL	0	1.7	0.254

Conf.: Confirmation Chrom. Ab.: Chromosomal Aberration
* Cytotoxicity = reduction in percentage Relative Population Doubling of respective negative control (MeOH)
N/A = Not Applicable

Reference ID: 4263670

Table 17. Chromosomal aberration main assay.

Chromosomal Aberrations Results (Non–Activated Assay)

Treatment	Replicate	#Cells Analyzed	# Cells with Aberrations*	Specific Aberrations observed**														
				Gaps		Breaks			ICRA					Other				
				TG	SG	TB	SB	D	TR	QR	CR	R	DC	DM	PV	PP	ER	AN
Negative Control (MeOH)	A	150	1	1	0	0	0	1	0	0	0	0	1	0	0	2	0	0
	B	150	0	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0
	Total	**300**	**1**	**3**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**4**	**0**	**0**
Negative Control (EtOH)	A	150	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
	B	150	0	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0
	Total	**300**	**1**	**2**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**0**	**0**
Positive Control	A	75	13	2	2	10	1	0	3	1	0	0	1	0	0	0	0	0
	B	50	22	2	0	19	0	4	8	2	1	1	1	3	0	0	0	0
	Total	**125**	**35**	**4**	**2**	**29**	**1**	**4**	**11**	**3**	**1**	**1**	**2**	**3**	**0**	**0**	**0**	**0**

Treatment	Replicate	#Cells Analyzed	# Cells with Aberrations*	Specific Aberrations observed**														
				Gaps		Breaks			ICRA					Other				
				TG	SG	TB	SB	D	TR	QR	CR	R	DC	DM	PV	PP	ER	AN
Test Article (1.70 mg/mL)	A	150	4	1	0	1	1	0	0	0	0	0	1	1	0	3	0	0
	B	150	4	2	0	1	3	1	0	0	0	1	2	0	0	3	0	2
	Total	**300**	**8**	**3**	**0**	**2**	**4**	**1**	**0**	**0**	**0**	**1**	**3**	**1**	**0**	**6**	**0**	**2**
Test Article (0.83 mg/mL)	A	150	6	1	0	1	0	0	0	0	0	2	2	1	0	4	0	2
	B	150	2	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0
	Total	**300**	**8**	**1**	**1**	**3**	**0**	**0**	**0**	**0**	**0**	**2**	**2**	**1**	**0**	**4**	**0**	**2**
Test Article (0.40 mg/mL)	A	150	3	2	0	1	0	0	0	0	0	0	1	1	0	4	0	0
	B	150	5	0	0	0	0	2	0	0	0	0	4	1	0	4	0	2
	Total	**300**	**8**	**2**	**0**	**1**	**0**	**2**	**0**	**0**	**0**	**0**	**5**	**2**	**0**	**8**	**0**	**2**

* Not including Gaps, PP, AN, or ER. ** Multiple aberrations can be observed within one cell N/A: Not Applicable

CODE FOR IDENTIFYING CHROMOSOMAL ABERRATIONS

TG = Chromatid Gap
SG = Chromosome Gap
CR = Complex Rearrangement
SB = Chromosome Break
ER = Endoreduplication
TR = Triradial
QR = Quadradial
TB = Chromatid Break
ICRA = Inter Chromosomal Rearrangement
AN = Aneuploidy
DM = Double Minute
DC = Dicentric Chromosome
PV = Pulverized
R = Ring
D = Deletion
PP = Polyploidy

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

Study title: Rodent Bone Marrow Micronucleus Assay-OECD 474 Cocaine

Study no: 11-4138-G3
Study report location: \\cdsesub1\evsprod\nda209575\0001\m4\42-stud-rep\423-tox\4233-genotox\42332-in-vivo\11-4138-g3\11-4138-g3-pre-clinical-study-report.pdf
Conducting laboratory and location: (b) (4) [redacted]
Date of study initiation: March 28, 2012
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Cocaine HCl, Lot 11330001, 100%

Key Study Findings

Under the conditions of the assay, cocaine hydrochloride was negative for the induction of micronucleus polychromatic erythrocytes in the bone marrow of mice and there was no evidence the drug was clastogenic or damaged the mitotic apparatus.

Methods

Doses in definitive study: 24.69, 74.07, and 222.22 mg/kg
Frequency of dosing: Once for 1 day
Route of administration: Oral gavage
Dose volume: 20 mL/kg
Formulation/Vehicle: Solution/sterile water for injection
Species/Strain: Mice/Swiss Albino (*Mus musculus*), animals were at least 6-weeks old, weighing between 23.0-35.0 g at time of dosing
Number/Sex/Group:

Group	Number of Animals	Timepoint
Test Substance (per dose)	10 (5 males and 5 females)	24 hours
	10 (5 males and 5 females)	48 hours
Negative Control	10 (5 males and 5 females)	24 hours
	10 (5 males and 5 females)	48 hours
Positive Control	10 (5 males and 5 females)	24 hours

Satellite groups: None
Basis of dose selection: Doses selection was based upon a dose range-finding assay in which cocaine hydrochloride was evaluated at 24.69, 74.07, 222.22, 666.66, and 2000 mg/kg. Test article dose was administered as a single dose by oral route. The highest dose of 222.22 was selected as the MTD based on observed mortality. 100% mortality was observed in the 2000 and 666.6 mg/kg groups; 60% (3/5 animals) mortality was observed in the 222.2 mg/kg group. Clinical signs of toxicity nor significant bone marrow toxicity were not observed in the animals in the

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24.69 and 74.07 mg/kg groups.
Negative control: Sterile water for injection
Positive control: Cyclophosphamide, 25 mcg/kg in water

Study Validity

The micronucleus assay performed was considered acceptable as if met the following criteria:

- The number of micronucleated polychromatic erythrocytes for positive and negative controls were within the historical control range.
- The frequency of micronucleated PCEs in the positive control group was statistically significant greater than the negative control group.

Results

No cocaine-related mortalities were observed at 24.69 mg/kg. Mortalities were observed in the 74.07 (1/5 male) and 222.22 (5/5 males, 4/5 females) mg/kg groups. No adverse clinical signs were attributed to cocaine treatment.

Analysis of the bone marrow cells collected at 24 and 48 hours after treatment is presented in the Sponsor's tables below. As expected, cyclophosphamide at a dose of 25 mcg/kg significantly increased the frequency of micronucleated cells and polychromatic erythrocyte (MNC:PCE ratio) compared to negative control. There was no treatment-related increase in the percent micronuclei polychromatic erythrocyte: polychromatic erythrocyte ratio which demonstrated that cocaine did not induced micronuclei in the bone marrow cells of male and female mice.

Table 18. Ratio of Polychromatic to Normochromatic Erythrocytes at 24 hours.

Test Article	Dose (mg/kg)	Number of Animals	Sacrifice Time (hrs)	MNC/PCE Erythrocytes (Mean ± SD)	
				M	F
WFI	0	5M, 5F	24	0.0020 ± 0.0008	0.0017 ± 0.0007
Cocaine HCl*	74.07	5M, 5F	24	0.0021 ± 0.0006	0.0013 ± 0.0014
	24.69	5M, 5F	24	0.0021 ± 0.0007	0.0014 ± 0.0013
CP	0.025	5M, 5F	24	0.0151 ± 0.0016	0.0126 ± 0.0025

*Mortality at highest dose (222 mg/Kg) precluded evaluation, so only two dose levels are shown.
Positive Control: CP = Cyclophosphamide. PCE = Polychromatic erythrocytes. MNC = Micronucleated Cells

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Study title: Evaluation of Micronuclei in B6C3F1 Mice and DNA Damage In Sprague Dawley Rats Administered [redacted] (b) (4).

Study no:	54873.0001
Study report location:	\\cdsesub1\evsprod\nda209575\0001\m4\42-stud-rep\423-tox\4233-genotox\42332-in-vivo\54873-0001\54873-0001-pre-clinical-study-report.pdf
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	March 30, 2017
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	[redacted] (b) (4), 99.98%

Key Study Findings

Under the study conditions, [redacted] (b) (4) did not produce a statistically significant increase in the mean % micronucleated polychromatic erythrocytes; and the mean % tail DNA or tail moment measured in blood or liver samples obtained from animals administered [redacted] (b) (4) via intravenous injection for two consecutive days when compared to control. These data demonstrated that [redacted] (b) (4) when tested at the maximum tolerated dose (MTD) was negative for the induction of micronuclei in blood and the induction of DNA damage in the liver and blood mice and rats, respectively. Thus, [redacted] (b) (4) tested negative in the in vivo micronucleus assay and Comet assay.

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Methods

Doses in definitive study:	0, 125, 250 and 500 mg/kg/day for both micronucleus and Comet assay
Frequency of dosing:	Two consecutive days
Route of administration:	Test Article: Intravenous; EMS: Oral
Dose volume:	Test Article: 5 mL/kg; EMS: 10 mL/kg
Formulation/Vehicle:	Test article was provided as a white crystalline powder that was dissolved in a vehicle consisting of sterile buffered water.
Species/Strain:	Mice/B6C3F1 Rats/Hsd Sprague Dawley
Number/Sex/Group:	Mice: 30/male/group (Micronucleus assay) Rats: 30/female/group (Comet assay)
Satellite groups:	None
Basis of dose selection:	**Micronucleus Assay** A dose-finding study was conducted in mice (male and female) administered 400, 500, 625, and 750 mg/kg/day for two consecutive days. Moribundity and mortality was observed in both genders at 625 and 750 mg/kg/day. "Due to the observed greater sensitivity to the test article, male mice were chosen for the definitive study." Based on mortality and transient adverse signs observed, the maximum tolerated dose for the definitive micronucleus assay was set at 400 to 500 mg/kg/day. **Comet Assay** A dose-finding study was conducted in rats (male and female) administered 400, 500, 625, 750, and 1000 mg/kg/day for two consecutive days. Moribundity and mortality was observed in females at 625 to 1000 mg/kg/day. Males survived up to and including 750 mg/kg/day. "Due to the observed greater sensitivity to the test article, female rats were chosen for the definitive study." Based on moribundity and mortality observed, the maximum tolerated dose for the definitive comet assay was set at 500 mg/kg/day.
Negative control:	Saline
Positive control:	Ethyl methanesulfonate (EMS)

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Incubation and sampling times

Micronucleus Assay. Mice were administered for two consecutive days with either vehicle control or test article via intravenous injection or received EMS (positive control) via oral gavage. Approximately 47 hours (± 30 minutes) following the second dose, the animals were euthanized. Immediately following the scheduled euthanasia, blood was collected for micronuclei frequency assessment.

Comet Assay. Rats received for two consecutive days either intravenous injection of vehicle control or test article or EMS via oral gavage. Approximately 3 hours (± 30 minutes) following the second dose, the animals were euthanized. Immediately following the scheduled euthanasia

Study Validity

The assay acceptance criteria:

1. Acceptable Controls
   - The vehicle control group MN-PCE and % tail DNA data from the Comet assay was within the historical control range. Concurrent negative controls should be within the 95% confidence interval of the distribution of the historical negative control data.

   - "EMS should induce an increase in the frequency of micronucleated PCE and an increase in % tail DNA at $p < 0.05$ versus negative controls."

2. Acceptable High Dose
   - "…the highest test article concentration evaluated for MN and DNA damage induction must, unless precluded by the use of the limit dose of 2000 mg/kg, induce bone marrow or animal toxicity or be a dose only slightly lower than that which would be expected to induce mortality."

   - The positive control group mean was elevated in a statistically significant manner relative to that of the vehicle control group and the positive control response was consistent with the historical positive control data.

Assay Evaluation Criteria

1. **Micronucleus Assay**
   - The criteria for a positive result was based on the observation of a statistically significant positive response in micronucleated PCEs and PCE for at least one dose, as well as a statistically significant dose-related response; otherwise, the result was deemed negative.

2. **Comet Assay**

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- The criteria for a positive result was based on the observation of a statistically significant positive response in the % Tail endpoint for at least one dose and/or a statistically significant dose-response; otherwise, the result was deemed negative.

Results

Mortality. All animals survived to schedule termination.

Clinical Signs. Immediately following dosing, transient observations of ataxia and apnea was observed in both controls and test article treated animals. One hour post dosing, all animals appeared normal.

Micronucleus Assay. As depicted in the Applicant's table below, (b) (4) in treated animals did not produce a statistically significant increase in the mean % PCE and mean frequency of MN-PCE in male mice when compared to control animals. The MN-PCE results for the vehicle control and all doses of (b) (4) were within the historical control 95% confidence intervals. The positive control EMS produce a statistically significant increase in the mean micronucleated polychromatic erythrocytes. No evidence of bone marrow toxicity was observed in (b) (4) treated animals.

Table 5. Summary of MN Frequency in Male Mice Administered (b) (4)

Dose Level (mg/kg/day)	Mean % PCE ± SEM	Mean MN-PCE/1000 ± SEM
0	1.51 ± 0.03	2.60 ± 0.25
125	1.56 ± 0.06	2.91 ± 0.25
250	1.68 ± 0.11	2.62 ± 0.18
500	1.61 ± 0.07	2.39 ± 0.16
EMS-200	1.19 ± 0.12	**10.6 ± 1.08***

Abbreviations: SEM - standard error of the mean; mg = milligram; kg = kilogram;
PCE = Polychromatic erythrocytes; MN-PCE = Micronucleated polychromatic erythrocytes
*Statistically significant compared to the concurrent control mean (*t*-test)

Comet Assay. Under the conditions of the Comet assay, no increase in DNA damage was observed. Relative to the vehicle control, no statistically significant increase in mean % tail DNA (% DNA fragments present in the tail) or tail moment was observed in liver and blood tissue collected from female rats (see Applicant's table below) in any of the ecgonine hydrochloride groups. The % tail DNA results for the vehicle control and all doses of ecgonine hydrochloride were within the historical control 95% confidence intervals. The positive control EMS produced the expected response. Compared to the

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vehicle control group, EMS induced a statistically significant increase in % tail DNA and tail moment in liver and blood. No cytotoxicity was observed as the percent hedgehog cells were similar across dosing groups and when compared to the vehicle control group.

Summary Comet data for blood

Dose Level (mg/kg/d)	Total no. cells scored	% Tail DNA		Olive Tail Moment		% Hedgehogs
		Mean	SEM	Mean	SEM	
EMS (150)	150	8.26	0.22	1.10	0.04	1.9
0	150	0.15	0.04	0.01	0.00	0.4
125	150	0.09	0.02	0.00	0.00	0.0
250	150	0.08	0.01	0.00	0.00	0.0
500	150	0.13	0.02	0.01	0.00	0.1

Summary Comet data for liver

Dose Level (mg/kg/d)	Total no. cells scored	% Tail DNA		Olive Tail Moment		% Hedgehogs
		Mean	SEM	Mean	SEM	
EMS (150)	150	14.43	0.48	1.76	0.05	0.9
0	150	5.58	0.65	0.45	0.04	2.0
125	150	5.48	0.91	0.52	0.10	1.7
250	150	5.97	0.51	0.48	0.04	2.0
500	150	5.85	0.43	0.46	0.04	1.9

7.4 Other Genetic Toxicity Studies

The Applicant submitted a publication that described results of a genotoxicity assay conducted with cocaine, which is reviewed below. Although not discussed by the Applicant, Salvadori et al, demonstrated that cocaine produced a small increase in micronucleated polychromatic erythrocytes, suggestive of clastogenic activity. However, as this study is not GLP and the source and impurity profile of the cocaine solution is not adequately described, and we have a GLP study to inform labeling, the data reported from this publication will not be included in labeling.

Study title: (Salvadori et al., 1998) Cocaine Mutagenicity and Hepatocarcinogenicity Evaluations in Rodents.

GLP compliance: No
QA statement: No
Drug, lot #, and % purity: Cocaine, seized lot by Brazilian Drug

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Enforcement Police, 99% pure

Key Study Findings

Cocaine did not result in a statistically significant increase in micronucleated polychromatic erythrocytes (MNPCE) compared to negative controls; however, there was a positive trend toward an increase in MNPCE.

Methods

Doses in definitive study:	0, 2, 4, and 8 mg/kg, IV 0, 18, 37, and 75 mg/kg IP
Frequency of dosing:	Single dose, sacrificed at 24 or 48 hours post dose
Route of administration:	IV and IP
Dose volume:	Not reported
Formulation/Vehicle:	Cocaine was diluted in saline
Species/Strain:	Male Swiss mice
Number/Sex/Group:	10 males/group
Basis of dose selection:	Doses were described as being 12.5, 25, and 50% of the cocaine LD_{50} for each route based on literature.
Negative control:	Saline
Positive control:	Cyclophosphamide, 50 mg/kg, IP

Study Validity

The high dose selected did appear to result in convulsions in some animals, therefore, the doses appear to have reached the maximum tolerated dose. The positive control was clearly positive. The study appears to be have employed an adequate number of animals and counted an adequate number of cells. The statistical analysis used a Chi Square test. Historical control data were not provided.

Results

The ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) in the bone marrow following IP and IV administration of cocaine are presented in the figures below, reproduced from the submission. According to the publication, the IP cocaine treated groups were not statistically different from the negative controls, although they report a significant trend for increased PCE at both sampling times. Likewise, in the IV cocaine studies, there was not a significant difference between cocaine treatment and the negative controls, but a trend was noted at the 24-hour time point.

Reference ID: 4263670

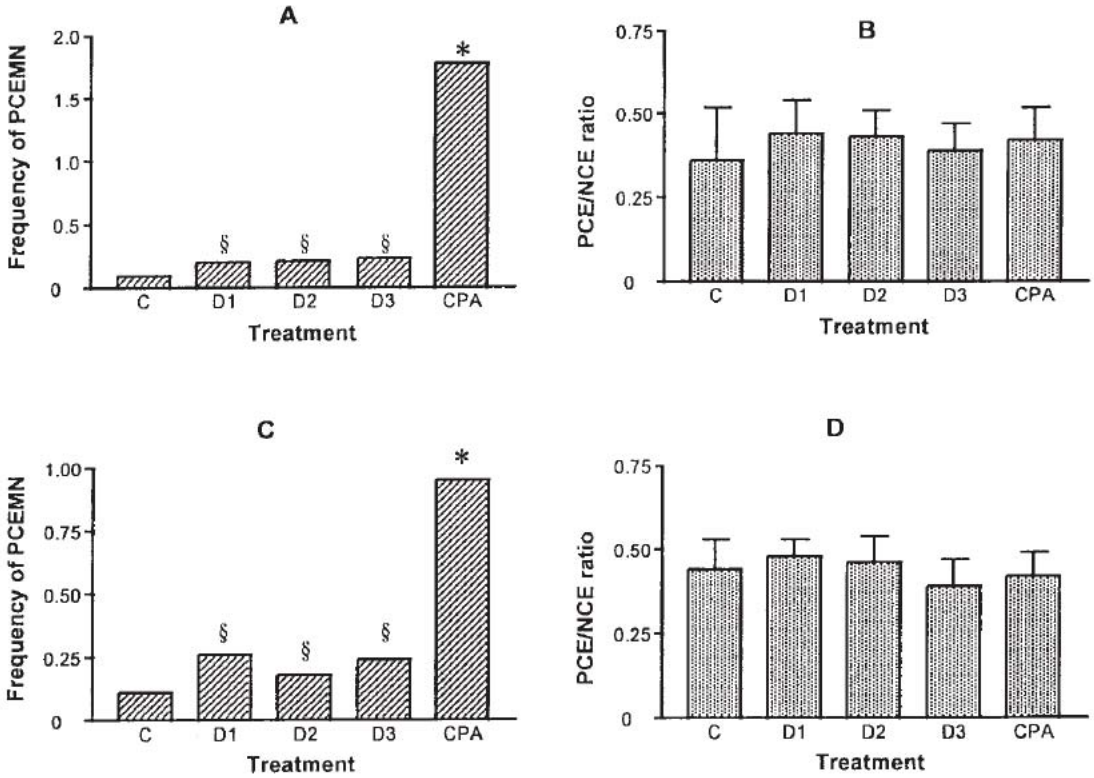

Fig. 1. Frequencies of PCENM (%) and PCE/NCE ratio (means ± SD) in mouse bone marrow cells 24 **(A,B)** and 48 hours **(C,D)** after i.p. treatment with cocaine. C, NaCl 0.9%; D1, D2, D3, cocaine 18, 37, and 75 mg/kg, respectively; CPA, cyclophosphamide (50 mg/kg). *, $P < .01$; §, $.05 < P < .10$.

Reference ID: 4263670

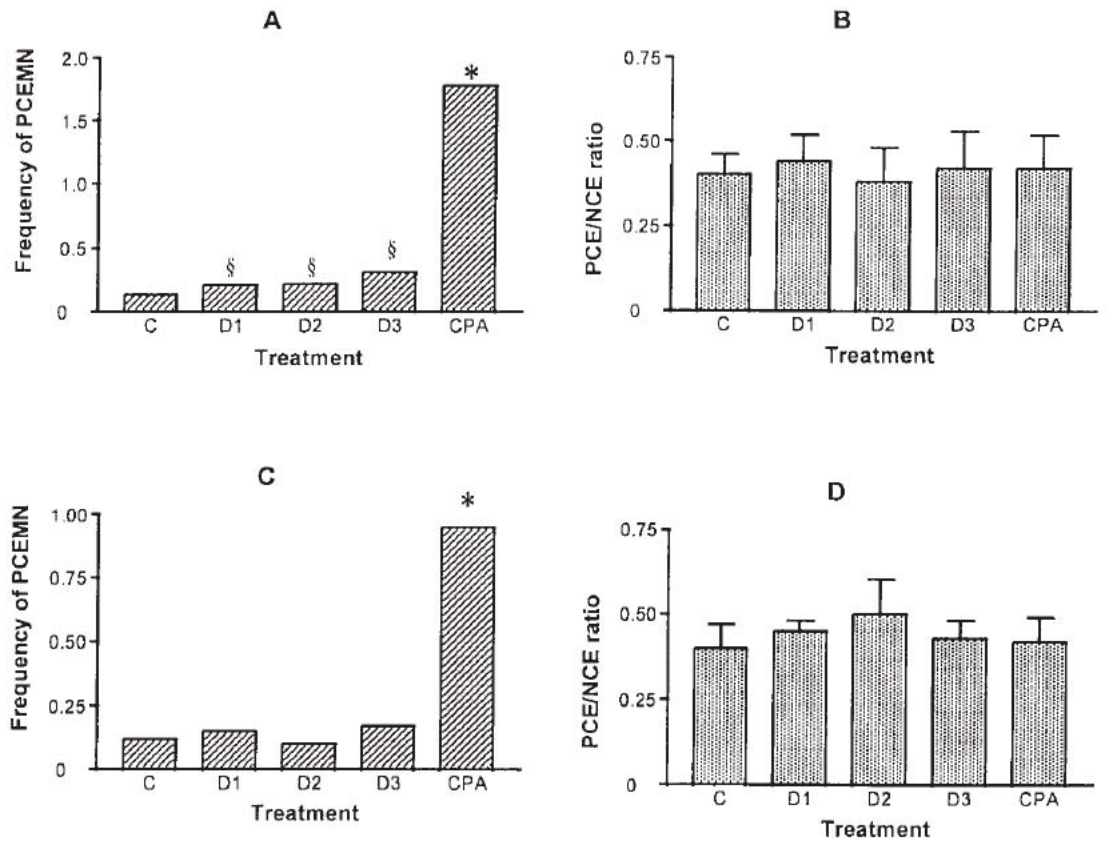

Fig. 2. Frequencies of PCENM (%) and PCE/NCE ratio (means ± SD) in mouse bone marrow cells 24 **(A,B)** and 48 hours **(C,D)** after i.v. treatment with cocaine. C, NaCl 0.9%; D1, D2, D3, cocaine 2, 4, and 8 mg/kg, respectively; CPA, cyclophosphamide (50 mg/kg); *, $P < .01$; §, $.05 < P < .10$.

8 Carcinogenicity

Carcinogenicity studies were not required to support the safety of cocaine hydrochloride topical solution since the product will be indicated for acute use. Long-term studies evaluating the carcinogenic potential of cocaine have not been conducted. In their literature review, the Applicant identified three published studies potentially relevant to the carcinogenic risk assessment of cocaine.

Salvadori et al. (1988) examined the effect of 6-week treatment with cocaine (5 or 10 mg/kg IV twice a week) on diethylnitrosamine promoted tumor growth. Although no effects on tumor promotion were noted (Salvadori et al., 1998), this study is not an adequate carcinogenicity study as it does not expose the animals to an adequate duration of treatment and should not be included in product labeling.

Rosenkranz and Klopman report that use of quantitative structure activity evaluations suggest that cocaine may have carcinogenic potential (Rosenkranz and Klopman, 1990); however, the Agency does not consider this approach to be adequately validated.

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Friere-Garbal et al. report that cocaine could function as a tumor promotor in mice (Fiere-Garbal et al., 1994). However, this study is also not an adequate carcinogenicity study as it does not expose the animals to an adequate duration of treatment and should not be included in product labeling.

The Applicant has not proposed to include results from these studies in their labeling. We concur.

9 Reproductive and Developmental Toxicology

(b) (4)

the submitted nonclinical information provided from the publish literature does not adequately address the endpoints for fertility and early development, embryo-fetal development and pre- and post-natal development but does provide some data and information to inform labeling. Therefore, the standard battery of reproductive and developmental toxicology studies will be recommended as a postmarketing requirement.

9.1 Fertility and Early Embryonic Development

Standard fertility and early embryonic development studies have not been conducted in animals with cocaine to understand the effects of cocaine on male and female reproductive functions (i.e., development and maturation of gametes, mating behavior and fertilization). Male reproductive toxicity studies should evaluate the potential of a drug to damage reproductive organs, gamete maturation and release, reduction in sperm count, alteration in sperm motility or morphology, but also evaluate functional effects such as libido/mating behavior. Female reproductive toxicity studies should evaluate the impact of a drug on the estrus cycle, tubal transport, implantation, development of preimplantation states of the embryo.

The Applicant summarized the existing literature to address these endpoints of male and female fertility. This Reviewer grouped the literature references into fertility and reproduction effects, hormonal effects, and testicular effects.

Fertility and Reproduction

Several published reports in the literature have studied the effects of cocaine on some endpoints typically covered by standard fertility studies. The table below summarizes the literature references supplied by the Applicant. Comments on their utility with respect to typical fertility and early embryonic development study endpoints are provided.

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Table 19. Effects of Cocaine on Female Fertility Endpoints

Effect Reported	Species	Treatment Period	Dose	HED (per 60 kg person)	Estimated Exposure Margin for % Cocaine Topical Solution Based on BSA‡		Citation	Comment
					4%	10%		
Cocaine delayed first estrus but did not impact fertility rate	Female Mice	PND 22 – 35 prior to mating	20 mg/kg BID SC (40 mg/kg/day) NOAEL not defined	195 mg/day	5.2x	1.5x	(Chen and Vandenbergh, 1994)	Really a juvenile animal study. Only includes fertility endpoint, not early embryonic development
Disrupted cyclicity and reduced ovulation and serum LH. Effect statistically significant at 10 and 20 mg/kg; trending at 5 mg/kg.	Female Rat	Several weeks; no mating	1 5 10 20 mg/kg SC (4 weeks)	9.7 mg 48 mg 97 mg 194 mg	0.26x 1.3x 2.6x 5.2x	0.073x 0.36x 0.73x 1.5x	(King et al., 1993)	No mating, not a fertility and early embryonic development study

‡: Referenced MDD is 37.5 mg/day for the 4% solution; mean of 23.44% of the total administered 160 mg dose is absorbed.
Referenced MDD is (b) (4) for the 10% solution; mean of 33.34% of the total administered 400 mg dose is absorbed.

As noted in the table above, none of the submitted studies included appropriate dosing intervals or endpoints to be considered adequate to address the fertility and early embryonic development requirements for cocaine.

Hormonal Effects

In terms of effects on female reproductive hormones, published studies suggest that cocaine can result in transient changes in progesterone and alterations in menstrual cyclicity.

Table 20. Effects on Cocaine on Female Reproductive Hormones

Effect Reported	Species	Dose	HED (per 60 kg person)	Estimated Exposure Margin for % Cocaine Topical Solution Based on BSA‡	Citation

Reference ID: 4363670

				4%	10%	
Reduced serum progesterone though not entirely dose dependent, follicular fluid progesterone decreased dose dependently at all doses.	Rabbits	0 10 20 40 80 mg/kg SC (5 days)	 194 mg 387 mg 774 mg 1548 mg	 5.2x 10.3x 20.6x 41.3x	 1.5x 2.9x 5.8x 11.6x	(Kaufmann et al., 1990)
Reduced estradiol levels and altered menstrual cyclicity and folliculogenesis	Monkey	0 or 4 mg/kg IV (13 days)	77 mg	2.1x	0.58x	(Potter et al., 1998)

‡: Referenced MDD is 37.5 mg/day for the 4% solution; mean of 23.44% of the total administered 160 mg dose is absorbed.
Referenced MDD is (b) (4) for the 10% solution; mean of 33.34% of the total administered 400 mg dose is absorbed.

Testicular Effects

The Applicant's literature search identified numerous articles examining the impact of cocaine administration on testes. These are summarized in the table below with more detailed analysis of several key studies presented thereafter.

Table 21. Effects of Cocaine on Male Fertility Endpoints

Effect Reported	Species	Dosing Regimen	HED (per 60 kg person)	Estimated Exposure Margin for % Cocaine Topical Solution Based on BSA‡		Citation	Comment
				4%	10%		
No change in sexual behavior (e.g., latency to first intromission, number of intromissions until ejaculation, latency to ejaculation) or fertility. No histopathological	Long Evans Rats (114-117 days old = ~ 16 weeks old) Allowed to mate with untreated females	15 or 30 mg/kg, SC (72 days)	145 mg 290 mg	3.9x 7.8x	1.1x 2.2x	(Abel et al., 1989)	Duration of treatment is appropriate; included fertility, sperm motility studies, testes histopathology data. Even have assessment of offspring for potential paternally mediated effects. A reasonable male fertility study. Rats older than typically

Reference ID: 4363670

Effect Reported	Species	Dosing Regimen	HED (per 60 kg person)	Estimated Exposure Margin for % Cocaine Topical Solution Based on BSA‡		Citation	Comment
				4%	10%		
changes to testes. Increased abnormal sperm morphology at 30 mg/kg. Increased hyperactivity of offspring.							used for fertility studies.
Necrosis of interstitial cells and seminiferous tubules at Day 15, progressed to Day 90. No histopathology at Day 7 reported.	Wistar Rats ("adult") (based on body weight reported, estimated to be ~6 weeks old)	30 mg/kg, IP (sacrificed on Days 7, 15, 30, 45, 60, 75, and 90)	290 mg	7.8x	2.2x	(Barroso-Moguel et al., 1994)	No NOAEL identified, suggests repeated exposure necessary to histopathological changes.
Reduced pregnancy rates; reduced birth weights of pups; reduced diameter of seminiferous tubules, reduced thickness of germinal epithelial cells, reduced Step VII spermatids	Sprague Dawley rats (30 days old)	15 mg/kg SC (Daily for 100 days & twice weekly for 150 days prior to mating)	145 mg	3.9x	1.1x	(George et al., 1996)	Duration appropriate, endpoints for male fertility reasonable. See below. This study seems adequate to substitute for a male fertility study. No NOAEL identified.
Reduced seminiferous tubule diameter and thickness of germinal epithelium, increased degeneration of germ cells, failure to release mature	Rats (25 days old)	15 mg/kg SC (100 days)	145 mg	3.9x	1.1x	(Li et al., 1997)	Assessed morphology of testes, appropriate duration of study. No NOAEL identified.

Reference ID: 4363670

Effect Reported	Species	Dosing Regimen	HED (per 60 kg person)	Estimated Exposure Margin for % Cocaine Topical Solution Based on BSA‡		Citation	Comment
				4%	10%		
spermatids							
Cocaine reduced testicular blood flow within 15 minutes and the effect lasted up to 60 minutes.	Sprague Dawley Rats (25 days old)	30 mg/kg, SC (single dose)	290 mg	7.8x	2.2x	(Li et al., 1999)	Not a fertility study. Measured testicular blood flow. Perhaps suggesting mechanism contributing to cocaine induced testicular toxicity. No NOAEL.

‡: Referenced MDD is 37.5 mg/day for the 4% solution; mean of 23.44% of the total administered 160 mg dose is absorbed.
Referenced MDD is (b) (4) for the 10% solution; mean of 33.34% of the total administered 400 mg dose is absorbed.

Collectively, the published literature on the effects of cocaine in adult male rats is not entirely consistent. Abel et al. report no adverse effects of adult males treated for 72 days via the SC route at doses up to 7.8 times the maximum daily dose based on body surface area. In contrast, Barroso-Moguel et al. (1994) report necrosis of male reproductive tissues after 7 days of this dose given IP. In theory, it is possible that IP could result in higher C_{max} values compared to SC, there could be strain differences, or the Wistar rats tested by Barroso-Moguel may have been younger than those tested by Abel.

The literature does report consistent adverse effects in peripubertal rats (George et al., 1996, Li et al., 1997). Specifically, in peripubertal male rats treated with cocaine, morphometric analysis of the testes showed a decrease in mean diameter of seminiferous tubules in peripubertal male rats administered cocaine daily for 100 days and twice weekly for 150 days. Similarly, the thickness of the germinal epithelium was decreased in peripubertal males administered cocaine daily for 100 days and twice weekly for 150 days. Cocaine also had significant effects on spermatogenesis in peripubertal rats; the number of spermatids was reduced (Step VII).

Abel et al., 1989

Abel and colleagues (1989) examined the effects of cocaine hydrochloride on reproductive function and sexual behavior of adult male rats and their offspring. Three groups of Long-Evan male rats were subcutaneously administered cocaine at doses of 0, 15, or 30 mg/kg for a minimum of 72 days. Rats in the 0 and 15 mg/kg groups were pair-fed with animals receiving the 30 mg/kg dose to account for reduced food intake. A

Reference ID: 4363670

fourth group served as a non-treated ad libitum-fed control. Male endpoints evaluations included: hormones (estradiol, LH, FSH, and T level), sexual behavior, fertility, sperm test (conducted 6 hours after the sexual behavior test), testis weight and histology, and effects on offspring. Offspring endpoint evaluation included: birth weight, litter size, postnatal mortality, weight at age Day 21, behavior (activity at 16 days of age, passive avoidance at 17 day of age, spontaneous alternation (T-maze test at 33 to 35 days of age, and sperm morphology at 58 days of age. Body weight and estradiol level and T/estradiol ratio were decreased in a dose-dependent manner by the subcutaneous administration of cocaine to male rats. Cocaine administration increased locomotor activity in a dose-dependent manner. Results from this study suggested that cocaine had no effects on male reproductive function, sexual behavior, or fertility. As depicted in the tables below reproduced from the publication, cocaine did affect sexual behavior or fertility as measured by the number of days to impregnate 2 females. Relative to control, a slight decrease in seminal vesicle (7.1%) weight was observed in the HD males (Table 2 below, page 8). An increase in the percentage of spermatozoa with heads separated from the tail was associated with the high dose of cocaine. Cocaine also did not affect weights of the testis or accessory organs, histology of the testis, number of implantations, resorptions, fetal or newborn weight of offspring, or offspring weight at 21 days of age. Cocaine had no effect on the behavior of the offspring as measured in the passive avoidance and T-maze models.

TABLE 1. Effects of Cocaine on Sexual Behavior of Male Rats*

	Dose			
	30 mg/kg N = 24	15 mg/kg N = 26	0 mg/kg N = 15	*Ad Libitum* N = 15
Latency to Intromission (sec)	90 ± 22	99 ± 23	119 ± 30	150 ± 32
Number of mounts	16 ± 3	11 ± 1	13 ± 1	15 ± 2
Latency to Ejaculate (sec)	895 ± 148	655 ± 97	849 ± 179	1206 ± 195
Post Ejaculation (sec)	419 ± 24	436 ± 24	472 ± 34	453 ± 46
Period				
Days to first plug	3.0 ± 0.6	3.3 ± 0.4	3.8 ± 0.6	†
Days to second plug	3.9 ± 0.8	3.5 ± 0.6	5.2 ± 1.3	†

*Mean ± SEM.
†Not recorded.

TABLE 2. Effects of Cocaine on the Testis and Seminal Vesicles of Rats*

	Dose			
	30 mg/kg N = 5	15 mg/kg N = 6	0 mg/kg N = 5	*Ad Libitum* N = 6
Body wt (g)	465 ± 8	524 ± 10	538 ± 21	603 ± 9
Testes (g)	3.8 ± 2.2	3.5 ± 0.1	4.1 ± 0.2	3.8 ± 0.2
Seminal vesicles (g)	1.5 ± 0.2	2.0 ± 0.1	2.1 ± 0.1	1.9 ± 0.1
Relative testis wt	8 ± 0	7 ± 0	8 ± 1	6 ± 0
Relative seminal vesicle wt†	3 ± 0	4 ± 0	4 ± 0	3 ± 0

*Mean ± SEM.
†(Organ wt/body wt) × 1000.

George et al., 1996

Reference ID: 4263670

The effects of chronic administration of cocaine on spermatogenesis and fertility were evaluated in prepubertal male Sprague-Dawley rats (George et al., 1996). Four groups of male rats (25 day of age) were subcutaneously administered a single dose of cocaine (0 or 15 mg/kg) either daily or twice weekly (Saturday and Sunday) for 100 days. Male's endpoints evaluation included: sexual behavior, fertility, sperm test (conducted 6 hours after the sexual behavior test), testis and epididymides weight and histology, hormonal analysis (follicle stimulating hormone, luteinizing hormone, and serum testosterone levels), histological examination of the testes (quantitative and qualitative morphologic assessment of the seminiferous tubules to assess the effect on the various stages of spermatogenesis), Sertoli cell count, and spermatid count. Pregnancy weight and birth weights were evaluated. Offspring endpoint evaluation included: birth weight and weight at 10 days of age.

Results from the study suggested that cocaine adversely affected male fertility after chronic administration. Compared to the control (86%) group ($p<0.05$), only 33% (3/9) of the female in the daily cocaine group became pregnant after 10 days of mating. After 20 days of mating, the pregnancy rate in the daily cocaine group was 50% compared to a pregnancy rate of 100% in the control group ($p<0.05$). Cocaine treatment on weekends only had no effect on fertility; pregnancy rate in the weekend cocaine group was not significantly different from the control group after 10 days (100% vs 100%) of dosing and 20 days of dosing (100% vs 86%). The birth weights of offspring from the group receiving daily cocaine were 12% less than that of controls ($p < 0.05$), but by 10 days there was no difference between groups. The weight of the testis and epididymis was not affected by cocaine exposure. There was no significant difference in the levels of testosterone, FSH, or LH among the cocaine treated groups and the controls. Morphologic analysis suggests that cocaine produced significant effects on spermatogenesis. Compared to the control group, the mean diameter of seminiferous tubules in the daily and twice weekly cocaine groups was significantly ($p<0.05$) reduced. The mean diameter of the tubules in the daily cocaine group was 213.16 ± 2.66 mcm and in the control group it was 272.95 ± 5.35 mcm. In the weekend-treated group the mean diameter was 230.36 ± 5.63 mcm, and controls showed 250.19 ± 4.26 mcm. Similarly, compared to the control, the thickness (height) of the germinal epithelium was also significantly ($p<0.05$) reduced in the cocaine-treated groups. The thickness of the germinal epithelium in the daily treated group was 70.80 ± 1.34 mcm. (control 89.71 ± 2.0 mcm.), and in the weekend treated group was 78.98 ± 1.53 mcm. (control 86.92 ± 1.76 mcm). However, there was no evidence of sloughed Sertoli cells or germ cells in the tubular lumen or epididymis. Cocaine treatment of prepubertal rats adversely affected spermatids. The number of step VII spermatids was reduced in both daily and twice weekly cocaine groups, a difference that was statistically significant ($p < 0.05$). Corrected true germ cell count (as reflected in counts of Step VII spermatids) in the daily cocaine group was 89.33 ± 3.92 compared with 110.83 ± 5.69 in the control ($p < 0.05$). In the weekend cocaine group the number was 80.57 ± 2.14, and in the controls it was 95.8 ± 4.63 ($p <0.05$).

Li et al., 1997

Reference ID: 4263670

The histological effects of chronic administration of cocaine on the testes were evaluated in prepubertal male Sprague-Dawley rats (Li et al., 1997). Four groups of male rats (25 day of age) were subcutaneously administered a single dose of cocaine (0 or 15 mg/kg) either daily or twice weekly (Saturday and Sunday) for 100 days. Male endpoint evaluations included: histological examination of the testes (quantitative and qualitative morphologic assessment of the seminiferous tubules to assess the effect on the various stages of spermatogenesis), Sertoli cell count, and spermatid count. Compared to the control group, the mean diameter of seminiferous tubules in the daily and twice weekly cocaine groups was significantly ($p<0.05$) reduced. The mean diameter of the tubules in the daily cocaine group was 70.80 ± 1.34 mcm compared to 89.71 ± 2.00 mcm from the control animals. In the weekend-treated group the mean diameter was 78.98 ± 2.54 mcm, and tubule thickness in control animals was 86.92. ± 1.76 mcm. Compared to the control, the thickness (height) of the germinal epithelium was also significantly ($p<0.05$) reduced in the cocaine-treated groups. The thickness of the germinal epithelium in the daily treated group was 70.80 ± 1.34 mcm. (control 89.71 ± 2.0 mcm.), and in the weekend treated group was 78.98 ± 1.53 mcm. (control 86.92 ± 1.76 µm). There was evidence that the number of degenerating germ cells was significantly greater in the cocaine-treated animals compared to the control animals. However, there was no evidence of sloughed Sertoli cells or germ cells in the tubular lumen or in the epididymis. The cocaine-treated animals also failed to release mature spermatids.

Li et al., 1999

The effects of subcutaneously administered cocaine on blood flow to the testes were evaluated in prepubertal male Sprague-Dawley rats (Li et al., 1999). Two groups of male rats (25 day of age) were subcutaneously administered a single dose of cocaine (0 or 30 mg/kg). Testicular blood blow was measured via Xenon clearance. Xenon-133 wash out experiments were carried out at 5, 10, 15, 20, 30, 45, 60, 90, and 270 minutes after cocaine or saline administration; individual animals were used for each time point. Compared to the control, subcutaneous injections of cocaine (30 mg/kg) to rats resulted in significantly ($p<0.05$) reduced testicular blood flow 15 to 60 minutes post-injection.

Summary

As per ICH S5(R2), fertility and early embryonic development studies are intended to test for toxic effects of a compound resulting from treatment prior to mating (males and females) through mating and implantation. For females, the studies should characterize effects on the estrus cycle, tubal transport, implantation, and development of preimplantation states of the embryo as well as mating behavior and fertility assessments. The acceptable dosing regimen is to dose females at least 2 weeks prior to mating through implantation (for rats, implantation is generally by Gestation Day 6-7 or pregnancy). For males, the studies should evaluate the impact of a drug on functional effects (e.g., libido, epididymal sperm maturation) that may not be detected via histopathological examination of the male reproductive tissues. Although spermatogenesis in the rat lasts 63 days, acceptable dosing regiments require that the

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rats be tested for at least 28 days prior to mating. Male rodents are considered sexually mature at approximately 50 days of age (7 weeks). However, male rodents do not have sperm in the cauda epididymis until Day 70 (Week 7). As such most standard study protocols test males between 10 and 12 weeks of age. As noted in the table above, most of the published studies actually tested juvenile animals and as such, these findings are relevant to pediatric indications and should be included in Section 8.4.

Evaluation of the dosing regimens and endpoints examined in the literature cited above indicates that a GLP female fertility and early embryonic development study with cocaine is warranted because the appropriate endpoints have not been fully characterized. Nonetheless, some of the information in the literature is appropriate for labeling. In terms of male fertility, histopathological evaluation of the testes, as well as fertility assessment and sperm morphology data do appear in the published literature. These studies do not clearly define a NOAEL but do characterize potential toxicity for peripubertal rats. These data clearly suggest a greater risk for younger male rats than older male rats.

Embryonic Fetal Development

Applicant did not conduct standard embryonic fetal development studies. However, the Applicant selected several embryo-fetal development studies that characterize cocaine induced toxicity, which included studies conducted in mice, rats, sheep, and primates. As per ICH S5(R2), embryo-fetal development studies are intended to detect adverse effects on the pregnant female and development of the embryo and fetus consequent to test article exposure of the female implantation to closure of the hard palate. The studies should characterize the toxicity profile of the test article compared to nonpregnant females as well as assess embryofetal death, altered growth and structural changes (including soft tissue and skeletal examination). The acceptable dosing regimen is to dose pregnant females from implantation to the closure of the hard palate (GD 6 to 18 for mice and rats; GD 6-20 for rabbits) and then perform Cesarean section on females one day prior to parturition (GD 18 mice; GD 20-21 rats; GD 29 rabbits). At terminal examination, the following endpoints are recorded: macroscopic examination of all adults (histological evaluation), corpora lutea number, number of live and dead implantations, individual fetal body weight, fetal abnormalities (soft tissue and skeletal changes), and gross evaluation of the placenta.

Several studies summarized by the Applicant for this section were not deemed relevant by this Reviewer given that several citations examined nonclinical animal models that do not conform to ICH S5 study designs (e.g. in vitro assays, chick embryo, sheep studies), did not examine standard embryofetal endpoints, or were not peer-reviewed journal articles but rather were conference abstracts that lacked sufficient detail. Only those published nonclinical literature submitted by the Applicant that contained sufficient details and data to permit substantive review and evaluated standard embryofetal study endpoints are summarized below.

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Mice

The developmental toxicity of cocaine administration was evaluated in several strains of mice using subcutaneous and intraperitoneal routes during the early period of organogenesis. These studies show that cocaine at low multiples of clinically relevant doses produced skeletal and visceral malformations in mice. The primary malformations observed included urogenital defects (i.e. enlarged renal pelvis, hydronephrosis and cryptorchidism), ocular defects (i.e. anophthalmia and microphthalmia), and various defects in skeletal ossification.

Mahalik et al., 1980

Mahalik et al. (1980) evaluated the teratogenic effects of cocaine once daily by subcutaneous injection in pregnant CF-1 mice that were divided into one of thirteen groups of eight mice (Mahalik et al., 1980). Six groups were subcutaneously administered a single dose of 60 mg/kg cocaine on Day 7, 8, 9, 10, 11, or 12. Dose selection was based on dose range-finding studies that demonstrated that doses of 70 mg/kg and higher resulted in convulsions and doses of 100 mg and higher resulted in mortality. Six groups received a single administration of saline on Day 7, 8, 9, 10, 11, or 12. One group of mice was untreated and served as a control group. Pregnant mice were sacrificed on Day 18 prior to the termination of gestation. After Cesarean section, uterine horns were examined grossly for fetal swellings and resorption sites. Fetal endpoint evaluation included body weight, sex, gross examination for external fetal malformations, visceral examination in every other fetus. Relative to vehicle control, no significant difference in maternal body weight was observed. There were no maternal mortalities or convulsions. The only clinical signs reported at this dose were described as mild excitatory responses and a Straub tail. Significant differences were noted in resorption on Day 10 and sex ratio on Day 9 when compared to vehicle control. Results from the study are presented in the Applicant's tables below. Compared to controls, soft tissue abnormalities were observed on each day of cocaine treatment. On Days 7 to 9, cocaine showed a significant increase in skeletal abnormalities compared with the vehicle control. Following subcutaneous administration of cocaine hydrochloride (2%) at 60 mg/kg/day, a significant incidence of fetuses with exencephaly, cryptorchidism, hydronephrosis, anophthalmia, malformed or missing lenses, delayed ossification of the skull, paws or centrum, extra ribs, malformed or crankshaft sternebrae and split or butterfly xiphoid processes were reported. Cocaine alone group showed a significant increase in visceral malformations as compared to control group, such as cerebral hemorrhage, hydrocephalus, intra-abdominal hemorrhage, and anomalies of the limb.

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Table I—Mean Values of the Test Groups

Treatment[a]	Day	Maternal Weight Ratio, S/T[b]	Fetal Ratio, Right Horn/ Left Horn	Resorption Ratio, Right Horn/ Left Horn	Mean Fetal Weight, g	Sex Ratio, M/F	Soft Tissue Abnormalities	Skeletal Abnormalities
Untreated	—	28.2/50.9	5.6/5.3	0.3/0.2	1.13	4.7/6.2	0.0	1.3
Saline	7	30.2/56.3	6.1/5.6	0.0/0.2	1.16	8.0/3.7	0.1	3.5
	8	28.1/54.2	6.3/5.3	0.2/0.2	1.14	7.0/4.7	0.2	1.7
	9	28.4/53.3	6.3/5.1	0.3/0.0	1.17	8.3/3.1	0.1	1.6
	10	30.7/55.6	5.7/5.7	0.0/0.6	1.15	6.8/4.6	0.0	4.0
	11	30.6/53.0	5.8/5.5	0.0/0.5	1.11	6.1/5.2	0.0	2.6
	12	27.4/52.1	6.5/4.6	0.5/0.0	1.15	7.1/4.0	0.0	3.8
Cocaine	7	29.5/54.8	5.5/6.2	0.2/0.3	1.14	5.6/6.1	6.0[c]	7.7[c]
	8	28.6/50.9	5.7/5.0	0.5/0.2	1.10	6.2/4.5	2.1[c]	7.3[c]
	9	29.8/54.4	6.7/4.7	0.7/0.2	1.12	6.3[c]/5.1[c]	2.2[c]	8.6[c]
	10	30.1/52.0	5.7/4.3	1.1[c]/1.0	1.13	6.2/3.8	2.1[c]	5.2
	11	30.4/53.6	6.5/4.3	0.6/0.7	1.13	6.6/4.2	2.6[c]	6.0
	12	28.6/53.3	5.8/5.2	0.1/0.2	1.19	6.0/5.0	1.0[c]	8.1

[a] The dose of saline was 0.3 ml sc, and the dose of cocaine was 60 mg/kg sc. [b] S = starting weight, and T = terminal weight. [c] Statistically significant in comparison with respective saline controls, $p < 0.05$.

Table II—Occurrence of Exencephaly, Cryptorchidism, and Hydronephrosis on Day of Treatment (Compared with Saline)

		Exencephaly				Cryptorchidism				Hydronephrosis			
		Litters		Fetuses		Litters		Fetuses		Litters		Fetuses	
Treatment[a]	Day	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Untreated	—	8	0	43	0	8	0	43	0	8	0	43	0
Saline	7	8	0	45	0	7	1	44	1	8	0	45	0
	8	8	0	46	0	7	1	45	1	8	0	46	0
	9	8	0	44	0	7	1	43	1	8	0	44	0
	10	8	0	44	0	8	0	44	0	8	0	44	0
	11	8	0	44	0	8	0	44	0	8	0	44	0
	12	8	0	43	0	8	0	43	0	8	0	43	0
Cocaine	7	7	1	44	1	6	2	42	3	4	4[b]	37	8[b]
	8	7	1	39	2	4	4	35	6[b]	7	1	40	1
	9	4	4[b]	41	5[b]	5	3	42	4	4	4[b]	41	5[b]
	10	7	1	39	1	4	4[b]	33	7[b]	6	2	37	3
	11	8	0	44	0	5	3	39	5[b]	6	2	41	3
	12	8	0	44	0	6	2	42	2	7	1	43	1

[a] The dose of saline was 0.3 ml sc, and the dose of cocaine was 60 mg/kg sc. [b] Statistically significant in comparison with respective saline controls, $p < 0.05$.

Table III—Occurrence of Anophthalmia, Malformed Lenses, and Missing Lenses on Day of Treatment (Compared with Saline)

		Anophthalmia				Malformed Lenses				Missing Lenses			
		Litters		Fetuses		Litters		Fetuses		Litters		Fetuses	
Treatment[a]	Day	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Untreated	—	8	0	43	0	8	0	43	0	8	0	43	0
Saline	7	8	0	45	0	8	0	45	0	8	0	45	0
	8	8	0	46	0	8	0	46	0	8	0	46	0
	9	8	0	44	0	8	0	44	0	8	0	44	0
	10	8	0	44	0	8	0	44	0	8	0	44	0
	11	8	0	44	0	8	0	44	0	8	0	44	0
	12	8	0	43	0	8	0	43	0	8	0	43	0
Cocaine	7	5	3	41	4[b]	3	5[b]	31	14[b]	4	4[b]	39	6[b]
	8	6	2	39	2	5	3	38	3	6	2	39	2
	9	6	2	44	2	6	2	44	2	8	0	46	0
	10	5	3	37	3	7	1	39	1	7	1	39	1
	11	5	3	40	4[b]	6	2	42	2	5	3	41	3
	12	6	2	42	2	8	0	44	0	5	3	41	3

[a] The dose of saline was 0.3 ml sc, and the dose of cocaine was 60 mg/kg sc. [b] Statistically significant in comparison with respective saline controls, $p < 0.05$.

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Table IV—Occurrence of Delayed Ossification of the Skull, Paws, and Centrum on Day of Treatment (Compared with Saline)

		Delayed Ossification Skull				Delayed Ossification Paws				Delayed Ossification Centrum			
		Litters		Fetuses		Litters		Fetuses		Litters		Fetuses	
Treatment[a]	Day	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Untreated	—	8	0	45	0	3	5	36	9	8	0	45	0
Saline	7	8	0	50	0	3	5	34	16	8	0	50	0
	8	8	0	48	0	4	4	41	7	8	0	48	0
	9	8	0	48	0	7	1	43	5	8	0	48	0
	10	8	0	48	0	2	6	32	16	8	0	48	0
	11	8	0	47	0	4	4	33	14	8	0	47	0
	12	7	1	45	1	2	6	32	14	8	0	46	0
Cocaine	7	8	0	49	0	2	6	26	23	8	0	49	0
	8	7	1	42	3	2	6	22	23[b]	8	0	46	0
	9	7	1	45	1	1	7[b]	16	30[b]	7	1	45	1
	10	7	1	40	1	1	7	20	21	8	0	41	0
	11	6	2	38	5[b]	2	6	24	19	8	0	43	0
	12	2	5[b]	34	10[b]	0	7	19	25[b]	6	1	40	4[b]

[a] The dose of saline was 0.3 ml sc, and the dose of cocaine was 60 mg/kg sc. [b] Statistically significant in comparison with respective saline controls, $p < 0.05$.

Table V—Occurrence of Split Sternebrae, Malformed Sternebrae, and Crankshaft Sternebrae on Day of Treatment (Compared with Saline)

		Split Sternebrae				Malformed Sternebrae				Crankshaft Sternebrae			
		Litters		Fetuses		Litters		Fetuses		Litters		Fetuses	
Treatment[a]	Day	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Untreated	—	7	1	44	1	8	0	45	0	7	1	44	1
Saline	7	3	5	40	10	8	0	50	0	7	1	49	1
	8	5	3	43	5	8	0	48	0	7	1	47	1
	9	5	3	44	4	6	2	46	2	6	2	46	2
	10	3	5	41	7	4	4	43	5	7	1	47	1
	11	6	2	45	2	8	0	47	0	6	2	45	2
	12	4	4	41	5	4	4	41	5	6	2	43	3
Cocaine	7	2	6	39	10	2	6[b]	36	14[b]	6	2	46	3
	8	4	4	38	7	4	4[b]	37	8[b]	4	4	39	6[b]
	9	1	7[b]	33	13[b]	5	3	39	7	3	5	37	9[b]
	10	5	3	37	4	1	7	29	12[b]	7	1	40	1
	11	5	3	38	5	3	5[b]	35	8[b]	6	2	41	2
	12	3	4	34	10	2	5	38	6	6	1	43	1

[a] The dose of saline was 0.3 ml sc, and the dose of cocaine was 60 mg/kg sc. [b] Statistically significant in comparison with respective saline controls, $p < 0.05$.

A developmental NOAEL was not identified in this study as only one dose, which produced adverse embryofetal effects, was tested. This dose of 60 mg/kg/day is 7.8-times the human reference dose of 37.5 mg of cocaine via the 4% solution.

Ohnaka et al., 2001

Ohnaka et al evaluated the effects of cocaine alone, alcohol alone, and the combination of cocaine in combination with alcohol (Ohnaka et al., 2001). Pregnant ICR mice were divided into 4 groups as follows: Group A received saline solution IP on Gestational Days 7, 8, 9. Group B received sucrose solution diluted in distilled water with a caloric value similar to that of the 10% ethanol solution. Group C received 10% ethanol solution given ad libitum. Group D received 50 mg/kg cocaine IP on GD 7, 8, and 9. Tap water was given ad libitum. Group E received 50 mg/kg/day cocaine IP on GD 7, 8, 9 with 10% ethanol solution given ad libitum. Mean number of implants, mean fetal body weights, mean placental weights were not different between the cocaine group compared to the control group.

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Group	Treatment	Fetal Mortality rate (%)	External Malformation rate (%)
A	Control	5.2	0
B	Sucrose	15.9	1.7
C	Ethanol p.o.	15.3	0
D	Cocaine i.p.	6.8	1.6
E	Ethanol p.o. +Cocaine i.p.	27.0*	1.6

*Significantly different from Group A and D (P<0.05)

Group	Treatment	No. of live fetuses	Cleft palate	polydactyly	Tail anomaly or anal atresia
A	Control	139	0	0	0
B	Sucrose p.o.	93	1	1	0
C	Ethanol p.o.	132	0	0	0
D	Cocaine i.p.	131	1	1	0
E	Ethanol p.o. +Cocaine i.p.	105	1	0	1

Cleft palate, polydactyl and tail anomaly were observed in fetuses exposed to cocaine but the rate of malformations were not significantly higher in any group. However, in dams that received both cocaine and alcohol, increased fetal mortality and fetal weight loss were noted. Results suggest that 50 mg/kg IP cocaine administered at early stages of organogenesis did not exhibit embryotoxicity.

Hunter et al., 1995

Hunter et al. evaluated the teratogenic effects of cocaine administrated to ICR mice during early organogenesis (Hunter et al., 1995). On Gestation Day 9, cocaine was intraperitoneally administered at doses ranging from 20 to 100 mg. Control animals were injected (IP) with saline. The developmental effects of 60 mg/kg cocaine on different days of gestation were evaluated following administration on Day 8, 9, or 10 or on all three days (8-10). Results from the study are presented in the tables below.

Reference ID: 4263670

TABLE 1
Effects of Intraperitoneal Cocaine Administration on Day 9 of Gestation: Maternal

Treatment (mg/kg)	Maternal deaths % (N)	Maternal body weight change (g)	
		Days 9–10	Days 9–19
Control	0 (0/28)	1.0 ± 0.1 (11)	22.3 ± 1.1
20	0 (0/12)	0.8 ± 0.3 (7)	23.3 ± 1.2
30	0 (0/12)	1.6 ± 0.7 (9)	20.8 ± 2.3
40	0 (0/11)	−0.5 ± 0.2 (3)	19.2 ± 2.3
50	0 (0/10)	0.0 ± 0.1 (5)	22.6 ± 1.3
60	12 (2/16)	−0.9 ± 0.4* (4)	21.3 ± 0.8
70	48 (22/46)	−4.2 ± 0.7* (9)	18.8 ± 1.1
80	75 (9/12)	—	21.8 ± 0.4
100	100 (3/3)	—	—
Fasted	0 (0/11)	−4.4 ± 0.3* (9)	22.7 ± 1.3

* $p < 0.05$ compared to control.

TABLE 2
Effects of Cocaine Administration on Day 9 of Gestation: Developmental[a]

Treatment (mg/kg)	No. litters	Implants	Live pups	Nonlive conceptuses			Pup weights (g)
				Total	Early	Late	
Control	28	12.7 ± 0.5	11.7 ± 0.5	1.1 ± 0.2	0.9 ± 0.2	0.2 ± 0.1	1.24 ± 0.02
Intraperitoneal administration							
20	12	13.9 ± 0.7	12.5 ± 0.9	1.4 ± 0.5	1.2 ± 0.4	0.2 ± 0.1	1.28 ± 0.04
30	11	12.7 ± 1.5	12.0 ± 1.4	1.0 ± 0.3	0.8 ± 0.3	0.2 ± 0.2	1.24 ± 0.03
40	11	11.7 ± 1.5	10.3 ± 1.4	1.5 ± 0.7	1.3 ± 0.7	0.2 ± 0.1	1.28 ± 0.05
50	10	14.3 ± 1.0	12.4 ± 0.8	1.9 ± 0.7	1.6 ± 0.6	0.3 ± 0.2	1.24 ± 0.03
60	14	12.9 ± 0.3	12.5 ± 0.4	0.4 ± 0.3	0.2 ± 0.2	0.2 ± 0.2	1.17 ± 0.04
70	9	12.9 ± 0.6	11.3 ± 0.9	1.6 ± 0.5	0.8 ± 0.2	0.8 ± 0.5*	1.18 ± 0.03
Fasted	11	12.9 ± 0.9	12.3 ± 0.9	0.6 ± 0.2	0.5 ± 0.2	0.1 ± 0.1	1.34 ± 0.03*
Subcutaneous administration							
70	13	13.5 ± 0.7	12.7 ± 0.8	0.7 ± 0.2	0.4 ± 0.1	0.4 ± 0.2	1.29 ± 0.02

[a] The mean ± SEM are presented on a per litter basis.
* $p < 0.05$ compared to control.

Maternal lethality occurred at doses ≥ 60 mg/kg. One hundred percent lethality occurred at the 100 mg/kg dose of cocaine. Cocaine administration on Day 9, no significant effect was noted on the number of implantation or number of live pups/litter when evaluated on Day 19. However, a significant increase in dead conceptus during late gestation was observed at higher dose that also produced maternal deaths.

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TABLE 3
Effects of Intraperitoneal Cocaine Administration on Day 9 of Gestation: Pup Abnormalities[a]

Treatment (mg/kg)	External abnormalities		Visceral abnormalities	
	Fetuses	Litters	Fetuses	Litters
Control	0 (0/298)	0 (0/25)	7.4 (22/298)	32 (8/25)
20	0.7 (1/146)	8 (1/12)	15.8 (23/146)*	83 (10/12)*
30	0 (0/132)	0 (0/11)	11.4 (15/132)	55 (6/11)
40	0.9 (1/112)	9 (1/11)	15.2 (17/112)*	64 (7/11)
50	1.6 (2/123)	20 (2/10)	18.7 (23/123)*	100 (10/10)*
60	1.5 (2/132)	18 (2/11)	15.9 (21/132)*	64 (7/11)
70	1.3 (1/79)	14 (1/7)	43.0 (34/79)*	100 (7/7)*
Fasted	0 (0/119)	0 (0/10)	3.4 (4/119)	40 (4/10)

[a] Data are presented as percentage affected (n affected/n evaluated).
* $p < 0.05$ compared to control.

No cocaine-induced external malformations were noted. However, a statistically significant increase incidence of visceral abnormalities was noted, namely enlarged renal pelvis. Incidences of enlarged renal pelvis were comparable whether cocaine was administered on Days 8, 9, or 10 or on all 3 days.

TABLE 4
Effect of Cocaine Administration on Day 9 of Gestation: Enlarged Renal Pelvis

Treatment (mg/kg)	Pups[a]	Litters[a]	% pups/litter[b]	Kidney morphological score[b]
Control	5.4 (16/298)	32.0 (8/25)	11.4 ± 4.1	1.08 ± 0.02
Intraperitoneal administration				
20	11.0 (16/146)*	58.3 (7/12)	11.6 ± 4.0	1.15 ± 0.04
30	9.8 (13/132)	45.5 (5/11)	18.1 ± 9.2	1.14 ± 0.04
40	11.6 (13/112)*	45.5 (5/11)	20.9 ± 11.1	1.16 ± 0.05
50	13.8 (17/123)*	80.0 (8/12)*	14.2 ± 3.9	1.18 ± 0.05*
60	13.6 (18/132)*	63.6 (7/11)	13.6 ± 4.5	1.22 ± 0.06*
70	38.0 (30/79)*	100 (7/7)*	40.3 ± 7.3*	1.51 ± 0.09*
Fasted	2.5 (3/119)	30.0 (3/10)	2.8 ± 1.4	1.04 ± 0.03
Subcutaneous administration				
70	2.2 (3/139)	27.3 (3/11)	2.2 ± 1.1	1.05 ± 0.02

[a] Data are presented as percentage affected (n affected/n evaluated).
[b] Mean ± SEM.
* $p < 0.05$ compared to control.

Results suggest that cocaine exposure at early stages of organogenesis resulted in an increase in the percentage of litters presenting with an enlarged renal pelvis. However,

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when pups from cocaine-treated mothers were allowed to develop to PND 21 and then evaluated, the kidneys were normal, suggesting that morphological alterations observed on GD 19 represent a delay of normal development and not a malformation.

Al-Motabagani and Mohamed, 2005

Al-Motabagani and colleagues (2005) evaluated the teratogenic effects of cocaine, alcohol or the combination of alcohol and cocaine administration (Al-Motabagani and Mohamed, 2005). Pregnant female mice were administered either 50% ethanol alone (25 mg/kg; oral) twice daily, cocaine (20 mg/100 g body weight or 200 mg/kg; IP) daily, 50% ethanol and cocaine, or vehicle control from Gestational Days 6 to 15. Pregnant mice were sacrificed on Day 20 and uterine contents were evaluated and the number of dead and live fetuses were determined as well as the number of resorption. The fetuses were examined for soft tissue anomalies. No significant effect was noted on the number of implantation or number of live pups/litter. An increase in anomalies in the head and neck (i.e., partial cleft palate and hydrocephaly), limb, and kidneys (i.e., unilateral hydronephrosis) were observed as shown in the table below (Table 2 from the article). A significant increase in some anomalies of the head and neck was observed in both alcohol and cocaine groups alone.

Parameter/group	Control group	Alcohol group	Cocaine group	Combined group
No. of foetuses examined for anomalies	57	54	53	63
No. of foetuses with head and neck anomalies	3	18	16	35
Complete cleft palate	1	3	1	7
Partial cleft palate	0	8	8	1
Excencephaly	1	0	0	18
Hydrocephaly	1	7	7	9
No. of foetuses with limb anomalies	3	14	9	25
Phocomelia	0	0	0	1
Wrist drop	1	5	0	9
Clubbing of the foot	1	6	8	10
Syndactly	0	3	1	3
Webbing of toes	1	0	0	1
Ectrodactly of the hind limb	0	0	0	1
No. of fetuses with renal anomalies	4	11	10	25
Unilateral hydronephrosis	2	1	9	3
Bilateral hydronephrosis	1	1	0	11
Ectopic kidney	1	9	1	11
No. of foetuses with kink tail	1	1	1	12

These findings suggest the potential for teratogenicity in fetuses exposed to cocaine during critical periods of early gestation at high doses of cocaine. It is notable that the

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dose of 200 mg/kg appears to exceed the lethal IP dose of cocaine that has been reported in several other publications that utilize the mouse model.

Rats

Several embryo-fetal development studies were identified in the published literature that were conducted in Sprague-Dawley and Long-Evans rats evaluating subcutaneous, and intraperitoneal cocaine administration during organogenesis. These studies demonstrated that cocaine was teratogenic in rats and are described in more detail below.

Study title: The Teratogenicity of Cocaine (Alan G. Fantel and Bonita J. Macphail, 1982)

Key Study Findings

Female rats were treated with cocaine hydrochloride (0, 50, 60, and 75 mg/kg/day) on Gestation Days 8-12 with the following findings:

1. Treatment-related mortalities were observed in maternal rats intraperitoneally administered cocaine at 75 mg/kg/day.
2. Maternal and fetal body weight was significantly reduced at 60 mg/kg/day.
3. Fetal edema, increased resorption and single incidences of cleft palate and hypertrophic left ventricle, and severe autolysis of the fetus were observed at 60 mg/kg/day of cocaine.
4. Maternal and developmental NOAEL = 50 mg/kg/day (HED = 8.1 mg/kg or 484 mg/60 kg person based on body surface area) was defined (based on decreased fetal body weight, resorption and edema findings at the 60 mg/kg dose).
5. Based on a body surface area comparison, the dose of 50 mg/kg in a rat is 8.1-times the maximum recommended dose of 160 mg/60 kg person in which 23.4% of cocaine (37.5 mg) is completely absorbed from the nasopharynx following intranasal administration. For the 10% cocaine topical solution, the dose of 50 mg/kg is 3.6-times the maximum recommended dose of 400 mg/60 kg person in which 33.4% of cocaine is absorbed completely from the pledgets (133.6 mg)

Methods

Doses:	0, 50, 60, 75 mg/day (rats); 60 mg/kg/day (mice)
Frequency of dosing:	Daily on Gestation Days 8-12 (rats); Daily on Gestation Days 7-16 (mice)
Dose volume:	Unknown
Route of administration:	Intraperitoneal

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Formulation: Cocaine HCl was dissolved in Hank's Balanced Salt Solution
Species/Strain: Rats/Sprague-Dawley; Mice/Swiss Webster
Number/Sex/Group: Rats
0 mg – 8 litters
50 mg – 12 litters
60 mg – 9 litters
75 mg – 0 litters (lethal)

Mice
0 mg – 8 litters
60 mg – 8 litters

Observations and Results

Examination of Embryo and Fetuses	
Cesarean/Necropsy	Day 20 of gestation (rats); Day 18 of gestation (mice)
Cesarean Section	The number of corpora lutea, early and late resorptions, dead and live fetuses, placental weight of live fetuses
Fetal Observations	Each fetus was weighed, sexed and examined for external alterations
Skeletal Examination	Examined for external malformations. Fetuses were fixed in a 3:1 ratio in Bouin's solution and 95% ethanol and subsequently cleared with potassium hydroxide and stained with alizarin red for skeletal examination.

Mortality
Cocaine was lethal at the high dose (75 mg/kg/day). All maternal dams died on or before Gestation Day 14.

Body Weight

The mean body weight of dams intraperitoneally administered cocaine at 50 mg/kg/day during the gestation period was comparable to the vehicle-treated dams. Treatment-related effects on the mean body weight were observed in the dams administered the high dose (60 mg/kg/day). Relative to control, the mean body weight was statistically ($p<0.025$) significantly decreased by 18% on Gestation Day 20.

Gestation Day 20 Laparohysterectomy Data

In the low-dose group, a total of 136 fetuses were delivered in 12 litters. Body weight was comparable in the fetuses from the low dose group. Nine resorptions occurred. In the mid-dose group, a total of 94 fetuses were delivered in nine litters; 79 of these fetuses were normal. Compared to control fetuses, fetal weight was reduced; the

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growth retardation was correlated with the reduced maternal weight at this dose. Relative to control, a significant number (p<0.001) of resorptions was observed; 38 resorptions were observed in fetuses from the mid-dose dams. As depicted in the table below, mean fetal weight was reduced approximately 27% in fetus born in the 60 mg/kg/day group.

TABLE 1. Average maternal and fetal weights (in grams) from cocaine-treated and control Sprague-Dawley rats

		Maternal weights		Fetal weights
		Day 8	Day 20	(day 20)
50 mg/kg	N	12	8	136
	$\bar{X}$	278.25	340.88	3.66
	Sx	33.97	43.05	0.40
60 mg/kg	N	9	8	94
	$\bar{X}$	286.33	284.50*	2.67**
	Sx	36.30	57.32	0.99
Control group	N	8	8	100
	$\bar{X}$	256.88	347.00	3.67
	Sx	22.90	33.04	0.33

*P < 0.025 compared to controls.
**P < 0.005 compared to controls.

Offspring (malformations, variations, etc)

No treatment-related effects in the type or incidence of gross external, soft tissue or skeletal malformations were noted following intraperitoneal administration of cocaine in the low dose group that received 50 mg/kg/day. Treatment-related effects were observed in fetuses from the mid-dose group that included single incidences of cleft palate and hypertrophic left ventricle, and severe autolysis of a fetus. It was also noted that in the mid-dose group that two litters had 13 fetuses with edema.

Study title: Effects of Cocaine on Rat Embryo Development in Vivo and in Cultures (Hiba el-Bizri, Ian Guest, and Daya Varma, 1991)

Key Study Findings

Pregnant SD rats were treated with cocaine hydrochloride (0, 6.25, 12.5, 25, 50, 100 mcmol/kg = 0, 2.1, 4.2, 8.5, 17, and 34 mg/kg, IP) on Gestation Days 0-19 with the following findings:

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1. Treatment-related mortalities were observed in maternal rats intraperitoneally administered cocaine 50 mcmol/kg/day and above.

2. Mean maternal body weight was reduced 4.8% at ≥ 25 mcmol/kg/day.

3. Cocaine caused a dose-dependent increase in soft tissue genitourinary tract findings, which include hydronephrosis and enlarged bladder at all doses.

4. Maternal NOAEL = 12.5 mcmol/kg/day (based on mortality and decreased body weight).

5. A developmental NOAEL was not identified as hydronephrosis was observed at all doses tested.

Methods

Doses:	0, 6.25, 12.5, 25, 50, and 100 mcmol/kg = 0 (saline), 2.1, 4.2, 8.5, 17 and 34 mg/kg/day
Frequency of dosing:	Once daily on Gestation Day 0 to 19
Dose volume:	Unknown
Route of administration:	Intraperitoneal
Formulation:	Cocaine was dissolved in saline
Species/Strain:	Rats/Sprague-Dawley
Number/Sex/Group:	5 to 13 litters/group

Observations and Results

Examination of Embryo and Fetuses	
Cesarean/Necropsy	Day 20 of gestation (rats)
Cesarean Section	The number of corpora lutea, resorption sites, dead and live fetuses, number of runts or edematous fetuses, and fetal and placental weights were recorded. A number of pregnant rats treated with saline or 15 mg/kg cocaine were allowed to deliver spontaneously.
Fetal Observations	Each fetus was weighed, sexed and examined for external alterations.
Fetal Examination	Day 20 fetuses were either fixed in Bouin's fluid for internal organ examination or in ethanol for skeletal examination after staining by Alizarin Red.

Overt cocaine-induced toxicity included excitation that occurred 15 to 30 minutes following treatment after dosing 5 to 6 days in the dose range of 6.25 to 50 mcmol/kg. In the 100 mcmol/kg group, the animals appeared lethargic, and 3 out of the 5 animals died within 15 to 30 minutes after dosing on Day 6 or 7 of treatment; convulsions

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preceded the death of these 3 animals. At 100 mcmol/kg, all implants were loss. Maternal and fetal results from the study report are presented in the author's table below. Relative to vehicle control, a small but significant decrease in food consumption and body weight was reported. Prenatal cocaine exposure did not affect gestational duration nor the growth pattern. At 100 mcmol/kg, three of the five dams died after six to seven injections.

Table 1. *Maternal and fetal effects of cocaine in rats**

	Cocaine (μmol/kg)					
Variables	0	6.25	12.5	25	50	100
n	13	6	6	6	17	5
Day 0 body wt (g)	231 ± 4	227 ± 5	235 ± 4	224 ± 6	234 ± 4	227 ± 4
Day 20 body wt (g)	375 ± 6	368 ± 8	374 ± 8	357 ± 10	357 ± 6†	
Food intake (g/d)	23.2 ± 0.2	23.7 ± 0.4	22.6 ± 0.4	22.1 ± 0.5	20.5 ± 0.4†	16 ± 0.4†
Maternal death (*n*)	0	0	0	0	2†	3†
Litter size (*n*)	13.1 ± 0.8	13.1 ± 0.8	14.8 ± 0.8	13.0 ± 0.9	13.3 ± 0.5	0†
Fetal body wt (g)	3.6 ± 0.1	3.4 ± 0.1	3.4 ± 0.1	3.6 ± 0.1	3.6 ± 0.1	
Placental wt (mg)	465 ± 14	440 ± 21	431 ± 17	476 ± 13	438 ± 7	
Edematous fetuses (*n*)	0/170	0/79	0/80	5/63	15/160	
Runts (*n*)	0/170	2/79	3/89†	2/63	12/160†	
Resorptions (*n*)	6	9†	1	5	12†	

* Cocaine was injected intraperitoneally once a day from d 0 to 19 of gestation inclusive and animals were killed on d 20; 0 dose refers to saline injections.

† Significantly ($p < 0.05$) different from the corresponding value for the saline-treated animals; data are either means ± SEM or total numbers.

Cocaine did not affect mean fetal and placental weights, although it increased the number of runts and edematous fetuses. Three pups born to one cocaine-treated animal died 20 days after birth. Brain catecholamine levels, which includes dopamine, epinephrine, and norepinephrine, were measured from Gestational Day 20 fetuses and newborn pups and were slightly elevated but were not significantly different compared to control animals. Cocaine caused a dose-dependent increase in soft tissue genitourinary tract findings, which include hydronephrosis and enlarged bladder as well as cerebral hemorrhage. Results from this study suggest that cocaine is a potential teratogen as evidenced by visceral malformations.

Table 2. *Fetal abnormalities after daily intraperitoneal injections of cocaine in rats from d 0 to 19 of gestation*

	Cocaine (μmol/kg)				
Variables*	0	6.25	12.5	25	50
Litters examined (*n*)	13	6	6	5	12
Fetuses examined (*n*)	37	19	21	18	34
Enlarged bladder	0	3†	6†	5†	9†
Hydronephrosis	2	7†	8†	8†	16†
Cerebral hemorrhage	0	0	0	1	4
Total abnormal fetuses (*n*)	2	8†	8†	9†	20†

* Refer to fetuses on d 20 of gestation.

† Significantly ($p < 0.05$) different from corresponding values for saline-treated (0 dose of cocaine) animals.

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Effects of cocaine (1 mcM – 1 mM) on the growth of embryos in culture were studied to find out if cocaine's fetal toxicity is correlated with the maternal toxicity. In the in vitro embryo culture study, rats were mated during a 2-hour period. Embryos (10-12 somite stage) was harvested from 10.5 day pregnant rats were cultured for 48 hours. Embryo size, mortality (absence of heart beat), and protein content were assessed at the end of the incubation period. Results are shown in the study report figure below. Cocaine elicited a concentration-dependent inhibition of embryo development in culture and was lethal to all embryos at a concentration of 300 mcM. Results from the in vitro embryo culture assay suggest that cocaine can exert direct fetal toxicity but may act independent of interactions with catecholamines as procaine a local anesthetic was also embryotoxic suggestive of a class effect.

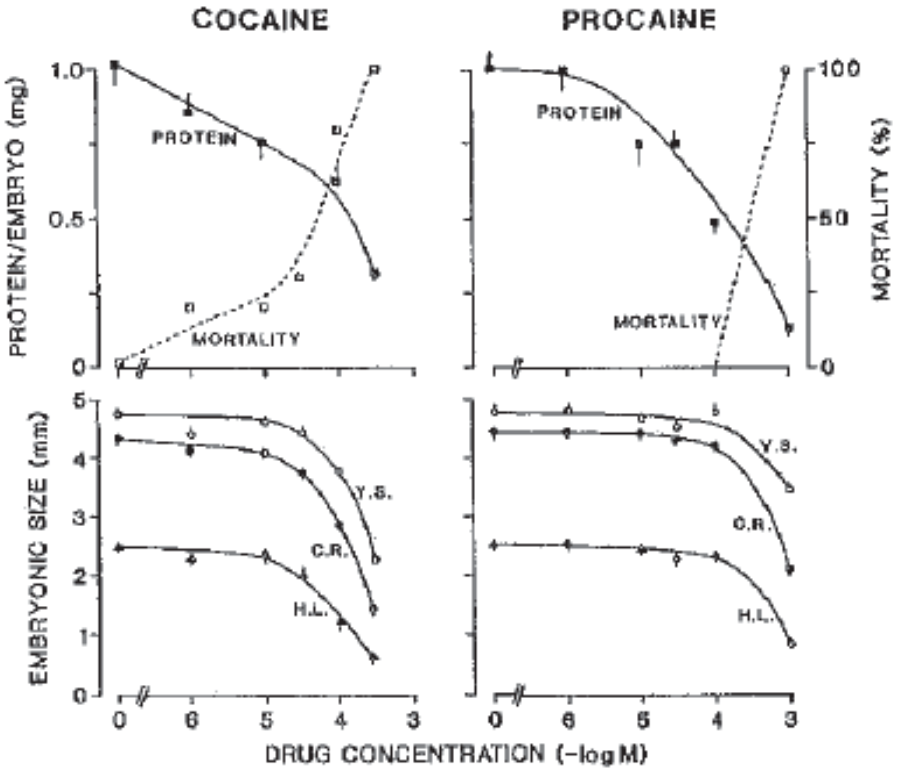

Fig. 1. Effects of cocaine and procaine on the development of rat embryos in culture. Each data point represents mean ± SEM of five to 10 embryos. *Y.S.*, yolk sac diameter; *C.R.*, crown rump; and *H.L.*, head length; absence of heart beats denoted mortality. Significant ($p < 0.05$) differences (not shown in the figure to avoid overcrowding): effects of cocaine on protein content and mortality were significant at all concentrations; procaine significantly inhibited protein content at $>10^{-5}$ M and was lethal to embryos only at 10^{-3} M; embryonic sizes at $>10^{-5}$ cocaine and $>10^{-4}$ procaine are significantly different from the corresponding values at 0 drug (saline) concentration.

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Webster and Brown-Woodman, 1990

Webster and Brown-Woodman (1990) evaluated the teratogenic effects of cocaine in pregnant Sprague-Dawley rats as a single or multiple doses following intraperitoneal administration in three different study aims (Webster and Brown-Woodman, 1990). Subsequently, the fetuses were examined at various times post-dose for evidence of hemorrhage and external malformations.

Dose-Response Study. Pregnant Sprague-Dawley rats received a single dose (0, (distilled water) 40, 50, 60, 70, or 80 mg/kg) on Gestational Day 16. Forty hours post-dosing, the fetuses were examined with a dissecting microscope for external malformations and cleft palate. As suggested in the table below, cocaine-induced maternal lethality occurred at the highest dose (80 mg/kg). Percent of implants resorbed was significant increased by cocaine exposure to 70 mg/kg relative to vehicle control. Teratogenicity was observed in a dose-dependent manner over a narrow dose range. A single dose of cocaine on Gestation Day 16 induced severe hemorrhage in the fetuses which primarily affected the footplates of both forelimbs and hindlimbs, and distal part of the tail. Hemorrhage was visible in the fetuses 2 hours after dosing. The hemorrhages were also observed in the genital tubercle, and the upper lip and adjacent part of the nose. "Less severe damage was restricted to the distal parts of the middle digits of the limb and the distal part of the tail."

TABLE 1. Effect of dose on cocaine teratogenesis on embryonic day 16 in the rat (fetuses examined 48 hours later)

Treatment (mg/kg)	No. of rats	No. of litters	Mean litter size	Percent of implants resorbed	Percent of live fetuses abnormal	No. of affected litters	Mean fetal weight (g)
40	5	5	17.2	1.1	0.0	0/5	1.23 ± 0.06*
50	7	7	14.0	5.0	7.3*	3/7	1.29 ± 0.08
60	6	5	16.0	3.4	21.5*	4/5	1.27 ± 0.16*
70	5	2	11.0	18.7*	45.8*	2/2	1.24 ± 0.09
80	4	0					
Controls	5	5	12.8	0.0	0.0	0/5	1.41 ± 0.10

*Significantly different from the control; $P < 0.05$.

Treatment Interval Study. On Gestation Day 16, rats were given two injections of 50 mg/kg cocaine at intervals of 1, 2, 3, or 4 hours. Forty hours post-dosing, the fetuses were weighed and examined. Percent of implants resorbed was significant increased by cocaine exposure to 70 mg/kg relative to vehicle control. Findings suggested that when two doses of cocaine (50 mg/kg) were administered 1 to 4 hours apart, the incidence of affected fetuses increases as the time interval between the two doses decreased. "When two doses were given 1 hour apart, the number of affected fetuses increased to 29.5%, two doses 2 hours apart affected 23%, and two doses 3 hours apart affected 17%."

Treatment Interval Study. On Gestation Day 16, rats were given two injections of 50 mg/kg cocaine at intervals of 1, 2, 3, or 4 hours. Forty hours post-dosing, the fetuses were weighed and examined. Percent of implants resorbed was significant increased by cocaine exposure to 70 mg/kg relative to vehicle control. Findings suggested that when two doses of cocaine (50 mg/kg) were administered 1 to 4 hours apart, the

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incidence of affected fetuses increases as the time interval between the two doses decreased. "When two doses were given 1 hour apart, the number of affected fetuses increased to 29.5%, two doses 2 hours apart affected 23%, and two doses **3** hours apart affected 17%."

TABLE 2. Effect of treatment interval on cocaine teratogenesis in the rat on embryonic day 16 (fetuses examined 48 hours later)

Dose (50 mg/kg)	No. of rats	No. of litters	Mean litter size	Percent of implants resorbed	Percent of live fetuses abnormal	No. of affected litters	Mean fetal weight (g)
1 injection	7	7	14.0	5.0	7.3	3/7	1.29 ± 0.08
2×1 hr	5	5	13.4	12.0*	29.5*	4/5	1.30 ± 0.09
2×2 hr	7	5	12.8	17.5*	23.1*	4/5	1.20 ± 0.19
2×3 hr	5	5	13.0	13.3	17.0	3/5	1.16 ± 0.12*
2×4 hr	5	4	11.0	13.1	1.8	1/4	1.30 ± 0.02
Controls	4	4	14.3	1.5	0.0	0/4	1.33 ± 0.02

*Significantly different from control; $P<0.05$.

Stage of Pregnancy Study. Pregnant rats were given two intraperitoneal injections of 50 mg/kg cocaine at 2 hour intervals on Gestation Day 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19. The pregnant rats were sacrificed on Gestational Day 21 and the fetuses were weighed and examined. Another group of pregnant rats were given two injections of 50 mg/kg cocaine at 1 hour apart on either Gestation Day 13, 14, 15, 16, 17, 18, or 19. Findings showed that this marginally teratogenic dose of cocaine (50 mg/kg) induced a low incidence of externally visible malformations, and was restricted to litters exposed to cocaine.

TABLE 3. Effect of stage of pregnancy on cocaine teratogenesis in the rat (2 i.p. doses 50 mg/kg 2 hours apart, killed on day 21)

Day of treatment	No. of rats	No. of litters	Mean litter size	Percent of implants resorbed	Percent of live fetuses abnormal	No. of affected litters	Mean fetal weight (g)
9	4	3	9.0	35.4	0.0	0/3	4.90 ± 0.79
10	6	1	18.0	0.0	0.0	0/1	4.34
11	4	1	15.0	0.0	0.0	0/1	4.58
12	3	3	14.3	0.0	0.0	0/3	4.53 ± 0.16
13	3	2	14.0	0.0	0.0	0/2	4.62 ± 0.16
14	3	3	10.7	6.3	0.0	0/3	5.20 ± 0.39*
15	4	4	15.0	0.0	7.8	1/4	4.31 ± 0.13
16	4	3	15.7	5.7	4.2	1/3	4.51 ± 0.41
17	5	4	11.8	17.3*	4.5	1/4	4.55 ± 0.16
18	5	4	12.3	9.9*	7.3	3/4	4.43 ± 0.66
19	5	2	3.0	12.5	16.7	1/2	5.13 ± 0.28
Controls	6	6	13.0	0.9	1.1	1/6	4.46 ± 0.79

*Significantly different from the control; $P<0.05$.

For this study, a NOAEL of 40 mg/kg was identified (HED = 6.5 mg/kg or 387 mg/60 kg person based on body surface area), which was based on hemorrhage, edema and external malformations at doses ≥ 50 mg/kg/day). Based on a body surface area comparison, the dose of 40 mg/kg in a rat is 10.4-times the maximum recommended dose of 160 mg/60 kg person in which 23.4% of cocaine (37.4 mg) is completely absorbed from the nasopharynx following intranasal administration. For the 10% cocaine topical solution, the dose of 40 mg/kg is 2.9-times the maximum recommended dose of 400 mg/60 kg person in which 33.4% of cocaine is absorbed completely from the pledgets (133.6 mg)

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Summary

The table below summarizes the literature references submitted by the Applicant. Reviewer's comments on the study design, adequacy with respect to typical embryofetal development study endpoints, and utility for labeling language are provided.

Table 22: Summary "Embryo-fetal Development" Studies Submitted by Applicant

Effect Reported	Species	Treatment Period	Doses*	HED (per 60 kg person) of NOAEL/ LOAEL	Estimated Exposure Margin for % Cocaine Topical Solution Based on BSA‡		Citation	Comment
					4%	10%		
Exencephaly, cryptorchidism, hydronephrosis, anophthalmia, delayed ossification	CF-1 mice	Single administration on GD 7, 8, 9, 10, 11 or 12	60 mg/kg (SC)	293 mg	7.8x	2.2x	(Mahalik et al., 1980)	Sample size is small. Maternal toxicity was not observed. Dosing period does not span organogenesis (GD 6-17) but study is informative as adverse effects are observed after single dose. Only a single dosing group was evaluated. As adverse findings were observed, a NOAEL was not identified.
Cocaine reduced crown rump length, increased vasodilation and hemorrhage and increased incidence of neural tube defects.	ICR mice	GD 8	78 mg/kg, IP	380 mg	10x	2.8x	(Fisher et al., 1994; Zimmerman et al., 1994)	Not a typical study design. Cocaine given on GD 8, embryos removed GD 10, 12, or 14
Single incidences of c eft palate, polydactyl and tail anomaly were reported but incidences were not s gnificantly different than control.	ICR mice	GD 7-9	50 mg/kg (IP)	244 mg	6.5x	1.8x	(Ohnaka et al., 2001)	Sample size is small. Maternal toxicity was not observed. Dosing period does not span organogenesis (GD 6-17).
Although external malformations	ICR mice	Single administration on	0 20 30	 97.6 mg 146 mg	 2.6x 3.9x	 0.73x 1.1x	(Hunter et al., 1995)	Sample size is small. Maternal deaths were observed at ≥ 60

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were not noted, higher incidences of v sceral abnormalities were observed in cocaine exposed pups. Enlarged renal pelvis, which may be indicative of hydronephrosis, was the most consistent abnormality reported.		GD 8, 9, or 10, or repeated administr ation from GD 8-10	40 50 60 70 80 100 mg/kg (IP)	194 mg 244 mg 293 mg 342 mg 390 mg 488 mg	5.2x 6.5x 7.8x 9.1x 10x 13x	1.5x 1.8x 2.2x 2.6x 2.9x 3.7x		mg/kg. Dosing period does not span organogenesis (GD 6-17).
Increases in incidences of partial cleft palate, hydrocephaly, c ubbing of the foot and hydronephrosis were reported in cocaine exposed pups.	Mice (strain not specifie d)	GD 6-15	20 mg/ 100 mg BW (or 200 mg/kg) (IP)	975.6 mg	26x	7.3x	(Al-Motabaga ni and Mohamed , 2005)	Sample size is small. A NOAEL was not identified. Maternal toxicity was not reported, which is surprising given the dose. Other studies have reported that cocaine IP administration is lethal at IP doses greater than 60 mg/kg.
Fetal edema, increased resorption, s ngle incidences of c eft palate and hypertrophic ventricle. Maternal toxicity was observed at the 60 (decreased body weight) and 75 mg/kg/day dose (death) groups.	Spragu e-Dawley rats	GD 8 - 12	0 **50** 60 75 mg/kg (IP)	484 mg 580 mg 726 mg	13x 15x 19x	3.6x 4.3x 5.4x	(Fantel and Macphail, 1982)	Sample size is small. Dosing period does not span organogenesis (GD 6-17). Maternal toxicity was observed at the 60 and 75 mg/kg/day dose groups.
Hemorrhage, fetal edema, limb defects	Spragu e-Dawley rats	Single administr ation on GD 9, 10, 11, 12, 13,14, 15, 16, 17, 18 or 19	0 **40** 50 60 70 80 mg/kg (IP)	387 484 581 677 774	10x 13x 15x 18x 20x	2.9x 3.6x 4.3x 5.1x 5.6x	(Webster and Brown-Woodman , 1990)	Cesarean section as well as skeletal and visceral examination were not performed. Sample size is small. Dosing period does not span organogenesis (GD 6-17) but study is informative as adverse effects are observed after single dose.
Hydronephrosis (all doses),	Spragu e-	GD 0 - 19	2.1 4.2	20.3 mg 40.6	0.54x 1.1x	0.15x 0.30x	(el-Bizri et al., 1991)	Sample size is small. Dosing started earlier

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cerebral hemorrhage, fetal edema (7.6, 15, and 30)	Dawley rats		8.5 17 34 mg/kg (IP)	82.2 164.5 329	2.2x 4.4x 8.8x	0.62x 1.2x 2.5x		than typical organogenesis period (GD 6-17). A developmental NOAEL was not identified as hydronephrosis was reported at all doses. Findings were observed at lower doses and may have been due to the initiation of dosing prior to implantation. Most EFD studies are initiated at GD 6.
Decreased litter weight and fetal weights, not entirely dose dependent.	Sprague-Dawley Rats	GD 7-19	20 30 40 50 mg/kg, BID, SC	194 290 387 484 mg BID	5.2x 7.7x 10x 13x Single dose	1.5x 2.2x 2.9x 3.6x Single dose	(Church et al., 1995)	Not an EFD study. Maternal lethality in HD, decreased BW in all doses. Unclear results to inform labeling.
Location in uterine horn impacts potential cocaine exposure and teratogenicity results.	Sprague Dawley Rat	GD 7-21	30 mg/kg, SC	146 mg	3.9x	1.1x	(Lipton et al., 1998)	Not an EFD study. Study examined impact of location in uterine horn on cocaine exposures.

‡: Referenced MDD is 37.5 mg/day for the 4% solution; mean of 23.44% of the total administered 160 mg dose is absorbed.
Referenced MDD is (b) (4) for the 10% solution; mean of 33.34% of the total administered 400 mg dose is absorbed.

Evaluation of the literature cited above indicates the dosing regimens and endpoints examined are not consistent with ICH S5A study designs and therefore GLP embryofetal development studies with cocaine are warranted. Nonetheless, some of the information in the literature are appropriate for labeling given the absence of definitive GLP studies. Collectively these studies demonstrate that cocaine can produce teratogenic effects at multiples of clinically relevant doses and it is also noted that many of the studies did not clearly define a developmental NOAEL. Taken together, these data suggest that cocaine administration during organogenesis as well as a single high exposure of cocaine can result in significant adverse effects to the fetus and mother. Therefore these data should be included into labeling until more definitive GLP studies are conducted.

Prenatal and Postnatal Development

GLP standard prenatal and postnatal development (PPD) studies have not been conducted. The Applicant submitted several published reports in the literature to characterize the effects of cocaine on pre- and postnatal development in rats and primates, which are summarized in the table below. The reviewer has commented on

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the utility of these studies with respect to typical prenatal and postnatal development study endpoints. As per ICH S5, PPD studies are intended characterize the effects of a drug on the pregnant/lactating female and on development of the conceptus and the offspring after exposure of the female from implantation through weaning. Endpoints in the maternal animals should include clinical signs and mortality, food intake and body weight changes, duration of pregnancy, and partition. Endpoints in the offspring include macroscopic examinations at necropsy, abnormalities, number of live/dead offspring, body weight (at birth, pre- and post-weaning), survival and growth, maturation and fertility of the offspring, physical development, sensory function and reflexes, and behavior. Specific functional testing methods are not outlined by ICH S5; however, studies that assess sensory function, motor activity, and learning and memory are recommended.

Table 23: Pre- and Postnatal Development Studies Identified by the Applicant

Effect Reported	Species	Treatment Period	Doses/NOAEL	HED (mg/day per 60 kg person)	Estimated Exposure Margin for % Cocaine Based on BSA		Citation	Comment
					4%	10%		
No material toxicity Infant toxicity included: Reduced body weight, overall body length, and crown c rcumference No change in infant reflexes when examined at birth	Pregnant Rhesus Monkeys (n-10 per group)	Prior to conception throughout gestation	Escalating doses up to 7.5 mg/kg IM TID, 5 consecutive days per week (22.5 mg/kg/day)	145 mg TID 435 mg/day	3.9x 12x	1.1x 3.3x	(Morris et al., 1997)	Not a typical PPD study, dosing earlier than typical and no lactational exposures. Detailed assessments of offspring.
Reduced offspring survival Of surviving offspring, no change in heart rate or Apgar scores.	Pregnant Rhesus Monkeys	GD 24 to term	0.3 mg/kg/h cocaine SC via mini-osmotic pump (7.2 mg/kg/day)	5.8 mg/h 139 mg/day	0.15x 3.7x	0.043x 1.0x	(Howell et al., 2001)	Not a standard PPD study, no lactational exposure. Endpoints included fetal survival, heart rate, body weight, biparietal diameter, body length and “Apgar scores”
No effect on offspring mortality, birthweights, postnatal	Female Wistar Rat	GD 8-22	30 mg/kg, PO 60 mg/kg, PO	290 mg 580 mg	7.7x 15x	2.2x 4.3x	(Hutchings et al., 1989)	Not a complete PPD study in terms of dosing or endpoints.

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growth and ontogeny of motor activity. HD pups demonstrated heightened motor activity on PND 20-23.								Dams has reduced food and water intake and BW deficits. Controls were pair fed to control for decreased food consumption. Pups were fostered by untreated dams.
No effect on offspring body weights, physical maturation, live births. Deficits in learning of an odor/milk association Increased locomotor activity, attenuated response to footshock ‡without change in sensitivity to shock suggesting cognitive deficit	Female Sprague Dawley Rat	GD 8-20 No maternal toxicity reported.	40 mg/kg SC NOAEL for effects on pups was not identified.	387 mg/day	10x	2.9x	(Spear et al., 1989)	Not a complete PPD study No dosing during lactation period No maternal toxicity reported.
Offspring of cocaine treated and pair fed dams demonstrated higher growth rats but constant deficit in forebrain weights compared to offspring from untreated control or saline dams.	Pregnant Wistar Rats	GD 8-22	60 mg/kg, SC	580 mg	15x	4.4x	(Silva et al., 1995)	Although not a PPD study, does suggest deficits in CNS development in offspring from cocaine treated dams like the results of food deprivation. A NOAEL was not determined.
Prenatal cocaine reduced striatal D2 receptor density in the first week of life but these returned to	Pregnant Sprague-Dawley rats	GD 7-21	60 mg/kg, PO	580 mg	15x	4.4x	(Stadlin et al., 1995)	Not a PPD study, but does suggest potential for CNS changes during rapid brain growth following in utero exposure to cocaine.

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normal by the second week.								
Study suggests differential toxicity during vs. after pregnancy may be due to differential biodistribution of cocaine	Fischer Rat	GD 16, GD 20, post delivery	20 mg/kg, IP	194 mg	5.2x	1.5x	(Dwivedi et al., 1993)	Biodistribution study Clinical significance unclear Not appropriate for labeling at this time
Offspring challenged on PND 20 with cocaine. Prenatal cocaine exposure reduced cocaine induced c-fos in brain and attenuated cocaine-induced behavioral responses	Pregnant Dutch Belted Rabbits	GD 8-29	4 mg/kg, IV BID	77 mg BID (154 mg/day)	2.1x 4.1x	0.58x 1.2x	(Tilakaratne et al., 2001)	Study supports conclusion that cocaine in utero can alter brain development and likely through changes in neurotransmitter receptors. Functional endpoints and species are not standard for a PPD study.

‡: Referenced MDD is 37.5 mg/day for the 4% solution; mean of 23.44% of the total administered 160 mg dose is absorbed. Referenced MDD is (b) (4) for the 10% solution; mean of 33.34% of the total administered 400 mg dose is absorbed.

Below
are some of the published nonclinical studies submitted by the Applicant that evaluated some of the standard pre- and postnatal developmental endpoints, summarized in greater detail.

Study title: The effect of chronic exposure during pregnancy on maternal and infant outcomes in the rhesus monkey (P. Morris, Z. Bintenda, M.P Gilliam, J Klein, K. McMartin, G. Korean, H.M. Duhart, W. Slikker JR. Paule, 1997. Neurotoxicology and Teratogenicity 19(1): 47-57)

GLP compliance: Unknown
Drug, lot #, and % purity: Cocaine, unknown, and 100%

Key Study Findings

Pregnant female monkeys were treated with escalating doses of cocaine hydrochloride up to 7.5 mg/kg three times per day, 5 consecutive days per week, prior to conception and throughout gestation with the following findings:
Cocaine and benzoylecgonine levels were detected in the maternal hair and infant hair at the end of the gestation period.

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1. Cocaine prenatal exposure during pregnancy had no significant effects on maternal indices.

2. Significant effects at birth were observed in the following infant indices: body weight, overall length, and crown circumference.

3. There were no changes in infant reflexes at birth.

4. Based on observed effects on fetal body weight, length, and crown circumference, the fetal LOEL was defined at 15.6 mg/kg (average dose at term).

5. Based on a body surface area comparison, the dose of 15.6 mg/kg in primates (HED of 302 mg/day) is 8.1-times the maximum recommended dose of 160 mg/60 kg person in which 23.44% of cocaine (37.5 mg) is completely absorbed. For the 10% cocaine topical solution, the dose of 15.6 mg is 2.3-times the maximum recommended dose of 400 mg mg/kg person in which 33.34% of cocaine is (133.6 mg) is completely absorbed.

Methods

Doses:	0 (saline), escalating doses was initially started at 1.0 mg/kg (1-2 weeks), followed by 1.5 mg/kg (1 to 2 weeks), the dose was gradually increased to as much as 2.5-3.0 mg/kg/injection in some monkeys
Frequency of dosing:	Prior to conception and throughout gestation, three times per day, 5 consecutive days per week
Dose volume:	0.1 mL/kg
Route of administration:	Intramuscular
Formulation/Vehicle:	Saline
Species/Strain:	Primates/Rhesus monkey
Number/Sex/Group:	10/female/group

Observations and Results

Clinical Observation (Female)	Body weight at term Body weight gain at term Body weight gain during pregnancy
Fetal Observations	Body weight Overall length Crown-to-rump length Rump-to-heel length Biparietal diameter Crown circumference
Infant's Behavioral Measures (at birth)	The following reflex measured once: vocalization (the presence of spontaneous vocalization by the infant), rooting (the infant directs

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	its mouth toward an index finger placed on its cheek near the corner of the mouth), eye blink to air puff (the infant blinks in response to a puff of air directed at its face), eye blink to a glabellar tap (the infant blinks in response to the tap of an index finger on its forehead between the eyes), pupillary response (the infant's pupils contract in response to a bright examination light), nystagmus (the presence of uncoordinated or asymmetric movements of the infant's eyeballs), visual orientation (the infant directs its gaze at different objects in the visual field), visual following (the infant follows the movement of an index finger across the visual field), auditory orientation (the infant turns its head toward the sound of a lips-smack made at the side of the head), auditory startle (the infant exhibits startling to a loud shout), placing (the infant places its hand/foot on the surface of the examining table in response to the stroke of an index finger on the dorsum of its hand/foot), prone progression (the infant attempts to move forward/backward when placed prone on the surface of the examining table), plantar grasp (the infant grasps an index finger placed on the palmar surface of its hand), Hoffman sign (the infant flexes its thumb in response to the stretch of a finger) Moro reflex (the infant struggles to remain upright when held supine and its head is suddenly unsupported), and Babinski reflex (the infant flexes its toes in response to the stroke of an index finger across the plantar surface of its foot).
Toxicokinetic Analysis	Not performed. However, at end of gestation, hair sample was collected from the mothers and infants to measure cocaine and benzoylecgonine level.

Mortality

No treatment mortality was observed in the dams.

Maternal Indices

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EFFECT OF CHRONIC COCAINE EXPOSURE ON MATERNAL OUTCOME: PHYSICAL MEASURES

Index of Outcome	Experimental Group: Control (N = 10)	Experimental Group: Cocaine (N = 10)
Body weight at term (kg)	8.14 ± 0.40	7.52 ± 0.34
Body weight gain during pregnancy (kg)	1.36 ± 0.20	1.15 ± 0.25
Length of pregnancy (days)	165.10 ± 2.13	163.00 ± 1.21

Rhesus monkeys were assigned to either a Control group (N = 10) or a Cocaine group (N = 10) that was administered (IM) escalating doses of cocaine, three times per day, 5 consecutive days per week, prior to conception and throughout gestation. The physical measures shown here were obtained from the mothers throughout gestation. Data represent means ± SEMs.

As depicted in the author's table (Table 5, page 54) above, no significant treatment-related effects in maternal primate indices were reported. "There were no significant correlations between any of the indices of maternal outcome and any of the indices of infant outcome."

Infant Indices

EFFECT OF CHRONIC COCAINE EXPOSURE ON INFANT OUTCOME: PHYSICAL MEASURES

Index of Outcome	Experimental Group: Control (N = 10)	Experimental Group: Cocaine (N = 10)
Body weight (kg)	0.49 ± 0.01	0.43 ± 0.01*
Overall length (cm)	29.86 ± 0.53	27.75 ± 0.70*
Crown-to-rump length (cm)	18.96 ± 0.48	17.50 ± 0.54
Rump-to-heel length (cm)	10.90 ± 0.41	10.25 ± 0.21
Biparietal diameter (cm)	5.13 ± 0.08	5.09 ± 0.10
Crown circumference (cm)	19.25 ± 0.20	18.45 ± 0.24*

Rhesus monkeys were assigned to either a Control group (N = 10) or a Cocaine group (N = 10) that was administered (IM) escalating doses of cocaine, three times per day, 5 consecutive days per week, prior to conception and throughout gestation. The physical measures shown here were obtained from their infants at birth. Data represent means ± SEMs.

*Cocaine significantly different from Control ($p < 0.05$, unpaired Student's t-test).

Infant indices data is presented in the authors' table (Table 6, page 54) above. At birth, body weight (-12.2%), overall length (-7.1%), and crown circumference (-4.2%) were significantly (p<0.05) reduced compared to control infants. No significant treatment-related effects in infant indices at birth with respect to: crown-to-rump length, rump-to-heel length, and biparietal diameter. The infant reflexes in infants exposed to cocaine

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during gestation were comparable to control infants. No significant differences in the cumulative score were observed.

EFFECT OF CHRONIC COCAINE EXPOSURE ON INFANT OUTCOME: BEHAVIORAL MEASURES

	Experimental Group	
Reflex	Control (N = 10)	Cocaine (N = 10)
Vocalization	1.00 ± 0.00	1.00 ± 0.00
Rooting	1.00 ± 0.00	1.00 ± 0.00
Eye blink (puff)	1.00 ± 0.00	1.00 ± 0.00
Eye blink (tap)	1.00 ± 0.00	1.00 ± 0.00
Pupillary response	1.00 ± 0.00	1.00 ± 0.00
Nystagmus	0.00 ± 0.00	0.00 ± 0.00
Visual orientation	1.00 ± 0.00	0.80 ± 0.13
Visual following	0.10 ± 0.09	0.10 ± 0.10
Auditory orientation	0.80 ± 0.13	0.80 ± 0.13
Auditory startle	0.50 ± 0.17	0.10 ± 0.10
Placing	1.00 ± 0.00	1.00 ± 0.00
Prone progression	0.90 ± 0.09	1.00 ± 0.00
Plantar grasp	1.00 ± 0.00	1.00 ± 0.00
Moro reflex	1.00 ± 0.00	1.00 ± 0.00
Babinski reflex	1.00 ± 0.00	1.00 ± 0.00
Cumulative reflex score	11.50 ± 0.27	11.10 ± 0.38

Rhesus monkeys were assigned to either a Control group (N = 10) or a Cocaine group (N = 10) that was administered (IM) escalating doses of cocaine, three times per day, 5 consecutive days per week, prior to conception and throughout gestation. The reflexes shown here were tested in their infants at birth. Assigning a value of "1" for the presence of the reflex and a value of "0" for the absence of the reflex, the algebraic sum of these values was considered a cumulative reflex score. Data represent means ± SEMs.

Toxicokinetic Analysis

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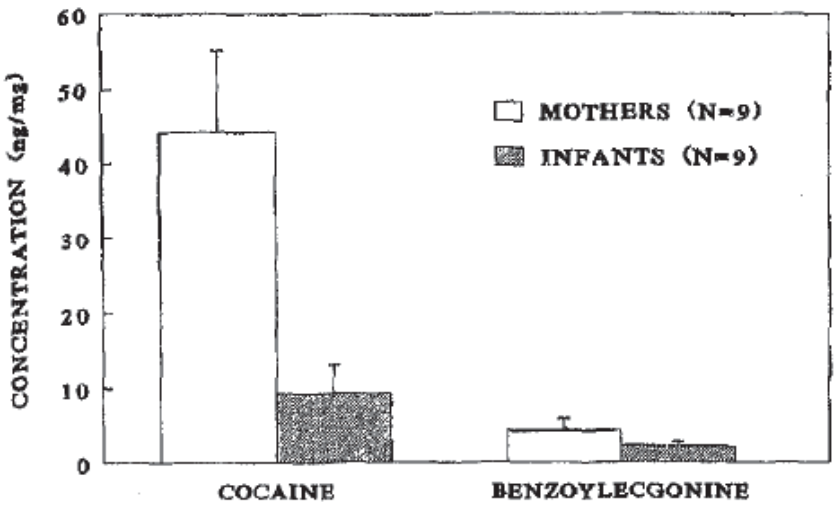

FIG. 2. Effect of chronic cocaine exposure on biochemical measures: maternal and infant hair cocaine and benzoylecgonine. Data represent the levels (ng/mg), as determined by RIA, of cocaine and benzoylecgonine in the first 1 cm (measured from the scalp) of maternal and infant hair samples taken at term, following chronic administration of cocaine to the mothers prior to conception and throughout gestation.

Hair analysis data is presented in the author's figure (Figure 1, page 2) above. Detectable levels of cocaine and benzoylecgonine were measured in all samples of maternal and infant hair from the cocaine treatment group. Hair cocaine levels were approximately 44 and 9 ng/mg for the mothers and the infants, respectively. Cocaine and benzoylecgonine were detected in maternal plasma. Plasma cocaine levels increased rapidly reaching C_{max} at approximately 175 ng/mL at 15 minutes following intramuscular injection; then steadily declined to approximately 30 ng/mL by 120 minutes after injection. The C_{max} level correspond to approximately 4 times the human plasma level of 43 ng/mL following use of this product. AUC data in the primate study were not provided.

Study title: Fetal development in rhesus monkeys exposed prenatally to cocaine (Howell LL, Schama KF, Ellis JE, Grimley PJ, Kitchens AJ, Byrd LD. 2001. Neurotoxicology and Teratology 23:133-140)

GLP compliance:	Unknown
Drug, lot #, and % purity:	Cocaine, unknown, and unknown

Key Study Findings

Pregnant female monkeys were treated with 0.3 mg/kg/h via SC osmotic minipump from postconception Day 24 to birth (Group 1) and conception through Day 42 of gestation (Group 2) with the following findings in the offspring delivered at term (Howell et al., 2001):

1. Cocaine exposure resulted in 22% reduced fetal survival

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2. No evidences that prenatal cocaine exposure were associated with abnormal physical growth in surviving fetuses.

3. No developmental toxicity was observed in surviving fetuses.

4. Survival rate of maternal animals was significantly affected in both early- and late-exposure to cocaine.

Methods

Doses:	Continuous SC infusion of Saline or cocaine 0.3 mg/kg/h via Alzet osmotic minipumps
Frequency of dosing:	Postconception Day 24 to gestation (Group 1); Conception through Day 42 of gestation (Group 2)
Dose volume:	Not specified
Route of administration:	Subcutaneous (osmotic minipump)
Formulation/Vehicle:	Saline
Species/Strain:	Primates/Rhesus monkey
Number/Sex/Group:	18/female/group 1; Group 2: n =12 (treatment group, n= 8 (vehicle control group)

Observations and Results

Clinical Observation (Female)	Food consumption (daily) Body weight (weekly) throughout gestation; Body weight at term
Fetal Observations	Diagnostic ultrasonography (at 28-day intervals) to visualize and confirm implantation of the fertilized ovum and to monitor utero development throughout gestation Implantation and development of the blastocyst 24 days after breeding, Development and positioning of the umbilical cord Visualization of the heart beat and cardiac activity Head circumference (biparietal diameter)
Postnatal Development at Birth (immediately after upon removal from their mothers and at two subsequent 30-min intervals)	Gestational age Body weight Biparietal diameter Body length (crown to rump) Apgar scale (heart rate, respiratory effect, muscle tone, behavioral state and color) was used to allow early assessment of neonate physical and behavioral states
Toxicokinetic Analysis	Maternal blood sample.

Mortality

No treatment mortality was observed in the dams.

Maternal Data

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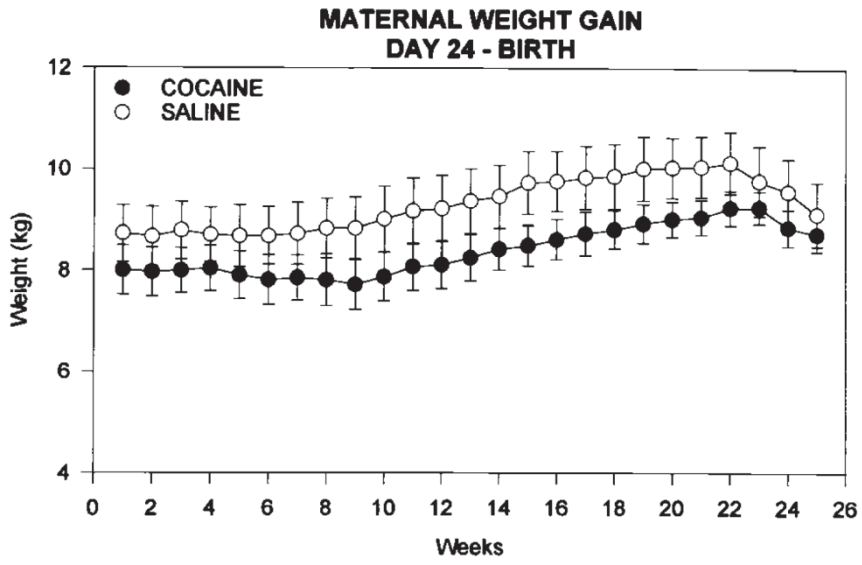

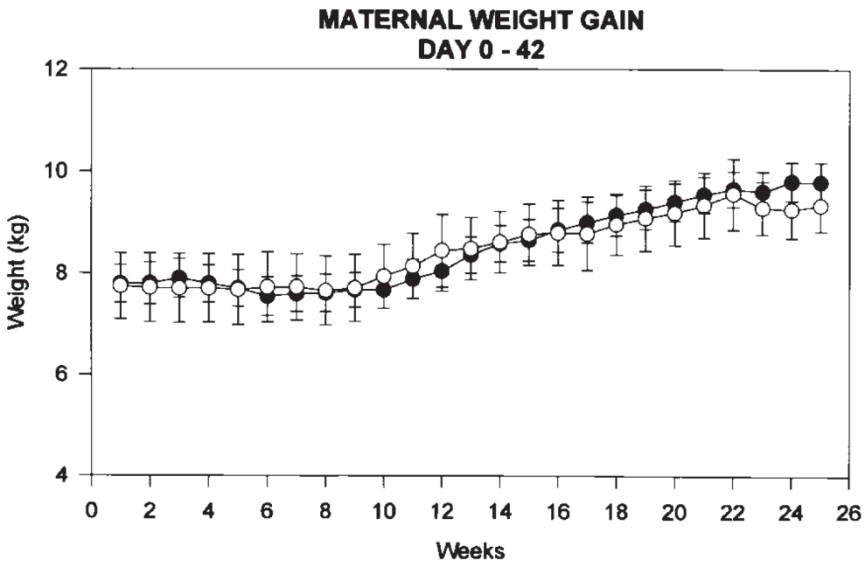

As depicted in the author's figure (Figure 3, Page 137) above, body weight increased during pregnancy (Gestation Day 24 to birth, Gestation Day 0-42). There were no significant differences in weight gain of the cocaine groups and saline groups. The mean body weight in animals exposed to cocaine was 9.2 ± 0.3 kg from Day 24 postconception through gestation. The average body weight in animals exposed to cocaine from conception through Day 42 of gestation was 9.8 ± 0.2 kg.

Fetuses Data

Survival rates were significantly lower in cocaine-exposed fetuses when results were combined for the late- and early-exposure groups. In the late-exposure group, 61% resulted in live births compared to 78% of live births in the control group. Similarly, in

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the early-exposure group, 67% resulted in live births compared to 75% in the pair-fed control group. Spontaneous abortion occurred in four fetuses and two fetuses in the cocaine-exposed group and control group, respectively. In the cocaine-exposed group, one fetus was spontaneously aborted, one was resorbed and two were born dead. One fetus was spontaneously aborted and one was born dead in the control group.

Pregnancy outcomes

	Total pregnancies	Total survived	% Survival
Saline (day 24–birth)	18	14	78
Cocaine (day 24–birth)	18	11	61
Saline (days 0–42)	8	6	75
Cocaine (days 0–42)	12	8	67

Chi-squared analysis was used to determine the statistical significance of survival rates. When data were combined for the late- and early-exposure groups, survival rates were significantly ($P < .05$) lower in cocaine-exposed fetuses.

As depicted in the author's table (Table 6, page 138) below, fetal heart rate was in the normal range. Also, fetal biparietal diameter increased normally during gestation.

Table 6
Fetal heart rate and biparietal diameter

	Month				
	2	3	4	5	6
Heart rate (beats/min)					
Saline (day 24 birth)	188±4	192±2	184±5	190±5	180±4
Cocaine (day 24 birth)	186±4	196±4	194±4	200±4	190±3
Saline (days 0 42)	177±4	187±6	189±6	181±6	174±7
Cocaine (days 0 42)	182±4	186±3	192±9	184±5	179±8
Biparietal diameter (cm)					
Saline (day 24 birth)		2.5±0.1	3.7±0.1	4.3±0.1	4.6±0.0
Cocaine (day 24 birth)		2.7±0.1	3.6±0.1	4.5±0.1	4.6±0.1
Saline (days 0 42)		2.3±0.1	3.7±0.1	4.4±0.1	4.7±0.0
Cocaine (days 0 42)		2.5±0.0	3.5±0.1	4.3±0.1	4.7±0.1

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Table 7
Physical growth measures at birth

	Gestational age (days)	Weight (g)	BPD (cm)	Crown-rump (cm)
Saline (day 24 birth)	170.5±2.2	524.9±23.2	5.1±0.1	19.9±0.4
Cocaine (day 24 birth)	163.9±2.6	499.9±28.4	5.0±0.1	19.2±0.4
Saline (days 0 42)	160.7±6.1	464.3±42.9	5.0±0.2	18.7±0.5
Cocaine (days 0 42)	174.1±1.9	531.0±21.9	5.1±0.1	19.6±0.3

As depicted in the author's table above (Table 7, page 138), mean gestational length was within normal range for both cocaine and saline groups. No significant differences between control and cocaine groups were observed on body weight, biparietal diameter, and body length.

At birth, no significant differences in Apgar scores were observed between cocaine and control groups.

Table 8
Apgar scores

Time	1	2	3
Saline (day 24–birth)	8.5±0.8	9.1±0.5	9.1±0.5
Cocaine (day 24–birth)	9.4±0.3	9.5±0.4	9.7±0.2
Saline (days 0–42)	8.2±1.0	8.2±1.3	8.0±1.2
Cocaine (days 0–42)	8.9±0.6	8.6±0.6	9.3±0.3

Apgar test — modified for rhesus monkeys[a]

Sign	0	1	2
Respiration	no respiration	<60/min	>60/min
Heart rate	no heart rate	150 bpm	>150 bpm
Muscle tone	flaccid	some tone	flexed
Skin color	gray	gray and pink	pink
State (irritability)	unconscious	passive	active

[a] Apgar scores are assigned at birth and at two subsequent 20-min intervals.

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Toxicokinetic Analysis

Mean maternal plasma level was 151 ng/mL, approximately 3.5 times the reported human plasma levels. However, AUC data were not provided.

PPD Overall Conclusions

As noted in the data summarized above, although the primate study conducted by NCTR was not a traditional PPD study, they did dose over the entire duration of gestation typically covered by a traditional PPD study. The existing data also suggest adverse effects on the offspring in terms of survival and growth. The existing data fail to test reproductive capacity of the offspring, a standard endpoint in a modern PPD study. Although the existing literature suggest adverse effects are likely to occur in a PPD study, given the lack of reproductive testing, lack of exposure via lactation, evidence for drug accumulation in milk, and lack of adequate toxicokinetic data to fully inform these endpoints, a dedicated rat PPD study is recommended as a postmarketing requirement.

10 Special Toxicology Studies

Juvenile Animal Studies

Juvenile animal studies were not completed by the Applicant to support this application. Rather, the Applicant conducted a literature review to address the safety of the drug product for use in pediatric patients. During the review of the proposed pediatric study plan (PSP) prior to submission of the NDA, the Applicant, the Division, and PeRC agreed that there is a potential use for this drug in children down to age 0. Further, it was agreed that the Applicant will conduct three juvenile studies to support their proposed clinical studies if the Applicant is unable to provide the Agency with adequate data to address the impact of cocaine hydrochloride on the central nervous development in adolescent and children pediatric subjects (0 to 17 years of age). The Division agreed to review the submitted literature and if the literature were deemed adequate, no new juvenile animal studies would be required. However, the literature would have to adequately define a NOAEL and characterize the toxicological profile of cocaine on neonatal and adolescent brain development or dedicated juvenile animal studies would be required.

During the course of the NDA review, the Division and PeRC agreed with the Applicant's partial waiver of pediatric patients under the age of 8 and deferral of pediatrics 8 to 17 years for the 4% topical cocaine solution. The Applicant was requested to provide adequate information to justify their waiver and deferral request. Until it is determined what studies are required, we cannot comment on the adequacy of the existing literature. The PeRC agreed with the Applicant April 25, 2018 request for

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(b) (4)

Juvenile animal studies are intended to obtain safety data for pediatric clinical dosing. They are generally targeted toxicity studies focusing on the developing organ systems of concern for a specific drug and are recommended if the target organ of toxicity for a given drug is undergoing significant changes in the indicated patient population. Cocaine is a CNS active drug. The CNS undergoes dramatic changes over the course of development and is potentially uniquely vulnerable over the entire age range from prior to birth to 20 plus years of age. Given the known CNS target of the drug, juvenile animal studies are recommended to characterize the impact of cocaine during critical periods of brain development.

The Applicant cited three abstracts and one publication in their summary for the Juvenile Toxicity section of the Toxicology Written Summary. Because abstracts are considered preliminary data, may not be reproducible, and are not peer reviewed, these will not be reviewed here. The single publication (NIDA monograph) did not dose juvenile animals and will also not be reviewed here.

The Applicant did reference a few published studies in their discussion of pre- and postnatal development studies that are actually juvenile animal studies. Magalhaes et al. doses male and female Wistar rat pups with 15 mg/kg cocaine hydrochloride via SC injection twice daily from PND 1 to 27 and examined forced swim behavior on PND 26 and 27 (Magalhaes et al., 2002). They report that cocaine treated animals were less active in the forced swim test and displayed clinical signs interpreted to be consistent with "behavioral despair" suggesting altered ability to cope with stressful situations.

Chen et al. (1993) doses Sprague Dawley rat pups with 40, 60, or 80 mg/kg cocaine from PND 4-9. Pups in the 60 and 80 mg/kg group demonstrated a dose-dependent reduction in survival. All doses produced reduced body weights. There were no reported effects on the mass of the whole brain, forebrain, cerebellum, or brainstem.

A summary of the reviewed literature cited by the Applicant is shown in the Table below. It is noted that a vast amount of literature is available for cocaine and brain development, and the number of publications submitted by the Applicant is not considered exhaustive. As the human pediatric dose is not yet determined and the size of the pledget will likely be smaller pending age of use, the total daily dose will likely be lower for some age groups. As such, exposure margin estimates are not provided at this time.

Table 24: Summary of Submitted "Juvenile Animal Studies"

Key Findings Reported	Species	Ages Tested	Dosing regimen and NOAEL if applicable	Citation	Comment

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Key Findings Reported	Species	Ages Tested	Dosing regimen and NOAEL if applicable	Citation	Comment
Dose-dependent reduction in survival rate was observed (60 and 80 mg/kg). All doses reduced body weight No effects on the mass of total brain or forebraln, cerebellum, or brainstem.	Sprague-Dawley Rats (Neonates)	PND 4 – PND 9/SC Corresponds to third trimester in humans	40, 60, 80 mg/kg SC No NOAEL based on body weight reductions	(Chen et al., 1993)	Dosed during brain growth spurt in rat. Measured survival rate, somatic growth, and brain development (brain weight only) Crude brain assessments, not appropriate for labeling.
Cocaine exposure during PND 1-10, but not PND 11-20, resulted in a small increased ASR amplitude on the second day of testing, a finding consistent with an alteration in long-term habituation.	Sprague–Dawley rats (female only)	PND 1–10 or PND 11–20 Corresponds to third trimester and first two years of life in terms of brain development	50 mg/kg, SC	(Dow-Edwards and Hughes, 1995)	Examined acoustic startle response (ASR)
Cocaine treated animals were less active in the forced swim test and displayed clinical signs interpreted to be consistent with "behavioral despair" suggesting altered ability to cope with stressful s tuations	Wistar rat pups	PND 1-27	15 mg/kg SC	(Magalhaes et al., 2002)	Examined forced swim test

The literature provided by the Applicant are inadequate to characterize the impact of cocaine on the developing brain. Should the application be approved, dedicated juvenile animal studies are recommended. The exact nature of the studies will be determined once the actual pediatric age range is established in the PSP.

11 Integrated Summary and Safety Evaluation

Cody Laboratories, Inc submitted a 505(b)(2) NDA for cocaine hydrochloride, a 4% and 10% topical solution formulation, for the indication of induction of local anesthesia when performing diagnostic procedures and surgeries on or through the mucous membranes of the nasal cavities in adults. The NDA is not relying on an Agency finding of safety and efficacy of any other FDA-approved product. Prior to this NDA submission, the Division informed the Applicant that any impurity or degradant that exceeded ICH Q3A(R2)/Q3B(R2) thresholds would have to be qualified for safety. Also, potential genotoxic impurities should be adequately controlled and adequate information on

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extractables and leachables must be provided to support the safety of the container closure system. In this NDA application, the Applicant submitted literature, a 14-day toxicity study in rats, and extractables/leachables data. A battery of genotoxicity assays, including Ames, in vitro chromosome aberration, in vivo bone marrow micronucleus, and Comet assays, was also submitted in the NDA.

General Toxicology

A 14-day local tolerance study (with a 14-day recovery period) with the drug product spiked with several impurities was conducted to establish local safety and qualify several impurities and excipients. Sprague-Dawley rats received intranasal instillation of test article vehicle (saline), 4% (5.4 to 9.6 mg/kg) and 10% (13.5 to 23.7 mg/kg) cocaine solution spiked with (b) (4) benzoate in rats for 14 consecutive days. No treatment-related mortalities or adverse treatment-related changes in hematology, clinical chemistry, or macroscopic findings at necropsy were reported. There were no test article-related microscopic changes observed in the nasal cavity or olfactory bulb after intranasal administration of the spiked cocaine solution. A local NOEL of 23.7 mg/kg/day for cocaine (10%) containing (b) (4) % benzoate was established due to the absence of adverse findings in the nasal cavity or olfactory bulb. The safety of these impurities was qualified via the intranasal route in this 14-day local tolerance study.

Impurities

A battery of genotoxicity assays, including Ames, in vitro chromosome aberration, in vivo bone marrow micronucleus, and comet assays were conducted to characterize the genotoxic potential of several drug substance impurities/degradants that were above qualification thresholds. The impurities (b) (4) are process impurities and degradation products. They were not genotoxic in the Ames assay. (b) (4) was not clastogenic in the chromosomal aberration assay; whereas (b) (4) was clastogenic in the chromosomal aberration assay. In the in vivo micronucleus study and Comet assay, (b) (4) was not genotoxic. The weight of evidence suggests that (b) (4) does not present a risk for genetic toxicity.

	Specification	
Impurity	**NDA Limit**	**Reviewer's Comment**
(b) (4)		• (b) (4) drug product degradant of cocaine and a process impurity. • Genotoxicity study with (b) (4) was negative in the Ames assay. • Not clastogenic: negative chromosomal aberration in vitro assay. • The local safety of this impurity was qualified via a 14-day local tolerance study. Negative findings in this study. • At NMT (b) (4)

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Impurity	Specification NDA Limit	Reviewer's Comment
		Acceptable as per ICH Q3A(R2)
(b) (4)		• Specs exceeds ICH Q3A(R2) qualification threshold • Not genotoxic: Weight of evidence indicates that (b) (4) was negative in genotoxicity studies. ○ Negative in the Ames assay. ○ Not genotoxic; positive in the in vitro chromosomal aberration assay but was negative in the in vivo Micronucleus and Comet assays. • The local safety of this impurity was qualified via a 14-day local tolerance study. Negative findings in this study. • At NMT (b) (4) *Acceptable as per ICH Q3A(R2)*
(b) (4)		• Specs exceeds ICH Q3A(R2) qualification threshold • A process impurity and drug product degradant. • Not genotoxic: Weight of evidence indicate that (b) (4) was negative in genotoxicity studies. ○ Genotoxicity study with (b) (4) was negative in the Ames assay. ○ Not clastogenic: negative chromosomal aberration in vitro assay. • The local safety of this impurity was qualified via a 14-day local tolerance study. Negative findings in this study. • At NMT (b) (4) *Acceptable as per ICH Q3A(R2)*

Likewise, (b) (4) is also a drug product degradant the Applicant has requested a specification of NMT (b) (4)%, which is clearly above ICH Q3B(R2) qualification thresholds. (b) (4). The local tolerance study and genetic toxicology studies provided adequate safety justification.

Safety of Container Closure System

The container closure system consists of an (b) (4) glass bottle ((b) (4) with a (b) (4) cap closure. (b) (4) ontainer/closure configurations are proposed for the two cocaine topical solutions strengths (4% and 10%). The 4% strength will be available in a 4-mL single-use bottles and 10-mL multi-dose bottles. (b) (4). The Applicant conducted an extraction study, two simulation leachables studies, and leachables evaluations. The Applicant also provided a toxicological risk assessment for those compounds that exceeded the qualification

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threshold of 5 mcg/day. Deficiencies identified by the Reviewer included: 1) An adequate leachable evaluation was not submitted. The conditions of the study design used in the leachables studies were not accurate. During the Pre-NDA meeting, the Applicant was advised that the leachables evaluation should be performed on three batches of the to-be-marketed drug product and include assessments at multiple timepoints over the course of the drug product stability. However, the leachables studies were performed with one batch for 16-months (end of the shelf-life). 2) The Applicant did not adequately characterize the potential leachables from the container closure system over the course of the stability studies to support the proposed expiration data. 3) The Applicant did not provide adequate information to demonstrate that the extractables data correlate with the leachables data. An adequate leachable evaluation was not submitted. It is unclear if the leachable data accurately reflect the levels of potential leachables in the final drug product; (b) (4)

> Conduct an adequate leachable safety assessment for your container closure system. This assessment must include leachable data from long-term studies testing at least three batches at multiple time points over the shelf-life of your drug product. Submit a toxicological assessment justifying the safety of the maximum level achieved over the course of stability for any leachable that exceeds 5 mcg/day, taking into consideration the maximum daily dose of the drug product.

Reproductive Toxicology

The standard battery of reproductive toxicology studies have not been conducted in animals with cocaine to fully characterize the effects of cocaine on reproduction and development. The Applicant addressed cocaine's potential reproductive effects from the published literature. Several published reports in the literature have studied the effects of cocaine on male fertility. Results from these studies have provided evidence that chronic administration of cocaine did not affect sexual behavior or fertility in adult male rats. Chronic cocaine administration to peripubertal rats did adversely affect the testes. Results from these studies provided evidence that cocaine administration can have deleterious effect on spermatogenesis and fertility although shorter term exposures are less likely to be adverse in males. These studies have also suggested that an acute dose of cocaine can alter female reproductive hormones and that cocaine may impact testicular function. However, the submitted studies did not adequately address cocaine effects on fertility and early embryonic development.

Select articles from the published literature that characterized cocaine exposure in mice and rats on embryo-fetal development endpoints were summarized. Cocaine was found to be teratogenic in mice and rats. Following intravenous, subcutaneous, and intraperitoneal administration, the primary malformations observed included urogenital defects such as hydronephrosis and cryptorchidism. The primary malformations

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observed following subcutaneous injection of cocaine included ocular defects such as anophthalmia and microphthalmia, and various defects in skeletal ossification. Embryo-fetal developmental studies were evaluated in Sprague-Dawley and Long-Evans rats using subcutaneous, and intraperitoneal routes during organogenesis. Findings from these studies support the conclusion that cocaine was found to be teratogenic in rats following subcutaneous, and intraperitoneal administration. The clinical significance of these studies are difficult to interpret without adequate toxicokinetic data.

Several published reports in the literature to characterize the effects of cocaine on pre- and postnatal development in rats and primates. Adverse fetal effects were noted at doses that exceed the predicted human exposure based on body surface area comparisons. Findings in primates suggest that prenatal cocaine exposure can induce subtle neurobehavioral deficits at doses that likely exceed the human exposures based on body surface area.

Juvenile Animal Studies

Juvenile animal studies have not been conducted. The Applicant addressed the effect of cocaine in juvenile animal from the published literature. The submitted studies did not adequately characterize the effect of cocaine on the developing brain or the safety of cocaine in pediatrics. The standard endpoints in juvenile studies were not addressed in most of the submitted published literature; the potential adverse effects in reflex ontogeny, sensory, motor, and cognitive function were not addressed. Also, the impact of cocaine during critical periods of brain development was not addressed. Although the current NDA submission is only seeking approval for use of this drug in adults, this drug will have a PREA required PMR. As noted in the agreed Pediatric Study Plan (PSP), juvenile animal studies will be required to support future clinical studies in pediatric patients. The Applicant has agreed to conduct juvenile animal studies to characterize the impact of cocaine on the developing brain and assess the potential for long-term cognitive/behavioral changes in animals following exposure during the critical periods of brain development.

12 Appendix/Attachments

Abel EL, Moore C, Waselewsky D, Zajac C and Russell LD (1989) Effects of cocaine hydrochloride on reproductive function and sexual behavior of male rats and on the behavior of their offspring. *J Androl* **10**:17-27.

Al-Motabagani MA and Mohamed AS (2005) Congenital malformations in mice induced by addiction to alcohol and cocaine. *East Afr Med J* **82**:433-438.

Barroso-Moguel R, Mendez-Armenta M and Villeda-Hernandez J (1994) Testicular lesions by chronic administration of cocaine in rats. *J Appl Toxicol* **14**:37-41.

Chen CJ and Vandenbergh JG (1994) Effect of chronic cocaine on reproduction in female house mice. *Pharmacol Biochem Behav* **48**:909-913.

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Chen WJ, Andersen KH and West JR (1993) Cocaine exposure during the brain growth spurt: studies of neonatal survival, somatic growth, and brain development. *Neurotoxicol Teratol* **15**:267-273.

Church MW, Morbach CA and Subramanian MG (1995) Comparative effects of prenatal cocaine, alcohol, and undernutrition on maternal/fetal toxicity and fetal body composition in the Sprague-Dawley rat with observations on strain-dependent differences. *Neurotoxicol Teratol* **17**:559-567.

Dow-Edwards DL and Hughes HE (1995) Adult reactivity in rats exposed to cocaine during two early postnatal periods. *Neurotoxicol Teratol* **17**:553-557.

Dwivedi C, Engineer FN and Vaughan SL (1993) Alterations in biodistribution of cocaine may explain differential toxicity in pregnant and postpartum rats. *Toxicol Appl Pharmacol* **118**:131-134.

el-Bizri H, Guest I and Varma DR (1991) Effects of cocaine on rat embryo development in vivo and in cultures. *Pediatr Res* **29**:187-190.

Fantel AG and Macphail BJ (1982) The teratogenicity of cocaine. *Teratology* **26**:17-19.

Fiere-Garbal M, Nunez MJ, Losada C, Pereiro D, Castro-Balano C, Saburido XL, Nunez R, Matos L, Varela-Patino MP and Rey-Mendez M (1994) Effects of cocaine on the development of moloney sarcoma virus-induced tumors in mice. *Research Communications in Substances of Abuse* **15**:105-112.

Fisher JE, Potturi RB, Collins M, Resnick E and Zimmerman EF (1994) Cocaine-induced embryonic cardiovascular disruption in mice. *Teratology* **49**:182-191.

George VK, Li H, Teloken C, Grignon DJ, Lawrence WD and Dhabuwala CB (1996) Effects of long-term cocaine exposure on spermatogenesis and fertility in peripubertal male rats. *J Urol* **155**:327-331.

Howell LL, Schama KF, Ellis JE, Grimley PJ, Kitchens AJ and Byrd LD (2001) Fetal development in rhesus monkeys exposed prenatally to cocaine. *Neurotoxicol Teratol* **23**:133-140.

Hunter ES, 3rd, Kotch LE, Cefalo RC and Sadler TW (1995) Effects of cocaine administration during early organogenesis on prenatal development and postnatal growth in mice. *Fundam Appl Toxicol* **28**:177-186.

Hutchings DE, Fico TA and Dow-Edwards DL (1989) Prenatal cocaine: maternal toxicity, fetal effects and locomotor activity in rat offspring. *Neurotoxicol Teratol* **11**:65-69.

Johanson CE and Schuster CR (2000) Cocaine, American College of Neuropsychopharmacology.

Kaufmann RA, Savoy-Moore RT, Sacco AG and Subramanian MG (1990) The effect of cocaine on oocyte development and the follicular microenvironment in the rabbit. *Fertil Steril* **54**:921-926.

King TS, Canez MS, Gaskill S, Javors MA and Schenken RS (1993) Chronic cocaine disruption of estrous cyclicity in the rat: dose-dependent effects. *J Pharmacol Exp Ther* **264**:29-34.

Kunko PM, Moyer D and Robinson SE (1993) Intravenous gestational cocaine in rats: effects on offspring development and weanling behavior. *Neurotoxicol Teratol* **15**:335-344.

Reference ID: 4263670

Li H, George VK, Bianco FJ, Jr., Lawrence WD and Dhabuwala CB (1997) Histopathological changes in the testes of prepubertal male rats after chronic administration of cocaine. *J Environ Pathol Toxicol Oncol* **16**:67-71.

Li H, George VK, Crawford SC and Dhabuwala CB (1999) Effect of cocaine on testicular blood flow in rats: evaluation by percutaneous injection of xenon-133. *J Environ Pathol Toxicol Oncol* **18**:73-77.

Lipton JW, Robie HC, Ling Z, Weese-Mayer DE and Carvey PM (1998) The magnitude of brain dopamine depletion from prenatal cocaine exposure is a function of uterine position. *Neurotoxicol Teratol* **20**:373-382.

Magalhaes A, Tavares MA and de Sousa L (2002) Postnatal cocaine exposure: effects on behavior of rats in forced swim test. *Ann N Y Acad Sci* **965**:529-534.

Mahalik MP, Gautieri RF and Mann DE, Jr. (1980) Teratogenic potential of cocaine hydrochloride in CF-1 mice. *J Pharm Sci* **69**:703-706.

Middaugh LD, Boggan WO, Bingel SA, Patrick KS and Xu W (1996) A murine model of prenatal cocaine exposure: effects on the mother and the fetus. *Pharmacol Biochem Behav* **55**:565-574.

Morris P, Binienda Z, Gillam MP, Klein J, McMartin K, Koren G, Duhart HM, Slikker W, Jr. and Paule MG (1997) The effect of chronic cocaine exposure throughout pregnancy on maternal and infant outcomes in the rhesus monkey. *Neurotoxicol Teratol* **19**:47-57.

Ohnaka H, Ukita K, Yamamasu S, Inoue M, Imanaka M, Ishiko O and Ogita S (2001) Effects of cocaine and ethanol on mouse fetuses. *Osaka City Med J* **47**:83-93.

Potter DA, Moreno A, Luther MF, Eddy CA, Siler-Khodr TM, King TS and Schenken RS (1998) Effects of follicular-phase cocaine administration on menstrual and ovarian cyclicity in rhesus monkeys. *Am J Obstet Gynecol* **178**:118-125.

Rosenkranz HS and Klopman G (1990) The carcinogenic potential of cocaine. *Cancer Lett* **52**:243-246.

Salvadori DM, Barbisan LF, Bazo AP, de Santana EQ, Denadai R, de Oliveira SV, Ribeiro LR and de Camargo JL (1998) Cocaine mutagenicity and hepatocarcinogenicity evaluations in rodents. *Teratog Carcinog Mutagen* **18**:199-208.

Scholz A (2002) Mechanisms of (local) anaesthetics on voltage-gated sodium and other ion channels. *Br J Anaesth* **89**:52-61.

Silva MC, Silva-Araujo A, Abreu S, Xavier MR, Monteiro LS and Tavares MA (1995) Effects of prenatal cocaine exposure on postnatal growth patterns of male Wistar rats. *Neurotoxicol Teratol* **17**:471-477.

Song J, Guan XW, Ren JQ and He W (2002) Developmental toxicity of cocaine exposure in mid-pregnancy mice. *Acta Pharmacol Sin* **23**:1029-1034.

Spear LP, Kirstein CL, Bell J, Yoottanasumpun V, Greenbaum R, O'Shea J, Hoffmann H and Spear NE (1989) Effects of prenatal cocaine exposure on behavior during the early postnatal period. *Neurotoxicol Teratol* **11**:57-63.

Stadlin A, Choi HL, Tsim KW and Tsang D (1995) Prenatal cocaine exposure revealed minimal postnatal changes in rat striatal dopamine D2 receptor sites and mRNA levels in the offspring. *Mol Neurobiol* **11**:67-76.

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Tilakaratne N, Cai G and Friedman E (2001) Attenuation of cocaine-induced genomic and functional responses in prenatal cocaine-exposed rabbits. *Pharmacol Biochem Behav* **69**:225-232.

Webster WS and Brown-Woodman PD (1990) Cocaine as a cause of congenital malformations of vascular origin: experimental evidence in the rat. *Teratology* **41**:689-697.

Zimmerman EF, Potturi RB, Resnick E and Fisher JE (1994) Role of oxygen free radicals in cocaine-induced vascular disruption in mice. *Teratology* **49**:192-201.

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/s/

BELINDA A HAYES
06/21/2018
PharmTox final review

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06/21/2018

RICHARD D MELLON
06/21/2018
I concur.