# CENTER FOR DRUG EVALUATION AND RESEARCH

***APPLICATION NUMBER:***

**22-474**

# PHARMACOLOGY REVIEW(S)

**ADDENDUM** **FOOD AND DRUG ADMINISTRATION**

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Division of Reproductive and Urologic Products
Center for Drug Evaluation and Research

Date: August 12, 2010

From: Jeffrey Bray, Ph.D., Pharmacologist

To: NDA 22-474

Subject: Final Labeling

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Pharm/Tox has reviewed the final submitted labeling and finds it acceptable.

| Application Type/Number | Submission Type/Number | Submitter Name | Product Name |
|---|---|---|---|
| NDA-22474 | ORIG-1 | LABORATOIRE HRA PHARMA | Ella , Ulipristal Acetate |

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/s/

JEFFREY D BRAY
08/12/2010

ALEXANDER W JORDAN
08/13/2010
I concur

**MEMO** **FOOD AND DRUG ADMINISTRATION**

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**Division of Reproductive and Urologic Products**
**Center for Drug Evaluation and Research**

**Date:** August 11, 2010

**Reviewer:** Alex Jordan, Ph.D.
Expert reviewer

**NDA #/SS#/date:** 22-474

**Sponsor:** HRA Pharma

**Drug Product:** Ulipristal acetate

**Indication:** Emergency Contraception

**Recommended Action:** Approval

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**Background:**

**Pharmacology:** Ulipristal acetate is a mixed progesterone receptor agonist/antagonist with anti-glucocorticoid activity that produced antiprogestational effects on female reproductive organs such as the ovary and uterus.

**Toxicology:** The findings in repeat dose toxicology studies in female rats and monkeys were predominately the expected effects of an antiprogesterone/antiglucocorticoid agent. The predominant organ systems affected in both species were reproductive and endocrine such as the ovary, mammary gland, uterus, adrenal and pituitary as well as the liver.

**Genotoxicity:** Ulipristal was not mutagenic in vitro in the reverse mutation bacterial assays or in the mouse lymphoma cell assay and was not clastogenic in peripheral human blood lymphocytes. Oral administration of ulipristal did not increase the incidence of micronuclei in the bone marrow of mice.

**Carcinogenicity:** Carcinogenicity studies have not been completed

**Reproductive toxicology:** When ulipristal acetate was given to rats prior to mating at doses giving exposures in the same range as in humans there were no adverse effects on fertility or the fetuses. When administered to pregnant rats and rabbits during the period of organogenesis at drug exposures ½ to 1/3 human exposure ulipristal caused embryofetal lethality. There were no malformations of the surviving fetuses in these studies. Administration of ulipristal to pregnant monkeys for four days during the first trimester caused pregnancy termination in 2/5 animals at drug exposures 3 times human exposure.

**Summary:** The nonclinical data support the approval of ulipristal acetate for emergency contraception.

**Outstanding nonclinical issues:** None

**Conclusion:** I concur with the primary nonclinical reviewer, Dr.Bray, in recommending an approval action for this NDA.

| Application Type/Number | Submission Type/Number | Submitter Name | Product Name |
|---|---|---|---|
| NDA-22474 | ORIG-1 | LABORATOIRE HRA PHARMA | Ella , Ulipristal Acetate |

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/s/

ALEXANDER W JORDAN
08/11/2010

Comments on NDA 22-474 ulipristal acetate
Date 7/29/10
From A. Jacobs, AD

1. I concur that there are no pharm/tox issues affecting approval and that the pregnancy category is appropriate.

2. I have discussed my comments with the supervisor and they will be addressed as appropriate.

| Application Type/Number | Submission Type/Number | Submitter Name | Product Name |
|---|---|---|---|
| NDA-22474 | ORIG-1 | LABORATOIRE HRA PHARMA | Ella , Ulipristal Acetate |

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/s/

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ABIGAIL ABBY C C JACOBS
07/29/2010

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

**PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION**

| | |
|---|---|
| Application number: | 22-474 |
| Supporting document/s: | 000 |
| Applicant's letter date: | October 14, 2009 |
| CDER stamp date: | October 15, 2009 |
| Product: | Ella (Ulipristal acetate) |
| Review Completion Date: | April 5, 2010 |
| Indication: | Women desiring emergency contraception for up to 120 h following unprotected sexual intercourse |
| Applicant: | HRA Pharma |
| Review Division: | Division of Reproductive and Urologic Products |
| Reviewer: | Jeffrey Bray, Ph.D., Pharmacologist |
| Supervisor/Team Leader: | Alex Jordan, Ph.D., Team Leader |
| Division Director: | Scott Monroe, M.D. |
| Project Manager: | Pamela Lucarelli |

## Disclaimer

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Any information or data necessary for approval of NDA 22-474 that HRA Pharma 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 described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that HRA Pharma does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 22-474.

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# 1 Executive Summary

## 1.1 Recommendations

### 1.1.1 Approvability

Yes. Pharmacology recommends approval for emergency contraception for up to 120 hours following unprotected sexual intercourse.

### 1.1.2 Additional Non Clinical Recommendations

None.

### 1.1.3 Labeling

Nonclinical labeling in sections 8.1, 8.3, 13.1 and 13.2 require modification. Below are preliminary suggested modifications in italics:

(b) (4)

(b) (4)



1 Page(s) of Draft Labeling has been withheld in full immediately following this page as B4 (CCI/TS)

## 1.2 Brief Discussion of Nonclinical Findings

Ulipristal acetate (CDB-2914) is a mixed progesterone receptor agonist/antagonist with anti-glucocorticoid activities that produced antiprogestational effects on female reproductive system organs, such as the ovary and the uterus. CDB-2914 was rapidly and highly absorbed, with elimination half-lives ~3 h and ~7 h for the rat and dog respectively; monkey was not determined. CDB-2914 was highly protein bound and had one major metabolite, an N-monodemethylated product (CDB-3877) in humans, rats, and monkeys, with other metabolites detected in reduced amounts. CDB-2914 was metabolized by CYP3A4 and can inhibit CYP3A4 substrates *in vitro*. CDB-2914 and its metabolites were primarily eliminated through the feces via the hepatobiliary route. The principle metabolite CDB-3877 demonstrated similar, but weaker, pharmacologic activity compared to CDB-2914.

The findings in repeat dose toxicology studies in the female rat and monkey were predominantly the expected effects of an antiprogestin/antiglucocorticoid agent. The predominant organ systems affected in both species were reproductive and endocrine such as the ovary, mammary gland, uterus, adrenal, and pituitary, as well as the liver. The incidence and severity of findings tended to increase with dose and/or duration, and reversibility was not assessed. In general, the findings in monkeys were milder than rats. Increases in ovarian effects such as cysts, ovarian weight and follicular atresia (in rats only) were noted. Increased serum prolactin was positively correlated with increased pituitary weight (with hyperplasia in rats only). Effects on the mammary gland, such as galactoceles or hyperplasia were noted in the rat likely due to increased prolactin levels and antiprogestin activity. Increased serum corticosteroids were positively correlated with increased adrenal weight and hypertrophy. Uterine glandular dilatation was noted with mononuclear cell infiltration in monkeys. Increased serum ALT was noted in both rats and monkeys, with liver weight increases and hepatocellular hypertrophy/hyperplasia in rats, and mononuclear cell infiltration in monkeys. Blood neutrophil counts were increased in both species. The NOAEL was 1 mg/kg in monkeys with an exposure multiple of 0.65 on a $mg/m^2$ basis compared to human clinical exposure. A NOAEL was not identified in rats. The single dose toxicology studies were inadequate since only one high dose was evaluated. There were no significant findings in in vitro and in vivo genotoxicity assays. The findings in reproductive toxicity studies were consistent with the pharmacology of an antiprogestin, such as marked reduction in pregnancy rates, reduced number of live pups delivered, loss of post-implantation fetuses early after treatment initiation, and early induction of parturition. These effects occurred at exposures equal to or less than human exposures based on $mg/m^2$. Nonclinical reproductive toxicology findings indicate a risk of pregnancy termination if administered to a pregnant woman.

# 2 Drug Information

## 2.1 Drug

### 2.1.1 CAS Registry Number (Optional)

126784-99-4

### 2.1.2 Generic Name

Ulipristal acetate

### 2.1.3 Code Name

CDB-2914, PGL-4001, VA2914

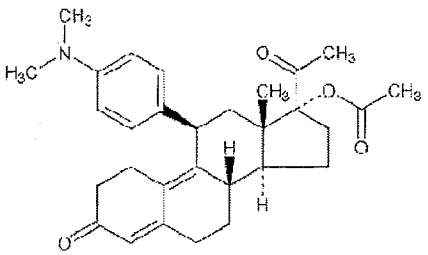
### 2.1.4 Chemical Name

17α-acetoxy-11α-(4-N,N-dimethylaminophenyl)-19-norpregna-4,9-diene-3,20- dione

### 2.1.5 Molecular Formula/Molecular Weight

$C_{30}H_{37}NO_4$/475.62

### 2.1.6 Structure



### 2.1.7 Pharmacologic class

Progesterone agonist/antagonist

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

IND 49,381 (HRA Pharma; CDB-2914 emergency contraception up to 5 days postcoital)
(b) (4) (HRA Pharma; CDB-2914 symptomatic relief of uterine fibroids)
(b) (4) (Population Council; CDB-2914 contraceptive vaginal ring)

## 2.3 Clinical Formulation

### 2.3.1 Drug Formulation

The drug product is a white to off-white, round, 9 mm diameter tablet engraved on both faces with "ella".

**Table 1 Ingredients and the Quantities in Ella 30 mg (Ulipristal acetate)**

| Ingredient | Function | Amount (mg/tablet) |
|---|---|---|
| Ulipristal acetate | API | 30.0 |
| Lactose monohydrate, NF | (b) (4) | |
| Povidone K30, USP | (b) (4) | |
| | | |
| Croscarmellose sodium, NF | (b) (4) | |
| Magnesium stearate (b) (4) NF | (b) (4) | |
| Total | | 300.0 |

*, (b) (4)

All inactive ingredients have been used in products previously FDA-approved at greater amounts.

### 2.3.3 Comments on Impurities/Degradants of Concern

The main impurity in the drug product is (b) (4) . (b) (4) is also the main degradant. The sponsor performed a series of nonclinical pharmacological and pharmacokinetic experiments to investigate (b) (4) and performed toxicokinetic analyses in pivotal toxicology studies. Since (b) (4) is present in animals and humans, this impurity/degradant is considered qualified. Another identified impurity (also a degradant) is a (b) (4) The amount present is <0.15%, which is the qualification threshold for a drug substance administered up to a maximum daily dose less than 2 g/day.

(b) (4)

(b) (4)

## 2.4 Proposed Clinical Population and Dosing Regimen

Women desiring emergency contraception for up to 120 h following unprotected sexual intercourse by a single oral administration of Ella 30 mg.

## 2.5 Regulatory Background

IND 49,381 was opened by NICHD on December 1, 1995 (FDA receipt date 12/5/1995). There was a change of sponsor on March 6, 2006 when HRA Pharma licensed Ulipristal acetate from NICHD.

## 3 Studies Submitted

### 3.1 Studies Reviewed

| Study Title | Study # | eCTD File Location # |
|---|---|---|
| Effects of VA2914 in the Irwin test in rats | DDFE1001 | 4.2.1.3.1.HRA2914-415 |
| Cardiovascular effects of VA-2914 in conscious, telemetered female Beagle dogs | DDFE1000 | 4.2.1.3.1.HRA2914-416 |
| Effects of VA-2914 on respiration rate and tidal volume in rats | DDFE1002 | 4.2.1.3.1.HRA2914-414 |
| Effects of VA-2914 on HERG tail current recorded from stably transfected HEK293 cells | DDFE1004 | 4.2.1.3.1.HRA2914-413 |
| Effects of VA-2914 on Action Potential parameters in dog isolated cardiac Purkinje fibres | DDFE1003 | 4.2.1.3.1.HRA2914-412 |
| ($^{14}$C)-VA2914: A study of absorption, distribution, metabolism and excretion following oral and intravenous administration to the rat and the determination of ($^{14}$C)-VA2914 concentration in mouse plasma following oral administration | 2109/001-D1145 | 4.2.2.2.1.HRA2914-425 |
| ($^{14}$C)-VA 2914: A study of absorption, metabolism and excretion following oral and intravenous administration to the cynomolgus monkey | 2109/002-D1145 | 4.2.2.2.1.HRA2914-426 |
| In vitro binding of [$^{14}$C]-VA2914 to mouse, rat, rabbit, dog, monkey and human plasma | PKFAC 0205 | 4.2.2.3.1.HRA2914-428 |
| In vitro binding of [$^{14}$C]-VA2914 to human plasma proteins and human blood distribution | PKFAC 0206 | 4.2.2.3.1.HRA2914-427 |
| PGL4002: Extent of binding to rat, monkey and human plasma proteins and partitioning between the plasma and cell fraction of human blood | PGL09-011 | 4.2.2.3.1.HRA2914.475 |
| ADME-Tox - Study of Compound 3877 | 15714 | 4.2.2.3.1.HRA2914-478 |
| Metabolism of ($^{14}$C)-VA 2914 in microsomes isolated from female mouse, rat, rabbit, dog, monkey and human | 2109/003 | 4.2.2.4.1.HRA2914-429 |
| Identification of the cytochrome P450 enzymes responsible for the in vitro metabolism of ($^{14}$C)- | 2109/004 | 4.2.2.4.2.HRA2914-430 |

| | | |
|---|---|---|
| VA 2914 and the effect of VA 2914 on the activity of specific human cytochrome P450 enzymes | | |
| Inhibition of PGL4002 on CYP Enzyme Activities in Pooled Human Liver Microsomes | PGL09-010 | 4.2.2.6.1.HRA2914-476 |
| Induction Effects of PGL4001 and PGL4002 on CYP1A2 and 3A4 Activities in Fresh Human Hepatocytes | PGL09-008 | 4.2.2.6.1.HRA2914-477 |
| Ulipristal acetate - Pre- and post-natal development study by the oral route (gavage) in the rat (Segment III) | AA75162 | 4.2.3.5.3.HRA2914-471 |
| VA2914: Bacterial reverse mutation test | 32775 MMO | 4.2.3.3.1.HRA2914-438 |
| Evaluation of the in vitro phototoxicity on Balb/c 3T3 fibroblats using the Neutral Red uptake assay | 2109/006 | 4.2.3.7.7.HRA2914-448 |

### 3.2 Studies Not Reviewed

| Study Title | Study # | eCTD File Location # |
|---|---|---|
| Biological activity of the monodemethyl (CDB3877) and the didemethyl (CDB3963) metabolites of CDB2914 | 403P | 4.2.1.1.6.HRA2914-450 |
| In vivo antiglucocorticoid activity of the monodemethyl (CDB-3877) metabolite of CDB-2914 | 123W | 4.2.1.1.1.HRA2914-468 |
| Validation Report – Development and validation of an LC-MS/MS technique for the determination of VA2914 and its metabolite 3877A in dog plasma | CP025032 | 4.2.2.1.1.HRA2914-418 |
| Validation Report – Development and validation of an LC-MS/MS technique for the determination of VA2914 and its metabolite 3877A in dog plasma | CP015097 | 4.2.2.1.1.HRA2914-419 |
| Short validation of an LC-MS/MS method for determination of Ulipristal acetate (VA2914) and its metabolite (3877A) in rat milk | CP085253 | 4.2.2.1.1.HRA2914-473 |
| Stability of Ulipristal Acetate (VA2914) and its metabolite (3877A) in rat plasma | CP085353 | 4.2.2.1.1.HRA2914-474 |
| Determination of VA-2914 and its main metabolite by LC-MS/MS in rat plasma samples collecting during the study DDFE1002 | CP025031 | 4.2.2.2.1.HRA2914-421 |
| Determination of VA-2914 and its main metabolite by LC-MS/MS | CP025370 | 4.2.2.2.1.HRA2914-422 |

| | | |
|---|---|---|
| in rat plasma samples collecting during the study DDFE1000 | | |
| Acute oral toxicity of CDB-2914 in the adult female rabbit | NICHD 002-95 | 4.2.2.2.1.HRA2914-452 |

### 3.3 Previous Reviews Referenced

Nonclinical studies to support the NDA filing have been previously reviewed in the following **Table** and will be summarized in the relevant sections of this review. Most of these studies were conducted in the 1990s but generally are considered to be adequate in this reviewer's judgment.

Pharmacology:

- Numerous published studies support the indication and mechanism of action

Toxicology:

- Acute Toxicology Study in Female Rats
- Acute Toxicology Study in Female Cynomolgus Monkeys
- 6-month Oral Toxicology Study in Female Rats
- 6-month Oral Toxicology Study in Female Cynomolgus Monkeys

Genetic Toxicology:

- Ames Reverse Bacterial Mutation Assay
- L5178Y Mouse Lymphoma Cell Mutation Assay
- Human Lymphocyte Metaphase Assay
- Mouse Micronucleus Assay

Reproductive Toxicology

- Rat Male Fertility Study, (see Appendix 1, References) [1]
- Embryofetal Toxicity Study in Rats (plus pilot Dose Range Finding)
- Embryofetal Toxicity Study in Rabbits (plus pilot Dose Range Finding)

| Application # | SN#/Review# | Submission Date | Reviewer | Date |
|---|---|---|---|---|
| IND 49,381 | 000/1 | December 1, 1995 | Dr. Krishan Raheja | December 26, 1995 |
| IND 49,381 | AR | January 27, 1998 | Dr. Krishan Raheja | February 3, 1998 |
| IND 49,381 | 001/1 | August 4, 1999 | Dr. Katherine Bonson | August 11, 1999 |
| IND 49,381 | 007/4 | January 25, 2001 | Dr. Suzanne Thornton | March 21, 2002 |

## 4 Pharmacology

The sponsor submitted a series of publications that described the primary and secondary pharmacology of Ulipristal acetate (will be referred to as CDB-2914 throughout this review) and its predominant metabolites *in vitro* and *in vivo*. CDB-2914 potently bound to the Progesterone Receptor (PR) and the Glucocorticoid Receptor

(GR) at low nanomolar concentration and exhibited anti-progestational and anti-glucocorticoid activities *in vitro*. CDB-2914 was a potent antiprogestin on the estrogen-primed rabbit endometrium. CDB-2914 was efficacious in preventing conception and ovulation in rats, rabbits, and monkeys. No significant adverse effects were observed in a series of safety pharmacology studies. The actions of CDB-2914 appear to be mediated through its effects on the ovaries and the endometrium.

## 4.1 Primary Pharmacology

CDB-2914 is an oral Selective Progesterone Receptor Modulator (SPRM) that derives its pharmacological activities through binding to the Progesterone Receptor (PR). Theoretically, a SPRM will have increased selectivity for PR over other steroid receptors and possibly have tissue-selective actions. CDB-2914 is a potent PR ligand exhibiting 4-10 nM affinity with significant (15-20 nM) affinity GR binding. The reported pharmacological activities of CDB-2914 are consistent with that of an antiprogestin with some anti-glucocorticoid activity:

Pharmacology relevant to mechanism of action

- Dose-dependent inhibition of ovulation when orally administered to rats at 1, 2, and 4 mg/animal (~ 6, 13, and 25 mg/kg, respectively), and mice (20 and 40 mg/kg, intraperitoneally)[2].
- Dose-dependent reduction in pregnancy, ovulation and implantation rates with increased resorptions in rabbits treated with a single oral dose of 32 and 64 mg/animal (~17.8 and 35.6 mg/kg, respectively)[3].
- Significantly reduced pregnancy and implantation rates in rats administered 0.5 mg ~(3.3 mg/k) for 7 days prior to mating with proven fertile males for 24 total days.
- In rats, oral or subcutaneous administration at 0.5, 1, and 2 mg/kg for varying numbers of days from (Gestation Day) GD 1-6 and sacrificed on GD 10-17 demonstrated dose-dependent reduction in conceptuses and increased number of resorptions. These activities could be reversed by progesterone. Oral treatment was slightly less effective[2].
- Dose-dependent pregnancy termination in guinea pigs administered subcutaneously 3, 10, and 30 mg/day (~ 7.5, 25, and 75 mg/kg) on GD 43 and 44. NOEL= 3 mg/day (~7.5 mg/kg)[4].
- CDB-2914 terminated pregnancies in macaques following oral (0.5 and 5.0 mg/kg) or intramuscular (0.5 mg/kg) administration on GD 23-26. Abortions were observed in 0/5 and 2/5 animals orally treated with 0.5 and 5.0 mg/kg. Intramuscular administration resulted in 4/5 pregnancy terminations. No malformations were observed in any surviving offspring.[5].
- Inhibition of proliferation and increased apoptosis in cultured human uterine leiomyoma cells[6].
- Inhibition of cultured human endometrial stromal cell proliferation[7].

Anti-PR

- Inhibition of R5020-stimulated alkaline phosphatase activity in T47D-CO human breast cancer cells with an $IC_{50}$ of 7-9 nM[8;9].
- Inhibition of R5020-stimulated transcriptional activity in T47D-CO breast cancer cells with an $IC_{50}$ of 2 nM[8;9].
- Inhibition (~35%) of ethinyl estradiol-stimulated effects in rat uterotrophic assay.
- Dose-dependent inhibition of progesterone-stimulated endometrial glandular proliferation of the estrogen primed rabbit uterus by intraluminal administration (0.25-1 µg/horn), or subcutaneous and oral administration (0.2-10 mg/rabbit/day).

Anti-GR

- Inhbition of dexamethasone-stimulated transcriptional activity in HepG2 human hepatocarcinoma cells with an $IC_{50}$ of 73 nM[8].
- Demonstrated anti-glucocortcoid activity by completely blocking methylprednsione-induced thymic involution at 100 mg/kg in rats, but was not able to completely block dexamethasone-induced involution at 75 mg/kg[3;9].

## 4.2 Secondary Pharmacology

- CDB-2914 did not exhibit significant androgen agonism/antagonism or estrogen agonism[9].
- CDB-2914 was not aromatized by CYP19[9].

The monodemethylated principle metabolite of CDB-2914, designated CDB-3877 or PGL4002, had a similar *in vitro* profile as the parent compound[9] but with reduced PR potency (see **Tables 2A and 2B** below); it was 4-fold less potent in the rabbit compared to CDB-2914. The didemethylated metabolite, designated CDB-3936 or PGL4004, had no significant *in vivo* activity. CDB-3877 had some anti-GR activity at ≥10 mg/kg in the adrenalectomized adult rat model of thymic involution (~10% of RU-486 at 10 mg/kg).

**Table 2 A) PR and B) GR Activities of CDB-2914 and its Metabolites *in vitro* with Relative Detection of Metabolites Based on RIA Method for Parent**

**A**

| Parent or Metabolite | PR Binding | | | | Transcriptional Ratio | % Serum Cross reactivity |
|---|---|---|---|---|---|---|
| | Human | | Rabbit Uterus | | | |
| | PRB (nM) | RBA (P4=100 %) | PRB (nM) | RBA (P4=100 %) | PR: anti-5020 (RU486=1) | |
| CDB-2914 (Parent) | 7.7 | 99 | 13.6 | 85 | 0.65 | 100 |
| CDB-3877 monodemethyl | 8.8 | 78 | 11.8 | 101 | 0.41 | 76 |
| CDB-3963 didemethyl | 83.2 | 9 | 17.5 | 60 | 0.022 | 59 |
| Aromatic A-ring | 19.5 | 36 | 72.7 | 16 | 0.0064 | <1 |
| 17 α-hydroxy | 91.1 | 8 | 77.5 | 15 | 0.0036 | <1 |

PRB, Progesterone Receptor B; RBA, Relative Binding Affinity; P4, Progesterone

B

| Parent or Metabolite | Rabbit Thymic GR Binding | | Transcriptional Ratio |
|---|---|---|---|
| | GR (nM) | RBA (Dex=100%) | GR: anti-Dex (RU486=1) |
| CDB-2914 (Parent) | 15.4 | 53 | 0.081 |
| CDB-3877 monodemethyl | 14.7 | 55 | 0.0045 |
| CDB-3963 didemethyl | 73.9 | 11 | 0.018 |
| Aromatic A-ring | 78.2 | 10 | 0.0024 |
| 17 α-hydroxy | 21.9 | 37 | 0.018 |

GR, Glucocorticoid Receptor; RBA, Relative Binding Affinity; Dex, Dexamethasone;

The sponsor further characterized potential off-target activities by applying a high-throughput screening method to investigate the potential interaction of CDB-2914 and its principle metabolite CDB-3877. Other than GR, which was previously known to bind to these compounds, no significant interaction was identified.

### 4.3 Safety Pharmacology

Overall, CDB-2914 had no remarkable findings in safety pharmacology studies performed.

**Table 3 Summary of Safety Pharmacology Findings for CDB-2914**

| Test | Dose (mg/kg) | Results |
|---|---|---|
| Functional Observational Battery in Rats | 5, 25, 125 | ↑urination |
| Respiration Rate and Tidal Volume in Rats | 5, 25, 125 | not remarkable |
| CV Effects in Conscious,Telemetered Female Beagle Dogs | 5, 25, 125 | ↑BP, MAP @ 25 mg/kg up to 6 h |
| hERG Tail Current in HEK293 Cells | 10 uM | ↓34.1% tail current (veh ↓27.2%) |
| Action Potential Parameters in Dog Isolated Cardiac Purkinje Fibres | 1,3,10 uM | not remarkable |

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Neurological effects: Not remarkable.

**Study title**: Effects of VA-2914 in the Irwin Test in rats

Study no.: DDFE1001
Volume #, and page #: EDR, 4.2.1.3.1.HRA2914-415
Conducting laboratory and location: (b) (4)
Date of study initiation: February 1, 2002
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: CDB-2914, 01031115, 99.8%
Animal species/strain/sex per dose/weight and age: Sprague-Dawley rats/6F per group age ~7 weeks, weight 176-203 g at study initiation.
Doses/vehicle: 5, 25, 125 mg/kg, ASV (0.5% w/v carboxymethylcellulose, 0.9% w/v sodium chloride, 0.9% v/v benzyl alcohol, and 0.4% v/v Tween 80), 10 mL/kg
Duration/route: single oral administration

**Methods**: Fasted animals were administered drug by oral gavage. Positive control was chlorpromazine at 20 mg/kg, and negative control was Aquesous Solution Vehicle (ASV) The parameters defined in the Irwin Test were systematically evaluated for each rat at 1, 2, 4, 6 and 24 h post dose. However, due to the large numbers of observations in the positive control group, the time taken to observe these animals exceeded the expected time of 10 min at the 1, 2, 4 and 6 h time points.

**Results**: Test-article, but not dose-dependent, increase in urination at 2+ h noted.

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Cardiovascular effects:

**Study title**: Cardiovascular Effects of VA-2914 in Conscious, Telemetered Female Beagle Dogs
Study no.: DDFE1000
Volume #, and page #: eCTD 4.2.1.3.1.HRA2914-416
Conducting laboratory and location: (b) (4)
Date of study initiation: February 8, 2002
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: CDB-2914, 01031115, 99.8%
Animal species/strain/sex per dose/weight and age: Beagle dogs/4F weight 9-12 kg at study initiation and 9.5-11.6 at termination.
Doses/vehicle: 5, 25, 125 mg/kg, ASV (0.5% w/v carboxymethylcellulose, 0.9% w/v sodium chloride, 0.9% v/v benzyl alcohol, and 0.4% v/v Tween 80), 5 mL/kg
Duration/route: single oral administration

**Methods**:
Non-naïve animals (last treatment was 11 weeks prior) that previously were surgically implanted (7 months prior) with telemetry transducers (pressure transducer with 6 chest ECG implant; (b) (4)) which are capable of measuring aortic blood pressure (from which heart rate was derived) were used. Habituation to jackets that contained electronics for telemetry recordings was performed for 3 times prior to

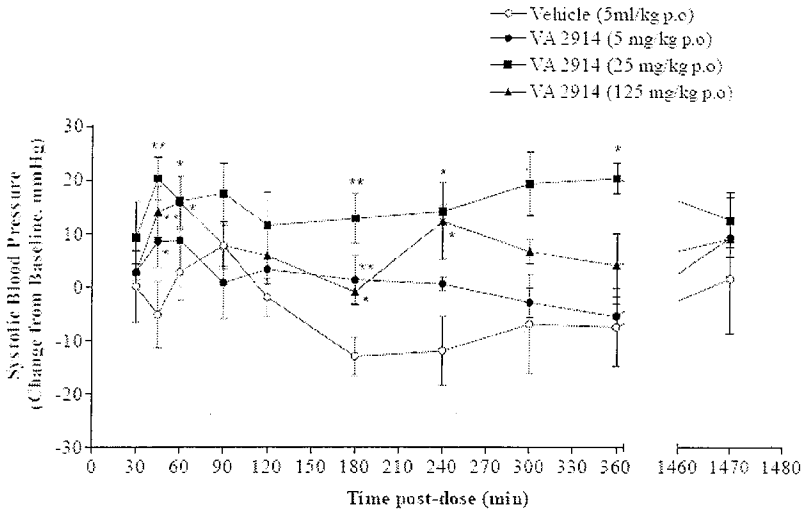
any dosing. A period of at least 10 min following placement of the animals in the jackets was allowed for stabilization prior to data acquisition. For each dose, data collection commenced at least 30 min before dosing and ended approximately 6 h after dosing. Further data collection periods were made for approximately 1 h at 23-25 h following each dose. The following parameters were measured: Systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR, measured from the blood pressure waveform) and 6 ECG leads will be measured continuously. Mean arterial blood pressure (MAP) was calculated as (DBP + 1/3(SBP-DBP)). PR interval, RR interval, QRS duration, QT interval, QTcF interval (calculated as QTcF = $QT/\sqrt[3]{RR}$, QTcQ interval (calculated as QTcQ = QT + #(1-RR). Ascending drug levels were administered to the same animals first for CV parameters and then for PK measurements according to the regimen below.

**Table 4 Regimen for Measurements Taken in Cardiovascular Study in Conscious Telemetered Female Beagle Dogs Following a Single Oral Dose of CDB-2914**

| Day | Dose (mg/kg) | Measurement |
|---|---|---|
| 1 | 0 | CV parameters |
| 3 | 5 | CV parameters |
| 10 | 25 | CV parameters |
| 17 | 125 | CV parameters |
| 31 | 5 | drug plasma level |
| 38 | 25 | drug plasma level |
| 45 | 125 | drug plasma level |

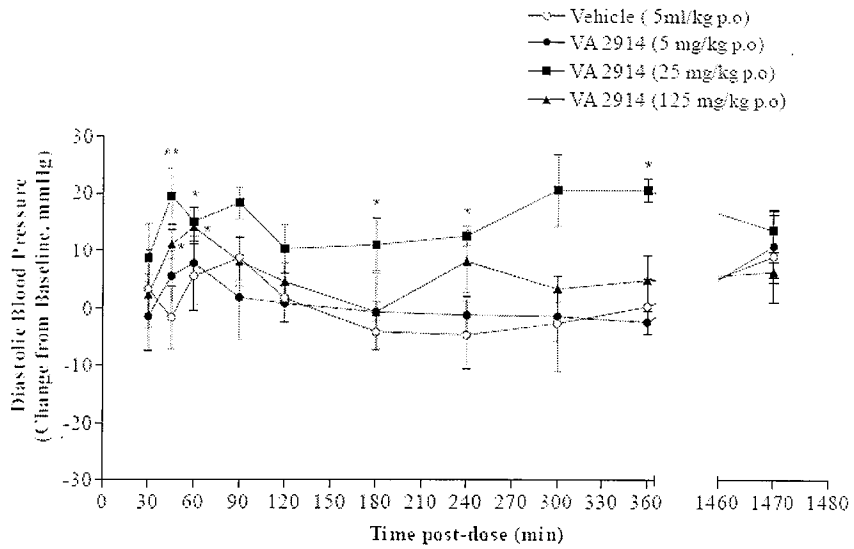
**Results**:
No clinical signs or behavioral effects were noted following administration of CDB-2914. Overall, there were minor statistical increases in BP (both systolic and diastolic) and MAP at ≥25 mg/kg at isolated time points up to 6 h, but no dose- or time- dependency compared to vehicle control, since the 25 mg/kg dose had the greatest effect on these parameters (see **Figures** below).

**Figure 1 The Effect of CDB-2914 on Systolic Blood Pressure Change in Conscious, Telemetered Female Beagle Dogs**



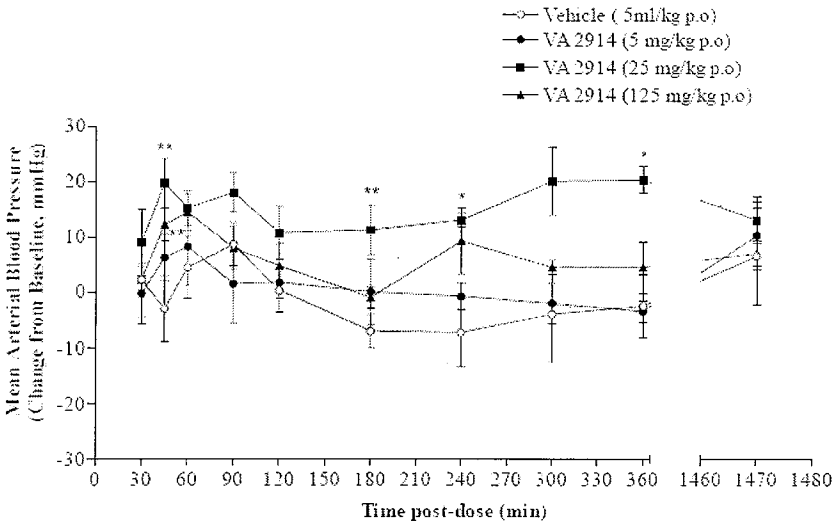

Sponsor Figure 1, pg. 24

**Figure 2 The Effect of CDB-2914 on Diastolic Blood Pressure Change in Conscious, Telemetered Female Beagle Dogs**




Sponsor Figure 2, pg. 25

**Figure 3 The Effect of CDB-2914 on Mean Arterial Blood Pressure Change in Conscious, Telemetered Female Beagle Dogs**



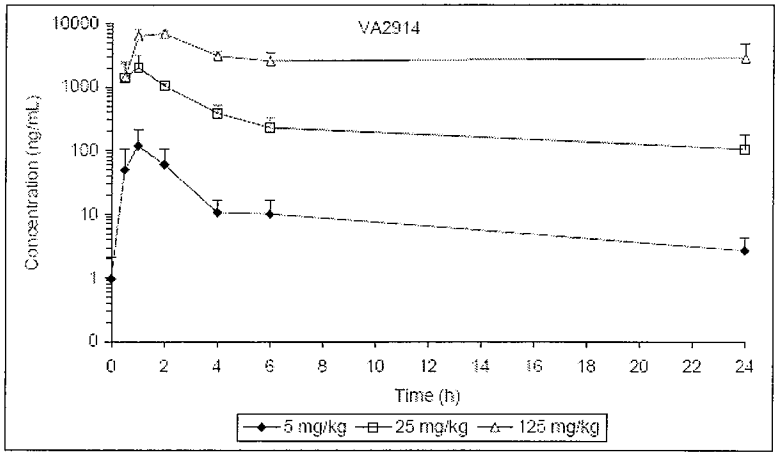

Sponsor Figure 3, pg. 26

There was no marked effect on RR, PR, QT, QTcF and QTcQ intervals or QRS duration for 24.5 h following administration of CDB-2914. No disturbances in rhythm or waveform morphology were noted in any of the ECG waveforms that were visually inspected. There were more incidences of supraventricular premature complexes in the treated groups than vehicle, but no dose-dependent relationship was noted, and these findings are common for beagle dogs. The sponsor concluded that no clear relationship existed.

This study also was used for Toxicokinetics of single dose exposure using the validated LC-MS/MS method to replace the previous RIA method of measurement. Determination of the PK parameters of CDB-2914 and CDB-3877 were described in Study #CP025370 (EDR 4.2.2.2.3.HRA2914.422). Overall, the exposures are more than dose proportional from 5 to 125 mg/kg range in beagle dogs. The CDB-2914/CDB-3877 exposure ratio increased based on AUC from 0.85 to 1.71 suggesting that metabolism of parent to metabolite was saturated with increased doses, but there was a large amount of variability in exposure. The half-life measurements were not very accurate with the range being from 7 to 29 h and 1 to 14 h for CDB-2914 and CDB-3877, respectively.

**Figure 4 Mean and SD Plasma Concentration-Time Profiles of CDB-2914 (A) and CDB-3877 (B) in Beagle Dogs**

**A** CDB-2914




**B** CDB-3877

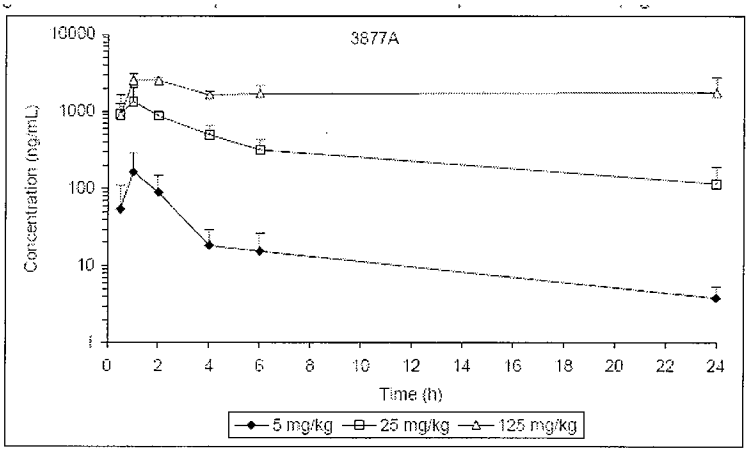



**Table 5 PK Parameters of CDB-2914 and CDB-3877 Following a Single Oral Administration to Female Beagle Dogs from Study DDFE1000**

| Parameter | Dose (mg/kg) | | | | | |
|---|---|---|---|---|---|---|
| | 5 | | 25 | | 125 | |
| | CDB-2914 | CDB-3877 | CDB-2914 | CDB-3877 | CDB-2914 | CDB-3877 |
| Mean $C_{max}$ (ng/mL) | 130.33 | 185.20 | 2153.33 | 1426.33 | 7360.0 | 2790.0 |
| SD | 81.81 | 101.86 | 976.54 | 600.93 | 340.44 | 202.24 |
| Mean $AUC_{0-t}$ (ng*h/mL) | 348 | 438 | 7727 | 7961 | 73199 | 42440 |
| SD | 229 | 356 | 1851 | 1650 | 27864 | 14322 |
| $t_{max}$ (h) | 1.00 | 1.00 | 1.00 | 1.00 | 2.00 | 1.00 |
| Range | 1.00 - 2.00 | 1.00 - 2.00 | 1.00 - 2.00 | 1.00 - 2.00 | 1.00 - 2.00 | 1.00 - 2.00 |
| | | | | | | |
| Exposure Multiple* | 0.7 | 1.8 | 13 | 33 | 125 | 2122 |

*Human exposure based on $AUC_{0-t}$ following administration of a single dose of 30 mg CDB-2914 is 584 ± 259 ng*h/mL ($AUC_{0-t}$ exposure of CDB-3877 was 240.0 ± 58.6 ng*h/mL).

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**Study title**: Effects of VA-2914 on Action Potential Parameters in Dog Isolated Cardiac Purkinje Fibres

Study no.: DDFE1003
Volume #, and page #: EDR, 4.2.1.3.1.HRA2914-412
Conducting laboratory and location: (b) (4)
Date of study initiation: April 4, 2002
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: CDB-2914, 01031115, 99.8%
Doses/vehicle: 1, 3, and 10 µM CDB-2914 dissolved in DMSO and diluted in physiological saline solution(PSS); vehicle was 0.1% DMSO in PSS

**Methods**: The following parameters were measured: action potential duration at 60% and 90% repolarization ($APD_{60}$ and $APD_{90}$), maximum rate of depolarization (MRD), upstroke amplitude (UA) and resting membrane potential (RMP). Animals were sacrificed by overdose of sodium pentobarbitone (200 mg/mL) and ventricular Purkinje fibers were isolated from the hearts. The fibers were first transferred to gassed PSS at room temperature, then individually to recording chambers. Electordes were positioned to electrically stimulate the fibers at 1 Hz until an action potential was evoked then increased to provide a suprathreshold stimulation voltage. The action potentials were then assessed and the fiber was accepted for experimental use if the acceptance criteria (APD90 ≥ 190 ms and ≤ 450 ms, MRD ≥ 300 V/s, UA ≥ 100 mV) had been met.

The fiber was equilibrated for 60 min to washout any residual sodium pentobarbitone and to obtain stable waveform readings. Stimulation frequency was decreased to 0.5 Hz and the previous 10 waveforms were analyzed and considered the baseline pre-treatment values.

**Results**: Test-article had no effect on respiration rate and tidal volume compared to vehicle control, but morphine significantly depressed both parameters.

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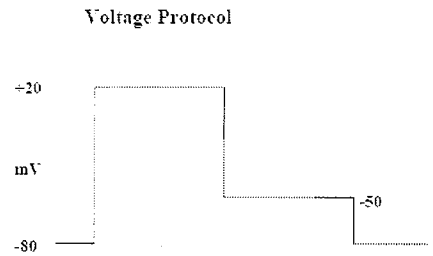
**Study title**: Effects of VA-2914 on HERG Tail Current Recorded from Stably Transfected HEK293 Cells
Study no.: DDFE1004
Volume #, and page #: EDR, 4.2.1.3.1.2.HRA2914-413
Conducting laboratory and location: (b) (4)
Date of study initiation: March 18, 2002
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: CDB-2914, 01031115, 99.8%

**Methods**: Treatments were: no treatment (control), 0.1% DMSO (vehicle control), 10 µM CDB-2914 in DMSO, and 100 nM E-4031 (positive control). Under standard cell culture conditions, HEK293 cells stably transfected with hERG cDNA were plated on to sterile glass coverslips in 35 $mm^2$ dishes at a density of 0.8-1.3 x $10^5$ cell/dish in MEM (supplemented with 10% FBS, 1% non-essential amino acids, and 0.4 mg/mL geneticin). Cells were transferred to the recording chamber and seals were formed between patch electrodes and individual cells to rupture membrane and establish a patch-clamp. Once a stable clamp was achieved, recording commenced in voltage-clamp mode, with the cell initially clamped at -80 mV. Treatments were applied to individual cells for 15 min and the voltage protocol below was used a minimum of 10 times.

**Figure 5 Voltage Protocol Used for Effects of CDB-2914 on hERG Tail Current**




The voltage was held for 4.8 s, 5s, and 5.2 s at +20 mV, -50 mV and -80 mV, respectively. The drop from test command voltage of +20 mV to -50 mV is the tail current.

**Results**: CDB-2914 had no effect on hERG tail current compared to vehicle control (34.1% decrease vs. 27.2% decrease for vehicle).

**Table 6 Effect of CDB-2914 (VA2914) on hERG Tail Current (Sponsor's Table 1, pg.8)**

Table 1

Effect of VA2914, DMSO and E-4031 on HERG Tail Current

| Treatment | Tail Current (% Control) |
|---|---|
| 10 μM VA2914 | 65.9 ± 2.4 |
| 0.1% DMSO | 72.8 ± 5.3 |
| 100 nM E-4031 | 3.9 ± 1.9 |

Data are given as mean ± s.e. mean for n = 4 cells for all treatment groups.

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Pulmonary effects: Not remarkable.

**Study title**: Effects of VA-2914 on Respiration Rate and Tidal Volume in Rats
Study no.: DDFE1002
Volume #, and page #: EDR, 4.2.1.3..1.HRA2914-414
Conducting laboratory and location: (b) (4)
Date of study initiation: February 1, 2002
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: CDB-2914, 01031115, 99.8%
Animal species/strain/sex per dose/weight and age: Sprague-Dawley rats/8F per group age ~11 weeks, weight 176-226 g at study initiation.
Doses/vehicle: 5, 25, 125 mg/kg, ASV (0.5% w/v carboxymethylcellulose, 0.9% w/v sodium chloride, 0.9% v/v benzyl alcohol, and 0.4% v/v Tween 80), 10 mL/kg
Duration/route: single oral administration

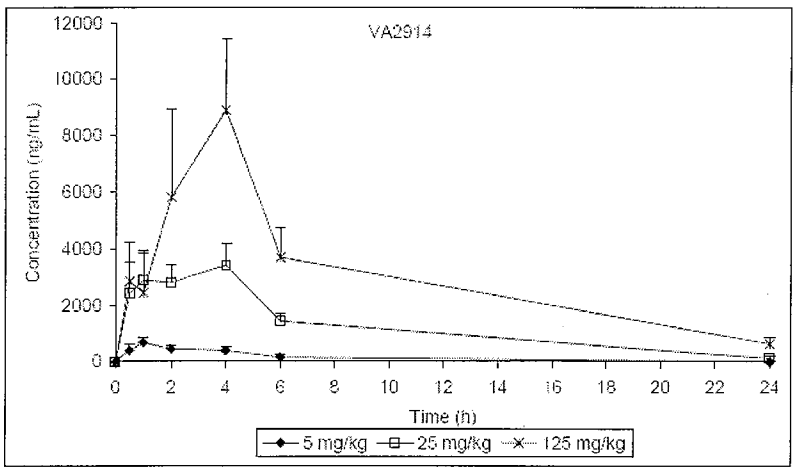
**Methods**: Animals were acclimatized to the plethysmography chambers on 3 separate sessions before use on study. Animals that struggled or vocalized were excluded at the Study Director's discretion, and those that were too small were removed to satellite PK groups (n=10/group). Fasted animals were administered drug by oral gavage. Positive control was morphine at 20 mg/kg (2 mL/kg, i.v.), and negative control was ASV. Animals were placed in the chamber 15 min before each timepoint (-10, 60 and 240 min postdose) to reacclimatize. To eliminate overbleeding, PK blood samples were drawn at -24, 1, 4 and 24 h from half the animals, and -24, 0.5, 2, and 6 h from the other half.

**Results**: Test-article had no effect on respiration rate and tidal volume compared to vehicle control, but morphine significantly depressed both parameters.

Determination of the PK parameters of CDB-2914 and CDB-3877 were described in Study #CP025031 (EDR 4.2.2.2.3.HRA2914.421). Overall, the exposures were dose proportional from 5 to 25 mg/kg, but less than dose proportional from 25 to 125 mg/kg range in rats. The CDB-2914/CDB-3877 exposure ratio increased based on AUC from 0.94 to 2.85 suggesting that metabolism of parent to metabolite was saturable with increased doses.

**Figure 6 Mean and SD Plasma Concentration-Time Profiles of CDB-2914 (A) and CDB-3877 (B)**

A CDB-2914




B) CDB-3877

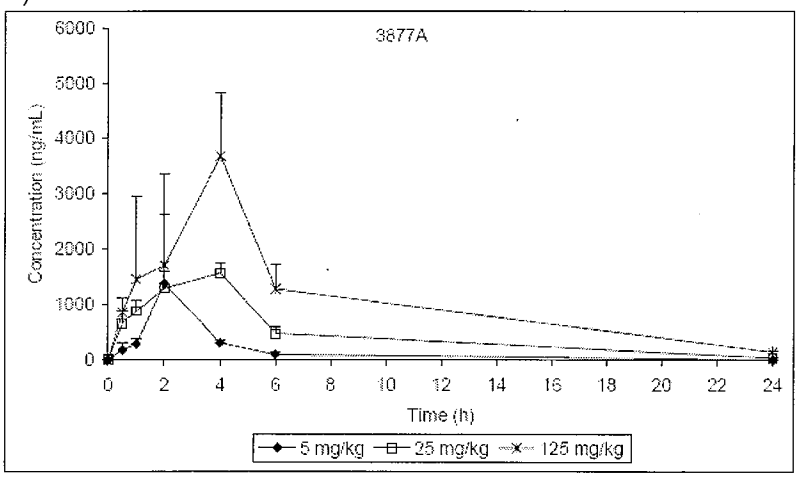



**Table 7 PK Parameters of CDB-2914 and CDB-3877 Following a Single Oral Administration to Female Rats from Study DDFE1002**

| Parameter | Dose (mg/kg) | | | | | |
|---|---|---|---|---|---|---|
| | 5 | | 25 | | 125 | |
| | CDB-2914 | CDB-3877 | CDB-2914 | CDB-3877 | CDB-2914 | CDB-3877 |
| $C_{max}$ (ng/mL) | 681 | 1375 | 3418 | 1562 | 8908 | 3684 |
| $AUC_{0-t}$ (ng*h/mL) | 3645 | 3876 | 29779 | 11064 | 72407 | 25443 |
| $t_{max}$ (h) | 1 | 2 | 4 | 4 | 4 | 4 |
| $t_{1/2}$ (h) | 2.99 | 2.63 | 4.38 | 4.00 | 5.84 | 4.72 |
| | | | | | | |
| Exposure Multiple* | 6.2 | 16 | 51 | 46 | 124 | 106 |

*Human exposure based on AUC following administration of a single dose of 30 mg CDB-2914 is 584 ± 259 ng*h/mL (AUC exposure of CDB-3877 was 240.0 ± 58.6 ng*h/mL).

Renal effects: Not reported

Gastrointestinal effects: Not reported

Abuse liability: Not reported

## 5 Pharmacokinetics/ADME/Toxicokinetics

The sponsor conducted a series of pharmacokinetic studies designed to investigate the absorption, distribution, metabolism, excretion and pharmacokinetic interactions of CDB-2914 in rats and monkeys and *in vitro*. Oral radiolabeled CDB-2914 was rapidly and highly absorbed in rats and monkeys with maximal concentrations reached at 1 and 4 h, respectively, and plasma bioavailability of 80% and 112%, respectively. Oral and intravenous exposure and half-life were comparable in rats; monkeys had similar exposure but the half-life was 3.5 days vs. 11 days for oral and intravenous administration, respectively. Mass balance studies demonstrated that tissue distribution in rats and monkeys was widespread with radiolabel concentrations highest in the liver, adrenal, and fatty tissues. There was very little blood brain barrier penetration. The retention time in tissues was fairly long and this was reflected in high Volume of Distribution and low Clearance values. Pigmented tissues in rats retained radiolabel compared to albinos suggesting an association with melanin. CDB-2914 was 96.7 to 99.5% protein bound in plasma of female rats, mice, rabbits, dogs, humans, and cynomolgus monkeys *in vitro*. CDB-2914 was highly bound to human blood and plasma with 4.9% bound to blood cells and 94.1% bound to plasma proteins with a free-fraction of 1.1%. CDB-2914 significantly bound to Human Serum Albumin (HSA), α1-acid

glycoprotein (AAG), and lipoprotein components of plasma *in vitro*. This suggests that distribution may be modulated during inflammation or infection when AAG levels are high.

CDB-2914 had one major metabolite, an N-monodemethylated product (CDB-3877, PGL4002) in humans, rats, and monkeys, with other metabolites detected in reduced amounts. *In vitro* metabolism of CDB-2914 demonstrated that rat liver microsomes produced a pattern closest to human in both a qualitative and quantitative manner, sharing the same 2 of 7 metabolites identified from all species tested. However, CDB-2914 and CDB-3877 were detected in plasma, bile and feces of rats and plasma, urine and feces of monkeys. CDB-2914 metabolism in human liver microsomes *in vitro* was blocked by CYP2E1 and CYP3A4 inhibitors. CYP activities mediated by CYP2C9, CYP2D6, and CYP3A4 were significantly inhibited by CDB-2914, and demonstrated some potential time dependent inhibition of CYP3A4 activity.

The major metabolite CDB-3877 was also investigated in a series of *in vitro* experiments. CDB-3877 was extensively metabolized in liver microsomal extracts of all species with ~1% remaining for all species, except human (18%). CDB-3877 at 1.5 µM was highly bound to female rats, mice, rabbits, dogs, humans, and cynomolgus monkeys plasma proteins at 93.5 to 98.0%, and was not highly bound to red cells *in vitro*. CDB-3877 at 10 µM was highly plasma protein bound for all species, except monkeys (47.1%) *in vitro*. The discrepancy in the monkey values may be due to the different concentrations of compound tested. CDB-3877 significantly bound to AAG and HSA, like the parent *in vitro*. CDB-3877 did not significantly inhibit or induce any CYP enzymatic activity tested *in vitro*.

Overall, the PK profile of CDB-2914 in rats and monkeys was similar. It is expected that CDB-2914 will be high distributed, and due to its lipophilic properties, will be associated with fatty tissues and lipids in blood with possible tissue retention due to the long half-lives detected for humans and monkeys. While the exposure of CDB-2914 may be affected by potent concomitant CYP3A4 inhibitors or inducers, it is unlikely to alter the exposure of other concomitant drugs.

## 5.1 PK/ADME

### 5.1.1 Methods of Analysis

The sponsor developed two methodologies for determining the concentration of CDB-2914 and its predominant metabolite CDB-3877 in plasma.

The first was a radioimmune-based assay ("Development of a Radioimmunoassay for CDB-2914", original NICHD Report dated April 27, 1994) that used rabbit antisera generated against the 3-carboxymethloxime-BSA conjugate. The assay was then validated using monkey serum spiked with CDB2914. This assay could not discern the parent and its mono- and di-demethylated metabolites with high precision. The details and uses of this quantification method were previously published[10].

The second was a LC-MS/MS method that was developed and validated for CDB-2914 and CDB-3877 in dog plasma (#CP025032, dated November 15, 2004), rat plasma

(#CP015097, dated 5/12/2006), and rat milk (#CP085253, dated 4/3/2006). This method used (b) (4) with a mixture of diethylether/hexane followed by a reversed phase) LC analysis of the extracts using tandem mass spectrometry detection on a 0.1 mL sample. The method was linear from 1.00 to 100 ng/ML in for rat and dog plasma using 7 calibration standards with 1.00 ng/mL being the LLOQ. The rat milk assay was not correctly validated for quantification since QC samples following freeze-thaw were not correct, but qualitative determination of CDB-2914 and CDB-3877 was permitted. All LC-MS/MS method validation reports were performed under GLP with a QA statement.

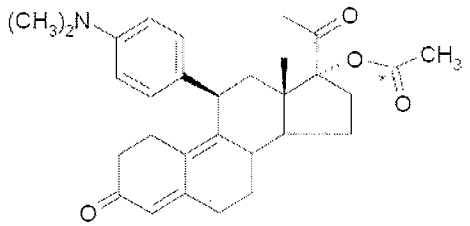
### 5.1.2 Absorption

**Title:** (14C)-VA2914: A study of absorption, distribution, metabolism and excretion following oral and intravenous administration to the rat and the determination of (14C)-VA2914 concentration in mouse plasma following oral administration

**Key study findings**:
- Oral [$^{14}$C]-CDB-2914 was rapidly absorbed with high bioavailability of 80%, and radiolabel elimination half-life of 6 h.
- Tissue concentrations were highest in the liver, the adrenal and fatty tissues.
- Elimination was predominantly through the feces via the hepatobiliary route

**Study no.**: 2109/001- D1145
**Volume #, and page #**: EDR, 4.2.2.2.1.HRA2914.425
**Conducting laboratory and location** (b) (4)

**Date of study initiation**: January 21, 2002
**GLP compliance**: Yes
**QA report**: Yes
**Drug, lot #, batch#, and % purity**: [$^{14}$C]-CDB-2914, 32/02-2109, 148-153-054, 98.9%
SA: 54 mCi/mmol, (Group D batch# 148-113-054)
CDB-2914, 108/02-2109, 010311115, >99.0%



* denotes position of $^{14}$C radiolabel

**Methods:** Female rats were administered 5 mg/kg [$^{14}$C]-CDB-2914 by oral (5 mL/kg) and intravenous (2 mL/kg) routes with the radioactive dose equivalent to 3.7 MBq/kg in ASV. Female mice were orally administered 5 mg/kg [$^{14}$C]-CDB-2914 (512 mL/kg). There were groups dosed by the following schedule:

| Dose Group | Investigation Type | Route | # Animals |
|---|---|---|---|
| A | PK | IV | 36 |
| B - not fasted | PK | oral | 32 |
| B1- fasted | PK | oral | 24 |
| C | Excretion balance | IV | 4 |
| D | Excretion balance | oral | 4 |
| E | Bile excretion | oral | 3 |
| F | QWBA Albino | oral | 18 |
| G | QWBA pigmented | oral | 2 |
| | | | |
| H (mouse) | PK | oral | 3 |

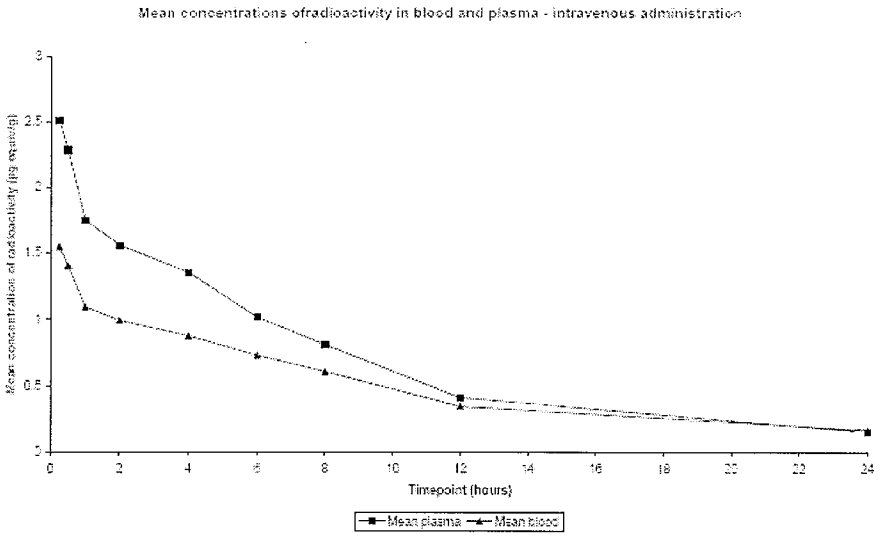
Radioactivity was directly measured by liquid scintillation counting (LSC) the blood, plasma, urine, cage wash/debris, and dose wipe samples. Following dosing, Group A, B, and B1 animals were sacrificed (4 and 3 animals/timepoint for intravenous oral, respectively) at 0.25 (IV only), 0.5 and 1, 2, 4, 6, 8, 12, and 24 h post-dose. Whole blood was collected in heparinized tubes and a portion analyzed for radioactivity, then the remainder was centrifuged to prepare plasma for radiolabel amount determination and metabolic profiling. For Groups C and D, urine was collected over the following time periods following administration of dose: 0-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 h with a collection vessel rinse of 2-5 mL of water added to the urine samples. Feces collection occurred over the following time periods: 0-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 h. After collection, feces were homogenized, combusted and then analyzed by LSC. Additionally, are expired air was trapped and counted until level reach <2x background with Group C collected/counted at 24 h intervals up to 168 h and Group D at 0-6, 6-12 and 12-24 hours, then at 24 h intervals up to 168 h. Cages were rinsed with water then methanol and counted after 24 and 48 h. For Group E, bile, feces, and urine were collected from bile duct cannulated animals were collected on the following time periods post-dose: Bile: 0-2, 2-4, 4-6, 6-12, 12-24, 24-48 h; Urine: 0-12, 12-24 and 24-48 h; Feces: 0-24 and 24-48 h. For Groups F and G following administration of single dose at the following timepoints: 1, 4, and 8 h, and 1, 2, and 3 days post-dose (3 and 1 animals/timepoint for albino and pigmented animals, respectively) were anaesthetized, blood was collected and then sacrificed. The carcasses were frozen in hexane cooled with dry ice, and then embedded in 2% CMC. Two holes were drilled in the block and filled with plasma samples collected at sacrifice. Five sagittal whole body sections (~40 µm thick) were sectioned by microtome and freeze-dried. Quantitative evaluation using phosphor imaging technologies was used to determine tissue concentrations of [$^{14}$C]-CDB-2914. One female mouse was sacrificed at 6, 24, and 48 h for PK to be used to support the micronucleus assay. Analysis and quantification of [$^{14}$C]-CDB-2914 pre- and post-dose aliquots was performed by a validated LC/MS method.

**Results**: Sponsor stated that doses delivered were between 4.9 and 5.26 mg/kg for rats with radioactive doses between 0.661 and 0.935 MBq and were considered acceptable for this study. The mice received a mean of 514 mg/kg with a mean

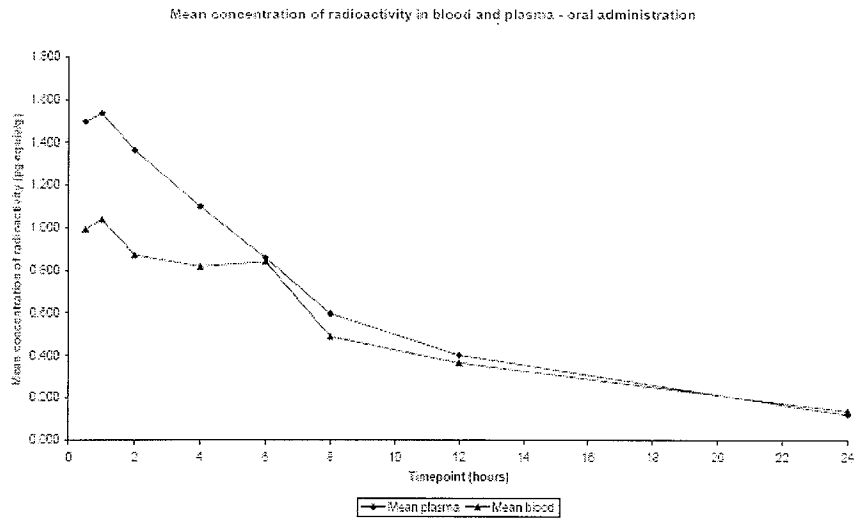
radioactive dose of 0.107 MBq. No clinical signs were observed for rats. A distended abdomen and piloerection was observed in one mouse.

**Figure 7 Concentration of Blood and Plasma Radioactivity Following a Single IV(A) or Oral (B1) Administration of 5 mg/kg ([$^{14}$C]-CDB-2914 to Female Rat Groups A and B1**

A) IV




B) Oral



There was rapid absorption of [$^{14}$C]-CDB-2914 in rats following oral administration with bioavailability of 80% and a $T_{max}$ of ~1 h. Following oral administration, the radiolabel was widely distributed in tissues and plasma with the peak concentrations detected at the 1 h (and first) sampling times and generally declining over the study course, but still quantifiable in most tissues at 3 days (last sample). The elimination half-life of [$^{14}$C]-CDB-2914 material was similar for both oral and i.v. administration at 6 h. There was a high Volume of Distribution and low Clearance consistent with the observed tissue retention. The ratio of blood to plasma radioactive concentrations ranged from 0.653 to 1.004 and 0.598 to 1.204 for oral and i.v. dosing, respectively, was comparable with the trend towards increased ratios over 24 h. The maximal levels of blood and plasma concentration were 35.7 and 48.85 µg equivalents/g, respectively at 24 h after single administration of 512 mg/kg to female mice.

**Table 8 PK Parameters of Blood and Plasma Radioactivity Concentration Following a Single Oral or IV Administration of 5 mg/kg [$^{14}$C]-CDB-2914 to Female Rat Group A & B1**

| PK Parameter | Mean Blood Value | | Mean Plasma Value | |
|---|---|---|---|---|
| | Oral (B) | IV (A) | Oral (B) | IV (A) |
| Cmax (µg equiv/g) | 1.042 | 1.549 | 1.535 | 2.510 |
| Tmax (h) | 1 | | 1 | |
| AUC0-t (µg equiv h/g) | 10.84 | 12.01 | 13.2 | 16.45 |
| AUC0-∞ (µg equiv h/g) | 12.43 | 13.99 | 14.29 | 17.84 |
| $t_{½elim}$ (h) | 8.01 | 8.279 | 6.28 | 6.359 |
| %F | 80.1 | | | |
| $V_d$ (L/kg) | | | 4.268 | 2.571 |
| Cl (L/h/kg) | | | 0.357 | 0.280 |

Tissues with the highest levels included the liver, the adrenal and fatty tissues (peri-renal fat), with plasma and blood having concentrations less than most tissues in albino rats. The concentrations in the liver and adrenals remained fairly high after 3 days (1.725 and 0.208 µg equiv/g, respectively), but the fatty tissues (peri-renal and inguinal fat) decreased to low levels. The central nervous system tissues, uvea, lens, and nasal mucosa had radiolabel concentration below the levels in plasma suggesting limited penetration of the blood-brain barrier. Certain tissues such as the bile duct and the contents of the gastrointestinal systems were qualitatively evaluated by visual examination and it was observed that the highest levels of radiolabel followed a pattern of excretion via the feces. Further, the bile ducts generally showed levels scored "moderate" or "high" through the 72 h period. The pigmented rats had a similar tissue pattern and concentrations. However, the uveal tract, pigmented skin and meninges had higher levels of radiolabel and were retained longer in these tissues compared to albino rats suggesting potential binding to melanin.

**Table 9 Relative Levels of Radioactivity in Selected Tissues of Albino Female Rats Following a Single Oral Administration of 5 mg/kg [$^{14}$C]-CDB-2914 (Sponsor Table 22)**

| | | | µg equivalents of VA2914/g of tissue | | | | | |
|---|---|---|---|---|---|---|---|---|
| Tissue type | Tissue | Kill time | 1 hour | 4 hours | 8 hours | 1 day | 2 days | 3 days |
| Vascular/ lymphatic | Plasma[1] | | 1.556 | 0.983 | 0.641 | 0.111 | 0.059 | 0.078 |
| | Blood | | 1.065 | 0.731 | 0.533 | 0.096 | 0.066 | 0.089 |
| | Aorta | | 2.629 | 1.754 | 2.149 | 0.300 | 0.102 | 0.126 |
| | Mandibular lymph nodes | | 2.104 | 2.235 | 1.291 | 0.155 | 0.081 | 0.119 |
| Metabolic/ excretory | Kidney cortex | | 3.577* | 2.928 | 2.245 | 0.327* | 0.174 | 0.225 |
| | Kidney medulla | | 2.580 | 1.981 | 1.410 | 0.158 | 0.083 | 0.089 |
| | Liver | | 16.61 | 11.67 | 9.895 | 2.379 | 1.298 | 1.725 |
| CNS | Brain | | 0.321 | 0.177 | 0.107 | BLQ | BLQ | 0.017 |
| | Pineal body | | 1.836 | 1.592 | 1.106 | 0.119 | 0.079 | 0.117 |
| | Choroid plexus | | 1.013 | 1.121 | 0.825 | 0.098 | 0.019 | 0.038 |
| | Meninges | | 0.540 | 0.469 | 0.301 | 0.052 | BLQ | 0.011 |
| | Spinal cord | | 0.328 | 0.191 | 0.131 | 0.017 | 0.008 | 0.019 |
| Endocrine | Adrenal | | 5.856 | 4.936* | 3.956* | 0.336 | 0.167 | 0.208 |
| | Thymus | | 1.509 | 1.266 | 0.903 | 0.128 | 0.098 | 0.136 |
| | Thyroid | | 2.650 | 2.046 | 1.665 | 0.315 | 0.203 | 0.192 |
| | Pituitary | | 2.100 | 2.144 | 1.518 | 0.172 | 0.096 | 0.129 |
| Secretory | Intra-orbital lachrymal gland | | 3.182 | 2.365 | 1.813 | 0.232 | 0.115 | 0.164 |
| | Harderian gland | | 5.167 | 3.719 | 2.682 | 0.446 | 0.148 | 0.150 |
| | Salivary glands | | 3.272 | 2.215 | 1.849 | 0.189 | 0.086 | 0.112 |
| Gonads | Clitoris | | 2.875 | 3.062 | 2.777 | 0.525 | 0.290 | 0.188 |
| | Ovary | | 4.350* | 3.069* | 2.616* | 0.329 | 0.164 | 0.204 |
| | Uterus | | 2.096 | 1.640 | 1.444 | 0.122 | 0.136 | 0.139 |
| Fatty | Brown fat | | 6.025 | 5.611 | 3.889 | 0.243 | 0.121 | 0.115 |
| | Inguinal fat | | 4.497 | 5.350 | 4.697 | 0.258 | 0.053 | 0.037 |
| | Peri-renal fat | | 6.384 | 6.047* | 6.579 | 0.530 | 0.102 | 0.040 |
| Muscular | Muscle | | 1.403 | 1.151 | 0.780 | 0.061 | 0.035 | 0.035 |
| | Myocardium | | 2.624 | 2.076 | 1.526 | 0.132 | 0.072 | 0.086 |
| | Tongue | | 2.411 | 1.874 | 1.267 | 0.115 | 0.067 | 0.084 |
| Ocular | Lens | | 0.110 | 0.109 | 0.292 | 0.098 | 0.042 | 0.047 |
| | Uveal tract | | 0.867 | 0.656 | 0.462 | 0.131 | 0.088 | 0.072 |
| Unclassified | Bone marrow | | 0.952 | 1.011 | 0.751 | 0.160 | 0.100 | 0.145 |
| | Lung | | 1.835 | 1.310 | 0.967 | 0.099 | 0.081 | 0.093 |
| | Nasal mucosa | | 0.665 | 0.679 | 0.569 | 0.109 | 0.057 | 0.066 |
| | Pancreas | | 3.734 | 2.973 | 2.150 | 0.213 | 0.105 | 0.135 |
| | Spleen | | 2.762 | 2.055 | 2.763* | 0.241 | 0.135 | 0.183 |
| | Tooth pulp | | 1.034 | 0.947 | 0.614 | 0.092 | 0.107 | 0.150 |
| | Peridontal membrane | | 0.894 | 0.752 | 0.725 | 0.085 | 0.084 | 0.111 |
| | Non pigmented skin | | 1.606 | 1.551 | 0.873 | 0.106 | 0.078 | 0.076 |
| Gastrointestinal | Stomach mucosa (fundic) | | 8.772* | 7.008* | 4.055* | 0.549 | 0.157 | 0.197 |
| | Stomach mucosa (non-fundic) | | 9.881* | 9.363* | 4.210* | 0.162 | 0.094 | 0.246 |
| | Small intestine mucosa | | 5.400 | 8.045* | 4.074* | 0.729 | 0.157 | 0.219 |
| | Caecum mucosa | | 2.625 | 4.948* | 5.543* | 0.398 | 0.083 | 0.380 |
| | Large intestine mucosa | | 3.041 | 2.159 | 4.280* | 2.356 | 0.135 | 0.242 |
| | Rectum mucosa | | 1.806 | 1.891 | 2.634* | 0.342 | 0.120 | 0.140 |

Upper limit of detection = 45.99 µg equiv/g
Lower limit of detection = 0.024 µg equiv/g

[1] - Plasma radioactivity concentrations determined by liquid scintillation counting methods
Limit of detection = 0.008 µg equiv/g.

Note: Tissue concentrations can be less than the lower limit of quantification as individual measurements below this limit taken as zero for the calculation of the mean.

BLQ - Tissue concentration below lower limit of quantification

* - Tissue measurement affected by flaring

**Table 10 Relative Levels of Radioactivity in Selected Tissues of Pigmented Female Rats Following a Single Oral Administration of 5 mg/kg [$^{14}$C]-CDB-2914 ((Sponsor Table 24)**

| | | µg equivalents of VA2914/g of tissue | |
|---|---|---|---|
| | Animal number and sex | 377F | 378F |
| Tissue type | Tissue Kill time | 1 day | 3 day |
| Vascular/ lymphatic | Plasma[1] | 0.137 | 0.021 |
| | Blood | 0.128 | 0.029 |
| | Aorta | 0.364 | 0.065 |
| | Mandibular lymph nodes | 0.232 | 0.041 |
| Metabolic/ excretory | Kidney cortex | 0.803 | 0.092 |
| | Kidney medulla | 0.336 | 0.041 |
| | Liver | 2.788 | 0.776 |
| CNS | Brain | 0.038 | BLQ |
| | Pineal body | NS | BLQ |
| | Choroid plexus | 0.109 | BLQ |
| | Meninges | 0.082 | 0.226 |
| | Spinal cord | 0.035 | BLQ |
| Endocrine | Adrenal | 0.694 | 0.079 |
| | Pituitary | 0.318 | 0.064 |
| | Thymus | 0.219 | 0.046 |
| | Thyroid | 0.458 | 0.091 |
| Secretory | Intra-orbital lachrymal gland | 0.417 | 0.066 |
| | Harderian gland | 0.731 | 0.113 |
| | Salivary glands | 0.447 | 0.055 |
| Gonads | Clitoris | 0.910 | 0.154 |
| | Ovary | 1.025 | 0.074 |
| | Uterus | 0.781 | BLQ |
| Fatty | Brown fat | 1.046 | 0.074 |
| | Inguinal fat | 1.425 | 0.026 |
| | Peri-renal fat | 1.875 | 0.049 |
| Muscular | Muscle | 0.150 | 0.026 |
| | Myocardium | 0.324 | 0.042 |
| | Tongue | 0.286 | 0.039 |
| Ocular | Lens | 0.101 | 0.070 |
| | Uveal tract | 2.414 | 1.007 |
| Unclassified | Bone marrow | 0.251 | 0.068 |
| | Lung | 0.267 | 0.038 |
| | Nasal mucosa | 0.208 | 0.039 |
| | Pancreas | 0.611 | 0.047 |
| | Periodontal membrane | 0.135 | NS |
| | Spleen | 0.315 | 0.069 |
| | Tooth pulp | 0.142 | NS |
| | Non pigmented skin | 0.253 | 0.036 |
| | Pigmented skin | 1.239 | 0.253 |
| Gastrointestinal | Stomach mucosa (fundus) | 1.047 | 0.068 |
| | Stomach mucosa (non fundic) | 1.810 | 0.054 |
| | Large intestine mucosa | 1.032 | 0.063 |
| | Caecum mucosa | 1.032 | 0.278 |
| | Small intestine mucosa | 1.560 | 0.098 |
| | Rectum mucosa | 0.369 | 0.113 |
| | Upper limit of detection = | 45.99 | µg equiv/g |
| | Lower limit of detection = | 0.024 | µg equiv/g |

[1] - Plasma radioactivity concentrations determined by liquid scintillation counting methods Limit of detection = 0.007 µg equiv/g.

BLQ - Tissue concentration below lower limit of quantification

NS - Tissue not sectioned

**Table 11 Comparison of Radioactivity Distribution to Selected Tissues of Albino and Pigmanted Female Rats Following a Single Oral Administration of 5 mg/kg [$^{14}$C]-CDB-2914**

| | µg equivalents of VA2914/g of tissue | | | |
|---|---|---|---|---|
| Sampling time | 1 day | | 3 days | |
| Tissue | Albino animals | Pigmented animal | Albino animals | Pigmented animal |
| Uveal tract | 0.131 | 2.414 | 0.072 | 1.007 |
| Pigmented skin | NA | 1.239 | NA | 0.253 |
| Non-pigmented skin | 0.106 | 0.253 | 0.076 | 0.036 |
| Meninges | 0.052 | 0.082 | 0.011 | 0.226 |

Sponsor's Table

CDB-2914 is extensively metabolized and the metabolic profiles of oral and i.v. administration are similar. The metabolic profiling performed on the excreta and plasma demonstrated that CDB-2914 and CDB-3877 were detected in plasma, bile and feces.

Radioactivity excreted following oral or intravenous administration occurred predominantly via the feces in intact rats. The majority of the radioactivity (~90%) was in the excreta over 0-72 h post-dose, and feces content accounted for ~70% of dose and renal accounted for 2 and 8% for i.v. and oral, respectively. Expiration elimination was low ~1% and was associated with $CO_2$, not organic solvents. About 2% of the radioactivity remained in the carcasses of intact rats. Biliary elimination accounted for~65% of the administered dose over 0-48 h post-dose, while renal excretion accounted for up to 4% of the dose.

**Table 12 Summary of % Total Recovery of Radiolabled Materials in Excreta and Carcasses Following a Single IV or Oral Administration of 5 mg/kg [$^{14}$C]-CDB-2914 to Female Rat Groups C, D and E**

| Sample | % of Administered Dose | | | | | |
|---|---|---|---|---|---|---|
| | IV (C) | | Oral (D) | | Oral (bile duct cannulated, E) | |
| | Mean | SD | Mean | SD | Mean | SD |
| Urine | 5.197 | 1.868 | 2.385 | 0.315 | 4.014 | 1.459 |
| Feces | 71.4 | 11.69 | 83.27 | 9.05 | 28.83 | 3.826 |
| Cage Wash | 9.995 | 5.646 | 2.875 | 3.483 | 0.269 | 0.142 |
| Final Wash | 0.013 | 0.015 | 0.016 | 0.015 | 0.127 | 0.034 |
| Cage Debris | ND | ND | 0.136 | 0.072 | 0.022 | 0.038 |
| CO2 Trap 1 | 1.141 | 0.697 | 1.626 | 0.475 | 3.685 | 1.912 |
| CO2 Trap 2 | 1.158 | 0.529 | 1.013 | 0.412 | 4.014 | 1.459 |
| Carcass | 2.012 | 0.393 | 1.879 | 0.291 | 28.83 | 3.826 |
| Bile | | | | | 61.48 | 3.36 |
| Total | 90.92 | 5.581 | 93.19 | 5.835 | 98.43 | 1.208 |

ND, Not Detected

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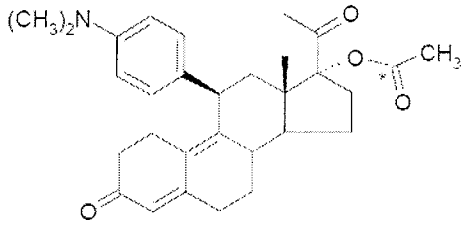
**Title:** (14C)-VA 2914: A study of absorption, metabolism and excretion following oral and intravenous administration to the cynomolgus monkey

**Key study findings**:

- Oral [$^{14}$C]-CDB-2914 was rapidly absorbed with high plasma bioavailability (F) of 112% and moderate blood F of 30%.
- Widespread but moderate levels radiolabel distribution with long elimination $t_{1/2}$s observed (~3.5 and 11 days, for oral plasma and i.v. plasma, respectively).
- Tissues with the highest levels included the liver, kidney cortex and the adrenal.
- Radioactivity excretion was predominantly via the feces.

**Study no.**: 2109/002-D1145
**Volume #, and page #**: EDR, 4.2.2.2.1.HRA2914.426
**Conducting laboratory and location** (b) (4)

**Date of study initiation**: June 20, 2003
**GLP compliance**: Yes
**QA report**: Yes
**Drug, lot #, batch#, and % purity**: Group A: [$^{14}$C]-CDB-2914, 32/02-2109, 148-113-054,
95.03% SA: 54 mCi/mmol
Group B: [$^{14}$C]-CDB-2914, 32/02-2109, 148-105-054,
95.03% SA: 54 mCi/mmol
CDB-2914, 108/02-2109, 010311115, 98.7%




**Methods:** Female monkeys were administered 5 mg/kg [$^{14}$C]-CDB-2914 by oral (3.2 mL/kg) and intravenous (1 mL/kg) routes in ASV. The animals were administered test article by the oral route, then by intravenous administration with a washout period of 10 days.

| Dose Group | Study Type | Route | Dose Volume (mg/mL) | Radioactive Equivalent Dose Concentrations | | | # Animals |
|---|---|---|---|---|---|---|---|
| | | | | MBq/mL | MBq/kg | mg/g | |
| A | PK/excretion | oral | 1.67 | 5 | 3.7 | 1.55 | 4* |
| B | PK/excretion | IV | 1.6 | 6.5 | 2.107 | 1.624 | 4* |

*Same animals used

Radioactivity was directly measured by liquid scintillation counting (LSC) the blood, plasma, urine, cage wash/debris, and dose wipe samples. Following oral dosing, blood was sampled at 0.5, 1, 2, 4, 6, 8, 12 and 24 h post-dose. Two additional blood samples were taken on Days 11 and 16 post-dose. Whole blood was collected in heparinized tubes and a portion analyzed for radioactivity, then the remainder was centrifuged to prepare plasma for radiolabel amount determination and metabolic profiling. Urine was collected over the following time periods following administration of dose: 0-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 h with a collection vessel rinse of 2-5 mL of water added to the urine samples. Feces collection occurred over the following time periods: 0-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 h. After collection, feces were homogenized, combusted and then analyzed by LSC. Cages were rinsed with water then methanol and counted after 24, 48 and 168 h.

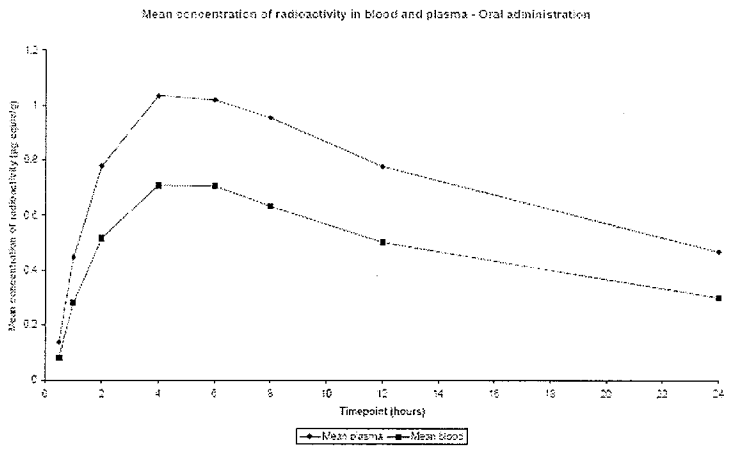
Due to low recovery of radioactivity following oral administration, blood and excreta collections were extended during the intravenous stage with animals housed in metabolic cages. The radioactive dose administered was also increased to allow the analysis of tissues and/or Quantitative Whole Body Autoradiography (QWBA), with the changes documented in Protocol Amendment 1. Blood was sampled at 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 h and 2, 3, 4, 5, 7, 9, and 11 days post-dose. Urine and feces were collected over the time intervals of 0-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 h and every 24 h, then after until 336 h (Day 14). Three animals were held in until Day 15 and excreta were collected with documentation in the Protocol Deviation section. Cages wash was collected after 24, 48 and 336 h.

The smallest animal was sedated and sacrificed followed by exsanguination for QWBA analysis (a smaller animal freezes faster and is easier to perform sectioning). The legs and tail were removed and the carcass was frozen in hexane cooled with dry ice, and then embedded in 2% CMC. Two holes were drilled in the block and filled with plasma samples collected at sacrifice. Sagittal whole body sections (~30 µm thick) were sectioned by microtome and freeze-dried. The removed parts were digested in KOH, neutralized, then counted by liquid scintillation. Quantitative evaluation using phosphor imaging technologies was used to determine tissue concentrations of [$^{14}$C]-CDB-2914. Analysis and quantification of [$^{14}$C]-CDB-2914 pre- and post-dose aliquots was performed by a validated LC/MS method. The 3 remaining animals were sedated and sacrificed followed by exsanguination on Day 15. The duodenum, caecum, colon, heart, lung, spleen, liver, kidney, stomach and the small intestine were removed and prepared for determination of radioactivity amounts in each.

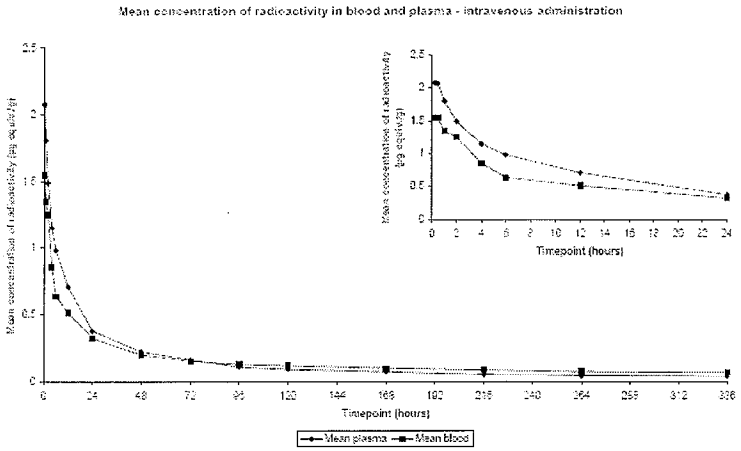
**Results**: Sponsor stated that doses delivered were between 5.283 and 5.331 mg/kg for the monkeys, with radioactive doses received being between 13.31 and 13.84 MBq. For intravenous administration, doses were between 5.068 and 5.089 mg/kg and between 17.75 and 19.03 MBq. These results were deeded acceptable by the sponsor.

**Figure 8 Concentration of Blood and Plasma Radioactivity Following a Single Oral (A) or IV (B) Administration of 5 mg/kg ([$^{14}$C]-CDB-2914 to Female Monkeys**

A) Oral




B) IV




The absorption of [$^{14}$C]-CDB-2914 in female monkeys following oral administration was high with $T_{max}$ of ~4 h in both plasma and blood and bioavailability of 112% in plasma. The bioavailability was only 30% in blood suggesting that there was little binding to blood cells. The ratio of plasma to blood radioactive concentrations ranged from 0.593 to 0.692 and 0.751 to 1.779 for oral and i.v. dosing, respectively, with the trend towards increased ratios over 24 h. Over time, the concentrations in blood were higher than in the plasma, suggesting that radioactive-containing material was beginning to binding to the blood cells. Overall, the oral AUC exposure, %F, and half-life values were considerably higher for plasma than blood, while the i.v. dosing values was comparable. Also, these blood values were higher for i.v. than oral dosing, but comparable for the plasma. The plasma elimination half-life of [$^{14}$C]-CDB-2914 material was for an extended duration for both oral and i.v. administration at ~3.5 and ~11 days, respectively. There was a high Volume of Distribution and moderately low Clearance values suggesting tissue retention.

**Table 13 Parameters of Whole Blood and Plasma Radioactivity Concentration Following a Single Oral (A) or IV (B) Administration of 5 mg/kg [$^{14}$C]-CDB-2914 to Female Monkeys**

| PK Parameter | Mean Blood Value | | Mean Plasma Value | |
|---|---|---|---|---|
| | Oral (A) | IV (B) | Oral (A) | IV (B) |
| Cmax (µg equiv/g) | 0.769 ± 0.074 | 1.552 ± 0.177 | 1.132 ± 0.113 | 2.081 ± 0.334 |
| Tmax (h) | 4.5 ± 1.915 | | 4 ± 633 | |
| AUC0-t (µg equiv h/g) | 11.45 ± 1.732 | 48.85 ± 8.559 | 64.45 ± 13.30 | 47.27 ± 6.408 |
| AUC0-∞ (µg equiv h/g) | 18.04 ± 2.688 | 77.78 ± 13.45 | 68.16 ± 14.18 | 61.09 ± 7.80 |
| $t_{½elim}$ (h) | 15.28 ± 1.530 | 313.7 ± 44.85 | 86.75 ± 5.365 | 262.6 ± 130 |
| %F | 23.49 ± 4.019 | | 112.7 ± 23.7 | |
| $V_d$ (L/kg) | | 29726 ± 6393 | | 30655 ± 12456 |
| Cl (L/h/kg) | | 65.61 ± 10.32 | | 82.86 ± 10.63 |

Radioactivity was widespread and detected in all tissues that were sectioned in the single QWBA monkey, albeit at fairly modest levels. Tissues with the highest levels included the liver, kidney cortex and the adrenal (cortex and medulla). The central nervous system tissues had radiolabel concentration below the levels in blood (0.064 µg equiv/g) and plasma (0.036 µg equiv/g) suggesting limited penetration of the blood-brain barrier. However, uvea and nasal mucosa levels were higher than blood and plasma levels. Only the liver had a recovery of >1% of radiolabel from the harvested organs; all others had 50x less recovery.

**Table 14 Relative Levels of Radioactivity in Selected Tissues from Female Cynomolgus Following a Single Intravenous Administration of 5 mg/kg [$^{14}$C]-CDB-2914**

**Mean concentrations of radioactivity in the tissues of a cynomolgus monkey after a single oral administration of ($^{14}$C)-VA2914 at a nominal dose level of 5 mg/kg bodyweight (Dose group B)**

| | µg equivalents of VA2914/g of Tissue | | | | |
|---|---|---|---|---|---|
| **Animal number** | | | **201F** | | |
| **Level** | **A** | **B** | **C** | **D** | **E** |
| Blood | | | 0.067 | | |
| Aorta | | | 0.055 | | |
| Gall bladder | | | 1.077 | | |
| Kidney cortex | | 0.213 | | | |
| Kidney medulla | | 0.077 | | | |
| Liver | | | 1.318 | | |
| Brain | | | | | 0.027 |
| Choroid plexus | | | | | 0.038 |
| Spinal cord | | | | | 0.025 |
| Adrenal cortex | | 0.486 | | | |
| Adrenal medulla | | 0.315 | | | |
| Pituitary | | | | | 0.063 |
| Thymus | | | | 0.066 | |
| Thyroid | | | | 0.154 | |
| Parotid salivary gland | 0.109 | | | | |
| Salivary glands | | 0.109 | | | |
| Brown fat | | | 0.131 | | |
| White fat | 0.039 | | | | |
| Clitoris | | | | | 0.106 |
| Uterus | | | | | 0.066 |
| Muscle | 0.083 | | | | |
| Myocardium | | 0.090 | | | |
| Tongue | | | | | 0.066 |
| Skin | | | | | 0.088 |
| Lens | | | | 0.033 | |
| Uveal tract | | | | 0.501 | |
| Bone marrow | 0.077 | | | | |
| Lung | | 0.054 | | | |
| Spleen | 0.176 | | | | |
| Nasal mucosa | | | | | 0.122 |
| Stomach mucosa (fundus) | 0.096 | | | | |
| Stomach mucosa (non-fundic) | 0.068 | | | | |
| Small intestine mucosa | | 0.120 | | | |
| Caecum mucosa | 0.137 | | | | |
| Large intestine mucosa | | | 0.096 | | |
| Rectum mucosa | | | | | 0.078 |
| Artefact | | | 0.523 | | |
| **Lower Limit of Quantification** | **0.011** | **0.011** | **0.008** | **0.011** | **0.008** |

Sponsor's Table 12.

**Table 15 Recovery of Radioactivity in Selected Tissues from Female Cynomolgus Following a Single Intravenous Administration of 5 mg/kg [$^{14}$C]-CDB-2914**

Recovery of radioactivity in tissues following intravenous administration of ($^{14}$C)-VA2914 to female monkeys at a nominal dose level of 5 mg/kg bodyweight (Dose group B)

| Sample | Percent of administered radioactivity | | | | |
|---|---|---|---|---|---|
| | 202F | 203F | 204F | Mean | SD |
| Duodenum | 0.002 | 0.003 | 0.002 | 0.002 | 0.001 |
| Caecum | 0.005 | 0.005 | 0.006 | 0.005 | 0.001 |
| Colon | 0.020 | 0.030 | 0.042 | 0.031 | 0.011 |
| Heart | 0.006 | 0.009 | 0.007 | 0.007 | 0.002 |
| Lung | 0.012 | 0.010 | 0.012 | 0.011 | 0.001 |
| Spleen | 0.005 | 0.007 | 0.006 | 0.006 | 0.001 |
| Liver | 0.963 | 1.387 | 0.951 | 1.100 | 0.248 |
| Kidney | 0.036 | 0.047 | 0.029 | 0.037 | 0.009 |
| G.I. Tract Contents | 0.034 | 0.069 | 0.100 | 0.068 | 0.033 |
| Stomach | 0.007 | 0.014 | 0.009 | 0.010 | 0.004 |
| Small Intestine | 0.024 | 0.030 | 0.025 | 0.026 | 0.003 |
| Total | 1.114 | 1.612 | 1.189 | 1.305 | 0.269 |

Sponsor's Table 11.

CDB-2914 is extensively metabolized with up to 20 metabolites, and the metabolic profiles of oral and i.v. administration are similar. The metabolic profiling performed on the excreta and plasma demonstrated that CDB-2914, CDB-3877, and CDB-3936 (didemethylated metabolite) were detected in plasma, urine and feces.

**Table 16 Summary of Radiolabeled Peaks Detected in Plasma, Urine and Feces Following Oral or Intravenous Administration of [$^{14}$C]-CDB-2914 to Female Cynomolgus Monkeys Present at ≥10% Total Radioactivity from a Source Material**

| Group | Source | Peak # | % of Total Radioactivity from Source Material | Note |
|---|---|---|---|---|
| Oral | Plasma | P11 | 25.9 | |
| Oral | Plasma | P13 | 9.6 | |
| Oral | Plasma | P16 | NA | Parent |
| IV | Plasma | P11 | 17.8 | |
| IV | Plasma | P16 | 22.52 | Parent |
| Oral | Urine | U10 | 14.1 | |
| Oral | Urine | U13 | 22.7 | |
| IV | Urine | U6 | 10.9 | |
| IV | Urine | U10 | 14.3 | |
| IV | Urine | U13 | 22.0 | |
| Oral | Feces | F10 | 18.9 | |
| Oral | Feces | F13 | 16.2 | |
| Oral | Feces | F16 | 11.9 | |
| IV | Feces | F10 | 26.8 | |
| IV | Feces | F13 | 13.2 | |
| IV | Feces | F16 | 11.2 | |

Radioactivity excretion following oral or i.v. administration occurred predominantly via the feces in female monkeys. Recovery of radioactivity from excreta was 78.42 ± 2.9% and 69.71 ± 2.43% for i.v. and oral administration, respectively. The majority of the radioactivity was in the excreta over 0-96 h post-dose, and feces content accounted for 67% and 45% of the i.v. and oral dose, respectively. Renal excretion via urine accounted for 7.3% and 6.3% for i.v. and oral, respectively, by 48 h. A significant amount was detected in the cage washes, debris, and swabs (17.9% for oral, and 1.4% for i.v.), and 1.2% of the radioactivity remained in the carcasses.

**Table 17 Summary of % Total Recovery of Radiolabeled Materials in Excreta and Carcasses Following a Single IV or Oral Administration of 5 mg/kg [$^{14}$C]-CDB-2914 to Female Monkeys**

| Sample | % of Administered Dose | | | |
|---|---|---|---|---|
| | IV | | Oral | |
| | Mean | SD | Mean | SD |
| Urine | 7.31 | 1.035 | 6.273 | 0.699 |
| Feces | 66.90 | 3.167 | 44.67 | 5.18 |
| Cage Wash | 1.437 | 0.564 | 11.34 | 1.918 |
| Final Wash | 0.047 | 0.016 | 0.475 | 0.27 |
| Cage Debris | 0.603 | 0.335 | 0.879 | 0.214 |
| Swabs | 0.223 | 0.248 | 6.087 | 1.797 |
| Carcass | 1.224 | 0.143 | -- | -- |
| Tissues | 1.305 | 0.269 | -- | -- |
| | | | | |
| Total | 78.42 | 2.919 | 69.71 | 2.431 |

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### 5.1.3 Distribution

**Title:** In Vitro Binding of [$^{14}$C]-VA2914 to Mouse, Rat, Rabbit, Dog, Monkey and Human Plasma

**Key study findings**:

- [$^{14}$C]-CDB-2914 was highly protein bound to plasma from rats, mice, rabbits, dogs, monkeys, and humans.

**Study no.**: PKFAC 0205
**Volume #, and page #**: EDR, 4.2.2.3.1.HRA2914.428
**Conducting laboratory and location**: (b) (4)

**Date of study initiation**: February 6, 2002
**GLP compliance**: No
**QA report**: Yes
**Drug, lot #, batch#, and % purity**: [$^{14}$C]-CDB-2914, 32/02-2109, 148-105-054, 98.7%
SA: 54 mCi/mmol

**Methods:** Plasma from at least 3 female rats, mice, rabbits, dogs, and cynomolgus monkeys was obtained from blood collected in heparinized tubes and stored at 4°C until plasma was isolated, then plasma was stored at -20°C until used. Plasma was obtained by centrifugation of the blood samples and pooled. Total protein was measured from the pooled plasma samples. Human plasma was obtained from 3 healthy, consenting women donors aged 18-50 years. Blood was collected and plasma prepared as above, with HSA also being measured in plasma. Equilibrium dialysis was used to determine the amount of plasma protein binding over a concentration range of 0.01 - 10 µg/mL by direct liquid scintillation measurement of radiolabeled material. The concentrations of [$^{14}$C]-CDB-2914 tested were 0.03 - 20 µM.

**Results: Table** below lists the results for protein binding of [$^{14}$C]-CDB-2914 to plasma when tested over the range of 0.03 - 20 µM. CDB-2914 was highly protein bound in plasma of all species examined with 96-7 - 99.46% bound. The % bound remained constant over the range of CDB-2914 tested for all species. There were statistically significant differences between some species, but not likely to be biologically significant.

**Table 18 Mean in Vitro Plasma Protein Binding of [$^{14}$C]-CDB-2914**

| Species Plasma Tested | % Bound |
|---|---|
| Mouse (CD) | 99.39 ± 0.34 |
| Rat (Wistar) | 99.46 ± 0.11 |
| Rabbit (NZW) | 97.86 ± 0.51 |
| Dog (Beagle) | 98.67 ± 1.22 |
| Monkey (Cynomolgus) | 96.68 ± 0.70 |
| Human | 98.24 ± 0.12 |

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**Title:** In vitro binding of [$^{14}$C]-VA2914 to human plasma proteins and human blood distribution

**Key study findings**:

- [$^{14}$C]-CDB-2914 was highly bound to human blood and plasma with 4.86% bound to blood cells and 94.09% bound to plasma proteins with a free-fraction of 1.1%.

- [$^{14}$C]-CDB-2914 significantly bound to HSA, AAG, and lipoprotein components of plasma.

**Study no.**: PKFAC 0206
**Volume #, and page #**: EDR, 4.2.2.3.1.HRA2914.427
**Conducting laboratory and location**: (b) (4)
**Date of study initiation**: May 27 2002
**GLP compliance**: No
**QA report**: Yes
**Drug, lot #, batch#, and % purity**: [$^{14}$C]-CDB-2914, 32/02-2109, 148-105-054, 98.7%
SA: 54 mCi/mmol

**Methods:** Human plasma from blood collected in heparinized tubes was obtained from 3 healthy, consenting women donors aged 18-50 years. Plasma was obtained by centrifugation of the blood samples, pooled and stored at -20°C until used. Human plasma proteins/components measured: HSA (human serum albumin), AAG (α1-acid glycoprotein), GG (γ-globulins), HDL, LDL, VLDL (high, low, and very low density lipoproteins), NEFA (non-esterified fatty acids).
Total protein, HSA, NEFA, NEFA/HSA, AAG, GG, and lipoproteins were measured from the pooled plasma samples and used to create simulated human plasma (pH adjusted to 7.4). Blood cell binding was determined by blood partitioning methodology over a concentration range of 0.02 - 21 µM [$^{14}$C]-CDB-2914 after 2 h at 37°C by direct liquid scintillation measurement of radiolabeled material. Binding was measured using both cells in native blood with plasma and washed cells in glucose saline buffer. Equilibrium dialysis was used to determine the amount of plasma protein binding after 2 h at 37°C over a concentration range listed in the **Table** below specific for each component by direct liquid scintillation measurement of radiolabeled material. The concentrations of [$^{14}$C]-CDB-2914 tested are listed in **Table** below

**Results:** Binding of CDB-2914 to blood cells in buffer (~83%) and plasma (~17%) remained constant over the concentration range tested using cells from the 3 women, with the plasma to blood distribution ratios being ~0.15 and ~4.1, respectively. Binding of CDB-2914 to pooled plasma is high in the absence of blood cells (98.3% vs. 18.7%). Binding of CDB-2914 to pooled plasma and to selected components remained constant over the concentration ranges tested, except for AAG, which decreased from 97.42% to 88.25%. Binding to AAG was significant and reported to be saturable and binds 1:1 to drug. The presence of NEFA significantly reduced binding of CDB-2914 to HSA from 22.2% to 14.7%. CDB-2914 associates with lipoproteins such as LDL and HDL due to its high hydrophobicity.

**Table 19 Binding of [$^{14}$C]-CDB-2914 to Simulated Human Plasma Components and Blood Cells**

| | CDB-2914 Concentrations (µM) | Measured NKa (Mean ± SD) | Simulated Blood Distribution (%) |
|---|---|---|---|
| Unbound fraction (fu) | | | 1.06 |
| Bound fraction: | | | (98.95) |
| Blood cells in plasma | 0.04-22 | 0.27± 0.12 | 4.86 |
| Blood cells in buffer | 0.04-22 | 6.36 ± 1.41 | |
| Plasma | 0.02-18 | 54.85 ± 4.34 | 94.09 |
| HSA w/ NEFA | 0.02-18 | 14.70 ± 1.74 | 15.51 |
| AAG | 0.02-16 | 34.85 ± 13.59 | 28.99 |
| GG | 0.02-10 | 0.40 ± 0.06 | 0.43 |
| VLDL | 0.001-7 | 0.45 ± 0.02 | 0.47 |
| LDL | 0.02-20 | 18.26 ± 0.65 | 19.26 |
| HDL | 0.02-20 | 27.91 ± 1.92 | 29.44 |

Modified from Sponsor's Table pg. 5.

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**Title:** PGL4002: Extent of binding to rat, monkey and human plasma proteins and partitioning between the plasma and cell fraction of human blood

**Key study findings**:

- CDB-3877 at 1.5 µM was highly bound to rat, human and monkey plasma proteins at 98.0%, 96.9%, and 93.5%, respectively, and was not highly bound to red cells *in vitro*.

**Study no.**: PGL09-011
**Volume #, and page #**: EDR, 4.2.2.3.1.HRA2914.475
**Conducting laboratory and location**: (b) (4)
**Date of study initiation**: None provided. Report Issued: September 8, 2009
**GLP compliance**: No
**QA report**: No
**Drug, lot #, batch#, and % purity**: CDB-3877(PGL-4002), IN-AKS-A-76, 97.5%

**Methods:** Human blood, Wistar rat, cynomolgus monkey, and human heparanized plasma were purchased for use. The amount of plasma protein binding at 0.15 and 1.5 µM CDB-3877 after 22 h at 37°C was analyzed by LC-MS/MS using equilibrium dialysis methodology. Blood partitioning analysis after 1 h at 37°C was performed and analyzed by LC-MS/MS to obtain the blood-to-plasma ratio. Reference compounds were used to validate the assays.

**Results**: CDB-3877 was highly bound to plasma proteins regardless of species examined (92.5% to 98.0%) with rat>human>monkey. Increasing the concentration from 0.15 to 1,5 µM had little effect on binding percentage. CDB-3877 had a blood cell: plasma ratio of 0.32 showing little binding to red cells compared to moderate and highly bound drugs. Stability after 1 h at 37°C in human plasma was 91%.

**Table 20 Protein Binding via Equilibrium Dialysis at 1.5 µM CDB-3877**

| Test Compound ID | Species | Concentration (µM) | | | | | | % Bound | % Recovery |
|---|---|---|---|---|---|---|---|---|---|
| | | Receiver | | | Donor | | | | |
| | | R1 | R2 | R3 | R1 | R2 | R3 | | |
| PGL4002 | Human | 0.037 | 0.033 | 0.035 | 1.16 | 1.07 | 1.16 | 96.9 | 78 |
| | Rat | 0.023 | 0.024 | 0.023 | 1.12 | 1.21 | 1.21 | 98.0 | 80 |
| | Monkey | 0.081 | 0.075 | 0.087 | 1.16 | 1.06 | 1.02 | 92.5 | 77 |

Sponsor Table 3.1, pg. 7

**Table 21 Blood Partitioning and Stability of CDB-3877**

| Test Compound ID | Red Blood Cell to Plasma Partitioning ($K_{RBCP}$) | Stability | | |
|---|---|---|---|---|
| | | Compound Response Ratio (Control Plasma) | | |
| | 60 minute | 2 minute | 60 minute | % Remaining at 60 minute |
| PGL4002 | 0.32 (24%) | 6.7 | 6.0 | 91 |
| Metoprolol | 1.17 (54%) | 63 | 60 | 93 |
| Chlorthalidone | 16.5 (94%) | 3.9 | 3.5 | 89 |

Sponsor Table 3.3, pg. 7

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**Title:** ADME-Tox - Study of Compound 3877

**Key study findings**:

- CDB-3877 was highly plasma protein bound for all species, except monkeys (47.1%) *in vitro.*
- CDB-3877 was extensively metabolized in liver microsomal extracts of all species with ~1% remaining for all species, except human (18%).

**Study no.**: 15714
**Volume #, and page #**: EDR, 4.2.2.3.1.HRA2914.478
**Conducting laboratory and location** (b) (4)
**Date of study initiation**: November 26, 2008
**GLP compliance**: No
**QA report**: Yes
**Drug, lot #, batch#, and % purity**: CDB-3877(PGL-4002), IC0605-01-WS002

**Methods:** Human blood, heparanized plasma from mouse, rat, rabbit, dog, cynomolgus monkey, and human, and human HSA and AAG proteins were purchased for use. The amount of protein binding of 10 µM CDB-3877 after 8 h at 37°C was analyzed by HPLC-MS/MS using equilibrium dialysis methodology. Blood partitioning analysis after 8 h at 37°C was performed and analyzed by HPLC-MS/MS to obtain the blood-to-plasma ratio. Metabolic stability of 1 µM CDB-3877 plus β-NADPH (1 mM), G6P (5mM), G6PDHase (1U/mL) after 0 and 1 h at 37°C was examined using liver microsomes at 0.3 mg/mL protein concentration from CD-1 mice, SD rats, beagle dogs, cynomolgus monkeys, and humans. Reference compounds were used to validate the assays.

**Results**: CDB-3877 was highly bound to plasma proteins (83 - 96%), except cynomolgus monkey which was 47.1%. The rank order was rat>mouse>human>rabbit, dog>>monkey. CDB-3877 was highly bound to HSA and AAG protein components of plasma with 70.1% and 97.4%, respectively. CDB-3877 had a Partition Coefficient of 1.5 (n=2) showing moderate binding to red cells compared to the reference highly bound drug, chlothalidone (10.8). CDB-3877 remaining after incubation with liver microsomes was ~1% with all species except human; for human, 18% CDB-3877 remained.

**Table 22 Mean % Plasma Protein Binding and %CDB-3877 Remaining After Liver Microsome Incubation (n=2)**

| Assay | Mean %Protein Bound | Mean % Recovery | Mean % CDB-3877 Remaining |
|---|---|---|---|
| Plasma protein binding | | | |
| human | 88.3 | 87.0 | 18 |
| rat | 96.2 | 103.9 | >1 |
| mouse | 92.9 | 99.8 | 1 |
| dog | 83.2 | 102.2 | >1 |
| monkey | **47.1** | 90.6 | >1 |
| rabbit | 84.5 | 110.2 | NT |
| HSA binding | 70.1 | 97.8 | NA |
| AAG binding | 97.4 | 92.2 | NA |

NT, Not Tested; NA Not Applicable

### 5.1.4 Metabolism

**Title:** Metabolism of ($^{14}$C)-VA 2914 in microsomes isolated from female mouse, rat, rabbit, dog, monkey and human

**Key study findings**:

- *In vitro* metabolism of CDB-2914 by rat liver microsomes produced a pattern closest to human in both a qualitative and quantitative manner, sharing 2 of 7 metabolites identified from the species tested.

**Study no.**: 2109/003
**Volume #, and page #**: EDR, 4.2.2.4.1.HRA2914.429
**Conducting laboratory and location** (b) (4)

**Date of study initiation**: July 2, 2002
**GLP compliance**: No
**QA report**: Yes
**Drug, lot #, and % purity**: CDB-2914, 108/02-2109, 010311115, 98.7%
[$^{14}$C]-CDB-2914, 148-153-054, 98.7%
SA: 54 mCi/mmol

**Methods:** Liver microsomes from female SD rats, CD-1 mice, NZW rabbits, beagle dogs, cynomolgus monkey, and pooled human from 10 female donors were purchased for use. A reaction contained 1 mg microsomal protein, 0.8 mM β-NADPH in Tris buffer, pH 7.4 and test article and was performed in duplicate. A pilot study using 1, 10 and 50 µM [$^{14}$C]-CDB-2914 incubated with rat liver microsomes for 0, 10, 30, 60, and 120 min at 37 °C was used with and without β-NADPH to determine the optimal conditions for the definitive experiments. The use without β-NADPH was to determine the dependence of the cofactor on metabolism of test article. After stopping the reaction with acetonitrile, an aliquot of the sample was analyzed using liquid scintillation to determine recovery of radiolabel. Another aliquot was analyzed by radio-HPLC methodology to determine quantitative and qualitative amounts of parent compound and metabolites produced.

The definitive study was similar to the pilot study except it only used 0, 10 and 60 min timepoints with 10 µM [$^{14}$C]-CDB-2914.

**Results**: Recoveries of radioactivity from the pilot rat study ranged from 83.0% to 108.8%, and radioactivity recoveries from negative controls in the absence of β-NADPH or microsomal protein ranged from 101.3% to 105.6%. The recoveries from definitive incubations with rat and the other species at 10 µM showed recoveries ranging from 83.4% to 108.1%. The sponsor states that the 10 min timepoint was more useful for comparative purposes due to the completeness of reactions demonstrated by loss of parent at 60 min. The Figure below demonstrates that rat and rabbit have similar amounts of parent metabolized after 10 min.

**Table 23 Percent 10 µM [$^{14}$C]-CDB-2914 Turnover in Microsomes in Each Species (n=2)**

| Species | % Parent Compound Metabolized | |
|---|---|---|
| | 10 min | 60 min |
| Rat | 13.6 | 31.7 |
| Mouse | 46.3 | 66.7 |
| Rabbit | 22.6 | 63.2 |
| Dog | 38 | 78.6 |
| Monkey | 75 | 79 |
| Human | 24.3 | 47.9 |

There were 7 metabolites reported that were present at ≥1% of total radioactivity. No significant metabolism occurred in the absence of β-NADPH; the highest was rabbit and monkey with 98% of parent recovered. Rat microsomes have a pattern closest to human in a both qualitative and quantitative manner with Metabolites 3 and 4. The structures of the putative metabolites were not reported.

**Table 24 Metabolic Profile Resulting from Incubation of 10 µM [$^{14}$C]-CDB-2914 in Microsomes in Each Species (n=2)**

| Species | Time (min) | CDB2914 (mean %) | Metabolite (mean %) | | | | | | | Mean % Recovery |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Rat | 0 | 100 | | | | | | | | 100.4 |
| | 10 | 86.4 | | | 1.5 | 12.2 | | | | 103.0 |
| | 60 | 68.3 | | | 8.6 | 23.2 | | | | 104.4 |
| Mouse | 0 | 98.8 | | | | 1.2 | | | | 106.6 |
| | 10 | 53.7 | | | 18.8 | 24.5 | ~1 | 2.5 | | 103.9 |
| | 60 | 33.3 | 1.2 | | 38.7 | 18.3 | 1.2 | 2.8 | 4.8 | 83.4 |
| Rabbit | 0 | 99.0 | | | | 1.0 | | | | 108.1 |
| | 10 | 77.4 | | | 2.5 | 20.1 | | ~1 | | 100.6 |
| | 60 | 36.8 | 2.0 | 3.1 | 15.3 | 41.9 | | 1.1 | | 104.4 |
| Dog | 0 | 100 | | | | | | | | 101.8 |
| | 10 | 62.0 | | | 18.6 | 19.5 | | | | 96.5 |
| | 60 | 21.4 | | | 65.8 | 11.6 | 1.3 | | | 100.0 |
| Monkey | 0 | 99.3 | | | | ~1 | | | | 102.9 |
| | 10 | 25.0 | | | 46.0 | 28.3 | | | | 103.6 |
| | 60 | 21.0 | 3.9 | | 69.2 | 4.0 | | | 2.1 | 93.0 |
| Human | 0 | 99 | | | | 1.1 | | | | 101.1 |
| | 10 | 75.7 | | | | 18.4 | | | | 103.2 |
| | 60 | 52.1 | | | 19.2 | 28.2 | | | | 100.3 |

**Title:** Identification of the cytochrome P450 enzymes responsible for the in vitro metabolism of (14C)-VA 2914 and the effect of VA 2914 on the activity of specific human cytochrome P450 enzymes

**Key study findings**:

- CDB-2914 was metabolized to two metabolites designated M1 and M2 in human liver microsomes that was blocked by CYP2E1 and CYP3A4 inhibitors.
- CYP activities mediated by CYP2C9, CYP2D6, and CYP3A4 were significantly inhibited by CDB-2914, and CDB-2914 demonstrated some potential time dependent inhibition for CYP3A4 activity only.

**Study no.**: 2109/004
**Volume #, and page #**: EDR, 4.2.2.4.2.HRA2914.430
**Conducting laboratory and location** (b) (4)

**Date of study initiation**: July 2, 2002
**GLP compliance**: No
**QA report**: No
**Drug, lot #, batch#, and % purity**: CDB-2914, Not provided, 98%
[$^{14}$C]-CDB-2914, Not provided, >98.5%
SA: 54 mCi/mmol

**Methods:** Microsomes were prepared from insect cells infected with CYP1A2, CYP2A6, CYP2C9(Arg144), CYP2C19, CYP2D6(Val374), CYP2E1 and CYP3A4 - containing baculoviruses. Pooled microsome preparation from 10 female donor livers was purchased. All reference inhibitors were dissolved in acetonitrile except quinidine which was dissolved in Tris Buffer (50 mM, pH 7.4). Conditions were optimized for both kinetic parameter determinations and for co-incubations with selective inhibitors.

For $V_{max}$ and $K_m$ determination using pooled human liver microsomes, a reaction contained 1 mg/mL microsomal protein, with or without 0.8 mM β-NADPH in 50 mM Tris buffer, pH 7.4 and [$^{14}$C]-CDB-2914 at 1, 2, 5, 7.5, 10, 25, 50, 75 and 100 µM and was performed for 5 min at 37°C with 5 min pre-incubation before addition of β-NADPH in duplicate. The use without β-NADPH was to determine the dependence of the cofactor on metabolism of test article. After stopping the reaction with acetonitrile, an aliquot of the sample was analyzed using HPLC with liquid scintillation to determine amount of radiolabel. Sponsor used commercially available software to conduct analyses.

For inhibition of CDB-2914 metabolism, pooled human liver microsomes were assayed as above, except that incubations were 15 min at 20 µM [$^{14}$C]-CDB-2914 with inhibitors listed in the **Table** below. The metabolism of 20 µM [$^{14}$C]-CDB-2914 on using insect cell microsomes expressing human CYP enzymes (Supersomes™) at 50 pmol CYP/mL were performed as outlined for the kinetic experiments.

**Table 25 Inhibitors Used to Determine Possible CYP Enzymes Involved in CDB-2914 Metabolism**

| CYP Isozyme | Inhibitor | Concentration (μM) |
|---|---|---|
| | | |
| CYP1A2 | furafylline | 50 |
| CYP2A6 | 8-methoxypsoralen | 10 |
| CYP2C9 | sulfaphenazole | 20 |
| CYP2C19 | tranylcypromine | 20 |
| CYP2D6 | quinidine | 3 |
| CYP2E1 | disulfiram | 200 |
| CYP3A4 | ketoconazole | 2 |

For inhibition of CYP enzyme activity by CDB-2914, the **Table** below lists the amount of pooled human liver microsomes, the CYP-specific substrates/reaction assayed with concentration, control inhibitor, incubation times and analytical method when incubated with [$^{14}$C]-CDB-2914 (at 10 and 100 μM), with and without 2 mM β-NADPH in 50 mM Tris buffer, pH 7.4 at 37°C pre-incubation for 5 min before addition of β-NADPH in duplicate. All reactions were stopped by the addition of methanol. For Time-Dependent Inhibition (TDI) analyses, CDB-2914 at 10 μM was incubated with microsomes as above except that the pre-incubation time increased to 15 min for TDI analyses. Positive control inhibitors for TDI reactions were not reported.

**Table 26 Human CYP Enzymes Tested for Activity Inhibition by CDB-2914 and the Substrates Used with Assay-Specific Parameters**

| CYP | Substrate Used / Reaction (μM) | Control Inhibitor (μM) | [Microsome Protein], mg/mL | Incubation Time (min) | Analytical Method |
|---|---|---|---|---|---|
| CYP1A2 | phenacetin O-deethylation (30) | Furafylline (50) | 0.5 | 20* | LC-MS |
| CYP2C9 | tolbutamide methyl-hydroxylation (100) | Sulfaphenazole (20) | 1.0 | 30 | HPLC w/ radio detection |
| CYP2C19 | S-mephenytoin 4-hydroxylation (80) | Tranylcypromine (100) | 1.0 | 20 | LC-MS |
| CYP2D6 | bufuralol 1-hydroxylation (10) | Quinidine (3) | 1.0^ | 15 | LC-MS |
| CYP2E1 | lauric acid 11-hydroxylation (100) | Disulfiram (200) | 1.0 | 30 | HPLC w/ radio detection |
| CYP3A4 | midazolam 1-hydroxylation (10) | Ketoconazole (3) | 0.25 | 10 | LC-MS |

*Pre-incubation time was 10 min, not 5 min.

^ Concentration decreased to 0.25 mg/mL for TDI

**Results**: CDB-2914 was metabolized to two metabolites designated M1 and M2 in human liver microsomes. The definitive identification of these metabolites was not carried out in this study. The $K_m$ values for the metabolism of CDB-2914 in the pooled human liver microsomes to M1 and M2 were 9.27 and 40.7 µM respectively, with corresponding $V_{max}$ values of 301 and 2007 pmol/mg/min, respectively. Almost complete inhibition of CDB-2914 metabolism to M1 and M2 occurred with incubation of CYP3A4 and CYP2E1 inhibitors. Minor inhibition to M1 occurred with CYP1A2 and CYP2D6 inhibitors. Determination of radioactivity associated with the metabolites showed that only CYP3A4 had levels above control when M1 and M2 were produced.

**Table 27 Effects of Selective Inhibitors on *in vitro* M1 and M2 Metabolite Production from CDB-2914 in Pooled Human Liver Microsomes or Expressed CYPs in Insect Cells (n=2)**

| CYP Isozyme | Inhibitor | Pooled Human | | Expressed | |
|---|---|---|---|---|---|
| | | % Inhibition to M1 | % Inhibition to M2 | % Radioactivity Associated with M1 | % Radioactivity Associated with M2 |
| Control | | 0 | 0 | nd | 0.88 |
| Acetonitrile* | | 0 | 0 | nd | 1.27 |
| CYP1A2 | furafylline | 35.8 | 0 | nd | 1.08 |
| CYP2A6 | 8-methoxypsoralen | 37.5 | 0 | nd | 0.73 |
| CYP2C9 | sulfaphenazole | 15.4 | 0 | nd | 1.00 |
| CYP2C19 | tranylcypromine | 13.6 | 4.15 | nd | 1.06 |
| CYP2D6 | quinidine | 2.96 | 6.73 | nd | 1.79 |
| CYP2E1 | disulfiram | 100 | 87.8 | nd | 1.16 |
| CYP3A4 | ketoconazole | 100 | 82.6 | 1.08 | 6.55 |

*Quinidine was dissolved in 50 mM Tris 7.4; nd, Not Detected

CYP activities mediated by CYP2C9, CYP2D6, and CYP3A4 were significantly inhibited by CDB-2914, but inhibition of CYP2C9 was only observed at 100 µM CDB-2914 (47.6 µg/mL). There was no inhibition of CYP1A2, CYP2C19, pr CYP2E1. No significant TDI was observed, except midazolam 1-hydroxylase activity mediated by CYP3A4 was inhibited by 10 µM CDB-2914.

**Table 28 Inhibition of in vitro CYP-Mediated Activities by CDB-2914 in Pooled Human Liver Microsomes (n=2)**

| CYP Isozyme | % Inhibition from Relative Control | | | | | | |
|---|---|---|---|---|---|---|---|
| | Positive Control | CDB-2914 (10 µM) | CDB-2914 (100 µM) | Ethanol (1%) | Acetonitrile (1%) | Time Dependent Inhibition | |
| | | | | | | CDB-2914 (10 µM) | Ethanol (1%) |
| CYP1A2 | 83.7 | 10.1 | 5.5 | 14.9 | 25.8 | 0.0 | 10.1 |
| CYP2C9 | 82.8 | 0.0 | **50.4** | 32.8 | 0.0 | 6.81 | 30.1 |
| CYP2C19 | 91.5 | 0.0 | 3.0 | 73.6 | 4.1 | 15.0 | 60.0 |
| CYP2D6 | 83.6 | **17.9** | **55.9** | 55.6 | -- | 11.8 | 0.0 |
| CYP2E1 | 82.3 | 0.0 | 10.6 | 34.6 | 0.0 | 11.1 | 10.1 |
| CYP3A4 | 96.2 | **31.2** | **54.0** | 3.1 | 8.8 | **44.7** | 10.8 |

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**Title:** Inhibition of PGL4002 on CYP Enzyme Activities in Pooled Human Liver Microsomes

**Key study findings**:

- CDB-3877 at 4 µM had minimal effect on *in vitro* inhibition of CYP enzyme activities.

**Study no.**: PGL09-010
**Volume #, and page #**: EDR, 4.2.2.6.1.HRA2914-476
**Conducting laboratory and location**: (b) (4)
**Date of study initiation**: None provided. Report Issued: September 4, 2009
**GLP compliance**: No
**QA report**: No
**Drug, lot #, batch#, and % purity**: CDB-3877(PGL-4002), FB5-272, 97.7%

**Methods:** Pooled human liver microsomes (46 donors) were purchased from (b) (4) (b) (4) for the cocktail of inhibitor experiments. Pooled human liver microsomes (11 donors) were supplied by conducting laboratory (b) (4)) for the individual inhibitor reactions. For the pooled CYP cocktail inhibition assay, reactions contained 0.8 mg microsomal protein, 1 mM β-NADPH in 0.1 M $KPO_4$ buffer, pH 7.4 with 5 mM $MgCl_2$, substrate (see **Table** below) and 4 µM CDB-2914 with a 37°C pre-incubation for 5 min before addition of β-NADPH in triplicate. The reaction was incubated for 20 min. After stopping the reaction with acetonitrile, an aliquot of the sample was analyzed using LC-MS/MS methodology. The individual assays were performed as above, except contained 0.25 mg/mL of microsomal protein. Positive inhibitor reactions were

performed as above, but with addition of control inhibitors were used at 10 µM without test article present.

**Table 29 Human CYP Enzymes Tested for Activity Inhibition by CDB-3877 and the Substrates and Inhibitors Used**

| CYP | Substrate Used and Reaction (µM) | Inhibitor (10 µM) |
|---|---|---|
| **Cocktail Inhibition Study** | | |
| CYP1A2 | 7-Ethoxyresorufin O-deethylation (0.85) | α-Naphthoflavone* |
| CYP2A6 | Coumarin 7-hydroxylation (3.6) | Tranylcypromine |
| CYP2C9 | Tolbutamide methyl-hydroxylation (166) | Sulfaphenazole |
| CYP2C19 | S-mephenytoin 4-hydroxylation (80) | Ticlopidine |
| CYP2D6 | Bufuralol 1-hydroxylation (30) | Quinidine |
| CYP2E1 | Chlorzoxazone6-hydroxylation (100) | 4-Methylpyrazole |
| CYP3A4 | Midazolam 1-hydroxylation (10) | Ketoconazole |
| CYP3A4 | Testosterone 6β-hydroxylation (20) | Ketoconazole |
| **Individual Inhibition Study** | | |
| CYP2B6 | Bupropion hydroxylation (100) | Thio-TEPA |
| CYP2C8 | Paclitaxel 6α -hydroxylation (6) | Quercetin |

*Concentration is 20 µM

**Results:** CDB-3877 at 4 µM had minimal effect on *in vitro* inhibition of CYP enzyme activities in the cocktail inhibition study with only CYP2C19, CYP2E1, CYP2B6, and CYP2C8 demonstrating inhibition >10% , and none >40% (see **Table** below). This concentration equates to 1.9 µg/mL of CDB-3877, and is expected to be 20x the MRHD.

**Table 30 Inhibition of PGL4002 on CYP Enzyme Activities**

| CYP | Metabolite Formation (nM, mean ± SD, n=3) | | % of Control Activity | % of Inhibition |
|---|---|---|---|---|
| | Without PGL4002 | With PGL4002 | | |
| CYP1A2 | 103 ± 13 | 112 ± 2.9 | 109 | |
| CYP2A6 | 3270 ± 164 | 3063 ± 146 | 94 | 6 |
| CYP2C9 | 1793 ± 331 | 1683 ± 85 | 94 | 6 |
| CYP2C19 | 634 ± 47 | 544 ± 25 | 86 | 14 |
| CYP2D6 | 871 ± 77 | 799 ± 45 | 92 | 8 |
| CYP2E1 | 3097 ± 338 | 2453 ± 167 | 79 | 21 |
| CYP3A4 (Midazolam) | 2200 ± 506 | 2557 ± 110 | 116 | |
| CYP3A4 (Testosterone) | 1550 ± 296 | 1440 ± 255 | 93 | 7 |
| CYP2B6 | 411 ± 58 | 257 ± 10 | 63 | 37 |
| CYP2C8 | 547 ± 29 | 329 ± 14 | 60 | 40 |

Sponsor's Table 8, pg. 9.

The sponsor-provided **Table** below listed the results of CYP inhibition in the cocktail inhibitor study. Since CYP2B6 and CYP2C8 were performed as individual reactions, $IC_{50}$ values of 4.7 and 2.6 µM, respectively were obtained when control inhibitors were evaluated. One concern is that the individually run CYP assays also demonstrated the greatest % inhibition, so that cocktail method may be underestimating potential CDB-3877-mediated CYP activity inhibition.

**Table 31 CYP Inhibition by Known Chemical Inhibitors in Cocktail Inhibition Study**

| Known Inhibitor | Percent of CYP Inhibition Relative to Controls (n = 4)* | | | | | | |
|---|---|---|---|---|---|---|---|
| | CYP1A2 | CYP2A6 | CYP2C9 | CYP2C19 | CYP2D6 | CYP2E1 | CYP3A4 |
| α-Naphthoflavone (20 µM) | **94 (0.5)** | 15 (12) | 45 (6.7) | 46 (3.5) | 8 (11) | 6 (11) | -75 (22) |
| Tranylcypromine (10 µM) | -1 (13) | **>99 (0)** | 47 (7.7) | 52 (5.9) | 30 (6.8) | 63 (4.1) | 17 (7.8) |
| Sulfaphenazole (10 µM) | -22 (12) | 0 (16) | **87 (1.9)** | 11 (15) | -3 (7.8) | 3 (7.8) | -5 (7.3) |
| Ticlopidine (10 µM) | 16 (13) | 19 (13) | 20 (10) | **82 (2.3)** | 57 (3.9) | 16 (11) | 44 (5.8) |
| Quinidine (10 µM) | -8 (13) | 5 (15) | 8 (10) | 21 (11) | **91 (1.7)** | 18 (10) | 6 (8.7) |
| 4-Methylpyrazole (10 µM) | -5 (13) | 9 (14) | 15 (10) | 16 (6.5) | 45 (5.4) | **65 (5.6)** | 12 (4.5) |
| Ketoconazole (10 µM) | -1 (10) | -8 (15) | 38 (9.8) | 45 (5.9) | 30 (12) | 19 (10) | **92 (0.4)** |

*Experiments were performed and resulting data were retained at (b). Data were expressed as the mean of % inhibition relative to the controls without inhibitors (RSD). Testosterone was used as the probe substrate of CYP3A4. Bold font indicates the relative specificity of the inhibitor to individual CYPs.

Sponsor's Table 9, pg. 9.

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**Title:** Induction Effects of PGL4001 and PGL4002 on CYP1A2 and 3A4 Activities in Fresh Human Hepatocytes

**Key study findings**:

- CDB-2914 and CDB-3877 demonstrated no induction of CYP1A2 and CYP3A4 enzyme activity in fresh human hepatocytes.

**Study no.**: PGL09-008
**Volume #, and page #**: EDR, 4.2.2.6.1.HRA2914-477
**Conducting laboratory and location**: (b) (4)
**Date of study initiation**: None provided. Report Issued: September 4, 2009
**GLP compliance**: No
**QA report**: No
**Drug, lot #, batch#, and % purity**: CDB-2914, IN-AKS-A-76, 97.5%
CDB-3877(PGL-4002), FB5-272, 97.7%

**Methods:** Fresh human hepatocytes from 2 donors were purchased from (b) (4) (b) (4)) and stored at 4°C until use. Hepatocytes were plated in triplicate at 1.5x10$^6$ cells/cm$^2$ and incubated with hepatocyte media for 24 h prior to experiments. CDB-2914 at 0.5 and 5 µM and CDB-3877 at 0.2 and 2 µM were incubated for 3 days at 37°C with daily replacement of media containing compounds. Positive control CYP inducers were β-naphthoflavone at 10 µM (CYP1A2) and rifampicin at 50 µM (CYP3A4).

CYP1A2 enzyme activity was determined using the O-deethylation reaction of 7-ethoxyresorufin to resorufin; and CYP3A4 enzyme activity was determined using formation of 6β-hydroxytestosterone from the hydroxylation of testosterone. The hepatocytes were washed with PBS then treated with 7-ethoxyresorufin at 20 µM or 250 µM testosterone in Williams' Media E for 1 h at 37°C. An aliquot was removed and the reaction stopped with acetonitrile and resorufin and 6β-hydroxytestosterone was measured by LC-MS/MS methods.

Cell viability was then examined using Aqueous ONE Solution Cell proliferation Assay (CellTiter 96®). This method uses conversion of MTS tetrazolium salt into formazan in viable cells at a rate proportional to the # living cells and this salt is spectroscopically measured.

**Results:** There was no treatment-related effect on cell viability for any condition or treatment for either donor ranging from 83-124% of negative controls. Minor statistically significant differences occurred but no pattern was observed. CYP1A2 and CYP3A4 enzyme activity were not induced by CDB-2914 or CDB-3877 compared to the positive controls with either donor (see **Tables** below).

**Table 32 Induction of PGL4001 and PGL4002 on CYP1A2 Activity in Human Hepatocytes**

| Donor | Compound | Treatment | Resorufin Formation (fmol/well/min)[a] | % of Solvent-Treated Cells[b] | % of BNF-Treated Cells[c] |
|---|---|---|---|---|---|
| 1 | Control | Solvent | 103 ± 3.7 | 100 | 0 |
| | | 10 µM BNF | 1452 ± 163** | 1411 | 100 |
| | PGL4001 | 0.5 µM | 100 ± 5.7 | 97 | -0.2 |
| | | 5 µM | 87 ± 3.8 | 84 | -1.2 |
| | PGL4002 | 0.2 µM | 93 ± 5.6 | 90 | -0.8 |
| | | 2 µM | 88 ± 4.6 | 85 | -1.1 |
| 2 | Control | Solvent | 77± 4.4 | 100 | 0 |
| | | 10 µM BNF | 545 ± 29** | 704 | 100 |
| | PGL4001 | 0.5 µM | 66 ± 5.7 | 85 | -2.5 |
| | | 5 µM | 75 ± 6.4 | 97 | -0.4 |
| | PGL4002 | 0.2 µM | 66 ± 4.4 | 85 | -2.5 |
| | | 2 µM | 76 ± 4.4 | 98 | -0.3 |

[a]: Data were expressed as the mean ± SD of three individual measurements (**p<0.01, compared with the vehicle-treated group).
[b]: Percentage of activity relative to the vehicle-treated cells.
[c]: Percentage of activity relative to the positive inducer-treated cells (USA FDA Draft Guidance: Drug Interaction Studies. September 2006; Hewitt NJ. et al.. Chem Biol Interact. 2007, 168:51-56).

Sponsor's Table 2, pg. 7.

**Table 33 Induction of PGL4001 and PGL4002 on CYP3A4 Activity in Human Hepatocytes**

| Donor | Compound | Treatment | 6β-OH Testosterone Formation (pmol/well/min)[a] | % of Solvent-Treated Cells[b] | % of RIF-Treated Cells[c] |
|---|---|---|---|---|---|
| 1 | Control | Solvent | 4.6 ± 1.4 | 100 | 0 |
| | | 50 μM RIF | 59 ± 19** | 1273 | 100 |
| | PGL4001 | 0.5 μM | 1.4 ± 0.6 | 31 | -5.9 |
| | | 5 μM | 0.5 ± 0.1 | 10 | -7.6 |
| | PGL4002 | 0.2 μM | 3.3 ± 0.8 | 71 | -2.4 |
| | | 2 μM | 2.6 ± 0.3 | 56 | -3.7 |
| 2 | Control | Solvent | 27± 3.1 | 100 | 0 |
| | | 50 μM RIF | 415 ± 100** | 1520 | 100 |
| | PGL4001 | 0.5 μM | 18 ± 1.0 | 67 | -2.3 |
| | | 5 μM | 1.9 ± 0.0 | 7 | -6.5 |
| | PGL4002 | 0.2 μM | 25 ± 1.4 | 90 | -0.7 |
| | | 2 μM | 8.8 ± 0.3 | 32 | -4.8 |

[a]: Data were expressed as the mean ± SD of three individual measurements (**p<0.01, compared with the vehicle-treated group).
[b]: Percentage of activity relative to the vehicle-treated cells.
[c]: Percentage of activity relative to the positive inducer-treated cells (USA FDA Draft Guidance: Drug Interaction Studies. September 2006; Hewitt NJ, et al.. Chem Biol Interact. 2007. 168:51-56).

Sponsor's Table 3, pg. 8.

## 5.2 Toxicokinetics

The toxicokinetic profiles obtained from the pivotal repeat-dose toxicology studies in rats and monkeys do not permit an accurate comparison to human exposure. The $C_{max}$ values below were based on terminal exposure and the AUC values were only for monkeys based on the whole 6 month study period.

**Table 34 Female Rat and Monkey TK Parameters from Main Toxicology Studies Using RIA Methodology that Measures Both CDB-2914 and CDB-3877**

**Table 6: Summary of serum levels of ulipristal acetate and its main metabolites CDB-3877 during main toxicology studies**

| Species | Studies | Dose (mg/kg) | Plasma Levels(ng/mL) | AUC @ (ng.h/mL) |
|---|---|---|---|---|
| Rat[2,3] | 14 day | 4 | 7.0 ± 2.1 | |
| | | 20 | 47.0 ± 9.6 | |
| | | 100 | 150.3 ± 47.0 | |
| Rat[4] | 6 months | 1 | 11.2 ± 0.7 | |
| | | 5 | 75.8 ± 4.9 | |
| | | 25 | 147.3 ± 9.5 | |
| Monkey[4] | 14 days | 20 | 122 ± 47-354 ± 80 | |
| | | 100 | 529 ± 219-2384 ± 918 | |
| Monkey[5] | 26 week | 1 | 89 ±12.5 | 14861 ± 1073 |
| | | 5 | 672 ±64.5 | 124702 ± 13266 |
| | | 25 | 2920 ±772 | 492775 ± 32478 |

[1]Steady state, [2]Serum level at terminal euthanasia (24h after last administration), [3]ulipristal acetate plasma exposure only, [4]Mean daily plasma level value, [5]Estimated steady state levels @ AUC 0-180days

## 6 General Toxicology

The sponsor conducted the pivotal toxicology studies in rats and cynomolgus monkeys. The 2-week studies were reviewed by Dr. Krishan Raheja, D.V.M. on December 5, 1995 (original IND) and the 6-month studies were reviewed by Dr. Suzanne Thornton, Ph.D. (Review #4, March 21, 2002). Single dose toxicology was inadequate since only one very high dose at 1250 mg/kg was evaluated in rats and monkeys (405x and 810x human exposure based on mg/m$^2$, respectively). The repeat-dose findings are summarized below.

Rats:
In a 14-day study, female rats were administered oral CDB-2914 at 4, 20, and 100 mg/kg/day (HEDs are approximately 39, 194, and 968 mg/day, respectively). There were increased serum amylase levels, increased liver and kidney weights, mammary gland lobular hyperplasia, and ovarian cysts at ≥20 mg/kg. Additionally, there were increased body weight gain, reduced reticulocytes and hemoglobin levels, increased lymphocytes, increased ALT levels with hepatocyte hyperplasia, increased pituitary and thyroid weights, hypertrophy of the adrenal cortex, and increased spleen weights with extramedullary hematopoiesis at 100 mg/kg.

In a 6-month study, female rats were administered oral CDB-2914 at 1, 5, and 25 mg/kg/day (HEDs are approximately 10, 48, and 242 mg/day). There was treatment- but not dose-related increases in neutrophils counts. There was a dose-dependent

increase in ovarian cysts and follicular atresia at ≥1 mg/kg. There was a dose-dependent increase in serum prolactin levels (significant at ≥1 mg/kg) with increases in numbers and severity of palpable galactoceles (≥1 mg/kg) in mammary glands macroscopically observed at necropsy. Histopathology findings were consistent with clinical chemistry and gross findings with increased galactoceles (≥1 mg/kg), hyperplasia, fibrosis, mineralization, and inflammation (≥5 mg/kg) observed in the mammary glands. Dose-dependent increases in pituitary gland weights (significant at ≥5 mg/kg) with increases in numbers and severity of pituitary hyperplasia consistent with the increase in serum prolactin. There were dose-dependent increases in serum corticosterone (statistically significant at 25 mg/kg) with increased adrenal gland weights (statistically significant at ≥5 mg/kg). Histopathological findings included dose-dependent increases in numbers and severity of adrenal cortex hypetrotrophy in the zona fasiculata and angiectasis. Minimal necrosis was observed in one animal at 25 mg/kg. Liver weight increases (statistically significant at ≥5 mg/kg) and hepatocellular hypertrophy in the centrilobular region was observed in 12/20 animals at 25 mg/kg. Spleen congestion was noted at 25 mg/kg.

Monkeys:
In a 14-day study, female monkeys were administered oral CDB-2914 at 20, and 100 mg/kg/day (HEDs are approximately 387 and 1935, respectively), there were increases in serum LDH, decreased ALP levels, increased organ weights of the spleen, ovaries, kidneys, and adrenals (without histological observations), and increased cervical mucous cells at ≥20 mg/kg. At 100 mg/kg, there were also decreased body weights, decreased serum cholesterol and calcium levels, and increased liver and thyroid weights.

In a 6-month study, female monkeys were administered oral CDB-2914 at 1, 5, and 25 mg/kg/day (HEDs are approximately 19, 97, and 484 mg/day. Neutrophils were increased at ≥1 mg/kg up and lymphocytes were increased at ≥5 mg/kg. Serum ALT was increased at ≥1 mg/kg. A single female had a moderate ovarian cyst at 25 mg/kg. Uterine dilatation and neutrophilic infiltration was observed at ≥5 mg/kg, and a uterine squamous metaplasia was noted in one animal at 25 mg/kg. Dose-dependent increases in serum cortisol levels at ≥1 mg/kg and increased adrenal weights with adrenal cortex hypertrophy at 25 mg/kg, and increases in serum prolactin levels with increased pituitary gland weights at ≥5 mg/kg, decreased thymus weights with mineralization and increased neutrophils at 25 mg/kg. There was no NOAEL due to findings at all doses.

## 7 Genetic Toxicology

These studies were previously reviewed by Dr. Krishan Raheja on December 5, 1995 (original IND) and January 27, 1998 (Annual Report). CDB-2914 was not mutagenic in the reverse mutation bacterial system at concentrations up to 5000 µg/plate in the presence or absence of the S9 activation system. The bacterial reverse mutation assay was repeated with the micronized clinical batch of drug product, and no mutagenicity was observed. CDB-2914 did not show any mutagenic activity in L5178Y mouse lymphoma cells at concentrations up to 200 µg/mL in the presence or absence of the S9

activation system. CDB-2914 was not associated with clastogenicity in peripheral human blood lymphocytes at concentrations up to 120 µg/mL. Oral administration of CDB-2914 up to 512 mg/kg (HED is approximately 2500 mg/day) did not increase the incidence of micronuclei in the bone marrow of male or female mice.

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

Study title: VA2914: Bacterial reverse mutation test

| | |
|---|---|
| Study no.: | 32775 MMO |
| Study report location: | EDR 4.2.3.3.1.2.hra2914-438 |
| Conducting laboratory and location: | (b) (4) |
| Date of study initiation: | January 17, 2007 |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity: | micronized CDB-2914, C610V010, 99.4% |

**Key Study Findings**

Methods

| | |
|---|---|
| Strains: | Salmonella typhimurium TA 98, TA100, TA102, TA1535, TA1537 |
| Concentrations in definitive study: | 15.6, 31.3, 62.5, 125, 250, and 500 µg/plate |
| Basis of concentration selection: | Maximum concentration achievable with test article Preliminary study up to 5000 µg/plate demonstrated that >500 µg/plate made plates unreadable due to precipitation of test article. |
| Negative control: | DMSO |
| Positive control: | See below: from sponsor's Study Report pg.11 |
| Formulation/Vehicle: | CDB-2914 dissolved in DMSO and diluted with water |

Metabolic activation system: S9 microsomal fraction from rat livers induced with Aroclor 1254 purchased from (b) (4) and validated by for its ability to activate benzo(a)pyrene and 2-aminoanthracene to mutagenic intermediates.

Positive controls:

**Table 35 Positive Controls Used in CDB-2914 Bacterial Reverse Mutation Test**

| | Dose-levels (µg/plate) | Strains |
|---|---|---|
| Without S9 mix | | |
| . sodium azide (NAN3) | 1 | TA 1535 - TA 100 |
| . 9-Aminoacridine (9AA) | 50 | TA 1537 |
| . 2-Nitrofluorene (2NF) | 0.5 | TA 98 |
| . Mitomycin C (MMC) | 0.5 | TA 102 |
| With S9 mix | | |
| . 2-Anthramine (2AM) | 2 | TA 1535 - TA 1537 - TA 98 |
| . Benzo(a)pyrene | 5 | TA 100 |
| . 2-Anthramine (2AM) | 10 | TA 102 |

Incubation and sampling times: Plate incorporation method was used for the first assay. The test article±metabolic activation, liquid culture, and top agar were mixed then plated onto minimal agar plates with $10^8$ cells/plate (3 plates/concentration/strain). Negative controls were performed on 6 plates. Plates were incubated 48-72 h at 37°C, and revertant colonies were counted. If positive, the assay was repeated as above. If negative, the repeat test was performed using a preincubation method + metabolic activation, and a direct plate incorporation method –metabolic activation. For preincubation, the test article, liquid culture, soft agar, and S9 mix were mixed, incubated for 60 min at 37°C then plated onto minimal agar plates, the assayed as for plate incorporation method. For TA1575 and TA1537, # revertants that was 3x the negative control value was considered positive, and for all other strains it was 2x the negative control value.

**Study Validity**

**Valid**

- Adequate strain selection.
- Doses were limited by concentration of test article.
- CDB-2914 formed a precipitate > 500 µg/plate in a preliminary study.
- CDB-2914 was not cytotoxic to any tester strain ± metabolic activation.
- No bacterial contamination in sterility tests of S9 mixes and test articles sham dilutions.
- Rates of revertants were within historical data range for positive and negative controls.

**Results**

No test article-dependent increase in revertant colonies was noted ± S9 metabolic activation system up to the top dose of 500 µg/plate. The mutant frequencies were within the historical range provided by the sponsor, and the positive controls all performed within the range of reported historical data. This was the second time this type of study was performed and produced the same conclusion. It was likely re-run

with a micronized clinically relevant batch of drug substance due to the >10 years' previous testing.

## 8 Carcinogenicity

CDB-2914 carcinogenicity testing in rats at daily doses of 1, 3, and 10 mg/kg has not been completed.

A computational toxicity consult was requested to evaluate carcinogenicity. CDB-2914 was not predicted to be carcinogenic to rodents.

**To:** Jeffrey Bray
**cc:** Alex Jordan
**From:** CDER/OPS/SRS/ICSAS
**Re:** NDA 22-474
**Date:** 02/16/2010

Ulipristal acetate was evaluated by CDER/OPS/SRS/ICSAS for rodent carcinogenicity using four (quantitative) structure-activity relationship [(Q)SAR] computational toxicology software programs[1]. The results of the predictions from all four software programs were weighted equally and the analysis was optimized for sensitivity (minimize false negatives) to reach the overall conclusions.

---

Rodent Carcinogenicity[2]

| Software | Rat | Mouse |
|---|---|---|
| Derek for Windows | no structural alerts | no structural alerts |
| Leadscope | – | – |
| MC4PC | – | Eqv |
| MDL-QSAR | – | – |
| **Overall ICSAS Prediction** | – | – |

In considering the entire weight of evidence, ICSAS concludes that Ulipristal acetate is predicted to be negative for both rat and mouse carcinogenicity.

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This report has been reviewed and finalized by the Informatics and Computational Safety Analysis Staff.

## 9 Reproductive and Developmental Toxicology

CDB-2914 administered only at 10 mg/kg had no effect on male reproductive organs, sperm parameters, mating behavior and fertility when administered 14, 35, or 70 days before mating[1].

The embryofetal toxicology studies were previously reviewed by Dr. Katherine Bonson (August 11, 1999; SN#0001, August 4, 1999). In rat embryofetal toxicity studies, use of doses low enough to not completely block pregnancy (0.1, 0.3 and 1 mg/kg) resulted in reduction in the pregnancy rate to 84% and there was a significant increase in post-implantation loss at 1 mg/kg. These were early resorption sites suggesting that fetal death occurred shortly after treatment initiation. Also, there were fewer live fetuses at 1 mg/kg compared to control. No teratogenicity was observed. In rabbit embryofetal toxicity studies, use of doses low enough to not completely block pregnancy (0.1, 0.3 and 1 mg/kg) resulted in reduction in the pregnancy rate to 75%, and there was a significant increase in early resorption sites and decrease in live fetuses at 1 mg/kg. No teratogenicity was observed.

Three peri-/postnatal studies were performed. The first study was conducted in pregnant rats on days 17-19 of gestation at 2, 4, and 8 mg/kg. These doses induced premature parturition with dose dependent increases in delivery on GD 18, and all animals delivered by GD 20, compared to GD 21 for all control animals. All pups delivered prematurely were found dead in their placental membranes. In a second study, female rats administered CDB-2914 at 0.5 and 1 mg/kg on Days 1-3 postcoitum had gestation durations dose-dependently increased. The number of pregnant females decreased and litter size was reduced at 1 mg/kg. In dams where implantation was not affected, the $F_1$ offspring developed normally and were fertile. (n=5). Pregnant rats treated with CDB-2914 at 0.03, 0.1, and 0.3 mg/kg from GD 17 to PND 21 demonstrated decreased viability of fetuses and pups (decreased gestation time, increased fetal resorptions, increased stillborns, and increased pup death up to PND 4) at 0.3 mg/kg. There were no other findings in either $F_0$ or $F_1$ animals.

### 9.3 Prenatal and Postnatal Development

Study title: Ulipristal acetate - Pre- and post-natal development study by the oral route (gavage) in the rat (Segment III)

| | |
|---|---|
| Study no: | AA75162 |
| Study report location: | EDR 4.2.3.5.3 - HRA2914-471 |
| Conducting laboratory and location: | (b) (4) |
| Date of study initiation: | September 3, 2008 |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity: | CDB-2914, C610X020, 100.7% |

**Key Study Findings**

- At 0.3 mg/kg, there was increased resorption of fetuses, increased stillborn pups, and increased pup loss extending out to PND 4 compared to control.
- Significant decrease in gestation period at 0.3 mg/kg compared to control group.
- NOAEL= 0.1 mg/kg

Methods

| | |
|---|---|
| Doses: | 0.03, 0.1, 0.3 mg/kg (based on Dose Range Finding: 1 mg/kg exceeded MTD due to total litter resorption- no maternal mortality/morbidity) |
| Frequency of dosing: | Daily |
| Dose volume: | 5 ml/kg |
| Route of administration: | Oral gavage |
| Formulation/Vehicle: | ASV (0.9% NaCl, 0.9% benzyl alcohol, 0.4% Tween-80, carboxymethylcellulose 0.5% in distilled water, |
| Species/Strain: | Sprague-Dawley rats Crl:OFA(SD) |
| Number/Sex/Group: | 25 virgin mated F/group |
| Satellite groups: | 6 virgin mated F/group |

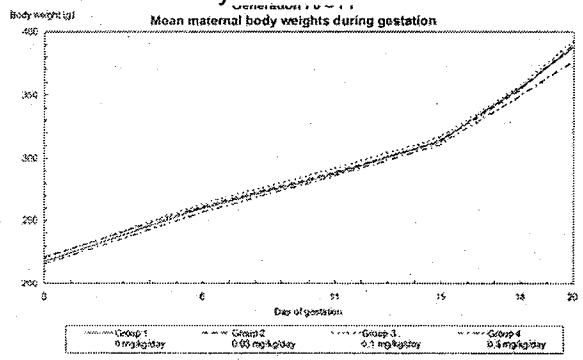
Study design: $F_0$ females aged 10-12 weeks weighing 182-257g were treated from Gestation Day (GD) 6 to Lactation Day 20 (L20) inclusive; the $F_1$ animals were not directly treated at any point, but exposure may have occurred *in utero* and likely through milk consumption. On Post-natal Day (PND) 4, litter size reduction/standardization occurred with gross necropsy on any dead or euthanized pups (4/sex/litter were randomly selected). $F_1$ animals were mated 1:1 for 21 days and females were sacrificed on GD 13 and necropsied. Necropsy occurred on the following schedule: $F_0$ females after weaning of the $F_1$ pups; $F_0$ females that failed to produce a viable litter by Day 26 *post-coitus*; unselected $F_1$ pups on PND 4 or after weaning on PND 21; mated $F_1$ males, after necropsy of the $F_1$ females; mated $F_1$ females: on GD 13. No post-partum evaluation of $F_0$ males or $F_2$ fetuses.

Parameters and endpoints evaluated:
$F_0$: Clinical signs, body weights, food consumption, pregnancy duration, parturition, number live/dead pups, pups/litter. On lactation day 21, or post-mating day 25, gross necropsy and selected organ weight with possible evaluation of uterine parameters, such as implantation loss. TK samples were taken at 0.5, 1, 2, 4, and 6 h (2 animals/timepoint) on GD 19 and 1 and 4 h LD 8 (3 animals/timepoint). Milk samples were taken prior to first TK on L8 for exposure evaluation. Evaluation included CDB 3877A (main metabolite).
$F_1$: Clinical signs, body weights, numbers, sex, viability on PND 1, 4, 7, 14 and 21. Pre-weaning physical development defined by onset and duration of pinna unfolding, incisor eruption and eye opening. Behavioral and functional tests in all pups as follows: surface righting reflex (PND 8), gripping reflex (PND 17), pupil reflex and auditory reflex (PND 21). Post-weaning physical development defined by vaginal opening (PND 28), balano-preputial skinfold cleavage (PND 38), water maze (5 to 6 weeks), open field (~7 weeks). TK samples were taken at 1 and 4 h after maternal dosing on L7 (4 pups/3 satellite litters).

**Observations and Results**

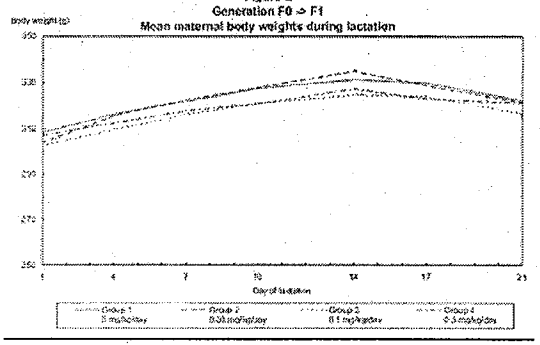
$F_0$ in-life:
Mortality: None.

Clinical signs: Unremarkable.
Body weight: Within historical range. There was a 3% decrease in BWt gain at 0.3 mg/kg compared to control on GD 20 with decrease occurring GD 18-20 possibly due to increased fetal mortality.




Unremarkable during lactation period (within historical range).

Figure 2
Generation F0 -> F1
Mean maternal body weights during lactation



Food consumption: Unremarkable (within historical range).

Mating/Fertility: Overall, a slight dose-dependent decrease in parameters likely due to the pharmacological effects of the test article, such as number of females pregnant, completing delivery, and with liveborn litters. At 0.3 mg/kg, there was significantly decreased gestation period and increased fetal mortality due to resorption of fetuses compared to the control group. In the HD group, one dam had total litter resorption which contributed to the 27.2% increase in pre-birth loss.

**Table 36 Mating and Fertility Parameters in Pregnant Rats Administered Oral CDB-2914 from Gestation Day 6 to Lactation Day 20**

| Parameter | Dose (mg/kg) | | | |
|---|---|---|---|---|
| | 0 | 0.03 | 0.1 | 0.3 |
| No. Mated | 25 | 25 | 25 | 25 |
| Mating Index (%) | 100 | 100 | 100 | 100 |
| No. Pregnant | 25 | 24 | 23 | 23 |
| Fertility Index (%) | 100 | 96 | 92 | 92 |
| No. Completing Delivery | 25 | 24 | 23 | 22 |
| Delivery (%) | 100 | 96 | 92 | 88 |
| No. Litters with Liveborn | 25 | 24 | 23 | 21 |
| Gestation Index (%) | 100 | 100 | 100 | 91.3 |
| Gestation (Days) | 21.9 | 22 | 21.7 | 21.5** |
| Mean Implantation Sites | 13.6 | 14.3 | 14.1 | 13.1 |
| Mean Pre-birth loss | 1 | 1.1 | 1 | 3.4 |
| Pre-birth loss (%) | 7.6 | 7.8 | 7 | 27.2*** |

**, P<0.01; ***, P<0.001

Toxicokinetics: CDB 2914 or CDB 3877A were not detectable in plasma of the 0.03 mg/kg group. $C_{max}$ and AUC exposure were slightly less than dose-proportional (~2.2-2.5) from 0.1 to 0.3 mg/kg. There were slightly higher levels of CDB 2914 than CDB 3877A and the metabolite:parent ratio was ~0.6 for $C_{max}$ and 0.759 for AUC. On Lactation Day 8, the plasma concentration of CDB 2914 or CDB 3877A was slightly lower than GD 20 when measured around, but not at $T_{max}$.

**Table 37 CDB-2914 Toxicokinetic Parameter from Pregnant Rats on Gestation Day 19**

Toxicokinetic parameters from dams on G19

| Compound | Test item dose (mg/kg/day) | $C_{max}$ (ng/mL) | $T_{max}$ (h) | t (h) | $AUC_{0-t}$ (ng.h/mL) | $AUC_{0-6h}$ (ng.h/mL) |
|---|---|---|---|---|---|---|
| Ulipristal acetate | 0.03 | NA | NA | NA | NA | NA |
| | 0.1 | 3.01 | 0.5 | 2 | 4.16 | NA |
| | 0.3 | 7.30 | 0.5 | 6 | 23.2 | 23.2 |
| Metabolite 3877A | 0.03 | NA | NA | NA | NA | NA |
| | 0.1 | 1.91 | 2 | 4 | 5.97 | 7.00 |
| | 0.3 | 4.26 | 2 | 6 | 17.6 | 17.6 |

Print date: 06 January 2009

NA: not applicable

Sponsor's Table (pg. 671)

## Table 38 CDB-2914 Plasma Concentrations on Lactation Day 8

Mean dam plasma concentrations on L8

| Compound | Dose (mg/kg/day) | Time (h) | Mean concentration (ng/mL) | SD | CV (%) | n |
|---|---|---|---|---|---|---|
| Ulipristal acetate | 0 | 1 | 0 | 0 | NA | 3 |
| | | 4 | 0 | 0 | NA | 3 |
| | 0.03 | 1 | 0 | 0.681 | NA | 3 |
| | | 4 | 0 | NA | NA | 2 |
| | 0.1 | 1 | 2.88 | 0.507 | 17.6 | 3 |
| | | 4 | 0 | 0 | NA | 3 |
| | 0.3 | 1 | 5.37 | NA | 135¤ | 2 |
| | | 4 | 0 | NA | NA | 2 |
| Metabolite 3877A | 0 | 1 | 0 | 0 | NA | 3 |
| | | 4 | 0 | 0 | NA | 3 |
| | 0.03 | 1 | 0 | 0 | NA | 3 |
| | | 4 | 0 | NA | NA | 2 |
| | 0.1 | 1 | 2.09 | 0.146 | 6.96 | 3 |
| | | 4 | 0 | 0.693 | NA | 3 |
| | 0.3 | 1 | 4.08 | NA | 119¤ | 2 |
| | | 4 | 1.22 | NA | 200¤ | 2 |

Print date: 19 January 2009

¤ As n = 2, delta % was calculated instead of SD and CV %
NA: not applicable

Sponsor's Table (pg. 678)

$F_0$ necropsy: One female at 0.1 mg/kg had a movable mass on the ventral side of the neck.

$F_1$ physical development:

There was a significant decrease in mean number of pups delivered and mean live litter size at 0.3 mg/kg compared to the control group. This is reflected by an increase in number of litters with stillborn pups at the HD, with one litter being completely stillborn. At PND 4 culling, there was a significant decrease in % pup survival at 0.3 mg/kg compared to the control group, with 2 dams having total litter loss by PND 2. The number of pups found dead or cannibalized was significant greater in the HD group than all other groups. There was no further pup loss through weaning and maturation.

## Table 39 $F_1$ Litter Parameters

| Parameter | Dose (mg/kg) | | | |
|---|---|---|---|---|
| | 0 | 0.03 | 0.1 | 0.3 |
| No. Litters | 25 | 24 | 23 | 22 |
| Mean Pups Delivered | 12.6 | 13.3 | 13.2 | 10.1** |
| Viable pups % | 100 | 98.1 | 99 | 96.9 |
| No. Litters with Stillborn Pups | 0 | 3 | 1 | 3^ |
| Mean Litter Size (live) | 12.6 | 13.0 | 13.0 | 9.7** |
| No. Found Dead/Missing/Cannibalized | 4 | 3 | 2 | 12** |
| % Males | 52.9 | 47.8 | 54.7 | 48.3 |
| % Survival at PND4 | 99.4 | 99 | 100 | 94.4** |
| % Survival at PND4 - PND21 | 99 | 100 | 98.9 | 100 |

**P<0.01; ***P<0.001; ^1 litter was fully stillborn

Nothing remarkable was noted for mean pup body weight at birth and through weaning with any group. There was a slight trend toward delayed onset of pinna unfolding, incisor eruption and eye opening at 0.3 mg/kg, but not significantly different; all animals had completed these milestones by the same day. No effect was noted on surface righting, gripping, auditory or pupil reflexes in any group.

$F_1$ behavioral evaluation: Nothing remarkable was noted for exploratory behavior and general movement in the open field test and learning capacity, memory or motor activity in the water maze test.

$F_1$ reproduction: Sexual maturation, body weight, mating performance and fertility parameters were unremarkable and within range of historical controls.

**Table 40 Mating and Fertility Parameters in $F_1$ Rats**

| Parameter | Dose (mg/kg) | | | |
|---|---|---|---|---|
| | 0 | 0.03 | 0.1 | 0.3 |
| No. Paired | 25 | 25 | 25 | 25 |
| No. Mated | 25 | 25 | 25 | 25 |
| Copulation Index (%) | 100 | 100 | 100 | 100 |
| No. Pregnant | 25 | 24 | 22 | 24 |
| Fertility Index (%) | 100 | 96 | 88 | 96 |
| Mean Corpora Lutea | 17 | 17 | 18 | 18 |
| Implantations (% of CL) | 95.0 | 93.7 | 91.6 | 90.8 |
| Mean Viable Embryos | 16 | 15 | 16 | 15 |
| Viable Embryos (% of Implantations) | 96.1 | 94.5 | 96.6 | 95.4 |
| Mean Pre-implantation Loss | 1 | 1 | 2 | 2 |
| Pre-implantation Loss (% of CL) | 5.0 | 6.3 | 8.4 | 9.2 |
| Mean Post-implantation Loss | 1 | 1 | 1 | 1 |
| Post-implantation Loss (% of CL) | 3.9 | 5.5 | 3.4 | 4.6 |

$F_2$ findings: $F_2$ not examined.

**Summary**: A preliminary DRF showed that 1 mg/kg exceeded MTD due to total litter resorption in all dams due to the pharmacological actions of the test agent. At 0.3 mg/kg (1/24 of the MRHD based on AUC exposure), there were significant reductions in gestation period, gestation index (litters with live births), mean pups delivered, mean live litter size, and survival to PND 4. Also, there were significant increases in resorptions (pre-birth loss), litters with stillborn pups, and pups found dead in the HD group. There were no physical, behavioral, or fertility effects noted in the $F_1$ progeny for any group after culling at PND 4. Exposure was slightly less than dose-proportional from 0.1 to 0.3 mg/kg, with 0.03 mg/kg exposure below the limit of quantification. Test article was qualitatively detected in milk on PND 7, but was below the limit of quantification. The NOAEL for this study was set at 0.1 mg/kg (1/132 of the MRHD based on AUC exposure).

## 10 Special Toxicology Studies

**Study title**: Evaluation of in vitro phototoxicity on Balb/c 3T3 fibroblasts using the Neutral Red uptake assay

**Key study findings**:

- Ulipristal acetate was not phototoxic up to 30 µg/mL (6.3 µM).

**Study no.**: 2109/006
**Volume #, and page #**: EDR 4.2.3.7.7 - HRA2914-448
**Conducting laboratory and locatior** (b) (4)
**Date of study initiation**: August 7, 2007
**GLP compliance**: Yes
**QA reports**: yes ( x ) no ( )
**Drug, lot #, and % purity**: CDB-2914 (micronized), C610V010, 99.4%
**Formulation/vehicle**: DMSO

**Methods**

Doses: 2.47, 3.53, 5.04, 7.20, 10.29, 14.70, 21.00, 30.00 µg/mL

Study design: Negative control is DMSO diluted in PBS and the positive control is chlorpromazine at 0.1 – 1000 ug/mL. Duplicate plates of Balb/c 3T3 clone A31 fibroblast cells at $1x10^4$ cells/well ($1x10^5$ cells/mL) were incubated in DMEM media for 42 h at 37°C. Medium was removed and controls and test article at above concentrations were added to the wells for 1 h in the dark at 37°C. After the 1 h incubation, one plate each of test substance and negative control and one plate containing the positive control were irradiated using the UV-A light source for 96 min 25 sec, to achieve a UVA dose of 5 J/$cm^2$. The remaining plates were kept at room temperature in the dark for the same time period. At the end of this period all plates were washed and new medium was added and the plates were incubated for 20 ± 2 h at 37°C. Cells were visually assessed for cytotoxicity, then washed. Then, Neutral Red solution (50 µg/mL in DMEM) was added to each well. The plates were incubated at 37°C for 3 h, washed twice then destained for 40 min. ODs of each well were read at 540 nm and absorbance were expressed in terms of Absolute $OD_{540}$ and $IC_{50}$ values were calculated for test article and positive control. Photo-irritation Factor was calculated: PIF = $IC_{50}$ no UVA/$IC_{50}$ plus UVA.

Acceptance Criteria
The assay was considered valid if the following criteria were met:
1. Irradiated vehicle controls showed a viability of approximately 80% of the non-irradiated vehicle control.
2. OD540 in the untreated unirradiated controls > 0.4.
3. The positive controls showed a clearly cytotoxic response in the presence of UV-A light, compared to the response seen in the absence of UV-A light, such that the PIF for the positive control was > 6.

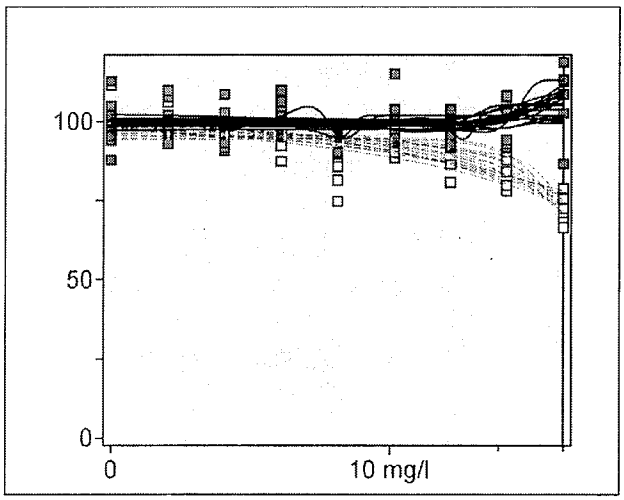
Evaluation criteria
For valid data:
1. The test article was considered 'phototoxic' in this assay if PIF values of > 5 were obtained.
2. The test article was considered 'non-phototoxic' in this assay if PIF values of < 2 were obtained.
3. The test article was considered equivocal 'probably phototoxic' in this assay if PIF values of >2 and <5 were obtained.

**Results**: The sponsor stated, "*Chlorpromazine induced an acceptable positive response with a PIF value of 62.098. In the untreated controls, $OD_{540}$ values were greater than 0.4. The irradiated vehicle control showed a viability of approximately 80% of the non-irradiated vehicle control. The assay was therefore considered valid.*"

The test article was limited by solubility to a high dose of 30 µg/mL. Test article phototoxicity $IC_{50}$ and PIF values could not be calculated since the cell survival at the highest dose was more than 50%. The test article was considered not phototoxic in this *in vitro* assay.

**Figure 9 CDB-2914 Survival Curve + and - UV-A**

Figure 1 - VA2914 Survival curve: - and + UV-A




| | |
|---|---|
| Solid lines and solid squares | in the absence of UV-A (-UV-A) |
| Dotted lines and open squares | in the presence of UV-A (+UV-A) |
| Y-axis | Percentage survival compared to vehicle controls |
| X-axis | Concentration in mg/L or ug/mL on a logarithmic scale |

## 11 Integrated Summary and Safety Evaluation

**Table 41 Calculated Exposure Multiples Based on Toxicity Observed in Females Based on Most Sensitive Finding**

| Organ System | Finding(s) | Species | NOAEL (mg/kg) | Exposure Multiple Based on $mg/m^2$ |
|---|---|---|---|---|
| Ovary | cysts | Rat | <1 | NA |
| | cysts | Monkey | 5 | 3.2 |
| Mammary gland | Galactoceles, hyperplasia | Rat | <1 | NA |
| | None | Monkey | 25 | 16 |
| Uterus | glandular dilatation | Rat | 5 | 1.6 |
| | glandular dilatation | Monkey | 1 | 0.7 |
| Adrenal | ↑weight, angiectasis | Rat | 1 | 0.3 |
| | ↑weight, hypertrophy | Monkey | 5 | 3.2 |
| Pituitary | ↑weight, hyperplasia | Rat | <1 | NA |
| | None | Monkey | 25 | 16 |
| Liver | ↑weight, hypertrophy | Rat | 1 | 0.3 |
| | neutrophil infiltration | Monkey | 1 | 0.7 |
| Spleen | congestion | Rat | 5 | 1.6 |
| | None | Monkey | 25 | 16 |
| Hematology | ↑leukocytes, lymphocytes | Rat | 1 | 0.3 |
| | ↑segmented neutrophils | Monkey | 1 | 0.7 |
| Clinical Chemistry | ↑ALT, PRL, globulin, corticosterone, cholesterol | Rat | <1 | NA |
| | ↓ALT, ↑cortisol | Monkey | 1 | 0.7 |
| Embryofetal lethality | ↑adsorptions, ↓live fetuses | Rat Embryofetal Toxicity study | 0.3 | 0.1 |
| | | Rabbit Embryofetal Toxicity study | 0.3 | 0.2 |
| | | Rat (Peri-Postnatal Development Study) | 0.1 | 0.008 Based on $AUC_{(0\text{-}24h)}$ |

NA, Not Applicable

**Table 42 Species Comparison of Single Oral Dose Administration of CDB-2914 to Females Using LC/MS-MS Methodology (CDB-2914 Only Measurement) for Rats, Dogs and Humans, and RIA Methodology for Monkeys and Humans (CDB-2914 plus Metabolites Measurement)**

| Species | Dose (mg/kg) | $C_{max}$ (ng/mL) | AUC (ng*h/mL) | $T_{max}$ (h) | $T_{1/2}$ (h) |
|---|---|---|---|---|---|
| Rat | 5 | 681 | 3645 | 1 | 2.99 |
| | 25 | 3418 | 29779 | 4 | 4.38 |
| | 125 | 8908 | 72407 | 4 | 5.84 |
| Dog | 5 | 130 | 348 | 1 | |
| | 25 | 2153 | 7727 | 1 | |
| | 125 | 7360 | 73199 | 2 | |
| Human 30 mg | 0.5 | 176 ± 89 | 548 ± 259 | 0.75 - 4 | 32 |
| **RIA Methodology** | | | | | |
| Monkey (ASV) | 5 | 180.2 | 2965 | 6 | 7.6 |
| (solid) | 5 | 129.1 | 2745 | 6 | 11.8 |
| Human 10 mg | 0.17 | 130 | 680 | 1.2 | |
| 50 mg | 0.83 | 440 | 3580 | 1.2 | |
| 100 mg | 1.7 | 760 | 5380 | 1.9 | |
| 200 mg | 3.3 | 570 | 7330 | 1.3 | |

**Table 43 Species Comparison of Single Oral Dose Administration of CDB-3877 to Females Using LC/MS-MS Methodology**

| Species | Dose (mg/kg) | $C_{max}$ (ng/mL) | AUC (ng*h/mL) | $T_{max}$ (h) | $T_{1/2}$ (h) |
|---|---|---|---|---|---|
| Rat | 5 | 1375 | 3876 | 2 | 2.63 |
| | 25 | 1562 | 11064 | 4 | 4.00 |
| | 125 | 3684 | 25443 | 4 | 4.72 |
| Dog | 5 | 185 | 438 | 1 | 7.01 |
| | 25 | 1426 | 7961 | 1 | 13.94 |
| | 125 | 2790 | 42440 | 1 | 1.19 |
| Human 30 mg | 0.5 | 176 ± 88.9 | 548 ± 259 | 0.88 | 32.4±6.33 |

ND, Not Determined

Ulipristal acetate (CDB-2914) is indicated for emergency contraception up to 120 h after unprotected sexual intercourse for women of child bearing potential that do not wish to become pregnant. CDB-2914 is a Selective Progesterone Receptor Modulator (mixed agonist/antagonist) with Glucorticoid Receptor antagonist activities. To support this indication the sponsor conducted a series of nonclinical pharmacology studies that demonstrated that CDB-2914 is predominantly an antiprogestin with antiglucocorticoid activity. CDB-2914 was contraceptive in rodents, guinea pigs, rabbits and monkeys

with antiprogestational effects noted on the ovary and the uterus. CDB-2914 was also effective at terminating pregnancies in nonclinical species.

CDB-2914 generally had a good pharmacokinetic profile with proportional exposures, reasonable half-life, and rapid and large distribution in rats, dogs, and monkeys. A distribution study showed that radiolabel is present in quantifiable levels after 3 days; but this may be because CDB-2914 is highly protein bound in all species examined. The in vitro metabolism of CDB-2914 is qualitatively and quantitatively similar in rats, mice, humans, dogs, and monkeys, with rats having the most comparable profile to humans. CDB-2914 has one major metabolite resulting from N-monodemethylation (CDB-3877), and a few minor metabolites of which the N-didemethylated product (PGL4004) is most significant. CDB-3877 and PGL4004 have similar but reduced pharmacological activities as the parent, and also cross-react with the monoclonal antibody used in the Radioimmune Assay (RIA) assay developed for drug measurements in earlier studies. The RIA and LC-MS/MS methods have differing sensitivities, with the LC-MS/MS method detecting higher concentrations of CDB-2914, making direct comparisons of drug levels between studies somewhat difficult since equivalent doses were not used. Further complicating the issue is that the RIA method detects the N-demethylated metabolites. CDB-2914 is a substrate for, and predominantly metabolized by, human CYP3A4 producing two products. These findings are consistent with other drugs that contain steroid backbone moieties. CDB-2914 and its metabolites are primarily eliminated through the feces via the hepatobiliary route.

The major toxicological findings reported were related to the dose- and duration-dependent anti-PR/anti-GR pharmacological actions of CDB-2914. The anti-PR findings in target organs occurred at lower doses, suggesting that CDB-2914 may be more selective for PR than GR at non-saturating exposures. The findings at 6 months were similar between the species, but generally milder in monkeys than rats (Table 41); in general, findings in the same organs were noted at 2 weeks. There were increased ovarian cysts at all doses in rats and monkeys, and follicular atresia noted in rats. Uterine findings of glandular dilatation were related to the anti-PR activities, and monkeys additionally had neutrophilic infiltration. There were dose-dependent increases in serum prolactin (reaching 12-fold in rats and 9-fold in monkeys) and pituitary weights in both species, and rats had findings of pituitary hyperplasia. The effects on the pituitary are likely indirect from both the anti-PR and anti-GR activities blocking normal negative feedback mechanisms resulting in increased prolactin production. There were mammary gland findings such as galactoceles, lobular hyperplasia, and inflammation in rats at all doses related to both the anti-PR effects and increased prolactin levels. Significant dose-dependent increases in serum corticosterone in rats (3.2x, maximum) and cortisol (2.5x, maximum) in monkeys and adrenal weight with adrenal cortex hypertrophy were noted and likely due to the anti-GR activity. Mild serum liver enzyme increases were noted in both species, and liver weight increases with hyperplasia/hypertrophy were seen at the high doses in rats. Further, increased liver weight, hepatocellular hypertrophy, and decreased ALT were observed in the 14-day rat study. The increase in neutrophils appear to be test article-related since these changes were observed in both species and monkeys had neutrophilic

infiltration in organs such as the uterus and liver. The NOAEL was <1 mg/kg in rats and 1 mg/kg in monkeys. Overall, the findings at 6 months were mild, expected and pharmacological in nature. The reversibility of these findings, and effects in males were not assessed.
The radioimmune assay methodology to quantify drug exposure, and $C_{trough}$ measurements (not AUC) make estimations of exposure multiples difficult. Single dose PK in rats at 5 mg/kg using LC/MS-MS methodology provided exposure multiples of 6.2x compared to clinical exposure (Table 42). Extrapolation suggests that the low dose of 1 mg/kg in rats would be equivalent to human clinical exposure. Also, single dose rat exposure levels and the exposure from the rat peri-postnatal study suggest that CDB-3877 is present at levels comparable to CDB-2914. CDB-3877 single dose exposure at 5 mg/kg was 16x human clinical exposure levels (Table 43).

In a series of published non-GLP female fertility studies, rats, mice and rabbits administered CDB-2914 had ovulation inhibited at exposures comparable to the human exposure based on $mg/m^2$. Pregnant rats and rabbits given CDB-2914 orally during the period of organogenesis had dose-dependent increased fetal losses with decreased number of live fetuses and increased post-implantation sites at ≥1 mg/kg. The embryofetal toxic effects were expected due to exaggerated antiprogestational pharmacology and occurred at doses comparable to the human clinical dose based on $mg/m^2$ (ranging from 1/3x to 1.5x). Pregnant rats administered ≥2 mg/rat of CDB-2914 orally on Gestation Days (GD) 17-19 had a dose-dependent increase in premature parturition starting as early as GD 18 with all delivering by GD20, compared to GD21 for controls. All pups delivered prematurely were found dead in placental sacs. Pregnant rats administered 0.1 mg/kg CDB-2914 during the period of organogenesis through weaning had decreased gestation time, increased fetal resorptions, increased stillborns, and increased pup death up to Postnatal Day 4. The fertility of the $F_1$ offspring was not impaired. The NOAEL was 0.1 mg/kg, a dose with an exposure that was 1/132x of the clinical drug exposure based on AUC. Teratogenicity was not detected in any study, but assessment was inadequate due to the low exposure levels required for evaluation of viable offspring. Administration of 10 mg/kg CDB-2914 only to male rats had no effect on reproductive organs, sperm parameters, mating behavior or fertility.

CDB-2914 was not mutagenic in the reverse mutation bacterial assays or in the L5178Y mouse lymphoma cell assay, and was not clastogenic in peripheral human blood lymphocytes. Oral administration of CDB-2914 did not increase the incidence of micronuclei in the bone marrow of male or female mice. The lack of genotoxicity and teratogenicity (albeit at low exposures relative to humans) suggest that potential emergency contraceptive failures with this drug will pose a low risk of fetal teratogenicity.

Overall, the pharmacology, pharmacokinetics, and toxicology effects of CDB-2914 were consistent between rats and monkeys and were similar to the effects seen in humans. CDB-2914 is a potent antiprogestin with anti-glucocorticoid activity and most of the nonclinical findings in the toxicology studies appear to be due to exaggerated antihormone pharmacology of CDB-2914. The nonclinical reproductive toxicology

findings suggest that there is a fetal risk if this drug is administered to a pregnant woman, specifically interruption of an established pregnancy. The ability to interrupt an established pregnancy with a single dose was not directly investigated in the pivotal nonclinical studies, but the data from published reports and one reproductive toxicology study (GD17-19 dosing at 4x the human dose based on $mg/m^2$) suggest that the potential exists. From a pharm/tox perspective, the nonclinical data support approval for emergency contraception.

## 12 Appendix/Attachments

Reference List

1. Wang C, Sinha-Hikim A, Leung A. The anti-progestin CDB 2914 has no antifertility effect in male rats. Contraception 1995; 51:215-8.

2. Reel JR, Hild-Petito S, Blye RP. Antiovulatory and postcoital antifertility activity of the antiprogestin CDB-2914 when administered as single, multiple, or continuous doses to rats. Contraception 1998; 58:129-36.

3. Hild SA, Reel JR, Hoffman LH, Blye RP. CDB-2914: anti-progestational/anti-glucocorticoid profile and post-coital anti-fertility activity in rats and rabbits. Hum Reprod 2000; 15:822-9.

4. Poyser NL, Forcelledo ML. A comparison of the pregnancy-terminating potencies of three anti-progestins in guinea-pigs, and the effects of sulprostone. Prostaglandins Leukot Essent Fatty Acids 1994; 50:245-7.

5. Tarantal AF, Hendrickx AG, Matlin SA, Lasley BL, Gu QQ, Thomas CA et al. Effects of two antiprogestins on early pregnancy in the long-tailed macaque (Macaca fascicularis). Contraception 1996; 54:107-15.

6. Xu Q, Takekida S, Ohara N, Chen W, Sitruk-Ware R, Johansson ED et al. Progesterone receptor modulator CDB-2914 down-regulates proliferative cell nuclear antigen and Bcl-2 protein expression and up-regulates caspase-3 and poly(adenosine 5'-diphosphate-ribose) polymerase expression in cultured human uterine leiomyoma cells. J Clin Endocrinol Metab 2005; 90:953-61.

7. Wu Y, Guo SW. Inhibition of proliferation of endometrial stromal cells by trichostatin A, RU486, CDB-2914, N-acetylcysteine, and ICI 182780. Gynecol Obstet Invest 2006; 62:193-205.

8. Attardi BJ, Burgenson J, Hild SA, Reel JR, Blye RP. CDB-4124 and its putative monodemethylated metabolite, CDB-4453, are potent antiprogestins with reduced antiglucocorticoid activity: in vitro comparison to mifepristone and CDB-2914. Mol Cell Endocrinol 2002; 188:111-23.

9. Attardi BJ, Burgenson J, Hild SA, Reel JR. In vitro antiprogestational/antiglucocorticoid activity and progestin and glucocorticoid

receptor binding of the putative metabolites and synthetic derivatives of CDB-2914, CDB-4124, and mifepristone. J Steroid Biochem Mol Biol 2004; 88:277-88.

10. Larner JM, Reel JR, Blye RP. Circulating concentrations of the antiprogestins CDB-2914 and mifepristone in the female rhesus monkey following various routes of administration. Hum Reprod 2000; 15:1100-6.

| Application Type/Number | Submission Type/Number | Submitter Name | Product Name |
|---|---|---|---|
| NDA-22474 | ORIG-1 | LABORATOIRE HRA PHARMA | Ella , Ulipristal Acetate |

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

/s/

JEFFREY D BRAY
06/25/2010

ALEXANDER W JORDAN
06/28/2010

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA/BLA

**NDA Number: 22-474** **Applicant: HRA Pharma** **Stamp Date: October 15, 2009**

**Drug Name: Ella** **NDA Type: 505(b)1**
**(Ulipristal acetate 30 mg)**

**45-Day Filing Review Date:** **November 29, 2009**
**74-Day Letter Date:** **December 28, 2009**
**Expected Date of Draft Review:** **March 1, 2009**
**PDUFA Goal date:** **August 13, 2010**

On **initial** overview of the NDA application for RTF:

| | Content Parameter | Yes | No | Comment |
|---|---|---|---|---|
| 1 | On its face, is the pharmacology/toxicology section of the NDA organized (in accord with 21 CFR 314 and current guidelines for format and content) in a manner to allow substantive review to begin? | X | | |
| 2 | Is the pharmacology/toxicology section of the NDA indexed and paginated in a manner allowing substantive review to begin? | X | | |
| 3 | On its face, is the pharmacology/ toxicology section of the NDA legible so that substantive review can begin? | X | | |
| 4 | Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted in this NDA (carcinogenicity, mutagenicity*, teratogenicity*, effects on fertility, juvenile studies, acute and repeat dose adult animal studies*, animal ADME studies, safety pharmacology, etc)? | X | | This drug product is indicated for single dose, intermittent use. A mouse micronucleus, a PPN study, all safety pharmacology studies, and a number of PK studies have been submitted without previous review. No carcinogenicity testing has been completed, but the sponsor has initiated studies for chronic indications. |
| 5 | If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA). | X | | Sponsor states that the drug substance used early in development by NICHD were not fully characterized. Material used in studies conducted by current sponsor is representative of clinical batches. |
| 6 | On its face, does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted a rationale to justify the alternative route? | X | | The appropriate route of administration was used for the studies. |

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA/BLA

| | Content Parameter | Yes | No | Comment |
|---|---|---|---|---|
| 7 | Has the sponsor submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations? | X | | |
| 8 | Has the sponsor submitted all special studies/data requested by the Division during pre-submission discussions with the sponsor? | X | | |
| 9 | Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57? | | X | Sponsor did not provide dosing multiples, and will need to be revised. Content will need to be revised. Potential class labeling for antiprogestin should be discussed with Division and sponsor. |
| 10 | If there are any impurity – etc. issues, have these been addressed? (New toxicity studies may not be needed.) | X | | (b) (4) |
| 11 | Has the sponsor addressed any abuse potential issues in the submission? | NA | | Mechanism of action has no known classical abuse potential. |
| 12 | If this NDA is to support a Rx to OTC switch, have all relevant studies been submitted? | NA | | |
| 13 | From a pharmacology/toxicology perspective, is the NDA fileable? If ``no`` please state below why it is not. | X | | |

Any Additional Comments: Ella (Ulipristal acetate) is a NME being developed as an Emergency Contraceptive for use up to 120 h post sexual intercourse. It is a selective progesterone receptor modulator with anti-progestational and some anti-glucocorticoid activities. The IND was submitted in 1995, and most of the nonclinical studies submitted to support the NDA application were performed in the 1990s. The pharmacology and some toxicology results have been published in peer-reviewed journals. There are at minimum a number of PK studies, all safety pharmacology, and 2 toxicology studies to be reviewed for this NDA application.

Jeffrey Bray, Ph.D. 10/28/2009

Reviewing Pharmacologist Date

Alex Jordan, Ph.D.

Team Leader/Supervisor Date

| Application Type/Number | Submission Type/Number | Submitter Name | Product Name |
|---|---|---|---|
| NDA-22474 | ORIG-1 | LABORATOIRE HRA PHARMA | Ella , Ulipristal Acetate |

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/s/

JEFFREY D BRAY
11/25/2009

ALEXANDER W JORDAN
11/25/2009