# CENTER FOR DRUG EVALUATION AND RESEARCH

*APPLICATION NUMBER:*

**22-575**

# PHARMACOLOGY REVIEW(S)

Comments on NDA 22-575 VRIV velaglucerase.
From A. Jacobs
Date: 2/19/10

1. I concur that there are no pharm-tox issues with approval with this NDA and that the pregnancy category is appropriate.

2. Regarding the review:

a. The conclusion that the drug causes adverse effects on sperm in the study on male rat fertility (p.70) conflicts with the overall conclusion and proposed labeling. I agree with the overall conclusion and proposed labeling.

b. Regarding labeling: The peripostnatal study that was conducted should be described in section 8.1.

I have discussed these comments with the Supervisor, who will address the comments as appropriate.

| Application Type/Number | Submission Type/Number | Submitter Name | Product Name |
|---|---|---|---|
| NDA-22575 | ORIG-1 | SHIRE HUMAN GENETIC THERAPIES INC | VELAGLUCERASE ALFA |

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/s/

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ABIGAIL ABBY C C JACOBS
02/22/2010

# ADDENDUM TO PHARMACOLOGIST'S REVIEW OF NDA 22-575
## Original Submission (000) dated August 31, 2009

**Reviewer Name:** Tamal K. Chakraborti, Ph.D.
Pharmacologist, DGP

**Date of Review:** February 19, 2010

**Drug:** Velaglucerase (VPRIV®)

**Category:** VPRIV (Velaglucerase alfa for injection) is a hydrolytic lysosomal glucocerebroside-specific enzyme indicated for long-term enzyme replacement therapy (ERT) for pediatric and adult patients with type 1 Gaucher disease.

This addendum is to incorporate the findings of the pre- and postnatal development study in rats to Section 8.1 of the Label. The recommended version in the review dated January 28, 2010 of the original submission is shown below, which does not contain the findings of the above study.

b(4)

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**Comments**: The findings of the pre- and postnatal development (Segment III) study in rats were not incorporated in the above section. So, we recommend the following revised labeling for "8.1 Pregnancy".

**Proposed Revised Version**:

"8.1 Pregnancy

Pregnancy Category B

Reproduction studies with Velaglucerase have been performed in pregnant rats at intravenous doses up to 17 mg/kg/day (102 mg/m2/day, about 1.8 times the recommended human dose of 60U/kg/day or 1.5 mg/kg/day or 55.5 mg/m2/day based on the body surface area). Reproduction studies have been performed in pregnant rabbits at intravenous doses up to 20 mg/kg/day (240 mg/m2/day, about 4.3 times the recommended human dose of 60U/kg/day based on the body surface area). These studies did not reveal any evidence of impaired fertility or harm to the fetus due to Velaglucerase alfa.
A pre- and postnatal development study in rats showed no evidence of any adverse effect on pre- and postnatal development at doses up to 17 mg/kg (102 mg/m2/day, about 1.8 times the recommended human dose of 60U/kg/day based on the body surface area). There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, VPRIV should be used during pregnancy only if clearly needed."

Tamal K. Chakraborti, Ph.D. Date
Pharmacologist

Comment:

Sushanta K. Chakder, Ph.D., Date
Supervisory Pharmacologist

| Application Type/Number | Submission Type/Number | Submitter Name | Product Name |
|---|---|---|---|
| NDA-22575 | ORIG-1 | SHIRE HUMAN GENETIC THERAPIES INC | VELAGLUCERASE ALFA |

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/s/

TAMAL K CHAKRABORTI
02/19/2010

SUSHANTA K CHAKDER
02/19/2010


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

# PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

| | |
|---|---|
| NDA NUMBER: | **22-575** |
| SERIAL NUMBER: | **000** |
| DATE RECEIVED BY CENTER: | **8/31/09** |
| PRODUCT: | **VPRIV® (Velaglucerase Alfa)** |
| INTENDED CLINICAL POPULATION: | **Patients with Type 1 Gaucher Disease** |
| SPONSOR: | **Shire Human Genetic Therapies** |
| DOCUMENTS REVIEWED: | **Nonclinical studies** |
| REVIEW DIVISION: | **Division of Gastroenterology Drug Products** |
| PHARM/TOX REVIEWER: | **Tamal K. Chakraborti, Ph.D.** |
| PHARM/TOX SUPERVISOR: | **Sushanta K. Chakder, Ph.D.** |
| DIVISION DIRECTOR: | **Donna Griebel, M.D.** |
| PROJECT MANAGER: | **Wes Ishihara** |

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# *EXECUTIVE SUMMARY*

## I. Recommendations

A. Recommendation on approvability: From a nonclinical standpoint, this NDA is recommended for approval.

B. Recommendation for nonclinical studies: None

C. Recommendations on labeling: The draft labeling of VIPRIV® generally conforms to the format specified under 21CFR 201.57(c)(14) Requirements for PLR (Physician's Labeling Rule) Prescription Drug Labeling. However, the following changes should be incorporated.

**8.1 Pregnancy**

**Sponsor's Version**:

b(4)

[redacted]

**Evaluation**: The text is in accordance with 21CFR 201.57(c)(14). However, the text should be modified as follows.

**Recommended Version**:

**"8.1 Pregnancy**

Pregnancy Category B

Reproduction studies with Velaglucerase have been performed in pregnant rats at intravenous doses up to 17 mg/kg/day (102 mg/m$^2$/day, about 1.8 times the recommended human dose of 60U/kg/day or 1.5 mg/kg/day or 55.5 mg/m$^2$/day based on the body surface area). Reproduction studies have been performed in pregnant rabbits at intravenous doses up to 20 mg/kg/day (240 mg/m$^2$/day, about 4.3 times the recommended human dose of 60U/kg/day based on the body surface area). These studies did not reveal any evidence of impaired fertility or harm to the fetus due to Velaglucerase alfa. There are, however, no adequate and well-controlled studies in pregnant women. Because

animal reproduction studies are not always predictive of human response, VIPRIV should be used during pregnancy only if clearly needed."

**8.3. Nursing Mothers**

**Sponsor's Version**

b(4)

[redacted]

**Evaluation**: The text is in accordance with 21CFR 201.57(c)(14).

**Recommended Version**: None

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

**Sponsor's Version**

b(4)

[redacted]

**Evaluation:** The text is not in accordance with 21CFR 201.57(c)(14)(i). The text should be modified as proposed below.

**Recommended Version**:

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

Long-term studies in animals to evaluate carcinogenic potential or studies to evaluate mutagenic potential have not been performed with Velaglucerase alfa.

In a male and female fertility study in rats, Velaglucerase alfa did not cause any significant adverse effect on male or female fertility parameters up to a maximum dose of 17 mg/kg/day (102 mg/m$^2$/day, about 1.8 times the recommended human dose of 60U/kg/day based on the body surface area)."

## II. Summary of nonclinical findings

A. Brief Overview of Nonclinical Findings: Velaglucerase alfa was adequately tested in a series of toxicology studies using bolus intravenous (IV) dose administration. These studies included an acute single-dose study, and 3- and 6-month repeat-dose toxicology studies in Sprague Dawley (SD) rats, and a 6-month toxicology study in Rhesus monkeys. The IV route of administration was used in all toxicology studies to conform to the intended clinical route of administration. In addition, reproductive and developmental toxicology studies (Segment I male and female fertility and early embryonic development in rats, Segment II teratology studies in rats and rabbits and Segment III pre- and postnatal development study in rats) were also conducted with Velaglucerase alfa.

In an IV acute toxicology study in rats, the maximum nonlethal dose was 20 mg/kg. In a 3-month IV toxicology study in rats at 0.85, 3.4 and 17 mg/kg (bi-weekly), there were no toxicologically-significant treatment-related findings up to 17 mg/kg. The target organs could be the lung (granulomatous inflammation), liver (small focal cluster of mononuclear cells in sinusoids near a blood vessel) and testes (decrease weight and tubule formation). The no-observed-adverse-effect-level (NOAEL) could not be determined as treatment-related effects were observed at all doses. Anti-DRX008A antibody was formed at all doses. In a 25-Week IV injection toxicology study in rats, animals were treated with DRX008A at 0.84, 3.4 and 17 mg/kg once every 2 weeks. The target organ could not be identified in the absence of any significant histopathology findings in any organ or tissue. The NOAEL could not be determined as treatment-related effects were observed at all doses. Anti-DRX008A antibody was formed at all doses. In a 6-Month IV injection toxicology study in Rhesus monkeys, animals were treated with DRX008A at 0.84, 3.4 and 17 mg/kg on a bi-weekly basis. The target organ could not be identified in the absence of any significant histopathology findings. The NOAEL was considered as 17 mg/kg. Significant anti-DRX008A antibody was formed at the high dose at Week 4 and 13. Overall, Velaglucerase alfa appeared to be well tolerated in rats and monkeys up to 17 mg/kg when administered on a bi-weekly basis.

Reproductive and developmental toxicology studies (Segment I fertility and early embryonic development in rats, Segment II teratology studies in rats and rabbit and Segment III pre- and postnatal study in rats) were also conducted with Velaglucerase alfa. In a Segment I male fertility and early embryonic

development to implantation study in rats, at 1.5, 5 and 17 mg/kg (twice weekly), there were no significant treatment-related adverse effects on male fertility parameters. However, two males (each at 1.5 mg/kg and 17 mg/kg) had too few sperm to assess motility. These animals also had relatively high incidences of abnormal sperm and low epididymal sperm concentrations. At necropsy, small testes and epididymides were noted for both animals and while each treated male showed positive confirmation of mating to an untreated female, neither resulted in a pregnancy. The relationship to the treatment was not clear. In a Segment I female fertility and early embryonic development to implantation study in SD rats, at 1.5, 5, and 17 mg/kg (twice weekly), Velaglucerase did not cause any significant adverse effect on female fertility parameters. In an IV Segment II teratology study in rats at 1.5, 5 and 17 mg/kg/day, Velaglucrease alfa was not teratogenic. In an IV Segment II teratology study in rabbits at 1.5, 10 and 20 mg/kg/day, Velaglucerase was not teratogenic. In a Segment III pre- and post-natal development study in rats, Velaglucerase alfa did not cause any significant adverse effect on pre- and post-natal development in rats when tested at 1.5, 5 and 17 mg/kg, IV.

Overall, Velaglucerase alfa has been adequately tested in general toxicology and reproductive toxicology studies. The nonclinical toxicology studies conducted with Velaglucerase alfa adequately support marketing approval of VIPRIV for the intended patient population at the recommended doses. Nonclinical toxicology studies conducted with Velaglucerase alfa provided adequate assurance of safety for its proposed use. Therefore, from a nonclinical standpoint, this NDA is recommended for approval.

B. Pharmacologic Activity: A mouse model of Gaucher disease, the D409V/null Mouse (9V/null) was developed for animal efficacy studies. The 9V/null mice exhibit many of the disease features typical of human Type 1 (non-neuronopathic) Gaucher disease, including accumulation of "storage cells" (glucocerebroside-laden macrophages) in the lung, liver, and spleen, and a lack of pathology in the brain. The 9V/null mouse model was used to compare the effects of Velaglucerase alfa to those of Imiglucerase (Cerezyme®), with regard to decrease of accumulated glucocerebroside and decrease in number of storage cells in visceral organs. The IV route of administration was used in all experiments as this is the intended route of administration in human patients. The 9V/null mice were administered once-weekly IV injections of either Velaglucerase alfa or Imiglucerase at dose levels of either 5, 15, or 60 U/kg for a total of either four or eight weeks, after which time the mice were sacrificed. Levels of glucocerebroside in the liver, lung, and spleen were determined and storage cells in the liver tissue were identified histologically. The results showed that Velaglucerase alfa and Imiglucerase similarly restored normal lipid (glucocerebroside) content in the liver, while the lipid content in the spleen was reduced to a lesser extent. In contrast, neither enzyme affected the lipid content of the lung. Both enzyme treatments

comparably reduced the number of storage cells (also called Gaucher cells) in the liver.

C. Nonclinical Safety Issues Relevant to Clinical Use: None

# *2.6 PHARMACOLOGY/TOXICOLOGY REVIEW*

## 2.6.1 INTRODUCTION AND DRUG HISTORY

**NDA number**: 22-575
**Review number**: 001
**Sequence number/date/type of submission**: 000/August 31, 2009/Original
**Information to sponsor**: Yes ( ) No (X)
**Sponsor and/or agent**: Shire Human Genetic Therapies, Cambridge, MA
**Manufacturer for drug substance**: Shire Human Genetic Therapies, Cambridge, MA

**Reviewer name**: Tamal K. Chakraborti, Ph.D.
**Division name**: Division of Gastroenterology Products (DGP)
**Review completion date**: January 28, 2010

**Drug**:

Trade name: VPRIV®

Generic name: Velaglucerase Alfa

Code name: GA-GCB (DRX008A)

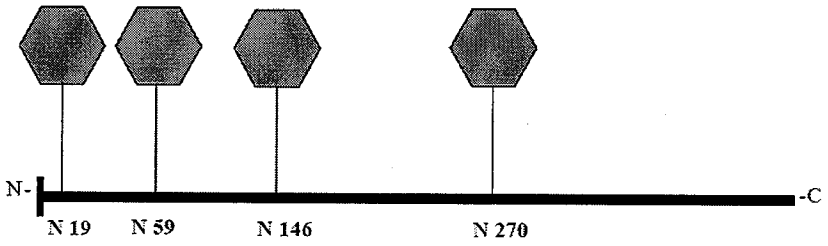
Chemical name: Glucocerebrosidase

CAS registry number: 37228-64-1

Molecular formula/molecular weight: Based on the amino acid sequence, the relative molecular mass of Velaglucerase alfa would be 56 kDa. However, following post-translation modification, such as glycosylation, the relative molecular mass of Velaglucerase alfa is 63 kDa.

Structure: Velaglucerase alfa is human glucocerebrosidase secreted from a transfected continuous human cell line (HT-1080), generated using Shire's gene activation technology. A schematic diagram illustrating the structure of Velaglucerase alfa is shown in the following Figure 3.2.S.1.2-1 (from the sponsor's submission). A 26 amino acid signal peptide derived from human growth hormone is cleaved when Velaglucerase alfa is secreted from cells. There are 5 potential N-linked glycosylation sites. While 4 of these sites are occupied as shown by peptide map analysis, N462 is unoccupied. In addition, there are two disulfide linkages near the N-terminus, consistent with the crystal structures published for human glucocerebrosidase.

**Figure 3.2.S.1.2-1 Schematic of Velaglucerase Alfa Structure**




The following (from the sponsor's submission) Figure 3.2.S.1.2-2 and Figure 3.2.S.1.2-3 present the 497 amino acid sequence of the secreted glucocerebrosidase, predicted from the mRNA and isolated from the 13E-16-V cell line and confirmed by peptide mass mapping and N-terminal sequence analysis. Velaglucerase alfa undergoes rapid reversible self-association between monomer and dimer, and is predominantly a dimer under the formulation conditions in solution (2.5 mg/mL protein concentration).

**Figure 3.2.S.1.2-2 Amino Acid Sequence of Secreted Velaglucerase alfa using Three-letter Code**

```
  1 Ala Arg Pro Cys Ile Pro Lys Ser Phe Gly Tyr Ser Ser Val Val Cys Val Cys Asn Ala   20
 21 Thr Tyr Cys Asp Ser Phe Asp Pro Pro Thr Phe Pro Ala Leu Gly Thr Phe Ser Arg Tyr   40
 41 Glu Ser Thr Arg Ser Gly Arg Arg Met Glu Leu Ser Met Gly Pro Ile Gln Ala Asn His   60
 61 Thr Gly Thr Gly Leu Leu Leu Thr Leu Gln Pro Glu Gln Lys Phe Gln Lys Val Lys Gly   80
 81 Phe Gly Gly Ala Met Thr Asp Ala Ala Ala Leu Asn Ile Leu Ala Leu Ser Pro Pro Ala  100
101 Gln Asn Leu Leu Leu Lys Ser Tyr Phe Ser Glu Glu Gly Ile Gly Tyr Asn Ile Ile Arg  120
121 Val Pro Met Ala Ser Cys Asp Phe Ser Ile Arg Thr Tyr Thr Tyr Ala Asp Thr Pro Asp  140
141 Asp Phe Gln Leu His Asn Phe Ser Leu Pro Glu Glu Asp Thr Lys Leu Lys Ile Pro Leu  160
161 Ile His Arg Ala Leu Gln Leu Ala Gln Arg Pro Val Ser Leu Leu Ala Ser Pro Trp Thr  180
181 Ser Pro Thr Trp Leu Lys Thr Asn Gly Ala Val Asn Gly Lys Gly Ser Leu Lys Gly Gln  200
201 Pro Gly Asp Ile Tyr His Gln Thr Trp Ala Arg Tyr Phe Val Lys Phe Leu Asp Ala Tyr  220
221 Ala Glu His Lys Leu Gln Phe Trp Ala Val Thr Ala Glu Asn Glu Pro Ser Ala Gly Leu  240
241 Leu Ser Gly Tyr Pro Phe Gln Cys Leu Gly Phe Thr Pro Glu His Gln Arg Asp Phe Ile  260
261 Ala Arg Asp Leu Gly Pro Thr Leu Ala Asn Ser Thr His His Asn Val Arg Leu Leu Met  280
281 Leu Asp Asp Gln Arg Leu Leu Leu Pro His Trp Ala Lys Val Val Leu Thr Asp Pro Glu  300
301 Ala Ala Lys Tyr Val His Gly Ile Ala Val His Trp Tyr Leu Asp Phe Leu Ala Pro Ala  320
321 Lys Ala Thr Leu Gly Glu Thr His Arg Leu Phe Pro Asn Thr Met Leu Phe Ala Ser Glu  340
341 Ala Cys Val Gly Ser Lys Phe Trp Glu Gln Ser Val Arg Leu Gly Ser Trp Asp Arg Gly  360
361 Met Gln Tyr Ser His Ser Ile Ile Thr Asn Leu Leu Tyr His Val Val Gly Trp Thr Asp  380
381 Trp Asn Leu Ala Leu Asn Pro Glu Gly Gly Pro Asn Trp Val Arg Asn Phe Val Asp Ser  400
401 Pro Ile Ile Val Asp Ile Thr Lys Asp Thr Phe Tyr Lys Gln Pro Met Phe Tyr His Leu  420
421 Gly His Phe Ser Lys Phe Ile Pro Glu Gly Ser Gln Arg Val Gly Leu Val Ala Ser Gln  440
441 Lys Asn Asp Leu Asp Ala Val Ala Leu Met His Pro Asp Gly Ser Ala Val Val Val Val  460
461 Leu Asn Arg Ser Ser Lys Asp Val Pro Leu Thr Ile Lys Asp Pro Ala Val Gly Phe Leu  480
481 Glu Thr Ile Ser Pro Gly Tyr Ser Ile His Thr Tyr Leu Trp Arg Arg Gln  497
```

Asn = potential N-linked glycosylation site
Cysteine residues are highlighted

**Figure 3.2.S.1.2-3 Amino Acid Sequence of Secreted Velaglucerase alfa using One-letter Code**

```
ARPCIPKSFG YSSVVCVCNA TYCDSFDPPT FPALGTFSRY ESTRSGRRME  50
LSMGPIQANH TGTGLLLTLQ PEQKFQKVKG FGGAMTDAAA LNILALSPPA 100
QNLLLKSYFS EEGIGYNIIR VPMASCDFSI RTYTYADTPD DFQLHNFSLP 150
EEDTKLKIPL IHRALQLAQR PVSLLASPWT SPTWLKTNGA VNGKGSLKGQ 200
PGDIYHQTWA RYFVKFLDAY AEHKLQFWAV TAENEPSAGL LSGYPFQCLG 250
FTPEHQRDFI ARDLGPTLAN STHHNVRLLM LDDQRLLLPH WAKVVLTDPE 300
AAKYVHGIAV HWYLDFLAPA KATLGETHRL FPNTMLFASE ACVGSKFWEQ 350
SVRLGSWDRG MQYSHSIITN LLYHVVGWTD WNLALNPEGG PNWVRNFVDS 400
PIIVDITKDT FYKQPMFYHL GHFSKFIPEG SQRVGLVASQ KNDLDAVALM 450
HPDGSAVVVV LNRSSKDVPL TIKDPAVGFL ETISPGYSIH TYLWRRQ   497
```

Potential N-glycosylation sites are underlined
Cysteine residues are highlighted

**Relevant IND**: IND 61,220 (Velaglucerase alfa, Shire)

**Drug class**: Enzyme replacement therapy (ERT)

**Intended clinical population**: Velaglucrease alfa is indicated for long-term enzyme replacement therapy (ERT) for pediatric and adult patients with Type 1 Gaucher disease (GD).

**Clinical formulation**: Velaglucerase alfa drug product is a sterile, white to off-white, preservative-free lyophilized powder in single-use vials. It is reconstituted with sterile Water for Injection (USP) resulting in a solution, which is diluted into normal saline for IV infusion. The 200 unit (200 U) presentation contains ~ Velaglucerase alfa per vial, and the 400 unit (400 U) presentation contains ~ Velaglucerase alfa per vial. The composition of the two presentations is shown in Table 3.2.P.1-1 (from the sponsor's submission).

**Table 3.2.P.1-1 Velaglucerase alfa Drug Product Composition**

| Names of Ingredients | Nominal Content per 200 U Vial[a] | Nominal Content per 400 U Vial[b] | Function | Reference to Standards |
|---|---|---|---|---|
| Velaglucerase alfa | ~ (200 unit)[c] | ~ (400 unit)[c] | Active Ingredient | In-house monograph |
| Sucrose | 100 mg | 200 mg | | NF, Ph Eur |
| Sodium citrate, dihydrate | 25.88 mg | 51.76 mg | ~ | USP, Ph Eur |
| Citric acid, monohydrate | 2.52 mg | 5.04 mg | | USP, Ph Eur |
| Polysorbate 20 | 0.22 mg | 0.44 mg | | NF, Ph Eur |

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**Route of administration**: Intravenous (IV) infusion

**Disclaimer**: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

**Studies reviewed within this submission:** The following table lists the studies reviewed in this submission.

| STUDY | REPORT NO. | | | REV. PAGE # |
|---|---|---|---|---|
| **PHARMACOLOGY** | | | | 13 |
| **ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION (ADME)** | | | | 27 |
| **ABSORPTION** | | | | 28 |
| Pharmacokinetics of DRX008A following IV administration to male SD rats | TKT-1U0-01-003 | | | 28 |
| Pharmacokinetics of DRX008A following IV administration to female SD rats | TKT-1U0-01-007 | | | 29 |
| Pharmacokinetics of DRX008A following IV administration to male Beagle dogs | 690-1U0-02-346 | | | 31 |
| **DISTRIBUTION** | | | | 32 |
| Tissue distribution of radioactivity following single IV injection of $^{125}$I-DRX008A in the SD rat | TKT-1U0-01-008 | | | 32 |
| Tissue distribution of radioactivity following single IV injection of $^{125}$I-DRX008A in the SD rat | TKT-1U0-01-001 | | | 34 |
| **OTHER ADME STUDIES** | | | | 43 |
| An *in vivo* comparison of [redacted] reference standard RE200-002 and [redacted] lot RE200-005 in ICR mice | 720-1U0-03-488 | | | 44 |
| Pharmacokinetics of DRX008A Lot 13E-16V- 0201 in Rats | 690-1U0-02-370 | | | 44 |
| A Crossover pharmacokinetic study of two lots of DRX008A administered by IV bolus injection to Cynomolgus monkeys | TKT-1U0-02-001 | | | 45 |
| **TOXICOLOGY** | | | | 47 |
| **Acute** | | | | 49 |
| ***Rat*** | | | | 49 |
| IV | TKT-1U0-00-004 | | | 49 |
| **Subacute/Subchronic** | | | | 50 |
| ***Rat*** | | | | 50 |
| 3-Month, IV | TKT-1U0-00-005 | | | 50 |
| 25-Week, IV | TKT-1U0-00-006 | | | 56 |
| ***Monkey*** | | | | 62 |
| 6-Month, IV | TKT-1U0-00-003 | | | 62 |
| **REPRODUCTIVE TOXICOLOGY** | | | | 70 |
| ***Rat*** | | | | 70 |
| Segment I fertility and early embryonic development, IV, Male | SHGT-1U0-06-010 | | | 70 |
| Segment I fertility and early embryonic development, IV, Female | SHGT-1U0-06-011 | | | 74 |
| Segment II teratology, IV | SHGT-1U0-06-013 | | | 78 |
| Segment III pre- and postnatal development, IV | SHGT-1U0-06-016 | | | 101 |
| ***Rabbits*** | | | | 85 |
| Segment II teratology, IV | SHGT-1U0-06-015 | | | 85 |
| **SPECIAL TOXICOLOGY** | | | | 109 |
| 2-Week, Rat, IV | SHGT-1U0-06-017 | | | 109 |


b(4)

**Studies not reviewed within this submission:** N/A

## 2.6.2 PHARMACOLOGY

### 2.6.2.1 Brief summary

A mouse model of Gaucher disease, the D409V/null Mouse (9V/null) was developed for animal efficacy studies. The 9V/null mice exhibit many of the disease features typical of human Type 1 (non-neuronopathic) Gaucher disease, including accumulation of "storage cells" (glucocerebroside-laden macrophages) in the lung, liver, and spleen, and a lack of pathology in the brain. Gaucher disease is caused by loss-of-function mutations in the GBA gene, encoding the enzyme acid-β-glucosidase. The D409V (aspartate at position 409 changed to valine) mutant strain was produced by homologous recombination with a replacement vector containing the D409V point mutation as well as a neomycin resistance gene, followed by cre-lox excision of extraneous sequences. A GBAnull mutant mouse was created similarly by deletion of genomic nucleotides g.5330 to g.5728 in exons 9 to 10. Since the GBAnull-and D409V-homozygous strains each possess extreme phenotypes rendering them impractical for research purposes (GBAnull homozygotes die a few hours after birth, while D409V homozygotes lack a disease phenotype), GBAnull-heterozygous mice were bred to D409V mice to produce the D409V/null compound heterozygote. D409V/null mice survive for over one year, reproduce normally, and have grossly normal behavior. However, the D409V allele encodes an unstable protein that is maintained at approximately 20% protein levels of wild type enzyme. Visceral organs of D409V/null mice have acid-β-glucosidase enzyme activity ranging from ~4% to 7% relative to wild type mice. D409V/null mice are well-suited to a study of type 1 Gaucher Disease since pathogenesis is relatively slow, mimicking human type 1 disease and providing ample opportunity for the application of therapeutics. Additionally, there is little if any CNS involvement in D409V/null mice. In this case, the 9V/null mouse model was used to compare the effects of Velaglucerase alfa to those of Imiglucerase (Cerezyme®), with regard to decrease of accumulated glucocerebroside and decrease in number of storage cells in visceral organs. The IV route of administration was used in all experiments as this is the intended route of administration in patients.

The 9V/null mice were given once-weekly IV injections of either Velaglucerase alfa or Imiglucerase at dose levels 5, 15, or 60 U/kg (0.0017, 0.005, and 0.02 U/μl, respectively) for a total of four or eight weeks, after which time the mice were sacrificed. Levels of glucocerebroside in the liver, lung, and spleen were determined by liquid chromatography/mass spectrometry (LC/MS), and storage cells in the liver tissue were identified histologically as large, CD68-positive cells. The results showed that Velaglucerase alfa and Imiglucerase similarly restored normal lipid (glucocerebroside) content in the liver, while the lipid content in the spleen was reduced to a lesser extent. In contrast, neither enzyme affected the lipid content of the lung. Both enzyme treatments comparably reduced the number of storage cells (also called Gaucher cells) in the liver.

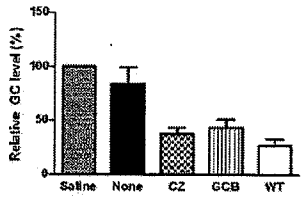
### 2.6.2.2 Primary pharmacodynamics

**Substrate Clearance (Report No. 751-1U0-09-1222, 751-1U0-09-1223, 751-1U0-09-1224)**

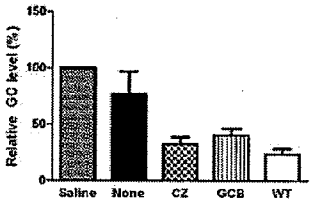
The objective of this study was to compare the biological/biochemical effects of Velaglucerase alfa and Imiglucerase in a mouse model of Gaucher disease at dose levels of 5, 15, or 60 U/kg. In this study, the clearance of accumulated glucosylceramide from the tissues of the 9V/null mouse was determined following repeated administration of either Velaglucerase alfa or Imiglucerase. The 9V/null mouse is a well characterized point-mutant model of non-neuronopathic (Type 1) Gaucher disease that accumulates glucosylceramide in a predictable manner in the viscera and that, as a result, has histologic abnormalities in the liver, spleen and lungs consistent with Gaucher disease. To determine improvements in tissue phenotypes by these enzymes, these studies evaluated the glucosylceramide levels and number of Gaucher cells (storage cells) following treatment with different glucocerebrosidase enzymes. The major objective of this study was to evaluate the therapeutic effect of Velaglucerase alfa or Imiglucerase on visceral lipid accumulation and lipid-laden tissue macrophages in the liver, spleen, and lung of 9V/null mice.

In these studies, nine-to-fourteen 9V/null mice (20-week old) per treatment group received once-weekly doses of 5 U Velaglucerase alfa/kg, 5 U Imiglucerase/kg, saline, or were untreated controls. As an added control, some experiments included untreated wild type (non-Gaucher) mice. Mice were treated for 8 or 4 weeks.

Figures 1-3 (from page 4-7 of the study report) show the results of tissue lipid contents after 4-week or 8-week treatments with 5 units/kg Velaglucerase and Imiglucerase. Both enzymes similarly restored normal lipid content in the liver (Figure 1), while the lipid content of the spleen (Figure 2) and lung (Figure 3) were unaffected. Liver was further analyzed histologically for the presence of storage cells (Gaucher cells). As shown in Figure 4, both enzyme treatments similarly reduced the number of storage cells relative to saline-treated controls. Velaglucerase and Imiglucerase groups were comparable to each other. Overall, the results demonstrated that Velaglucerase alfa was comparable to Imiglucerase in terms of its ability to clear accumulated glucosylceremide from the liver, and in terms of its ability to reduce the number of storage cells in this organ.

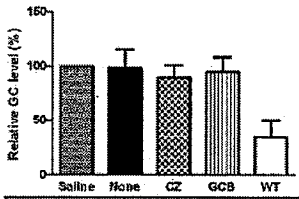



| | Saline | None | CZ | GCB | WT |
|---|---|---|---|---|---|
| Number of values | 10 | 9 | 10 | 10 | 2 |
| Minimum | 100.0 | 36.40 | 15.30 | 11.10 | 22.00 |
| Maximum | 100.0 | 167.0 | 73.20 | 83.20 | 33.00 |
| Mean | 100.0 | 83.62 | 37.58 | 43.37 | 27.50 |
| Std. Deviation | 0.0 | 46.95 | 18.43 | 24.21 | 7.778 |
| Std. Error | 0.0 | 15.52 | 5.827 | 7.655 | 5.500 |
| Sum | 1000 | 752.6 | 375.8 | 433.7 | 55.00 |

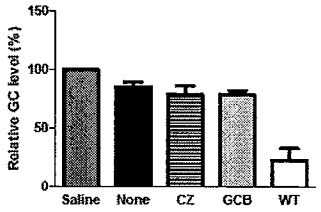



| | Saline | None | CZ | GCB | WT |
|---|---|---|---|---|---|
| Number of values | 10 | 9 | 10 | 10 | 2 |
| Minimum | 100.0 | 31.00 | 14.30 | 20.90 | 19.20 |
| Maximum | 100.0 | 223.0 | 61.10 | 70.70 | 28.80 |
| Mean | 100.0 | 76.83 | 33.10 | 40.98 | 24.00 |
| Std. Deviation | 0.0 | 61.80 | 18.41 | 18.31 | 6.788 |
| Std. Error | 0.0 | 20.60 | 5.823 | 5.789 | 4.800 |
| Sum | 1000 | 691.5 | 331.0 | 409.8 | 48.00 |

**Figure 1. Relative glucosylceramide levels in liver after 4-week *(top panel)* or 8-week *(bottom panel)* treatment of 9V/null mice with 5 units/kg vela-a (GCB), imi (CZ), and untreated (None) or Saline-treated 9V/null controls. WT= untreated wildtype mice. All treatment groups *except* "WT" were performed on 9v/null mice. [Notebook refs: *Top panel:* Lipid binder Ying Sun, Pages 3,4,7,8,16; injection record in TKT Study binder YHX Therapeutic 5U/kg/wk, Pages 35-49; *Bottom panel:* Lipid binder Ying Sun, Pages 3,4,7,8, injection record in TKT Study binder YHX Therapeutic 5U/kg/wk, Pages 8-34, 50-58]**

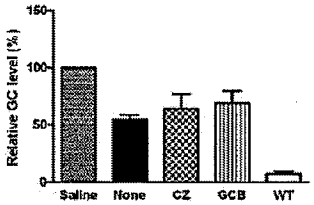



| | Saline | None | CZ | GCB | WT |
|---|---|---|---|---|---|
| Number of values | 8 | 8 | 8 | 9 | 2 |
| | | | | | |
| Minimum | 100.0 | 46.40 | 44.50 | 35.10 | 19.40 |
| Maximum | 100.0 | 192.5 | 129.9 | 140.1 | 50.30 |
| | | | | | |
| Mean | 100.0 | 98.29 | 86.70 | 95.40 | 34.85 |
| Std. Deviation | 0.0 | 48.03 | 32.49 | 39.07 | 21.85 |
| Std. Error | 0.0 | 16.98 | 11.49 | 13.02 | 15.45 |
| | | | | | |
| | | | | | |
| Sum | 800.0 | 786.3 | 717.6 | 858.6 | 69.70 |

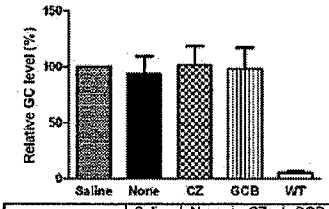



| | Saline | None | CZ | GCB | WT |
|---|---|---|---|---|---|
| Number of values | 9 | 6 | 7 | 8 | 2 |
| | | | | | |
| Minimum | 100.0 | 75.10 | 44.30 | 60.80 | 12.70 |
| Maximum | 100.0 | 99.70 | 101.9 | 97.20 | 32.80 |
| | | | | | |
| Mean | 100.0 | 85.85 | 78.97 | 78.85 | 22.75 |
| Std. Deviation | 0.0 | 9.321 | 19.84 | 10.38 | 14.21 |
| Std. Error | 0.0 | 3.805 | 7.498 | 3.670 | 10.05 |
| | | | | | |
| | | | | | |
| Sum | 900.0 | 515.1 | 552.8 | 630.8 | 45.50 |

**Figure 2. Relative glucosylceramide levels in spleen after 4-week *(top panel)* or 8-week *(bottom panel)* treatment of 9V/null mice with 5 units/kg vela-a (GCB), imi (CZ), and untreated (None) or Saline-treated 9V/null controls. WT= untreated wildtype mice. All treatment groups *except* "WT" were performed on 9v/null mice. [Notebook refs: *Top panel:* Lipid binder Ying Sun, Pages 1, 2, 26, 27; *Bottom panel:* Lipid binder Ying Sun, Pages 1, 2, 26, 27. Injection records same as Figure 1]**

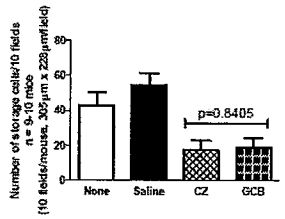



| | Saline | None | CZ | GCB | WT |
|---|---|---|---|---|---|
| Number of values | 7 | 6 | 7 | 7 | 3 |
| Minimum | 100.0 | 42.90 | 41.36 | 37.50 | 4.800 |
| Maximum | 100.0 | 71.20 | 138.8 | 115.8 | 11.40 |
| Mean | 100.0 | 54.78 | 64.22 | 68.89 | 7.033 |
| Std. Deviation | 0.0 | 10.28 | 33.76 | 29.37 | 3.782 |
| Std. Error | 0.0 | 4.195 | 12.76 | 11.10 | 2.184 |
| Sum | 700.0 | 328.7 | 449.5 | 482.2 | 21.10 |

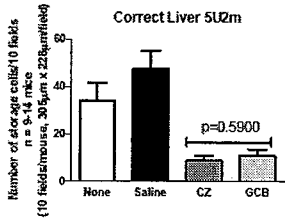



| | Saline | None | CZ | GCB | WT |
|---|---|---|---|---|---|
| Number of values | 10 | 9 | 10 | 9 | 3 |
| Minimum | 100.0 | 37.90 | 39.80 | 37.50 | 3.600 |
| Maximum | 100.0 | 151.6 | 172.4 | 182.7 | 8.600 |
| Mean | 100.0 | 94.37 | 101.7 | 98.46 | 5.300 |
| Std. Deviation | 0.0 | 46.06 | 54.60 | 56.83 | 2.858 |
| Std. Error | 0.0 | 15.35 | 17.26 | 18.94 | 1.650 |
| Sum | 1000 | 849.3 | 1017 | 886.1 | 15.90 |

**Figure 3. Relative glucosylceramide levels in lung after 4-week *(top panel)* or 8-week *(bottom panel)* treatment of 9V/null mice with 5 units/kg vela-a (GCB), imi (CZ), and untreated (None) or Saline-treated 9V/null controls. WT= untreated wildtype mice. All treatment groups *except* "WT" were performed on 9v/null mice. [Notebook refs: *Top panel:* Lipid binder Ying Sun, Pages 5, 6, 12, 13; *Bottom panel:* Lipid binder Ying Sun, Pages 5, 6, 12, 13. Injection records same as Figure 1]**



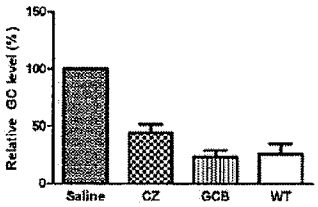

| | None | Saline | CZ | GCB |
|---|---|---|---|---|
| Number of values | 9 | 10 | 10 | 10 |
| Minimum | 7.000 | 7.000 | 1.000 | 0.0 |
| Maximum | 88.00 | 80.00 | 52.00 | 48.00 |
| Mean | 43.00 | 54.80 | 17.40 | 16.00 |
| Std. Deviation | 23.20 | 21.54 | 17.58 | 17.49 |
| Std. Error | 7.733 | 6.811 | 5.552 | 5.532 |
| Sum | 387.0 | 548.0 | 174.0 | 160.0 |



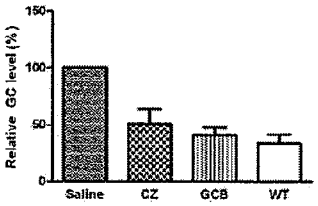

| | None | Saline | CZ | GCB |
|---|---|---|---|---|
| Number of values | 9 | 11 | 14 | 10 |
| Minimum | 5.000 | 13.00 | 0.0 | 0.0 |
| Maximum | 69.00 | 82.00 | 27.00 | 24.00 |
| Mean | 34.11 | 47.64 | 8.857 | 10.90 |
| Std. Deviation | 22.91 | 25.89 | 9.080 | 8.937 |
| Std. Error | 7.637 | 7.748 | 2.427 | 2.826 |
| Sum | 307.0 | 524.0 | 124.0 | 109.0 |

**Figure 4. Comparison of the number of Storage Cells in liver after 4-week *(top panel)* or 8-week *(bottom panel)* treatment of 9V/null mice with 5 units/kg vela-a (GCB), imi (CZ), and untreated (None) or Saline-treated controls. 9-14 mice are reported for each test condition, and ten 305 µm x 228 µm fields were evaluated per mouse. The values displayed on the graphs are the means of the total number of storage cells observed per ten fields. All treatment groups were performed on 9v/null mice. P values were by Student's t-test). [Notebook refs: *Top panel:* Binder TKT Cell Count 1, Section: 5U/1m; *Bottom panel:* Binder TKT Cell Count 1, Section: 5U/2m. Injection records same as Figure 1]**

The following figures (from page 4-7 of the study report) show the results for 15 U/kg dose.

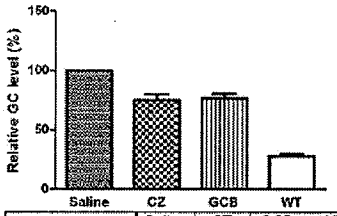



| | Saline | CZ | GCB | WT |
|---|---|---|---|---|
| Number of values | 10 | 10 | 10 | 4 |
| Minimum | 100.0 | 17.40 | 7.300 | 12.80 |
| Maximum | 100.0 | 80.70 | 70.20 | 52.30 |
| Mean | 100.0 | 44.49 | 23.23 | 26.03 |
| Std. Deviation | 0.0 | 23.65 | 18.59 | 17.98 |
| Std. Error | 0.0 | 7.480 | 5.879 | 8.959 |
| Sum | 1000 | 444.9 | 232.3 | 104.1 |

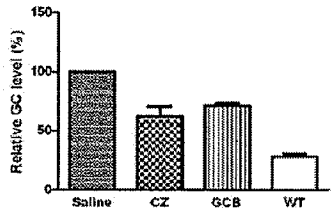



| | Saline | CZ | GCB | WT |
|---|---|---|---|---|
| Number of values | 10 | 10 | 10 | 4 |
| Minimum | 100.0 | 20.80 | 17.10 | 15.30 |
| Maximum | 100.0 | 153.2 | 95.60 | 48.70 |
| Mean | 100.0 | 50.51 | 40.63 | 33.85 |
| Std. Deviation | 0.0 | 42.39 | 23.01 | 15.99 |
| Std. Error | 0.0 | 13.40 | 7.276 | 7.997 |
| Sum | 1000 | 505.1 | 406.3 | 135.4 |

**Figure 1. Relative glucosylceramide levels in liver after 4-week *(top panel)* or 8-week *(bottom panel)* treatment of 9V/null mice with 15 units/kg vela-a (GCB), or imi (CZ), and in Saline-treated 9V/null controls. WT= untreated wildtype mice. All treatment groups *except* "WT" were performed on 9v/null mice. [Notebook refs: *Top panel:* Lipid Binder Ying Sun, Pages 2, 4, 9, 26, 27, injection record in TKT Study binder YHX Therapeutic 15 U/kg/wk, Pages 30-44; *Bottom panel:* Lipid Binder Ying Sun, Pages 3, 4, 9, 26, 27, injection record in TKT Study binder YHX Therapeutic 15 U/kg/wk, Pages 7-29]**

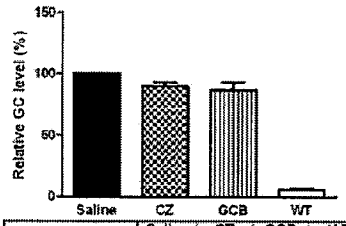



| | Saline | CZ | GCB | WT |
|---|---|---|---|---|
| Number of values | 9 | 8 | 9 | 3 |
| Minimum | 100.0 | 61.30 | 62.10 | 24.20 |
| Maximum | 100.0 | 96.40 | 96.70 | 32.70 |
| Mean | 100.0 | 74.98 | 76.72 | 27.77 |
| Std. Deviation | 0.0 | 12.99 | 11.27 | 4.412 |
| Std. Error | 0.0 | 4.592 | 3.758 | 2.547 |
| Sum | 900.0 | 599.8 | 690.5 | 83.30 |

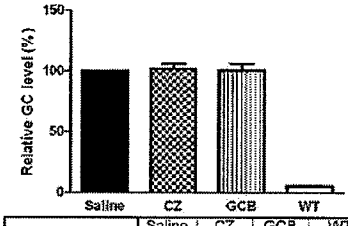



| | Saline | CZ | GCB | WT |
|---|---|---|---|---|
| Number of values | 10 | 10 | 9 | 3 |
| Minimum | 100.0 | 27.60 | 63.60 | 24.50 |
| Maximum | 100.0 | 100.4 | 83.20 | 33.10 |
| Mean | 100.0 | 62.49 | 71.42 | 28.13 |
| Std. Deviation | 0.0 | 26.35 | 6.779 | 4.452 |
| Std. Error | 0.0 | 8.332 | 2.260 | 2.571 |
| Sum | 1000 | 624.9 | 642.8 | 84.40 |

**Figure 2. Relative glucosylceramide levels in spleen after 4-week *(top panel)* or 8-week *(bottom panel)* treatment of 9V/null mice with 15 units/kg vela-a (GCB), or imi (CZ), and in Saline-treated 9V/null controls. WT= untreated wildtype mice. All treatment groups *except* "WT" were performed on 9v/null mice. [Notebook refs: *Top panel:* Lipid Binder Ying Sun, Pages 1, 2, 23, 24; *Bottom panel:* Lipid Binder Ying Sun, Pages 1, 2, 23, 24. Injection Records same as in Figure 1]**

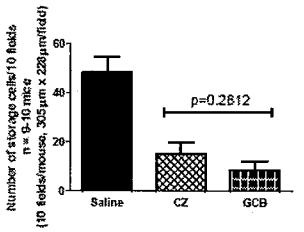



| | Saline | CZ | GCB | WT |
|---|---|---|---|---|
| Number of values | 10 | 10 | 10 | 4 |
| | | | | |
| Minimum | 100.0 | 76.00 | 54.40 | 4.500 |
| Maximum | 100.0 | 103.5 | 128.8 | 6.800 |
| | | | | |
| Mean | 100.0 | 89.75 | 86.71 | 5.925 |
| Std. Deviation | 0.0 | 11.35 | 21.48 | 1.044 |
| Std. Error | 0.0 | 3.589 | 6.794 | 0.5218 |
| | | | | |
| | | | | |
| Sum | 1000 | 897.5 | 867.1 | 23.70 |

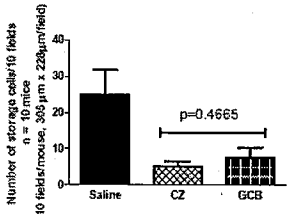



| | Saline | CZ | GCB | WT |
|---|---|---|---|---|
| Number of values | 10 | 10 | 10 | 4 |
| | | | | |
| Minimum | 100.0 | 85.70 | 82.50 | 4.300 |
| Maximum | 100.0 | 129.0 | 137.8 | 6.500 |
| | | | | |
| Mean | 100.0 | 102.1 | 100.9 | 5.625 |
| Std. Deviation | 0.0 | 13.55 | 16.98 | 0.9777 |
| Std. Error | 0.0 | 4.284 | 5.368 | 0.4888 |
| | | | | |
| | | | | |
| Sum | 1000 | 1021 | 1009 | 22.50 |

**Figure 3. Relative glucosylceramide levels in lung after 4-week *(top panel)* or 8-week *(bottom panel)* treatment of 9V/null mice with 15 units/kg vela-a (GCB), or imi (CZ), and in Saline-treated 9V/null controls. WT= untreated wildtype mice. All treatment groups *except* "WT" were performed on 9v/null mice. [Notebook refs: *Top panel:* Lipid Binder Ying Sun, Pages 5, 6, 17, 18, 19, 20, 21; *Bottom panel:* Lipid Binder Ying Sun, Pages 5, 6, 17, 18, 19, 20, 21. Injection Records same as in Figure 1]**



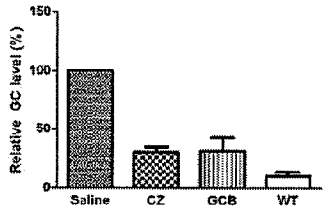

| | Saline | CZ | GCB |
|---|---|---|---|
| Number of values | 10 | 10 | 9 |
| | | | |
| Minimum | 14.00 | 1.000 | 0.0 |
| Maximum | 73.00 | 49.00 | 26.00 |
| | | | |
| Mean | 48.30 | 15.30 | 8.667 |
| Std. Deviation | 20.32 | 14.74 | 10.64 |
| Std. Error | 6.426 | 4.660 | 3.547 |
| | | | |
| | | | |
| Sum | 483.0 | 153.0 | 78.00 |



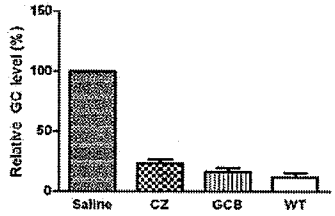

| | Saline | CZ | GCB |
|---|---|---|---|
| Number of values | 10 | 10 | 10 |
| | | | |
| Minimum | 4.000 | 0.0 | 0.0 |
| Maximum | 64.00 | 13.00 | 31.00 |
| | | | |
| Mean | 25.00 | 5.000 | 7.500 |
| Std. Deviation | 21.16 | 5.228 | 9.253 |
| Std. Error | 6.692 | 1.653 | 2.926 |
| | | | |
| | | | |
| Sum | 250.0 | 50.00 | 75.00 |

**Figure 4. Comparison of the number of Storage Cells in liver after 4-week *(top panel)* or 8-week *(bottom panel)* treatment with 15 units/kg vela-a (GCB), or imi (CZ), and in Saline-treated negative controls. 9-10 mice are reported for each test condition, and ten 305 µm x 228 µm fields were evaluated per mouse. The values displayed on the graphs are the means of the total number of storage cells observed per ten fields. All treatment groups were performed on 9v/null mice. P values were by Student's t-test. [Notebook refs: *Top panel:* Binder TKT Cell Count-2, Section 15U/1m ; *Bottom panel:* Binder TKT Cell Count-2, Section 15U/2m. Injection Records same as in Figure 1.]**

The following figures (from page 4-7 of the study report) show the results for the 60 U/kg dose.

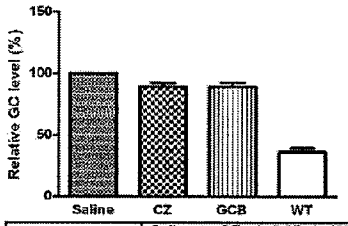



| | Saline | CZ | GCB | WT |
|---|---|---|---|---|
| Number of values | 10 | 10 | 10 | 2 |
| Minimum | 100.0 | 13.00 | 10.20 | 6.400 |
| Maximum | 100.0 | 54.00 | 116.3 | 13.50 |
| Mean | 100.0 | 30.17 | 31.62 | 9.950 |
| Std. Deviation | 0.0 | 15.61 | 35.29 | 5.020 |
| Std. Error | 0.0 | 4.936 | 11.16 | 3.550 |
| Sum | 1000 | 301.7 | 316.2 | 19.90 |

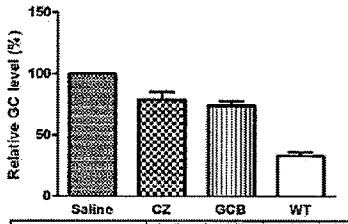



| | Saline | CZ | GCB | WT |
|---|---|---|---|---|
| Number of values | 10 | 10 | 10 | 2 |
| Minimum | 100.0 | 9.000 | 7.400 | 7.400 |
| Maximum | 100.0 | 40.60 | 32.60 | 15.60 |
| Mean | 100.0 | 23.14 | 16.45 | 11.50 |
| Std. Deviation | 0.0 | 11.39 | 9.788 | 5.798 |
| Std. Error | 0.0 | 3.602 | 3.095 | 4.100 |
| Sum | 1000 | 231.4 | 164.5 | 23.00 |

**Figure 1. Relative glucosylceramide levels in liver after 4-week *(top panel)* or 8-week *(bottom panel)* treatment of 9V/null mice with 60 units/kg vela-a (GCB), or imi (CZ), and in Saline-treated 9V/null controls. WT= untreated wildtype mice. All treatment groups *except* "WT" were performed on 9v/null mice. [Notebook refs: Lipid Binder Ying Sun, Pages 3,4,17-22. Injection Record in TKT Study binder YHX Therapeutic: 60U/kg/wk, Pages 32-36, 71-79]**

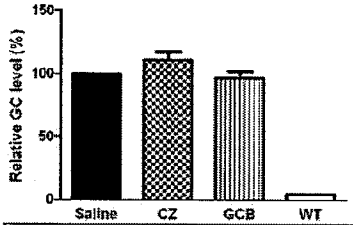



| | Saline | CZ | GCB | WT |
|---|---|---|---|---|
| Number of values | 10 | 10 | 10 | 3 |
| Minimum | 100.0 | 73.20 | 73.10 | 31.30 |
| Maximum | 100.0 | 103.3 | 109.1 | 42.30 |
| Mean | 100.0 | 89.07 | 89.18 | 35.93 |
| Std. Deviation | 0.0 | 9.907 | 11.98 | 5.701 |
| Std. Error | 0.0 | 3.133 | 3.790 | 3.292 |
| Sum | 1000 | 890.7 | 891.8 | 107.8 |

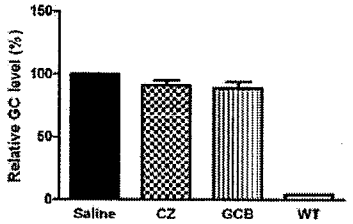



| | Saline | CZ | GCB | WT |
|---|---|---|---|---|
| Number of values | 10 | 10 | 10 | 3 |
| Minimum | 100.0 | 53.20 | 56.20 | 26.60 |
| Maximum | 100.0 | 129.7 | 99.00 | 39.00 |
| Mean | 100.0 | 78.66 | 73.93 | 33.10 |
| Std. Deviation | 0.0 | 20.37 | 11.86 | 5.285 |
| Std. Error | 0.0 | 6.441 | 3.749 | 3.051 |
| Sum | 1000 | 786.6 | 739.3 | 99.30 |

**Figure 2. Relative glucosylceramide levels in spleen after 4-week *(top panel)* or 8-week *(bottom panel)* treatment of 9V/null mice with 60 units/kg vela-a (GCB), or imi (CZ), and in Saline-treated 9V/null controls. WT= untreated wildtype mice. All treatment groups *except* "WT" were performed on 9v/null mice. [Notebook refs: Lipid Binder Ying Sun, Pages 1,2,23-25. Injection Record same as Figure 1.]**

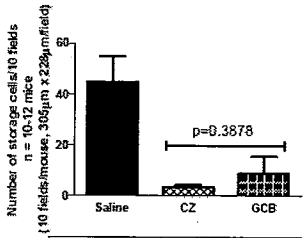



| | Saline | CZ | GCB | WT |
|---|---|---|---|---|
| Number of values | 10 | 10 | 10 | 2 |
| Minimum | 100.0 | 71.40 | 75.00 | 4.800 |
| Maximum | 100.0 | 137.0 | 132.9 | 4.800 |
| Mean | 100.0 | 110.5 | 96.94 | 4.800 |
| Std. Deviation | 0.0 | 21.27 | 15.59 | 0.0 |
| Std. Error | 0.0 | 6.725 | 4.930 | 0.0 |
| Sum | 1000 | 1105 | 969.4 | 9.600 |

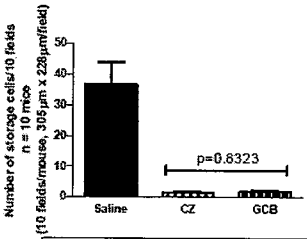



| | Saline | CZ | GCB | WT |
|---|---|---|---|---|
| Number of values | 10 | 10 | 10 | 2 |
| Minimum | 100.0 | 63.60 | 67.10 | 4.000 |
| Maximum | 100.0 | 109.3 | 118.1 | 4.000 |
| Mean | 100.0 | 90.93 | 88.56 | 4.000 |
| Std. Deviation | 0.0 | 12.69 | 16.12 | 0.0 |
| Std. Error | 0.0 | 4.012 | 5.096 | 0.0 |
| Sum | 1000 | 909.3 | 885.6 | 8.000 |

**Figure 3. Relative glucosylceramide levels in lung after 4-week *(top panel)* or 8-week *(bottom panel)* treatment of 9V/null mice with 60 units/kg vela-a (GCB), or imi (CZ), and in Saline-treated 9V/null controls. WT= untreated wildtype mice. All treatment groups *except* "WT" were performed on 9v/null mice. [Notebook refs: Lipid Binder Ying Sun, Pages 5,6,9-11 Injection Record same as Figure 1.]**




| | Saline | CZ | GCB |
|---|---|---|---|
| Number of values | 12 | 10 | 10 |
| | | | |
| Minimum | 4.000 | 0.0 | 0.0 |
| Maximum | 128.0 | 9.000 | 66.00 |
| | | | |
| Mean | 45.00 | 3.400 | 9.100 |
| Std. Deviation | 35.16 | 3.373 | 20.09 |
| Std. Error | 10.15 | 1.067 | 6.352 |
| | | | |
| | | | |
| Sum | 540.0 | 34.00 | 91.00 |




| | Saline | CZ | GCB |
|---|---|---|---|
| Number of values | 10 | 10 | 10 |
| | | | |
| Minimum | 7.000 | 0.0 | 0.0 |
| Maximum | 82.00 | 5.000 | 8.000 |
| | | | |
| Mean | 36.80 | 1.600 | 1.800 |
| Std. Deviation | 23.48 | 1.713 | 2.394 |
| Std. Error | 7.419 | 0.5416 | 0.7572 |
| | | | |
| | | | |
| Sum | 368.0 | 16.00 | 18.00 |

**Figure 4. Comparison of the number of Storage Cells in liver after 4-week *(top panel)* or 8-week *(bottom panel)* treatment with 60 units/kg vela-a (GCB), or imi (CZ), and in Saline-treated negative controls. 10-12 mice are reported for each test condition, and ten 305 µm x 228 µm fields were evaluated per mouse. The values displayed on the graphs are the means of the total number of storage cells observed per ten fields. All treatment groups were performed on 9v/null mice. P values were by Student's t-test. [Notebook refs: *Top panel:* Binder TKT Cell Count-2, Section 60U/1m ; *Bottom panel:* Binder TKT Cell Count-2, Section 60U/2m. Injection Records same as in Figure 1.]**

### 2.6.2.3 Secondary pharmacodynamics

None

### 2.6.2.4 Safety pharmacology

None

### 2.6.2.5 Pharmacodynamic drug interactions

None

## 2.6.3 PHARMACOLOGY TABULATED SUMMARY

None

## 2.6.4 PHARMACOKINETICS/TOXICOKINETICS

### 2.6.4.1 Brief summary

Animal pharmacokinetic studies were conducted to explore the pharmacokinetics, biodistribution, and excretion of Velaglucerase alfa (GA-GCB or DRX008A). Pharmacokinetic (PK) studies were conducted in SD rats, Rhesus monkeys (as part of 6-month toxicology study), and Beagle dogs. Biodistribution studies were conducted in SD rats with $^{125}$I-Velaglucerase alfa. The formulation and the route of administration (IV) used in the PK studies were identical to the intended clinical use. Additionally, a series of studies were conducted to assess the comparability of Velaglucerase alfa produced from a ——— process. These studies included a single dose PK study in rats, biodistribution study in ICR mice, and a crossover PK study in Cynomolgus monkeys. b(4)

The PK properties of Velaglucerase alfa in SD rats and Rhesus monkeys were similar in males and females for both species. An additional PK study was conducted in male Beagle dogs. Following a single IV dose, Velaglucerase alfa was rapidly cleared from the serum in all three species. At the low dose (0.84 mg/kg), distribution half-lives were approximately 2, 4, and 5 minutes in rats, dogs, and monkeys, respectively. While Cmax was proportional to dose in all three species, area under the curve (AUC) was not dose-proportional. At the higher doses of 3.4 and 17 mg/kg, distribution half-lives increased and clearance was decreased. Following repeated administration of IV doses in Rhesus monkeys, Velaglucerase alfa did not appear to accumulate in the serum following biweekly administration. Following repeated IV administration, four of the ten high dose monkeys developed antibodies to Velaglucerase alfa (IgG class).

Two biodistribution studies in male SD rats were conducted using $^{125}$I-Velaglucerase alfa. Animals received bolus IV injections at 1 or 10 mg/kg and were sacrificed at time-points between 20 minutes and 48 hours post dose. The greatest amount of radioactivity was found in the liver 20 minutes after dosing (approximately 70% of administered dose at 1.1 mg/kg). At the high dose only 24% and 38% of the administered dose was found in the liver and circulation, respectively. About 1.5% was detected in the spleen at 20 minutes (1.1 mg/kg dose), and 3% was detected in the kidney. From 0 to 48 hours post dose, the cumulative recovery of radioactivity in the urine was 91%-106%. The radioactivity was also associated with the contents of the small and large intestine (3%-8% in the small intestine and 1%-2% in the large intestine).

To optimize manufacture of Velaglucerase alfa, the manufacturing process was modified b(4)
. As part of the manufacturing comparability program, an ICR mouse biodistribution and a Cynomolgus monkey crossover PK study were conducted to evaluate the PK profiles following administration of drug substance produced from either manufacturing process. An additional rat PK study utilizing material was also conducted. In these studies, the concentration of protein found in the liver and spleen of ICR mice, 20 minutes after IV administration of 1.6 mg/kg of either material, was found to be comparable. Additionally, Cmax and AUC values were also comparable. Based upon these data, material produced via the was considered comparable to that produced via the manufacturing process.

### 2.6.4.2 Methods of Analysis

[Discussed under individual study reviews]

### 2.6.4.3 Absorption

**Pharmacokinetics of DRX008A Following Intravenous Administration to Male Sprague Dawley Rats (TKT-1U0-01-003)**

**Methods:** The serum pharmacokinetics of DRX008A was determined following IV administration to SD rats. In this study, 18 male rats were used, with five assigned to each of three treatment groups and 3 assigned to the vehicle group. The test article (DRX008A) or vehicle (50 mM sodium citrate, 3% sorbitol, 0.01% Tween 20, pH 6.0) was administered IV once on Day 1. Groups 2, 3 and 4 received DRX008A at dose levels of 0.83, 3.3 and 17 mg/kg, respectively, and Group 1 received the vehicle. Blood samples were collected from animals in Group 1 prior to dosing and at 1 and 3 hours post-dose, Group 2 prior to dosing and at 1, 3, 5, 10, 15, 20, 25, 30, 35, 40, 50, and 60 minutes postdose, Group 3 prior to dosing and at 1, 3, 5, 10, 15, 20, 25, 30, 40, 50, 60, 90 and 120 minutes postdose and Group 4 prior to dosing and at 1, 3, 5, 10, 20, 30, 45, 60, 75, 90, 105 120 and 180 minutes postdose.

**Results**: DRX008A was completely eliminated by 30 to 90 minutes postdose. Mean $T_{1/2}$ increased with dose from 2.3 minutes at the low dose to 3.9 and 4.9 minutes at the two higher doses, respectively. $C_{max}$ values were dose proportional, but AUC was not dose proportional. AUC increased approximately 7-9 fold compared to the 4-5 fold increase in doses. Mean serum clearance decreased as dose increased (13.8, 7.7 and 4.7 mL/min/kg at 0.83, 3.3 and 17 mg/kg, respectively). Mean apparent volume of distribution values ranged from 4.5% to 5.4%. The following table (from page 41 of the study report) shows the PK parameters in male SD rats.

**Table 1: Pharmacokinetic Parameters Following IV Dose of DRX008A in Male Sprague-Dawley Rats**

| Group No. | Animal No. | Body Weight (Kg) | Cmax (U/mL) | AUC (min* U/mL) | $T_{1/2}$ (λz) (min) | MRT (min) | Cl (mL/min) | Cl (mL/min/kg) | $V_{ss}$ (mL) | $V_{ss}$ (% BW) |
|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 4 | 0.270 | 38.8 | 155 | 2.8 | 4.0 | 3.27 | 12.1 | 13.2 | 4.9% |
| | 5 | 0.285 | 50.9 | 147 | 2.0 | 2.9 | 3.53 | 12.4 | 10.2 | 3.6% |
| 0.85 mg/kg | 6 | 0.296 | 36.2 | 106 | 2.1 | 3.0 | 4.85 | 17.0 | 14.4 | 5.0% |
| | 7 | 0.290 | 38.9 | 132 | 2.8 | 3.4 | 4.10 | 14.1 | 13.8 | 4.7% |
| | 8 | 0.294 | 41.2 | 134 | 2.3 | 3.3 | 3.89 | 13.2 | 12.7 | 4.3% |
| | | | | | | | | | | |
| Mean | | 0.285 | 41.2 | 135 | 2.3 | 3.3 | 3.93 | 13.8 | 12.8 | 4.5% |
| SD | | 0.009 | 5.7 | 19 | 0.32 | 0.5 | 0.61 | 2.0 | 1.6 | 0.6% |
| N | | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |

| Group No. | Animal No. | Body Weight (Kg) | Cmax (U/mL) | AUC (min* U/mL) | $T_{1/2}$ (λz) (min) | MRT (min) | Cl (mL/min) | Cl (mL/min/kg) | $V_{ss}$ (mL) | $V_{ss}$ (% BW) |
|---|---|---|---|---|---|---|---|---|---|---|
| 3 | 9 | 0.304 | 121 | 909 | 2.1 | 5.4 | 2.80 | 9.2 | 17.9 | 5.9% |
| | 10 | 0.277 | 84.7 | 845 | 2.2 | 7.4 | 2.42 | 8.7 | 17.9 | 6.5% |
| 3.3 mg/kg | 11 | 0.292 | 121 | 1269 | 5.0 | 7.8 | 1.69 | 5.8 | 12.9 | 4.4% |
| | 12 | 0.294 | 122 | 853 | 4.6 | 5.6 | 2.48 | 8.4 | 13.9 | 4.7% |
| | 13 | 0.291 | 135 | 1127 | 5.7 | 8.6 | 1.86 | 6.4 | 12.9 | 4.2% |
| | | | | | | | | | | |
| Mean | | 0.292 | 117 | 981 | 3.9 | 6.7 | 2.25 | 7.7 | 15.0 | 5.1% |
| SD | | 0.010 | 19.0 | 205 | 1.68 | 0.9 | 0.48 | 1.5 | 3 | 1.0% |
| N | | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |

| Group No. | Animal No. | Body Weight (Kg) | Cmax (U/mL) | AUC (min* U/mL) | $T_{1/2}$ (λz) (min) | MRT (min) | Cl (mL/min) | Cl (mL/min/kg) | $V_{ss}$ (mL) | $V_{ss}$ (% BW) |
|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 14* | 0.285 | 706 | 8795 | 5.2 | 12.0 | 1.16 | 4.1 | 14.1 | 4.9% |
| | 15* | 0.286 | 407 | 5619 | 5.1 | 11.4 | 1.87 | 6.5 | 21.4 | 7.4% |
| 17 mg/kg | 16* | 0.278 | 436 | 5781 | 4.5 | 10.7 | 1.79 | 6.4 | 19.1 | 6.9% |
| | 17* | 0.257 | 569 | 9601 | 5.2 | 10.9 | 1.06 | 3.8 | 11.6 | 4.1% |
| | 18* | 0.283 | 750 | 12771 | 4.4 | 12.7 | 0.83 | 2.9 | 10.5 | 3.7% |
| | | | | | | | | | | |
| Mean | | 0.284 | 579 | 8513 | 4.9 | 11.6 | 1.35 | 4.7 | 15.4 | 5.4% |
| SD | | 0.004 | 155 | 2968 | 0.4 | 0.8 | 0.45 | 1.6 | 4.7 | 1.7% |
| N | | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |

## Pharmacokinetics of DRX008A Following Intravenous Administration to Male and Female Sprague Dawley Rats (TKT-1U0-00-007)

**Methods:** The objective of this study was to investigate the PK of DRX008A following a single IV administration to male and female SD rats. Three animals per sex were placed into Group 1 and five animals per sex were placed into Groups 2, 3, and 4. Animals in Group 1 were dosed with the vehicle (50 mM Na Citrate, pH 6.0, 0.01% Tween-20, 3% Sorbitol) at a dose volume of 1.5 mL/kg. Animals in Groups 2, 3, and 4 were dosed with DRX008A at a dose volume of 1.5, 1.5 and 7.5 mL/kg, respectively, and at doses of 0.85, 3.4 and 17 mg/kg, respectively. Blood samples were collected at the following time points: pre-dose, 5, 15, 30 minutes and 1, 1.5, 2, 3, 4, 6, 8, and 24 hours postdose from the animals in Groups 1, 2, 3 and 4.

**Results**: Pharmacokinetic profiles were similar between male and female rats. Group mean elimination half-lives, $T_{1/2}$ ranged from 2.5 to 7 8 minutes, and DRX008A was eliminated from serum by 1-2 hours after dosing for all animals. Cmax was dose proportional for both male and female rats, but AUC was not dose proportional and increased approximately 9-fold compared to the 5-fold increase in dose from 3.4 to 17 mg/kg. Mean serum clearance decreased from 7.0 to 4.2 mL/min/kg at 3.4 and 17 mg/kg, respectively. Mean apparent volume of distribution ranged from 3.4% to 5.5% (3.4 and 17 mg/kg dose groups). The following table (from page 51 of the study report) shows the PK data from in male SD rats.

**Table 1: Single Dose IV Pharmacokinetic Parameters in Sprague-Dawley Rats Males**

| Group No. | Animal No. | Body Weight (Kg) | Cmax (U/mL) | AUC (min* U/mL) | $T_{1/2}$ (λz) (min) | MRT (min) | Cl (mL/min) | Cl (mL/min/kg) | $V_{ss}$ (mL) | $V_{ss}$ (% BW) |
|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 7 | 0.307 | 69 | 267 | 2.0 | 1.9 | 1.65 | 5.4 | 3.1 | 1.0% |
| | 8 | 0.314 | 121 | 448 | 3.4 | 1.8 | 1.00 | 3.2 | 1.8 | 0.6% |
| 0.85 mg/kg | 9 | 0.323 | 74 | 268 | 1.8 | 1.6 | 1.71 | 5.3 | 2.8 | 0.9% |
| | 10 | 0.306 | 88 | 329 | 3.6 | 1.9 | 1.26 | 4.1 | 2.4 | 0.8% |
| | 11 | 0.306 | 88 | 314 | 1.8 | 1.7 | 1.41 | 4.6 | 2.3 | 0.8% |
| | | | | | | | | | | |
| Mean | | 0.311 | 88 | 325 | 2.5 | 1.8 | 1.41 | 4.5 | 2.5 | 0.8% |
| SD | | 0.007 | 20.3 | 74 | 0.89 | 0.1 | 0.29 | 0.9 | 0 | 0.2% |
| N | | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| | | | | | | | | | | |
| 3 | 17 | 0.310 | 144 | 1245 | 4.3 | 6.3 | 1.43 | 4.6 | 9.0 | 2.9% |
| | 18 | 0.317 | 157 | 1282 | 3.3 | 5.7 | 1.40 | 4.4 | 8.1 | 2.5% |
| 3.4 mg/kg | 19 | 0.311 | 93 | 949 | 3.9 | 7.0 | 1.90 | 6.1 | 13.2 | 4.2% |
| | 20 | 0.309 | 130 | 1073 | 3.1 | 5.8 | 1.63 | 5.3 | 9.4 | 3.0% |
| | 21 | 0.317 | 91 | 1173 | 4.2 | 6.2 | 1.72 | 5.4 | 14.0 | 4.4% |
| | | | | | | | | | | |
| Mean | | 0.313 | 123 | 1144 | 3.7 | 6.6 | 1.62 | 5.2 | 10.7 | 3.4% |
| SD | | 0.004 | 29.9 | 135 | 0.52 | 1.0 | 0.21 | 0.7 | 3 | 0.8% |
| N | | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| | | | | | | | | | | |
| 4 | 28 | 0.303 | 273 | 5813 | 7.1 | 14.6 | 1.53 | 5.0 | 22.4 | 7.4% |
| | 29 | 0.301 | 523 | 7408 | 9.3 | 13.9 | 1.19 | 4.0 | 16.6 | 5.5% |
| 17.0 mg/kg | 30 | 0.316 | 540 | 7234 | 7.0 | 12.0 | 1.29 | 4.1 | 15.5 | 4.9% |
| | 31 | 0.308 | 492 | 12210 | 7.8 | 17.7 | 0.74 | 2.4 | 13.2 | 4.3% |
| | | | | | | | | | | |
| Mean | | 0.307 | 457 | 8166 | 7.8 | 14.6 | 1.19 | 3.9 | 16.9 | 5.5% |
| SD | | 0.007 | 124 | 2789 | 1.1[4] | 2.4 | 0.33 | 1.1 | 3.9 | 1.3% |
| N | | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |

**Pharmacokinetics of DRX008A Following Intravenous Administration to Male Beagle Dogs (690-1U0-02-346)**

**Methods**: In this study, DRX008A was evaluated in a single dose IV pharmacokinetic study in male Beagle dogs. Animals received a bolus IV dose at 0.83 or 3.3 mg/kg ( n = 3/dose). Serum samples were collected from each animal serially over 3 hours and analyzed for DRX008A concentration using an enzyme activity assay.

**Results**: At 0.83 mg/kg, the elimination half-life was approximately 4 minutes, and administered DRX008A was completely eliminated from all low dose animals by 45 minutes after dosing. At 3.3 mg/kg, the $T_{1/2}$ was also approximately 4 minutes, and DRX008A was completely eliminated from serum by 75 minutes after dosing. Cmax was nearly dose proportional and increased approximately 5-fold compared to the 4-fold increase in dose. AUC, however, was not dose-proportional and increased approximately 10-fold compared to the 4-fold increase in dose. Mean serum clearance decreased from 22.3 to 8.9 mL/min/kg as the dose increased from 0.83 to 3.3 mg/kg. Mean apparent volume of distribution values were 7.9% to 11.5% at 0.83 and 3.3 mg/kg, respectively. The following table (from page 7 of the study report) shows the PK parameters in Beagle dogs.

| Group No. | Animal No. | Body Weight (kg) | Cmax (U/mL) | AUC (min* U/mL) | $T_{1/2}$ (el) (min) | MRT (min) | Cl (mL/min) | Cl (mL/min/kg) | $V_{ss}$ (mL) | $V_{ss}$ (% BW) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1001 | 7.6 | 19.8 | 90.5 | 3.2 | 4.6 | 153 | 20.2 | 699 | 9.2% |
| | 1002 | 10.1 | 18.2 | 99.5 | 3.8 | 5.5 | 186 | 18.4 | 1015 | 10.1% |
| 0.83 mg/kg | 1003 | 10.2 | 12.0 | 64.7 | 3.7 | 5.4 | 289 | 28.3 | 1552 | 15.2% |
| | | | | | | | | | | |
| Mean | | 9.3 | 16.7 | 84.9 | 3.6 | 5.1 | 209 | 22.3 | 1089 | 11.5% |
| SD | | 1.5 | 4.1 | 18.1 | 0.34 | 0.5 | 71 | 5.3 | 431 | 3.3% |
| N | | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | | | | | | | | | | |
| Group No. | Animal No. | Body Weight (kg) | Cmax (U/mL) | AUC (min* U/mL) | $T_{1/2}$ ($\lambda$z) (min) | MRT (min) | Cl (mL/min) | Cl (mL/min/kg) | $V_{ss}$ (mL) | $V_{ss}$ (% BW) |
| 2 | 2001 | 10.6 | 100 | 1029 | 3.9 | 9.5 | 74.6 | 7.0 | 711 | 6.7% |
| | 2002 | 10.7 | 94.2 | 855 | 3.9 | 8.6 | 91.0 | 8.5 | 783 | 7.3% |
| 3.3 mg/kg | 2003 | 10.2 | 62.9 | 616 | 4.0 | 8.7 | 114 | 11.2 | 994 | 9.7% |
| | | | | | | | | | | |
| Mean | | 10.5 | 85.8 | 833 | 3.9 | 9.0 | 93 | 8.9 | 829 | 7.9% |
| SD | | 0.3 | 20.0 | 207 | 0.1 | 0.5 | 20 | 2.1 | 147 | 1.6% |
| N | | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | | | | | | | | | | |

### 2.6.4.4 Distribution

**Tissue Distribution of Radioactivity Following Single Intravenous Injection of $^{125}$I-DRX008A in the Male Sprague Dawley Rat (TKT-1U0-01-008)**

**Methods:** The purpose of this study was to determine the tissue distribution of $^{125}$I-DRX008A following single IV injection in male SD rats. In this study, a total of 40 male rats (approximately 6-7.5 weeks of age with body weights ranging from 211 to 237 g) were administered $^{125}$I-DRX008A (specific activity = 1.11 x $10^9$ dpm/mg of labeled protein) by IV injection at the following dose levels: Group 2 (20 males) 1 mg/kg (4.4 µCi/animal) and Group 3 (20 males) at 10 mg/kg (4.3 µCi/animal). A single control animal (Group 1) was treated with the vehicle (50mM Citrate, 3% Sorbitol, 0.01% Tween-20, pH 6.0) only. Terminal blood, plasma and tissues were collected from 5 rats/time point from Groups 2 and 3 at 20 minutes, 1, 2 and 4 h postdose. Urine was also collected at 20 minutes, 1, 2 and 4 h postdose from 5 animals/time point from Groups 2 and 3. The radioactivity concentration and content of the collected samples were determined by liquid scintillation spectroscopy (LSC). The following table (from page 13 of the study report) shows the study design.

The animals were assigned to the study groups as follows:

| Group No. | Test Article | Target Dose Level (mg/kg) | Animal Number |
|---|---|---|---|
| 1 | Vehicle | 0 | 1001 |
| 2 | $^{125}$I-DRX008A | 1 | 2001-2020 |
| 3 | $^{125}$I-DRX008A | 10 | 3001-3020 |

**Results**: Quantifiable levels of radioactivity were observed up to 4 h postdose in both the plasma and blood in both groups treated with DRX008A. The highest radioactivity concentrations were observed at 20 min postdose for both groups. For both plasma and blood, there was an increase in radioactivity concentration with dose level at all time points. This increase was not always dose proportional. Blood to plasma radioactivity ratios ranged from approximately 0.6 to 0.8 at all time points for the low dose group and from 0.5 to 0.7 at all time points for the high dose group, indicating that radioactivity was more associated with plasma than with blood at most time points for both groups.

The highest radioactivity concentrations for both the low and high dose groups were observed in the thyroid gland, lungs, liver, kidneys, spleen, bone marrow and adrenal glands. In the high dose group, high amounts of radioactivity were also observed in the heart and large and small intestines. The radioactivity concentration in most collected tissues remained relatively constant at the 20 minute and 1 h time points. However for some tissues, the radioactivity concentration either gradually decreased over the course of

the study or peaked at one of the later time points. The tissue to plasma radioactivity ratios observed for both the low and high dose groups in the thyroid gland, lungs, liver, spleen, kidneys, bone marrow and small intestine indicated that radioactivity was cleared more rapidly from plasma than from the tissues. Tissue radioactivity (as percent of dose) was mainly associated with the liver, followed by the small intestine, lungs and kidneys. The amount of radioactivity recovered in the urine of animals at the low dose was almost double that recovered from the high dose group. The recoveries were as follows: 20 min, 0 %; 1 h, 0-3%, 2 h, 4-7%; and 4 h, 13-20%. The cumulative recovery of radioactivity at 4 h postdose was approximately 29% for the low dose group and 17% for the high dose group. The following Table (from page 29 of the study report) shows the mean concentration of radioactivity in the blood, plasma and tissues.

TABLE NO. 4 Mean Concentration of Radioactivity in Blood, Plasma and Tissues of Male Sprague-Dawley Rats Following Single Intravenous Injection of $^{125}$I-DRX008A PROJECT NO. 45789

**Group 2: At a Mean Dose of 0.99 mg/kg**

| Sample | Concentration of Radioactivity, µg eq/g [a] | | | |
|---|---|---|---|---|
| | 20 min | 1 h | 2 h | 4 h |
| Blood [b] | 0.782 ± 0.053 | 0.632 ± 0.049 | 0.362 ± 0.049 | 0.482 ± 0.135 |
| Plasma [b] | 1.245 ± 0.082 | 0.942 ± 0.068 | 0.475 ± 0.061 | 0.730 ± 0.243 |
| Adipose Tissue (Epididymal) | 0.056 ± 0.016 | 0.068 ± 0.013 | n/a | n/a |
| Adrenal Glands | 2.317 ± 0.544 | 0.956 ± 0.206 | n/a | n/a |
| Bone (Femur) | 1.397 ± 0.137 | 0.795 ± 0.066 | 0.497 ± 0.030 | 0.324 ± 0.108 |
| Bone Marrow | 5.739 ± 0.772 | 2.872 ± 0.314 | 1.766 ± 0.100 | 1.142 ± 0.442 |
| Brain | 0.011 ± 0.007 | 0.017 ± 0.004 | n/a | n/a |
| Epididymides | 0.131 ± 0.039 | 0.225 ± 0.031 | n/a | n/a |
| Eyes | 0.087 ± 0.019 | 0.108 ± 0.027 | n/a | n/a |
| Harderian Glands | 0.143 ± 0.027 | 0.162 ± 0.036 | n/a | n/a |
| Heart | 0.304 ± 0.111 | 0.251 ± 0.071 | 0.139 ± 0.017 | 0.150 ± 0.055 |
| Kidneys | 3.589 ± 0.449 | 5.531 ± 0.520 | 1.712 ± 0.098 | 1.679 ± 0.271 |
| Large Intestine | 0.097 ± 0.025 | 0.134 ± 0.025 | 0.077 ± 0.010 | 1.302 ± 0.466 |
| Liver | 16.167 ± 1.068 | 7.027 ± 0.462 | 3.451 ± 0.340 | 2.058 ± 0.615 |
| Lungs | 10.203 ± 2.204 | 9.772 ± 4.251 | 8.897 ± 2.764 | 7.739 ± 7.387 |
| Lymph Nodes (Mesenteric) | 0.272 ± 0.153 | 0.361 ± 0.067 | n/a | n/a |
| Muscle (Leg Adductor) | 0.064 ± 0.010 | 0.074 ± 0.007 | n/a | n/a |
| Pancreas | 0.235 ± 0.023 | 0.414 ± 0.053 | n/a | n/a |
| Pituitary Gland | 0.352 ± 0.077 | 0.288 ± 0.051 | n/a | n/a |
| Prostate Gland | 0.134 ± 0.034 | 0.200 ± 0.033 | n/a | n/a |

[a] Mean ± S.D., N=5, N=4 (Heart 20 min). The values for the individual animals are presented in Appendices 2, 3 and 4.
[b] Plasma and blood concentration units are µg eq/mL.
n/a Not applicable.

Mean blood and tissue to plasma radioactivity ratios are shown in the following Table (from page 33 of the study report).

TABLE NO. 5 Mean Blood and Tissue to Plasma Radioactivity Ratios of Male Sprague-Dawley Rats Following Single Intravenous Injection of $^{125}$I-DRX008A PROJECT NO. 45789

**Group 2: Dose 0.99 mg/kg**

| Sample | Tissue/Blood to Plasma Ratio [a] | | | |
|---|---|---|---|---|
| | 20 min | 1 h | 2 h | 4 h |
| Blood | 0.634 ± 0.012 | 0.676 ± 0.008 | 0.769 ± 0.008 | 0.677 ± 0.043 |
| Adipose Tissue (Epididymal) | 0.048 ± 0.016 | 0.078 ± 0.020 | n/a | n/a |
| Adrenal Glands | 1.975 ± 0.462 | 1.077 ± 0.223 | n/a | n/a |
| Bone (Femur) | 1.199 ± 0.192 | 0.894 ± 0.030 | 1.120 ± 0.102 | 0.476 ± 0.130 |
| Bone Marrow | 4.894 ± 0.654 | 3.232 ± 0.271 | 3.994 ± 0.507 | 1.634 ± 0.480 |
| Brain | 0.009 ± 0.006 | 0.020 ± 0.005 | n/a | n/a |
| Epididymides | 0.113 ± 0.036 | 0.253 ± 0.023 | n/a | n/a |
| Eyes | 0.074 ± 0.018 | 0.122 ± 0.028 | n/a | n/a |
| Harderian Glands | 0.122 ± 0.028 | 0.183 ± 0.042 | n/a | n/a |
| Heart | 0.259 ± 0.098 | 0.282 ± 0.077 | 0.315 ± 0.056 | 0.216 ± 0.028 |
| Kidneys | 3.084 ± 0.544 | 6.222 ± 0.395 | 3.875 ± 0.548 | 2.608 ± 0.752 |
| Liver | 13.828 ± 1.423 | 7.935 ± 0.749 | 7.812 ± 1.215 | 3.022 ± 0.399 |
| Lungs | 8.708 ± 1.902 | 10.946 ± 4.567 | 19.944 ± 5.929 | 10.566 ± 9.008 |
| Lymph Nodes (Mesenteric) | 0.234 ± 0.136 | 0.406 ± 0.068 | n/a | n/a |
| Muscle (Leg Adductor) | 0.055 ± 0.013 | 0.084 ± 0.009 | n/a | n/a |
| Pancreas | 0.202 ± 0.030 | 0.466 ± 0.052 | n/a | n/a |
| Pituitary Gland | 0.299 ± 0.061 | 0.324 ± 0.045 | n/a | n/a |
| Prostate Gland | 0.114 ± 0.028 | 0.228 ± 0.049 | n/a | n/a |
| Salivary Glands | 0.171 ± 0.029 | 0.264 ± 0.015 | n/a | n/a |

[a] Mean ± S.D., N=5, N=4 (Heart 20 min). The values for individual animals are presented in Appendices 3 and 4.
n/a Not applicable: no sample collected as per protocol.

## Tissue Distribution of Radioactivity Following Single Intravenous Injection of $^{125}$I-DRX008A in the Sprague Dawley Rat (TKT-1U0-01-001)

**Methods:** The previous study was conducted in male rats and the time points for tissue and blood collections were 1, 2 and 4 h postdose. The purpose of this study was to determine the tissue distribution of $^{125}$I-DRX008A following single IV injection in male and female SD rats. In this study, a total of 44 rats (7 weeks of age with body weights ranging from 180 g to 236 g) were administered $^{125}$I-DRX008A (specific activity = 2.78 x $10^9$ cpm/mg protein) by IV injection at the following dose levels: Group 2 (10 males /12 females) 1 mg/kg (3-4 µCi/animal) and Group 3 (10 males/12 females) at 10.2 mg/kg (3 µCi/animal). A single control animal (Group 1) was treated with the vehicle (50 mM Citrate, 3% Sorbitol, 0.01% Tween-20, pH 6.0) only. Blood, plasma and tissues were collected from 4 rats/sex/time point at 4, 24 and 48 h post dose. Urine was collected at 4, 24 and 48 h postdose. The radioactivity concentration and content of the collected samples were determined by LSC. The following table (from page 16 of the study report) shows the study design.

The animals were assigned to the study groups as follows:

| Group No. | Test Article | Target Dose Level mg/kg | Animal Number | |
|---|---|---|---|---|
| | | | males | females |
| 1 | Vehicle | 0 | 1001 | |
| 2 | $^{125}$I-DRX008A | 1.0 | 2001-2012 | 2501-2512 |
| 3 | $^{125}$I-DRX008A | 10.0 | 3001-3012 | 3501-3512 |

**Results**: Quantifiable levels of radioactivity were observed up to 48 h post dose in both plasma and blood for both groups and both sexes. The highest radioactivity concentrations were observed at 4 h post dose for both groups and both sexes. The values decreased at 24 and 48 h post dose. For both plasma and blood, there was an increase in radioactivity concentration with dose level at all time points. However, this increase was not dose proportional and not always consistent between males and females. The rate of clearance decreased with increased dose in males but not in females. The blood to plasma radioactivity ratios ranged from approximately 0.5 to 0.9 at all time points for both groups and sexes. Clearance from the blood was similar to that from the plasma.

The highest radioactivity concentrations were observed in the thyroid gland, liver, spleen, kidneys, large intestine, small intestine, bone marrow and lungs. The radioactivity concentration in most tissues decreased at 24 h post dose compared to 4 h post dose and continued to decrease at 48 h post dose, except for the thyroid gland. The tissue to plasma ratios of these tissues indicated that radioactivity was cleared more rapidly from the plasma than from the tissues. For both dose levels, the highest radioactivity content was observed in the liver (5-11% at 4 h post dose) followed by the small intestine (3-5%), skin (3-5%), large intestine (1- 2%), thyroid gland (1%), kidneys (1%), lungs (0.2-0.8%), spleen (0.2-0.5%) and contents of the gastrointestinal tract [(large intestine (0-32%), small intestine (20-29%) and stomach (0-2%)]. The radioactivity content in most tissues declined at 24 h and 48 h post dose, except for the thyroid gland in which it approximately doubled at 24 h and was sustained at 48 h post dose. The mean recovery of radioactivity in the urine is as follows: 0-4 h, 14-16 %; 4-24 h, 69-86 % and 24-48 h, 4-6 %. The cumulative recovery of radioactivity at 48 h post dose for these groups was approximately 91-106%, indicating that a large proportion of radioactivity was excreted by the renal route. The radioactivity associated with the contents of the small and large intestine also suggested some radioactivity elimination by billiary excretion. The following table (from page 37-40 of the study report) shows the mean concentration of radioactivity in the blood, plasma and tissues.

Mean Concentrations of Radioactivity in Blood, Plasma and Tissues of Male Sprague-Dawley Rats Following Single Intravenous Injection of $^{125}$I-DRX008A

**Group 2: At a Mean Dose of 0.99 mg/kg**

| Sample | Concentration of Radioactivity, µg eq/g [a] | | |
|---|---|---|---|
| | 4 h | 24 h | 48 h |
| Blood [b] | 0.398 ± 0.122 | 0.069 ± 0.006 | 0.023 ± 0.000 |
| Plasma [b] | 0.556 ± 0.198 | 0.098 ± 0.008 | 0.032 ± 0.001 |
| Adrenal Glands | 0.451 ± 0.120 | 0.107 ± 0.049 | -- |
| Bone (Femur + Bone Marrow) | 0.385 ± 0.035 | 0.052 ± 0.003 | 0.018 ± 0.008 |
| Bone Marrow (Femur) | 1.165 ± 0.217 | 0.231 ± 0.043 | -- |
| Brain | 0.013 ± 0.003 | 0.001 ± 0.001 | -- |
| Adipose Tissue (Epididymal) | 0.126 ± 0.042 | 0.050 ± 0.011 | -- |
| Epididymides | 0.113 ± 0.017 | 0.025 ± 0.006 | -- |
| Eyes | 0.058 ± 0.011 | 0.015 ± 0.005 | -- |
| Harderian Gland | 0.109 ± 0.018 | 0.011 ± 0.012 | -- |
| Heart | 0.143 ± 0.039 | 0.024 ± 0.005 | 0.005 ± 0.004 |
| Kidneys | 1.333 ± 0.281 | 0.180 ± 0.009 | 0.063 ± 0.017 |
| Large Intestine | 1.205 ± 0.505 | 0.042 ± 0.010 | -- |
| Liver | 2.706 ± 0.234 | 0.731 ± 0.067 | 0.333 ± 0.080 |
| Lungs | 0.399 ± 0.098 | 1.053 ± 1.215 | 0.063 ± 0.089 |
| Lymph Nodes (Mesenteric) | 0.184 ± 0.062 | 0.030 ± 0.004 | -- |
| Muscle (Leg adductor) | 0.052 ± 0.020 | 0.008 ± 0.007 | -- |
| Pancreas | 0.140 ± 0.050 | 0.007 ± 0.006 | -- |
| Pituitary Gland | 0.024 ± 0.048 | 0.000 ± 0.000 | -- |
| Prostate Gland | 0.504 ± 0.390 | 0.035 ± 0.010 | -- |
| Salivary Glands | 0.141 ± 0.034 | 0.023 ± 0.008 | -- |
| Skin (Whole body) | 0.280 ± 0.150 | 0.030 ± 0.015 | -- |
| Small Intestine | 0.846 ± 0.298 | 0.041 ± 0.013 | -- |
| Spleen | 2.120 ± 0.158 | 0.364 ± 0.062 | 0.178 ± 0.041 |
| Stomach | 0.424 ± 0.047 | 0.000 ± 0.000 | -- |
| Testes | 0.119 ± 0.019 | 0.024 ± 0.001 | 0.007 ± 0.001 |
| Thymus | 0.060 ± 0.014 | 0.005 ± 0.008 | -- |
| Thyroid Gland(and Parathyroid Glands) | 122.668 ± 72.464 | 367.510 ± 67.699 | 291.758 ± 47.872 |

[a] Mean ± SD, N=4 (4h), N=3 (24h and 48h). The values for the individual animals are presented in Appendices 2, 3 and 4.

[b] Plasma and blood concentration units are µg eq/mL.

TABLE NO. 4

PROJECT NO. 45745

Mean Concentrations of Radioactivity in Blood, Plasma and Tissues of Female Sprague-Dawley Rats Following Single Intravenous Injection of $^{125}$I-DRX008A

**Group 2: At a Mean Dose of 1.03 mg/kg**

| Sample | Concentration of Radioactivity, µg eq/g [a] | | |
|---|---|---|---|
| | 4 h | 24 h | 48 h |
| Blood [b] | 0.404 ± 0.274 | 0.065 ± 0.039 | 0.025 ± 0.006 |
| Plasma [b] | 0.600 ± 0.461 | 0.101 ± 0.063 | 0.026 ± 0.002 |
| Adrenal Glands | 0.452 ± 0.090 | 0.082 ± 0.030 | -- |
| Bone (Femur + Bone Marrow) | 0.315 ± 0.013 | 0.046 ± 0.008 | 0.012 ± 0.010 |
| Bone Marrow (Femur) | 0.941 ± 0.482 | 0.173 ± 0.123 | -- |
| Brain | 0.011 ± 0.006 | 0.001 ± 0.003 | -- |
| Eyes | 0.054 ± 0.018 | 0.010 ± 0.004 | -- |
| Harderian Gland | 0.078 ± 0.006 | 0.008 ± 0.016 | -- |
| Heart | 0.123 ± 0.066 | 0.016 ± 0.008 | 0.000 ± 0.000 |
| Kidneys | 1.207 ± 0.532 | 0.286 ± 0.047 | 0.133 ± 0.038 |
| Large Intestine | 1.253 ± 0.572 | 0.045 ± 0.018 | -- |
| Liver | 2.721 ± 0.222 | 0.501 ± 0.191 | 0.303 ± 0.039 |
| Lungs | 0.602 ± 0.309 | 0.372 ± 0.561 | 0.015 ± 0.003 |
| Lymph Nodes (Mesenteric) | 0.168 ± 0.092 | 0.018 ± 0.013 | -- |
| Muscle (Leg adductor) | 0.034 ± 0.018 | 0.000 ± 0.000 | -- |
| Ovaries | 0.459 ± 0.161 | 0.057 ± 0.004 | 0.025 ± 0.007 |
| Pancreas | 0.102 ± 0.028 | 0.000 ± 0.000 | -- |
| Periovarian Fat | 0.018 ± 0.037 | 0.000 ± 0.000 | -- |
| Pituitary Gland | 0.103 ± 0.069 | 0.000 ± 0.000 | -- |
| Salivary Glands | 0.116 ± 0.057 | 0.013 ± 0.007 | -- |
| Skin (Whole body) | 0.198 ± 0.082 | 0.057 ± 0.026 | -- |
| Small Intestine | 1.046 ± 0.833 | 0.042 ± 0.021 | -- |
| Spleen | 1.882 ± 0.237 | 0.255 ± 0.121 | 0.178 ± 0.007 |
| Stomach | 0.361 ± 0.151 | 0.013 ± 0.003 | -- |
| Thymus | 0.048 ± 0.017 | 0.002 ± 0.004 | -- |
| Thyroid Gland (and Parathyroid Glands) | 83.873 ± 32.515 | 260.322 ± 86.214 | 250.576 ± 76.054 |

[a] Mean ± SD, N=4. The values for the individual animals are presented in Appendices 2, 3 and 4.

[b] Plasma and blood concentration units are µg eq/mL.

Mean Concentrations of Radioactivity in Blood, Plasma and Tissues of Male Sprague-Dawley Rats Following Single Intravenous Injection of $^{125}$I-DRX008A

**Group 3: At a Mean Dose of 10.2 mg/kg**

| Sample | Concentration of Radioactivity, µg eq/g [a] | | |
|---|---|---|---|
| | 4 h | 24 h | 48 h |
| Blood [b] | 4.140 ± 0.715 | 0.836 ± 0.133 | 0.363 ± 0.104 |
| Plasma [b] | 5.849 ± 1.337 | 1.198 ± 0.138 | 0.558 ± 0.180 |
| Adrenal Glands | 3.167 ± 0.513 | 0.685 ± 0.064 | -- |
| Bone (Femur + Bone Marrow) | 2.356 ± 0.126 | 0.433 ± 0.033 | 0.034 ± 0.059 |
| Bone Marrow | 4.694 ± 3.155 | 1.230 ± 0.219 | -- |
| Brain | 0.132 ± 0.013 | 0.009 ± 0.015 | -- |
| Adipose Tissue (Epididymal) | 2.095 ± 0.580 | 0.394 ± 0.101 | -- |
| Epididymides | 0.000 ± 0.000 | 0.000 ± 0.000 | -- |
| Eyes | 0.576 ± 0.023 | 0.230 ± 0.218 | -- |
| Harderian Gland | 0.952 ± 0.089 | 0.000 ± 0.000 | -- |
| Heart | 1.313 ± 0.103 | 0.290 ± 0.036 | 0.000 ± 0.000 |
| Kidneys | 14.351 ± 3.401 | 2.362 ± 0.129 | 0.945 ± 0.128 |
| Large Intestine | 12.964 ± 3.950 | 0.422 ± 0.124 | -- |
| Liver | 12.910 ± 0.869 | 3.210 ± 0.538 | 1.688 ± 0.418 |
| Lungs | 9.766 ± 5.738 | 6.765 ± 6.751 | 3.043 ± 3.684 |
| Lymph Nodes (Mesenteric) | 2.589 ± 0.558 | 0.395 ± 0.060 | -- |
| Muscle (Leg adductor) | 0.427 ± 0.053 | 0.000 ± 0.000 | -- |
| Pancreas | 1.328 ± 0.403 | 0.105 ± 0.113 | -- |
| Pituitary Gland | 0.190 ± 0.380 | 0.000 ± 0.000 | -- |
| Prostate Gland | 1.859 ± 0.851 | 0.705 ± 0.202 | -- |
| Salivary Glands | 1.229 ± 0.100 | 0.249 ± 0.066 | -- |
| Skin (Whole body) | 1.880 ± 0.209 | 0.278 ± 0.240 | -- |
| Small Intestine | 15.771 ± 3.419 | 0.459 ± 0.028 | -- |
| Spleen | 8.080 ± 0.857 | 1.862 ± 0.226 | 0.920 ± 0.250 |
| Stomach | 0.054 ± 0.037 | 0.000 ± 0.000 | -- |
| Testes | 1.220 ± 0.070 | 0.231 ± 0.029 | 0.099 ± 0.031 |
| Thymus | 0.628 ± 0.046 | 0.000 ± 0.000 | -- |
| Thyroid Gland (and Parathyoid Glands) | 1245.835 ± 902.463 | 3318.285 ± 532.388 | 3393.499 ± 1166.225 |

[a] Mean ± SD, N=4 (4h), N=3 (24h and 48h). The values for the individual animals are presented in Appendices 2, 3 and 4.

[b] Plasma and blood concentration units are µg eq/mL.

Mean Concentrations of Radioactivity in Blood, Plasma and Tissues of Female Sprague-Dawley Rats Following Single Intravenous Injection of $^{125}$I-DRX008A

**Group 3: At a Mean Dose of 10.2 mg/kg**

| Sample | Concentration of Radioactivity, µg eq/g [a] | | |
|---|---|---|---|
| | 4 h | 24 h | 48 h |
| Blood [b] | 6.362 ± 1.487 | 0.789 ± 0.029 | 0.311 ± 0.063 |
| Plasma [b] | 9.879 ± 2.672 | 1.190 ± 0.048 | 0.472 ± 0.063 |
| Adrenal Glands | 4.139 ± 0.220 | 0.828 ± 0.141 | -- |
| Bone (Femur + Bone Marrow) | 2.221 ± 0.136 | 0.352 ± 0.051 | 0.000 ± 0.000 |
| Bone Marrow (Femur) | 4.140 ± 2.742 | 1.348 ± 0.262 | -- |
| Brain | 0.158 ± 0.029 | 0.006 ± 0.011 | -- |
| Eyes | 0.650 ± 0.088 | 0.099 ± 0.015 | -- |
| Harderian Gland | 1.279 ± 0.254 | 0.000 ± 0.000 | -- |
| Heart | 1.681 ± 0.528 | 0.253 ± 0.026 | 0.000 ± 0.000 |
| Kidneys | 16.126 ± 4.063 | 3.162 ± 0.467 | 1.319 ± 0.215 |
| Large Intestine | 21.782 ± 4.226 | 0.321 ± 0.111 | -- |
| Liver | 13.910 ± 1.064 | 3.047 ± 0.487 | 1.303 ± 0.077 |
| Lungs | 18.460 ± 19.082 | 12.566 ± 11.512 | 0.643 ± 0.447 |
| Lymph Nodes (Mesenteric) | 2.981 ± 0.837 | 0.444 ± 0.382 | -- |
| Muscle (Leg adductor) | 0.441 ± 0.113 | 0.000 ± 0.000 | -- |
| Ovaries | 4.855 ± 1.014 | 0.704 ± 0.130 | 0.353 ± 0.056 |
| Pancreas | 1.285 ± 0.065 | 0.000 ± 0.000 | -- |
| Periovarian Fat | 0.311 ± 0.359 | 0.000 ± 0.000 | -- |
| Pituitary Gland | 3.872 ± 3.988 | 0.000 ± 0.000 | -- |
| Salivary Glands | 1.351 ± 0.297 | 0.188 ± 0.055 | -- |
| Skin (Whole body) | 1.946 ± 0.293 | 0.368 ± 0.285 | -- |
| Small Intestine | 11.948 ± 6.967 | 0.361 ± 0.062 | -- |
| Spleen | 8.373 ± 0.483 | 1.933 ± 0.106 | 1.005 ± 0.016 |
| Stomach | 0.170 ± 0.024 | 0.000 ± 0.000 | -- |
| Thymus | 0.697 ± 0.141 | 0.000 ± 0.000 | -- |
| Thyroid Gland (and Parathyroid Glands) | 728.205 ± 312.469 | 1586.351 ± 592.998 | 1433.813 ± 412.637 |

[a] Mean ± SD, N=4. The values for the individual animals are presented in Appendices 2, 3 and 4.

[b] Plasma and blood concentration units are µg eq/mL.

Mean blood and tissue to plasma radioactivity ratios are shown in the following Table (from page 41-44 of the study report).

Tissue/Blood to Plasma Radioactivity Ratio in Male Sprague-Dawley Rats
Following Single Intravenous Injection of $^{125}$I-DRX008A

**Group 2: At a Mean Dose of 0.99 mg/kg**

| Sample | Blood and Tissue to Plasma Ratio [a] 4 h | 24 h | 48 h |
|---|---|---|---|
| Blood | 0.729 ± 0.034 | 0.715 ± 0.032 | 0.720 ± 0.021 |
| Adrenal Glands | 0.925 ± 0.392 | 1.159 ± 0.480 | -- |
| Bone (Femur + Bone Marrow) | 0.784 ± 0.193 | 0.567 ± 0.050 | 0.583 ± 0.247 |
| Bone Marrow (Femur) | 2.454 ± 1.026 | 2.511 ± 0.458 | -- |
| Brain | 0.026 ± 0.007 | 0.021 | -- |
| Epididymides | 0.227 ± 0.043 | 0.272 ± 0.057 | -- |
| Adipose Tissue (Epididymal) | 0.269 ± 0.154 | 0.548 ± 0.134 | -- |
| Eyes | 0.119 ± 0.034 | 0.160 ± 0.046 | -- |
| Heart | 0.281 ± 0.066 | 0.254 ± 0.043 | 0.234 |
| Harderian Glands | 0.216 ± 0.036 | 0.183 | -- |
| Kidneys | 2.627 ± 0.358 | 1.969 ± 0.200 | 2.038 ± 0.502 |
| Large Intestine | 2.275 ± 0.247 | 0.456 ± 0.104 | -- |
| Liver | 5.574 ± 1.578 | 7.971 ± 0.928 | 10.862 ± 2.431 |
| Lungs | 0.780 ± 0.093 | 11.007 ± 12.548 | 2.072 ± 2.932 |
| Lymph Nodes (Mesenteric) | 0.365 ± 0.124 | 0.326 ± 0.028 | -- |
| Muscle (Leg adductor) | 0.100 ± 0.006 | 0.132 | -- |
| Pancreas | 0.280 ± 0.107 | 0.103 | -- |
| Pituitary Gland | 0.120 | n/a ± n/a | -- |
| Prostate Gland | 0.863 ± 0.474 | 0.371 ± 0.076 | -- |
| Salivary Glands | 0.279 ± 0.058 | 0.242 ± 0.077 | -- |
| Skin (Whole body) | 0.558 ± 0.325 | 0.336 ± 0.178 | -- |
| Small Intestine | 1.803 ± 0.842 | 0.445 ± 0.116 | -- |
| Spleen | 4.378 ± 1.279 | 3.941 ± 0.423 | 5.793 ± 1.285 |
| Stomach | 0.867 ± 0.229 | n/a ± n/a | -- |
| Testes | 0.236 ± 0.040 | 0.266 ± 0.031 | 0.244 ± 0.024 |
| Thymus | 0.118 ± 0.021 | 0.143 | -- |
| Thyroid Gland (and Parathyroid Glands) | 286.943 ± 212.548 | 3967.149 ± 418.075 | 9505.922 ± 1359.542 |

[a] Mean ± SD, N=4 (4h), N=3 (24h and 48h). The values for the individual animals are presented in Appendices 3 and 4.
n/a Not applicable.

Tissue/Blood to Plasma Radioactivity Ratio in Female Sprague-Dawley Rats
Following Single Intravenous Injection of $^{125}$I-DRX008A

**Group 2: At a Mean Dose of 1.03 mg/kg**

| Sample | Blood and Tissue to Plasma Ratio [a] 4 h | 24 h | 48 h |
|---|---|---|---|
| Blood | 0.712 ± 0.069 | 0.660 ± 0.032 | 0.940 ± 0.193 |
| Adrenal Glands | 1.060 ± 0.553 | 1.121 ± 0.661 | -- |
| Bone (Femur + Bone Marrow) | 0.754 ± 0.367 | 0.635 ± 0.326 | 0.663 ± 0.282 |
| Bone Marrow (Femur) | 2.640 ± 1.756 | 3.507 ± 0.415 | -- |
| Brain | 0.021 ± 0.005 | 0.027 | -- |
| Eyes | 0.128 ± 0.087 | 0.109 ± 0.014 | -- |
| Heart | 0.243 ± 0.063 | 0.171 ± 0.061 | n/a ± n/a |
| Harderian Glands | 0.228 ± 0.037 | 0.167 | -- |
| Kidneys | 2.446 ± 0.569 | 3.620 ± 1.341 | 5.465 ± 1.697 |
| Large Intestine | 2.528 ± 0.813 | 0.510 ± 0.135 | -- |
| Liver | 6.399 ± 2.881 | 7.160 ± 4.068 | 12.363 ± 1.125 |
| Lungs | 1.251 ± 0.643 | 4.789 ± 7.601 | 0.584 ± 0.100 |
| Lymph Nodes (Mesenteric) | 0.344 ± 0.146 | 0.260 ± 0.105 | -- |
| Muscle (Leg adductor) | 0.068 ± 0.016 | n/a ± n/a | -- |
| Ovaries | 0.996 ± 0.415 | 0.758 ± 0.363 | 0.994 ± 0.260 |
| Pancreas | 0.228 ± 0.098 | n/a ± n/a | -- |
| Periovarian Fat | 0.060 | n/a ± n/a | -- |
| Pituitary Gland | 0.312 ± 0.198 | n/a ± n/a | -- |
| Salivary Glands | 0.239 ± 0.084 | 0.142 ± 0.051 | -- |
| Skin (Whole body) | 0.481 ± 0.392 | 0.691 ± 0.403 | -- |
| Small Intestine | 3.099 ± 3.646 | 0.460 ± 0.129 | -- |
| Spleen | 4.494 ± 2.348 | 3.725 ± 2.303 | 7.264 ± 0.485 |
| Stomach | 0.853 ± 0.656 | 0.173 ± 0.084 | -- |
| Thymus | 0.103 ± 0.043 | 0.043 | -- |
| Thyroid Gland (and Parathyroid glands) | 203.271 ± 150.791 | 3147.255 ± 1227.428 | 10213.973 ± 3099.429 |

[a] Mean ± SD, N=4 (4h, 24h and 48h). The values for the individual animals are presented in Appendiices 3 and 4.
n/a Not applicable.

Tissue/Blood to Plasma Radioactivity Ratio in Male Sprague-Dawley Rats
Following Single Intravenous Injection of $^{125}$I-DRX008A

**Group 3: At a Mean Dose of 10.2 mg/kg**

| Sample | Blood and Tissue to Plasma Ratio [a] Point | 24 h | 48 h |
|---|---|---|---|
| Blood | 0.722 ± 0.045 | 0.702 ± 0.031 | 0.662 ± 0.022 |
| Adrenal Glands | 0.612 ± 0.228 | 0.610 ± 0.074 | -- |
| Bone (Femur + Bone Marrow) | 0.448 ± 0.125 | 0.385 ± 0.036 | 0.081 |
| Bone Marrow (Femur) | 1.091 ± 0.310 | 1.111 ± 0.324 | -- |
| Brain | 0.025 ± 0.005 | 0.023 | -- |
| Epididymides | n/a ± n/a | n/a ± n/a | -- |
| Adipose Tissue (Epididymal) | 0.379 ± 0.043 | 0.346 ± 0.061 | -- |
| Eyes | 0.108 ± 0.024 | 0.203 ± 0.190 | -- |
| Heart | 0.246 ± 0.048 | 0.257 ± 0.016 | n/a ± n/a |
| Harderian Glands | 0.178 ± 0.034 | n/a ± n/a | -- |
| Kidneys | 2.608 ± 0.327 | 2.117 ± 0.364 | 1.877 ± 0.443 |
| Large Intestine | 2.369 ± 0.496 | 0.372 ± 0.100 | -- |
| Liver | 2.426 ± 0.541 | 2.831 ± 0.205 | 3.271 ± 0.532 |
| Lungs | 1.872 ± 1.318 | 6.104 ± 5.912 | 7.112 ± 8.966 |
| Lymph Nodes (Mesenteric) | 0.485 ± 0.150 | 0.352 ± 0.060 | -- |
| Muscle (Leg adductor) | 0.080 ± 0.015 | n/a ± n/a | -- |
| Pancreas | 0.262 ± 0.136 | 0.134 | -- |
| Pituitary Gland | 0.124 | n/a ± n/a | -- |
| Prostate Gland | 0.362 ± 0.209 | 0.621 ± 0.156 | -- |
| Salivary Glands | 0.233 ± 0.063 | 0.219 ± 0.046 | -- |
| Skin (Whole body) | 0.356 ± 0.097 | 0.349 | -- |
| Small Intestine | 3.049 ± 1.147 | 0.408 ± 0.026 | -- |
| Spleen | 1.541 ± 0.466 | 1.653 ± 0.178 | 1.771 ± 0.215 |
| Stomach | 0.012 ± 0.002 | n/a ± n/a | -- |
| Testes | 0.232 ± 0.063 | 0.205 ± 0.011 | 0.190 ± 0.014 |
| Thymus | 0.120 ± 0.034 | n/a ± n/a | -- |
| Thyroid Gland (and Parathyroid glands) | 238.071 ± 215.302 | 2964.327 ± 580.727 | 6537.413 ± 1843.013 |

[a] Mean ± SD, N=4 (4h), N=3 (24h and 48h). The values for the individual animals are presented in Appendices 3 and 4.
n/a Not applicable.

Tissue/Blood to Plasma Radioactivity Ratio in Female Sprague-Dawley Rats
Following Single Intravenous Injection of $^{125}$I-DRX008A

**Group 3: At a Mean Dose of 10.2 mg/kg**

| | Blood and Tissue to Plasma Ratio [a] | | |
|---|---|---|---|
| Sample | 4 h | 24 h | 48 h |
| Blood | 0.655 ± 0.026 | 0.670 ± 0.027 | 0.662 ± 0.054 |
| Adrenal Glands | 0.466 ± 0.114 | 0.740 ± 0.141 | -- |
| Bone (Femur + Bone Marrow) | 0.250 ± 0.059 | 0.314 ± 0.047 | n/a ± n/a |
| Bone Marrow (Femur) | 0.450 ± 0.335 | 1.206 ± 0.258 | -- |
| Brain | 0.017 ± 0.002 | 0.021 | -- |
| Eyes | 0.073 ± 0.016 | 0.088 ± 0.011 | -- |
| Heart | 0.180 ± 0.020 | 0.226 ± 0.027 | n/a ± n/a |
| Harderian Glands | 0.142 ± 0.031 | n/a ± n/a | -- |
| Kidneys | 1.760 ± 0.292 | 2.814 ± 0.362 | 2.988 ± 0.473 |
| Large Intestine | 2.396 ± 0.397 | 0.287 ± 0.098 | -- |
| Liver | 1.553 ± 0.307 | 2.725 ± 0.511 | 2.972 ± 0.441 |
| Lungs | 2.335 ± 2.927 | 11.130 ± 9.952 | 1.426 ± 0.941 |
| Lymph Nodes (Mesenteric) | 0.325 ± 0.075 | 0.401 ± 0.353 | -- |
| Muscle (Leg adductor) | 0.048 ± 0.006 | n/a ± n/a | -- |
| Ovaries | 0.533 ± 0.089 | 0.631 ± 0.139 | 0.328 ± 0.052 |
| Pancreas | 0.145 ± 0.037 | n/a ± n/a | -- |
| Periovarian Fat | 0.064 | n/a ± n/a | -- |
| Pituitary Gland | 0.378 ± 0.287 | n/a ± n/a | -- |
| Salivary Glands | 0.147 ± 0.013 | 0.168 ± 0.051 | -- |
| Skin (Whole body) | 0.215 ± 0.036 | 0.434 ± 0.159 | -- |
| Small Intestine | 1.422 ± 0.993 | 0.324 ± 0.066 | -- |
| Spleen | 0.948 ± 0.257 | 1.722 ± 0.055 | 2.292 ± 0.314 |
| Stomach | 0.019 ± 0.003 | n/a ± n/a | -- |
| Thymus | 0.076 ± 0.010 | n/a ± n/a | -- |
| Thyroid Gland (and Parathyroid glands) | 82.980 ± 47.483 | 1418.435 ± 529.912 | 3249.835 ± 929.662 |

[a] Mean ± SD, N=4. The values for the individual animals are presented in Appendices 3 and 4.
n/a Not applicable.

## 2.6.4.5 Metabolism

None

## 2.6.4.6 Excretion

None

## 2.6.4.7 Pharmacokinetic drug interactions

None

## 2.6.4.8 Other Pharmacokinetic Studies

**An *In Vivo* Comparison of [redacted] Reference Standard RE200-002 and Lot RE200-005 in ICR Mice (720-1U0-03-488)** b(4)

**Methods:** In this study female ICR mice were used to compare the biodistribution of two lots of GA-GCB (DRX008A) material manufactured using either a [redacted] process. The lots used were [redacted] reference standard RE200-002 and [redacted] lot RE200-005. The animals (n = 3/group) were treated intravenously with 1.6 mg/kg of GA-GCB or vehicle (sterile phosphate buffered saline/PBS with 0.02% Tween 20, pH 6.5). All mice were sacrificed 20 minutes post injection. Tissue levels of GA-GCB were determined from the liver, kidney, lung, spleen and serum by enzyme-linked immunosorbent assay (ELISA).

**Results:** The concentrations of GA-GCB in the liver and spleen were comparable for protein lots RE200-002 and RE200-005. The amount of administered protein found in the liver (20 minutes post dosing) was 27.5 ± 10.0 % and 41.2 ± 11.5% for RE200-002 and RE200-005, respectively. The concentration of GA-GCB found in the spleen (20 minutes post dosing) was 0.79% and 0.96% for RE200-002 and RE200-005, respectively. These mean values were also comparable. Serum concentrations were also measured 20 minutes after dosing, as a control, to confirm that all mice had received comparable doses. The concentration of GA-GCB in the serum was 0.36% and 0.48% for RE200-002 and RE200-005, respectively. These mean values were comparable.

**Pharmacokinetics of DRX008A Lot 13E-16V-0201 in Rats (690-1U0-02-370)**

**Methods:** The objective of this analysis was to determine PK parameters of DRX008A lot 13E-16V-0201 following a single IV dose in male SD rats. This is a non-GLP (Good Laboratory Practice) study. DRX008A lot 13E-16V-0201 (produced using [redacted] conditions) was evaluated in this study. The animals (n = 3/group) received a single, bolus IV dose at 0.75 or 3.0 mg/kg. Serum samples were collected from each animal serially over 90 minutes for measuring DRX008A concentrations. b(4)

**Results**: At 0.75 mg/kg, the mean $T_{1/2}$ was 2.2 minutes, and the drug was eliminated completely by 25 minutes after dosing. At 3.0 mg/kg, the $T_{1/2}$ was 2.6 minutes and the drug was completely eliminated by 50 minutes after dosing. Cmax was proportional to dose and increased approximately 3-fold compared to the 4-fold increase in dose. AUC, however, was not dose-proportional and increased approximately 6-fold compared to the 4-fold increase in dose. Mean serum clearance decreased from 15.0 to 8.8 mL/min/kg as the dose increased from 0.75 to 3.0 mg/kg. Mean apparent volume of distribution values were 5.8% and 6.6% of body weight at 0.75 and 3.0 mg/kg, respectively. Overall, DRX008A was rapidly eliminated from rat serum with a half-life of approximately 2-3 minutes.

**A Crossover Pharmacokinetic Study of Two Lots of DRX008A Administered by Intravenous Bolus injection to Cynomolgus Monkeys (TKT-1U0-02-001)**

**Methods**: The objective of this study was to perform a comparative PK evaluation of two drug product lots of DRX008A when administered by IV bolus injection on Day 1 and Day 8 in a crossover design in Cynomolgus monkeys. In this study, 16 Cynomolgus monkeys (8/sex, 2.8 to 3.6 years of age for the males and 2.0 to 4.0 years of age for the females) were assigned to two treatment groups as shown in the table below (from page 7 of the study report). The two lots of test article DRX008A were Lot No. RE200-002 and Lot No. RE200-005. The vehicle was 50 mM citrate, pH 6.0, 3.0% sorbitol, 0.01% Tween 20.

| Group No. | Number of M/F | Nominal Dose Level (mg/kg) | Dose Volume (mL/kg) | Nominal Dose Solution Conc. (mg/mL) | Dose Administration (Lot Numbers) Day 1 | Day 8 |
|---|---|---|---|---|---|---|
| 1 | 4/4 | 2.0 | 1 | 2 | IV (RE200-002) | IV (RE200-005) |
| 2 | 4/4 | 2.0 | 1 | 2 | IV (RE200-005) | IV (RE200-002) |

IV = intravenous
M/F = male/female

Each animal received a single IV bolus dose of one lot of DRX008A at 2.0 mg/kg (1 mL/kg) on Day 1 and the other lot of DRX008A at 2.0 mg/kg (1 mL/kg) on Day 8 in a crossover design. Blood samples for PK analysis were collected prior to each dose administration (Days 1 and 8) and at the following time points following dosing: 2, 5, 10, 20, 30, 40, 50, 60, 75, 90, 120, and 150 minutes post dose. The animals were observed twice daily for clinical signs.

**Results**: No treatment-related clinical signs were observed. DRX008A lots RE200-005 and RE200-002 had comparable PK parameters. The following table (from page 63 of the study report) shows the PK parameters.

Table 3: Summary of Pharmacokinetic Parameters in Cynomolgus Monkeys Dosed with 2 Lots of DRX008A

| Lot | | Body Wt (Kg) | $C_{max}$ (U/mL) | AUC (min*U/mL) | $T_{1/2}$(el) (min) | MRT (min) | Cl (mL/min) | Cl (mL/min/kg) | Vss (mL) | Vss % BW |
|---|---|---|---|---|---|---|---|---|---|---|
| RE200-002 | Mean | 3.0 | 93.1 | 714 | 5.3 | 7.6 | 18.50 | 6.30 | 136 | 4.6% |
| | SD | 0.3 | 12.7 | 174 | 1.0 | 1.5 | 4.93 | 1.72 | 17 | 0.6% |
| | CV (%) | 9% | 14% | 24% | 19% | 19% | 27% | 27% | 13% | 13% |
| RE200-005 | Mean | 3.0 | 126.1 | 1049 | 5.8 | 8.4 | 16.99 | 5.70 | 137 | 4.6% |
| | SD | 0.2 | 22.7 | 261 | 1.1 | 1.7 | 4.31 | 1.36 | 24.9 | 0.8% |
| | CV (%) | 8% | 18% | 25% | 20% | 20% | 25% | 24% | 18% | 17% |
| | P value | | N/A | N/A | 0.18 | 0.19 | 0.37 | 0.28 | N/A | N/A |

N/A, not applicable
P values calculated using Student's t-test

### 2.6.4.9 Discussion and Conclusions

Pharmacokinetic properties of Velaglucerase alfa in SD rats and Rhesus monkeys were found to be similar in both sexes. Following a single IV dose, Velaglucerase alfa appeared to be rapidly cleared from the rat, dog, and monkey serum. At the low dose (0.84 mg/kg), $T_{1/2}$ values were approximately 2, 4, and 5 minutes in rats, dogs, and monkeys, respectively. While Cmax was proportional to dose in all three species, AUC was not dose-proportional. At higher doses, 3.4 and 17 mg/kg, $T_{1/2}$ values were increased and clearance values were decreased. In Rhesus monkeys, antidrug antibody was formed at the high dose of 17mg/kg. Tissue distribution studies in rats, using $^{125}$I-labeled Velaglucerase alfa, highest amount of radioactivity was found in the liver by 20 minutes postdose (~70% at 1 mg/kg) with lesser amounts localized in other organs (spleen, kidney and bone/bone marrow). Tissue elimination of Velaglucerase alfa from the liver and spleen appeared to follow a biphasic elimination profile. Initial half-lives were approximately 1 hour in both organs, and elimination half lives ranged from 13 hours (spleen) to 17 hours (liver). A large proportion of the radioactivity was excreted by the renal route over 48 hours post-dose in tissue distribution studies in rats. The radioactivity associated with the contents of the small and large intestines also suggested some radioactivity elimination by billiary excretion.

### 2.6.4.10 Tables and figures to include comparative TK summary

None

## 2.6.5 PHARMACOKINETICS TABULATED SUMMARY

None

## 2.6.6 TOXICOLOGY

### 2.6.6.1 Overall toxicology summary

General Toxicology: Velaglucerase alfa was tested in a series of toxicology studies using bolus IV dose administration. These studies included an acute single-dose, and 3- and 6-month repeat-dose studies in SD rats, and a 6-month study in Rhesus monkeys. The IV route of administration was used in all toxicology studies to conform to the intended clinical route of administration. In addition, reproductive and developmental toxicology studies (Segment I fertility and early embryonic development in rats, Segment II teratology in rats and rabbits and Segment III pre- and postnatal development in rats) were also conducted with Velaglucerase alfa.

Acute toxicology study was conducted in the rat after IV doses of 1, 5, and 20 mg/kg. the maximum nonlethal dose was 20 mg/kg. There were no treatment-related clinical signs. There were no significant treatment-related changes in body weight, hematology or serum chemistry parameters. There were no other treatment-related changes in organ weights. No significant treatment-related macroscopic or histopathological alterations were observed in any tissues.

In a 3-month IV injection toxicology study in rats, animals were treated with DRX008A at 0.85, 3.4 and 17 mg/kg on a bi-weekly basis. There was no mortality. The target organs appeared to be the lung (granulomatous inflammation), liver (small focal cluster of mononuclear cells in sinusoids near a blood vessel) and testes (decrease weight and tubule formation). The no-observed-adverse-effect-level (NOAEL) could not be determined as treatment-related effects were observed at all doses. Anti-DRX008A antibody was formed at all doses.

In a 25-Week IV injection toxicology study in rats, animals were treated with DRX008A at 0.85, 3.4 and 17 mg/kg once every 2 weeks. The target organ could not be identified in the absence of any significant histopathology findings in any organ or tissue.. The NOAEL could not be determined as treatment-related effects were observed at all doses. Anti-DRX008A antibody was formed at all doses.

In a 6-Month IV injection toxicology study in Rhesus monkeys, animals were treated with DRX008A at 0.85, 3.4 and 17 mg/kg on a bi-weekly basis. The target organ could not be identified in the absence of any significant histopathology findings. The NOAEL may be considered as 17 mg/kg. Significant anti-DRX008A antibody was formed in four animals at the high dose at Weeks 4 and 13.

Reproductive Toxicology: In a Segment I male fertility and early embryonic development to implantation study in rats, animals were treated intravenously with GA-GCB every three to four days (twice weekly) beginning 4 weeks prior to pairing and continued through the 3-week mating and 2-week postmating periods at 0 (5% sucrose, 0.01% Polysorbate-20, 50 mM sodium citrate, pH 6.0), 1.5, 5 and 17 mg/kg. There was no mortality. Treatment-related clinical signs included swollen limbs/paw and lips or face

at 5 and 17 mg/kg. There were no significant treatment-related adverse effects on male fertility parameters. However, two males (each at 1.5 mg/kg and 17 mg/kg) had too few sperm to assess motility. These animals also had relatively high incidences of abnormal sperm and low epididymal sperm concentrations. At necropsy, small testes and epididymides were noted for both animals and while each treated male showed positive confirmation of mating to an untreated female, neither resulted in a pregnancy.

In a Segment I female fertility and early embryonic development to implantation study in SD rats, animals were treated intravenously with GA-GCB every three to four days (twice weekly) beginning 14 days prior to pairing and continued through mating and until mating was confirmed at 0 (5% sucrose, 0.01% Polysorbate-20, 50 mM sodium citrate, pH 6.0), 1.5, 5, and 17 mg/kg. There was no mortality. Treatment-related clinical signs included swollen limbs/paw and lips or face at 5 and 17 mg/kg. There were no significant treatment-related effects on female fertility parameters.

In an IV Segment II teratology study in rats, timed pregnant females (n = 25/dose) were treated with GA-GCB at 1.5, 5 and 17 mg/kg/day from gestation day 6 (GD 6) through GD 17. There was no mortality. Treatment-related clinical signs in dams included swollen limbs, paws, face and lips. There was no significant effect on maternal body weight, food consumption and uterine or ovarian parameters. There were no significant treatment-related effects on fetal body weights or fetal sex ratio, fetal external, visceral or skeletal parameters. GA-GCB was not teratogenic in rats at the tested doses under the conditions of the experiment.

In an IV Segment II teratology study in rabbits, timed pregnant females (n = 23/dose) were treated with GA-GCB at 1.5, 10 and 20 mg/kg/day from GD 6 through GD18. There was no treatment-related mortality or clinical signs. There was no significant effect on maternal body weight, food consumption and uterine or ovarian parameters. There were no significant treatment-related effects on fetal body weights or fetal sex ratio, fetal external, visceral or skeletal parameters. GA-GCB was not teratogenic in rabbits at the tested doses under the conditions of the experiment.

In a Segment III pre- and postnatal development study in rats, pregnant females were treated at 1.5, 5 and 17 mg/kg. The test article or the vehicle was administered to all groups by bolus IV injection on GD 6, 9, 13, 16, and 20, and on LD 1, 5, 8, 12, 15, and 19. One F0 female at 1.5 mg/kg died on study Day 33. The cause of death was not apparent from necropsy findings; however, a small amount of red fluid was found in the abdominal cavity. There were no significant treatment-related effects on F0 necropsy parameters. There were no significant treatment-related clinical signs in F1 pups. There were no significant treatment-related effects on F1 growth, survival, and sexual maturation. No significant effect of treatment with GA-GCB was evident from motor activity testing or learning and memory assessments of the F1 pups. There were no significant treatment-related effects on F1 reproductive performance or fertility. Overall, GA-GCB did not cause any significant adverse effect on pre- and postnatal development in rats when tested at 1.5, 5 and 17 mg/kg, IV.

### 2.6.6.2 Single-dose toxicity

**Acute Intravenous Toxicology Study of DRX008A in the Rat**

| Report No. | Testing Laboratory | Species/ Route | Date Started | Date Completed | Batch No. |
|---|---|---|---|---|---|
| 88005TKT-1U0-00-004 | [redacted] | SD Rat | 9/8/2000 | 5/22/2002 | RE200-001 |

b(4)

**GLP Compliance:** Statements of compliance with GLP regulations and the quality assurance unit (QAU) were included.

**Methods:** Rats (10/sex/group) received DRX008A at doses of 0 (50 mM citrate, 3% sorbitol, 0.01% Tween-20), 1, 5, and 20 mg/kg by IV route, the dose volume was 10 mL/kg. Animals were observed daily for 14 days post dose. Parameters evaluated included mortality/moribundity, clinical observation/ physical examination findings, body weight, clinical pathology, organ weight, gross necropsy and histopathological examination. The study design is shown below (from page 12 of the study report).

| Group | Dose Level[a] (mg/kg) | Dose Solution Concentration (mg/mL) | Number of Animals[b] | | Number Sacrificed | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Day 2 | | Day 15 | |
| | | | Male | Female | Male | Female | Male | Female |
| 1 | 0 | 0 | 10 | 10 | 5 | 5 | 5 | 5 |
| 2 | 1 | 0.1 | 10 | 10 | 5 | 5 | 5 | 5 |
| 3 | 5 | 0.5 | 10 | 10 | 5 | 5 | 5 | 5 |
| 4 | 20 | 2.0 | 10 | 10 | 5 | 5 | 5 | 5 |

[a] On Day 14 of study, additional rats (3 rats/sex/group) of approximately the same age and weight as those used initially were dosed intravenously with 0, 1, 5, or 20 mg/kg of DRX008A to obtain additional hematology and coagulation samples due to clotting of an abnormally large number of specimens collected on Day 2.

[b] Protocol listed doses of 1, 5 and 20 mg/kg were based on a test article concentration of 2 mg/mL (enzyme activity assay). Actual doses were 1.1, 5.7, and 23 mg/kg based on the measured extinction coefficient of 1.63 $(mg/mL)^{-1}$ $(cm)^{-1}$ (see Certificate of Analysis in Appendix 8).

**Results:** The maximum nonlethal dose was 20 mg/kg. There were no significant treatment-related clinical signs or body weight changes. Evaluation of serum chemistry, hematology and coagulation parameters did not reveal any changes related to the administration of DRX008A. There were no treatment-related changes in organ weights at either Day 2 or Day 15. No treatment-related microscopic alterations were evident from gross necropsy, and no treatment-related histopathological alterations were identified in any of the tissues examined. The following table summarizes the results of the acute toxicity study in the rat.

Acute Toxicity Study with DRX008A in the Rat

| Species | Route | Dose (mg/kg) | Maximum Nonlethal Dose (mg/kg) | | Minimum Lethal Dose (mg/kg) | | Time to death |
|---|---|---|---|---|---|---|---|
| | | | Male | Female | Male | Female | |
| SD Rats, 10/sex/dose | IV | 1, 5, 20 | 20 | 20 | NA | NA | NA |

NA: Not applicable

**Summary**: In an acute IV toxicology study in rats, animals were treated with DRX008A at 1, 5, and 20 mg/kg. The maximum nonlethal dose was 20 mg/kg. There were no clinical signs that were considered to be treatment related. There were no test article-related changes in body weight, serum chemistry, and hematology parameters. There were no treatment-related changes in organ weights. There were no significant treatment-related macroscopic or histopathological changes in any organ or tissues.

### 2.6.6.3 Repeat-dose toxicity

**Study title: 3-Month Intravenous Injection Toxicology Study in the Rat**

**Key study findings:**

- Animals were treated at 0.85, 3.4 and 17 mg/kg/day on a bi-weekly basis
- There was no mortality
- Treatment-related clinical signs included facial and/or forepaw swelling.
- In males, blood urea nitrogen (BUN) values significantly decreased (89%, 85% and 83% of control at 0.85, 3.4 and 17 mg/kg, respectively) at all doses (0.85, 3.4 and 17 mg/kg). A significantly higher (104% of control at 0.85 mg/kg and 107% of control at 3.4 mg/kg) mean albumin value was observed at 0.85 and 3.4 mg/kg in males.
- Urine pH was significantly higher at 17 mg/kg in males.
- At Week 13, antibodies to DRX008A were detected in 7 of 10 low dose animals, 6 of 10 mid-dose animals, and in 1 of 10 high dose animals.
- There was a decrease (10%) in the relative weight of testes at the high dose.
- Perivascular hemorrhage and/or perivascular chronic active inflammation were seen at the injection site including control. One control and one high dose male animal had a small intravascular focal cluster of chronic inflammatory cells mixed with a fragment of foreign material. Two high dose males and one high dose female had chronic granulomatous inflammation in the lung. A few livers (control and high dose males) had a small focal cluster of mononuclear cells in sinusoids near a blood vessel. One male had tubules in the testes at the high dose.

- The target organs could be the lung (granulomatous inflammation), liver (small focal cluster of mononuclear cells in sinusoids near a blood vessel) and testes (decrease weight and tubule formation).
- The no-observed-adverse-effect-level (NOAEL) could not be determined as treatment-related effects were observed at all doses.
- The number of animal (5/sex/dose) was not adequate. The sponsor should have used at least 10 animals /sex/dose. The basis of dose selection was not provided.

**Study no.**: TKT-1U0-00-005
**Volume #, and page #**: N/A
**Conducting laboratory and location**: [redacted] b(4)

**Date of study initiation**: September 8, 2000
**Date of study completion**: August 29, 2002
**GLP compliance**: A statement of compliance was included.
**QA report**: yes ( X ) no ( )
**Drug, lot #, and % purity**: DRX008A, Lot RE200-001, 99.8%

**Methods:**

Doses: 0 (50 mM citrate, 3% sorbitol, 0.01% Tween-20, pH of 6.0), 0.85, 3.4 and 17 mg/kg, IV, Bi-weekly
Basis of dose selection: Not provided
Species/strain: SD Rats
Number/sex/group or time point: 5/sex/group
Route, formulation, and volume: Intravenous injection, solution, 7.5 mL/kg
Satellite groups used for toxicokinetics or recovery: None
Age: 5 weeks old
Weight: 116-154 g
Study design or methodology: The drug was administered on a bi-weekly basis. The study design is shown below (from page 12 of the study report).

| Group | Dose Level[a] (mg/kg) | Dose Solution Concentration (mg/mL) | Number of Animals | | Animal Numbers | |
|---|---|---|---|---|---|---|
| | | | Male | Female | Male | Female |
| 1 | 0 | 0 | 5 | 5 | 11758-11762 | 11763-11767 |
| 2 | 0.75 | 0.1 | 5 | 5 | 11768-11772 | 11773-11777 |
| 3 | 3.0 | 0.4 | 5 | 5 | 11778-11782 | 11783-11787 |
| 4 | 15.0 | 2.0 | 5 | 5 | 11788-11792 | 11793-11797 |

[a]Protocol listed doses of 0.75, 3.0 and 15 mg/kg were based on a test article concentration of 2 mg/mL (enzyme activity assay). Actual doses were 0.85, 3.4 and 17 mg/kg based on the measured extinction coefficient of 1.63 $(mg/mL)^{-1}$ $(cm)^{-1}$ (see Certificate of Analysis in Appendix 9).

**Observations and times:**

Mortality: Twice daily

Clinical signs: Twice daily

Body weights: Individual body weights were recorded at randomization, prior to dosing, and weekly thereafter.

Food consumption: Not recorded

Hematology: At necropsy

Clinical chemistry: At necropsy

Urinalysis: At necropsy

Antibody analysis: Serum samples were collected one day after the last dose to determine antibody levels to DRXOOSA and to bovine IgG (a low-level impurity from cell culture serum used to grow the cells that produce DRX008A. The final concentration of IgG in lot RE200-001 was O.2 pg/mg of DRXOOSA).

Gross pathology: At necropsy

Organ weights: Following (from page 15 of the study report) organs were weighed from all animals.

adrenals
brain
heart
kidneys
liver
lungs
ovaries/testes
pituitary (post-fixation)
prostate
seminal vesicles
spleen
thymus
thyroid/parathyroids
uterus

Histopathology: The following organs/tissues were examined for histopathology from all control and high dose animals.

| | |
|---|---|
| aorta | testes |
| heart | epididymides |
| salivary gland (mandibular) | prostate |
| tongue | seminal vesicles |
| esophagus | ovaries with oviducts |
| stomach | fallopian tubes |
| duodenum | cervix |
| ileum | vagina |
| jejunum | uterus |
| cecum | adrenals |
| colon | pituitary |
| rectum | thyroid/parathyroids |
| pancreas | skin/mammary glands |
| liver | bone (sternum) and (femur) |
| trachea | skeletal muscle (gastrocnemius) |
| lungs | eyes with optic nerve |
| bone marrow (sternum) | sciatic nerve |
| thymus | brain (3 levels) |
| spleen | spinal cord (3 levels) |
| mandibular lymph nodes | gross lesions |
| mesenteric lymph nodes | injection site(s) |
| kidneys | femorotibial joint |
| urinary bladder with ureters | larynx |

**Results:**

Mortality: All rats survived to the terminal sacrifice.

Clinical signs: Clinical signs were observed at 1-4 hours following the fifth, sixth and seventh doses of DRX008A, which included facial and/or forepaw swelling [in the majority of high-dose males and females (Group 4) and in one mid-dose male (Group 3], This was subsided by 4 hours after dosing.

Body weights: The mean initial (Day 1) and final (Day 85) body weights of the control males were 154 and 395 g, respectively. The mean initial (Day 1) and final (Day 85) body weights of the control females were 116 and 245 g, respectively. There were no significant treatment-related changes.

Hematology: There were no significant treatment-related changes.

Clinical chemistry: Significantly lower mean BUN values were observed in Group 2, 3, and 4 [89%, 85% and 83% of control (19.8 mg/dL) at 0.85, 3.4 and 17 mg/kg, respectively] males, and a significantly higher mean albumin value in Group 2 [104% of control (4.14 g/dL] at 0.85 mg/kg) and (107% of control at 3.4 mg/kg) males.

Urinalysis: The pH was significantly higher in group 4 (17 mg/kg) males. The urine pH data not provided.

Antibody analysis: After 13 weeks of biweekly dosing, antibodies to DRX008A were detected in 7 of 10 low dose animals, 6 of 10 mid-dose animals, and in 1 of 10 high dose

animals. The lower antibody response seen in high dose animals is presumably due to the fact that the highest dose, 17 mg/kg, was sufficient to bind the circulating antibodies and prevent their detection when serum was collected one day after the last dose of DRX008A. None of the rats dosed with DRX008A had antibodies to bovine IgG. None of the animals in the control group had antibodies to either DRX008A or to bovine IgG since the vehicle consisted of 50 mM sodium citrate, 3% sorbitol and 0.01% Tween-20 with a pH of 6.0. The results of the antibody analysis are shown below (from page 127 and 128 of the study report).

**Table 1: Analysis of Sprague Dawley Rat Serum Samples for Antibodies**
**Males**

**Group 1: Vehicle Control**

| Animal | Anti DRX008A | Anti-Bovine IgG |
|---|---|---|
| 11758 | 0.049 | 0.045 |
| 11759 | 0.079 | 0.084 |
| 11760 | 0.059 | 0.057 |
| 11761 | 0.044 | 0.040 |
| 11762 | 0.048 | 0.073 |

**Group 2: 0.75 mg/kg**

| Animal | Anti DRX008A | Anti-IgG |
|---|---|---|
| 11768 | 0.037 | 0.030 |
| 11769 | 0.674* | 0.074 |
| 11770 | 0.245* | 0.059 |
| 11771 | 0.254* | 0.082 |
| 11772 | 2.484* | 0.059 |

**Group 3: 3.0 mg/kg**

| Animal | Anti DRX008A | Anti-IgG |
|---|---|---|
| 11778 | 0.149* | 0.055 |
| 11779 | 0.048 | 0.044 |
| 11780 | 0.837* | 0.040 |
| 11781 | 0.227* | 0.047 |
| 11782 | 0.093 | 0.071 |

**Group 4: 15.0 mg/kg**

| Animal | Anti DRX008A | Anti-IgG |
|---|---|---|
| 11788 | 0.039 | 0.040 |
| 11789 | 0.045 | 0.060 |
| 11790 | 0.051 | 0.050 |
| 11791 | 0.033 | 0.040 |
| 11792 | 0.052 | 0.062 |

Mean absorbance value at 1:50 dilution is reported.

* Positive antibody result

Average baseline absorbance values were 0.037 and 0.049 for detection of anti-DRX008A and anti-bovine IgG antibodies, respectively.

**Table 1: Analysis of Sprague Dawley Rat Serum Samples for Antibodies Females**

**Group 1: Vehicle Control**

| Animal | Anti DRX008A | Anti-Bovine IgG |
|---|---|---|
| 11763 | 0.057 | 0.047 |
| 11764 | 0.039 | 0.076 |
| 11765 | 0.041 | 0.054 |
| 11766 | 0.041 | 0.048 |
| 11767 | 0.055 | 0.061 |

**Group 2: 0.75 mg/kg**

| Animal | Anti DRX008A | Anti-IgG |
|---|---|---|
| 11773 | 0.031 | 0.036 |
| 11774 | 1.786* | 0.061 |
| 11775 | 2.370* | 0.058 |
| 11776 | 0.107 | 0.038 |
| 11777 | 1.641* | 0.101 |

**Group 3: 3.0 mg/kg**

| Animal | Anti DRX008A | Anti-IgG |
|---|---|---|
| 11783 | 0.040 | 0.042 |
| 11784 | 0.759* | 0.049 |
| 11785 | 1.207* | 0.050 |
| 11786 | 0.106 | 0.085 |
| 11787 | 1.182* | 0.073 |

**Group 4: 15.0 mg/kg**

| Animal | Anti DRX008A | Anti-IgG |
|---|---|---|
| 11793 | 0.043 | 0.056 |
| 11794 | 0.033 | 0.050 |
| 11795 | 0.034 | 0.057 |
| 11796 | 0.223* | 0.073 |
| 11797 | 0.076 | 0.078 |

Mean absorbance value at 1:50 dilution is reported.

* Positive antibody result

Average baseline absorbance values were 0.037 and 0.049 for detection of anti-DRX008A and anti-bovine IgG antibodies, respectively.

Gross pathology: There were no significant treatment-related macroscopic observations.

Organ weights: There was a decrease in the relative weight of testes. The decrease in testes weight of approximately 10% in the high-dose males was statistically significant when expressed as testes/body weight ratio. However, there was no histopathological correlate.

Histopathology: Some injection sites reactions in the treated and control animals included perivascular hemorrhage and/or perivascular chronic active inflammation. One control

and one high dose male animal had a small intravascular focal cluster of chronic inflammatory cells mixed with a fragment of foreign material. Two high dose males and one high dose female had chronic granulomatous inflammation in the lung. A few livers (control and high dose males) had a small focal cluster of mononuclear cells in sinusoids near a blood vessel. One male had tubules in the testes at the high dose. The following tables (from page 152-155) show the histopathological changes.

Toxicokinetics: None

**Summary**: In a 3-month IV injection toxicology study in rats, animals were treated with DRX008A at 0.85, 3.4 and 17 mg/kg on a bi-weekly basis. There was no mortality. The target organs could be the lung (granulomatous inflammation), liver (a small focal cluster of mononuclear cells in sinusoids near a blood vessel) and testes (decrease weight and tubule formation). The no-observed-adverse-effect-level (NOAEL) could not be determined as treatment-related effects were observed at all doses. Anti-DRX008A antibody was formed at all doses. It is to be mentioned here the number of animal (5/sex/dose) was not adequate. The sponsor should have used at least 10 animals /sex/dose. The basis of dose selection was also not provided.

**Study title: 25-Week Intravenous Injection Toxicology Study in the Rat with a 4-Week Recovery Period**

**Key study findings:**

- Animals were treated at 0.84, 3.4 and 17 mg/kg once every two weeks
- One high dose male died following dosing on Day 1; the cause of death was considered likely treatment-related. One high dose male was euthanized shortly after dosing on Day 29. One mid dose male died following blood sampling on Day 169. The cause of death was considered to be due to the bleeding procedure.
- Clinical signs were seen at all doses including the control, which included swollen paws, swollen muzzle, red paws and red muzzle. In addition, labored breathing, reduced activity, and/or lateral recumbency were observed less frequently.
- Total protein (high dose), albumin (high dose) and BUN (all doses) were increased in females at Week 25.
- Hematuria was observed in mid and high dose males at Week 24 and in a single high dose male and female at Week 28.
- Antibodies to DRXOOSA (IgG class) were detected in a majority of male and female rats dosed with DRX008A.
- There was no significant gross or histopathology findings.
- The target organ could not be identified in the absence of any significant histopathology findings in any organ or tissue
- The NOAEL could not be determined as treatment-related effects were observed at all doses.

**Study no.**: TKT-1U0-00-006 and Amendment (histopathology for Gr. 2 and 3 animals)

**Volume #, and page #:** N/A
**Conducting laboratory and location:** _____ b(4)
**Date of study initiation:** November 1, 2000
**Date of study completion:** February 25, 2003
**GLP compliance:** A statement of compliance was included.
**QA report:** yes ( X ) no ( )
**Drug, lot #, and % purity:** DRX008A, Lot RE200-001/002, 99.8/99.7%

**Methods:**

Doses: 0 (50 mM citrate, 3% sorbitol, 0.01% Tween-20, pH of 6.0), 0.84, 3.4 and 17 mg/kg, IV, once every two weeks
Basis of dose selection: Not provided
Species/strain: SD Rats
Number/sex/group or time point: 5-10/sex/group
Route, formulation, and volume: Intravenous injection, solution, 7.5 mL/kg
Satellite groups used for toxicokinetics or recovery: None
Age: 6 weeks old
Weight: Male: 152-175 g; Female: 127-148 g
Study design or methodology: The drug was administered once every 2 weeks. The study design is shown below (from page 5 of the study report).

| Group No. | Identification | Dose Level* (mg/kg/dose) | Dose Volume (mL/kg/dose) | No. of Animals Males | Females |
|---|---|---|---|---|---|
| 1 | Vehicle Control | 0 | 7.5 | 10 | 10 |
| 2 | DRX008A | 0.75 | 7.5 | 5 | 5 |
| 3 | DRX008A | 3.0 | 7.5 | 10 | 10 |
| 4 | DRX008A | 15.0 | 7.5 | 10 | 10 |

* Protocol listed doses of 0.75, 3.0 and 15 mg/kg were based on test article concentrations of 2 and 2.3 mg/mL (using an enzyme activity assay) for lot nos. RE200-001 and RE200-002, respectively. Actual doses were 0.84, 3.4 and 17 mg/kg based on the measured extinction coefficient of 1.63 $(mg/mL)^{-1}(cm)^{-1}$ (see Revised Certificates of Analysis in Appendix 18).

**Observations and times:**

Mortality: Twice daily

Clinical signs: Twice daily

Body weights: Individual body weights were recorded at randomization, prior to dosing, and weekly thereafter.

Food consumption: Weekly

Ophthalmoscopy: Once prior to the start of treatment and again during Weeks 13, 23 and 28

Hematology: At necropsy and at the end of the recovery period

Clinical chemistry: At necropsy

Urinalysis: At Week 24 and 28

Antibody analysis: Blood samples for antibody analysis were collected from all Group 2 animals during Weeks 4 and 15 and from all animals in Groups 1 to 4 during Week 8. In addition, all Group 2 animals and those animals in Groups 1, 3 and 4 assigned to the main sacrifice groups (5 animals/sex/group) were bled for antibody analysis during Week 24 and recovery animals in Groups 1, 3 and 4 were bled during Week 26 and again at the end of the 4-week recovery period (Week 29).

Gross pathology: At necropsy

Organ weights: Following (from page 11 of the study report) organs were weighed from all animals.

adrenal glands
brain
heart
kidneys
liver
lungs
ovaries/testes
* pituitary
prostate
seminal vesicles
spleen
thymus
thyroid lobes and parathyroid glands
uterus

Histopathology: The following organs/tissues were examined for histopathology from all animals.

| | |
|---|---|
| | abnormalities |
| a | animal identification |
| | adrenals |
| | aorta (thoracic) |
| ** | bone and marrow (sternum) |
| | brain (cerebrum, cerebellum, midbrain and medulla oblongata) |
| | cecum |
| | colon |
| | duodenum |
| * | epididymides |
| | esophagus |
| * | eyes |
| | femorotibial joint |
| | Harderian glands |
| | heart (including section of aorta) |
| | ileum |
| | injection site(s) |
| | jejunum |
| | kidneys |
| | lacrimal glands |
| | larynx |
| | liver (sample of 2 lobes) |
| ++ | lungs (with bronchi and bronchioles) |
| | lymph nodes (mandibular and mesenteric) |
| | mammary gland (inguinal) |
| * | optic nerves |
| | ovaries |
| | oviducts |
| | pancreas |
| | pituitary |
| | prostate |
| a | rectum |
| | salivary glands |
| | sciatic nerve |
| | seminal vesicles |
| | skeletal muscle |
| | skin (inguinal) |
| | spinal cord (cervical) |
| | spleen |
| | stomach |
| * | testes |
| | thymus |
| | thyroid lobes (and parathyroids) |
| | tongue |
| | trachea |
| | ureters |
| | urinary bladder |
| | uterus (horns, body and cervix) |
| | vagina |

## Results:

Mortality: One high dose male (No. 4006) died following dosing on Day 1 and was subsequently replaced; the cause of death was considered likely treatment-related. One high dose male (No. 4007) was euthanized shortly after dosing on Day 29. Prior to death the animal showed reduced activity, increased muscle tone, swelling of the muzzle and

lateral recumbency. One mid dose male (No. 3003) died following blood sampling on Day 169. The cause of death was considered to be due to the bleeding procedure.

Clinical signs: Clinical signs were seen at all doses including the control, which included swollen paws, swollen muzzle, red paws and red muzzle. In addition, labored breathing, reduced activity, and/or lateral recumbency were observed less frequently. In general, signs occurred shortly following dosing and were transient, with the swelling and reddening being most persistent, but resolving within approximately 24 hours. Similar clinical signs were observed in low dose animals, although the severity and incidence was similar to controls. In a few animals, the response was more severe, and included convulsions in mid dose female Nos. 3510, 3506 and 3507 following dosing on Days 43, 85 and 85, respectively. Swelling and reddening of the muzzle/extremities appeared indicative of a histamine-type reaction, however; blood sampling for histamine analysis conducted following dosing on Day 169 did not confirm this hypothesis. Summary of clinical observations are shown in the following table (from page 14 of the study report, table shows the target doses).

Text Table 1: Summary of Significant Clinical Observations Following Dosing

| Observation | Dose No. | Dose Group | | | |
|---|---|---|---|---|---|
| | | Vehicle | 0.75 mg/kg | 3 mg/kg | 15 mg/kg |
| Swollen paws, swollen muzzle, red paws, or red muzzle | 1 | - | - | - | 20/20 |
| | 2 | - | - | - | 20/20 |
| | 3 | 4/20 | - | 5/20 | 20/20 |
| | 4 | - | 1/10 | 7/20 | 19/19 |
| | 5 | 2/20 | 1/10 | 6/20 | 19/19 |
| | 6 | - | 1/10 | 4/20 | 18/19 |
| | 7 | 8/20 | 5/10 | 12/20 | 19/19 |
| | 8 | 7/20 | 4/10 | 8/20 | 18/19 |
| | 9 | 5/20 | 5/10 | 9/20 | 18/19 |
| | 10 | 4/20 | 5/10 | 8/20 | 19/19 |
| | 11 | 4/20 | 6/10 | 11/20 | 18/19 |
| | 12 | 15/20 | 9/10 | 17/20 | 19/19 |
| | 13 | 15/20 | 9/10 | 19/20 | 19/19 |

Observations for males and females are combined

Body weights: The mean initial (Week -1) and final (Week 28) body weights of the control males were 162.6 and 625.4 g, respectively. The mean initial (Week -1) and final (Week 28) body weights of the control females were 139.1 and 339.8 g, respectively. There were no significant treatment-related changes.

Food Consumption: The mean initial (Week -1) and final (Week 28) food consumption of the control males were 13.94 and 22.8 g/animal/day, respectively. The mean initial (Week -1) and final (Week 28) food consumption of the control females were 12.42 and 16.88 g/animal/day, respectively. There were no significant treatment-related changes.

Ophthalmoscopy: There were no significant treatment-related changes.

Hematology: There were no significant treatment-related changes.

Clinical chemistry: Total protein (115% of control at high dose, control = 6.8 g/dL), albumin (115% of control at high dose, control = 4.7 g/dL) and BUN (122%, 113% and 115% of control at 0.75, 3.0 and 15.0 mg/kg, respectively) were increased in females at Week 25. There were no other significant treatment-related changes.

Urinalysis: A moderate to large amount of blood was detected in the urine (hematuria) of several mid and high dose males at Week 24 and in a single high dose male and female at Week 28. There were no treatment-related effects on urinalysis parameters.

Antibody analysis: Antibodies to DRXOOSA (IgG class) were detected in a majority of male and female rats dosed with DRX008A. The maximum number of antibody positive rats was seen at Week 8 (6 of 10 animals at low dose, 15 of 20 animals at mid dose and 14 of 19 animals at high dose). The results of the antibody analysis are shown below (from page 652 of the study report).

- J11 -

APPENDIX NO. 18 PROJECT NO. 56879

**Table 2: Incidence of Anti-DRX008A Antibody Response in Rats**

| Group (mg/kg) | Study Week | | | | |
|---|---|---|---|---|---|
| | 4 | 8 | 15 | 24/26 | 29* |
| Males | | | | | |
| Vehicle | - | 0/10 | - | 1/10 | 1/5 |
| 0.84 mg/kg | 1/5 | 3/5 | 3/5 | 2/5 | NA |
| 3.4 mg/kg | - | 6/10 | - | 4/10 | 0/5 |
| 17 mg/kg | - | 8/9 | - | 4/9 | 3/4 |
| Females | | | | | |
| Vehicle | - | 0/10 | - | 0/10 | 0/5 |
| 0.84 mg/kg | 2/5 | 3/5 | 3/5 | 3/5 | NA |
| 3.4 mg/kg | - | 9/10 | - | 9/10 | 4/5 |
| 17 mg/kg | - | 6/10 | - | 6/10 | 4/5 |

- not determined

NA, not applicable (animals previously sacrificed)

* Vehicle, mid and high dose groups had 5 animals/sex/group in recovery groups (sacrificed during week 29, 4 weeks after last dose).

Gross pathology: There were no significant treatment-related macroscopic observations.

Organ weights: There were no significant treatment-related changes.

Histopathology: There were no significant treatment-related microscopic observations.

**Summary**: In a 25-Week IV injection toxicology study in rats, animals were treated with DRX008A at 0.84, 3.4 and 17 mg/kg once every 2 weeks. Mortality was observed at mid and high doses. The target organ could not be identified in the absence of any significant histopathology findings in any organ or tissue. The NOAEL could not be determined as treatment-related effects were observed at all doses. Ant-DRX008A antibody was formed at all doses.

**Study title: 6-Month Intravenous Injection Toxicology Study in the Rhesus Monkey with a 4-Week Recovery Period**

**Key study findings:**

- Animals were tested at 0.84, 3.4 and 17 mg/kg on a bi-weekly basis
- There was no mortality
- There was no significant treatment-related clinical signs
- There were no significant treatment-related effects on body weight, ophthalmology, ECG, hematology or serum chemistry, urinalysis, gross and histopathology parameters.
- Anti-drug antibody was formed at the high dose at Week 4 and 13
- The target organ could not be identified in the absence of any significant histopathology observations in any organ/tissue
- The NOAEL appeared to be 17 mg/kg

**Study no.**: TKT-1U0-00-003 and Amendment (histopathology for Gr. 2 and 3 animals)
**Volume #, and page #**: N/A
**Conducting laboratory and location**: [redacted] b(4)
**Date of study initiation**: September 6, 2000
**Date of study completion**: October 29, 2003
**GLP compliance**: A statement of compliance was included.
**QA report**: yes ( X ) no ( )
**Drug, lot #, and % purity**: DRX008A, Lot RE200-001/002, 99.8/99.7%

**Methods:**

Doses: 0 (50 mM citrate, 3% sorbitol, 0.01% Tween-20, pH of 6.0), 0.84, 3.4 and 17 mg/kg, IV, Bi-weekly
Basis of dose selection: Not provided
Species/strain: Rhesus monkeys
Number/sex/group or time point: 4-5/sex/group
Route, formulation, and volume: Intravenous injection, solution, 7.5 mL/kg
Satellite groups used for toxicokinetics or recovery: None
Age: 2-5 years
Weight: Male: 2.6-3.6 kg; Female: 2.4-3.5 kg

Study design or methodology: The drug was administered on a bi-weekly basis. The study design is shown below (from page 15 of the study report).

***2. Group Assignments and Dose Levels***

| Group No. | Number of Males/Females | Dose Level* (mg/kg/dose) | Dose Vol. (mL/kg) | Dose Solution Conc. (mg/mL) | Number Sacrificed on: Day 86 | Day 170 | Day 198 |
|---|---|---|---|---|---|---|---|
| 1 | 5/5 | 0 (control) | 7.5 | 0 | 2/2 | 2/2 | 1/1 |
| 2 | 4/4 | 0.75 | 7.5 | 0.1 | 2/2 | 2/2 | -- |
| 3 | 5/5 | 3.0 | 7.5 | 0.4 | 2/2 | 2/2 | 1/1 |
| 4 | 5/5 | 15.0 | 7.5 | 2.0 | 2/2 | 2/2 | 1/1 |

*Protocol listed doses of 0.75, 3.0 and 15 mg/kg were based on test article concentrations of 2.0 and 2.3 mg/mL (using an enzyme activity assay) for Lots RE200-001 and RE200-002, respectively. Actual doses were 0.84, 3.4 and 17 mg/kg based on the measured extinction coefficient of 1.63 $(mg/mL)^{-1}$ $(cm)^{-1}$ (see Revised Certificates of Analysis).

**Observations and times:**

Mortality: Twice daily

Clinical signs: Twice daily

Body weights: Individual body weights were recorded at prior to dosing and weekly thereafter.

Food consumption: Daily, qualitatively

Electrocardiography (ECG): ECGs were recorded (using Leads I, II, III, aVR, aVL, and aVF) from all available animals prior to the study and in Weeks 12 and 24 (on a non-dosing day), and from recovery animals in Week 28.

Ophthalmoscopy: An ophthalmic examination was conducted on all animals prior to initiation of the study and in Weeks 12 and 24 and from recovery animals in Week 28.

Hematology: Blood samples for hematology from all animals at pre-study, on Day 2, on Weeks 4, 12, 20, and 24, and from recovery animals in Week 28.

Clinical chemistry: Blood samples for clinical chemistry were collected from all animals at pre-study, on Day 2, on Weeks 4, 12, 20, and 24, and from recovery animals in Week 28.

Urinalysis: Urine samples were collected from all animals at pre-study, in Weeks 4, 12, and 24, and from recovery animals in Week 28.

Antibody analysis: Blood was collected for analysis of antibodies to DRX008A. Blood samples were collected from all surviving animals: pre-study, and on a non-dosing day in Weeks 2, 4, 13, 25, and 29.

Gross pathology: At necropsy

Organ weights: Following (from page 19 of the study report) organs were weighed from all animals.

| Organs Weighed | |
|---|---|
| Adrenals | Brain |
| Epididymides | Heart |
| Kidneys | Liver |
| Lungs | Spleen |
| Pituitary (post fixation) | Thymus |
| Testes | Thyroid with parathyroids |
| Salivary Glands (mandibular) | Prostate |
| Ovaries | |

Histopathology: The following organs/tissues were examined for histopathology from all animals.

| Tissues Collected | |
|---|---|
| Cardiovascular | Urogenital |
| Aorta | Kidneys |
| Heart | Urinary Bladder |
| Digestive | Ureters |
| Salivary Glands | Testes |
| Mandibular | Epididymis |
| Sublingual | Prostate |
| Parotid | Seminal Vesicles |
| Tongue | Ovaries |
| Larynx | Oviducts |
| Esophagus | Uterus |
| Stomach | Cervix |
| Small Intestine | Vagina |
| Duodenum | Endocrine |
| Jejunum | Adrenals |
| Ileum | Pituitary |
| Peyer's Patch (Ileum x 2) | Thyroid/Parathyroids |
| Large Intestine | Skin/Musculoskeletal |
| Cecum | Skin/Mammary Gland |
| Colon | Bone (femoral head) |
| Rectum | Bone (7th rib) |
| Pancreas | Joint, femoral-tibial |
| Liver | Skeletal Muscle (thigh) |
| Gallbladder | Nervous/Special Sense |
| Respiratory | Eyes with optic nerve |
| Trachea | Sciatic Nerve |

| Tissues Collected | |
|---|---|
| Lung | Brain |
| Lymphoid/Hematopoietic | Spinal Cord (thoracic) |
| Bone Marrow (sternum) | Other |
| Thymus | Animal Number Tattoo |
| Spleen | Gross Lesions |
| Lymph Nodes | Injection Site(s)* |
| Mandibular | |
| Mesenteric | |

* Both right and left cephalic and saphenous vein injection sites were collected and evaluated (Note: injections were primarily administered in the right or left saphenous veins. The cephalic veins were rarely used)

Toxicokinetics: Blood was collected from each animal at the following times (from page 18 of the study report):

| | |
|---|---|
| Days 1 and 15: | Pre-dose and 5, 15, 30, 60, 120, 180, 240, 360, 480, 600, 1440 minutes postdose |
| Days 29 and 57: | 30 minutes postdose |
| Day 85: | Pre-dose and 5, 15, 30, 60, 120, 180, 240, 360, 480, 600, 1440 minutes postdose |
| Day 113: | 30 minutes postdose |
| Days 141 and 169: | Group 1: Pre-dose and 30 minutes postdose |
| | Group 2: Pre-dose and 2, 5, 10, 20, 30, 45, 60, 75, 90, 120, and 150 minutes postdose |
| | Group 3: Pre-dose and 2, 5, 15, 30, 45, 60, 75, 90, 120, 150 and 180 minutes postdose |
| | Group 4: Pre-dose and 2, 5, 15, 30, 45, 60, 90, 120, 150, 180, 240 and 300 minutes postdose |

**Results:**

Mortality: None

Clinical signs: Clinical signs included watery-liquid stool during the study for animals from all groups, including the control group. These observations were not considered treatment-related since their incidence was similar across groups.

Body weights: The mean initial (Week 1) and final (Week 24) body weights of the control animals (male and female) were 3.0 and 3.3 kg, respectively. There were no significant treatment-related changes.

Electrocardiogram: There were no significant treatment-related changes.

Ophthalmoscopy: There were no significant treatment-related changes.

Hematology: There were no significant treatment-related changes.

Clinical chemistry: There were no significant treatment-related changes.

Urinalysis: There were no significant treatment-related changes.

Antibody analysis: Four high-dose animals (R14407M, R14443M, R14274F and R14490F) developed antibodies (IgG class) to DRX008A by Week 4 (two animals) or Week 13 (two animals). Anti-drug antibodies were not detected at low and mid dose. The following table (from page 326 and 327 of the study report) shows the antibody analysis data.

**Table 8: Analysis of Rhesus Monkey Serum Samples for Anti-DRX008A Antibodies.**

**Group 1: Vehicle Control**

| Animal | Pre-Study | Week 2 | Week 4 | Week 13 | Week 25 | Week 29 |
|---|---|---|---|---|---|---|
| R14412M | 0.077 | 0.068 | 0.068 | 0.071 | | |
| R14440M | 0.089 | 0.080 | 0.097 | 0.051 | | |
| R14662M | 0.059 | 0.056 | 0.056 | 0.051 | 0.054 | |
| R14885M | 0.062 | 0.179 | 0.096 | 0.068 | 0.068 | 0.062 |
| R98E031M | 0.057 | 0.046 | 0.047 | 0.052 | 0.051 | |
| R14251F | 0.022 | 0.045 | 0.030 | 0.024 | | |
| R14853F | 0.089 | 0.079 | 0.093 | 0.078 | 0.072 | |
| R14465F | 0.113 | 0.154 | 0.135 | 0.121 | | |
| R14467F | 0.062 | 0.065 | 0.068 | 0.058 | 0.060 | 0.067 |
| R14868F | 0.092 | 0.094 | 0.111 | 0.074 | 0.136 | |

**Group 2: 0.84 mg/kg/week**

| Animal | Pre-Study | Week 2 | Week 4 | Week 13 | Week 25 | Week 29 |
|---|---|---|---|---|---|---|
| R14428M | 0.030 | 0.040 | 0.040 | 0.025 | | |
| R14429M | 0.050 | 0.080 | 0.065 | 0.064 | 0.065 | |
| R14430M | 0.208 | 0.142 | 0.149 | 0.112 | | |
| R98E081M | 0.093 | 0.100 | 0.111 | 0.105 | 0.089 | |
| R14296F | 0.042 | 0.039 | 0.050 | 0.034 | | |
| R14452F | 0.034 | 0.044 | 0.034 | 0.025 | | |
| R14494F | 0.047 | 0.048 | 0.048 | 0.046 | 0.045 | |
| R14852F | 0.080 | 0.073 | 0.076 | 0.080 | 0.071 | |

**Group 3: 3.4 mg/kg/week**

| Animal | Pre-Study | Week 2 | Week 4 | Week 13 | Week 25 | Week 29 |
|---|---|---|---|---|---|---|
| R14232M | 0.033 | 0.032 | 0.035 | 0.030 | | |
| R14415M | 0.036 | 0.035 | 0.050 | 0.032 | | |
| R14423M | 0.048 | 0.047 | 0.049 | 0.045 | 0.044 | |
| R14884M | 0.072 | 0.070 | 0.078 | 0.057 | 0.069 | 0.072 |
| R98E140M | 0.050 | 0.050 | 0.051 | 0.049 | 0.048 | |
| R14290F | 0.025 | 0.024 | 0.016 | 0.012 | | |
| R14453F | 0.027 | 0.020 | 0.022 | 0.025 | | |
| R14755F | 0.064 | 0.074 | 0.063 | 0.062 | 0.057 | |
| R14860F | 0.051 | 0.052 | 0.052 | 0.074 | 0.087 | 0.089 |
| R14872F | 0.060 | 0.058 | 0.053 | 0.081 | 0.084 | |

Mean of 2 values at 1:50 reported.

**Group 4: 17.0 mg/kg/week**

| Animal | Pre-Study | Week 2 | Week 4 | Week 13 | Week 25 | Week 29 |
|---|---|---|---|---|---|---|
| R14407M | 0.023 | 0.019 | 0.021 | 0.124* | | |
| R14443M | 0.024 | 0.044 | 0.439* | 0.129* | | |
| R14875M | 0.064 | 0.059 | 0.068 | 0.096 | 0.094 | 0.094 |
| R14879M | 0.048 | 0.053 | 0.052 | 0.048 | 0.049 | |
| R14888M | 0.055 | 0.055 | 0.054 | 0.054 | 0.051 | |
| R14274F | 0.032 | 0.045 | 0.173* | 0.167* | | |
| R14472F | 0.059 | 0.058 | 0.054 | 0.052 | 0.050 | |
| R14490F | 0.049 | 0.051 | 0.121 | 0.402* | | |
| R14762F | 0.067 | 0.073 | 0.088 | 0.066 | 0.108 | |
| R14869F | 0.062 | 0.062 | 0.062 | 0.099 | 0.125 | 0.131 |

Mean of 2 values at 1:50 reported.

* Positive antibody result

Since antibodies to DRX008A might affect serum clearance of subsequently administered doses of DRX008A, this relationship was examined for the four antibody-positive animals. Mean serum clearance decreased on Day 15 (4.5 mL/min/kg) and Day 85 (6.3 mL/min/kg) from that of Day 1 (9.75 mL/min/kg). It appears that antibody formation in theses animals decreased serum clearance on Day 15 and Day 85.

**Table 6: Effect of Anti-DRX008A Antibodies on Serum Clearance**

| Animal No. | Antibody Response | | | Serum Clearance (mL/min/kg) | | |
|---|---|---|---|---|---|---|
| | Day 10 | Day 25* | Day 86 | 1st Dose (Day 1) | 2nd Dose (Day 15) | 7th Dose (Day 85) |
| R14407M | - | - | + | 9.0 | 4.1 | 7.2 |
| R14443M | - | + | + | 12.9 | 4.8 | 6.1 |
| R14274F | - | + | + | 14.4 | 5.9 | 8.2 |
| R14490F | - | - | + | 2.7 | 3.1 | 3.8 |

* Measured on Day 27 for R14443M

Antibodies were only found in these four high dose animals.

Toxicokinetics: Toxicokinetic (TK) parameters were similar between male and female animals. Mean serum elimination mean half-lives ranged from 5-11 minutes, and the drug was completely removed from the serum of all animals at 2-3 hours after dosing. DRX008A did not appear to accumulate in the serum following biweekly dosing. Cmax was proportional to dose, however, AUC was not dose-proportional. At 0.84 mg/kg, mean AUC values were 143, 127, 138, 133 and 76 min* U/mL on Days 1, 15, 85, 141 and 169, respectively. At 3.4 mg/kg, mean AUC values were 811, 725, 847, 606 and 438 min* U/mL on Days 1, 15, 85, 141 and 169, respectively. At 17 mg/kg, mean AUC values were 6192, 6553, 6908, 4686 and 2393 min* U/mL on Days 1, 15, 85, 141 and 169, respectively. The drug exposure was decreased on Day 169 when compared to earlier time points. The TK parameters are shown in the following table.

| Parameter | Dose (mg/kg) | Males + Females | | | | | |
|---|---|---|---|---|---|---|---|
| | | Day 1 | Day 15 | Day 85 | Day 141 | Day 169 | |
| Cmax (U/ml) | 0.84 | 22.5 | 18.7 | 22.4 | 21.9 | 14.8 | |
| Cmax (U/ml) | 3.4 | 81.6 | 71.6 | 90.5 | 68.3 | 54.1 | |
| Cmax (U/ml) | 17.0 | 412 | 413 | 495 | 290 | 226 | |
| AUC (U*min/ml) | 0.84 | 143 | 127 | 138 | 133 | 76 | |
| AUC (U*min/ml) | 3.4 | 811 | 725 | 847 | 606 | 438 | |
| AUC (U*min/ml) | 17.0 | 6192 | 6553 | 6908 | 4686 | 2393 | |
| $T_{1/2}$ (min) | 0.84 | 4.7 | 4.7 | 4.4 | 4.1 | 4.0 | |
| $T_{1/2}$ (min) | 3.4 | 7.0 | 7.1 | 6.6 | 6.6 | 5.7 | |
| $T_{1/2}$ (min) | 17.0 | 11.0 | 10.9 | 9.7 | 11.3 | 11.1 | |

Gross pathology: There were no significant treatment-related macroscopic observations.

Organ weights: There were no significant treatment-related changes.

Histopathology: There were no significant treatment-related microscopic observations.

**Summary**: In a 6-Month IV injection toxicology study in Rhesus monkeys, animals were treated with DRX008A at 0.84, 3.4 and 17 mg/kg on a bi-weekly basis. The target organ could not be identified in the absence of any significant histopathology findings. The NOAEL was considered as 17 mg/kg. Anti-DRX008A antibody was formed at the high dose at Week 4 and 13.

### 2.6.6.4 Genetic toxicology

None

### 2.6.6.5 Carcinogenicity

None

### 2.6.6.6 Reproductive and developmental toxicology

## Fertility and Early Embryonic Development

The sponsor submitted final reports of the Segment I fertility and early embryonic development study in male and female rats under IND 61,220 SDN 075 dated March 25, 2008. The following reviews are incorporated below from the pharmacology review of IND 61,220 dated February 6, 2009.

**Study title**: Study for the Effects of GA-GCB on Male Fertility and Early Embryonic Development to Implantation in Rats (Segment I)

**Key study findings**: In a Segment I male fertility and early embryonic development to implantation study in rats, animals (n = 25/dose) were treated intravenously with GA-GCB every three to four days (twice weekly) beginning 4 weeks prior to pairing and continued through the 3-week mating and 2-week postmating periods at 0 [5% sucrose, 0.01% P20 (Polysorbate-20), 50mM sodium citrate, pH 6.0], 1.5, 5.0, and 17 mg/kg/day (2 mL/kg). There was no mortality. Treatment-related clinical signs included swollen limbs/paw and lips or face at 5 and 17 mg/kg. There were no significant treatment-related effects on male fertility parameters. However, two males (each at 1.5 mg/kg and 17 mg/kg) had too few sperm to assess motility. These animals also had relatively high incidences of abnormal sperm and low epididymal sperm concentrations. At necropsy, small testes and epididymides were noted for both animals and while each treated male showed positive confirmation of mating to an untreated female, neither resulted in a pregnancy. GA-GCB appears to cause adverse effects on sperms.

**Study no.**: 1255-003
**Volume #, and page #**: Report No. SHGT-IU0-06-010, EDR
**Conducting laboratory and location**: [redacted] b(4)
**Date of study initiation**: September 15, 2006
**GLP compliance**: A statement of compliance was included
**QA reports**: yes ( X ) no ( )
**Drug, lot #, and % purity**: GA-GCB, DARTEP06-001002, 97-98%

**Methods:**

Doses: 1.5, 5.0 and 17 mg/kg

Species/strain: Sprague Dawley (SD) rats
Number/sex/group: 25 males/dose
Route, formulation, and volume: Intravenous (IV) bolus injection, solution in the vehicle [5% sucrose, 0.01% P20 (Polysorbate-20), 50mM sodium citrate, pH 6.0], 2 mL/kg.
Satellite groups used for toxicokinetics: None
Study design: The test article was administered every three to four days (twice weekly) beginning 4 weeks prior to pairing and continued through the 3-week mating and 2-week postmating periods. Three treatment groups of 25 male rats/group were administered the test article at respective dose levels of 1.5, 5.0, and 17 mg/kg/dose. Control animals received vehicle (5% sucrose, 0.01% P20 [Polysorbate-20], 50mM sodium citrate, pH 6.0). The vehicle or test article was administered to all male animals via intravenous (IV) bolus injection at a dose volume of 2 mL/kg. The study design is shown below (from page 14 of the report).

**Group Assignments**

| Group Number | Dose Level (mg/kg/dose) | Number of Animals | |
|---|---|---|---|
| | | Male | Female[a] |
| 1 | 0 | 25 | 25 |
| 2 | 1.5 | 25 | 25 |
| 3 | 5.0 | 25 | 25 |
| 4 | 17 | 25 | 25 |

[a]Untreated and used only for pairing with the males

Parameters and endpoints evaluated: Observations included clinical signs, food consumption, and body weights. After 4 weeks of treatment, 25 untreated female rats were paired to a control or treated male (1:1) until evidence of mating (sperm and/or vaginal plug) was confirmed [Gestation Day (GD) 0] or for 21 days. Mated females were euthanized on GD13, uterine examinations were conducted, and the location of embryos, resorptions, and the total number of implantations were recorded. The number of corpora lutea on each ovary was also recorded. Sperm evaluations (motility, caudal epididymal sperm concentrations, and morphology) were performed for all males.

**Results:**

Mortality: None

Clinical signs: Treatment-related clinical signs included swollen paws, swollen limbs (fore- and/or hindlimbs), and/or swollen face or lips at the 30 minute postdose at 5 and 17 mg/kg. At 1.5 mg/kg/dose, swollen forelimbs were seen in nine animals on Day 22.

Body weight: The mean initial (Day 1) and final (Day 25) body weights of control animals were 248 g and 380 g, respectively. No significant treatment-related changes were observed.

Food consumption: The mean initial (Week 1-2) and final (Week 4-5) food consumption in control males were 25.8 and 26.4 g/animal/day, respectively. No significant treatment-related changes were observed.

Necropsy: There were no significant treatment-related changes.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): No significant treatment-related effect was observed on the reproductive performance and fertility indices. Male mating indices were 100% in the control and each treated group. Fertility and fecundity indices were 96% in the control, 1.5, and 5.0 mg/kg groups, and 84% in the 17 mg/kg/dose group. The fertility and fecundity index at 17 mg/kg was lower compared to control, however, this was not statistically significant, and was not considered indicative of a treatment-related effect in the absence of a dose-response. One male in each of the control, 1.5, and 5.0 mg/kg groups failed to successfully inseminate a female and three males at 17 mg/kg were also unsuccessful. There were 24, 22, 24, and 25 females with a confirmed mating date in the control, 1.5, 5, and 17 mg/kg, respectively. The mean number of days-to-mating (copulatory interval) ranged from 2.4 to 3.1 days in the treated groups and was comparable to 2.3 days in controls. The following Table (from page 46 of the report) shows the summary of the reproductive and fertility parameters.

Study Number 1255-003
Study for Effects of GA-GCB on Male Fertility and Early Embryonic Development to Implantation in Rats

Table 7 Summary of Reproductive and Fertility Parameters

| Endpoint | 0 mg/kg/dose | 1.5 mg/kg/dose | 5.0 mg/kg/dose | 17 mg/kg/dose |
|---|---|---|---|---|
| No. Males on Study | 25 | 25 | 25 | 25 |
| No. Males Paired | 25 | 25 | 25 | 25 |
| No. Males Mated | 25 | 25 | 25 | 25 |
| No. Males Impregnating a Female | 24 | 24 | 24 | 21 |
| Male Mating Index | 100.0 | 100.0 | 100.0 | 100.0 |
| Male Fertility Index | 96.0 | 96.0 | 96.0 | 84.0 |
| Male Fecundity Index | 96.0 | 96.0 | 96.0 | 84.0 |
| Females with Confirmed Mating Day | 24 | 22 | 24 | 25 |
| Copulatory Interval (Days) | | | | |
| Mean | 2.3 | 2.4 | 3.1 | 3.0 |
| SD | 1.12 | 1.74 | 3.18 | 2.36 |
| N | 24 | 22 | 24 | 25 |

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No.- Number
N- Number of measures used to calculate mean
SD- Standard Deviation

No effect of treatment with GA-GCB was evident from uterine implantation data for females mated to treated males as the mean number of uterine implantations, embryos, resorbing embryos, pre- and postimplantation loss indices in these dams were comparable to data for females mated to control males. There were 23, 21, 23, and 21 pregnancies in the control, 1.5, 5, and 17 mg/kg, respectively. The following Table (from page 48 of the study report) shows the uterine data.

b(4)

Study Number 1255-003
Study for Effects of GA-GCB on Male Fertility and Early Embryonic Development to Implantation in Rats

Table 8 Summary of Maternal and Developmental Observations at Uterine Examination

| Endpoint | 0 mg/kg/dose | 1.5 mg/kg/dose | 5.0 mg/kg/dose | 17 mg/kg/dose |
|---|---|---|---|---|
| No. Females on Study | 25 | 25 | 25 | 25 |
| No. Not Pregnant | 1 | 1 | 1 | 4 |
| No. Pregnant | 24 | 24 | 24 | 21 |
| Pregnancy Index Percent | 96.0 | 96.0 | 96.0 | 84.0 |
| No. Died Pregnant | 0 | 0 | 0 | 0 |
| No. Abortions | 0 | 0 | 0 | 0 |
| No. Females Pregnant with No Mating Date | 1 | 3 | 1 | 0 |
| No. Females with All Resorptions | 0 | 0 | 0 | 0 |
| No. Females with Viable Embryos Day 13 Gestation | 23 | 21 | 23 | 21 |



No.- Number

Sperm motility, caudal epididymal sperm concentrations (total and relative to per gram tissue), and percent abnormal sperm for the treated groups were comparable to controls. Two males (animal numbers 450 at 1.5 mg/kg and animal number 490 at 17 mg/kg) had too few sperm to assess motility. These animals also had relatively high incidences of abnormal sperm and low epididymal sperm concentrations. At necropsy, small testes and epididymides were noted for both animals and while each treated male showed positive confirmation of mating to an untreated female, neither resulted in a pregnancy. The following Table (from page 52 of the study report) shows the summary of sperm evaluations.

Study Number 1255-003
Study for Effects of GA-GCB on Male Fertility and Early Embryonic Development to Implantation in Rats

b(4)

Table 9 Summary of Sperm Evaluation

| Endpoint | 0 mg/kg/dose Mean | SD | N | 1.5 mg/kg/dose Mean | SD | N | 5.0 mg/kg/dose Mean | SD | N | 17 mg/kg/dose Mean | SD | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sperm Motility Percent Motility | 89.2 | 6.59 | 25 | 86.8 | 8.99 | 24 | 85.8 | 8.27 | 25 | 84.9 | 12.38 | 24 |
| Total Sperm Concentration per Cauda Epididymis x $10^6$ | 3.830 | 0.2968 | 25 | 3.841 | 0.7828 | 25 | 3.820 | 0.1821 | 25 | 3.823 | 0.7702 | 25 |
| Sperm Concentration per gram Cauda Epididymis x $10^6$ | 12.870 | 1.3832 | 25 | 12.304 | 2.8073 | 25 | 12.933 | 0.9728 | 25 | 12.506 | 2.9386 | 25 |
| Percent Abnormal | 3.56 | 2.224 | 25 | 3.40 | 1.251 | 24 | 3.00 | 1.594 | 25 | 2.73 | 1.588 | 24 |

N- Number of measures used to calculate mean
SD- Standard Deviation

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**Study title:** Study for the Effects of GA-GCB on Female Fertility and Early Embryonic Development to Implantation in Rats (Segment I)

**Key study findings:** In a Segment I female fertility and early embryonic development to implantation study in SD rats, animals were treated intravenously with GA-GCB every three to four days (twice weekly) beginning 14 days prior to pairing and continued through mating and until mating was confirmed at 0 [5% sucrose, 0.01% P20 (Polysorbate-20), 50mM sodium citrate, pH 6.0], 1.5, 5, and 17 mg/kg (n = 25/dose, 2 mL/kg). There was no mortality. Treatment-related clinical signs included swollen limbs/paw and lips or face at 5 and 17 mg/kg. There were no significant treatment-related effects on female fertility parameters. The NOAEL appeared to be 1.5 mg/kg.

**Study no.:** 1255-004
**Volume #, and page #:** Report No. SHGT-1U0-06-011, EDR
**Conducting laboratory and location:**
**Date of study initiation:** September 15, 2006
**GLP compliance:** A statement of compliance was included
**QA reports:** yes ( X ) no ( )
**Drug, lot #, and % purity:** GA-GCB, DARTEP06-001002, 97-98%

b(4)

**Methods:**

Doses: 1.5, 5 and 17 mg/kg
Species/strain: SD rats
Number/sex/group: 25 females/dose
Route, formulation, and volume: Intravenous bolus injection, solution in the vehicle (5% sucrose, 0.01% P20 [Polysorbate-20], 50mM sodium citrate, pH 6.0), and 2 mL/kg
Satellite groups used for toxicokinetics: None
Study design: The test article was administered every three to four days (twice weekly) beginning 14 days prior to pairing and continued through the mating period until mating was confirmed. Three treatment groups of 25 female rats/group were administered the test article at 1.5, 5, and 17 mg/kg. Control animals received vehicle (5% sucrose, 0.01% P20 [Polysorbate-20], 50mM sodium citrate, pH 6.0). The vehicle or test article was administered to all male animals via intravenous bolus injection at a dose volume of 2 mL/kg. The study design is shown below (from page 14 of the report).

**Group Assignments**

| Group Number | Dose Level (mg/kg/dose) | Number of Animals | |
|---|---|---|---|
| | | Male[a] | Female |
| 1 | 0 | 25 | 25 |
| 2 | 1.5 | 25 | 25 |
| 3 | 5.0 | 25 | 25 |
| 4 | 17 | 25 | 25 |

[a]Males were untreated and used for breeding purposes only.

Parameters and endpoints evaluated: Females were observed for clinical signs, body weights, and food consumption. Males and females of the same treatment group were paired for mating (1:1) until evidence of mating (sperm or copulatory plug) was confirmed or for up to 21 days. Females were examined daily for estrous cycle determination during the premating and mating periods until evidence of mating was confirmed or the cohabitation period ended. Mated females were euthanized on GD13, uterine examinations were conducted, and the location of normally developing implants (embryos), resorptions, and the total number of implantations were recorded. The number of corpora lutea on each ovary was also recorded.

**Results:**

Mortality: None

Clinical signs: Treatment-related clinical signs included swollen paws, swollen limbs (fore- and/or hindlimbs), and/or swollen face or lips on Day 8 at 5 and 17 mg/kg.

Body weight: The mean initial (Day 1) and final (Day 18) body weights of control animals were 187 g and 226 g, respectively. No significant treatment-related changes were observed.

Food consumption: The mean initial (Week 1-2) and final (Week 2-3) food consumption in control males were 18.3 and 18.8 g/animal/day, respectively. No significant treatment-related changes were observed.

Necropsy: There were no significant treatment-related changes.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): There was no significant treatment-related effect on estrous cycles. No significant effect of treatment with GA-GCB was evident from female reproductive indices. Mating, fertility, and fecundity indices were 100% in the treated females and were comparable to control indices (92 to 100%). The copulatory interval (i.e. mean number of days to mating evidence) in the treated groups was also comparable to controls. Reproductive and fertility indices are shown in the following Table (page 54 of the report) below.

b(4)

Study Number 1255-004
Study for Effects of GA-GCB on Female Fertility and Early Embryonic Development to Implantation in Rats

Table 12 Summary of Reproductive and Fertility Parameters

| Endpoint | | 0 mg/kg/dose | 1.5 mg/kg/dose | 5.0 mg/kg/dose | 17 mg/kg/dose |
|---|---|---|---|---|---|
| No. Females on Study | | 25 | 25 | 25 | 25 |
| No. Females Paired | | 25 | 25 | 25 | 25 |
| No. Females Mated | | 25 | 25 | 25 | 25 |
| No. Pregnant | | 23 | 25 | 25 | 25 |
| Female Mating Index (%) | | 100.0 | 100.0 | 100.0 | 100.0 |
| Female Fertility Index (%) | | 92.0 | 100.0 | 100.0 | 100.0 |
| Female Fecundity Index (%) | | 92.0 | 100.0 | 100.0 | 100.0 |
| Females with Confirmed Mating Day | | 25 | 21 | 24 | 24 |
| Copulatory Interval (Days) | | | | | |
| | Mean | 2.8 | 2.2 | 2.6 | 2.6 |
| | SD | 1.38 | 1.00 | 1.61 | 1.25 |
| | N | 25 | 21 | 24 | 24 |

Best Possible Copy

No. - Number
SD - Standard Deviation
N - Number

No effect of treatment with GA-GCB was evident from uterine implantation data. The mean number of corpora lutea, normally developing implants (i.e. embryos), resorptions, uterine implantations, and pre- and post-implantation loss indices per dam in the treated groups were

comparable to controls. There were 23, 21, 24 and 24 pregnancies in the control, 1.5, 5, and 17 mg/kg, respectively. The following Table (from page 56 of the report) shows the uterine data.

Study Number 1255-004
Study for Effects of GA-GCS on Female Fertility and Early Embryonic Development to Implantation in Rats

b(4)

Table 13 Summary of Maternal and Developmental Observations at Uterine Examination

| Endpoint | 0 mg/kg/dose | 1.5 mg/kg/dose | 5.0 mg/kg/dose | 17 mg/kg/dose |
|---|---|---|---|---|
| No. Females on Study | 25 | 25 | 25 | 25 |
| No. Not Pregnant | 2 | 0 | 0 | 0 |
| No. Pregnant | 23 | 25 | 25 | 25 |
| Pregnancy Index Percent | 92.0 | 100.0 | 100.0 | 100.0 |
| No. Died Pregnant | 0 | 0 | 0 | 0 |
| No. Abortions | 0 | 0 | 0 | 0 |
| No. Early Deliveries | 0 | 0 | 0 | 0 |
| No. Females with All Resorptions | 0 | 0 | 0 | 0 |
| No. Females with Viable Embryos Day 13 Gestation | 23 | 21 | 24 | 24 |
| No. Females Pregnant with No Confirmed Mating Date | 0 | 4 | 1 | 1 |



No. - Number

# Embryofetal Development

The sponsor submitted final reports of the Segment II teratology studies in rats and rabbits under IND 61,220 SDN 075 dated March 25, 2008. The following reviews are incorporated below from the pharmacology review of IND 61,220 dated February 6, 2009.

**Embryofetal Development (Segment II Teratology)**

**Study title**: Segment II Intravenous Teratology Study in Rats

**Key study findings**: In an intravenous Segment II teratology study in rats, timed pregnant females (n = 25/dose) were treated with GA-GCB at 1.5, 5 and 17 mg/kg/day (2 mL/kg) from GD6 through GD17. There was no mortality. Treatment-related clinical signs in dams included swollen limbs, paws, face and lips. There was no significant effect on maternal body weight, food consumption and uterine or ovarian parameters. There were no significant treatment-related effects on fetal body weights or fetal sex ratio, fetal external, visceral or skeletal parameters. GA-GCB was not teratogenic in rats at the tested doses under the conditions of the experiment.

**Study no.**: 1255-007
**Volume #, and page #**: Report No. SHGT-1U0-06-013, EDR

**Conducting laboratory and location:** [redacted]
**Date of study initiation:** December 5, 2006 b(4)
**GLP compliance:** A statement of compliance was included
**QA reports:** yes ( X ) no ( )
**Drug, lot #, and % purity:** GA-GCB, Lot DARTEP-06-001002, 97-98%

**Methods:**

Doses: 0 [5% sucrose, 0.01% P20 (polysorbate-20), 50 mM sodium citrate, pH 6.0], 1.5, 5 and 17 mg/kg. The vehicle or test article was administered to all groups by bolus intravenous injection once on Gestation Days (GD) 6, 9, 12, 15, and 17 at a dose volume of 2 mL/kg.

Basis of dose selection: Doses were selected based on the results of an IV dose ranging study (Study No. 1255-005, Report No. SHGT-1U0-06-012) in rats. In this dose ranging study, five treatment groups of five time-mated female SD rats were administered GA-GCB at 1.5, 3, 5, 11, and 17 mg/kg. The control group animals received the vehicle [5% sucrose, 0.01% P20 (polysorbate-20), 50 mM sodium citrate, pH 6.0]. The vehicle or test article was administered to all groups by bolus intravenous injection once on Gestation Days (GD) 6, 9, 12, 15, and 17 at a dose volume of 2 mL/kg. Clinical signs at 11 and 17 mg/kg included swollen paws, swollen limbs (fore- or hind-limbs) and swollen face or lips. Pregnancy rates were 100% in the control, 1.5, 3, 5, and 11 mg/kg, and 80% in the 17 mg/kg. No significant effect of treatment was evident from uterine implantation data or fetal parameters (body weights, sex ratios, or external evaluations). On the basis of these data, dose levels of 1.5, 5, and 17 mg/kg were selected for the current Segment II study.

Species/strain: SD rats
Number/group: 25/dose
Route, formulation, and volume: Intravenous, solution, 2 mL/kg
Satellite groups used for toxicokinetics: None
Study design: The study design is shown in the following Table (from page 13 of the report).

| Group Assignments | | | |
|---|---|---|---|
| Group Number | Dose Level (mg/kg/dose) | Test Article Concentration (mg/mL) | Number of Time-mated Females |
| 1 | 0 | 0.0 | 25 |
| 2 | 1.5 | 0.75 | 25 |
| 3 | 5.0 | 2.5 | 25 |
| 4 | 17 | 8.5 | 25 |

Parameters and endpoints evaluated: Observations included clinical signs, body weights, and food consumption. On GD20, all animals were euthanized and subjected to a complete necropsy, including a uterine examination in which early and late resorptions, viable and nonviable fetuses for each uterine horn, the total number of implantations, and the position of the cervix were recorded. The total number of corpora lutea on each ovary was also recorded. Gravid uterine weights were recorded. All fetuses were weighed, sexed externally, given an external examination, and processed for visceral or skeletal examination. Malformations and developmental variations were also recorded.

**Results:**

Mortality (dams): None

Clinical signs (dams): Treatment-related clinical signs at 5 and 17 mg/kg included swelling of lips, jaw, or muzzle and/or swelling of the extremities and paws. At 5 mg/kg, there was sporadic occurrence of animals with swollen fore- and hind-limbs and/or swollen forepaws. However, swelling of the lips, face or muzzle was not seen among these animals. These findings and the swollen extremities and paws were most prevalent at 17 mg/kg. All animals at 17 mg/kg had some swelling of the extremities, paws and muzzle on one or more treatment days. These clinical signs were seen in some animals on GD6 and continued in most of the animals on subsequent treatment days.

Body weight (dams): The mean initial (Day 0) and final (Day 20) body weights were 206 and 364 g, respectively. There were no treatment-related effects.

Food consumption (dams): The mean initial (0-6) and final (6-20) food consumption in control animals were 19.4 and 23.2 g/animal/day, respectively. There were no treatment-related effects

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.): No significant treatment-related effects were observed in C-section parameters. The mean number of corpora lutea, uterine implantations, viable fetuses, resorptions (early, late, and total), and pre- and postimplantation loss indices per dam in the treated groups were comparable to control. Gravid uterine weights were also comparable to controls and no effect of treatment was evident from these data. The following Table (from page 40 of the study report) shows the C-section data.

b(4)

[redacted] Study Number 1255-007
Study for Effects on Embryo-Fetal Development in Rats with GA-GCB

Table 6 Summary of Maternal and Developmental Observations at Uterine Examination

| Endpoint | 0 mg/kg/dose | 1.5 mg/kg/dose | 5.0 mg/kg/dose | 17 mg/kg/dose |
|---|---|---|---|---|
| No. Females on Study | 25 | 25 | 25 | 25 |
| No. Not Pregnant | 0 | 0 | 0 | 0 |
| No. Pregnant | 25 | 25 | 25 | 25 |
| Pregnancy Index Percent | 100.0 | 100.0 | 100.0 | 100.0 |
| No. Died Pregnant | 0 | 0 | 0 | 0 |
| No. Abortions | 0 | 0 | 0 | 0 |
| No. Early Deliveries | 0 | 0 | 0 | 0 |
| No. Females with All Resorptions | 0 | 0 | 0 | 0 |
| No. Females with Viable Fetuses Day 20 Gestation | 25 | 25 | 25 | 25 |

b(4)

[redacted] Study Number 1255-007
Study for Effects on Embryo-Fetal Development in Rats with GA-GCB

Table 6 Summary of Maternal and Developmental Observations at Uterine Examination

| Endpoint | | 0 mg/kg/dose | 1.5 mg/kg/dose | 5.0 mg/kg/dose | 17 mg/kg/dose |
|---|---|---|---|---|---|
| Corpora Lutea | | | | | |
| No. per Animal | Mean | 14.0 | 13.8 | 13.9 | 13.5 |
| | SD | 1.99 | 1.89 | 1.88 | 1.68 |
| | N | 25 | 25 | 25 | 25 |
| Implantation Sites | | | | | |
| No. per Animal | Mean | 12.4 | 12.7 | 12.6 | 12.8 |
| | SD | 1.78 | 2.14 | 1.80 | 1.53 |
| | N | 25 | 25 | 25 | 25 |
| Preimplantation Loss | | | | | |
| % per Animal | Mean | 10.7 | 8.0 | 9.0 | 8.9 |
| | SD | 11.3 | 10.9 | 11.6 | 9.4 |
| | N | 25 | 25 | 25 | 25 |
| Viable Fetuses | | | | | |
| No. per Animal | Mean | 11.7 | 12.0 | 12.0 | 12.3 |
| | SD | 2.03 | 2.35 | 1.90 | 1.65 |
| | N | 25 | 25 | 25 | 25 |
| Fetal Sex Ratio | | | | | |
| % Males per Animal | Mean | 42.5 | 48.7 | 52.0 | 49.5 |
| | SD | 11.94 | 16.05 | 15.06 | 15.51 |
| | N | 25 | 25 | 25 | 25 |

b(4)

Study Number 1255-007
Study for Effects on Embryo-Fetal Development in Rats with GA-GCB

Table 6 Summary of Maternal and Developmental Observations at Uterine Examination

| Endpoint | | 0 mg/kg/dose | 1.5 mg/kg/dose | 5.0 mg/kg/dose | 17 mg/kg/dose |
|---|---|---|---|---|---|
| Postimplantation Loss | | | | | |
| % Implants per Animal | Mean | 5.5 | 5.7 | 4.6 | 2.1 |
| | SD | 6.2 | 6.7 | 7.4 | 3.8 |
| | N | 25 | 25 | 25 | 25 |
| Nonviable Fetuses | | | | | |
| No. per Animal | Mean | 0.0 | 0.0 | 0.0 | 0.0 |
| | SD | 0.00 | 0.00 | 0.00 | 0.00 |
| | N | 25 | 25 | 25 | 25 |
| Litter Size | | | | | |
| No. per Animal | Mean | 11.7 | 12.0 | 12.0 | 12.3 |
| | SD | 2.03 | 2.35 | 1.90 | 1.65 |
| | N | 25 | 25 | 25 | 25 |
| Resorptions: Early + Late | | | | | |
| No. per Animal | Mean | 0.6 | 0.7 | 0.6 | 0.2 |
| | SD | 0.76 | 0.75 | 1.00 | 0.44 |
| | N | 25 | 25 | 25 | 25 |
| Resorptions: Early | | | | | |
| No. per Animal | Mean | 0.6 | 0.7 | 0.6 | 0.2 |
| | SD | 0.71 | 0.75 | 1.00 | 0.44 |
| | N | 25 | 25 | 25 | 25 |
| Resorptions: Late | | | | | |
| No. per Animal | Mean | 0.0 | 0.0 | 0.0 | 0.0 |
| | SD | 0.20 | 0.00 | 0.00 | 0.00 |
| | N | 25 | 25 | 25 | 25 |

No. - Number
SD - Standard Deviation
N - Number of measures used to calculate mean



Offspring (malformations, variations, etc.): There were no treatment-related effects on fetal body weights. Fetal sex ratios (% male fetuses/litter) in the treated groups ranged form 48.7 to 52.0% and were higher than the 42.5% in controls. These differences were not statistically significant and considered unrelated to treatment with GA-GCB.

No significant treatment-related effects were seen from the fetal external examinations. The only external observation was filamentous tail. This was seen in a single fetus in each of the control and 1.5 mg/kg. The only developmental variation seen was dermal hypoplasia. This was seen in three fetuses from a single litter at 5 mg/kg. However, there was no dose-response relationship or findings at the highest dose. In the absence of a dose-response and similar finding among the fetuses at the 17 mg/kg, these findings were considered unrelated to treatment. The following Tables (from page 48, 50 and 52 of the study report) show the fetal external, visceral and skeletal observations.

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Study Number 1255-007
Study for Effects on Embryo-Fetal Development in Rats with GA-GCB

Table 9 Summary of Individual Fetal External Observations

| Observation | Classification | 0 mg/kg/dose | 1.5 mg/kg/dose | 5.0 mg/kg/dose | 17 mg/kg/dose |
|---|---|---|---|---|---|
| No. Litters Evaluated | | 25 | 25 | 25 | 25 |
| No. Fetuses Evaluated | | 293 | 300 | 293 | 308 |
| **Body** | | | | | |
| Skin, Dermal hypoplasia | V | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 1 (4.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 3 (1.0) | 0 (0.0) |
| **Tail** | | | | | |
| Entire, Filamentous | M | | | | |
| No. Litters (%) | | 1 (4.0) | 1 (4.0) | 0 (0.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 1 (0.3) | 1 (0.3) | 0 (0.0) | 0 (0.0) |

Best Possible Copy

M - Malformation
V - Variation

[1]Not statistically analyzed
No. - Number

b(4)

Study Number 1255-007
Study for Effects on Embryo-Fetal Development in Rats with GA-GCB

**Table 10 Summary of Individual Fetal Visceral Observations**

| Observation | Classification | 0 mg/kg/dose | 1.5 mg/kg/dose | 5.0 mg/kg/dose | 17 mg/kg/dose |
|---|---|---|---|---|---|
| No. Litters Evaluated | | 25 | 25 | 25 | 26 |
| No. Fetuses Evaluated | | 147 | 150 | 150 | 154 |
| **Abdominal cavity** | | | | | |
| Kidney, Increased renal pelvic cavitation | V | | | | |
| No. Litters (%) | | 2 (8.0) | 2 (8.0) | 0 (0.0) | 1 (4.0) |
| No. Fetuses (%)[1] | | 2 (1.4) | 2 (1.3) | 0 (0.0) | 1 (0.6) |
| Ureter, Dilated | V | | | | |
| No. Litters (%) | | 2 (8.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 2 (1.4) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Uterus, Short | V | | | | |
| No. Litters (%) | | 1 (4.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 1 (0.7) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| **Thoracic cavity** | | | | | |
| Innominate artery, Absent | V | | | | |
| No. Litters (%) | | 1 (4.0) | 0 (0.0) | 1 (4.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 1 (0.7) | 0 (0.0) | 1 (0.7) | 0 (0.0) |

V - Variation

[1]Not statistically analyzed
No. - Number

b(4)

Study Number 1255-007
Study for Effects on Embryo-Fetal Development in Rats with GA-GCB

**Table 11 Summary of Individual Fetal Skeletal Observations**

| Observation | Classification | 0 mg/kg/dose | 1.5 mg/kg/dose | 5.0 mg/kg/dose | 17 mg/kg/dose |
|---|---|---|---|---|---|
| No. Litters Evaluated | | 25 | 25 | 25 | 25 |
| No. Fetuses Evaluated | | 146 | 150 | 149 | 154 |
| **Caudal vertebra(e)** | | | | | |
| All, Absent | V | | | | |
| No. Litters (%) | | 0 (0.0) | 1 (4.0) | 0 (0.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 1 (0.7) | 0 (0.0) | 0 (0.0) |
| **Cervical vertebra(e)** | | | | | |
| Neural arch(es), Additional ossification center | V | | | | |
| No. Litters (%) | | 1 (4.0) | 0 (0.0) | 2 (8.0) | 1 (4.0) |
| No. Fetuses (%)[1] | | 1 (0.7) | 0 (0.0) | 2 (1.3) | 1 (0.6) |
| Neural arch(es), Incompletely ossified | V | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 1 (4.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 1 (0.7) | 0 (0.0) |
| **Forelimb(s)** | | | | | |
| Metacarpals, Not ossified | V | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 2 (8.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 3 (2.0) | 0 (0.0) |

V - Variation
[1]Not statistically analyzed
No. - Number

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**Study title:** Segment II Intravenous Teratology Study in Rabbits

**Key study findings:** In an intravenous Segment II teratology study in rabbits, timed pregnant females (n = 23/dose) were treated with GA-GCB at 1.5, 10 and 20 mg/kg/day (2 mL/kg) from GD6 through GD18. There was no treatment-related mortality or clinical signs. There was no significant effect on maternal body weight, food consumption and uterine or ovarian parameters. There were no significant treatment-related effects on fetal body weights or fetal sex ratio, fetal external, visceral or skeletal parameters. GA-GCB was not teratogenic in rabbits at the tested doses under the conditions of the experiment.

**Study no.:** 1255-008
**Volume #, and page #:** Report No. SHGT-1U0-06-015. EDR
**Conducting laboratory and location:** b(4)
**Date of study initiation:** January 15, 2007
**GLP compliance:** A statement of compliance was included.
**QA reports:** yes ( X ) no ( )
**Drug, lot #, and % purity:** GA-GCB, Lot DARTEP-06-005, 98%

**Methods:**

Doses: 0 [5% sucrose, 0.01% P20 (polysorbate-20), 50 mM sodium citrate, pH 6.0], 1.5, 10 and 20 mg/kg. The vehicle or test article was administered to all groups by bolus intravenous injection once on Gestation Days (GD) 6, 9, 12, 15, and 18 at a dose volume of 2 mL/kg.

Basis of dose selection: Doses were selected based on the results of an IV dose ranging study (Study No. 1255-006, Report No. SHGT-1U0-06-014) in rabbits at 0.5, 1.5, 5, 10, and 20 mg/kg. There was no mortality or treatment-related clinical signs. No treatment-related effects were seen on gestation body weights and body weight change, gestation food consumption, or macroscopic findings. Pregnancy rates ranged from 83.3-100% in the treated groups and 100% in controls. One female at 20 mg/kg group initiated delivery on GD29 prior to scheduled euthanasia. No effects of treatment were seen from uterine implantation data, fetal body weights, or fetal external examinations. Based on the results of this dose ranging study, the highest dose for the current Segment II teratology study was selected as 20 mg/kg.

Species/strain: New Zealand white rabbits
Number/group: 23/dose
Route, formulation, and volume: Intravenous, solution, 2 mL/kg
Satellite groups used for toxicokinetics: None
Study design: The study design is shown in the following Table (from page 14 of the report).

| Group Assignments | | | |
|---|---|---|---|
| Group Number | Dose Level (mg/kg/dose) | Test Article Concentration (mg/mL) | Number of Time-mated Females |
| 1 | 0 | 0.0 | 23 |
| 2 | 1.5 | 0.75 | 23 |
| 3 | 10 | 5.0 | 23 |
| 4 | 20 | 10.0 | 23 |

Parameters and endpoints evaluated: Observations included clinical signs, body weights, and food consumption. On GD29, all animals were euthanized and subjected to a complete necropsy, including a uterine examination in which early and late resorptions, viable and nonviable fetuses for each uterine horn, the total number of implantations, and the position of the cervix were recorded. The total number of corpora lutea on each ovary was also recorded. Gravid uterine weights were recorded. All fetuses were weighed, sexed externally and examined for external, visceral and or skeletal changes.

**Results:**

Mortality (dams): There was no treatment-related mortality. One animal at 1.5 mg/kg died on GD23. The cause of death was not determined. One animal at 20 mg/kg was noted with

impaired hind-limb function during the treatment period and was euthanized *in extremis*. All other animals (control and treated) survived to scheduled termination.

Clinical signs (dams): None

Body weight (dams): The mean initial (Day 0) and final (Day 29) body weights were 3.2 and 3.7 kg, respectively. There were no treatment-related effects.

Food consumption (dams): The mean initial (0-6) and final (18-29) food consumption in control animals were 142.5 and 140.1 g/animal/day, respectively. There were no treatment-related effects.

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.): Pregnancy rates were 95.7%, 100%, 87.0% and 100% in the control, 1.5, 10, and 20 mg/kg, respectively. A total of four females were not pregnant (one control and three at 10 mg/kg). No animals aborted or delivered early; however, one female at 1.5 mg/kg had an early resorption. No effect of treatment was evident from uterine implantation data. The mean number of corpora lutea, uterine implantations, viable fetuses, and resorptions sites (early, late and total), and post-implantation loss indices in the treated groups were comparable to controls. Pre-implantation loss was higher in controls compared to other treatment groups. Gravid uterine weights at 10 and 20 mg/kg animals were comparable to controls. At 1.5 mg/kg, the gravid uterine weight was statistically higher than controls. This was not considered toxicologically meaningful or indicative of a treatment related response in the absence of this effect at higher doses. The following Table (from page 42 of the study report) shows the uterine data.

b(4)

[redacted] th Study Number 1255-008
Study for Effects on Embryo-Fetal Development in Rabbits with GA-GCB

**Table 6 Summary of Maternal and Developmental Observations at Uterine Examination**

| Endpoint | 0 mg/kg/dose | 1.5 mg/kg/dose | 10 mg/kg/dose | 20 mg/kg/dose |
|---|---|---|---|---|
| No. Females on Study | 23 | 23 | 23 | 23 |
| No. Not Pregnant | 1 | 0 | 3 | 0 |
| No. Pregnant | 22 | 23 | 20 | 23 |
| Pregnancy Index Percent | 95.7 | 100.0 | 87.0 | 100.0 |
| No. Died Pregnant | 0 | 1 | 0 | 1 |
| No. Abortions | 0 | 0 | 0 | 0 |
| No. Early Deliveries | 0 | 0 | 0 | 0 |
| No. Females Pregnant by Stain | 0 | 1 | 0 | 0 |
| No. Females with All Resorptions | 0 | 0 | 0 | 0 |
| No. Females with Viable Fetuses Day 29 Gestation | 22 | 21 | 20 | 22 |

b(4)

[redacted] Study Number 1255-008
Study for Effects on Embryo-Fetal Development in Rabbits with GA-GCB

**Table 6 Summary of Maternal and Developmental Observations at Uterine Examination**

| Endpoint | | 0 mg/kg/dose | 1.5 mg/kg/dose | 10 mg/kg/dose | 20 mg/kg/dose |
|---|---|---|---|---|---|
| Corpora Lutea No. per Animal | Mean | 10.1 | 10.7 | 9.8 | 9.4 |
| | SD | 1.52 | 1.80 | 1.62 | 1.92 |
| | N | 22 | 21 | 20 | 22 |
| Implantation Sites No. per Animal | Mean | 8.6 | 9.6 | 8.7 | 8.8 |
| | SD | 1.84 | 2.59 | 1.38 | 1.86 |
| | N | 22 | 22 | 20 | 22 |
| Preimplantation Loss % per animal | Mean | 14.9 | 5.6 * | 10.1 | 6.0 * |
| | SD | 14.40 | 7.58 | 10.50 | 6.22 |
| | N | 22 | 21 | 20 | 22 |
| Viable Fetuses No. per Animal | Mean | 8.0 | 9.2 | 8.2 | 8.3 |
| | SD | 1.84 | 2.68 | 1.58 | 1.52 |
| | N | 22 | 22 | 20 | 22 |
| Fetal Sex Ratio % Males per animal | Mean | 46.6 | 47.7 | 48.5 | 49.6 |
| | SD | 19.60 | 16.08 | 21.66 | 16.14 |
| | N | 22 | 21 | 20 | 22 |

b(4)

No. - Number
SD - Standard Deviation
N - Number of measures used to calculate mean
*Significantly different from control ($p<0.05$)

b(4)

Study Number 1255-008
Study for Effects on Embryo-Fetal Development in Rabbits with GA-GCB

**Table 6 Summary of Maternal and Developmental Observations at Uterine Examination**

| Endpoint | | 0 mg/kg/dose | 1.5 mg/kg/dose | 10 mg/kg/dose | 20 mg/kg/dose |
|---|---|---|---|---|---|
| Postimplantation Loss | | | | | |
| % Implants per Animal | Mean | 7.2 | 8.5 | 5.8 | 4.4 |
| | SD | 12.37 | 21.28 | 9.29 | 7.18 |
| | N | 22 | 22 | 20 | 22 |
| Norvable Fetuses | | | | | |
| No. per Animal | Mean | 0.0 | 0.0 | 0.0 | 0.0 |
| | SD | 0.00 | 0.00 | 0.00 | 0.00 |
| | N | 22 | 22 | 20 | 22 |
| Litter Size | | | | | |
| No. per Animal | Mean | 8.0 | 9.2 | 8.2 | 8.3 |
| | SD | 1.94 | 2.68 | 1.58 | 1.52 |
| | N | 22 | 22 | 20 | 22 |
| Resorptions: Early + Late | | | | | |
| No. per Animal | Mean | 0.8 | 0.5 | 0.5 | 0.5 |
| | SD | 1.05 | 0.60 | 0.76 | 0.80 |
| | N | 22 | 22 | 20 | 22 |
| Resorptions: Early | | | | | |
| No. per Animal | Mean | 0.4 | 0.2 | 0.4 | 0.4 |
| | SD | 0.91 | 0.39 | 0.49 | 0.66 |
| | N | 22 | 22 | 20 | 22 |
| Resorptions: Late | | | | | |
| No. per Animal | Mean | 0.2 | 0.3 | 0.2 | 0.1 |
| | SD | 0.66 | 0.55 | 0.49 | 0.29 |
| | N | 22 | 22 | 20 | 22 |

No. - Number
SD - Standard Deviation
N - Number of measures used to calculate mea

Offspring (malformations, variations, etc.): There were no significant treatment-related effects on fetal body weights or sex-ratio. No external malformation was seen at 1.5 and 10 mg/kg. At 20 mg/kg, external malformations were seen in two fetuses. One fetus from the litter of animal number 271 had an apparent jaw malformation (i.e. micrognathia). The other external malformation seen in this group was edema of the entire body in one fetus from the litter of animal number 278. The low incidence of these malformations at 20 mg/kg/dose group was considered spurious, spontaneous in nature, and unrelated to treatment in the absence of a dose-response. No external variations were seen among the control and treated fetuses. In the 10 mg/kg/dose group, two fetuses from a single litter (fetuses 8 and 10 from the litter of animal number 269) had multiple nodules on the surface of the skin. The following Table (from page 49 of the study report) shows the fetal external malformations.

b(4)

Study Number 1255-008
Study for Effects on Embryo-Fetal Development in Rabbits with GA-GCB

Table 9 **Summary of Individual Fetal External Observations**

| Observation | Classification | 0 mg/kg/dose | 1.5 mg/kg/dose | 10 mg/kg/dose | 20 mg/kg/dose |
|---|---|---|---|---|---|
| No. Litters Evaluated | | 22 | 21 | 20 | 22 |
| No. Fetuses Evaluated | | 177 | 202 | 164 | 183 |
| **Body** | | | | | |
| Entire, Edema | M | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (0.5) |
| Entire, Loose skin | P | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (0.5) |
| Skin, Nodule | P | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 1 (5.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 2 (1.2) | 0 (0.0) |
| **Head** | | | | | |
| Jaw(s), Micrognathia | M | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (0.5) |

M - Malformation
P - Pathological
[1]Not statistically analyzed
No. - Number

Best Possible Copy

No effect of treatment with GA-GCB was evident from fetal visceral examinations. Two fetuses from a single litter at 10 mg/kg had similar heart and aortic arch malformations (i.e. aortic arch dilated, ventricular septum discontinuous, pulmonary arch small or constricted, and small ventricles) but in the absence of similar malformations among the fetuses at 20 mg/kg/dose, their occurrence was considered spurious and unrelated to treatment. The following Table (from page 52-56 of the study report) shows the fetal visceral observations.

Study Number 1255-008
Study for Effects on Embryo-Fetal Development in Rabbits with GA-GCB

b(4)

**Table 10 Summary of Individual Fetal Visceral Observations**

| Observation | Classification | 0 mg/kg/dose | 1.5 mg/kg/dose | 10 mg/kg/dose | 20 mg/kg/dose |
|---|---|---|---|---|---|
| No. Litters Evaluated | | 22 | 21 | 20 | 22 |
| No. Fetuses Evaluated | | 177 | 202 | 164 | 183 |
| **Abdominal cavity** | | | | | |
| Abdomen, Fluid filled | P | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (0.5) |
| Gallbladder, Absent | M | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 1 (5.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 1 (0.6) | 1 (0.5) |
| Gallbladder, Smaller than normal | V | | | | |
| No. Litters (%) | | 1 (4.5) | 0 (0.0) | 3 (15.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 1 (0.6) | 0 (0.0) | 3 (1.8) | 0 (0.0) |
| Kidney, Increased renal pelvic cavitation | V | | | | |
| No. Litters (%) | | 0 (0.0) | 2 (9.5) | 0 (0.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 3 (1.5) | 0 (0.0) | 0 (0.0) |
| Spleen, Discolored | P | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 1 (5.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 1 (0.6) | 0 (0.0) |
| Ureter, Malpositioned | V | | | | |
| No. Litters (%) | | 1 (4.5) | 3 (14.3) | 1 (5.0) | 2 (9.1) |
| No. Fetuses (%)[1] | | 1 (0.6) | 4 (2.0) | 1 (0.6) | 3 (1.6) |

M - Malformation
V - Variation
P - Pathological
[1]Not statistically analyzed
No. - Number

Study Number 1255-008
Study for Effects on Embryo-Fetal Development in Rabbits with GA-GCB

b(4)

**Table 10 Summary of Individual Fetal Visceral Observations**

| Observation | Classification | 0 mg/kg/dose | 1.5 mg/kg/dose | 10 mg/kg/dose | 20 mg/kg/dose |
|---|---|---|---|---|---|
| No. Litters Evaluated | | 22 | 21 | 20 | 22 |
| No. Fetuses Evaluated | | 177 | 202 | 164 | 183 |
| **Head** | | | | | |
| Eye(s), Hemorrhagic | P | | | | |
| No. Litters (%) | | 1 (4.5) | 1 (4.8) | 2 (10.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 2 (1.1) | 1 (0.5) | 2 (1.2) | 1 (0.5) |
| Eye(s), Microphthalmia | M | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 1 (5.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 1 (0.6) | 0 (0.0) |
| **Thoracic cavity** | | | | | |
| Aortic arch, Dilated | M | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 1 (5.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 2 (1.2) | 0 (0.0) |
| Aortic arch, Retroesophageal | M | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 1 (5.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 1 (0.6) | 1 (0.5) |
| Both lungs, Smaller than normal | M | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (0.5) |

M - Malformation
P - Pathological
[1]Not statistically analyzed
No. - Number

Study Number: 1255-008
Study for Effects on Embryo-Fetal Development in Rabbits with GA-GCS

b(4)

**Table 10 Summary of Individual Fetal Visceral Observations**

| Observation | Classification | 0 mg/kg/dose | 1.5 mg/kg/dose | 10 mg/kg/dose | 20 mg/kg/dose |
|---|---|---|---|---|---|
| No. Litters Evaluated | | 22 | 21 | 20 | 22 |
| No. Fetuses Evaluated | | 177 | 202 | 164 | 183 |
| **Thoracic cavity cont.** | | | | | |
| Common carotid artery, Arising from innominate artery | V | | | | |
| No. Litters (%) | | 1 (4.5) | 0 (0.0) | 0 (0.0) | 2 (9.1) |
| No. Fetuses (%)[1] | | 1 (0.6) | 0 (0.0) | 0 (0.0) | 2 (1.1) |
| Ductus arteriosus, Smaller than normal | V | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 1 (5.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 1 (0.6) | 0 (0.0) |
| Innominate artery, Absent | V | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 2 (10.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 2 (1.2) | 0 (0.0) |
| Interventricular septum, Discontinuous | M | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 1 (5.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 2 (1.2) | 0 (0.0) |
| Pulmonary artery, Arising from aortic arch | M | | | | |
| No. Litters (%) | | 1 (4.5) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 1 (0.6) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Pulmonary trunk, Constricted | M | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 1 (5.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 1 (0.6) | 0 (0.0) |

M - Malformation
V - Variation
[1] Not statistically analyzed
No. - Number

Best Possible Copy

b(4)

Study Number 1255-008
Study for Effects on Embryo-Fetal Development in Rabbits with GA-GCB

**Table 10 Summary of Individual Fetal Visceral Observations**

| Observation | Classification | 0 mg/kg/dose | 1.5 mg/kg/dose | 10 mg/kg/dose | 20 mg/kg/dose |
|---|---|---|---|---|---|
| No. Litters Evaluated | | 22 | 21 | 20 | 22 |
| No. Fetuses Evaluated | | 177 | 202 | 184 | 183 |
| **Thoracic cavity cont.** | | | | | |
| Pulmonary trunk, Smaller than normal | M | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 1 (5.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 1 (0.6) | 0 (0.0) |
| Right lung, Azygous lobe absent | V | | | | |
| No. Litters (%) | | 3 (13.6) | 1 (4.8) | 4 (20.0) | 4 (18.2) |
| No. Fetuses (%)[1] | | 3 (1.7) | 1 (0.5) | 4 (2.2) | 4 (2.2) |
| Subclavian artery, Arising from innominate artery | V | | | | |
| No. Litters (%) | | 1 (4.5) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 1 (0.6) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Subclavian artery, Extra | V | | | | |
| No. Litters (%) | | 1 (4.5) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 2 (1.1) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Subclavian artery, Retroesophageal | V | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 1 (5.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 1 (0.6) | 1 (0.5) |
| Thoracic cavity, Fluid filled | P | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (0.5) |

M - Malformation
V - Variation
P - Pathological

[1]Not statistically analyzed
No. - Number

Best Possible Copy

b(4)

Study Number 1255-008
Study for Effects on Embryo-Fetal Development in Rabbits with GA-GCB

**Table 10 Summary of Individual Fetal Visceral Observations**

| Observation | Classification | 0 mg/kg/dose | 1.5 mg/kg/dose | 10 mg/kg/dose | 20 mg/kg/dose |
|---|---|---|---|---|---|
| No. Litters Evaluated | | 22 | 21 | 20 | 22 |
| No. Fetuses Evaluated | | 177 | 202 | 164 | 183 |
| **Thoracic cavity cont.** | | | | | |
| Thymus, Hemorrhagic | P | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 1 (5.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 1 (0.6) | 0 (0.0) |
| Ventricle, Smaller than normal | M | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 1 (5.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 2 (1.2) | 0 (0.0) |

M - Malformation
P - Pathological
[1]Not statistically analyzed
No. - Number

No significant effect of treatment was evident from the fetal skeletal examinations. The following Table (from page 57 of the study report) shows the skeletal observations.

Study Number 1255-008
Study for Effects on Embryo-Fetal Development in Rabbits with GA-GCB

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**Table 11** Summary of Individual Fetal Skeletal Observations

| Observation | Classification | 0 mg/kg/dose | 1.5 mg/kg/dose | 10 mg/kg/dose | 20 mg/kg/dose |
|---|---|---|---|---|---|
| No. Litters Evaluated | | 22 | 21 | 20 | 22 |
| No. Fetuses Evaluated | | 177 | 202 | 164 | 183 |
| **Cervical vertebra(e)** | | | | | |
| Centra, Additional ossification center | V | | | | |
| No. Litters (%) | | 1 (4.5) | 0 (0.0) | 0 (0.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 1 (0.6) | 0 (0.0) | 0 (0.0) | 1 (0.5) |
| Centra, Hemicentric | V | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 1 (5.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 1 (0.6) | 0 (0.0) |
| Centra, Misaligned | V | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 1 (5.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 1 (0.6) | 0 (0.0) |
| Neural arch(es), Additional ossification center | V | | | | |
| No. Litters (%) | | 2 (9.1) | 0 (0.0) | 2 (10.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 5 (2.8) | 0 (0.0) | 2 (1.2) | 0 (0.0) |
| Neural arch(es), Cervical ribs | V | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 1 (5.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 1 (0.6) | 0 (0.0) |

V - Variation

[1]Not statistically analyzed
No. - Number

Study Number 1255-008
Study for Effects on Embryo-Fetal Development in Rabbits with GA-GCB

b(4)

**Table 11 Summary of Individual Fetal Skeletal Observations**

| Observation | Classification | 0 mg/kg/dose | 1.5 mg/kg/dose | 10 mg/kg/dose | 20 mg/kg/dose |
|---|---|---|---|---|---|
| No. Litters Evaluated | | 22 | 21 | 20 | 22 |
| No. Fetuses Evaluated | | 177 | 202 | 164 | 183 |
| **Hind limb(s)** | | | | | |
| Talus, Not ossified | V | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 2 (1.1) |
| **Lumbar vertebra(e)** | | | | | |
| Centra, Extra | M | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (0.5) |
| Centra, Fused | M | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (0.5) |
| Centra, Hemicentric | V | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (0.5) |
| Neural arch(es), Extra | M | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (0.5) |

M - Malformation
V - Variation
[1]Not statistically analyzed
No. - Number

b(4)

Study Number 12SS-008
Study for Effects on Embryo-Fetal Development in Rabbits with GA-GCB

**Table 11 Summary of Individual Fetal Skeletal Observations**

| Observation | Classification | 0 mg/kg/dose | 1.5 mg/kg/dose | 10 mg/kg/dose | 20 mg/kg/dose |
|---|---|---|---|---|---|
| No. Litters Evaluated | | 22 | 21 | 20 | 22 |
| No. Fetuses Evaluated | | 177 | 202 | 184 | 183 |
| **Lumbar vertebra(e) cont.** | | | | | |
| Neural arch(es), Misaligned | M | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (0.5) |
| **Rib(s)** | | | | | |
| Costal cartilage, Branched | M | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 1 (5.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 1 (0.6) | 0 (0.0) |
| Costal cartilage, Fused | M | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 1 (5.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 1 (0.6) | 0 (0.0) |
| Costal cartilage, Misaligned | V | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (0.5) |
| Rib(s), Rudimentary | V | | | | |
| No. Litters (%) | | 18 (81.8) | 21 (100.0) | 15 (75.0) | 20 (90.9) |
| No. Fetuses (%)[1] | | 39 (22.0) | 68 (33.7) | 38 (22.0) | 61 (33.3) |

M - Malformation
V - Variation
[1]Not statistically analyzed
No. - Number

Study Number 1255-008
Study for Effects on Embryo-Fetal Development in Rabbits with GA-GCB

b(4)

Table 11 Summary of Individual Fetal Skeletal Observations

| Observation | Classification | 0 mg/kg/dose | 1.5 mg/kg/dose | 10 mg/kg/dose | 20 mg/kg/dose |
|---|---|---|---|---|---|
| No. Litters Evaluated | | 22 | 21 | 20 | 22 |
| No. Fetuses Evaluated | | 177 | 202 | 164 | 183 |
| **Rib(s) cont.** | | | | | |
| Rib(s), Unilateral full rib | V | | | | |
| No. Litters (%) | | 11 (50.0) | 15 (71.4) | 13 (65.0) | 16 (72.7) |
| No. Fetuses (%)[1] | | 17 (9.6) | 26 (12.9) | 22 (13.4) | 29 (15.8) |
| **Skull** | | | | | |
| Frontal bone, Fused | M | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 1 (5.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 1 (0.6) | 0 (0.0) |
| Hyoid arch, Bent | V | | | | |
| No. Litters (%) | | 3 (13.6) | 3 (14.3) | 9 (45.0) | 5 (22.7) |
| No. Fetuses (%)[1] | | 4 (2.3) | 3 (1.5) | 12 (7.3) | 5 (2.7) |
| Hyoid body, Not ossified | V | | | | |
| No. Litters (%) | | 1 (4.5) | 1 (4.8) | 0 (0.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 1 (0.6) | 1 (0.5) | 0 (0.0) | 1 (0.5) |
| Interparietal bone, Bipartite | V | | | | |
| No. Litters (%) | | 1 (4.5) | 0 (0.0) | 1 (5.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 1 (0.6) | 0 (0.0) | 1 (0.6) | 0 (0.0) |

M - Malformation
V - Variation
[1]Not statistically analyzed
No. - Number

Study Number 1255-008
Study for Effects on Embryo-Fetal Development in Rabbits with GA-GDB

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**Table 11 Summary of Individual Fetal Skeletal Observations**

| Observation | Classification | 0 mg/kg/dose | 1.5 mg/kg/dose | 10 mg/kg/dose | 20 mg/kg/dose |
|---|---|---|---|---|---|
| No. Litters Evaluated | | 22 | 21 | 20 | 22 |
| No. Fetuses Evaluated | | 177 | 202 | 164 | 183 |
| **Skull cont.** | | | | | |
| Jugal, Bipartite | V | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 1 (5.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 1 (0.6) | 0 (0.0) |
| Jugal, Fused | M | | | | |
| No. Litters (%) | | 1 (4.5) | 1 (4.8) | 2 (10.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 1 (0.6) | 1 (0.5) | 2 (1.2) | 3 (1.6) |
| Nasal bone, Additional ossification center | V | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 1 (5.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 1 (0.6) | 0 (0.0) |
| Parietal bone, Additional ossification center | V | | | | |
| No. Litters (%) | | 0 (0.0) | 1 (4.8) | 1 (5.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 1 (0.5) | 1 (0.6) | 0 (0.0) |
| **Sternum** | | | | | |
| Sternebra(e), Additional ossification center | V | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 1 (5.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 1 (0.6) | 2 (1.1) |

M - Malformation
V - Variation

[1]Not statistically analyzed
No. - Number

Study Number 1255-008
Study for Effects on Embryo-Fetal Development in Rabbits with GA-GCB

b(4)

**Table 11 Summary of Individual Fetal Skeletal Observations**

| Observation | Classification | 0 mg/kg/dose | 1.5 mg/kg/dose | 10 mg/kg/dose | 20 mg/kg/dose |
|---|---|---|---|---|---|
| No. Litters Evaluated | | 22 | 21 | 20 | 22 |
| No. Fetuses Evaluated | | 177 | 202 | 164 | 183 |
| **Sternum cont.** | | | | | |
| Sternebra(e), Extra | M | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 1 (5.0) | 0 (0.0) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 1 (0.6) | 0 (0.0) |
| Sternebra(e), Fused | M | | | | |
| No. Litters (%) | | 2 (9.1) | 3 (14.3) | 2 (10.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 2 (1.1) | 3 (1.5) | 4 (2.4) | 1 (0.5) |
| Sternebra(e), Misaligned | V | | | | |
| No. Litters (%) | | 4 (18.2) | 2 (9.5) | 5 (25.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 5 (2.8) | 2 (1.0) | 5 (3.0) | 1 (0.5) |
| Sternebra(e), Not ossified | V | | | | |
| No. Litters (%) | | 14 (63.6) | 10 (47.6) | 10 (50.0) | 8 (36.4) |
| No. Fetuses (%)[1] | | 25 (14.1) | 22 (10.9) | 19 (11.6) | 19 (10.4) |
| **Thoracic vertebra(e)** | | | | | |
| Centra, Extra | M | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (0.5) |

M - Malformation
V - Variation
[1]Not statistically analyzed
No. - Number

Study Number 1255-008
Study for Effects on Embryo-Fetal Development in Rabbits with GA-GCB

b(4)

**Table 11 Summary of Individual Fetal Skeletal Observations**

| Observation | Classification | 0 mg/kg/dose | 1.5 mg/kg/dose | 10 mg/kg/dose | 20 mg/kg/dose |
|---|---|---|---|---|---|
| No. Litters Evaluated | | 22 | 21 | 20 | 22 |
| No. Fetuses Evaluated | | 177 | 202 | 164 | 183 |
| **Thoracic vertebra(e) cont.** | | | | | |
| Neural arch(es), Extra | M | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (0.5) |
| Neural arch(es), Misaligned | M | | | | |
| No. Litters (%) | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (4.5) |
| No. Fetuses (%)[1] | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (0.5) |

M - Malformation
[1]Not statistically analyzed
No. - Number

# Prenatal and Postnatal Development

**Study title: Pre- and Post-natal Development Study in Rats Following Intravenous Administration of GA-GCB**

**Key study findings:**

- Animals were treated at 1.5, 5 and 17 mg/kg on gestation day (GD) 6, 9, 13, 16 and 20 and on lactation day (LD) 1, 5, 8, 12, 15 and 19
- One F0 generation female (animal number 240) at 1.5 mg/kg/dose died on study Day 33. The cause of death was not apparent from necropsy findings. A small amount of red fluid was found in the abdominal cavity of this animal.
- There were no significant treatment-related effects on F0 necropsy.
- There were no significant treatment-related clinical signs in F1 pups. No significant treatment-related effects were evident for growth, survival, and sexual maturation of F1 pups.
- No significant effect of treatment with GA-GCB was evident from motor activity testing of the F1 pups (basic movements, fine movements, rearing). No significant effect of treatment with GA-GCB was evident from learning and memory assessments of the F1 pups (passive avoidance test).
- No significant effect of treatment with GA-GCB was evident from reproductive performance or fertility of the F1 animals.
- No significant treatment-related adverse effects were observed on pre- and post-natal development

**Report No.**: SHGT-1U0-06-016
**Volume #, and page #**: N/A
**Conducting laboratory and location**: [redacted] b(4)
**Date of study initiation**: January 30, 2007
**Date of study completion**: April 11, 2008
**GLP compliance**: A statement of compliance was included.
**QA reports**: yes ( X ) no ( )
**Drug, lot #, and % purity**: GA-GCB, Lot No. DARTEP06-001002, DARTEP06-005, 98%

**Methods**: Three treatment groups of time-mated female SD rats (n = 25/group) were administered the test article at 1.5, 5.0, or 17 mg/kg (dose volume of 2 mL/kg). One additional group of 25 time-mated females served as the control and received the vehicle, 5% sucrose, 0.01% P20 (Polysorbate-20), 50 mM sodium citrate, pH 6.0. The test article or vehicle was administered to all groups by bolus IV injection on GD 6, 9, 13, 16, and 20, and on LD 1, 5, 8, 12, 15, and 19.

Doses: 0 (5% sucrose, 0.01% P20 (Polysorbate-20), 50 mM sodium citrate, pH 6.0), 1.5, 5.0 and 17 mg/kg
Basis of dose selection: Not provided

Species/strain: SD pregnant female rats
Number/group: 25/group
Route, formulation, and volume: Intravenous injection (bolus), solution, 2 mL/kg
Satellite groups used for toxicokinetics: None
Study design: The study design is shown below (from page 15 of the study report)

| Group Assignments | | |
|---|---|---|
| Group Number | Dose Level (mg/kg/dose) | Number of Time-mated Females |
| 1 | 0 | 25 |
| 2 | 1.5 | 25 |
| 3 | 5.0 | 25 |
| 4 | 17 | 25 |

Parameters and endpoints evaluated: Observations of the parental (F0/P) dams included clinical signs, body weights, and food consumption during gestation and lactation, parturition and F1 litter data, and success in rearing F1 offspring to weaning. Observations of the offspring (F1) included survival at birth and during lactation, individual pup body weights and sex at birth and during lactation, gross abnormalities and physical development, including pre-weaning reflex and sensory evaluations. Litters were weaned on LD 21. On Postnatal Day (PND) 28, 25 male and 25 female F1 pups were randomly selected from each group to continue on study for evaluation of sexual maturation (vaginal opening, preputial separation), behavioral [motor activity and learning and memory (step-through passive avoidance)], and reproductive and fertility assessments. The latter was evaluated by mating animals within treatment groups (1 male:1 female). Mated F1 females were euthanized on GD 13 and evaluated for number of implantations, resorptions, and embryos. F1 males were euthanized after the GD 13 F1 pregnancies had been evaluated. All F0 and F1 animals were subjected to a complete necropsy.

**Results:**

$F_0$ in-life: One F0 female (animal number 240) at 1.5 mg/kg died on Day 33. The cause of death was not apparent from necropsy findings; however, a small amount of red fluid was found in the abdominal cavity. Clinical signs in this animal included decreased activity, rapid breathing, and pale appearance. All other F0 animals survived to scheduled necropsy. In addition, swollen paws, swollen limbs (fore- and/or hindlimbs), and/or swollen face or lips at the 30 minute post dose examination were seen commonly among the F0 females at 15 mg/kg. These were observed after treatment on the first day

of study (GD 6) and continued to be seen in some animals in this group for the remainder of gestation and lactation periods, occurring primarily on treatment days. There were no significant treatment-related effects on body weight or food consumption.

$F_0$ necropsy: There were no significant treatment-related effects. Pregnancy rate was 96% in controls and 100% in each of the treated groups. The gestation index was 100% in each of the control and treated groups. No effect of treatment with GA-GCB was evident from parturition data or F1 litter size data. Mean gestation length, mean number of pups (live, dead, and total) at birth, and mean stillbirth indices in the treated groups were comparable to controls. Similarly, the mean number of live pups per litter at birth and at LD 4, 7, 14, and 21 in the treated groups was comparable to controls. The following table (from page 61 of the study report) shows the summary of F0 parturition and litter data.

Study Number 1255-009
GA-GCB: A Study for Effects on Pre- and Postnatal Development, Including Maternal Function in Rats Following Intravenous Administration

b(4)

Table 10 Summary of P Natural Delivery and Litter Data

| Endpoint | | 0 mg/kg/dose | 1.5 mg/kg/dose | 5.0 mg/kg/dose | 17 mg/kg/dose |
|---|---|---|---|---|---|
| No. Females on Study | N | 25 | 25 | 25 | 25 |
| No. Females Pregnant | N | 24 | 25 | 25 | 25 |
| Females Delivering Litters[1] | N | 24 | 25 | 25 | 25 |
| | % | 96.0 | 100.0 | 100.0 | 100.0 |
| With Stillborn Pups[1] | N | 1 | 1 | 2 | 4 |
| | % | 4.2 | 4.0 | 8.0 | 16.0 |
| With All Stillborn[1] | N | 0 | 0 | 0 | 0 |
| | % | 0.0 | 0.0 | 0.0 | 0.0 |
| Gestation Length (Days) | Mean | 21.9 | 21.8 | 21.8 | 22.0 |
| | SD | 0.34 | 0.41 | 0.37 | 0.35 |
| | N | 24 | 25 | 25 | 25 |
| **No. of Pups at Day 0** | | | | | |
| (Total Pups Born/Litter) | Mean | 11.7 | 11.1 | 11.6 | 11.2 |
| | SD | 1.94 | 1.51 | 1.70 | 1.68 |
| | N | 24 | 25 | 25 | 25 |
| Liveborn/Litter | Mean | 11.7 | 11.0 | 11.6 | 11.0 |
| | SD | 1.93 | 1.62 | 1.79 | 1.88 |
| | N | 24 | 25 | 25 | 25 |

SD - Standard Deviation
N - Number of measures used to calculate mean
No. - Number
[1]Not statistically analyzed

Study Number 1255-009
GA-GCS: A Study for Effects on Pre- and Postnatal Development, Including Maternal Function in Rats Following Intravenous Administration

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Table 10 Summary of P Natural Delivery and Litter Data

| Endpoint | | 0 mg/kg/dose | 1.5 mg/kg/dose | 5.0 mg/kg/dose | 17 mg/kg/dose |
|---|---|---|---|---|---|
| **No. of Pups at Day 0 cont.** | | | | | |
| Stillborn/Litter | Mean | 0.0 | 0.1 | 0.1 | 0.2 |
| | SD | 0.20 | 0.40 | 0.28 | 0.37 |
| | N | 24 | 25 | 25 | 25 |
| Gestation Index | % | 100.0 | 100.0 | 100.0 | 100.0 |
| | N | 24 | 25 | 25 | 25 |
| Stillborn Index | Mean %/Litter | 0.32 | 0.80 | 0.76 | 1.45 |
| | SD | 1.57 | 4.00 | 2.86 | 3.49 |
| | N | 24 | 25 | 25 | 25 |
| Total Implantation Scars/Litter | Mean | 12.0 | 12.0 | 12.3 | 11.9 |
| | SD | 1.76 | 1.38 | 1.74 | 1.24 |
| | N | 24 | 25 | 25 | 25 |

SD - Standard Deviation
N - Number of measures used to calculate mean
No. - Number

Study Number 1255-009
GA-GCS: A Study for Effects on Pre- and Postnatal Development, Including Maternal Function in Rats Following Intravenous Administration

b(4)

Table 10 Summary of P Natural Delivery and Litter Data

| Endpoint | | 0 mg/kg/dose | 1.5 mg/kg/dose | 5.0 mg/kg/dose | 17 mg/kg/dose |
|---|---|---|---|---|---|
| **No. Live Pups/Litter** | | | | | |
| Day 4 (Preculling) | Mean | 11.5 | 11.0 | 11.4 | 10.9 |
| | SD | 1.82 | 1.55 | 2.51 | 1.83 |
| | N | 24 | 24 | 25 | 25 |
| Day 4 (Postculling) | Mean | 8.0 | 8.0 | 7.8 | 7.9 |
| | SD | 0.20 | 0.00 | 1.00 | 0.28 |
| | N | 24 | 24 | 25 | 25 |
| Day 7 | Mean | 8.0 | 8.0 | 7.8 | 7.9 |
| | SD | 0.20 | 0.00 | 1.00 | 0.28 |
| | N | 24 | 24 | 25 | 25 |
| Day 14 | Mean | 8.0 | 8.0 | 7.8 | 7.9 |
| | SD | 0.20 | 0.21 | 1.00 | 0.28 |
| | N | 24 | 23 | 25 | 25 |
| Day 21 | Mean | 7.9 | 8.0 | 7.8 | 7.9 |
| | SD | 0.28 | 0.21 | 1.00 | 0.28 |
| | N | 24 | 23 | 25 | 25 |

SD - Standard Deviation
N - Number of measures used to calculate mean
No. - Number

Study Number 1255-009
GA-GCB: A Study for Effects on Pre- and Postnatal Development, Including Maternal Function in Rats Following Intravenous Administration

b(4)

Table 10 Summary of P Natural Delivery and Litter Data

| Endpoint | | 0 mg/kg/dose | 1.5 mg/kg/dose | 5.0 mg/kg/dose | 17 mg/kg/dose |
|---|---|---|---|---|---|
| **Sex Ratio (% Males per Animal)** | | | | | |
| Pups Day 0 | Mean %/Litter | 52.21 | 46.39 | 54.92 | 49.37 |
| | SD | 13.39 | 14.94 | 15.15 | 18.14 |
| | N | 24 | 25 | 25 | 25 |
| Pups Day 4 (Preculling) | Mean %/Litter | 52.58 | 48.65 | 54.75 | 49.132 |
| | SD | 13.50 | 15.24 | 15.23 | 18.09 |
| | N | 24 | 24 | 25 | 25 |
| Pups Day 4 (Postculling) | Mean %/Litter | 48.68 | 49.48 | 54.67 | 48.50 |
| | SD | 9.05 | 9.38 | 9.65 | 14.10 |
| | N | 24 | 24 | 25 | 25 |
| Pups Day 21 | Mean %/Litter | 48.38 | 49.15 | 54.67 | 48.50 |
| | SD | 9.12 | 9.69 | 9.65 | 14.10 |
| | N | 24 | 23 | 25 | 25 |
| **Pup Survival Indices** | | | | | |
| Viability Index (LD 4) | Mean %/Litter | 99.41 | 84.94 | 96.11 | 99.36 |
| | SD | 2.84 | 19.99 | 14.11 | 2.22 |
| | N | 24 | 25 | 25 | 25 |
| Lactation Index (LD 21) | Mean %/Litter | 99.48 | 95.31 | 100.00 | 100.00 |
| | SD | 2.55 | 20.48 | 0.00 | 0.00 |
| | N | 24 | 24 | 25 | 25 |

SD - Standard Deviation
N - Number of measures used to calculate mean

$F_1$ physical development: There were no significant treatment-related clinical signs or mortality. No significant treatment-related effects were evident from postweaning evaluations of the F1 pups for growth (body weight), survival, and sexual maturation. Overall, there were no significant treatment-related effects on F1 physical development.

$F_1$ behavioral evaluation: No significant effect of treatment with GA-GCB was evident from motor activity testing of the F1 pups (basic movements, fine movements, rearing, and total distance was evaluated over a 20-minute testing intervals). No effect of treatment with GA-GCB was evident from learning and memory assessments of the F1 pups with passive avoidance testing. Overall, there was no significant treatment-related effect on F1 behavioral evaluations.

$F_1$ reproduction: No effect of treatment with GA-GCB was evident from reproductive performance or fertility of the F1 animals. Mating, fertility, and fecundity indices for the F1 treated animals were comparable to controls. The mean number of days-to-mating (copulatory interval) ranged from 2.7 to 4.3 days for the treated groups and was comparable to the 4.0 days in controls. Overall, reproductive performance of the F1 treatment group animals was (24 pregnancies in the control group and 23 pregnancies in each of the treated groups) comparable to the control group. The following table (from page 118-119 of the study report) shows the F1 reproductive and fertility parameters.

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GA-GCB: A Study for Effects on Pre- and Postnatal Development, Including Maternal Function in Rats Following Intravenous Administration

**Table 29 Summary of Reproductive and Fertility Parameters**

| Endpoint | 0 mg/kg/dose | 1.5 mg/kg/dose | 5.0 mg/kg/dose | 17 mg/kg/dose |
|---|---|---|---|---|
| No. Females on Study | 25 | 25 | 25 | 25 |
| No. Females Paired | 25 | 25 | 25 | 25 |
| No. Females Mated | 25 | 23 | 24 | 24 |
| No. Pregnant | 24 | 23 | 23 | 23 |
| Female Mating Index | 100.0 | 92.0 | 96.0 | 96.0 |
| Female Fertility Index | 96.0 | 92.0 | 92.0 | 92.0 |
| Female Fecundity Index | 96.0 | 100.0 | 95.8 | 95.8 |
| No. Males on Study | 25 | 25 | 25 | 25 |
| No. Males Paired | 25 | 25 | 25 | 25 |
| No. Males Mated | 25 | 23 | 24 | 24 |
| No. Males Impregnating a Female | 24 | 23 | 23 | 23 |
| Male Mating Index | 100.0 | 92.0 | 96.0 | 96.0 |
| Male Fertility Index | 96.0 | 92.0 | 92.0 | 92.0 |
| Male Fecundity Index | 96.0 | 100.0 | 95.8 | 95.8 |

No. - Number

b(4)

Study Number 1255-009
GA-GCB: A Study for Effects on Pre- and Postnatal Development, Including Maternal Function in Rats Following Intravenous Administration

**Table 29 Summary of Reproductive and Fertility Parameters**

| Endpoint | | 0 mg/kg/dose | 1.5 mg/kg/dose | 5.0 mg/kg/dose | 17 mg/kg/dose |
|---|---|---|---|---|---|
| Females with Confirmed Mating Day | | 24 | 21 | 22 | 22 |
| Copulatory Interval (Days) | | | | | |
| | Mean | 4.0 | 2.7 | 4.3 | 3.9 |
| | SD | 2.94 | 0.97 | 2.35 | 3.56 |
| | N | 24 | 21 | 22 | 22 |

N - Number of measures used to calculate mean
SD - Standard Deviation

F1 uterine implantation data are summarized in the following table (from page 121 of the study report).

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Table 30 Summary of Maternal and Developmental Observations at Uterine Examination

| Endpoint | 0 mg/kg/dose | 1.5 mg/kg/dose | 5.0 mg/kg/dose | 17 mg/kg/dose |
|---|---|---|---|---|
| No. Females on Study | 25 | 25 | 25 | 25 |
| No. Not Pregnant | 1 | 2 | 2 | 2 |
| No. Pregnant | 24 | 23 | 23 | 23 |
| Pregnancy Index Percent | 96.0 | 100.0 | 95.8 | 95.8 |
| No. Died Pregnant | 0 | 0 | 0 | 0 |
| No. Abortions | 0 | 0 | 0 | 0 |
| No. Early Deliveries | 0 | 0 | 0 | 0 |
| No. Females with All Resorptions | 0 | 0 | 0 | 0 |
| No. Females Pregnant with No Mating Date | 1 | 2 | 2 | 2 |
| No. Females with Viable Embryos Day 13 Gestation | 23 | 21 | 21 | 21 |

No. - Number

[redacted] Study Number 1255-009
GA-GCB: A Study for Effects on Pre- and Postnatal Development, Including Maternal Function in Rats Following Intravenous Administration

b(4)

**Table 30 Summary of Maternal and Developmental Observations at Uterine Examination**

| Endpoint | | 0 mg/kg/dose | 1.5 mg/kg/dose | 5.0 mg/kg/dose | 17 mg/kg/dose |
|---|---|---|---|---|---|
| Corpora Lutea | | | | | |
| No. per Animal | Mean | 17.8 | 18.2 | 18.7 | 17.5 |
| | SD | 2.23 | 2.45 | 3.65 | 2.29 |
| | N | 23 | 21 | 21 | 21 |
| Implantation Sites | | | | | |
| No. per Animal | Mean | 16.0 | 15.5 | 16.1 | 16.4 |
| | SD | 3.17 | 4.11 | 4.54 | 2.52 |
| | N | 23 | 21 | 21 | 21 |
| Preimplantation Loss | | | | | |
| % per animal | Mean | 9.7 | 15.3 | 14.0 | 6.5 |
| | SD | 15.0 | 18.6 | 19.7 | 8.9 |
| | N | 23 | 21 | 21 | 21 |
| Viable Embryos | | | | | |
| No. per Animal | Mean | 15.0 | 14.5 | 14.8 | 15.2 |
| | SD | 2.88 | 3.92 | 5.03 | 2.47 |
| | N | 23 | 21 | 21 | 21 |

No. - Number
SD - Standard Deviation
N - Number of measures used to calculate mean

[redacted] Study Number 1255-009
GA-GCB: A Study for Effects on Pre- and Postnatal Development, Including Maternal Function in Rats Following Intravenous Administration

b(4)

**Table 30 Summary of Maternal and Developmental Observations at Uterine Examination**

| Endpoint | | 0 mg/kg/dose | 1.5 mg/kg/dose | 5.0 mg/kg/dose | 17 mg/kg/dose |
|---|---|---|---|---|---|
| Postimplantation Loss | | | | | |
| % Implants per Animal | Mean | 5.3 | 6.0 | 7.9 | 6.9 |
| | SD | 4.8 | 6.0 | 16.8 | 6.5 |
| | N | 23 | 21 | 21 | 21 |
| Litter Size | | | | | |
| No. per Animal | Mean | 15.0 | 14.5 | 14.8 | 15.2 |
| | SD | 2.88 | 3.92 | 5.03 | 2.47 |
| | N | 23 | 21 | 21 | 21 |
| Resorptions: Early + Late | | | | | |
| No. per Animal | Mean | 0.9 | 1.0 | 1.4 | 1.1 |
| | SD | 0.85 | 0.97 | 3.02 | 1.15 |
| | N | 23 | 21 | 21 | 21 |

No. - Number
SD - Standard Deviation
N - Number of measures used to calculate mean

There were no significant treatment-related macroscopic findings in the F1 offspring.

$F_2$ findings: Not conducted

**Summary**: In a Segment III pre- and post-natal development study in rats, pregnant females were treated at 1.5, 5 and 17 mg/kg. The test article or the vehicle was administered to all groups by bolus IV injection on GD 6, 9, 13, 16, and 20, and on LD 1, 5, 8, 12, 15, and 19. One F0 female at 1.5 mg/kg died on study Day 33. The cause of death was not apparent from necropsy findings; however, a small amount of red fluid was found in the abdominal cavity. There were no significant treatment-related effects on F0 necropsy parameters. There were no significant treatment-related clinical signs in F1 pups. There were no significant treatment-related effects on F1 growth, survival, and sexual maturation. No significant effect of treatment with GA-GCB was evident from motor activity testing or learning and memory assessments of the F1 pups. There were no significant treatment-related effects on F1 reproductive performance or fertility. Overall, GA-GCB did not cause any significant adverse effect on pre- and postnatal development in rats when tested at 1.5, 5 and 17 mg/kg, IV.

### 2.6.6.7 Local tolerance

None

### 2.6.6.8 Special toxicology studies

**2-Week Toxicity Study in Male Rats (SHGT-1U0-06-017)**

**Methods**: This non-GLP study was conducted to evaluate the swelling of the limbs (fore- and/or hind) and/or face/muzzle seen soon after dosing with GA-GCB in the Segment I male fertility study. In this study, two treatment groups of four male SD rats/group were treated with GA-GCB at a dose level of 17 mg/kg/dose (dose volume of either 2 mL/kg as used in the male fertility study or 7.5 mL/kg). A third group of four male rats received diphenhydramine (DPH) at a dose level of 5 mg/kg (0.4 mL/kg) 5 to 10 minutes prior to receiving GA-GCB. A fourth group of four male rats received DPH alone at a dose level of 5 mg/kg, and a fifth group of four males served as the control and received vehicle (5% sucrose, 0.01% Polysorbate-20, 50 mM sodium citrate, pH 6.0). The test article, DPH, and vehicle were administered *via* IV injection twice weekly at 3 and 4 day intervals for two weeks (total of five doses). Clinical signs and mortality were observed twice daily. Body weights were recorded daily. Food consumption was recorded on a weekly basis. Blood samples were collected for histamine and complement (CH50) analyses from all animals at pre-dose and at 15 minutes following each dose. At study termination, necropsy examinations were performed, organ weights were recorded, and selected tissues were collected and preserved for possible further evaluation.

**Results**: All animals survived to scheduled necropsy. Some GA-GCB-treated rats showed swelling of the fore- and/or hindlimbs and/or swelling of the face/muzzle. This response was seen shortly after dosing. Body weights, food consumption, organ weights and gross pathology were not affected by the treatment. Total complement (CH50) levels were unaffected by the treatment. However, histamine increased substantially 15 minutes post dose (2 mL/kg). Histamine increased only after every other dose (Days 1, 8, and 15), and no increase of histamine was seen following administration of DPH alone. The elevated histamine levels following treatment with GA-GCB indicated an allergic type reaction to the GA-GCB.

### 2.6.6.9 Discussion and Conclusions

Velaglucerase alfa was adequately tested in a series of toxicology studies using bolus intravenous (IV) dose administration. These studies included an acute single-dose study, and 3- and 6-month repeat-dose toxicology studies in Sprague Dawley (SD) rats, and a 6-month toxicology study in Rhesus monkeys. The IV route of administration was used in all toxicology studies to conform to the intended clinical route of administration. In addition, reproductive and developmental toxicology studies (Segment I male and female fertility and early embryonic development in rats, Segment II teratology studies in rats and rabbits and Segment III pre- and postnatal development study in rats) were also conducted with Velaglucerase alfa as per the Division's recommendations.

In an IV acute toxicology study in rats, the maximum nonlethal dose was 20 mg/kg. In a 3-month IV toxicology study in rats at 0.85, 3.4 and 17 mg/kg (bi-weekly), there were no significant treatment-related findings up to 17 mg/kg dose, the target organs appeared to be the lung (granulomatous inflammation), liver (small focal cluster of mononuclear cells in sinusoids near a blood vessel ) and testes (decreased weight and tubule formation). The no-observed-adverse-effect-level (NOAEL) could not be determined as treatment-related effects were observed at all doses. Anti-DRX008A antibody was formed at all doses. The basis of dose selection was also not provided. In a 25-Week IV injection toxicology study in rats, animals were treated with DRX008A at 0.84, 3.4 and 17 mg/kg once every 2 weeks. The target organ could not be identified in the absence of any significant histopathology findings. The NOAEL could not be determined as treatment-related effects were observed at all doses. Anti-DRX008A antibody was formed at all doses. In a 6-Month IV injection toxicology study in Rhesus monkeys, animals were treated with DRX008A at 0.84, 3.4 and 17 mg/kg on a bi-weekly basis. The target organ could not be identified in the absence of any significant histopathology findings. The NOAEL may be considered as 17 mg/kg. Significant anti-DRX008A antibody was formed at the high dose at Week 4 and 13. Overall, it appears that Velaglucerase alfa was well tolerated up to 17 mg/kg in rats and monkeys.

Reproductive and developmental toxicology studies (Segment I fertility and early embryonic development in rats, Segment II teratology studies in rats and rabbits and Segment III pre- and postnatal development study in rats) were also conducted with Velaglucerase alfa. In a Segment I male fertility and early embryonic development to

implantation study in rats, at 1.5, 5 and 17 mg/kg (twice weekly), there were no significant treatment-related adverse effects on male fertility parameters. However, two males (each at 1.5 mg/kg and 17 mg/kg) had too few sperm to assess motility. These animals also had relatively high incidences of abnormal sperm and low epididymal sperm concentrations. At necropsy, small testes and epididymides were noted for both animals and while each treated male showed positive confirmation of mating to an untreated female, neither resulted in a pregnancy. The relationship to the treatment was not clear. In a Segment I female fertility and early embryonic development to implantation study in SD rats, at 1.5, 5, and 17 mg/kg (twice weekly), Velaglucerase did not cause any significant adverse effect on female fertility parameters. In an IV Segment II teratology study in rats at 1.5, 5 and 17 mg/kg/day, Velaglucrease alfa was not teratogenic. In an IV Segment II teratology study in rabbits at 1.5, 10 and 20 mg/kg/day, Velaglucerase alfa was not teratogenic. In a Segment III pre- and postnatal development study in rats, Velaglucerase alfa did not cause any significant adverse effect on pre- and postnatal development when tested at 1.5, 5 and 17 mg/kg, IV.

Overall, Velaglucerase alfa has been adequately tested in general toxicology and reproductive toxicology studies. The nonclinical toxicology studies conducted with Velaglucerase alfa adequately support marketing approval of VIPRIV for the intended patient population at the recommended doses. Nonclinical toxicology studies conducted with Velaglucerase alfa provided adequate assurance of safety for its proposed use. Therefore, from a nonclinical standpoint, this NDA is recommended for approval.

### 2.6.6.10 Tables and Figures

Tables and figures were incorporated in the appropriate sections of this review

## 2.6.7 TOXICOLOGY TABULATED SUMMARY

Please see the table under the section above "**Studies reviewed within this submission".**

## OVERALL CONCLUSIONS AND RECOMMENDATIONS

**Conclusions**: This NDA application contains adequate nonclinical studies for the marketing approval of VIPRIV® for the intended patient population at the recommended doses and the nonclinical studies conducted with Velaglucerase alfa provided adequate assurance of safety for its proposed use. Therefore, from a nonclinical standpoint, this NDA is recommended for approval.

**Unresolved toxicology issues (if any)**: None

**Recommendations**: From a nonclinical standpoint, this NDA is recommended for approval.

**Suggested labeling**: The draft labeling of VIPRIV® generally conforms to the format specified under 21CFR 201.57(c)(14) Requirements for PLR (Physician's Labeling Rule) Prescription Drug Labeling. However, the recommended changes should be incorporated as mentioned under "Recommendations on labeling" in the "Executive Summary" section.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

**APPENDIX/ATTACHMENTS**

None

| Application Type/Number | Submission Type/Number | Submitter Name | Product Name |
|---|---|---|---|
| NDA-22575 | ORIG-1 | SHIRE HUMAN GENETIC THERAPIES INC | VELAGLUCERASE ALFA |

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

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/s/

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TAMAL K CHAKRABORTI
01/28/2010

SUSHANTA K CHAKDER
01/28/2010
I concur with the contents of the review and the recommendations.