

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
125293

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology
OND IO *Paul C. Brown 9-10-10*

BLA: 125293

Submission date: March 15, 2010 (complete response to action letter)

Drug: pegloticase (Krystexxa) monomethoxy polyethylene glycol (PEG) modified recombinant mammalian uricase (urate oxidase)

Applicant: Savient Pharmaceuticals

Indication: treatment-refractory gout

Reviewing Division: Division of Pulmonary, Allergy and Rheumatology Products

Introductory Comments: This BLA was originally submitted on October 13, 2008 but it was not approved at that time due to product quality deficiencies. The pharm/tox reviewer and supervisor for the original submission noted several deficiencies and concerns in the nonclinical information submitted to support this BLA but concluded that the BLA could be approved from a pharm/tox perspective. However, the reviewer and supervisor recommended that four additional nonclinical studies be conducted after approval to address the deficiencies and concerns. The pharm/tox reviewer and supervisor for the resubmission also found the information adequate to support approval from the pharm/tox perspective. The reviewer of the resubmission differed slightly in the recommended additional studies. The supervisor for the resubmission agreed with the originally recommended studies.

Conclusions and Recommendations:

General toxicity:

As after review of the original submission, I agree that additional information on the functional consequences of tissue vacuolation induced by pegloticase would be useful. The vacuolation appears to develop over time. There is no understanding whether these vacuoles would continue to expand or spread to additional tissues such that prolonged exposure exceeding that currently assessed nonclinically or clinically would lead to functional consequences not yet observed. Previous experience with pegylated products may not adequately inform about the risk with pegloticase since other products may differ in the degree of pegylation. The development of vacuoles in cells other than macrophages such as was observed in the dog adrenal gland and aortic endothelia is concerning since functional deficits in these tissues could lead to adverse effects. This also may differ from other pegylated products.

The applicant has proposed a study in dogs to address the possible cumulative and functional effects of vacuole formation. Such a study may provide useful information that can not be easily obtained in humans.

I agree that such a study can be a postmarketing requirement.

Reproductive and developmental toxicity:

I agree that the applicant should provide information on the impact of pegloticase on fertility, embryofetal development (in a second species) and pre/postnatal development. These studies were also recommended after review of the original submission.

Overall:

I agree that the nonclinical information supports approval of this BLA, but that it is appropriate to collect additional information on the reproductive and long term toxicity of pegloticase after approval.

Specifically, the following items may be postmarketing requirements:

1. a study in dogs to assess the functional consequences of adrenal and aortic endothelial vacuolation,
2. a study in rats of the effect of pegloticase on fertility,
3. a study in rabbits of the effect of pegloticase on embryofetal development, and
4. a study in rats of the effect of pegloticase on pre-postnatal development.

I agree with the labeling suggestions outlined in the supervisor's secondary review.

INTEROFFICE MEMO

TO: BLA 125293
KRYSTEXXA™ (pegloticase)

FROM: Molly E. Topper, Ph.D.
Pharmacology/Toxicology Supervisor
Division of Pulmonary, Allergy and Rheumatology Products

DATE: August 13, 2010

Savient Pharmaceuticals, Inc (the applicant) resubmitted their BLA 125293 on March 15, 2010 for KRYSTEXXA™ (pegloticase) for the proposed indication of the treatment of chronic gout in patients refractory to conventional therapy. The proposed recommended dose and regimen for adult patients is 8 mg given as an intravenous infusion every two weeks. Pegloticase is a monomethoxypoly (ethylene glycol)-modified (PEGylated) homotetramer recombinant porcine/baboon chimeric uricase enzyme. The uricase enzyme catalyzes the conversion of uric acid to allantoin. There are approximately 9 ± 1 strands of 10 kDa mPEG per uricase monomeric subunit.

The resubmitted application was in response to the Complete Response (CR) action taken on July 31, 2009 to the applicant's original application submitted October 31, 2008 for the same indication. As described in detail in the Complete Response letter dated July 31, 2009, the CR action was taken due to product quality deficiencies. There were no nonclinical or clinical deficiencies preventing approval of the original application.

The original BLA submission was reviewed in the Division of Anesthesia, Analgesia and Rheumatology Products (DAARP). Dr. BeLinda Hayes was the nonclinical reviewer and Dr. R. Daniel Mellon was the Pharmacology/Toxicology Supervisor (see Dr. Hayes' original review and Dr. Mellon's original secondary review for details of the submissions). Dr. Hayes recommended approval of the BLA pending agreements on labeling and post-marketing studies. The original recommendations in the primary review for post-marketing studies included the following:

1. Conduct a fertility study in the rat model
2. Conduct an embryo-fetal development study in the rabbit model (Segment 2)
3. Conduct a peri-natal and post-natal development study in the rat model (Segment 3)
4. Conduct in vitro and/or ex vivo assessments of aortic endothelial cell and adrenal function following co-culture with pegloticase

Following completion of the primary review, a teleconference between DAARP and Savient Pharmaceuticals was held on July 9, 2009 to discuss the post-marketing study number 4 above and the safety concerns of vacuole accumulation in multiple tissues with specific concern focusing on the adrenal cortical cells and endothelial cells of the aorta. The applicant proposed an 18-month dog study (12 months dosing and 6 month recovery) to evaluate the impact of cytoplasmic vacuoles in the adrenal gland and the aortic outflow tract of the heart. These discussions and the Division's agreement with the outlined chronic toxicology study proposed by the Sponsor were captured in Dr. Hayes' Memo to File dated July 20, 2009. This proposal supercedes the original post-marketing recommendation number 4 above. Dr. Paul Brown's tertiary review of the original application concurred with the Division's recommendations. The regulatory project manager for this application, Ms. Ramani Sista contacted the sponsor July 29, 2010 and confirmed that the 18-month dog study has not been initiated to date; however, the applicant does have a protocol for this study.

The Division of Pulmonary, Allergy and Rheumatology Products (DPARP) reviewed the resubmission of the BLA 125293 as the rheumatology products were transferred from DAARP to DPAP (now DPARP) in March 2010. Dr. Asoke Mukherjee was the primary nonclinical reviewer of the resubmission. No new nonclinical data were submitted in the resubmission. However, updated labeling was included in the resubmission. Dr. Mukherjee recommended approval of the BLA based on the data submitted and reviewed in the original application with recommendations of post-marketing studies and minor changes to the applicant's labeling. Dr. Mukherjee's recommended post-marketing studies were identical to those recommended by Dr. Hayes in the original BLA review with the exception of study number 4. Dr. Mukherjee did not agree with this proposed post-marketing study as his interpretation of the 6-month dog study showed no toxicity related to the vacuole formation in the adrenal gland and heart. The following are Dr. Mukherjee's post-marketing requirement recommendations:

1. Conduct a fertility study in the rat model (segment 1)
2. Conduct a segment 2 reproductive safety study in pregnant rabbits.
3. Conduct a peri-natal and post-natal development study in the rat model (segment 3).

Recommendation on approvability

From the nonclinical perspective, I concur with Dr. Mukherjee's recommendation for approval of the resubmission of BLA 125293 pending agreement on the proposed labeling and on post-marketing requirement studies (see below).

Recommendation for nonclinical studies

I concur with the three post-marketing requirements recommended by both Dr. Hayes and Dr. Mukherjee. In addition to these three, a fourth post-marketing

requirement study that was proposed by the sponsor July 9, 2009 is recommended. This proposed study was not supported by Dr. Mukherjee. As was summarized in Dr. Hayes' original BLA review, vacuolation in the splenic red pulp, liver Kuppfer cells, duodenum, jejunum, adrenal cortex, and the great vessels of the heart in the 39-week dog toxicology study with no reversibility after a 12-week recovery period was observed. The lack of demonstrated clearance of these vacuoles and the potential continuous accumulation of PEG in the vacuoles in these tissues raise questions of potential adverse effects on the function of the adrenal gland and the heart with chronic clinical dosing. Therefore, I support Dr. Hayes' and Dr. Mellon's recommendation for the applicant to complete a fourth nonclinical post-marketing study in dogs to evaluate chronic toxicity of this product. The following total post-marketing requirements should be relayed to the sponsor:

1. Conduct a fertility study in the rat model
2. Conduct an embryo-fetal development study in the rabbit model (Segment 2)
3. Conduct a peri-natal and post-natal development study in the rat model (Segment 3)
4. Conduct an 18-month study in dog to evaluate the impact of cytoplasmic vacuoles in the adrenal gland and the aortic outflow tract of the heart.

Recommendations on labeling: I concur with some of the labeling recommendations proposed by Dr. Mukherjee. However, I recommend the following recommended modifications from the Sponsor's proposed labeling. These changes are presented as track changes to the Applicant's label in column 2. NOTE: The final language for the product label may change, following discussions with the review team and input from the tertiary pharmacology toxicology reviewer, Dr. Paul Brown.

(b) (4)



Molly E. Topper August 13, 2010
Molly E. Topper, Ph.D.
Pharmacology/Toxicology Supervisor

0037DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number: 125293
Supporting document/s: 0037
Applicant's letter date: March 15, 2010
CDER stamp date: March 15, 2010
Product: Krystexxa
Indication: Chronic gout
Applicant: Savient Pharmaceuticals Inc.
Review Division: Division of Pulmonary, allergy and rheumatology
Drug Products
Reviewer: Asoke Mukherjee Ph.D.
Supervisor/Team Leader: Molly Topper, Ph.D.
Division Director: Badrul Chowdhury, M.D., Ph.D.
Project Manager: Ramani Sista

Asoke Mukherjee
8/10/2010
Molly E Topper 8/10/2010

Template Version: December 7, 2009

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TABLE OF CONTENTS

1	EXECUTIVE SUMMARY	3
1.1	RECOMMENDATIONS	3
1.2	BRIEF DISCUSSION OF NONCLINICAL FINDINGS	5
2	DRUG INFORMATION	6
3	STUDIES SUBMITTED.....	16
4	PHARMACOLOGY.....	17
4.1	PRIMARY PHARMACOLOGY:	17
9	REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	17
9.1	FERTILITY AND EARLY EMBRYONIC DEVELOPMENT:	17
9.2	EMBRYONIC FETAL DEVELOPMENT.....	17
9.3	PRENATAL AND POSTNATAL DEVELOPMENT	18
11	INTEGRATED SUMMARY AND SAFETY EVALUATION.....	19
12	APPENDIX/ATTACHMENTS: NIL	21

Table of Tables

Table 1	8
Table 2	11

Table of Figures

Figure 1	10
Figure 2	10
Figure 3	12
Figure 4	13

1 Executive Summary

1.1 Recommendations: The BLA can be approved based on the non-clinical data reviewed in the review dated Oct 31, 2009 and the present review. Specific non-clinical post-marketing requirements (PMR) for the approval of the product are shown under section 1.1.2. The reviewer also recommends non-clinical portion of the label as shown under section 1.1.3.

1.1.1 Approvability: Yes, on the basis of non-clinical reviews

1.1.2 Additional Non-Clinical Recommendations:

Post-Marketing Requirements (PMR):

1. Conduct a fertility study in the rat model (segment 1)
2. Conduct a segment 2 reproductive safety study in pregnant rabbits.
3. Conduct a peri-natal and post-natal development study in the rat model (segment 3).

1.1.3 Labeling:

The following non-clinical recommendation was given to the package insert. The difference from the applicant's label is **bolded**. Recommendation for the package insert in the present review is shown below.

(b) (4)

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immediately following
this page as B4 (CCI/TS)

1.2 Brief Discussion of Nonclinical Findings:

Pegloticase is a pegylated uricase enzyme developed for the treatment of hyperuricemia in gout patients. The original BLA submission was October 13, 2008. Dr. Belinda Hayes was the original nonclinical reviewer for this application. The original non-clinical review was completed on Oct 31, 2009 and details of the non-clinical findings are referred to in the present review. However, the product was not approved previously due to chemistry and manufacturing deficiencies. The present review summarized non-clinical recommendations as PMRs related to reproductive safety of the product. These recommendations are part of the approval of the product.

Non-clinical toxicity findings in Pharmacology/Toxicology review dated Oct 31, 2009 are summarized below.

The non-clinical toxicity assessment was made on the basis of a 39-week toxicity study in dogs. The treatment induced immunogenic and antigenic responses following repeated injections for 39 weeks by IV route resulted in the development of non-neutralizing antibodies to the product. In addition vacuolation in several systemic organs e.g., adrenal cortex, duodenum, jejunum, liver, spleen and intimal cell of aortic outflow (mostly in male dogs) was noted in the absence of other organ system toxicity. No segment 2 reproductive toxicity was noted in pregnant rats.

2 Drug Information

2.1 Drug:

Krystexxa is expressed in E. coli system by recombinant DNA technology.

2.1.1 CAS Registry Number (Optional): 885051-90-1

2.1.2 Generic Name:

Pegloticase Injection, PEG-Uricase

2.1.3 Code Name: Nil

2.1.4 Chemical Name:

Oxidase, Urate (synthetic *Sus scrofa* variant pigKS-ΔN subunit), homotetramer, amide with α-carboxy-ω-methoxypoly(oxy-1,2-ethanediyl)

des-(1-6)-[7-threonine,46-threonine,291-lydsine,301-serine]uricase (EC 1.7.3.3, urate oxidase) *Sus scrofa* (pig) tetramer, non acetylated, carbamates with α-carboxy-ω-methoxypoly(oxyethylene)

Molecular Formula/Molecular Weight:

(b) (4) Where (b) (4)

MW: approximately 497 kDa (b) (4)

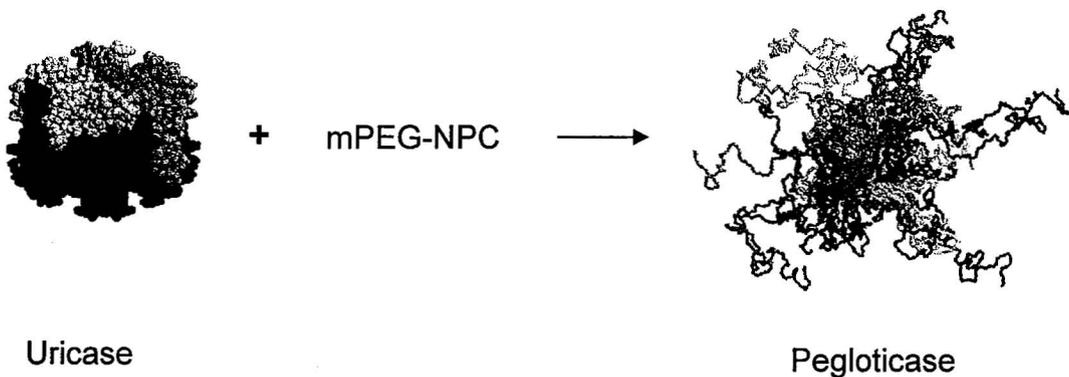
2.1.6 Structure:

```
{ [H3C-O- (CH2CH2-O)m-CO-]n-NH- [TYKKNDEVEF VRTGYGKDMI KVLHIQRDGG YHSIKEVATT  
VQLTLSSKKD  
YLHGDNSDVI PTDTIKNTVN VLAKFKGIKS IETFAVTICE HFLSSFKHVI  
RAQVYVEEVP WKRFEKNGVK HVHAFIYPTPT GTHFCEVEQI RRGPPVIHSG  
IKDLKVLKTT QSGFEGFIKD QFTTLPEVKD RCFATQVYCK WRYHQGRDVD  
FEATWDTVRS IVLQKFAGPY DKGEYSPSVQ KTLYDIQVLT LGQVPEIEDM  
EISLPNIHYL NIDMSKMGLI NKEEVLLPLD NPYGKITGTV KRKLSSRL] }4
```

Wherein, (b) (4) and each uricase monomeric subunit having the amino acid sequence listed above.

Pegylation:

On the average 9 ± 1 strands of methoxypoly(ethylene glycol) (10kDa) are attached to Lysine(K) residues per uricase monomeric subunit or 36 (b) (4) strands of mPEG per pegloticase monomer.



2.1.7 Pharmacologic class:

Bio-uricolytic agent (therapeutic protein)

Enzyme

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

IND#	Drug	Status	Division	Indication	Status Date	Sponsor
10122	Uricase (recombinant E. coli Bio Technology General Corp.) PEG conjugate	Active	DAARP	Treatment of symptomatic gout	12/14/2001	Savient Pharmaceuticals

2.3 Clinical Formulation

2.3.1 Drug Formulation:

The drug product is a colorless liquid at (b) (4) concentration of Pegloticase containing 8 mg/ml of uricase protein. The composition of a single dose drug product is shown from the applicant's table 1 below. About (b) (4) of drug product is supplied in a 2 ml vial for IV infusion at pH 7.0 to 7.6.

Table 1

Ingredient	Quantity/ ml	Specification	Concentration W/W	Function
Pegloticase	(b) (4)	In house specification	(b) (4)	Active Pharmaceutical Ingredient
Disodium hydrogen phosphate dihydrate	2.18 mg	USP	(b) (4)	(b) (4)
Sodium dihydrogen phosphate dihydrate	0.43 mg	USP	(b) (4)	(b) (4)
Sodium Chloride	8.77 mg	USP	(b) (4)	(b) (4)
Water for injection	(b) (4)	USP		(b) (4)

2.3.2 Comments on Novel Excipients:

There was no novel excipient used in the drug product formulation.

2.3.3 Comments on Impurities/Degradants of Concern:

Leachables and extractables were examined from the container closure systems to identify possible impurities in the drug substance and drug product as referred in the Complete Response.

The container closure system of the drug substance is consisted of a blue (b) (4) screw cap (b) (4).

The stopper and vial cap used for the proposed commercial batch (process C) consisted of a 13 mm gray Teflon-faced (b) (4) rubber injection stopper with aluminum crimp and flip-off cap. Both supplied by (b) (4). The applicant stated that leachable and extractable data for the container closure system was provided in the (b) (4). However, no letter of authorization was submitted in the BLA. A report 2009003788 titled "Screening study using drug product placebo on Savient's drug product and drug substance packaging system" was submitted for a review.

Study Report:

Title: Screening study using drug product placebo on Savient drug product and drug substance packaging systems.

The (b) (4) cap used in a 100 ml glass bottle containing phosphate buffered saline was used to examine the presence of leachables and extractables from the screw cap. In addition, 13 mm (b) (4) gray Teflon (b) (4) rubber stopper used for a glass vial containing the phosphate buffered saline was used for the determination of leachables and extractables of the unit dosage form. The applicant stated that the use of placebo solutions ensured interference in the analytical methods. The contract laboratory stated that the assay was conducted over a 4-week period instead of 8-week period routinely used for this type of study.

Buffer filled bottles and vials were placed invertedly. Leachable and extractable data were obtained at the baseline on day 0 and subsequently on at the end of week 4. The leachable and extractables were examined on day 3 for the drug substance. Additionally, any extractable impurity identified in the incubation system was spiked with the placebo solutions to determine the possibility of assay interference.

The caps and stoppers were treated with 0.5N sodium hydroxide and washed thoroughly before the use. Extractables from (b) (4) caps were conducted from pieces cut from the caps using phosphate buffered saline and isopropanol as solvents. Extracts were analyzed using HPLC method by a UV detector system and mass spectrometric (MS) detection methods.

HPLC and MS data did not show extractable residues in the phosphate buffered saline from (b) (4) caps. These chromatograms are shown in following figures from the applicant's submission below.

Figure 1



Figure 2

(b) (4)



Therefore, (b) (4) caps did not show extractable impurities in the phosphate buffered saline used as the vehicle for the drug substance.

Data for the isopropyl alcohol extracts suggests that HPLC system detected impurities using UV detection systems as listed by the applicant with respect to the retention time below.

Table 2

(b) (4)



The applicant provided following chromatogram from replicated assays as shown below.

Figure 3

(b) (4)



Among the above peaks, (b) (4) at (b) (4) retention time were of importance with respect to binding with the drug substance protein or modifying the protein based on the RED-OX activities of impurities. The structure for the peak at (b) (4) was identified by mass spec fragment technique as (b) (4).

However, presence of these extractable impurities was not detected from the mass spectrometric detection system as shown in the figure provided in the applicant's submission.

Figure 4



For the accelerated extractable study, bottles with (b) (4) caps and vials containing rubber stoppers were filled with 25 and 1.25 ml of phosphate buffered saline, respectively, and representatives for the drug substance and drug product systems. These containers were stored at 50 °C for one-month. The applicant stated that leachables were detected qualitatively on day 0 and four weeks thereafter. Presence of the following components of leachables was looked for detection.

For stoppers: (b) (4)

For caps: (b) (4)

Results of the study did not show leachable impurities in the phosphate buffered saline from the (b) (4) caps on days 0, 3 and week 4. Similarly, no leachables were present in the phosphate buffered saline from the rubber stoppers on day 0 and week 4.

Above experiments signified that no leachables and extractables were expected in the drug substance and product from (b) (4) caps and rubber stoppers, respectively, on storage. **Leachable and extractable data also needs to relate to the product stability testing data that is under review by a product quality team.** A summary of data indicating that no extractable and leachable substances were present in the buffer used for the drug substance and drug product is shown from the applicant's table below.

(b) (4)



Applicant's conclusion of the leachable and extractable study report:

Extractables and leachables from the (b) (4) caps and rubber stoppers were not expected in the drug substance and drug products, respectively.

Discussion and recommendation by the reviewer:

The method developed by the (b) (4) did not provide data on impurities in the (b) (4) caps and rubber stoppers. The analytical methods, sensitivity of the method and validation of the method applied in the extractable and leachable study # 2009003788 needs to be further reviewed by the drug product reviewers. Therefore, the reviewer could not comment on the appropriateness of the qualitative method applied for the study # 2009003788 especially in the absence of impurities when mass spectrometric detection system was used. Due to this deficiency, the reviewer recommends that the applicant consider providing the following data for the development of methods for the post approval stability protocol for IV infusion identified as document # PRT-QA-077. For the time being, the present study would be accepted for the approval from Pharmacology/Toxicology point of view pending the required data.

The following post-marketing commitments (PMC) were sent to product quality reviewer on July 12, 2010 to consider. However, these comments were not part of the Pharm/Tox review and needs to be incorporated in the approval letter only if the product reviewer considers appropriate.

Proposed Post Marketing commitments (PMC) to the product reviewer:

1. Identify potential extractable and leachable impurities based on the manufacturing of the caps and stoppers from the supplier's DMF, develop reference standards for the impurities, limit of detection and analytical methods.
2. Apply these methods for the proposed post-marketing study # PRT-QA-077 according to its proposed time line to determine leachables and extractables quantitatively. A time line for the study needs to be determined.
3. Based on the data obtained from #1 and #2 items above, determine the structural alert for mutagenicity for each impurity. If any impurity poses a structural alert for mutagenicity, in vitro genetic toxicology study evaluating the potential mutagenicity of the impurity would be needed. In case a mutagenic impurity is detected, the limit of mutagenic impurity present as leachables and extractables in the drug product needs to confirm the CDER guidelines of exposure not to exceed 1.5 ug daily for the chronic treatment.

2.4 Proposed Clinical Population and Dosing Regimen:

Krystexxa is indicated for the treatment of gout for patients who are refractory to the conventional treatment up to 8 mg/IV dose as infusion every 2 weeks.

2.5 Regulatory Background

Pharmacology/Toxicology data for the BLA was reviewed for the submission of the original BLA dated Oct 31, 2009. A complete response (CR) was issued on July 31, 2009 due to CMC issues. The CR letter stated that the batch used for the Phase 3 studies was not represented in the proposed commercial batch. The (b) (4) for the clinical study and intended commercial lots were different. Therefore, further clinical studies would be needed to establish the efficacy and safety of the product. Alternatively, validation of similarities between the clinical batch and commercial batch would be necessary. The applicant was asked to respond to several deficiencies related to the stability and the presence of

several leachables and extractable impurities in the drug product. The applicant was also asked to submit a modified draft label for the Complete Response.

The pharmacology/toxicology review of data indicated several deficiencies that would need Post Marketing Requirements (PMR) to address the deficiencies. However, these deficiencies were officially not conveyed to the applicant because the approval letter was not issued. The applicant submitted a response to the CR and a draft package insert in this amendment of the BLA. The guidelines on container closure systems for packaging human drugs and biologics 1999 stated that the degree of concern for leachables and extractables are high from the injectable dosage form. In addition, the following issues were raised in the guideline were considered:

1. Packaging system found acceptable for one drug product is not automatically assumed to be appropriate for another.

Based on the review, appropriate recommendations for the approval from the non-clinical perspective would be made in this review (see above under impurities and degradants).

3 Studies Submitted

The following study was submitted in the CR response.

1. Screening study using drug product placebo on Savient's Drug Product and Drug Substance packaging system.

3.1 Studies Reviewed

1. Screening study using drug product placebo on Savient's Drug Product and Drug Substance packaging system.

The data were reviewed for this review to determine if any safety issues were raised from the leachables and extractables from the stoppers and caps. However, a detail review would be done by the product reviewer.

3.3 Previous Reviews Referenced

The Pharmacology / Toxicology review dated Oct 31, 2009 was referenced to this review.

4 Pharmacology

4.1 Primary Pharmacology:

The summary of product data indicated that comparison of IV and SC route of administration had high bioavailability of the formulation for uricase activity in the non-clinical model. However, the subcutaneous route was not chosen due to high immunogenicity and lower bioavailability in the initial Phase 1 clinical trial. No new Pharmacology studies were submitted to the BLA. A Pharmacology/Toxicology review written in Oct 31, 2009 is referred for a full detail.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development:

No segment 1 data in rats were provided in the BLA. The review of Pharmacology/Toxicology data dated Oct 31, 2009 recommended the conduct of segment 1 reproductive toxicity study in rats as one of the Post Marketing Requirements (PMR). The present reviewer concurs with the recommendation considering that about 10 to 50% of the drug is distributed in the testes and ovaries compared to the plasma following a single IV dose of the drug in rats. Therefore, conduct of the fertility study in male and female rats is an appropriate recommendation as one of the PMRs.

9.2 Embryonic Fetal Development

The reviewer for the Pharmacology/Toxicology Review dated Oct 31, 2009 recommended for the conduct of a segment 2 reproductive safety study in rabbits to fulfill the requirement of ICH M3 guidelines for non-clinical study. The applicant conducted a segment 2 reproductive safety study in rats that did not show any toxicity to embryos, malformation and teratogenicity. However, extramedullary hematopoiesis was noted in the spleen in all treated pregnant rats along with the severe depletion of uric acid in the maternal blood. Extramedullary hematopoiesis could be related to the mechanism of the drug and metabolism of uric acid. The reviewer concluded for this review that the increase uric acid metabolism would potentiate the cell cycle and cell turnover of the circulating cells that could enhance the activity of splenocytes to induce extramedullary hematopoiesis. Therefore, apart from the pharmacodynamic-related effects, there was no defined maternal toxicity observed in the segment 2 reproductive safety study in rats. The extramedullary hematopoiesis observed in the teratogenicity study in rats was also associated with persistent vacuolation of splenocytes with a reduction of TNF secretion in response to LPS. These data clearly demonstrate a

pharmacodynamic activity related to the drug. A slow dissociation of PEG from drug substance in the splenocytes could also be responsible for the vacuolation process. This is evident from the recovery data that after 8 weeks of treatment free period, number of vacuoles were reduced.

The fetal body weight change at 10 mg/kg might not be related to the treatment although it was statistically significant due to a small change in the values and absent of the similar effect in the high dose group.

The reviewer agreed with the conclusion of the previous primary reviewer that there was no statistically significant increase in the malformation and variations due to the treatment. The pharmacokinetic data in pregnant rats indicated treatment related increase in the exposure and uricase activity in the maternal blood. However, it is stated in the previous review that the drug was not transferred to the fetuses across the placental barrier based on the absence of the drug in fetal blood. Therefore, it is important to establish if Pegloticase crossed the placental barrier in the rabbit model before conducting a rabbit segment 2 study as previously recommended by the reviewer.

The review dated Oct 31, 2009 recommended the following segment 2 study as one of the post-marketing requirements:

Conduct a segment 2 reproductive toxicity study in rabbit model.

Recommendation for the present review as a PMR :

The reviewer recommends the sponsor conduct a segment 2 reproductive safety study in rabbits.

9.3 Prenatal and Postnatal Development

The applicant did not conduct a pre-natal and post-natal study as recommended in the ICH M3 guidelines on non-clinical safety of drugs. Therefore, the review dated Oct 31, 2009 recommended that the sponsor "Conduct a peri-natal and post-natal development study in the rat model (segment 3)".

Recommendation for the present review:

The present reviewer concurs with this recommendation to conduct a segment 3 pre-natal and post-natal study in rats because the recommended study would be an important safety evaluation on the effect of Pegloticase on the rapidly dividing cells of oral mucosa if it is secreted in the milk and transferred to new born pups through breast feeding during the weaning period. One can argue that this recommendation should only be given if Pegloticase is excreted in the milk of nursing mothers. However,

considering the variability of nursing habits and the amount excreted in the milk, a bioassay in rats would be appropriate to understand the role of Pegloticase for pre-natal and postnatal development.

11 Integrated Summary and Safety Evaluation

Pharmacology/Toxicology data were previously reviewed on Oct 31, 2009 and recommended approval of the product on the basis of non-clinical data with following post-marketing requirements (PMR):

1. Conduct a fertility study in the rat model (Segment 1)
2. Conduct a embryo-fetal development study in the rabbit model (Segment 2)
3. Conduct a peri-natal and post-natal development study in the rat model (Segment 3)
4. Conduct an *in vitro* assessment of aortic endothelial cell function following co-culture with pegloticase.

The above recommendations were made following a review of following major studies:

- 1. 12-week and 39-week toxicity studies in dogs.**
- 2. A 54-day toxicity study in rats**
- 3. A segment 2 reproductive toxicity study in rats.**

The 39-week toxicity study in dogs showed vacuolation in several systemic organs e.g., adrenal cortex, duodenum, jejunum, aortic outflow of the heart, liver and spleen. Although these vacuoles were present in the lymphoid organs and drug metabolizing systems, the presence of vacuoles in the intimal lining of the aortic outflow of the heart and adrenal glands was of concern as stated in the previous review dated Oct 31, 2010. However, female dogs did not show any vacuoles in the aortic outflow of the heart. Vacuolation was not completely reversible within 12 weeks of drug free period although the number of vacuoles was reduced during the recovery. Mechanistic staining techniques showed vacuoles were present in the macrophages in tissues as if macrophages phagocytized pegloticase with the exception of adrenal cortex. The reviewer concluded that there was no clinical, pathological attributes to the vacuole formation in the tissue and the vacuolation process represented a normal physiological function. However, a recommendation to conduct *in vitro* cell culture of endothelial cells in the presence of Pegloticase was made by Dr. Hayes considering activated endothelial cells could play a role on the development of atherosclerosis.

Following the issue of a Complete Response for the original application, a Memo was filed to this BLA dated July 20, 2009. This Memo reconsidered the originally proposed PMR to conduct *in vitro* and/or *ex vivo* assessments of aortic endothelial cell and adrenal function following co-culture with pegloticase. On July 13, 2009 the sponsor submitted a proposal to conduct an 18-month study in dog to evaluate the impact of

cytoplasmic vacuoles in the adrenal gland and the aortic outflow tract of the heart. This study proposed dosing the dog for 12-months with up to 5 fold the human dose every 14 days followed by a recovery period of 6 months. The Division accepted this proposal to address the potential accumulation of vacuoles with chronic dosing and to address the potential reversibility of the vacuolation. As of August 2, 2010, the proposed study has not been initiated.

The previous Pharmacology/Toxicology review also recommended the sponsor to conduct the segment 1 and 3 reproductive safety studies in rats and a segment 2 reproductive safety study in rabbits. The review dated Oct 31, 2009 recommended approval of the BLA on the basis of non-clinical data with post marketing recommendations stated above.

Conclusion of the present review:

It is considered and concluded in the present review that the applicant conducted the long-term toxicity study in a suitable species considering the PK-PD data presented in the BLA. The reviewer concluded that appearance of vacuoles is related to the uptake of the drug by phagocytic cells, a slow degradation process for the drug substance and altered cell turnover due to neutralization of uric acid. In the absence of clearly defined systemic toxicity, presence of vacuoles is not considered to be of toxicological importance and a similar non-clinical effect is expected from pegylated proteins.

The review dated Oct 31, 2010 discussed the relationship between vacuole formation and atherosclerosis. Development of atherosclerosis involves lipid accumulation, activation of platelets and deployment of a sensitive species to orchestrate the atherosclerotic process. The chronic toxicity data in the BLA did not indicate presence of any lesions in the major artery except vacuolation of intimal cells in a major artery mostly in male dogs at the high dose. The presence of inflammation in the intimal layer of the major artery was ruled out upon reexamination of the slide by an independent pathologist. Therefore, it is unlikely that any in vitro or in vivo non-clinical data would provide additional information on the prothrombotic events or cardiovascular safety concern in gout patients. The reviewer conceived that further cardiovascular risk assessment needs to be done from the clinical database available to date.

The reviewer agreed on the PMRs for conducting segment 1 and 3 reproductive toxicity studies in rats on the basis of the distribution of the drug in gonads and possibility of excretion of the drug in the breast milk. The reviewer also recommends conduct of a second segment 2 reproductive safety study in rabbits to evaluate direct and indirect toxicity to fetuses when pegloticase is injected to pregnant rabbits.

A study of extractable and leachable impurities was conducted according to the guidance for industry on container closure systems for packaging human drugs and biologics. Consult product manufacturing review for the safety related to the container closure systems.

On the basis of the review of data and previous Pharmacology/Toxicology review dated Oct 31, 2009, the present review recommends approval of the BLA with the post marketing requirements (PMR) as shown in the executive summary.

12 Appendix/Attachments: Original pharmacology/toxicology review completed on Oct 2009.

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology
OND IO *Paul Brown 7-31-09*

BLA: 125293

Submission date: October 31, 2008

Drug: pegloticase (Krystexxa) monomethoxy polyethylene glycol (PEG) modified recombinant mammalian uricase (urate oxidase)

Applicant: Savient Pharmaceuticals

Indication: treatment-refractory gout

Reviewing Division: Division of Anesthesia, Analgesia and Rheumatology Products

Introductory Comments: The pharm/tox reviewer and supervisor noted several deficiencies and concerns in the nonclinical information submitted to support this BLA but concluded that the BLA could be approved from a pharm/tox perspective largely because of previous agreements with the applicant on what constituted an adequate nonclinical package for approval. However, the reviewer and supervisor recommended that additional studies be conducted after approval to address the deficiencies and concerns.

General toxicity:

Toxicity of pegloticase was assessed in 12-week and 39-week studies in dogs in which pegloticase was administered intravenously every 5 days. Vacuolation was noted in both of these studies. More tissues showed vacuolation in the 39 week study compared to the 12 week study. Tissue vacuolation was dose-related with most vacuolation appearing at the high dose in the 39 week study of 10 mg/kg dose. Most vacuolation was associated with macrophages; however, at 10 mg/kg there was increased vacuolation in adrenal cortical cells and aortic endothelial cells. No functional consequences of the vacuolation were noted in these dog studies although a thorough assessment of the possible functional consequences, particularly in the adrenal and aortic cells, was not included in these studies. The reviewer and supervisor recommended additional evaluation of the functional consequences of vacuolation in these critical tissues.

Secretion of TNF in response to LPS was used as a measure of spleen macrophage function in cells collected from rats treated with pegloticase. A decrease in response to LPS stimulation was observed in vacuolated macrophages.

Reproductive and developmental toxicity:

Fertility:

The applicant did not conduct fertility studies. The reviewer and supervisor recommended that a fertility study be conducted in rats as a postmarketing requirement.

Embryofetal development:

The applicant conducted an embryofetal development study in rats. No teratogenic effects were noted at doses of 5, 10 or 40 mg/kg. The reviewer and supervisor recommended that

a second embryofetal development study be conducted in another species as a postmarketing requirement.

Pre/postnatal:

No studies were conducted to assess effects of pegloticase in the pre/postnatal timeframe. The reviewer and supervisor recommended that a pre/postnatal study be conducted in rats as a postmarketing requirement.

Carcinogenicity:

Long term carcinogenicity studies with pegloticase have not been conducted. In discussing the lack of carcinogenicity evaluation, the supervisor noted that the applicant was not previously asked to evaluate carcinogenicity and there do not appear to be effects on cell growth or survival or any clear immunosuppressive effects.

Labeling:

The applicant had not proposed a pregnancy category. The reviewer and supervisor recommended a pregnancy category of C primarily because the evaluation of the effect of pegloticase on reproduction and development was incomplete.

The applicant proposed wording that described the vacuolation in the animal toxicology section of labeling. The reviewer and supervisor agreed with describing the vacuolation in this section of labeling although they recommended some changes to the wording. They recommended including that vacuolation in macrophages appeared to be associated with a decreased functional response of macrophages to LPS.

Conclusions and Recommendations:

General toxicity:

I agree that additional information on the functional consequences of tissue vacuolation would be useful. The vacuolation appears to develop over time. There is no understanding whether these vacuoles would continue to expand or spread to additional tissues such that prolonged exposure exceeding that currently assessed nonclinically or clinically would lead to functional consequences not yet observed. The development of vacuoles in cells other than macrophages such as was observed in the dog adrenal gland and aortic endothelia is concerning since functional deficits in these tissues could lead to adverse effects.

The Agency's concern about the possible functional consequences of tissue vacuolation was discussed with the applicant in a telephone conference on July 9, 2009. The applicant was asked to propose a strategy for assessing the functional consequences of the vacuoles. In vitro and in vivo strategies were briefly discussed. The applicant has since proposed a study in dogs that are dosed every other week for 12 months with another 6 months of dose-free follow up. Parameters to assess adrenal and aortic function would be measured at various times during the study. Assessment of vacuolation in circulating monocytes would also be included and may provide a biomarker that could also be used in humans. Such a study may provide useful information that can not be easily obtained

in humans. The study may provide a clearer understanding of the long term consequences of vacuolation and a means of assessing whether vacuolation was occurring in humans. The results of such a study may be used to modify the labeling of the product and to alter how the product is used.

I agree that such a study can be a postmarketing requirement. Study details may be resolved once the applicant submits a complete protocol.

Reproductive and developmental toxicity:

I agree that the applicant should provide information on the impact of pegloticase on fertility, embryofetal development (in a second species) and pre/postnatal development. Rat and rabbit appear to be relevant species for this biologic product. It is unclear if pegloticase will transfer across the placenta; however, uricase is not normally present in human circulation and so its impact on fertility and fetal development are unknown. It is also not clear if the PEG moiety could be separately transferred to the fetus and if so what impact it would have. Normally, this information would be required prior to approval; however, considering the particular patient population and the previous agreements with the applicant, I agree that these may be postmarketing requirements. The recommendation for an assessment of pre/postnatal development may be reconsidered if the applicant provided convincing data that the number of women of childbearing potential likely to be treated with pegloticase was very small.

Carcinogenicity:

No hyperplasia or evidence of hormonal activity was observed in the nonclinical studies. Immunosuppression was not noted and there is no known mechanism by which uricase activity directly promotes cell growth. Some uncertainty remains about the possible long term effects of exposure to pegloticase although most of the concern is focused on the tissue vacuolation and not on carcinogenic potential. No additional evaluation of carcinogenicity is recommended at this time.

Overall:

I agree that the nonclinical information supports approval of this BLA, but that it is appropriate to collect additional information on the reproductive and long term toxicity of pegloticase after approval.

Specifically, the following items may be postmarketing requirements:

1. a study to assess the functional consequences of adrenal and aortic endothelial vacuolation,
2. a study of the effect of pegloticase on fertility,
3. a study in a second species of pegloticase on embryofetal development, and
4. a study of the effect of pegloticase on pre-postnatal development.

I agree with the labeling suggestions outlined in the supervisor's secondary review.



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

MEMO TO FILE

BLA Number: 125293
Sponsor: Savient Pharmaceuticals
Drug Name: KRYSTEXXA™ (pegloticase)
Reviewer name: BeLinda A. Hayes, Ph.D. *B.A. Hayes - 7/20/09*
Pharm/Tox Supervisor: R. Daniel Mellon, Ph.D. *R.D. Mellon 7-20-2009*
Division name: Division of Anesthesia, Analgesia, and Rheumatology Products
Date: July 20, 2009

RE: Amendment to Pharmacology Toxicology Review regarding proposed post-marketing non-clinical toxicology study.

Background

BLA 125293 for pegloticase was submitted by Savient Pharmaceuticals on October 31, 2008 to support marketing for the treatment of refractory gout. Pegloticase, a new molecular entity, is a monomethoxy polyethylene glycol (PEG) homotetramer recombinant porcine/baboon chimeric mammalian uricase (urate oxidase) enzyme.

Results from repeat-dose toxicity studies of 12 weeks or 39-weeks duration in the dogs revealed vacuolation as the toxicity finding associated with pegloticase administration. The incidence of vacuolation was treatment-duration and dose-related. After dosing up to 5 mg/kg for 12 weeks, vacuolation was only observed in the red pulp of the spleen. The repeat-dose toxicity study of 39 weeks duration demonstrated vacuolation of a greater number of tissues in the body including the splenic red pulp, liver Kuppfer cells, duodenum, jejunum, adrenal cortex, and the great vessels of the heart. A complete reversibility of the vacuolation was not demonstrated after a 12-week recovery period. The cells in the spleen, liver, duodenum, and jejunum appear to be macrophages and the vacuoles appear to contain PEG. In contrast, the vacuoles in the adrenal gland and intima of the heart were not shown to be associated with macrophages. While no functional changes and adverse pathology were observed in these organs, the potential long-term effects resulting from the accumulation of pegloticase in vacuoles are unknown.

The data suggest that with long-term exposure, PEG accumulates. The data raises several concerns; 1) will longer duration of treatment result in vacuolization in additional tissues; 2) what is the impact of accumulation PEG bioburden; and 3) what is the potential functional consequences, if any, of vacuolization in both the adrenal cortical cells and aortic endothelial cells of the heart.

On July 9, 2009, the Division held a teleconference with representatives from Savient Pharmaceutical. The purpose of the teleconference was to discuss potential post-marketing requirement studies to address the Agency concerns about the observed vacuolation in the adrenal gland and endothelial cells in the greater vessel of the heart. The dialog between the Agency and the sponsor focused on the functional consequences of the vacuoles in the adrenal cortical cells and endothelial cells of the aorta; that is, will the function and/or adhesive property of the endothelial cells be altered. Characterizing the time course of these vacuoles was also discussed; when do they occur, do they change over time and how quickly are the vacuoles eliminated/resolved. The Agency asked the sponsor to think about designing non-clinical studies that would evaluate the potential significant of these vacuoles and characterize the functional consequences of these vacuoles in these tissues over time. The Division asked the sponsor to submit a proposal that will address these concerns.

Savient Response

Per the Agency discussion with the sponsor on July 9, 2009, the sponsor submitted a proposal for an in vivo study to address the Division concerns. The sponsor is proposing to conduct an 18-month repeat-dose toxicity study in dogs. A dosing regimen similar to the clinical dosing will be employed; pegloticase will be administered at doses of 8 mg, 10 mg and 40 mg every 14 days. Both sequential in-life and postmortem evaluations will be conducted in order to determine if the cytoplasmic vacuoles occur in tissues, particularly if changes in circulating monocytes could be used to monitor such changes, and to characterize the functional consequences of these changes, should they occur under more clinically relevant dosing intervals. See sponsor proposal for details (Attached).

Reviewer Recommendation: The sponsor's proposal appears to be adequate to address the Agency concerns. The Division should accept the proposed post-marketing study design. Further comments may be provided following review of the submitted complete study protocols.

To the Sponsor. In general, your proposed 18-month dog study is reasonable to address the concerns raised by the nonclinical review staff on July 9, 2009. The complete detail study protocol should be submitted for review as per the timelines proposed. You should include justification for the functional assessments chosen, including any references to support the utility of the proposed studies.



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

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

BLA NUMBER: 125293
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: 10/31/08
PRODUCT: **KRYSTEXXA™ (pegloticase) injection**
INTENDED CLINICAL POPULATION: **Treatment Refractory Gout Patients**
SPONSOR: **Savient Pharmaceuticals, Inc.**
REVIEW DIVISION: **Division of Anesthesia, Analgesia, and
Rheumatology Products (HFD-170)**
PHARM/TOX REVIEWER: **BeLinda Hayes, Ph.D.**
PHARM/TOX SUPERVISOR: **R. Daniel Mellon, Ph.D.** *R. Daniel Mellon*
DIVISION DIRECTOR: **Bob A. Rappaport, M.D.** *6-23-09*
PROJECT MANAGER: **Diana Walker, Ph.D.**

Background: Savient Pharmaceuticals submitted BLA 125293 to support marketing of KRYSTEXXA™ (pegloticase) injection for the treatment of refractory gout. Pegloticase is a monomethoxypoly(ethylene glycol)-modified (PEGylated) homotetramer recombinant porcine/baboon chimeric uricase enzyme. The uricase enzyme catalyzes the conversion of uric acid to allantoin. There are approximately 9 ± 1 strands of 10 kDa mPEG per uricase monomeric subunit. The approximate molecular weight of pegloticase is 497 kDa (MW of unPEGylated homotetramer is 136.2 kDa). PEG represents almost (b) of this drug product's molecular weight. PEGylation of proteins is an established method used to increase solubility, decrease immunogenicity, reduce susceptibility to proteolytic enzymes and cells, and reduces renal elimination (Veronese and Mero, 2008).

As the uricase enzyme targets uric acid rather than a potentially species-specific protein, there are multiple relevant species that could be used to characterize the potential toxicity of this product. The only potential limiting factor would be the development of anti-product antibodies that could lead to accelerated clearance of the product from the test species, which was not demonstrated in the studies conducted to date. Due to the reduced immunogenicity and clearance of this product, standard nonclinical species are able to be used to characterize the potential toxicity of this drug product. Basic pharmacology studies were completed in normal and hyperuricemic animals to demonstrate that the PEGylated enzyme retains enzymatic activity. It should be noted that even in non hyperuricemic animals, the product reduced endogenous levels of uric acid. Toxicity studies were not conducted in an animal model of hyperuricemia.

The development program was completed under IND 10,122 (initial submission date 12/14/2001). The IND was originally submitted to CBER on November 15, 2001. The nonclinical development program was agreed upon prior to transfer of this IND to DAARP. The pivotal nonclinical toxicology studies completed to support the clinical development program and BLA submission consisted of a 12-week and 39-week repeat dose toxicology study in the dog model and a segment II embryofetal development study in the rodent model. At the End-of-Phase 2 meeting, held between CDER (ODE VI) and (b) (4) on July 26, 2005, the following nonclinical comment was provided by the FDA:

FDA: Based on the available information, no additional toxicology studies are needed. However, it is always possible that our review of the chronic dog study and/or data being obtained in ongoing clinical trials might identify concerns that need to be addressed through additional toxicology studies.

The IND was transferred to DAARP in the fall of 2005. A preNDA meeting was held on April 17, 2008. At that time DAARP provided the following nonclinical comments:

Following review of your submitted 39-week repeat-dose toxicology study in the dog, the Division has concerns regarding the finding of vacuolation in multiple tissues. Your BLA submission should include data to document the contents of the vacuoles to support your conclusion that the vacuoles contain PEG and do not result in adverse effects to those tissues. Of particular concern are the findings of vacuoles in tissues that may not contain reticuloendothelial cells, such as the great vessels of the heart

and the adrenal cortex. Your BLA submission should include an updated discussion of the potential significance of vacuoles in tissues following administration of PEGylated proteins and include copies of all referenced literature articles.

Although previously agreed to by the Agency in 2005 based on standards at that time, the nonclinical development program is not consistent with current nonclinical requirements for biologic products. For example, chronic repeat-dose toxicity studies were not conducted in two species, the standard battery of reproductive toxicity studies was not completed (fertility, embryo-fetal development study in a second species, peri- and post-natal development), and carcinogenicity assessment in two species was not completed. Although DAARP will honor the previous agreement, based on current nonclinical standards of biologic product development, reproductive and developmental toxicity studies to complete the standard battery should be completed in order to appropriately inform the product labeling. Due to the previous agreement, these studies may be completed post approval. I concur with Dr. Hayes that carcinogenicity studies for this product are not necessary given the lack of any predictable effect on cell growth/survival or evidence of clear immunosuppressive effects.

Overview of nonclinical findings

As noted in Dr. Hayes' review, repeated administration of pegloticase to the dog did not appear to result in adverse effects; however, there were histopathological changes noted in several tissues and the significance of these findings is not clear. In the 12-week repeat dose dog study, vacuoles were noted only in the red pulp of the spleen at all doses. The toxicology study of 39 weeks duration demonstrated vacuolation of a greater number of tissues in the body including the splenic red pulp, liver Kuppfer cells, duodenum, jejunum, adrenal cortex, and the great vessels of the heart. A 12-week recovery period failed to demonstrate complete reversibility of these vacuoles. The cells in the spleen, liver, duodenum, and jejunum appear to be reticulocytes and the vacuoles appear to contain PEG. In contrast, the vacuolated cells in the aortic endothelium and the adrenal cortex are not part of the reticuloendothelial system. To my knowledge, these tissues have not previously been reported to demonstrate vacuoles in any FDA-approved PEGylated products and may represent a unique finding for this highly PEGylated product.

Vacuolation of PEG via phagocytic cells has been previously reported with PEGylated products, and is believed to be the result of clearance of the PEGylated product from the circulation. For example, a published study in the literature reports vacuolation of renal cortical tubular epithelium following treatment with PEGylated tumor necrosis factor binding protein (TNF-bp) in a 3-month rat repeat dose toxicity study that were only partially reversible after a 2-month recovery period (Bendele, et al., 1998). Although the vacuoles were described as sufficiently large to distort the tubule profiles and compress the nuclei, there was no evidence of necrosis or altered clinical chemistry and urinalysis parameters. These authors report that single doses of the PEGylated product resulted in small discreet singular vacuoles with do not compress the nuclei or distort the tubules; however, repeated dosing lead to larger vacuoles which were associated with cellular distortion, indicating that

the findings progress with both dose and duration. Vacuoles were not reported by these authors in other tissues, and it was speculated that the lesions located in the kidney were most likely due to nonspecific uptake of the protein by pinocytosis. The authors also indicate that lysosomal proteases were able to process the protein portion of the PEGylated TNF- α but were not able to process the PEG. Breakdown of PEG would require an enzyme capable of breaking an ether linkage which is not common in mammalian systems (Bendele, et al., 1998). Published intravenous PK studies in rats with PEGylated poly-L-lysine dendrimers have reported that increasing the length and complexity of PEG chains results in slower elimination from the body and shifting of elimination from primarily renal to fecal routes. Further studies suggest that poly-L-lysine dendrimers demonstrated distribution into a variety of tissues including liver, spleen, kidney with lower levels found in the pancreas, heart, lungs, and even brain (Kaminskas, et al., 2008). Although the data reported by Kaminskas with the PEGylated dendrimers may not be representative of the pegloticase product, the data suggest that larger MW PEGylated products will likely accumulate in organs of the reticuloendothelial system over time and could theoretically be distributed to non-reticuloendothelial tissues.

The finding of vacuoles in reticuloendothelial system following pegloticase administration may be attributed to the expected clearance mechanisms for PEGylated products. However, it is not known why pegloticase produced vacuoles in the specific tissues reported in the 39-week dog study. The findings with pegloticase appears to be unusual since vacuolated cells were detected in tissues which are not considered to have a primary phagocytic function, specifically endothelial cells in the heart vasculature and cells in the adrenal cortex. It is not known if longer duration of treatment may result in vacuolization of cells in additional tissues nor has there been demonstration of complete reversibility of the vacuoles.

There are no data to determine if the presence of the vacuoles in the aortic endothelial cells or adrenal cortex cells alters cellular function. To date, the sponsor has determined that vacuole formation in rat splenic macrophages can result in decreased functional responses to LPS. Although the existing 9-month dog study and the clinical experience to date provide reassurance that these vacuoles do not appear to result in adverse effects, longer-term safety data are not available for the apparent dose- and time-dependent accumulation of PEG containing vacuoles in tissues. It is not clear what an appropriate duration of nonclinical toxicology study would be to determine the impact of accumulating PEG bioburden. As such, the long-term human data may provide the most useful information regarding the long-term safety of this product.

Recommendation on approvability

From a nonclinical perspective, I concur with Dr. BeLinda Hayes' recommendation that BLA 125293 may be approved pending agreement on the proposed labeling and with the following recommended post-marketing requirements. This conclusion is largely driven by the previous agreements made with the Sponsor and the lack of any clear evidence for adverse effects noted in tissues demonstrating vacuoles over the duration of standard toxicology studies in the dog combined with the clinical experience to date.

Recommendation for nonclinical studies

I concur with Dr. Hayes' recommendation that the Sponsor should conduct in vitro and/or ex vivo assessments of aortic endothelial cell and adrenal cell function following co-culture with pegloticase. Given the unusual findings and the high level of PEGylation with this particular product, the Sponsor should be required to conduct additional studies to characterize the potential functional consequences of vacuolization of both the aortic endothelial cells and the adrenal cortex cells. Such studies could be done either via ex vivo studies or in vitro studies, if co-cultures of cells with pegloticase are shown to demonstrate vacuoles. The studies should be designed to specifically address the potential for functional changes to the cells in order to determine if the histopathological changes noted in the repeat dose toxicology studies have functional consequences. These data are necessary in order to inform adequate clinical monitoring during the long-term clinical observational study that will be conducted post-marketing. Based on the results of these studies, further repeat dose toxicology studies of longer than 9 months duration may be needed, (b) (4)

2 Page(s) of Draft Labeling have been Withheld in Full immediately following this page as B4 (CCI/TS)

(b) (4)
Pharmaceuticals

Reference List

Bendele A, Seely J, Richey C, Sennello G and Shopp G (1998) Short communication: renal tubular vacuolation in animals treated with polyethylene-glycol-conjugated proteins. *Toxicol Sci* **42**:152-157.

Kaminskas LM, Boyd BJ, Karellas P, Krippner GY, Lessene R, Kelly B and Porter CJ (2008) The impact of molecular weight and PEG chain length on the systemic pharmacokinetics of PEGylated poly l-lysine dendrimers. *Mol Pharm* **5**:449-463.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
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CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

BLA NUMBER: 125293
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: 10/31/08
PRODUCT: KRYTEXXA™ (pegloticase)
INTENDED CLINICAL POPULATION: Treatment of failure gout to control hyperuricemia and to manage the signs and symptoms of gout
SPONSOR: Savient Pharmaceuticals
DOCUMENTS REVIEWED: Electronic Submission
REVIEW DIVISION: Division of Anesthesia, Analgesia and Rheumatology Drug Products (HFD-170)
PHARM/TOX REVIEWER: BeLinda A. Hayes, Ph.D. B.A.H. 6-23-09
PHARM/TOX SUPERVISOR: R. Daniel Mellon, Ph.D. RDM 6-23-09
DIVISION DIRECTOR: Bob A. Rappaport, M.D.
PROJECT MANAGER: Diana Walker, Ph.D.

TABLE OF CONTENTS

EXECUTIVE SUMMARY	3
2.6 PHARMACOLOGY/TOXICOLOGY REVIEW	13
2.6.1 INTRODUCTION AND DRUG HISTORY	13
2.6.2 PHARMACOLOGY	18
2.6.2.1 Brief summary	19
2.6.2.2 Primary pharmacodynamics.....	19
2.6.2.3 Secondary pharmacodynamics.....	22
2.6.2.4 Safety pharmacology	22
2.6.2.5 Pharmacodynamic drug interactions.....	23
2.6.3 PHARMACOLOGY TABULATED SUMMARY.....	23
2.6.4 PHARMACOKINETICS/TOXICOKINETICS	23
2.6.4.1 Brief summary	23
2.6.4.2 Methods of Analysis	24
2.6.4.3 Absorption	25
2.6.4.4 Distribution.....	27
2.6.4.5 Metabolism	32
2.6.4.6 Excretion.....	32
2.6.4.7 Pharmacokinetic drug interactions.....	35
2.6.4.8 Other Pharmacokinetic Studies.....	36
2.6.4.9 Discussion and Conclusions	46
2.6.4.10 Tables and figures to include comparative TK summary	46
2.6.5 PHARMACOKINETICS TABULATED SUMMARY.....	49
2.6.6 TOXICOLOGY.....	52
2.6.6.1 Overall toxicology summary.....	52
2.6.6.2 Single-dose toxicity	54
2.6.6.3 Repeat-dose toxicity	60
2.6.6.4 Genetic toxicology.....	100
2.6.6.5 Carcinogenicity.....	100
2.6.6.6 Reproductive and developmental toxicology.....	100
2.6.6.7 Local tolerance.....	111
2.6.6.8 Special toxicology studies	111
2.6.6.9 Discussion and Conclusions	116
2.6.6.10 Tables and Figures.....	116
2.6.7 TOXICOLOGY TABULATED SUMMARY	116
OVERALL CONCLUSIONS AND RECOMMENDATIONS.....	116
APPENDIX/ATTACHMENTS	118

EXECUTIVE SUMMARY

I. Background

Pegloticase, a new molecular entity, is a monomethoxy polyethylene glycol (PEG) modified recombinant mammalian uricase (urate oxidase). The proposed indication is for treatment failure gout to control hyperuricemia (b) (4)

by intravenous infusion over no less than 120 minutes at a dose of 8 mg every 2 weeks. Patients with treatment failure gout are defined as patients in whom conventional urate-lowering therapy failed to normalize the patients' serum uric acid and control the signs and symptoms of gout.

Hyperuricemia, the “cardinal biochemical feature and prerequisite for gout”, is defined as a serum uric acid concentration greater than 6.8 mg/dL (Mandell, 2008; Schumacher, 2008). There is a strong relationship between elevated uric acid levels and gouty arthritis. At serum uric acid levels greater than 6.8 mg/dL, uric acid exceed its solubility and uric acid crystals can precipitate out of solution and deposit in joints and other body tissues where they can produce an inflammatory response. In approximately 10% of patients with gout, hyperuricemia results from overproduction of uric acid due to a variety of metabolic derangements or medical disorders such as psoriasis. In approximately 90% of patients, hyperuricemia is the result of under excretion of uric acid due to alterations in renal function. Regardless of the cause of hyperuricemia, the primary goal of gout management is to decrease the concentration of serum uric acid. Lowering the serum uric acid to a level of less than 6.0 mg/dL has been shown to result in a reduction in the frequency of gout attacks. Uric acid, the final product of purine metabolism, is converted to allantoin by the enzyme urate oxidase. In most mammals, urate oxidase is found in liver peroxisomes and converts uric acid to the more soluble and easily excreted allantoin. However, in humans and higher primates, uricase is not expressed. As a consequence of a non-functional uricase enzyme, humans have relatively higher urate levels compared to that of other species. Therefore, in theory, a therapeutic with uricase activity for converting uric acid to allantoin will reduce serum uric acid levels; thus preventing, or at least reducing the frequency of, gout attacks.

The rationale for the development of pegloticase is the potential to lower serum uric acid level by capturing urate oxidase enzymatic activity (i.e., breakdown uric acid deposits); it catalyzes the conversion of uric acid plus oxygen and water to allantoin plus carbon dioxide and hydrogen peroxide. The Applicant has provided nonclinical and clinical support for the rationale that pegloticase has the potential to lower uric acid level. Additionally, pegloticase has a long half-life, thus allowing a longer duration of action with less frequent administration (i.e., once or twice a month).

Recommendations

A. Recommendation on approvability

From the pharmacologist's perspective, BLA 125293 may be APPROVED, pending agreement on the labeling outlined below and the post-marketing requirements/commitments.

B. Recommendation for nonclinical studies

No further nonclinical studies are needed for marketing approval. However, the following post-marketing studies should be required to be completed post-approval:

1. Conduct a fertility study in the rat model (Segment 1)
2. Conduct a embryo-fetal development study in the rabbit model (Segment 2)
3. Conduct a peri-natal and post-natal development study in the rat model (Segment 3)
4. Conduct in vitro and/or ex vivo assessments of aortic endothelial cell and adrenal function following co-culture with pegloticase.

C. Recommendations on labeling

The following revisions to the Proposed Labeling are recommended:

(b) (4)



III. Summary of nonclinical findings

A. Pharmacologic activity

Pharmacology:

Pegloticase, a monomethoxy polyethylene glycol (PEG) modified recombinant mammalian uricase (urate oxidase), is indicated for the treatment of treatment failure gout. Pegloticase is designed to capture urate oxidase enzymatic activity (i.e., breakdown uric acid deposits) and provide a sufficiently long half-life in patients to enable a convenient schedule of dosing (i.e., once or twice a month). Uric acid is the final oxidation product of purine metabolism in humans. In most mammals, urate oxidase is found in the liver and converts uric acid to allantoin. However, in humans and higher primates, uricase is not expressed. In humans, uric acid elimination is primary via the kidneys and enteric excretions.

Pharmacodynamics:

The Applicant did not conduct non-clinical pharmacodynamics studies to evaluate the pharmacological activity of pegloticase. To provide information on the biological activity of pegloticase in lowering uric acid levels, the effects of pegloticase on plasma level of uric acid were measured in the single-dose and repeated-dose toxicity studies conducted in rats and dogs and embryo/fetal development study. As part of the dose-range finding and definitive embryo-fetal development study in rats, serum uric acid measurements were measured. Results from the reproductive toxicity studies showed that pegloticase lowered serum uric acid levels in pegloticase treated pregnant rats. The lowering effects of pegloticase on serum uric acid were also demonstrated in rat and dogs following single intravenous administration or subcutaneous administration for 54 days and 53 days, respectively. At the end of treatment, uric acid was undetectable or at near-baseline levels in all pegloticase treatment groups.

Using normal (Uox+/+) and uricase-deficient knockout mice (Uox-/-) mice, the therapeutic potential of pegloticase for treating hyperuricemia was evaluated (Kelley *et al.*, 2001). Pegloticase (0.34 U or 0.08 mg) effectively lowered mean serum urate concentration in Uox-/- mice. The results from these toxicology studies and the study in uricase-deficient mice demonstrated that pegloticase was effective in lowering uric acid levels following intravenous and subcutaneous administration.

Safety Pharmacology:

Dedicated safety pharmacology studies are generally not required for investigation and registration of biologic agents; therefore were not conducted on pegloticase by the Applicant. However, evaluations of standard safety pharmacology parameters were included in the general toxicology studies in dogs. The effects of pegloticase on several behavioral parameters were assessed in the modified Irwin's behavioral screen conducted in dogs. Evaluation of subcutaneously and intravenously administered pegloticase in dogs demonstrated that pegloticase was void of CNS effects.

To assess the potential cardiac toxicity, the effects of pegloticase on heart rate and electrocardiographic parameters were measured in conscious dogs. Pegloticase did not reveal an ability to disrupt cardiovascular function. No alterations in ECG waveforms or heart rate were noted when pegloticase was tested in conscious dogs at doses up to 10 mg/kg/week for 39 weeks.

Pharmacokinetics:

The absorption, distribution, metabolism and excretion of pegloticase were evaluated in pharmacokinetic studies performed in rats, dogs and pigs after a single intravenous, intramuscular and subcutaneous injection. The intravenous route is the intended route of administration for the treatment of refractory gout. Pegloticase was rapidly and well absorbed after a single intravenous administration to rats. Peak plasma and whole blood levels of ^{125}I -pegloticase were observed at the first sampling point of 5 min and 1 hour, respectively. Plasma level declined slowly in a manner consistent with a single compartment model. Radioactivity levels were still detectable 7 days after dosing. Elimination half-life ranged between 22.7 hours (combined male and female) 35 hours (males) and 48 hours (females).

Pegloticase was well absorbed after subcutaneous administration. Plasma levels of radioactivity showed a gradual rise and decline. Peak plasma and whole blood of ^{125}I -pegloticase was observed 24-hours after dosing. Radioactivity levels were still detectable 7 days after dosing. The absolute bioavailability after a single dose of pegloticase was 26%.

Urine is the principal route of ^{125}I excretion following subcutaneous and intravenous of ^{125}I -pegloticase to rats. The excretion of the ^{125}I -pegloticase-derived material was TCA-soluble suggesting that it is pegloticase-like protein degradation product. Fecal elimination was a minor route of excretion for ^{125}I -pegloticase following both intravenous and subcutaneous administrations. There was no gender related difference in the excretion profile of ^{125}I -pegloticase.

The distribution of ^{125}I -pegloticase-derived radioactivity was characterized in rats following single intravenous and subcutaneous of ^{125}I -pegloticase. Distribution was extensive; all tissues collected were exposed to radioactivity at the first time

point (0.5 hours) after dosing. The highest concentrations of ^{125}I -pegloticase were observed 0.5 hours after dosing and had the propensity to be associated with organs with high blood perfusion. At 1 and 24 hours post-dosing, levels of radioactivity in the tissues were substantially lower than the radioactivity in blood. Distribution of radioactivity at 24 hours after dosing differed between male and female rats. With the exception of the spleen, the decline in the level of radioactivity paralleled that of the blood. Radioactivity was still measurable in the spleen 7-days after dosing.

Specific metabolism studies to address pegloticase metabolism were not performed. One would expect that the non-pegylated portions of the molecule will undergo typical protein catabolism. The metabolism of ^{125}I -pegloticase was evaluated in the pharmacokinetic studies in rats. The majority of the excreted ^{125}I -pegloticase-derived material was a pegloticase-like protein degradation product. The protein degradation products were not further identified. Whether small peptide with PEG attached can be further metabolized intact has yet to be determined.

B. Brief Overview of Nonclinical Findings:

The nonclinical toxicology program to support the clinical safety of pegloticase consisted of single dose and repeat-dose toxicity studies in rats and dogs administered either via subcutaneous or via intravenous route. Repeat-dose toxicity studies of up to 54-days duration in rats and 39 weeks in dogs were performed. The primary chronic long-term toxicity study to support BLA125,293 was conducted in one specie; the dog. This is in compliance to the agreement made between the Applicant and CBER Office of Therapeutic Research & Review during drug development. The Applicant additionally evaluated pegloticase in an embryo/fetal development toxicity study in rats.

The likely development of immunogenic and antigenic responses from biotechnology-derived pharmaceutical products is a potential concern. The results of the 12-week toxicity in dogs revealed that antibodies formed against pegloticase, and uricase and PEG in most dogs at the end of the 87 days treatment period with intravenous administration of PEG-uricase. Anti-uricase and anti-pegloticase were still present in some dogs after the recovery period. Most importantly, the antibodies were non-neutralizing; uricase enzymatic activity was still present. Similar results were observed in dogs following 39-weeks of intravenous administration of pegloticase. Measurable titers of anti-uricase and anti-pegloticase were obtained from some of the dogs in all treatment groups. The antibodies were non-neutralizing. It is generally accepted that the results of nonclinical immunogenicity evaluation do not always predict the occurrence of immunogenic effects by biotechnology-derived pharmaceuticals in humans. This was borne out with pegloticase; immunogenicity results from the nonclinical studies were not predictive of results in humans. The medical reviewer, Dr. Neuner,

reported in her review that approximately 88% of patients administered pegloticase every 2 weeks tested positive for anti-pegloticase antibodies on at least one time point over the course of the controlled studies. Treatment response rate decreased with increasing anti-pegloticase titer.

Repeat-dose toxicity studies of 12 weeks or 39-weeks duration in the dogs revealed vacuolation as the toxicity finding associated with pegloticase administration. The incidence of vacuolation was treatment-duration and dose-related. After dosing up to 5 mg/kg for 12 weeks, vacuolation was only observed in the spleen. The major target organ of toxicity was identified as adrenal cortex, duodenum, heart, jejunum, liver and spleen.

Repeat-dose toxicology:

In the 12-week repeat-dose toxicity study in dogs, pegloticase was intravenously administered at dose levels of 0.5, 1.5 and 5 mg/kg every 5 days. Vacuolation was observed within the red pulp area of the spleen from all terminal sacrifice male dogs in the MD (1.5 mg/kg) group and all males and females in the HD (5 mg/kg) group. The vacuolation was not reversible; vacuolation was still observed in both males and females in the mid- and high-dose groups at the end of the recovery period.

The 6-month chronic toxicology study in dogs was the primary toxicity study supporting the chronic clinical administration of pegloticase. Intravenous administration of pegloticase for 39-weeks in dogs revealed that pegloticase was well tolerated at doses up to 10 mg/kg; all dogs survived to the scheduled sacrifice. There were no treatment-related findings for clinical signs, body weight, food consumption, ophthalmic and electrocardiographic examinations, and clinical pathology. The treatment-related toxicological finding of clinical relevance/concerns is the identification of vacuoles in different organs following repeated (intermittent)-dosing. Formation of vacuoles appeared in a dose-dependent fashion. Histological analysis, using H&E staining, showed treatment-related vacuolation in the adrenal gland, duodenum, jejunum, liver, heart and spleen relative to controls at doses ≥ 0.4 mg/kg, depending on the tissue. Dose-dependent vacuolation in the spleen was identified in the red pulp from all terminal sacrifice dogs of the 10 mg/kg/week group and all but one male in the 1.5 mg/kg/week group. The vacuoles were clear, round and variable in size. Vacuolation within the adrenal cortex were observed with the 10 mg/kg/week dose. Vacuoles and peribasal histiocytic infiltrate were identified in the basal area of the lamina propria of the duodenum and jejunum of dogs in the high-dose group. Minimal to slight vacuolation of the liver (Kupffer cells) were observed in all high-dose males and females. Vacuolation of intimal cells in the aortic outflow area of the heart was observed in one male and 2 males in the LD and HD groups, respectively, after the end of the treatment period. Vacuolated cells were not observed in the heart in any of the females in the treatment groups. However,

in the females, the incidence of vacuolation increased during the recovery period. After the recovery period, vacuolated cells were observed in all males in the HD recovery group and in one female in the HD recovery group. H&E staining of tissues after a 12-week recovery period suggested that the vacuolation was not reversible; the presence of vacuolated cells remained essentially unchanged in the adrenal cortex, spleen, liver, duodenum and jejunum.

To qualitatively identify the vacuoles, immunohistochemical staining methods were applied to the paraffin sections. The immunohistochemical stain for macrophages, MAC387, revealed the presence of macrophages in the spleen, liver and lamina propria of the duodenum and jejunum in both control and PEG-uricase treated dogs. However, only vacuoles in the pegloticase treated dogs stained positive for PEG and/or uricase; thus suggesting that the vacuoles in these tissues were macrophages. No clinical pathology or functional markers were associated with the vacuoles present in these organs; thus suggesting that the vacuoles are representing the macrophages phagocytizing the uricase and PEG components of pegloticase. Cytoplasmic vacuoles identified in the adrenal gland and the heart of pegloticase-treated dogs did not stain positive for macrophages; however, they did stain positive for uricase and/or PEG. Immunostaining for uricase in the adrenal gland revealed positivity in vacuoles and cortical cells located within the zona reticularis and/or zona fasciculata in females in the high-dose group. Immunostaining for both uricase and PEG had evidence of staining of vacuoles located in the zona reticularis and/or zona fasciculata in one male in the high dose group and cortical cells in all males in the high dose groups. The intensity of immunostaining for uricase and PEG in vacuoles located in the heart ranged from slight to marked in high-dose males. Cells located in the intima, subendothelium also stained in the intensity range of slight to marked in high-dose males. Similar results were observed in females in the high-dose group.

While the results from the H & E histological analysis from the two separate pathologists varied in the scoring of the intensity and/or frequency of the vacuolation in the animals after treatment and after the recovery period, the results clearly demonstrated that vacuoles formation in selected organs is associated with repeated pegloticase administration. Vacuoles formation has been associated with other pegylated compounds (Conover *et. al.*, 1996). Results from the immunohistochemistry analysis clearly demonstrated that the vacuoles in the spleen, liver, duodenum and jejunum were located in macrophage and uricase and/or PEG were contained in these vacuoles. Hence, vacuolation in these organs appears to represent a normal physiological response to the foreign material; phagocytosis of the large pegloticase compound. In contrast, the vacuoles in the adrenal gland and intima of the heart were not shown to be associated with macrophages. While no functional changes and adverse pathology were observed in these organs, the potential long-term effects resulting from the accumulation of pegloticase in vacuoles are unknown.

Reproductive toxicology:

The effects of pegloticase on embryofetal development were studied in the Sprague-Dawley rat. Pegloticase was intravenously administered at 5, 10 and 40 mg/kg to pregnant rats on gestation days 6, 8, 10, 12, 14 and 16 with C-section on day 20. Resulting maternal and fetal effects in pegloticase-treated groups were compared to vehicle-treated dams and their fetuses. No maternal toxicity was noted which includes no observed effect on measures of fertility. Consistent with the microscopic findings revealed after repeated dosing with pegloticase, the spleen was identified as a target organ of toxicity in the dams. Relative to the control, increased spleen weight and vacuolation was observed in the high-dose group (40 mg/kg). NOAEL of 10 mg/kg ($AUC_{0-24} = 1,818.4 \mu\text{g}\cdot\text{day/mL}$) was established for the maternal toxicity. Pegloticase was not teratogenic in rats at the doses examined; no apparent drug-induced developmental toxicity was observed in rats. Therefore, 40 mg/kg was established as the NOAEL for the teratology in rat.

Approximate exposure margins for the human relative to the recommended human dose of 8 mg or 0.133 mg/kg in patients approximately 60 kg in weight are presented in the following table (by the reviewer):

		Exposure to Pegloticase at the Nonclinical NOAEL and the Predicted Human Exposure with Therapeutic Use of Pegloticase (8 mg every 2-weeks; 0.133 mg/kg (4.9 mg/m ²))				
		NOAEL			Multiple of Clinical Dose	
Species	Study Duration	(mg/kg/day)	(mg/m ²)	HED mg/kg	mg/kg	mg/m ²
Dog	12-weeks	5.0	100	2.8	37.6	20.4
Dogs	39-weeks*	10.0	200	5.55	75.2	40.8
Rat	Embryo/fetal dev. tox. study	40	240	6.48	300	48.9

C. Nonclinical safety issues relevant to clinical use

Nonclinical safety issues relevant to clinical use that were identified in the studies using pegloticase in dogs include the potential to form vacuoles in the intimal lining of the aortic outflow tract of the heart. According to the independent pathologist, the vacuoles were located in endothelial cells. Regarding reversibility, there was equivocal evidence for recovery from the pegloticase-induced vacuolation. The findings were not consistent from the two histological analyses. While results from the hematoxylin and staining analysis indicated that the vacuolation within the intima of the aortic outflow of the heart was not reversible at the end of the 12-week recovery period, result from the immunohistochemical analysis suggest partial recovery. These results may be a

reflection in the differences in the sensitivity of the methodology. Patients with gout, hyperuricemia, have a greater risk of cardiovascular diseases (Niskanen *et al.*, 2004; Feig, *et al.*, 2008). Also, hyperuricemia is often associated with features of the metabolic syndrome, which includes hypertension, type II diabetes mellitus, renal insufficiency, hyperlipidemia and obesity, all of which are associated with an increased risk of cardiovascular disease (Feig, *et al.*, 2008). Due to the high prevalence of cardiovascular diseases in the intended patient population, one may speculate that the accumulation of pegloticase in vacuoles in the heart could change the severity and susceptibility to cardiovascular disease in this patient population. For instance, the potential accumulation of pegloticase in vacuoles in the endothelial cells may theoretically increase the incidence of the patient developing a cardiovascular adverse event, such as atherosclerosis.

Injury to the endothelial cells has been linked to the development of atherosclerosis. While the primary function of the endothelial cells is to act as a selective barrier between vessel lumen and surrounding tissue, endothelial cells are also associated with phagocytosis. Endothelial cells attract macrophages when injured. Overtaxing the endothelial cells in the intimal layer of the aorta may lead to the development of atherosclerosis. The role of endothelial cells in atherosclerosis is briefly described: Injured endothelial cells release cytokines that attract circulating macrophages into the intima. Prolonged insult to the endothelial cells will result in the build up of macrophages in the arterial wall and eventually resulting in the formation of atherosclerotic plaques. One can predict a similar process resulting from the prolonged accumulation of pegloticase-in vacuoles within the endothelial cells. The nonclinical safety issues warrant clear and adequate precautionary statements in the product label; pegloticase should be used with caution in patients with pre-existing cardiovascular diseases.

Eight cardiac serious adverse events were observed during product development. Three patients randomized to pegloticase died; these deaths were attributed to cardiovascular events. Refer to the Clinical Review for BLA 125,293 for detailed review of the clinical study findings.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

BLA number: 125293
Review number: 1
Sequence number/date/type of submission: 000/October 31, 2008/Orginial BLA
Sequence number 0008/February 4, 2009 / Labeling Supplement
Sequence number 0017 /April 8, 2009 / Nonclinical Study Report:
 Immunohistochemistry Report
Information to sponsor: Yes () No ()
Sponsor and/or agent: Savient Pharmaceuticals, Inc.
 One Tower Center, 14th Floor
 East Brunswick, NJ 08816
Manufacturer for drug substance: Bio-technology General (Israel), Ltd.
 Be'er Tuvia Industrial Zone
 P.O. Box 571
 Kiryat Malachi 83104
 Israel

Reviewer name: **BeLinda A. Hayes, Ph.D.**
Division name: Division of Anesthesia, Analgesia
 and Rheumatology Products
HFD #: 170
Review completion date: **May 28, 2009 (draft 1);**
June 22, 2009 (final)

Drug:

Trade name: KRYSTEXXA
Generic name: pegloticase (USAN, INN), PEG-uricase,
 Puricase

Code name:
Chemical name:
Name 1 (USAN): Oxidase, Urate (synthetic *Sus scrofa* variant pigKS-ΔN
 subunit), homotetramer, amide with α-carboxy-ω-methoxypoly(oxy-1,2-
 ethanediyl)
Name 2 (USAN): des-(1-6)-[7-threonine,46-threonine,291-lydsine,301-
 serine]uricase (EC 1.7.3.3, urate oxidase) *Sus scrofa* (pig) tetramer, non
 acetylated, carbamates with α-carboxy-ω-methoxypoly(oxyethylene)
CAS registry number: 885051-90-1
Molecular formula/molecular weight:
 (b) (4) : : : : :
MW: approximately 497 kDa (b) (4)

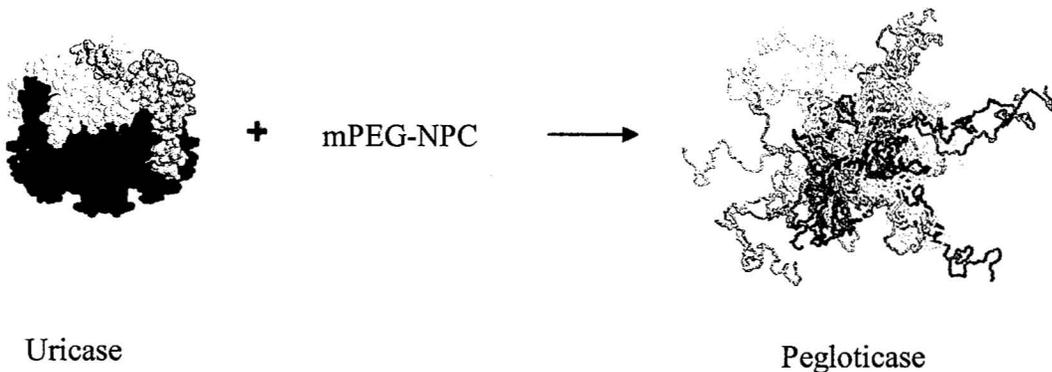
Structure:

{ [H₃C-O- (CH₂CH₂-O)_m-CO-]_n-NH- [TYKKNDEVEF VRTGYGKDMI KVLHIQRD GK YHSIKEVATT VQLTLSSKKD YLHGDNSDVI PTDTIKNTVN VLAKFKGIKS IETFAVTICE HFLSSFKHVI RAQVYVEEVP WKRFEKNGVK HVHAFIYTPT GTHFCEVEQI RNGPPVIHSG IKDLKVLKTT QSGFEGFIKD QFTTLPEVKD RCFATQVYCK WRYHQGRD VD FEATWDTVRS IVLQKFAGPY DKGEYSPSVQ KTLYDIQVLT LGQVPEI EDM EISLPNIHYL NIDMSKMGLI NKEEVLLPLD NPYGKITGT V KRKLS SRL] }₄

Wherein, (b) (4) and each uricase monomeric subunit having the amino acid sequence listed above.

Peylation:

On the average 9 ± 1 strands of methoxypoly(ethylene glycol) (10kDa) are attached to Lysine(K) residues per uricase monomeric subunit or 36 (b) (4) strands of mPEG per pegloticase monomer.



Relevant INDs:

IND#	Drug	Status	Division	Indication	Status Date	Sponsor
10122	Uricase (recombinant, <i>E. coli</i> , Bio-Technology General Corp.), PEG conjugate	Active	DAARP	Treatment of symptomatic gout	12/14/2001	Savient Pharmaceuticals

Drug class: Bio-uricolytic agent (therapeutic protein)

Intended clinical population: Patients diagnosed with treatment failure gout to control hyperuricemia and manage the signs and symptoms of gout in patients with refractory gout.

Clinical formulation: Puricase is supplied as a sterile, clear solution for dilution and intravenous infusion. Each single-use vial contains (b) (4) of pegloticase comprised of 8 mg/mL uricase protein conjugated to monomethoxypoly(ethylene glycol) (mPEG) in phosphate buffered saline. The composition of a single dose of Puricase is described in the table below.

Composition of Single Dose Strength of Puricase				
Ingredient	Quantity/mL ¹	Specification	Concentration (w/v %)	Function
Pegloticase	(b) (4)	In-house specification	(b) (4)	API
Disodium hydrogen phosphate dehydrate (Na ₂ HPO ₄ ·2H ₂ O)	2.18 mg	USP, Ph.Eur.		(b) (4)
Sodium dihydrogen phosphate dehydrate (Na ₂ H ₂ PO ₄ ·2H ₂ O)	0.43 mg	USP, Ph.Eur.		
Sodium chloride (NaCl)	8.77 mg	USP, Ph.Eur.		
Water for Injection (WFI)	(b) (4)	USP, Ph.Eur.		
				(b) (4)

2: (b) (4) of pegloticase correspond to 8 mg of uricase protein conjugated to (b) (4) of 10 kDa mPEG.

Route of administration: Intravenous

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

Studies reviewed within this submission:

Report №	Study Title	Module/CTD Description
Pharmacology		
Kelley et. al., 2001	Diabetes insipidus in uricase-deficient mice: a model for evaluating therapy with poly(ethylene glycol)-modified uricase.	4.2.1.1/Primary Pharmacodynamics
Pharmacokinetics/Toxicokinetics		
№ 00V258-1	Absorption, distribution, metabolism and excretion (ADME) of intravenously-administered ¹²⁵ I-labeled Puricase in rats.	4.2.2.2/Absorption
№ 00V258-2	Distribution, metabolism and excretion of intravenously-administered ¹²⁵ I-labeled Puricase™ in rats.	
№ 00V258-3	Absorption, distribution, metabolism and excretion (ADME) of subcutaneously versus intravenously administered ¹²⁵ I-labeled Puricase™ in rats.	
№ 00V258-4	Absorption, distribution, metabolism and excretion (ADME) of subcutaneously versus intravenously administered ¹²⁵ I-labeled Puricase™ in rats.	
№: 01V359-2	Report, pharmacokinetic study of Puricase in rats, treated with five weekly injections by the intravenous, intramuscular and subcutaneous routes.	
№ 01V365-1	Pharmacokinetic study of Puricase in beagle dogs.	
Toxicology		
№ (b) 441011	A single-dose toxicity and toxicokinetic study of intravenously administered PEG-uricase in juvenile beagle dogs with a four-week recovery period.	4.2.3.1/Single-Dose Toxicity
№ (b) 441012	A single-dose toxicity and toxicokinetic study of intravenously administered PEG-uricase in adult beagle dogs with a four-week recovery period.	
№ 6432-106	12-Week Repeated Dose Intravenous Injection Toxicity Study with Puricase® in Dogs.	4.2.3.2/Repeat-Dose Toxicity
№ 7533-100	39-Week Repeated Intravenous Injection Chronic Toxicity and Toxicokinetic Study with Puricase® in Dogs with a 12-Week Recovery.	
№ IM1678 ((b) (4) Study № 7533-100)	Processing and Evaluation of anti-PEG, anti-uricase, and on anti-macrophage marker in dog tissues in support of (b) (4) Study No. 7533-100, 39-Week Repeated Intravenous Injection Chronic Toxicity and Toxicokinetic Study with Puricase® in Dogs with a 12-Week Recovery.	
№ (b) (4)-441007	A Dose Range-Finding Intravenous Study of the Effects of Puricase on Embryo/Fetal Development in Rats.	4.2.3.5.2/Embryo-fetal Development
№ (b) 441008	An Intravenous Study of the Effects of Puricase on Embryo/Fetal Development in Rats.	
№ 01V378-2	The effect of repeated Puricase administration on splenic macrophage vacuole formation and disappearance, and on macrophage functionality.	4.2.3.7/Other Toxicity Studies
№ 01V378-4	The effect of repeated Puricase administration on splenic macrophage vacuole formation and disappearance, and on macrophage functionality.	

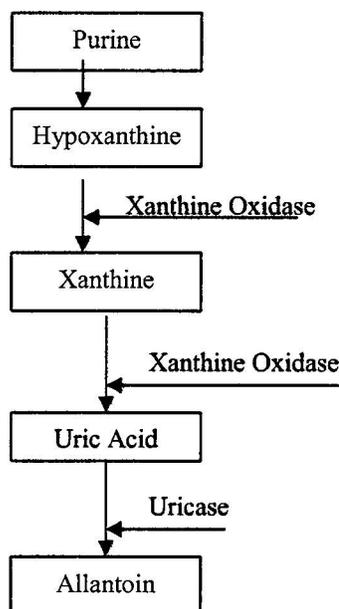
Studies not reviewed within this submission:

Report №	Study Title	Module/CTD Description
Pharmacokinetics		
№ 00V266-2	5-Day pharmacokinetic study of "Puricase" in young pigs.	4.2.2.2/Absorption
№ 00V283-1	Summary of the pharmacokinetic behavior of Puricase™ in the circulation of rat, rabbit, dog and pig, and its bioavailability after intramuscular and subcutaneous administration.	
№ 01V352-1	Pharmacokinetic study of Puricase in rabbits.	
№ 01V363-1	Report on the stability of Puricase in vials and working solutions during the subchronic toxicology study.	
№ 01V373-3	Toxicokinetics of plasma Puricase activity and antibody formation in the repeated dose toxicity study in rats.	
№ 02V526-2	Report, toxicokinetic profile of plasma Puricase® during intravenous subchronic toxicology study in beagle dogs.	
№ 20-2-0156-00	28-Day pharmacokinetic study of "Puricase™" in beagle dogs.	
Toxicology		
№ 10-4-0185-00	Acute subcutaneous toxicity test of "Puricase™" in the rat.	4.2.3.1.2/Single-Dose Toxicity
№ 10-4-0186-00	Acute intravenous toxicity test of "Puricase™" in the rat.	
№ 10-2-0187-00	Determination of the maximal tolerated dose of "Puricase™" in the dog.	
№ 20-4-0188-00	Repeated dose toxicity study of "Puricase™" in the rat following subcutaneous administration.	4.2.3/Repeat-Dose Toxicity
№ 20-4-0189-00	Repeated dose toxicity study of "Puricase™" in the dog following subcutaneous administration.	
№ 02V450-2	Report, comparative study in rats: The immunogenicity of the high molecular weight component <i>versus</i> the major tetrameric component of Puricase.	4.2.3.7.1/Other Toxicity Studies
№ 03V614-2	Report, comparative study in rats: The immunogenicity of Puricase preparations prepared with m-PEG-reagents from three different sources.	
№ 04C1116-1	Summary, activity and concentration of all test articles used in preclinical studies with Puricase® (From October 2000 to July 2003)	4.2.3.7.7/Other Toxicity Studies

2.6.2 PHARMACOLOGY

Pegloticase, a monomethoxy polyethylene glycol (PEG) modified recombinant mammalian uricase (urate oxidase), is indicated for the treatment of treatment failure gout. Pegloticase is designed to capture urate oxidase enzymatic activity (i.e., breakdown uric acid deposits) and provide a sufficient long half-life in patients to enable a convenient schedule of dosing (i.e., once or twice a month). Uric acid is the final oxidation product of purine metabolism in humans. In most mammals, urate oxidase is found in the liver and converts uric acid to allantoin. However, in humans and higher primates, uricase is non-functional. In humans, uric acid elimination is primary via the kidneys and enteric excretions.

Gout, a crystalline arthropathy hallmarked by elevated uric acid (hyperuricemia), is characterized by deposition of monosodium urate monohydrate crystals in the joints and periarticular structures. Hyperuricemia, the principal biochemical feature and prerequisite for gout, results from an imbalance between the rates of production and excretion of uric acid. Hyperuricemia is defined as a serum urate concentration greater than 6.8 mg/dL. At serum urate levels greater than 6.8 mg/dL, uric acid crystals can precipitate out of solution and deposit in joints and other body tissues where they can produce an inflammatory response. Management and prevention of gout entails normalizing serum urate level. Allopurinol, a potent inhibitor of xanthine oxidase, is the most widely used therapy for treatment of chronic gout. However, there is a subset of patients that are resistance to the urate lowering effects of allopurinol. Therefore, pegloticase has received orphan drug status.



2.6.2.1 Brief summary

2.6.2.2 Primary pharmacodynamics

There were no nonclinical pharmacodynamics (primary) studies conducted for this application. To provide information on the biological activity of pegloticase following intravenous administration, the effects of PEG-uricase on plasma level of uric acid were measured in the subcutaneous repeated-dose toxicity studies conducted in rats and dogs, and an embryo-fetal development study in rats. Also, in support of “proof of principle” for the clinical use of PEG-uricase, the sponsor submitted a published article by Kelley and colleagues (2001) to support the efficacy of pegloticase for the treatment of hyperuricemia. As indicated by the studies briefly described below, pegloticase was shown to be effective in lowering plasma uric acid levels in animals with normal uricase activity and in uricase-deficient knockout mice.

Drug activity related to proposed indication:

Study №: 20-4-0188-0

Objective of the study: To characterize the potential toxicity of PEG-uricase in rats following subcutaneous administration once daily every 2 days for 54 days (28 injections total) followed by a 14-day recovery period.

Method: Male and female Wistar/Hsd CpB:WU rats (n = 10/sex/group) were subcutaneously administered PEG-uricase once every 2 days for 54 days at a dose levels of 0, 3.4, 10.2, or 34 mg/kg. Blood samples were collected prior to dosing (day 0) and at the end of dosing (day 55).

Results: Plasma level of uric acid was undetectable or at near-baseline levels in all treatment groups at the end of the treatment phase in both male and female rats. Following the recovery period, plasma uric acid level remained undetectable in the high-dose group.

Dose (mg/kg)	Mean Plasma Uric Levels (µmol/L)					
	Week 0 (prior to treatment)		Day 53 (week 7) (end of treatment)		Week 9 (recovery)	
	Male	Female	Male	Female	Male	Female
0	-	-	25.2	35.0	13.0	17.6
3.4	31.8	24.0	0.2*	0.0*	-	-
10.2	-	-	0.0*	0.0*	-	-
34	-	-	0.1*	0.1*	0.0*	0.0*

*: Significantly different from controls , p≤ 0.05 (Dunnett)

Study №: 20-2-0189-00

Objective of the study: To characterize the potential toxicity of PEG-uricase in dogs following subcutaneous administration once daily every 4 days for 52 days (14 injections total) followed by a 28-day recovery period.

Method: Male and female Beagle/Hsd Bor:BEAG dogs (n = 3/sex/group) were subcutaneously administered PEG-uricase once every 4 days for 52 days at dose levels of 0, 1.7, 5.1, or 17 mg/kg.

Results: As depicted in the table below, pegloticase effectively reduced plasma levels of uric acid after repeated dosing for 7 weeks. At the end of the treatment period, plasma uric acid levels in all treatment groups were significantly reduced compared to the control group. After the 28-day recovery period, plasma uric acid was still reduced when compared to the control group.

Dose (mg/kg)	Mean Plasma Uric Levels (µmol/L)							
	Week 0 (prior to treatment)		Day 53 (Week 7) (end of treatment)		Week 9 (recovery)		Week 11 (recovery)	
	Male	Female	Male	Female	Male	Female	Male	Female
0	6.8	7.2	9.0	8.7	10.0	6.5	6.0	6.5
1.7	5.3	7.0	0.0*	0.0*	-	-	-	-
5.1	6.7	5.7	0.0*	0.0*	-	-	-	-
17	4.4	5.6	0.0*	0.0*	0.5	0.0	0.0	0.0

*: Significantly different from controls, $p \leq 0.05$ (Dunnett)

Study №: (b) (4)441007

Objective of the study: The serum uric acid levels were measured as part of the dose-range finding embryo-fetal study conducted in rats.

Method: Pregnant female Crl:CD®(SD)IGS BR rats (n = 8/group) were intravenously administered pegloticase at dose levels of 0, 5, 10, 20 or 40 mg/kg on gestation days 6, 8, 10, 12, 14 and 16. Plasma serum levels were measured prior to dosing and on day 17 (end of treatment).

Results: Consistent with the pharmacological activity of pegloticase, plasma level of uric acid was undetectable in all treatment groups at the end of treatment.

Dose (mg/kg)	Mean Plasma Uric Levels ($\mu\text{mol/L}$)	
	Day 5 (start of treatment)	Day 17 (end of treatment)
0	1.1	1.1
5	1.5	0.0
10	1.2	0.0
20	1.3	0.0
40	1.0	0.0

Study №: (b) (4)-441008

Objective of the study: The serum uric acid levels were measured as part of the dose-range finding embryo-fetal study conducted in rats.

Method: Pregnant female Crl:CD[®](SD)IGS BR rats (n = 8/group) were intravenously administered pegloticase at dose levels of 0, 5, 10, or 40 mg/kg on gestation days 6, 8, 10, 12, 14 and 16. Plasma serum levels were measured prior to dosing and on day 17 (end of treatment).

Results: Pegloticase decreased plasma level of uric acid to undetectable levels in all treatment groups at the end of treatment.

Dose (mg/kg)	Mean Plasma Uric Levels ($\mu\text{mol/L}$)	
	Day 5 (start of treatment)	Day 17 (end of treatment)
0	1.2	1.5
5	1.3	0.0
10	1.1	0.0
40	0.9	0.0

Reference: Kelley, S.J. *et. al.* (2001). Diabetes insipidus in uricase-deficient mice: a model for evaluating therapy with poly(ethylene glycol)-modified uricase. *J Am Soc Nephrol* 12(5):1001-1009.

Objective of the study: To evaluate the ability of PEG-uricase to reduce uric acid levels in uricase-deficient mice.

Method: Uricase-deficient mice were administered unmodified recombinant uricase or poly(ethylene glycol)-modified porcine uricase. Ten uricase-deficient mice received intraperitoneal injections of 0.34 U PEG-uricase every 5-days for 50 days. Twelve uricase deficient mice received intraperitoneal injection of 1 Unit (diluted to 0.23

mg/mL) unmodified (native) recombinant uricase. Endpoint measures included uric acid excretion, and anti-uricase antibody.

Results: Results indicated that PEG-uricase was effective in lowering uric acid levels. Compared to native uricase, PEG-uricase exhibited greater bioavailability and was more effective in lowering serum uric acid levels. Marked reduction in urinary level of uric acid was observed following each injection of PEG-uricase; the greatest reduction was noted 24-hours after injection. As depicted in the figure below, low level of urinary uric acid was also noted 72 and 120-hours after injection. PEG-uricase activity (0.053 ± 0.025 U/mL) was still evident 9 days after the last injection; serum uric acid concentration was 1.3 ± 2.1 mg/dl compared to 7.3 ± 0.8 mg/dl before dosing. Significant anti-uricase antibody was not measured.

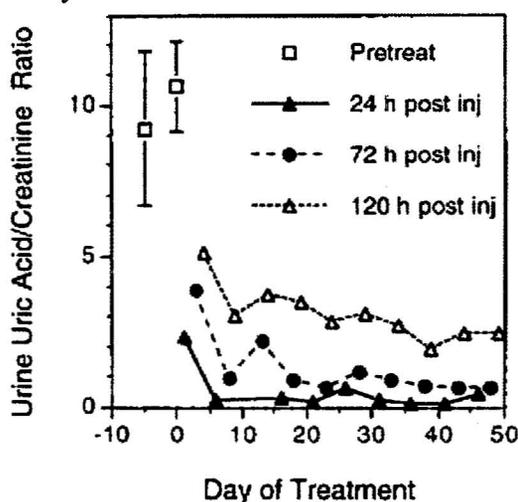


Figure 4. Effect of treatment with PEG-uricase on uric acid excretion. Ten 4-month-old *Uox*^{-/-} mice were given intraperitoneal injections of 0.34 U of PigK PEG-uricase every 5 d. The uric acid/creatinine molar ratio was assessed in urine samples from all mice on two occasions before treatment (Pretreat; bars, SD). During treatment, the uric acid/creatinine ratio was assessed for all mice in urine samples obtained approximately 24, 72, or 120 h after injection (post inj) (for clarity, error bars are not shown for these data points).

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2.6.2.3 Secondary pharmacodynamics

There were no nonclinical pharmacodynamics (secondary) studies conducted for this application.

2.6.2.4 Safety pharmacology

Safety pharmacology studies were not conducted. Dedicated safety pharmacology studies are typically not needed for biologics according to ICH S6 Guidance. Results from toxicology studies in dogs following intravenous administration provided evidences that Puricase did not produce remarkable behavioral changes, central nervous effects, cardiovascular effects nor clinical toxicities in dogs after intravenous administration. The

effects of puricase on general behavior and activity were studied in dogs according to the modified methods of Irwin following intravenous administration. No evidence of mortality, clinical signs of toxicity or effects on general activity or behavioral changes were observed at doses up to 10 mg/kg/day. In conscious dogs, intravenously administered puricase at doses up to 10 mg/kg did not induce noticeable cardiovascular effects (blood pressure, heart rate, and ECG parameters).

2.6.2.5 Pharmacodynamic drug interactions

There was no nonclinical pharmacodynamics studies conducted for this BLA application.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

N/A

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

The pharmacokinetic profile of puricase was evaluated in rats, dogs, rabbits, subcutaneous, or intramuscular routes; only studies conducted in rats and dogs were reviewed. In the rats and dogs pharmacokinetic studies, the pharmacokinetic profile of puricase was characterized following intravenous, subcutaneous and/or intramuscular administrations; however, the intravenous route is relevant to the intended clinical route. Pegloticase is recommended at a dose of 8 mg as an intravenous infusion every 2 weeks.

Pharmacokinetic studies in rats demonstrated that puricase is rapidly absorbed following intravenous administration; maximum plasma concentrations occurred within 5 minutes after injection. It had a long half-life in rats and dogs following intravenous administration. Systemic exposure in rats after intravenous administration, as calculated from the AUC₀₋₂₄ was increased in a dose-related fashion. Following a single intravenous dose of ¹²⁵I-PEG-uricase, ¹²⁵I-labeled PEG-uricase was extensively distributed with the exception to the brain. No ¹²⁵I-labeled PEG-uricase derived radioactivity was detected in the brain. In most tissues, the decline of radioactivity paralleled the decline in plasma level. The PEG-uricase derived radioactivity in the spleen declined at a slower rate. The PEG-uricase derived radioactivity in the spleen most likely represented the ¹²⁵I-PEG-uricase being sequestered within macrophages. The radioactivity in the thyroid most likely reflects the uptake of ¹²⁵I in the thyroid. Puricase was primarily excreted in the urine. Less than 10% of the administered ¹²⁵I-PEG-uricase was excreted in the feces.

2.6.4.1 Brief summary

The absorption, distribution, metabolism and elimination profiles of puricase were evaluated in pharmacokinetic studies in rats (Sprague-Dawley), dogs (Hsd:Bur:BEAG Beagle), rabbits and pigs after a single intramuscular, subcutaneous or intravenous

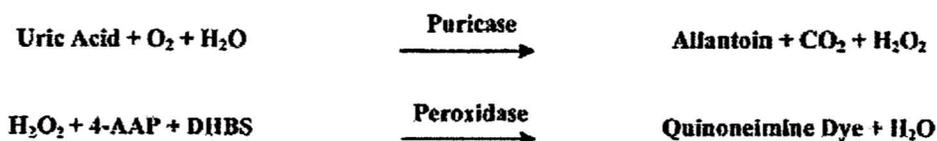
injection. The pharmacokinetic study conducted in the rabbit and pig was not reviewed. The key findings of these studies are listed below:

- Puricase is rapidly and well absorbed after a single intravenous administration to rats.
- Absolute subcutaneous bioavailability of puricase is low; 26% in rats at a 0.5 mg/kg dose.
- Urine is the principal route of ^{125}I excretion following [^{125}I]-PEG-uricase administration.
- Routes and rate of excretion of [^{125}I]-PEG-uricase were similar in male and female rats following subcutaneous, intramuscular and intravenous administration.
- The distribution of puricase was extensive in rats following single intravenous and subcutaneous administration of ^{125}I -labeled PEG-uricase. All tissues collected were exposed to radioactivity at first time point (i.e., 0.5 hours) after dosing.

2.6.4.2 Methods of Analysis

For evaluation of plasma and whole blood levels of puricase from rats and dog plasma samples were prepared from whole blood samples. These plasma samples were evaluated using a colorimetric method. In the rat, the lower limit of quantitation (LLOQ) ranged from 0.85 to 1.56 $\mu\text{g/mL}$. In the dog, the LLOQ for puricase ranged from 0.70 to 1.95 $\mu\text{g/mL}$. Using the data obtained from the colorimetric method, enzymatic activity and the TK parameters C_{max} , T_{max} and AUC could be determined. Gamma scintillation counting was used for radioactivity measurement when radioactive labeled drug was administered. ELISA method was used to measure antibodies to puricase.

Puricase levels in blood were quantified using a validated colorimetric assay measuring the enzymatic activity of Puricase[®] (performed by Bio-Technology General - Israel). The assay is a composite 2-step kinetic reaction in which H_2O_2 produced as a result of the oxidation of uric acid to allantoin by the enzymatic activity of uricase, reacts in the presence of 4-aminophenazone (4-AAP) and 3,5-dichloro-2-hydroxybenzene sulfonic (DHBS) to form quinoneimine, a colored dye that can be quantified by measuring absorbance at 530 nm.



The activity of Puricase determined by the colorimetric method is expressed as $\text{mAU}_{520}/\text{min}$.

2.6.4.3 Absorption

The pharmacokinetic profile of puricase was determined in rats, dogs and pigs following intravenous intramuscular or subcutaneous administration. Only the studies performed in rats and dogs were reviewed.

Study Title: Absorption, distribution, metabolism and excretion (ADME) of subcutaneously versus intravenously administered ¹²⁵I-labelled Puricase™ in rats.

Study №: 00V258-4 and 00V258-3 (details the procedure used in protocol № 00V258-4)

Study Objective: The objective of this study was to evaluate the pharmacokinetic profile and measure the absorption of ¹²⁵I-labeled PEG-uricase (Puricase) following a single subcutaneous and intravenous administration in Sprague Dawley rats.

Methods: The absorption and pharmacokinetic profile of ¹²⁵I-labeled PEG-uricase were studied in rats following a single intravenous or subcutaneous administration. Male (n = 2/time point/injection route) and female (n = 1/ time point/injection route) Sprague-Dawley rats received a single intravenous or subcutaneous injection of ¹²⁵I-labeled PEG-uricase at a volume of 0.5 mL (0.5 mg/kg equivalent; corresponding to 5.8x10⁶ cpm). At 5 minutes, 1 day and 3 days post-injection, 0.5 mL blood samples were drawn from the eye orbital sinus and quantification of radioactivity was determined in both plasma and whole blood. Total and specific radioactivity levels, as well as percent TCA-precipitated radioactivity are measured. Radioactivity levels were measured using a gamma scintillation counter. Seven days after dosing, all animals were anaesthetized; blood (10 mL drawn from the aorta) and tissues were collected. The following tissues were collected: adrenals, bone, caecum, esophagus, eyes, gonads, heart, kidneys, large intestine, liver, lungs, lymph nodes, pancreas, pituitary gland, salivary gland, skeletal muscle, skin, small intestine, spleen, stomach, thymus, trachea/thyroid and urinary bladder.

Key study findings:

Blood and plasma concentrations of radioactivity following a single intravenous and subcutaneous administration of ¹²⁵I-labeled PEG-uricase (Puricase) to male and female rats at a dose level equivalent to 0.5 mg/kg.

(b) (4)

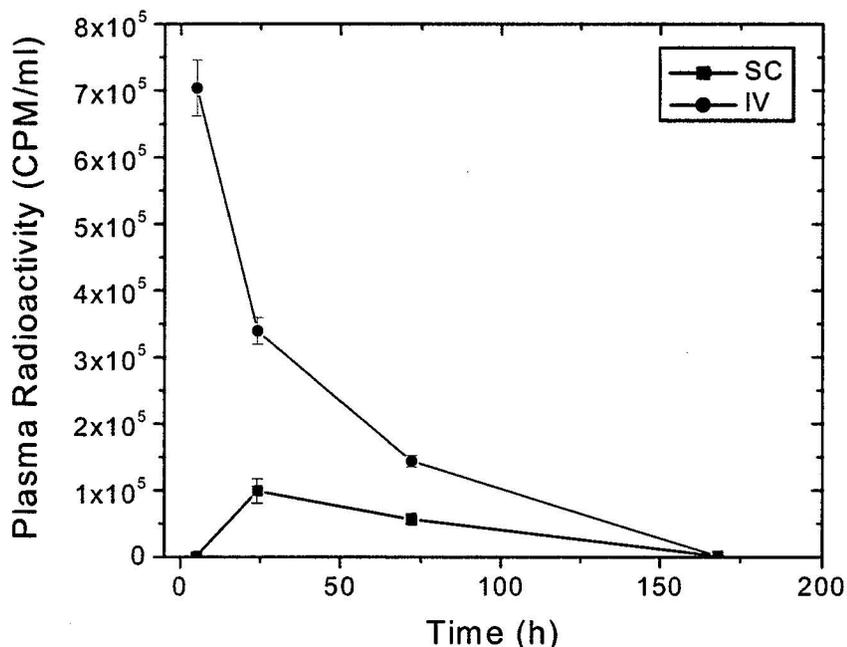


Figure 1. Mean Plasma Radioactivity Values Versus Time Combined for Both Sexes in Each Group

- Absorption of intravenously administered ¹²⁵I-labeled PEG-uricase was rapid; relatively high concentrations of radioactivity were measured in the plasma and whole blood at the first sampling time (5 minutes) after dosing.
- Following a single subcutaneous administration of ¹²⁵I-labeled PEG-uricase, peak levels of radioactivity was observed at 24 hours post-dosing in both blood and plasma and gradually declined thereafter.
- In contrast to the subcutaneous route, the levels of radioactivity in both blood and plasma declined slowly after intravenous administration of ¹²⁵I-labeled PEG-uricase.

Mean PK Parameters of ¹²⁵I-PEG-uricase in rats after intravenous and subcutaneous administration of 0.5 mg/kg of PEG-uricase.

PK Parameters	SC	IV
T _{max} (hour)	24	NC
T _{1/2} (hour) ± SEM	NC	22.7 ± 4.6*
C _{max} (µg/mL)	NA	NA
AUC _(0-∞) (µg·h/mL)	NC	NC
Bioavailability (%)	26	100
NA: Not Applicable NC: Not Calculated *: Male and female combined		

- Elimination half-life (for male and female combined) was 22.7 hours.

- Bioavailability of the PEG-uricase in rat following subcutaneous administration was low; bioavailability was 26% relative to the intravenous route.

2.6.4.4 Distribution

Tissue distribution of puricase was evaluated in rats following subcutaneous and intravenous administration in rats as part of the ADME studies.

Study Title: Distribution, metabolism and excretion of intravenously administered ¹²⁵I-labelled Puricase™ in rats.

Study №: 00V258-2 (non-GLP) and 00V258-1 (details the procedure used in protocol № 00V258-2)

Study Objective: To characterize the tissue distribution of ¹²⁵I-labeled PEG-uricase (Puricase) following a single intravenous administration in Sprague Dawley rats.

Methods: Male (n = 2/time point) and female (n = 2/ time point) Sprague-Dawley rats received a single intravenous or subcutaneous injection of ¹²⁵I-labeled PEG-uricase at a volume of 0.5 mL (0.5 mg/kg; corresponding to 0.39 µg (2x10⁶ cpm) of ¹²⁵I-labeled PEG-uricase + 0.1 mg puricase per rat). Two males and 2 females were anesthetized at 1, and 24 hours and at 2, 3, 4, 6 and 7 days after dosing. Prior to tissue collection, blood was drawn from each rat and the level of radioactivity in plasma and whole blood was measured.

The following tissues were collected: adrenals, brain, bone, caecum, esophagus, esophagus, eyes, gonads, heart, kidneys, large intestine, liver, lungs, lymph nodes, pancreas, pituitary gland, salivary gland, skeletal muscle, skin, small intestine, spleen, stomach, thymus, trachea/thyroid and urinary bladder. Large organs were weighed prior to measuring radioactivity levels. Radioactivity levels were measured using a gamma scintillation counter. Additionally, a piece of kidney, liver, lung and spleen was homogenized, counted and TCA precipitation was measured. Also, pieces of these organs were fixed in formalin for optional autoradiography evaluation.

Key Study Findings:

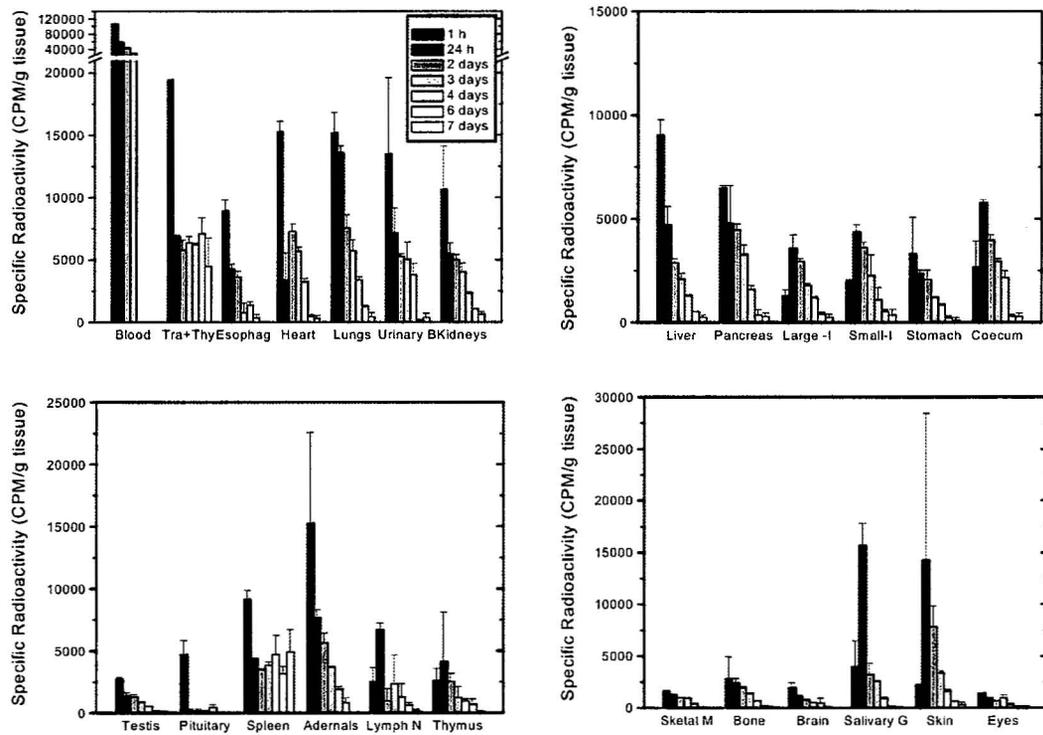


Figure 6. Tissue Values of Specific Radioactivity – Male Rats

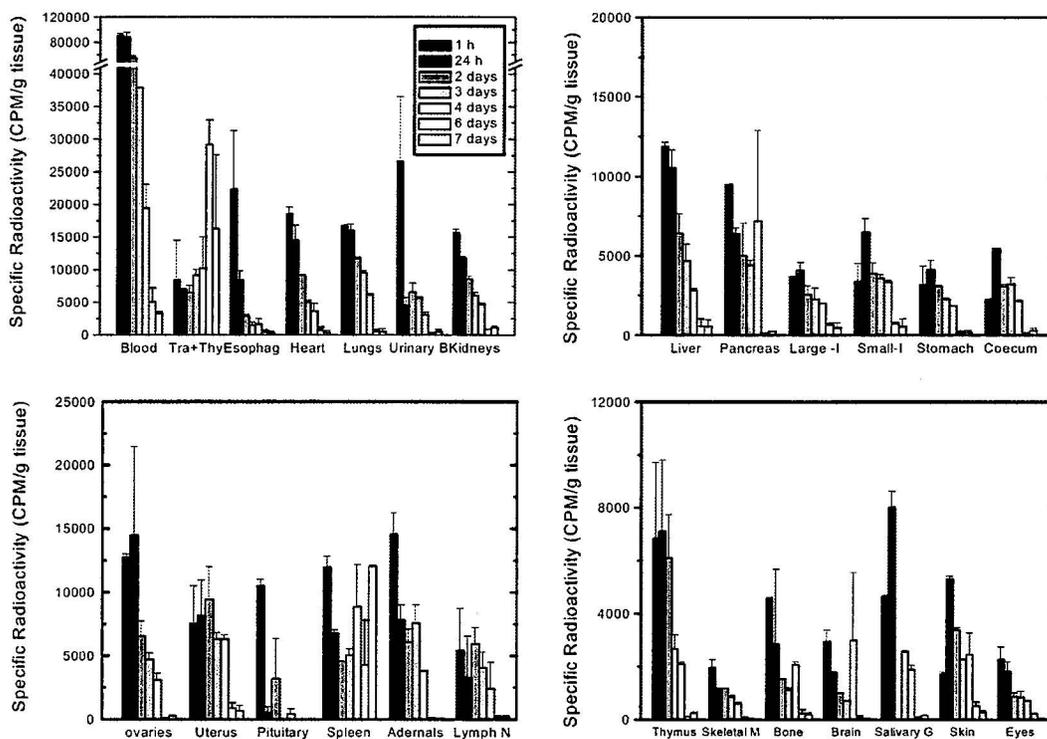


Figure 7. Tissue Values of Specific Radioactivity – Female Rats

- The specific radioactivity in blood was substantially higher than in any other tissues at all time points.
- The rank order of tissue distribution of ^{125}I -labeled PEG-uricase derived specific radioactivity in males at 24-hours post dosing was: blood > salivary gland > skin > lung > adrenal > lymph node.
- The rank order of tissue distribution of ^{125}I -labeled PEG-uricase derived specific radioactivity in females at 24-hours post dosing was: blood > lung > heart > ovaries = kidney = liver.
- As depicted in the figures above, the decline of specific radioactivity in most organs paralleled the decline in blood.
- Specific radioactivity in the spleen and thyroid/trachea did not decline with time; it basically remained the same over the study. The rate of clearance from the spleen was slower than the other tissues. The Sponsor concluded that “the retention of specific activity in the spleen likely represents sequestration of ^{125}I -pegloticase-like material by spleen macrophages with a slower rate of clearance than in other tissues.” *The reviewer concurs with the Sponsor’s interpretation of the results observed in the spleen because the results from the TCA precipitation assay suggest that the radioactivity in the spleen represented precipitated protein.*

- As depicted in the Sponsor's Table 4 below, results from the TCA precipitation assay suggests that the radioactivity in the kidney, liver, lungs and spleen was largely precipitated proteins and most likely the puricase-like material. Seven days post-dosing, high percentage of TCA precipitable radioactivity was still measurable in the spleen, liver and kidney.

Table 4. Percentage of TCA Precipitable Radioactivity in Tissue Homogenates at Various Times*

Group	Kidney		Liver		Spleen		Lungs	
	Male	Female	Male	Female	Male	Female	Male	Female
1 h	100.0 ± 0.0	100.0 ± 0.0	98.0 ± 2.8	100.0 ± 0.0	91.0 ± 12.7	84.0 ± 0.0	97.5 ± 3.5	97.0 ± 2.8
1 day	99.0 ± 1.4	76.5 ± 5.0	55.0 ± 28.3	96.8 ± 4.6	86.8 ± 7.1	85.7 ± 19.7	78.6 ± 10.5	74.3 ± 4.5
2 days	93.5 ± 9.2	92.5 ± 5.0	93.5 ± 9.2	96.0 ± 5.7	91.5 ± 12.0	98.0 ± 2.8	90.0 ± 14.1	100.0 ± 0.0
3 days	91.5 ± 0.7	95.5 ± 3.5	92.0 ± 5.0	94.3 ± 8.1	83.3 ± 16.8	75.3 ± 5.2	86.7 ± 12.4	78.5 ± 0.8
4 days	83.0 ± 14.1	90.5 ± 3.5	96.5 ± 5.0	67.0 ± 14.1	100.0 ± 0.0	78.0 ± 22.6	100.0 ± 0.0	87.0 ± 18.4
6 days	80.5 ± 13.4	89.7 ± 5.2	100.0 ± 0.0	95.0 ± 7.1	100.0 ± 0.0	100.0 ± 0.0	91.4 ± 12.2	81.6 ± 1.7
7 days	80.1 ± 4.0	85.7 ± 14.4	94.0 ± 7.8	82.5 ± 9.8	87.8 ± 3.2	90.7 ± 6.6	68.0 ± 14.1	79.0 ± 29.7

* Values represent the mean ± range

Study Title: Absorption, distribution, metabolism and excretion (ADME) of subcutaneously versus intravenously administered ¹²⁵I-labelled Puricase™ in rats.

Study №: 00V258-4 and 00V258-3 (details the procedure used in protocol № 00V258-4)

Study Objective: To characterize the tissue distribution of ¹²⁵I-labeled PEG-uricase (Puricase) following a single subcutaneous and intravenous administration in Sprague Dawley rats.

Methods: Male (n = 2/time point/injection route) and female (n = 1/ time point/injection route) Sprague-Dawley rats received a single intravenous or subcutaneous injection of ¹²⁵I-labeled PEG-uricase at a volume of 0.5 mL (0.5 mg/kg equivalent; corresponding to 5.8x10⁶ cpm). Seven days after dosing, all animals were anaesthetized; blood (10 mL drawn from the aorta) and tissues were collected. The following tissues were collected: adrenals, brain, bone, caecum, esophagus, eyes, gonads, heart, kidneys, large intestine, liver, lungs, lymph nodes, pancreas, pituitary gland, salivary gland, skeletal muscle, skin, small intestine, spleen, stomach, thymus, trachea/thyroid and urinary bladder. In addition, the skin area zone of the subcutaneously injected rats was excised and counted separately. Also, the muscle area under this skin area zone was excised and counted. Large organs were weighed prior to measuring radioactivity levels. Radioactivity levels were measured using a gamma scintillation counter.

Key Study Findings:

The tissue distribution of ¹²⁵I-labeled PEG-uricase is shown in the Sponsor's Figure 3 below.

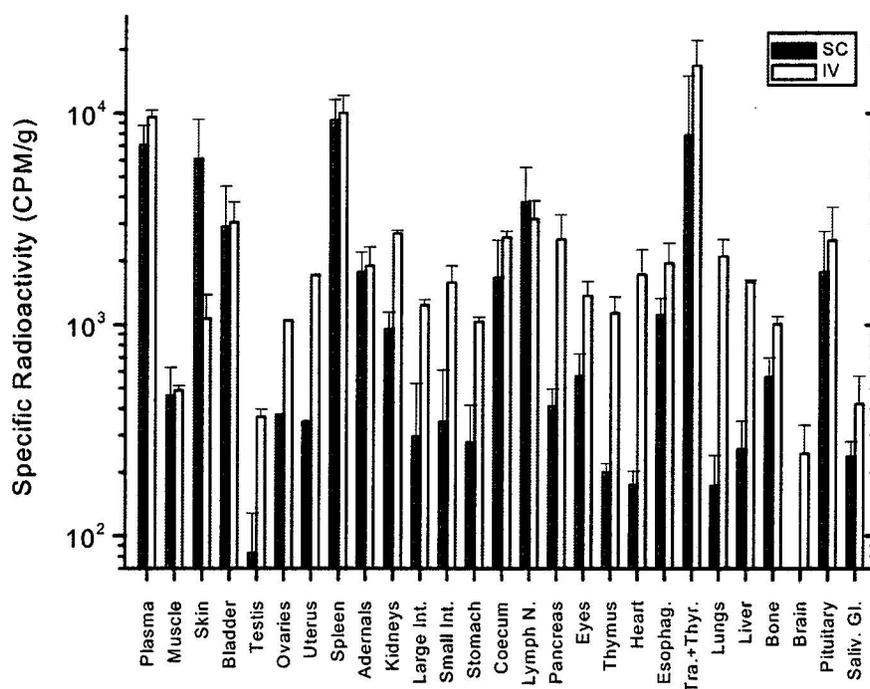


Figure 3. Mean Values of Specific Radioactivity (SR) Found in the Various Tissues and Organs on Day 7

- The results obtained were consistent with those observed in Study №: 00V258-2. Specific radioactivity of ^{125}I -puricase was detected in tissues 7-days after dosing. The measured specific radioactivity of ^{125}I -puricase in the tissues was lower than that measured in plasma.
- The distribution of radioactivity into tissues, with the exception of the brain, was similar following subcutaneous or intravenous administration of ^{125}I -puricase. Compared to intravenous administration, the accumulation of ^{125}I -puricase derived radioactivity was lower in basically all tissues following subcutaneous administration.
- Following subcutaneous administration, ^{125}I -labeled puricase was not distributed to the brain.
- Following subcutaneous administration, the rank order of distribution of ^{125}I -labeled PEG-uricase derived radioactivity was: spleen > trachea/thyroid > skin > lymph nodes.
- The specific radioactivity measured in the skin and muscle in the area of the injection site was low. According to the Sponsor, radioactivity measured in the skin above and adjacent to the injection site accounted for 0.11% of the injected dose in rat. In the muscle below and adjacent to the injection site, the quantity of radioactivity accounted for 0.014% of the dose injected in the rat.
- Following intravenous administration, the rank order of distribution of ^{125}I -labeled PEG-uricase derived radioactivity was: spleen > trachea/thyroid > skin > lymph nodes > pancreas = caecum.

2.6.4.5 Metabolism

No studies on the metabolism of puricase were submitted with this BLA. Due to the size of the PEG-uricase one would expect it to be metabolized by catabolism. While there were no direct metabolism studies performed, the metabolism of PEG-uricase was examined by measuring radioactivity precipitated by TCA. Results from the two ADME studies conducted in rats identified puricase protein degradation products in the urine. The data suggested that puricase underwent extensive degradation; approximately 89% of the radioactivity in the urine was TCA soluble.

2.6.4.6 Excretion

The excretion profile of radioactivity in urine and feces following subcutaneous and intravenous administration of ^{125}I -labeled PEG-uricase (puricase) was investigated in rats. Recovery of total radioactivity was examined at intervals up to 168 hours (7 days).

Study Title: Distribution, metabolism and excretion of intravenously administered ^{125}I -labelled PuricaseTM in rats.

Study №: 00V258-2 (non-GLP) and 00V258-1 (details the procedure used in protocol № 00V258-2)

Study Objective: To evaluate the excretion profile of ^{125}I -labeled PEG-uricase (Puricase) following a single intravenous administration in Sprague Dawley rats.

Methods: Male (n = 2/time point) and female (n = 2/ time point) Sprague-Dawley rats received a single intravenous or subcutaneous injection of ^{125}I -labeled PEG-uricase at a volume of 0.5 mL (0.5 mg/kg; corresponding to 0.39 μg (2×10^6 cpm) of ^{125}I -labeled PEG-uricase + 0.1 mg puricase per rat). Urine and feces samples was collected from the 7 day group from 0-8 hours, 8-24 hours, 2 days, 3 days, 4 days, 6 days, and 7 days after injection. Radioactivity was measured by gamma scintillation counting. Urine samples were also subjected to TCA precipitation.

Key Study Findings:

- As depicted in the Sponsor's figures below, the urinary route was the primary route of elimination of ^{125}I -labeled PEG-uricase derived radioactivity.
- Less than 10% of ^{125}I -labeled PEG-uricase derived radioactivity was excreted in the feces.
- The highest rate of excretion of ^{125}I -labeled PEG-uricase derived radioactivity was measured two days after dosing in both urine and feces.
- There were no gender-related differences in the excretion profile of ^{125}I -labeled PEG-uricase derived specific radioactivity.

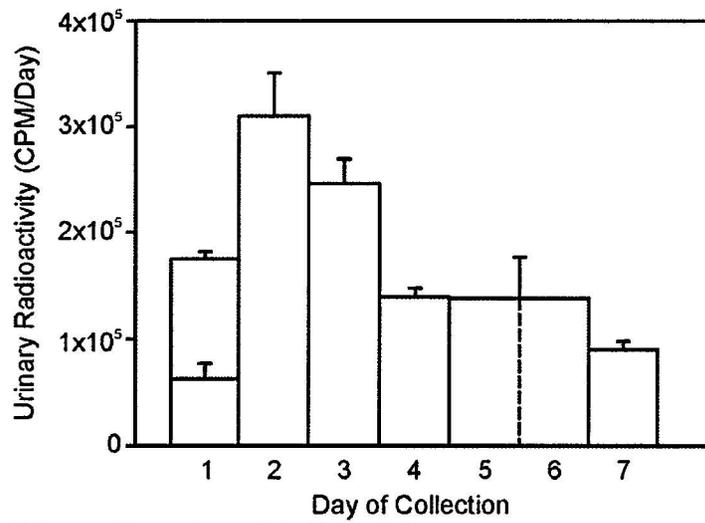


Figure 4. Urinary Excretion of Radioactivity

Note: The mean radioactivity of total urine collected on day 1 from 0 h through 24 h was 1.76×10^5 cpm (gray + unfilled portion of bar). Mean radioactivity of urine collected from 8 h to 24 h of day 1 was 6.19×10^4 cpm (unfilled portion of bar).

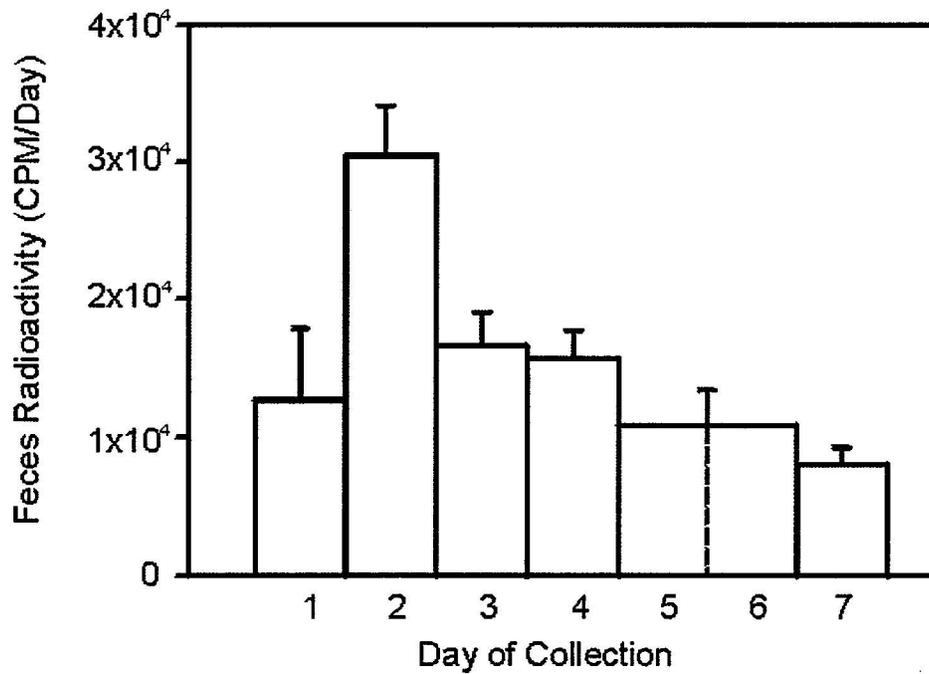


Figure 5. Fecal Excretion of Radioactivity

Study Title: Absorption, distribution, metabolism and excretion (ADME) of subcutaneously versus intravenously administered ¹²⁵I-labelled Puricase™ in rats.

Study №: 00V258-4 and 00V258-3 (details the procedure used in protocol № 00V258-4)

Study Objective: The objective of this study was to evaluate the excretion profile and measure the absorption of ¹²⁵I-labeled PEG-uricase (Puricase) following a single subcutaneous and intravenous administration in Sprague Dawley rats.

Methods: The excretion of ¹²⁵I-Puricase derived radioactivity was determined in urine and feces following a single intravenous or subcutaneous administration. Male (n = 2/time point/injection route) and female (n = 1/ time point/injection route) Sprague-Dawley rats received a single intravenous or subcutaneous injection of ¹²⁵I-labeled PEG-uricase at a volume of 0.5 mL (0.5 mg/kg equivalent; corresponding to 5.8x10⁶ cpm). Urine and feces samples were collected at 24 hours post injection (day of injection) and 2, 3, 4, 5, 6, and 7 days after injection. Radioactivity was measured by gamma scintillation counting. Urine samples were also subjected to TCA precipitation.

Key Study Findings:

Total radioactivity in urine and feces following subcutaneous administration.

Collection Day	Animal №/Sex	Radioactivity in Urine		Radioactivity in Feces
		Total cpm	% TCA-Precipitable	Total cpm
1	1/F	(b) (4)	13.3	(b) (4)
	2/M		15.0	
	3/M		10.6	
2	1/F		16.3	
	2/M		17.1	
	3/M		15.5	
3	1/F		21.7	
	2/M		21.1	
	3/M		17.3	
4	1/F		22.5	
	2/M	22.0		
	3/M	21.0		
5-6	1/F	22.0		
	2/M	19.3		
	3/M	22.7		
7	1/F	17.9		
	2/M	20.8		
	3/M	20.5		

Total radioactivity in urine and feces following intravenous administration.

Collection Day	Animal №/Sex	Radioactivity in Urine		Radioactivity in Feces
		Total cpm	% TCA-Precipitable	Total cpm
1	4/F	(b) (4)	16.4	(b) (4)
	5/M		14.4	
	6/M		13.5	
2	4/F		21.0	
	5/M		14.8	
	6/M		19.5	
3	4/F		19.7	
	5/M		17.8	
	6/M		20.0	
4	4/F		22.7	
	5/M		20.5	
	6/M		19.9	
5-6	4/F		20.5	
	5/M	21.7		
	6/M	17.5		
7	4/F	17.8		
	5/M	20.8		
	6/M	18.6		

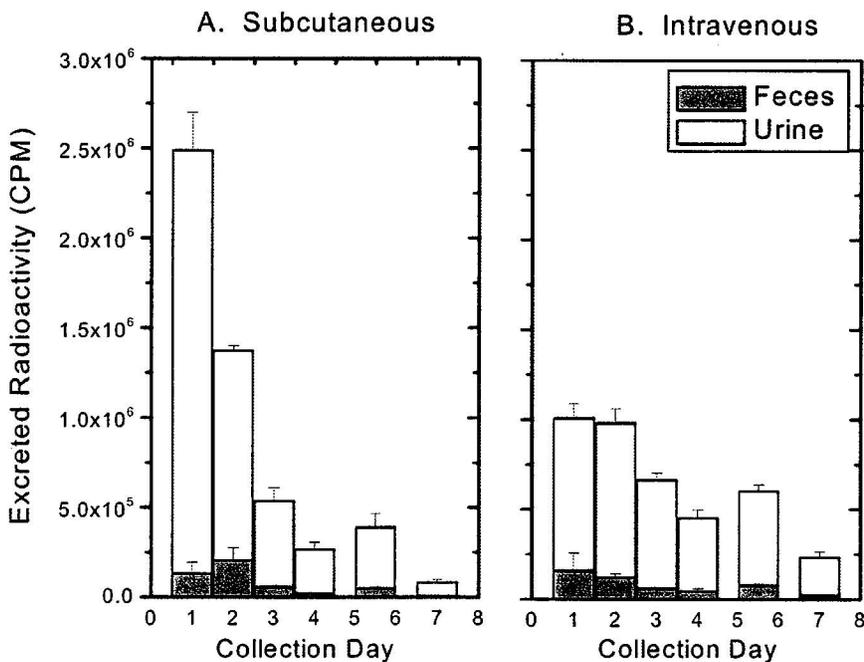


Figure 2. Mean Daily Accumulation of Radioactivity in the Urine and Feces Following Subcutaneous (A) or Intravenous (B) Administration

- As depicted in the tables and Sponsor's figure 2 above, radioactivity was excreted primarily in the urine following both subcutaneous and intravenous administration.
- Fecal elimination was a minor route of excretion of ¹²⁵I-puricase-derived radioactivity following both subcutaneous and intravenous administration. Less than 10% of radioactivity was excreted in feces.
- The rate and pattern of excretion differed between the two routes of administration. Following intravenous administration, the rate of excretion was gradual during the first 3 days after administration. In contrast, the rate of excretion was faster following subcutaneous administration, excreted radioactivity was highest on the first day of administration.

2.6.4.7 Pharmacokinetic drug interactions

No studies were conducted to explore potential pharmacokinetic drug interactions with uricase.

2.6.4.8 Other Pharmacokinetic Studies

Study Title: Pharmacokinetic study of Puricase in beagle dogs.

Study №: 01V365-1 and 01V365-2 (amendment to study report №: 01V365-1)

Supporting Study Reports:

№ 20-2-0156-00: Describes the administration of the test material, handling of the animals and shipment of samples from (b) (4) to BTG.

№ 00V262-1: Describes Colorimetric Assay used for the determination of uricase activity.

№ 00V293-1: Details the procedure used to evaluate immunogenicity.

Study Objective: The objectives of this study were:

1. To evaluate the total exposure (AUC) and clearance rate (i.e., elimination half-life) of Puricase™ after repeated intravenous administration.
2. To determine the bioavailability of Puricase™ following intramuscular and subcutaneous administration.
3. To evaluate the immunogenicity of the PEG-conjugated enzyme product.

Methods: The pharmacokinetics of Puricase™ was determined in male and female Hsd:Bur:BEAG beagle dogs (n = 2/sex/injection route) following intravenous, subcutaneous and intramuscular administration. Three doses of Puricase™ were administered following each route. In the first injection period (day 1), the dogs were administered 0.4 mg/kg of Puricase™ in a phosphate-buffered saline solution at a dose of 0.4 mg/kg. A second injection was administered 2-weeks later (day 15) at a dose level of 0.4 mg/kg). Data from this second injection was not utilized in the analysis due to

injection errors observed; activity profile (i.e., delayed appearance of uricase activity) of the intravenous group suggested that the puricase was injected paravenously instead of intravenously. Also, low level of uricase was noted in the subcutaneous group. Hence these animals were retested five weeks (day 65) after the first injection. Due to insufficient quantity of puricase available, these dogs were administered puricase at a dose of 0.348 mg/kg per route.

Blood samples were collected from the jugular vein at pre-dose, 5-min, 6, 24, 48, 72 (day 1), 120, 192 (days 15 & 65), 216 (day 1), 312 (days 15 & 65) and 336 (day 1) hours after the first and third following the first and third intravenous injection, and pre-dose, 1, 6, 24, 48, 72 (day 1), 120, 192 (days 15 & 65), 216 (day 1), 312 (days 15 & 65) and 336 (day 1) hours after the first and third intramuscular and subcutaneous injection. Plasma concentrations of puricase and antibodies to puricase were determined using an ELISA method. Using a colorimetric assay coupled with an enzymatic reaction, uricase activity was measured one month after the first injection of Puricase™. The rate of formation of the quinoneimine dye is proportional to the concentration and activity of the active puricase in the sample. Uricase concentrations as low as 300 ng per mL of plasma could be accurately detected in the assay.

Key Study Findings:

- There were no apparent clinical signs of toxicity observed following intravenous, intramuscular and subcutaneous administration of puricase. Also, there were no treatment-related effects on body weight.
- As indicated in the table below, puricase did not cause an immune response directed against free uricase, m-PEG or lightly PEGylated uricase following i.m., s.c., or i.v. administration.

Injection Group	Antigens →	Antibody Response to Antigens: № Positives/№ Tested		
		Uricase	m-PEG	Pegloticase
Intravenous		0/4	0/4	0/4
Subcutaneous		0/4	0/4	0/4
Intramuscular		0/4	0/4	0/4

Pharmacokinetic parameters of Puricase in male and females dogs following i.v., s.c. and i.m. administration.

Injection Number	Dose (mg/kg)	Route	PK Parameters								
			AUC ($\mu\text{g}\cdot\text{h}/\text{mL}$)			$T_{1/2}$ (hour) ^A mean \pm SEM			T_{max} (hour)	C_{max} ($\mu\text{g}/\text{mL}$)	F (%) ^B
			M	F	M + F	M	F	M + F	M + F	M + F	M + F
1	0.4	IV	1,042.4	930.9	986.8	108.35 \pm 47.73	79.3 \pm 13.43	93.8 \pm 16.57	NA	NA	100
		IM	678.3	663.7	671.6	NC	NC	NC	72	5.07	68.1
		SC	576.9	405.8	491.0	NC	NC	NC	72	4.04	49.8
3	0.348	IV	1052.5	1,169.1	1,110.8	174.6 \pm 65.05	150.8 \pm 62.5	162.7 \pm 26.94	NA	NA	100
		IM	963.06	939.9	951.5	NC	NC	NC	48	3.21	85.7
		SC	408.2	656.2	531.9	NC	NC	NC	24	4.37	47.9

A: Half-life values were calculated from active plasma puricase concentrations
B: F = bioavailability computed from AUC values obtained from male and female dogs (combined)
NA: Not Applicable to calculate for the intravenous route
NC: Not Calculated

- Bioavailability of puricase, as determined by the ratios of AUC after s.c. and i.v. or i.m. and i.v. was higher following intramuscular administration than after subcutaneous administration. Bioavailability was 68.1% (first injection) and 85.7% (third injection) and 49.8% (first injection) and 47.9% (third injection) following intramuscular and subcutaneous administration, respectively.
- A different pattern of exposure was observed following subcutaneous, intravenous and intramuscular injection of puricase.
- There were no apparent differences in the pharmacokinetic parameters observed in male and female dogs following intravenous, intramuscular and subcutaneous administrations. The AUC values in males and females were 1,042.4 and 986.8 $\mu\text{g}\cdot\text{h}/\text{mL}$ and 1052.5 and 1,169.1 $\mu\text{g}\cdot\text{h}/\text{mL}$ following the first and third intravenous injection, respectively. The AUC values in males and females were 678.3 and 663.7 $\mu\text{g}\cdot\text{h}/\text{mL}$ and 963.06 and 939.9 $\mu\text{g}\cdot\text{h}/\text{mL}$ following the first and third subcutaneous injection, respectively.
- The half-life for puricase following intravenous injection ranged from 3 to 6 days and 4.5 to 7 days for males and females, respectively.
- Mean half-life for the intravenous group (male and female combined) was 4 (93.8 hours) and 6.7 days (162.7 hours) after the first and third injection of puricase, respectively.
- After the first subcutaneous and intramuscular administration of a 0.4 mg/kg dose of puricase, C_{max} were reached at 72 hours of dosing, with a mean value of 4.04 $\mu\text{g}/\text{mL}$ (s.c.) and 5.07 $\mu\text{g}/\text{mL}$ (i.m.).
- After the third subcutaneous and intramuscular administration of a 0.348 mg/kg dose of puricase, C_{max} were reached at 48 and 24 hours of dosing, respectively with a mean value of 3.21 $\mu\text{g}/\text{mL}$ (s.c.) and 4.37 $\mu\text{g}/\text{mL}$ (i.m.).

Plasma uricase activity of PeGylated (9x 10) PigKS-ΔN Uricase in dogs following i.m., s.c. and i.v. injection

Day 0 Injection

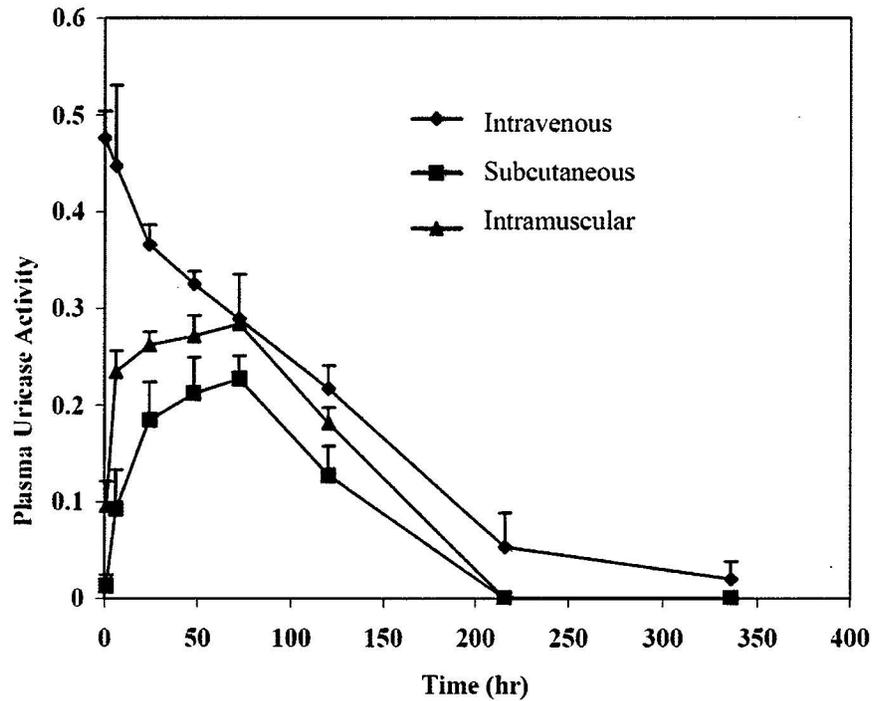


Figure 1. Pharmacokinetic Behavior of Puricase after the First Intravenous, Intramuscular and Subcutaneous Administration to Dogs

The points and vertical brackets represent the mean and SEM of plasma uricase activity of male and female dogs

Plasma uricase activity of PeGylated (9x 10) PigKS-ΔN Uricase in dogs following the third i.m., s.c. and i.v. injection

Day 65 Injection

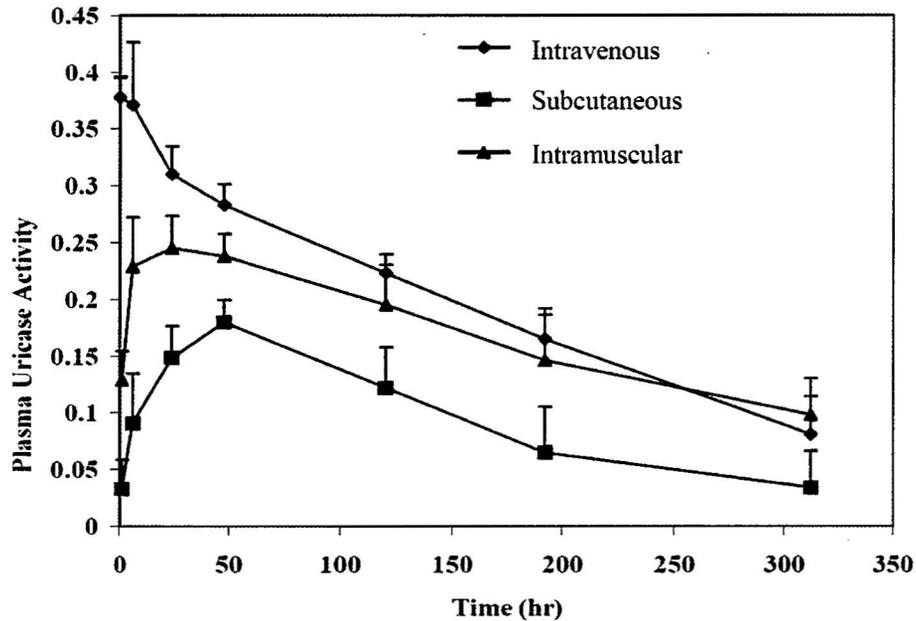


Figure 2. Pharmacokinetic Behavior of Puricase after the Third Intravenous, Intramuscular and Subcutaneous Administration to Dogs

The points and vertical brackets represent the mean and SEM of plasma uricase activity of male and female dogs

- Enzyme activity is still evident at 336, 120 and 120 hours after the first intravenous, subcutaneous and intramuscular injection, respectively.
- After the third intravenous, subcutaneous and intramuscular injection, enzyme activity is still presence at 312, 312 and 312 hours, respectively.

Study Title: Report, pharmacokinetic study of Puricase in rats, treated with five weekly injections by the intravenous, intramuscular and subcutaneous routes.**Study №: 01V359-2 and 01V365-2 (amendment to study report №: 01V365-1)****Supporting Study Reports:****№ 00V262-1: Describes Colorimetric Assay used for the determination of uricase activity.****№ 00V293-2: Details the ELISA method used to evaluate immunogenicity.****Study Objective:** The objectives of this study were:

1. To determine total exposure (AUC) and evaluate the clearance rate (i.e., elimination half-life) of Puricase™ after repeated (i.e., intermittent) intravenous administration.
2. To determine the bioavailability of Puricase™ following intramuscular and subcutaneous administration.
3. To evaluate the immunogenicity of the PEG-uricase.

Methods: The pharmacokinetics of Puricase™ was determined in male and female rats (n = 6/sex/injection route) following intravenous, subcutaneous and intramuscular administration. Puricase™ (lot № OPD-68-007-4) in phosphate buffered saline was administered once weekly for five weeks (days 0, 7, 14, 21 and 28) at an approximate dose of 0.7 mg/kg, 2.1 mg/kg or 2.1 mg/kg following intravenous and subcutaneous and intramuscular administration, respectively.

Blood samples were collected from the eye orbital sinus from 3/sex/time point according to the following schedule:

Day	Route		
	IV	SC	IM
0	5-min and 6-8 hours post-injection	1-hr and 6-8 hrs post-injection	1-hr and 6-8 hrs post-injection
1	24-hrs post-injection	24-hrs post-injection	24-hrs post-injection
2	48-hrs post-injection	48-hrs post-injection	48-hrs post-injection
3	72-hrs post-injection	72-hrs post-injection	72-hrs post-injection
4	96-hrs post-injection	96-hrs post-injection	96-hrs post-injection
5	120-hrs post injection	120-hrs post injection	120-hrs post injection
7	168-hrs post injection, before subsequent injection	168-hrs post injection, before subsequent injection	168-hrs post injection, before subsequent injection

Blood was drawn from the aorta of all animals 2-weeks after the 5th injection to test for an immune response. Plasma concentrations of puricase and antibodies to puricase were determined using an ELISA method.

Using a colorimetric assay coupled with an enzymatic reaction, uricase activity was measured one month after the first injection of Puricase™. The rate of formation of the quinoneimine dye is proportional to the concentration and activity of the active puricase

in the sample. Uricase concentrations as low as 300 ng per mL of plasma could be accurately detected in the assay.

Key Study Findings:

- There were no apparent clinical signs of toxicity observed following intravenous, intramuscular and subcutaneous administration of puricase. Also, there were no treatment-related effects on body weight.
- Two rats died during the study. Rat № 2 died on day 5 after the first subcutaneous injection. Rat № 18 died on the day that blood was being drawn for the immunogenicity test.

Pharmacokinetic parameters of Puricase in male and female rats (combined) after the day 1, 14 and 28 injection by the i.v., s.c. and i.m. route of administration.

Injection Day	Dose (mg/kg)	Route	PK Parameters						
			AUC (µg·h/mL)			T _{1/2} (hour) mean ± SEM	T _{max} (hour)	C _{max} (µg/mL)	F (%) ^B
			M	F	M + F	M + F	M + F	M + F	M + F
1	0.7	IV	426.6	324.8	375.6	34.0 ± 2.12	NA	NA	100
	2.1	IM	577.3	440.3	508.4	NC	48	5.94	45.1
	2.1	SC	576.9	262.5	337.6	NC	48	4.28	30.0
14	0.7	IV	534.5	297.8	416.5	31.0 ± 4.53	NA	NA	100
	2.1	IM	726.9	155.7	441.2	NC	24	5.17	35.3
	2.1	SC	395.4	39.0	177.9 ^A	NC	24	3.13	14.2
28	0.7	IV	630.6	505.5	567.4	43.2 ± 5.57	NA		100
	2.1	IM	796.3	545.7	671.0	NC	24	8.92	39.4
	2.1	SC	706.4	32.6	319.2	NC	24	4.18	18.8

A: Sponsor reported that the low AUV value reflects plasma concentrations in females that were largely below the LOQ at most time points (AUC = 39 µg·h/mL) compared to males (AUC = 395.4 µg·h/mL)
 B: F = bioavailability computed from AUC corrected for dose
 NA: Not Applicable to calculate for the intravenous route
 NC: Not Calculated

- Bioavailability of puricase, as determined by the ratios of AUC after s.c. and i.v. or i.m. and i.v. was higher following intramuscular administration than after subcutaneous administration. Bioavailability was 45.1%, 35.3% and 39.4% following the first, second and third intramuscular injection, respectively. Following the first, second and third subcutaneous injection, bioavailability was 30.0%, 14.2% and 18.8%, respectively.
- Exposure was higher in males compared to females following intravenous, intramuscular and subcutaneous administrations. The AUC values in males were 426.6, 534.5 and 630.6 µg·h/mL following the first, second and third intravenous injection, respectively. Whereas in females, the AUC values were 324.8, 297.8 and 505.5 µg·h/mL following the first, second and third intravenous injection, respectively.

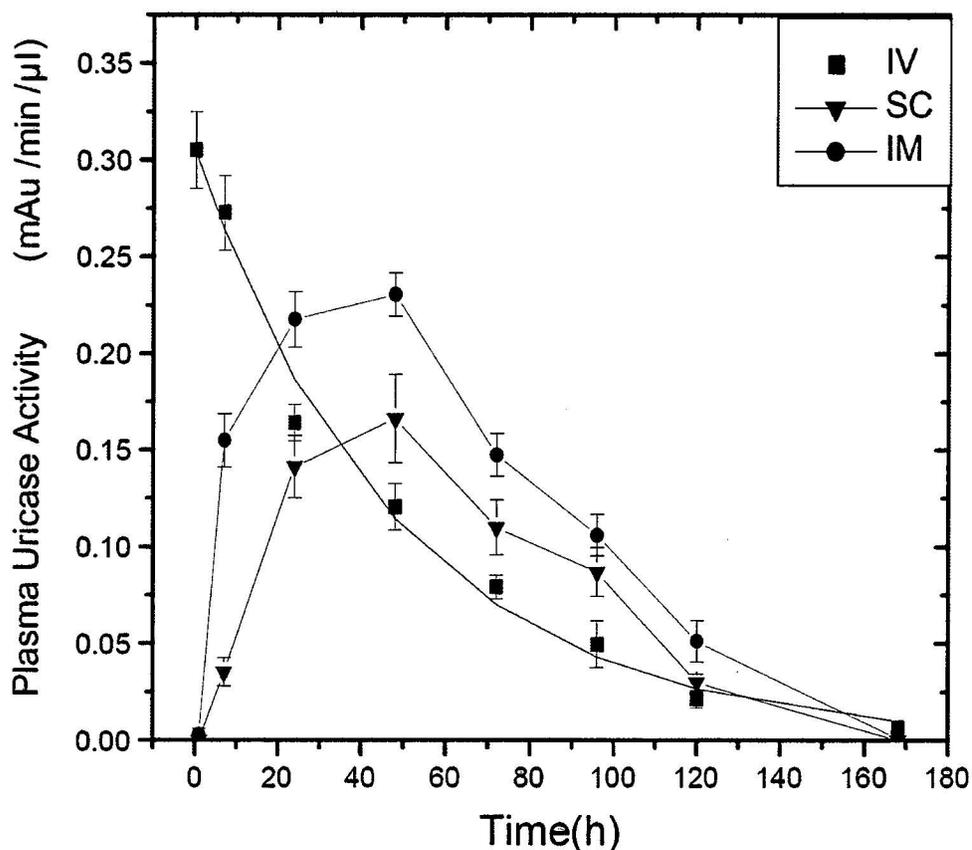
Plasma uricase activity of Puricase in rats following i.m., s.c. and i.v. injection.

Figure 1. Pharmacokinetic Behavior of Puricase after the First Intravenous, Intramuscular and Subcutaneous Administration to Rats

- Peak enzyme activity was observed at 5 minutes, 48 hours and 48 hours after the first intravenous, subcutaneous and intramuscular injection, respectively.
- Enzyme activity was still noted at 120 hours after the first injection following each route of administration.
- Higher puricase activity was measured following intramuscular administration compared to the subcutaneous route.

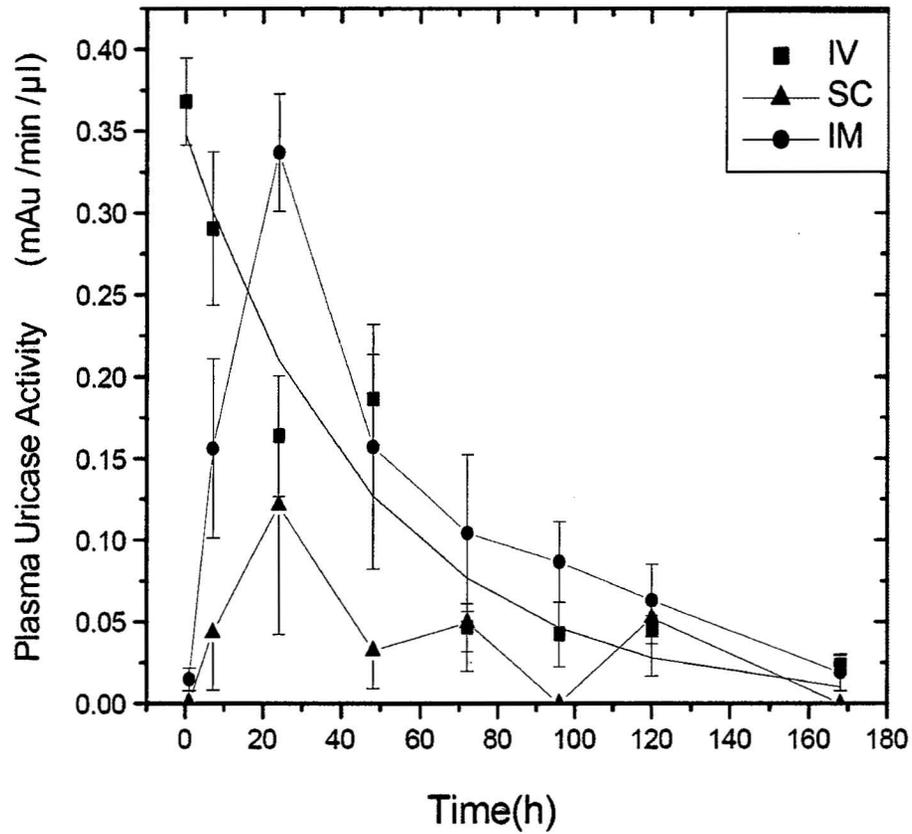


Figure 3. Pharmacokinetic Behavior of Puricase after the Third Intravenous, Intramuscular and Subcutaneous Administration to Rats

- In contrast to the results observed after the first injection of puricase, enzyme activity was variable after the third subcutaneous injection.

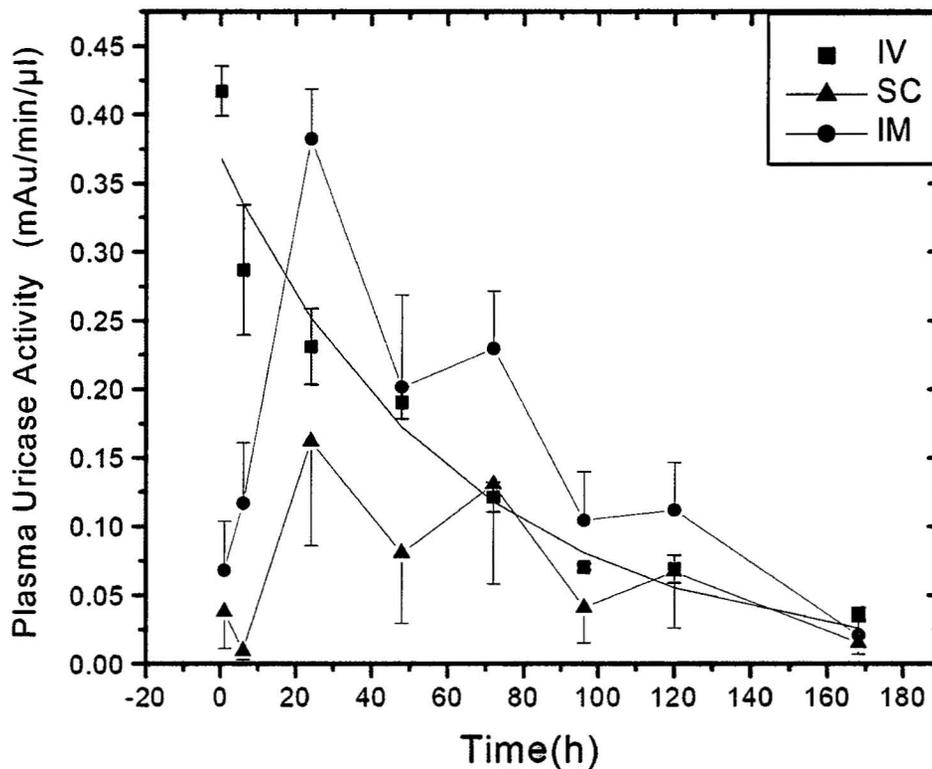


Figure 4. Pharmacokinetic Behavior of Puricase after the Fifth Intravenous, Intramuscular and Subcutaneous Administration to Rats

- Puricase activity was variable after the fifth subcutaneous and intramuscular injection.

Summary of antibody response in rats.

Injection Group	№ Positive/№ Tested		
	Uricase	PEG	Puricase
Intravenous	0/11	0/11	0/11
Intramuscular	0/10	5/10	7/10
Subcutaneous	2/10	8/10	8/10

- Antibodies directed against unmodified uricase, m-PEG and puricase was measured after 5 subcutaneous and intramuscular injections. Antibodies formation was higher following subcutaneous administration than after intramuscular injections.
- Antibodies directed against unmodified uricase, m-PEG and puricase were not detected in any of the animals administered puricase intravenously.
- The antibodies formed in rats were non-neutralizing. As depicted in the table below, the antibodies lacked the ability to neutralize the enzymatic activity of puricase.

Puricase Activity on rat plasma samples collected on study day 42	
Rat №	Average Activity* (mAU/min)
Control 1	2.78
Control 2	2.63
Control 3	3.53
4	3.52
9	2.17
10	2.63
11	2.58
12	2.50
17	3.13
19	3.39
20	2.28
27	3.08
*: Puricase activity in colorimetric units per 10 µl plasma sample.	

2.6.4.9 Discussion and Conclusions

The absorption, distribution, metabolism and elimination profiles of puricase were evaluated in pharmacokinetic studies in rats (Sprague-Dawley), dogs (Hsd:Bur:BEAG Beagle), rabbits and pigs after a single intramuscular, subcutaneous or intravenous injection. The pharmacokinetic study conducted in the rabbit and pig was not reviewed.

Two pharmacokinetic studies performed in rats showed that puricase was rapidly and well absorbed after a single intravenous administration to rats based on absorption of radioactivity from ^{125}I -labeled PEG-uricase. Systemic exposure in rats after intravenous administration, as calculated from the AUC increased in a dose-dependent fashion. Peak concentrations in both plasma and whole blood were observed at the first sampling time point of 5 minutes and 1 hour, respectively, and then declined in a manner consistent with a single compartment model. Radioactivity levels were still detectable 7 days after dosing. Elimination half-life of puricase was determined to be 22.7 hours (combined male and female). Results from the other single dose rat study showed a gender difference in elimination half-life; treatment of females with ^{125}I -labeled PEG-uricase resulted in a more prolonged exposure to ^{125}I -labeled PEG-uricase. An apparent elimination half-life of 35 hours and 48 hours was reported for male and female rats, respectively.

Following subcutaneous administration of 0.5 mg/kg of puricase to rats, plasma and whole blood concentrations of puricase peaked at 24 hours after dosing and gradually declined thereafter. ^{125}I -labeled PEG-uricase levels were still detectable 7 days after dosing. Relative to intravenous administration, bioavailability was low (26%).

As part of an acute toxicity study, the pharmacokinetic profile of puricase was determined in juvenile dogs (n=3/sex/dose) following intravenous administration of a

single dose (0.4, 1.5 and 10 mg/kg) with a four week recovery period. Plasma concentrations of puricase and AUC increased with increasing dose. Maximum plasma concentrations were reached at one hour (first sampling time) post-dosing. Exposure was similar in males and females. The half-life for puricase in plasma ranged from 65 to 99 hours in the males and 43 to 109 hours in the females. Systemic clearance was comparable between the genders.

The pharmacokinetic profile of puricase (0.4 mg/kg) was determined in dogs following three injections by the intravenous, intramuscular and subcutaneous routes. There were no differences in exposures between males and female dogs. Following intravenous administration, the half-life was 108 and 79 hours for male and female dogs, respectively. The second injection was administered 14 days after the first injection and the third injection was administered approximately 7 weeks after the second injection. After the first and third subcutaneous injection of puricase, C_{max} was reached at 72 and 48 hours, respectively. After the first and third intramuscular injection of puricase, C_{max} was reached at 72 and 24 hours after dosing, respectively. The bioavailability of puricase administered subcutaneously and intramuscularly was 47.9% (after 3rd injection) – 49.7% (after 1st injection) and 68.1% (after 1st injection) – 85.7% (after 3rd injection), respectively.

The distribution of puricase was evaluated in rats following single intravenous and subcutaneous administration of ^{125}I -PEG-uricase to rats. Following a single intravenous a dose of ^{125}I -PEG-uricase was extensively distributed with the exception to the brain. No ^{125}I -PEG-uricase derived radioactivity was detected in the brain. All tissues collected were exposed to radioactivity at first time point (i.e., 0.5 hours) after dosing. The highest concentrations of ^{125}I -PEG-uricase was observed at the first time point (1.0 hours) after dosing and had the propensity to be associated with organs with high blood perfusion. At 1 and 24 hours after dosing, levels of radioactivity in the tissues were substantially lower than the radioactivity in blood. The distribution of radioactivity at 24-hours post dosing differed between males and female rats. At 24-hours post dosing, the rank order of tissue distribution of radioactivity in males was: blood > salivary gland > skin > lung > adrenal > lymph node. The rank order of distribution of radioactivity in females at 24-hours post dosing was: blood > lung > heart > ovaries = kidney = liver. The radioactivity in the liver, lung, kidney and spleen was largely represented precipitated protein; thus suggesting that it represents puricase-like material. With the exception of the spleen and thyroid gland, the decline in the level of radioactivity paralleled that of the blood. Radioactivity was still measurable in these organs 7-days after dosing. The radioactivity in the spleen probably represents the sequestration of ^{125}I -puricase-like material in macrophages. The radioactivity in the thyroid most likely reflects the uptake of ^{125}I iodine in the thyroid. In a separate study that compared the distribution of ^{125}I -labeled PEG-uricase in rats 7 days after a single subcutaneous and intravenous administration, the distribution of radioactivity into tissues (with the exception of the brain) was similar following both routes. However, relative to the intravenous route, the level of radioactivity in these tissues was lower following subcutaneous administration.

The metabolism of puricase was evaluated as part of the two ADME studies conducted in rats. Using TCA precipitation assay, it was shown that pegloticase was excreted as a protein degradation product. The excretion of puricase was studied as part of the two ADME studies conducted in rats. The urinary route is the principal route of excretion following the intravenous and subcutaneous administration of ¹²⁵I-puricase. Less than 10% of the ¹²⁵I-puricase was excreted in the feces. The rate of excretion following intravenous administration was gradual compared to after subcutaneous administration. No gender-related difference in excretion was observed.

2.6.4.10 Tables and figures to include comparative TK summary

2.6.5.4.1 Pharmacokinetics: Toxicokinetics after Repeated Dosing

Study Number / CTD Section Number	01V373-3 / 4.2.2.2 01V373-5 / 4.2.2.2			01V373-2 / 4.2.2.2 01V373-4 / 4.2.2.2			02V526-2 / 4.2.2.2 02V526-5 / 4.2.2.2			7533-100 / Appendix 5 / 4.2.3.2 (Report 05C1360-2) / 4.2.3.2		
Species	Rat			Dog			Dog			Dog		
Gender/Number of animals	6/sex/dose			3 - 5/sex/dose			5/sex/dose			6/sex/dose		
Feeding condition	Fed			Fed			Fed			Fed		
Vehicle/Formulation	PBS			PBS			PBS			PBS		
Methods/Frequency of Administration	SC every 2 days x 54 days			SC every 4 days x 52 days			IV every 5 days x 86 days			IV every 7 days x 273 days		
Doses (mg/kg)	3.4, 10.2, 34			1.7, 5.1, 17			0.5, 1.5, 5			0.4, 1.5, 10		
Sample	Plasma			Plasma			Plasma			Plasma		
Analyte	Pegloticase			Pegloticase			Pegloticase			Pegloticase		
Assay	Colorimetric enzyme method			Colorimetric enzyme method			Colorimetric enzyme method			Colorimetric enzyme method		
Dose (mg/kg)	3.4	10.2	34	1.7	5.1	17	0.5	1.5	5	0.4	1.5	10
Pharmacokinetic Metrics (M+F combined)												
Mean Plateau Concentration (µg/mL)	22.9	61.5	228.5	38.2	138.1	465.9	-	-	-	-	-	-
Mean C _{max} (µg/mL)	-	-	-	-	-	-	23.5	75.7	286.0	14.9	72.5	472.8
Mean T _{max} or plateau (day)	31	15	15	15	15	15	86	86	86	84	84	84
Mean AUC _(0-∞) (µg·h/mL)*	953	3275	12488	1851 ^a	6770 ^a	22104 ^a	1516	4813	19885	3380	15088	99002
						28465 ^b						
Mean t _{1/2} (days)	2.76	2.78	3.32	NA	NA	8.3	7.1	6.9	9.5	NC	NC	NC

*Decimals rounded to the nearest whole number - = Not Applicable

^a At end of treatment

^b At end of treatment and recovery

NA = Not Available for these dose groups based on study design NC = Not Calculated PBS = Phosphate buffered saline pH 7.3 ± 0.3

2.6.5.4.2 Pharmacokinetics: Toxicokinetics after Repeated Dosing (Reproductive and Developmental)

Study Number / CTD Section Number	(b) 441007 (Appendix F) / 4.2.3.5.2				(b) 441008 (Appendix F) / 4.2.3.5.2			
Species	Rat				Rat			
Gender/Number of animals	6/dose				6/dose			
Feeding condition	Fed				Fed			
Vehicle/Formulation	PBS				PBS			
Methods/Frequency of Administration	IV/G6 - G16 q2d				IV/G6 - G18 q2d			
Doses (mg/kg)	5, 10, 20, 40				5, 10, 40			
Sample	Plasma				Plasma			
Analyte	Pegloticase				Pegloticase			
Assay	Colorimetric enzyme method				Colorimetric enzyme method			
Dose (mg/kg)	5	10	20	40	5	10	40	
Pharmacokinetic Metrics								
Mean AUC (µg·day/mL) ¹	778.7	1614.9	3733.4	6044.9	918.2	1818.4	7091.7	

- = Not Applicable

PBS = Phosphate buffered saline pH 7.3 ± 0.3

¹ Mean AUC determined from time points GD6 and 16 (1 h pre-dose, 1 h post-dose)

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Absorption:

2.6.5.3 PHARMACOKINETICS: ABSORPTION AFTER A SINGLE DOSE

Study Number:	(4.2.2.2) 00V258-2	(4.2.2.2) 00V258-4	(4.2.2.2) 00V266-2 (00V266-3)			
Species	Rat	Rat	Pig			
Gender /No. animals	2M+2F/time point	2M+1F/time point/injection route	2M+1F/injection route			
Feeding condition	Fed	Fed	Fed			
Vehicle/Formulation	PBS	PBS	PBS			
Dose	0.39 µg ¹²⁵ I-Pegloticase - 0.1 mg Pegloticase per rat = ~ 0.5 mg/kg	1.85 µg ¹²⁵ I-Pegloticase + 0.08 mg Pegloticase per rat = ~ 0.5 mg/kg	0.4 mg/kg Pegloticase			
Sample	Plasma	Plasma	Plasma			
Analyte	Total radioactivity	Total radioactivity	Pegloticase			
Assay	Gamma counting	Gamma counting	Colorimetric enzyme assay			
Administration Method	IV	IV	SC	IV	SC	IM
Pharmacokinetic Metrics:						
Mean C _{max} (µg/mL)	-	-	-	-	3.32	3.15
Mean T _{max} (h)	-	-	24	-	46	70
Mean AUC _(0-t) (µg·h/mL)	NC	NC	NC	925.1	709.9	662.6
Mean t _{1/2} (h)	35.3 (M) and 48.1 (F)	22.7 (M+F)	NC	178 (M+F)	NC	NC
Mean Bioavailability (%)	100	100	26	100	76.8	71.6

- = Not Applicable NC = Not Calculated PBS = Phosphate buffered saline pH 7.3 ± 0.3

Study Number / CTD Section Number	(b) 441011 (Appendix I) / 4.2.3.1			(b) 441012 (Appendix J) / 4.2.3.1		
Species	Dog			Dog		
Age	7-8 weeks			Approximately 20 weeks		
Gender/Number of animals	3/sex/dose			3/sex/dose		
Feeding condition	Fed			Fed		
Vehicle/Formulation	PBS			PBS		
Methods/Frequency of Administration	IV/single bolus dose			IV/single bolus dose		
Doses (mg/kg)	0.4, 1.5, 10			0.4, 1.5, 10		
Sample	Plasma			Plasma		
Analyte	Pegloticase			Pegloticase		
Assay	Colorimetric enzyme method			Colorimetric enzyme method		
Dose (mg/kg)	0.4	1.5	10	0.4	1.5	10
Pharmacokinetic Metrics – Male						
Mean AUC _{0-t} (µg·h/mL)	565	2707	24176	868	4984	30378
Mean AUC _{inf} (µg·h/mL)	733	2948	24488	1278	5307	33221
Mean C _{max} (µg/mL)	7.36	24.4	190	8.19	27.0	156
Mean t _{1/2} (h)	65	93	99	105	138	187
Mean CL (mL/h·mg)	0.55	0.51	0.41	0.32	0.28	0.30
Mean Vd (mL/kg)	52	68	59	47	56	81
Pharmacokinetic Metrics – Female						
Mean AUC _{0-t} (µg·h/mL)	446	2689	23719	812	4712	24785
Mean AUC _{inf} (µg·h/mL)	545	3018	24129	1230	5135	25784
Mean C _{max} (µg/mL)	7.62	24.5	187	7.75	27.6	141
Mean t _{1/2} (h)	43	98	109	112	140	129
Mean CL (mL/h·mg)	0.74	0.52	0.42	0.33	0.30	0.39
Mean Vd (mL/kg)	45	70	65	52	59	71

- = Not Applicable NC = Not Calculated PBS = Phosphate buffered saline pH 7.3 ± 0.3

2.6.5.4 PHARMACOKINETICS: ABSORPTION AFTER REPEATED DOSING

Study Number / CTD Number	01V359-2 (01V359-3) / 4.2.2.2			01V352-1 (01V352-2) / 4.2.2.2			01V365-1 (01V365-2) / 4.2.2.2		
Species	Rat			Rabbit			Dog		
Gender/Number of animals	6/sex/injection route			2/sex/injection route			2/sex/injection route		
Feeding condition	Fed			Fed			Fed		
Vehicle/Formulation	PBS			PBS			PBS		
Method/Frequency of Administration	IV/SC/IM every 7 days x 5 weeks			IV/SC/IM every 14 days x 8 weeks			IV/SC/IM every 14 days x 2 + week 9		
Dose (mg/kg)	0.7 (IV) or 2.1 (IM/SC)			0.4			0.4 or 0.35 (week 9)		
Sample	Plasma			Plasma			Plasma		
Analyte	Pegloticase			Pegloticase			Pegloticase		
Assay	Colorimetric enzyme method			Colorimetric enzyme method			Colorimetric enzyme method		
Pharmacokinetic Metrics (M+F combined)	IV	SC	IM	IV	SC	IM	IV	SC	IM
Mean C _{max} (µg/mL) [first/last injection]	-	4.28/4.18	5.94/8.92	-	4.46/5.65	5.16/6.24	-	4.04/3.21	5.07/4.37
Mean T _{max} (h) [first/last injection]	-	48/24	48/24	-	48/96	48/48	-	72/48	72/24
Mean AUC _(0-∞) (µg·h/mL) [first/last injection]	376/567	338/319	508/671	921/1474	776/1402	905/1296	987/1111	491/532	672/952
Mean t _{1/2} (h) [first/last injection]	34.0/43.2	NC	NC	88.5/153	NC	NC	93.8/162.7	NC	NC
Mean Bioavailability (%) [first/last injection]	100/100	30.0/18.8	45.1/39.4	100/100	84.3/95.1	98.3/87.9	100/100	49.8/47.9	68.1/85.7

* Decimals rounded to the nearest whole number
 -- Not Applicable NC = Not Calculated PBS = Phosphate buffered saline pH 7.3 ± 0.3

Study Number / CTD Section Number	01V373-3 / 4.2.2.2 01V373-5 / 4.2.2.2			01V373-2 / 4.2.2.2 01V373-4 / 4.2.2.2			02V526-2 / 4.2.2.2 02V526-5 / 4.2.2.2			7533-100 / Appendix 5 / 4.2.3.2 (Report 05C1360-2) / 4.2.3.2		
Species	Rat			Dog			Dog			Dog		
Gender/Number of animals	6/sex/dose			3 – 5/sex/dose			5/sex/dose			6/sex/dose		
Feeding condition	Fed			Fed			Fed			Fed		
Vehicle/Formulation	PBS			PBS			PBS			PBS		
Methods/Frequency of Administration	SC every 2 days x 54 days			SC every 4 days x 52 days			IV every 5 days x 86 days			IV every 7 days x 273 days		
Doses (mg/kg)	3.4, 10.2, 34			1.7, 5.1, 17			0.5, 1.5, 5			0.4, 1.5, 10		
Sample	Plasma			Plasma			Plasma			Plasma		
Analyte	Pegloticase			Pegloticase			Pegloticase			Pegloticase		
Assay	Colorimetric enzyme method			Colorimetric enzyme method			Colorimetric enzyme method			Colorimetric enzyme method		
Dose (mg/kg)	3.4	10.2	34	1.7	5.1	17	0.5	1.5	5	0.4	1.5	10
Pharmacokinetic Metrics (M+F combined)												
Mean Plateau Concentration (µg/mL)	22.9	61.5	228.5	38.2	138.1	465.9	-	-	-	-	-	-
Mean C _{max} (µg/mL)	-	-	-	-	-	-	23.5	75.7	286.0	14.9	72.5	472.8
Mean T _{max} or plateau (day)	31	15	15	15	15	15	86	86	86	84	84	84
Mean AUC _(0-∞) (µg·h/mL)*	953	3275	12488	1851 ^a	6770 ^a	22104 ^a	1516	4813	19885	3380	15088	99002
						28465 ^b						
Mean t _{1/2} (days)	2.76	2.78	3.32	NA	NA	8.3	7.1	6.9	9.5	NC	NC	NC

*Decimals rounded to the nearest whole number -- Not Applicable
^a At end of treatment
^b At end of treatment and recovery
 NA = Not Available for these dose groups based on study design NC = Not Calculated PBS = Phosphate buffered saline pH 7.3 ± 0.3

2.6.5.4.2 Pharmacokinetics: Toxicokinetics after Repeated Dosing (Reproductive and Developmental)

Study Number / CTD Section Number	(b) 441007 (Appendix F) / 4.2.3.5.2				(b) 441008 (Appendix F) / 4.2.3.5.2		
Species	Rat				Rat		
Gender/Number of animals	6/dose				6/dose		
Feeding condition	Fed				Fed		
Vehicle/Formulation	PBS				PBS		
Methods/Frequency of Administration	IV/G6 – G16 q2d				IV/G6 – G18 q2d		
Doses (mg/kg)	5, 10, 20, 40				5, 10, 40		
Sample	Plasma				Plasma		
Analyte	Pegloticase				Pegloticase		
Assay	Colorimetric enzyme method				Colorimetric enzyme method		
Dose (mg/kg)	5	10	20	40	5	10	40
Pharmacokinetic Metrics							
Mean AUC (µg·day/mL) ¹	778.7	1614.9	3733.4	6044.9	918.2	1818.4	7091.7

- = Not Applicable
 PBS = Phosphate buffered saline pH 7.3 ± 0.3
¹ Mean AUC determined from time points GD6 and 16 (1 h pre-dose, 1 h post-dose)

Excretion:

2.6.5.13 PHARMACOKINETICS: EXCRETION

Study No. / CTD Section Number	00V258-2 / 4.2.2.2		00V258-4 / 4.2.2.2			
Species	Rat		Rat			
Gender (M/F)/Number of animals	2M+2F/time point		2M+1F/time point/injection route			
Feeding condition	Fed		Fed			
Vehicle/Formulation	PBS (PBS = Phosphate buffered saline pH 7.4		PBS (PBS = Phosphate buffered saline pH 7.4			
Method of Administration	IV – single dose		IV or SC: single dose			
Dose (mg/kg)	0.39 µg ¹²⁵ I-Pegloticase + 0.1 mg Pegloticase per rat = ~0.5 mg/kg		1.85 µg ¹²⁵ I-Pegloticase + 0.08 mg Pegloticase per rat = ~0.5 mg/kg			
Analyte	Total radioactivity		Total radioactivity			
Assay	Gamma counting		Gamma counting			
Administration Route:	IV		IV		SC	
Excretion Route:	Urine	Feces	Urine	Feces	Urine	Feces
Collection Day	Mean Total Radioactivity (cpm)		Mean Total Radioactivity (cpm)			
0-8 h	113922	-	-	-	-	-
8-24h	61923	12972	-	-	-	-
Day 1	-	-	846546	526726	2360000	132240
Day 2	310200	30575	859178	211404	1170000	204627
Day 3	257090	17068	603828	60162	477506	57526
Day 4	141340	16165	405367	44356	244743	21978
Day 5-6	264118	23726	526726	76574	339352	50635
Day 7	90007	8677	211404	21506	77808	5974

- = Not Applicable

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: Toxicology studies with puricase were conducted in rats, and dogs following administration of single and multiple doses. The key repeat-dose toxicology studies to support the chronic indication were conducted in dogs up to 39 weeks in dogs. Intravenous administration of Puricase® to dogs for 12-weeks and 39-weeks was well tolerated without any treatment-related adverse effects.

Single intravenous dose at 72.5 mg/kg in rats were well tolerated. Beagle dogs tolerated doses up to 10 mg/kg (intravenous) as single dose. There were no treatment-related overt clinical signs in any of the single dose toxicity studies. In the two dog acute toxicity studies, no treatment-related macroscopic or microscopic findings were observed.

In a 12-week repeat dose dog study, dogs were administered Puricase® intravenously at doses of 0.5, 1.5, and 5 mg/kg every 5 days. There were no treatment-related mortalities or overt clinical signs. The main treatment-related finding was dose-dependent vacuolation in the spleen. Cytoplasmic vacuolation in splenic red pulp was noted at ≥ 0.5 mg/kg and 1.5 mg/kg in male and female dogs, respectively. The vacuolation appears to be a non-adverse event; no functional changes or adverse pathology were associated with the vacuolation. A NOAEL of 5.0 ($AUC_{\text{days}1-127} = 19,884.9 \mu\text{g}\cdot\text{day}/\text{mL}$; male and female combined) and was established in males and females, based on the presence of vacuolated cells.

A 39-week repeat-dose toxicity study with a recovery period of 12-weeks was conducted in dogs that dosed the animals once a week with 0 (vehicle), 0.4, 1.5 and 10 mg/kg/week. ECG data were obtained on pre-dosing and following dosing during weeks 12, 24, and 39 and during recovery period. Puricase did not induce clinically significant electrocardiographic abnormalities. No treatment-related changes were observed on heart rate, QT or QTc electrocardiograms in either male or females. The primary noteworthy toxicity findings from the scheduled sacrificed animals were characterized by cellular vacuolization in several organs, including the adrenal cortex (1.5 and 10 mg/kg group), lamina propria of the duodenum and jejunum (10 mg/kg group), intimal cells of the cardiac aortic outflow of the heart (0.4 and 10 mg/kg groups), hepatic Kupffer cells (1.5 and 10 mg/kg groups) and red pulp region of the spleen (1.5 and 10 mg/kg groups). These histological findings remained essentially unchanged following the recovery period. A NOEL was not defined. However, the sponsor established the NOAEL at 0.4 mg/kg/week. This was based on the misreporting of the vacuolation in the heart noted in the males at 1.5 mg/kg/week when it actually occurred at 0.4 mg/kg/day. The reviewer established a NOAEL at 10.0 mg/kg/week ($AUC = 99,002.1 \mu\text{g}\cdot\text{day}/\text{ml}$; male and female combined)

Genetic toxicology: There were no genetic toxicology studies submitted with this BLA.

Such studies are not needed with this product.

Carcinogenicity: No carcinogenicity studies were conducted with pegloticase. Such studies are not needed with this product.

Reproductive toxicology: The potential for maternal toxicity and developmental toxicity by PEG-uricase was evaluated in rats. Dose selection for the pivotal Segment II study was based on the results of a preliminary dose range-finding toxicity study, conducted in pregnant rats. In the dose-range finding study, PEG-uricase was administered intravenously once daily on gestation days 6, 8, 10, 12, 14 and 16 at dose levels of 0, 5, 10, 20 and 40 mg/kg. The animals were C-sectioned on gestation Day 20.

The results of the intravenous dose range-finding study in rats (Study No. (b) (4)-441007) showed no treatment-related effects clinical signs, no mortality and maternal mean body weight, gravid uterine weights and food consumption were comparable to the control group values. Treatment-related vacuolation and increased spleen weight were observed in the spleen of the high-dose (40 mg/kg) females. The AUC for active plasma puricase concentrations increased in a dose-proportional manner. No evidence of teratogenicity by PEG-uricase was found during the fetal examination. No external developmental variations were noted in any fetuses in this study. Based on the results of this study, dosage levels of 5, 10 and 40 mg/kg were selected for the definitive embryo/fetal development study.

The definitive Segment II embryo/fetal developmental study was conducted in pregnant CrI:CD[®] (SD)IGSBR rats with the PEG-uricase at dose levels of 0, 5, 10 or 40 mg/kg. In this study the pregnant rats were injected intravenously once daily on gestation days 6, 8, 10, 12, 14 and 16; the animals were C-sectioned on gestation Day 20. There was no evidence of maternal toxicity in the control group or at any dose of PEG-uricase tested. All females survived to the scheduled necropsy. All females gained weight throughout gestation and net body weights, gravid uterine weights, and food consumption were comparable between all groups. Treatment-related effects of PEG-uricase on maternal organ pathology were noted at scheduled necropsy. The spleen was identified as the target organ of toxicity. Relative to the control group, the mean absolute spleen weight was increased by 25%. Higher incidence of extramedullary hematopoiesis was observed in the spleen of all PEG-uricase treatment groups. Vacuolation was observed in the spleen of females in the high-dose group. There were no abortions. Based on the pathology findings in the spleen, the NOAEL for maternal toxicity is defined at 10 mg/kg ($AUC_{0-t} = 1,818.4 \mu\text{g}\cdot\text{day}/\text{mL}$). The exposure at the NOAEL for maternal toxicity represented approximately 40 times the exposure at the clinical dose of 8 mg every 2 weeks based on the AUC_{inf} of $45 \mu\text{g}\cdot\text{day}/\text{mL}$ in humans, and 12x based on mg/m^2 . This study was negative for external, visceral and skeletal malformations, and variations by intravenously administered PEG-uricase. The NOAEL for embryo-fetal toxicity (i.e., development delay) in this study was 40 mg/kg ($AUC_{0-t} = 7,091.7 \mu\text{g}\cdot\text{day}/\text{mL}$), representing exposures in the dams that were approximately 158 times the clinical

systemic exposure at the MRHD, on an AUC_{inf} basis, given intravenously every 2-weeks; and 49 times based on body surface area.

Special toxicology: A series of studies were completed to evaluate the immunogenicity of puricase, and on the effects of repeated subcutaneous administration of puricase on vacuole formation and disappearance. Studies characterized the formation of vacuoles following subcutaneous administration of puricase (34 mg/kg) to rats showed that vacuoles formed in splenic macrophages within 2 weeks of dosing and persisted up to 8 weeks after cessation of dosing. A deficit in macrophage function was associated with the vacuoles formation.

2.6.6.2 Single-dose toxicity

Single-dose toxicology studies were conducted in rats and dogs following subcutaneous and intravenous administration. Only the study performed using the intravenous route was reviewed, as the intravenous route is the intended clinical route. The following is a summary of these studies.

A single-dose toxicity and toxicokinetic study of intravenous administered PEG-uricase in juvenile beagle dogs with a four-week recovery period (Study Report № (b) (4)-441011, (b) (4) compliant).

The acute toxicity of PEG-uricase and toxicokinetic profile was characterized in juvenile Beagle dogs (7-8 weeks) after a single, intravenous administration of either vehicle or PEG-uricase. In addition, recovery from potential treatment-related effects or potential latent effects was evaluated during a 4-week recovery period. PEG-uricase (Lot № 7088) was administered to juvenile dogs (n = 3/sex/group) at a dose of 0, 0.4, 1.5, and 10.0 mg/kg. Clinical observations for signs of mortality or moribundity were observed twice daily (morning and afternoon). Clinical observations for signs of overt toxicities were performed prior to dosing, at time of dosing and between 1 hour and 1 hour and 15 minutes after-dosing; and once daily during the 28-day recovery period. Body weight was recorded twice weekly. Fasting peripheral blood samples for hematologic and serum chemistry profiles were obtained prior to group assignment for determination of baseline values, then and on study day 14 and prior to the scheduled necropsy (study day 28). Urinalysis and urine chemistries were recorded at these same time points. Ophthalmic examinations were performed once during the pre-dosing period, then during the last week of the recovery period. Electrocardiograms, heart rate and waveform intervals (PR, QRS, RR, QT and QTC) were recorded prior to group assignment, approximately 1 hour after dosing, then near the end of the recovery period (week 3). A complete necropsy and gross pathologic evaluation was performed on all dogs at the scheduled sacrificed, with organ weights recorded, and tissue samples taken and processed for histopathologic evaluation. Blood samples were also collected for toxicokinetic evaluation from all animals. PEG-uricase levels were measured at approximately 1 hour prior to dose administration and approximately 1 hour post-dosing on study day 0. In addition, PEG-uricase levels were also determined on study days 1, 2, 4, 7, 10, 14, 21 and 28, blood

samples were collected at approximately (\pm 1 hour) the same time of blood collection as study day 0. PEG-uricase concentration in plasma was determined using a validated spectrophotometric method.

There were no overt signs of clinical toxicity noted in animals from either the placebo control or the PEG-uricase treated groups. All animals survived to the scheduled sacrificed. Body weight gains were not appreciably different in the female dogs treated with PEG-uricase as compared to the group injected with the placebo control. Body weight gain was lower (-75%) in the high-dose male dogs.

There were no treatment-related effects on ophthalmoscopic parameters, electrocardiographic parameters and in the clinical laboratory parameters of hematology and urinalysis. However, as depicted in the table below, statistically significant alterations in several hematologic parameters were observed in both males and females. Statistically significant lower mean percentage reticulocyte counts were noted in the 1.5 mg/kg group male dogs on study day 14. Relative to the control, females in the low-dose, mid-dose and high dose group exhibited a statistically significant decrease in mean platelet counts on study day 28. On study day 14, relative to the control females, mean platelet counts were significantly lower in the mid-dose (28%) and high-dose (26.4%) groups. The lower platelet counts were not considered treatment-related because the decreases were relative to higher mean platelet counts in the control group. Although these hematology parameters showed statistically significant decreases, they appeared not to be toxicologically relevant.

Summary of hematology findings (% change) in males and females administered a single intravenously administered dose of PEG-uricase.

Percent Change from Control					
Parameters	Study Day	Dose mg/kg			
		0.0	0.4	1.5	10.0
Males					
Reticulocyte (%)	14	3.5	↓11.4%	↑37.1%*	↓31.4%
Females					
Platelet Count (thous/ μ L)	14	584	↓20.4%	↓28.1%*	↓26.4%*
	28	599	↓21.2%*	↓26.2%*	↓23.9%**

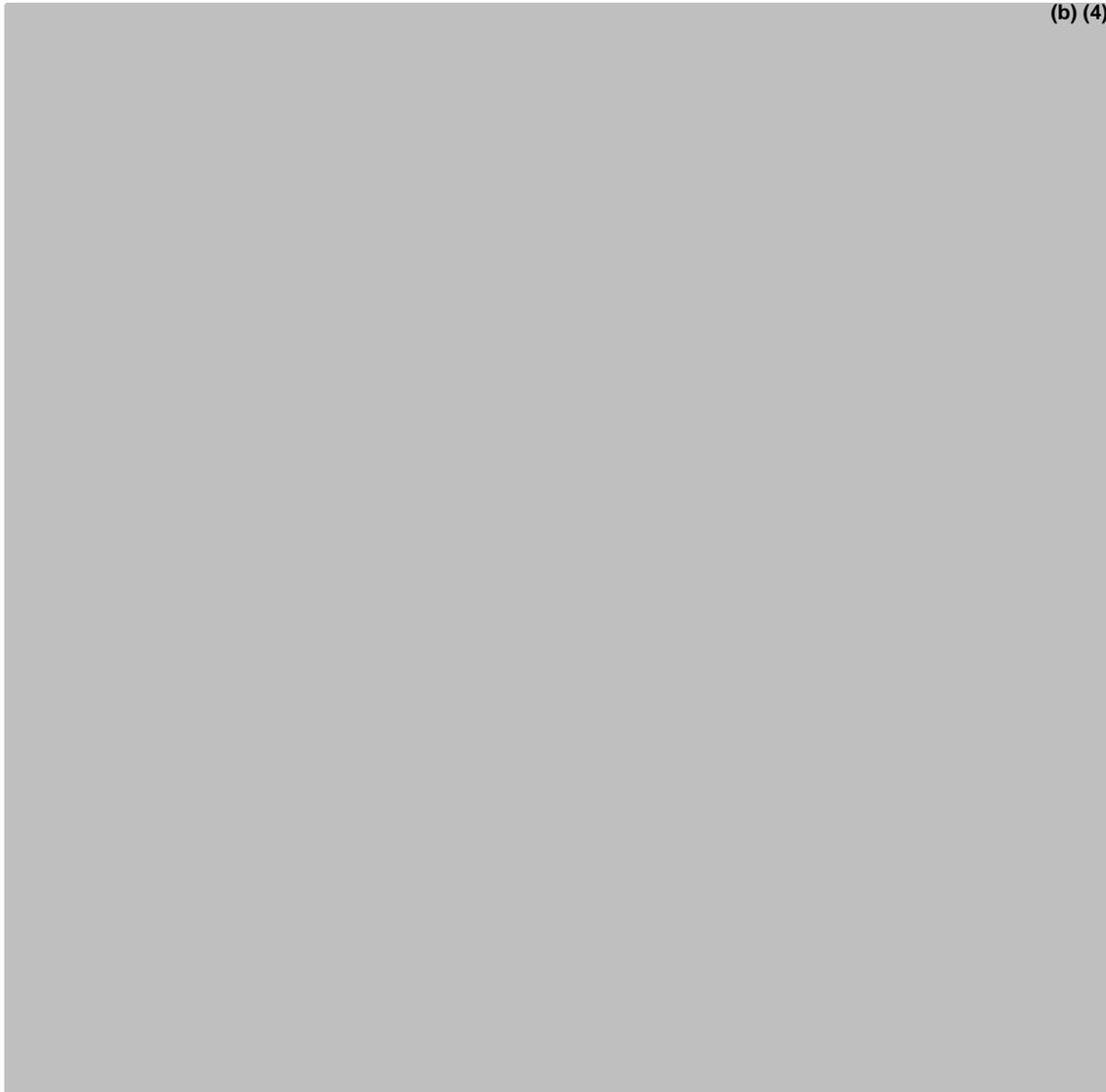
*: Significant different from control value (Dunnett-test), $p \leq 0.05$
 **: Significant different from control value (Dunnett-test), $p \leq 0.01$

Following a single intravenous dose of PEG-uricase, serum chemistry profile showed a treatment-related decrease in serum uric acid levels. As depicted in the sponsor's tables copied below, mean and individual serum uric acid levels of females in the 0.4, 1.5 and 10.0 mg/kg groups were significantly decreased on study days 14 and 28 compared to that of the control. Relative to the control, the mean serum uric acid levels of males in all treatment groups were significantly lower on day 14; whereas on day 28, only the males in the low- and high-dose groups mean serum uric acid levels were significantly reduced.

This effect is not considered to be an adverse effect of the test article but an effect consistent with the pharmacological activity of uricase. Uricase is an enzyme that catalyzes the conversion of uric acid to allantoin. There were no other test article-related effects on serum chemistry parameters.

Text Table 9A. Mean Uric Acid (mg/dL) Evaluation								
PEG-uricase (mg/kg)	Males				Females			
	0	0.4	1.5	10	0	0.4	1.5	10
No. of Dogs	3	3	3	3	3	3	3	3
Day -5/-6 ^a	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.2
Day 14	0.1	0.0**	0.0**	0.0**	0.1	0.0**	0.0**	0.0**
Day 28	0.1	0.0**	0.0	0.0**	0.1	0.1	0.0	0.0
^a = Males were evaluated on study day -5 and females were evaluated on study day -6 ** = Significantly different from the control group at 0.01 using Dunnett's test								

(b) (4)



At necropsy, no remarkable macroscopic and microscopic evidence of organ pathology were observed in any animals in either the control or PEG-uricase treated animals. No treatment-related effects on organ weights were noted. However, mean heart weight relative to body weight was decreased slightly in females at the 1.5 (↓12%) and 10.0 (↓12.8%) dose levels. No meaningful differences in mean absolute heart weights and mean heart weights relative to brain weights were observed in PEG-uricase treated females when compared to the control group. Thus, suggesting that the observed effect on heart weight was possibly not treatment-related.

The toxicokinetic data are presented in the sponsor's tables below. PEG-uricase was rapidly absorbed at all doses with mean maximum plasma concentration reached at 1 hour post-dosing (the first post-dosing collection time point). As can be seen in the sponsor's table, the mean half-life for PEG-uricase in plasma of ranged from 65 to 99 hours in the males and 43 to 109 hours in the females, with a trend toward longer half-lives with increased dosage in both genders. Apparent PEG-uricase volume of distribution ranged from 40 to 70 mL/kg, corresponding to the circulating plasma volume. Dose-dependent increase in both $AUC_{(0-last)}$ and C_{max} was observed in both males and females, but not quite dose-proportionally (variable).

Table 1. Mean ± SD Concentrations of PEG-Uricase in Plasma of Male Juvenile Dogs following Intravenous Administration of Puricase at 0.4, 1.5, or 10 mg/kg

Hours Post-Dosing	0.4 mg/kg		1.5 mg/kg		10 mg/kg	
	Mean (µg/mL)	SD	Mean (µg/mL)	SD	Mean (µg/mL)	SD
0	0.00	0.00	0.00	0.00	0.00	0.00
1	7.36	0.0490	24.2	0.458	190	23.4
24	5.22	0.680	20.9	3.86	152	4.36
48	5.18	0.882	16.9	0.808	117	5.03
96	2.92	0.397	9.66	0.626	74.9	10.1
168	0.960	0.863	6.07	0.193	46.4	1.54
240	0.00	0.00	3.13	0.518	30.8	4.41
336	0.00	0.00	1.77	0.250	22.5	1.65
504	0.00	0.00	0.00	0.00	6.66	0.747
672	0.00	0.00	0.00	0.00	2.17	0.344

N = 3

Table 2. Mean ± SD Concentrations of PEG-Uricase in Plasma of Female Juvenile Dogs following Intravenous Administration of Puricase at 0.4, 1.5, or 10 mg/kg

Hours Post-Dosing	0.4 mg/kg		1.5 mg/kg		10 mg/kg	
	Mean (µg/mL)	SD	Mean (µg/mL)	SD	Mean (µg/mL)	SD
0	0.00	0.00	0.00	0.00	0.00	0.00
1	7.62	0.199	24.5	1.00	187	10.4
24	5.15	0.618	18.5	1.96	137	6.66
48	5.03	0.329	16.1	2.30	107	23.8
96	1.94	0.100	9.73	1.51	72.2	5.01
168	0.0880	0.152	6.21	1.64	49.3	4.56
240	0.00	0.00	4.14	1.87	32.0	3.93
336	0.00	0.00	1.62	1.41	23.5	2.12
504	0.00	0.00	0.00	0.00	6.53	0.641
672	0.00	0.00	0.00	0.00	2.53	0.943

N=3

MEAN TOXICOKINETIC RESULTS FOR PEG-URICASE						
Intravenous Dosage	AUC _{last} (µg×h/mL)	AUC _{inf} (µg×h/mL)	C _{max} (µg/mL)	Half-life (h)	CL (mL/h×kg)	V _d (mL/kg)
MALES						
0.4 mg/kg	565	733	7.36	65	0.55	52
1.5 mg/kg	2707	2948	24.4	93	0.51	68
10 mg/kg	24176	24488	190	99	0.41	59
FEMALES						
0.4 mg/kg	446	545	7.62	43	0.74	45
1.5 mg/kg	2689	3018	24.5	98	0.52	70
10 mg/kg	23719	24129	187	109	0.42	65

A single-dose toxicity and toxicokinetic study of intravenous administered PEG-uricase in adult beagle dogs with a four-week recovery period (Study Report № (b) (4)-441012, (b) (4), compliant). The purpose of this study was to assess the acute toxic effects of PEG-uricase in adult dogs after a single oral dose followed by a 4- week recovery period. PEG-uricase (Lot № 7088) was administered to adult dogs (n = 3/sex/group, 20-weeks of age) at a dose of 0, 0.4, 1.5, and 10.0 mg/kg. Evaluation included twice daily (morning and afternoon) observations for mortality and moribundity, clinical signs (pretest, at time of dosing and between 1 hour and 1 hour and 15 minutes after-dosing; once daily during the 28-day recovery period), body weight (twice weekly beginning approx. 1 week prior to dosing), food consumption

(daily beginning approx. 1 week prior to dosing), clinical pathology (blood and urine collected prior to dosing, day 14 and day 28), ophthalmic examination (pre-dosing and near the end of the recovery period), ECGs recording (approx. 1 hour following dosing) and necropsy (macroscopic examination and organ weight). For toxicokinetic evaluation, blood samples were collected from all animals at 0 (predose) and at approximately 1 hour following dose administration and on study days 1, 2, 4, 7, 10, 14, 21, and 28 within 1 hour of the time that dosing occurred on the day of dosing.

There were no treatment-related deaths or overt signs of clinical toxicity in animals from the placebo control or PEG-uricase treated groups. Body weight gains were not appreciably different between the treatment groups and control groups. There were no changes in food consumption in rats treated with PEG-uricase as compared to the control group. There were no test article-related alterations in hematology and coagulation parameters. Consistent with the pharmacological activity of uricase, uric acid levels were lower in all PEG-uricase treatment groups on days 14 and 18 compared to that of the control. There were no significant treatment-related effects noted in the electrocardiographic data at the study day 0 and 26 evaluations. No pathological changes were evident on gross evaluation.

The toxicokinetic data are presented in the sponsor's table below. Systemic exposure, as defined by C_{max} and AUC_{0-last} , to PEG-uricase increased in a dose-dependent manner but not quite dose-proportional (variable). A 3.75-fold increase in dose (0.4 - 1.5 mg/kg) resulted in a 6-fold increase in AUC_{0-last} in both genders. A 25-fold increase in dose (0.4 - 10.0 mg/kg) resulted in a 35-fold and 31-fold increase in AUC_{0-last} in males (868 vs 30378 $\mu\text{g}\cdot\text{h}/\text{mL}$) and females (812 vs 24785 $\mu\text{g}\cdot\text{h}/\text{mL}$), respectively, after a single dose. Between LD and MD (6.66-fold increase in dose), change in PEG-uricase mean AUC_{0-last} levels increased in an approximate dose proportional manner for males and females. C_{max} , increased in an approximate dose-proportional fashion LD and MD and between MD and HD in both genders. A 25-fold increase in dose (0.4 - 10.0 mg/kg) resulted in a less than dose-proportional increase in C_{max} in both males (19-fold; 8.19 vs 156 $\mu\text{g}/\text{mL}$) and females (18-fold; 7.75 vs 141 $\mu\text{g}/\text{mL}$). Female dogs were generally exposed to slightly lower levels of PEG-uricase.

MEAN TOXICOKINETIC RESULTS FOR PEG-URICASE						
Intravenous Dosage	AUC_{last} ($\mu\text{g}\cdot\text{h}/\text{mL}$)	AUC_{inf} ($\mu\text{g}\cdot\text{h}/\text{mL}$)	C_{max} ($\mu\text{g}/\text{mL}$)	Half-life (h)	CL ($\text{mL}/\text{h}\cdot\text{kg}$)	V_d (mL/kg)
MALES						
0.4 mg/kg	868	1278	8.19	105	0.32	47
1.5 mg/kg	4984	5307	27.0	138	0.28	56
10 mg/kg	30378	33221	156	187	0.30	81
FEMALES						
0.4 mg/kg	812	1230	7.75	112	0.33	52
1.5 mg/kg	4712	5135	27.6	140	0.30	59
10 mg/kg	24785	25784	141	129	0.39	71

2.6.6.3 Repeat-dose toxicity

Study title: 12-Week Repeated Dose Intravenous Injection Toxicity Study with Puricase® in Dogs.

Key study findings: Puricase® (0.5, 1.5 and 5 mg/kg) was administered intravenously to dogs every 5 days for 12 weeks with the following results:

- Repeated-dosing with Puricase® for 12-weeks was well-tolerated. There were no treatment-related mortalities or overt clinical signs.
- Vacuolated cells were noted in the spleen at doses ≥ 0.5 mg/kg and 1.5 mg/kg in males and females, respectively.
- Antigenicity developed against Puricase® and uricase in both male and female dogs.
- The anti-uricase antibodies were non-neutralizing.
- A NOEL was not clearly established due to the findings of treatment-related vacuolation of the spleen.
- A NOAEL of 5.0 mg/kg was established, with the only notable finding being the presence of vacuolated cells in the spleen which are not considered adverse due to the lack of any evidence of cellular damage or inflammatory cell infiltrate.

Study no.:

(b) (4) Study № 6432-106

Volume #, and page #:

Electronic document

Conducting laboratory and location:

(b) (4)

Date of study initiation:

July 2, 2002

GLP compliance:

Yes

QA report:

yes (x) no ()

Drug, lot #, and % purity:

Puricase®. Lot № 06860080 (used Day –Day 81 (except for group 2)) and Lot № 16860010 (Day 81 (Group 2 only) through 86), 100% (assumed)

Methods

Doses: 0, 0.5, 1.5 and 5.0 mg/kg/week

Species/strain: Dog/Beagle

Number/sex/group or time point (main study):

Group	Test Article	Dosage Level (mg/kg/week)	Dosage Volume (mL/kg)	Dose Concentrations (mg/mL)	Number of Animals	
					Females	Males
Main						
1	Puricase	0	2.0.	-	3	3
2	Puricase	0.5	2.0	0.25	3	3
3	Puricase	1.5	2.0	0.75	3	3
4	Puricase	5.0	2.0	2.5	3	3
Recovery						
1	Puricase	0	2.0	-	2	2
2	Puricase	0.5	2.0	0.25	2	2

3	Puricase	1.5	2.0	0.75	2	2
4	Puricase	5.0	2.0	2.5	2	2
Dosing Schedule: Days 1, 6, 11, 16, 21, 26, 31, 36, 46, 51, 56, 61, 66, 71, 76, 81 and 86						

Route, formulation, volume, and infusion rate: Intravenous via the cephalic vein or a peripheral vein, dissolved in phosphate buffered saline solution, 2.0 mL/kg, infusion rate was not defined.

Satellite groups used for toxicokinetics or recovery: Yes

Age: approximately 4 months

Weight: Males: 6.9 to 10.3 kg; Females: 5.8 to 8.1 kg

Sampling times:

Unique study design or methodology: A recovery/challenge group was incorporated in the 12-week study. In this group, dogs were dosed for 4-weeks with 5.0 mg/kg of Puricase[®], followed by 6-weeks recovery period with no treatment. After the 4-week recovery period, the dogs were challenged with a single injection of the low dose of Puricase[®]. On day 74, 3 males and 3 females were sacrificed. The remaining 2 dogs per sex were sacrificed 2-weeks later.

Group	Test Article	Dosage Level (mg/kg/day)	Dosage Volume (ml/kg)	Dose Concentrations (mg/mL)	Number of Animals	
					Females	Males
4	Puricase [®]	5.0	2.0	2.5	5	5
Dosing Schedule: Days 1, 6, 11, 16, 21, 26, and 31						
Day 74: Challenged with a low dose (0.5 mg/kg) of Puricase [®]						

In addition, a modified Irwin's behavioral test and immunoglobulins and complement analysis were performed.

Observations and times:

Toxicokinetic: Blood samples (2 mL) were collected from all dogs/sex/group (including control dogs) on days 0, 3, 6, 21, 26, 41, 43, 80, 86, 87, 90, 94, 99, 113 and 127 for toxicokinetic evaluation at 1 hour pre-dose and 1 hour after dosing. Blood was collected from the recovery dogs prior to sacrifice during week 52. All animals were fasted overnight prior to the scheduled blood collection. Blood was collected from the jugular vein. Puricase levels in blood were quantified using a validated colorimetric assay measuring the enzymatic activity of Puricase[®] (performed by Bio-Technology General - Israel).

Mortality: Each dog was examined for mortality and moribundity twice daily (morning and afternoon).

Clinical signs: Each animal was examined daily for treatment-related clinical signs. In addition to the daily observation, the animals were checked once weekly for a more detailed observations. A modified Irwin's behavioral screening was performed prior to treatment and during weeks 6, 13 and 15 (recovery animals); and on day 74 prior to sacrifice of 3 male and 3 female dogs in the recovery/challenge group. The following parameters were evaluated:

Parameter	Observation
CNS	Activity/general reaction Pain Reaction Aggressiveness/irritability Stereotypy Tremor Twitches
Coordination	Abnormal gait Abnormal position
Reflex, tone	Abnormal body posture Skin turgor Plantary reflex Corneal reflex (lid)
Autonomic functions	Skin and membrane color Diarrhea Salivation Respiration

Body weights: Body weights were recorded at least once prior to dosing and weekly thereafter.

Food consumption: Food consumption was recorded weekly.

Ophthalmoscopy: Eyes of all animals were examined before the first dose and during weeks 5, 13 and 15 (recovery animals). On day 74, prior to sacrifice, three males and three females from the recovery challenge group were examined. An indirect ophthalmoscope was used to examine the eyes.

EKG: EKG was not taken.

Hematology: Blood was collected prior to first dosing and at the scheduled sacrifice. Blood, if possible, was also collected from animals sacrificed at an unscheduled interval. All animals were fasted overnight prior to the scheduled blood collection. Blood was collected from the jugular vein. The following parameters were examined:

Hematology Parameters		
Coagulation Parameters	White Blood Cell Parameters	Red Blood Cell Parameters
Activated partial thromboplastin time (APTT)	Differential leukocyte count (Absolute) <ul style="list-style-type: none"> - Neutrophil (NEUT) - Lymphocyte (LYM) - Monocyte (MONO) - Eosinophil (EOS) - Basophil (BASO) 	Erythrocyte count (RBC)
Prothrombin time (PT)	Leukocyte count (WBC)	Hemoglobin (HGB)
Platelet count (PLT)		Hematocrit (HCT)
		Mean corpuscular volume (MCV)
		Mean corpuscular hemoglobin (MCH)
		Mean corpuscular hemoglobin concentration (MCHC)
		Reticulocyte smear ^A
		Blood smear ^A
A: Prepared but not examined unless necessary to verify hematology results		

Clinical chemistry: Blood was collected prior to first dosing and at the scheduled sacrifice. Blood, if possible, was also collected from animals sacrificed at an unscheduled interval. All animals were fasted overnight prior to the scheduled blood collection. Blood was collected from the jugular vein. The following parameters were examined:

Serum Chemistry Parameters	
Alanine aminotransferase (ALT)	Inorganic Phosphorus (PHOS)
Albumin (ALB)	Potassium (K)
Aspartate aminotransferase (AST)	Sodium (Na)
Albumin/globulin ratio (AGR)	Total bilirubin (TBIL)
Alkaline phosphatase (ALP)	Cholesterol (CHOL)
Calcium (Ca)	Urea Nitrogen
Chloride (Cl)	Total Protein
Creatinine (CREA)	
Gamma glutamyl transferase (GGT)	
Glucose (GLU)	
Globulin (GLOB)	

Urinalysis: Urine, prior to collection of blood, was collected overnight from all surviving animals (fasted overnight). The following parameters were examined:

Urine Parameters
Urinalysis
Appearance: Color and clarity
Bilirubin
Blood
Glucose
Ketones
Microscopy of sediment
pH
Protein
Specific gravity
Urobilinogen
Volume

Gross pathology: Animals were euthanized *in extremis* or at termination by exsanguination under barbiturate anesthesia. Terminal body weight will be recorded. Macroscopic evaluation was performed. The external surface, all orifices, and the thoracic, abdominal and cranial cavities were examined.

Organ weights: The weights of the following organs were measured at necropsy:

Adrenals (2)	Prostrate
Brain	Spleen
Heart	Testes (2)
Kidneys (2)	
Liver with gallbladder (drained)	
Lung	
Ovaries (2)	
Pituitary gland	

Histopathology: The following tissues were collected from all animals

Dose: mg/kg	0	0.5	1.5	5.0
Species	Dogs			
Adrenal (2)	X	X	X	X
Bone Marrow smear				
Bone (femur with bone marrow – articular surface of the distal end)	X	X	X	X
Brain	X	X	X	X
Cecum	X	X	X	X
Colon	X	X	X	X
Duodenum	X	X	X	X
Epididymis (2)	X	X	X	X
Esophagus	X	X	X	X
Eye (2)	X	X	X	X
Gall bladder	X	X	X	X
Heart	X	X	X	X
Ileum	X	X	X	X
Injection site (all)	X	X	X	X
Jejunum	X	X	X	X
Kidney (2)	X	X	X	X
Lesions	X	X	X	X
Liver	X	X	X	X
Lungs with mainstem bronchi	X	X	X	X
Lymph nodes, mesenteric	X	X	X	X
Mammary Gland (females)	X	X	X	X
Ovary (2)	X	X	X	X
Pancreas	X	X	X	X
Pituitary gland	X	X	X	X
Prostate	X	X	X	X
Rectum	X	X	X	X
Salivary gland (mandibular -2)	X	X	X	X
Sciatic nerve	X	X	X	X
Skeletal muscle (thigh)	X	X	X	X
Skin	X	X	X	X
Spinal cord (cervical, thoracic & lumbar)	X	X	X	X
Spleen	X	X	X	X
Sternum with bone marrow	X	X	X	X
Stomach	X	X	X	X
Testis (2)	X	X	X	X
Thymus	X	X	X	X
Thyroid (2) with parathyroid	X	X	X	X
Tongue	X	X	X	X
Trachea	X	X	X	X
Urinary bladder	X	X	X	X
Uterus	X	X	X	X
Vagina	X	X	X	X

Tissues were fixed in 10% neutral-buffered formalin. Tissues from the control and all treatment groups were stained with hematoxylin and eosin stain and examined histologically.

Adequate Battery: yes (X), no ()—explain
Peer review: yes (), no (X)

Immunoglobulins and Complement Analysis: Blood samples were collected from all animals (fasted) according to the schedule presented below (copied from sponsor’s submission). The blood samples will be analyzed for total IgA, IgE, IgG, IgM; anti-PEG IgG antibodies; anti-uricase, anti-Puricase®, anti-PEG Ig3 antibodies and C3, C4 and CH50 complement factors.

BLOOD SAMPLING TABLE

The schedule of blood sampling for Puricase® activity (plasma), immunoglobulins and complement (serum) analyses are given in the table below:

Day of Study	Animal Groups	Blood Volume (mL)	Type (Plasma or Serum)*	Specific Comments
Day 0	All	2 7	Plasma Serum	Prior to dosing
Day 1	Main	2	Plasma	10 – 30 min post dose
Day 3	Main	2	Plasma	Prior to feeding
Day 6	Main	2	Plasma	Prior to dosing
Day 11	Main	7	Serum	Prior to dosing, immunoglobulins only.
Day 21	Main	2	Plasma	10 – 30 min post dose
Day 26	Main	2	Plasma	Prior to dosing
Day 32	Recovery+Challenge	7	Serum	Prior to feeding
Day 41	Main	2	Plasma	10 – 30 min post dose
Day 43	Main	2	Plasma	Prior to feeding
Day 73	Recovery+Challenge	7	Serum	Prior to dosing
Day 74	Recovery+Challenge	7	Serum	3 male + 3 female prior to sacrifice
Day 80	Recovery+Challenge	2 7	Plasma Serum	remaining 2 male + 2 female
Day 86	Main	2	Plasma	10 – 30 min post dose
Day 87	All	2 7	Plasma Serum	Day of Termination of the Main and Recovery+Challenge.
Day 90	Recovery	2	Plasma	Prior to feeding
Day 94	Recovery	2	Plasma	Prior to feeding
Day 99	Recovery	2 7	Plasma Serum	Prior to feeding
Day 113	Recovery	2 7	Plasma Serum	Prior to feeding
Day 127	Recovery	2 7	Plasma Serum	Day of Termination

* Unless otherwise indicated, plasma will be analyzed for Puricase® activity, while serum will be analyzed for total immunoglobulins, complement components and activity, and specific IgGs.

Results

Toxicokinetic: The toxicokinetic data are summarized in the table below. Puricase was detected in the plasma of all dogs and plasma puricase concentrations increased with increasing dose in both male and female dogs. The mean AUC values for puricase increased with dose but in a greater than dose-proportional manner with a half-life between 7.1 and 9.5 days. A 3-fold increase in dose (0.5-1.5 mg/kg) resulted in a 3.1-fold and 2.7-fold increase in AUC_{Days1-127} in males (1389.6 vs 5121.4 µg·day/mL) and females (1642.4 vs 4504.3 µg·day/mL), respectively. A 10-fold increase in dose (0.5-5.0 mg/kg) resulted in a 16-fold and 10.6-fold increase in AUC_{Days1-127} in males (1389.6 vs 22294.7 µg·day/mL) and females (1642.4 vs 17475.7 µg·day/mL), respectively.

Dose (mg/kg)	C _{max} (µg/mL)			T _{1/2} (Days)			AUC _{Days1-127} (µg·day/mL)		
	M	F	M+F	M	F	M+F	M	F	M+F
0.5	22.8	24.1	23.5	5.7	8.4	7.1	1389.6	1,642.4	1,516.0
1.5	79.8	71.5	75.7	4.8	9.1	6.9	5,121.4	4,504.3	4,812.9
5.0	316.1	255.9	286.0	10.2	8.7	9.5	22,294.7	17,475.7	19,884.9

Mortality: No treatment-related deaths occurred; all animals survived until schedule study termination.

Clinical signs: No treatment-related clinical signs were observed. However, it should be pointed out that clinical signs were observed in all treatment groups, including control group. These clinical signs included: excessive salivation, various forms of excretion (i.e., mucoid, liquid, discolored and nonformed feces), optic discharge, red and/or swollen conjunctivae, red skin (i.e, ears and generalized), vomitus and abnormal autonomic abnormalities (i.e., red skin, lips and ears). These clinical signs were still evidence during the recovery period.

Body weights: No treatment-related changes in body weights were observed.

Food consumption: Food consumption was measured weekly, consumption relative to control was evaluated. Compared to controls food intake a significant difference ($p \leq 0.01$) was noted in males (main study) at week 1. At week 1, main study males in both the 0.5 and 5 mg/kg/day groups displayed a 10% increase in food consumption. Food consumption was comparable to the control during the remaining of the dosing period and recovery period.

No treatment-related changes in food consumption were observed in the females; food consumption was comparable to the control throughout the 12 weeks of dosing and recovery period.

Ophthalmoscopy: No treatment-related changes were noted in the eye. However, incidence of palpebrae/conjunctiva, lacrimation/epiphora has been observed in a few animals. These ophthalmoscopic incidences are tabulated below:

Ophthalmoscopic Findings	Dose (mg/kg)							
	0		0.5		1.5		5.0	
	M	F	M	F	M	F	M	F
Main Study								
Palpebrae/Conjunctiva Lacrimation/Epiphora - Bilateral – Mild	0	0	1	0	0	0	0	0
Palpebrae/Conjunctiva Lacrimation/Epiphora - Unilateral (Right) -Mild	0	0	0	1	1	0	0	0
Palpebrae/Conjunctiva Lacrimation/Epiphora - Unilateral (Right) –Moderate - Unilateral (Left) - Moderate	0	0	0	0	0	0	1	0
Recovery Group								
Palpebrae/Conjunctiva Lacrimation/Epiphora - Bilateral – Mild	0	0	1	0	0	0	0	0
Palpebrae/Conjunctiva Lacrimation/Epiphora - Unilateral (Left) -Mild	0	1	0	0	0	0	0	0
Palpebrae/Conjunctiva Lacrimation/Epiphora - Unilateral (Left) –Mild - Unilateral (Left) - Moderate	0	0	0	0	0	1	0	0

EKG: Not performed.

Hematology: The standard battery of hematological parameters was assessed at terminal sacrifice (Day 87) in 3 animals/sex/group in the main study. No treatment-related changes were noted in the hematology parameters measured. Hematology parameters were comparable to the control group in the recovery and recovery/challenge groups.

Clinical chemistry: Statistically significant changes noted at the day 87 blood draws are summarized in the table below. Minor treatment-related decrease in alanine aminotransferase was observed in females in the 0.5, 1.5 and 5.0 mg/kg groups. Relative to the control, a 14.7%, 14.7% and 29.4% decrease was noted in the low-, mid- and high-dose groups, respectively. Inorganic phosphorous in males in the low-dose and high-dose groups was significantly higher than the control by 9.4% and 11.3%, respectively. Aspartate aminotransferase was statistically higher (17.2%) than the control in at 0.5 mg/kg. The increase in the mean ALAT level was the result of individual animals having an increase level and did not demonstrate a dose-dependent pattern. Therefore, this finding is of little toxicological significance. These clinical chemistry changes did not demonstrate a dose-dependent pattern. Therefore, these findings are of little toxicological significance.

Parameter	Gender	Dose (mg/kg/day)			
		0	0.5	1.5	5.0
Aspartate Aminotransferase (U/L)	Males	29.0 ± 2.0	34.0 ± 3.2* (+17.2%)	31.0 ± 1.2 (+6.9%)	34.0 ± 1.2 (+17.2%)
	Females	41.0 ± 9.5	31.0 ± 3.1 (-24.4%)	36.0 ± 173 (-12.2%)	31.0 ± 1.2 (-24.4%)
	Males	28.0 ± 3.2	28.0 ± 6.1	22.0 ± 2.9	28.0 ± 6.8

Parameter	Gender	Dose (mg/kg/day)			
		0	0.5	1.5	5.0
Alanine Aminotransferase (U/L)			NC	(-21.0%)	NC
	Females	34.0 ± 2.1	29.0 ± 1.2* (-14.7%)	29.0 ± 1.5* (-14.7%)	24.0 ± 0.6* (-29.4%)
Inorganic Phosphorous (mg/dL)	Males	5.3 ± 0.06	5.8 ± 0.35* (+9.4%)	5.9 ± 0.31* (+11.3%)	5.2 ± 0.10 (+9.3%)
	Females	4.9 ± 0.65	4.7 ± 0.06 (-4.1%)	4.7 ± 0.51 (-4.1%)	5.0 ± 0.35 (+2.0%)

*: Statistically significant (at p≤0.05) differences compared to controls

Urinalysis: No treatment-related alterations in urinalysis parameters were observed with analysis of urine or urine sediment.

Gross pathology: No drug-related changes were observed.

Organ weights: At the end of the 12 week study period, the group mean relative organ weight, organ-to-body weight and organ-to-brain weight ratio compared to the control was significantly (at p≤0.05) increased in the mid-dose females for the ovaries by 63.5%, 55.5% and 88.8%, respectively; but returned to control levels after the 4-week recovery period. Significant increase (20.6%) in absolute liver weight was observed in males at the 0.5 mg/kg dose level.

Histopathology: Treatment-related histological changes were noted in the spleen. Vacuolation of the reticuloendothelial cells, graded as minimal to moderate, was present within the red pulp area of the spleen from all terminal-sacrifice male dogs of 1.5 mg/kg dose group and all male and female dogs in the 5.0 mg/kg dose group. Vacuolation was not reversible. This microscopic finding was still noted in the mid-dose (1/2 males and 1/2 females) and high-dose (2/2 males and 2/2 females) groups at the end of the 4-week recovery period. Vacuolation of the splenic red pulp was also observed in all males and females administered 5.0 mg/kg of PEG-uricase in the recovery + challenge group. No other histological changes (i.e., necrosis or degeneration) were associated with the observed vacuolation. Injection site reactions were also noted in both the control and treatment groups. The local reactions included inflammation and hemorrhaging.

Parameter		Incidence of Microscopic Histological Changes (total occurrence/number of animals)							
		Males (mg/kg)				Females (mg/kg)			
		0.0	0.5	1.5	5.0	0.0	0.5	1.5	5.0
Injection Site									
Inflammation	Treatment	2/3	0/3	0/3	0/3	1/3	1/3	1/3	2/3
	Recovery	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
	Recovery + Challenge	-	-	-	0/5	-	-	-	0/5
Hemorrhage (Acute)	Treatment	2/3	2/3	3/3	0/3	2/3	3/3	2/3	2/2
	Recovery	1/2	0/2	1/2	0/2	1/2	1/2	1/2	2/2
	Recovery + Challenge	-	-	-	2/5	-	-	-	5/5
Epidermis/Dermis/Hair Shaft, Inflammation	Treatment	1/3	2/3	0/3	2/3	2/3	0/3	1/3	2/3
	Recovery	1/2	0/2	0/2	0/2	2/2	0/2	0/2	0/2
	Recovery + Challenge	-	-	-	1/5	-	-	-	2/5
Spleen									
Reticuloendothelial Cells,	Treatment	0/3	1/3	3/3	3/3	0/3	0/3	2/3	3/3

Parameter		Incidence of Microscopic Histological Changes (total occurrence/number of animals)							
		Males (mg/kg)				Females (mg/kg)			
		0.0	0.5	1.5	5.0	0.0	0.5	1.5	5.0
Vacuolation	Recovery	0/2	0/2	1/2	2/2	0/2	0/2	1/2	2/2
	Recovery + Challenge	-	-	-	5/5	-	-	-	5/5

Immunoglobulins and Complement Analysis: The immunogenicity of Puricase[®] in serum was evaluated from Day 0 to Day 127. As depicted in the sponsor's tables below, anti-uricase and anti-Puricase[®] antibodies developed in all Puricase[®] treatment groups. Antibodies developed in most of the animals in each treatment group and were not dose-dependent. The titer level of anti-uricase and anti-Puricase[®] decreased during the recovery period (day 127).

Table 5. Summary, the Number of Dogs with Positive Anti-Puricase[®] Antibodies in the Main and Recovery Studies⁽¹⁾

Dose Group (mg/kg)	Treatment Day			
	87	99	113	127
0.5	8/10	2/4	4/4	0/4
1.5	7/10	4/4	4/4	3/4
5.0	4/10	2/4	1/4	3/4

(1) Samples with response values higher than the 95% UCL of 0.018 A₄₀₅/µl of serum

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Table 6. Summary, the Number of Dogs with Positive Anti-m-PEG Antibodies in the Main and Recovery Studies⁽¹⁾

Dose Group (mg/kg)	Treatment Day			
	87	99	113	127
0.5	4/10	2/4	4/4	0/4
1.5	0/10	2/4	4/4	0/4
5.0	6/10	0/4	0/4	0/4

(1) Samples with response values higher than the 95% UCL of 0.281 A₄₀₅/µl of serum

Table 7. Summary, the Number of Dogs with Positive Anti-Uricase Antibodies in the Main and Recovery Studies⁽¹⁾

Dose Group (mg/kg)	Treatment Day			
	87	99	113	127
0.5	7/10	2/4	4/4	0/4
1.5	10/10	4/4	4/4	3/4
5.0	7/10	3/4	1/4	2/4

(1) Samples with response values higher than the 95% UCL of 0.340 A₄₀₅/µl of serum

Recovery + Challenge Study: Results from this study showed that the puricase challenge did not enhance the antibody response against m-PEG, pegloticase or uricase.

Study title: 39-Week Repeated Intravenous Injection Chronic Toxicity and Toxicokinetic Study with Puricase[®] in Dogs with a 12-Week Recovery.

Key study findings: Puricase[®] (0.4, 1.5 and 10 mg/kg) was administered intravenously to dogs every 5 days for 12 weeks with the following results:

- Repeated-dosing with Puricase[®] for 39-weeks was well-tolerated. There were no treatment-related mortalities or overt clinical signs.
- Vacuolated cells were noted in the adrenal cortex, duodenum, heart, jejunum, liver and spleen at doses ≥ 0.4 mg/kg, depending on the tissues.
- Immunohistochemistry analysis suggested that the vacuoles in the liver, spleen, duodenum and jejunum were macrophages. The contents of the vacuoles were uricase and/or PEG.
- Antigenicity developed against Puricase[®] and uricase in both male and female dogs.
- Only one high-dose dog showed antibodies against PEG.
- The anti-uricase antibodies were non-neutralizing.
- Based on the presence of vacuolated cells, a NOEL was not established.
- A NOAEL of 10.0 mg/kg was established, with the only notable findings being the formation of vacuoles in the several organs that the reviewer does not consider adverse.

Study no.:

(b) (4) Study № 7533-100

Volume #, and page #:

Electronic document

Conducting laboratory and location:

(b) (4)

Date of study initiation:

March 2, 2004

GLP compliance:

Yes

QA report:

yes (x) no ()

Drug, lot #, and % purity:

Puricase[®]. Lots № 26860050, № 36860010

and № 46860010, purity was not included on the Certificates of Analysis, it was assumed that it was purity was not 100%

Methods

Doses: 0, 0.4, 1.5 and 10.0 mg/kg/week

Species/strain: Dog/Beagle

Number/sex/group or time point (main study):

Group	Test Article	Dosage Level (mg/kg/week)	Dosage Volume (ml/kg)	Dose Concentrations (mg/mL)	Number of Animals	
					Females	Males
Main						
1	Puricase	0	2.0	-	6	6
2	Puricase	0.4	2.0	0.2	6	6
3	Puricase	1.5	2.0	0.75	6	6
4	Puricase	10.0	2.0	5.0	6	6
Recovery						

Group	Test Article	Dosage Level (mg/kg/week)	Dosage Volume (ml/kg)	Dose Concentrations (mg/mL)	Number of Animals	
					Females	Males
1	Puricase	0	2.0	-	3	3
2	Puricase	0.4	2.0	0.2	3	3
3	Puricase	1.5	2.0	0.75	3	3
4	Puricase	10.0	2.0	5.0	3	3

Route, formulation, volume, and infusion rate: Intravenous via the cephalic vein, dissolved in phosphate buffered saline, 2.0 mL/kg, administered slowly over a period of 30 to 60 seconds

Satellite groups used for toxicokinetics or recovery: Yes: Recovery: 3 dogs/dose from each group was used for recovery; Toxicokinetic: all animals were used for TK evaluation

Age: approximately 6 months old

Weight: Males: 5.7 to 9.7 kg; Females: 5.4 to 7.9 kg

Sampling times:

Unique study design or methodology: Yes: A modified Irwin's behavioral test and immunoglobulins and complement analysis were performed.

Observations and times:

Toxicokinetic: Blood samples (2 mL) were collected from all dogs/sex/group (including control dogs) on day 1, and weeks 2, 3, 4, 12, 24, 39 and 40 for toxicokinetic evaluation at 1 hour pre-dose and 1 hour after dosing. Blood was collected from the recovery dogs prior to sacrifice during week 52. All animals were fasted overnight prior to the scheduled blood collection. Blood was collected from the jugular vein or other appropriate artery or vein, if necessary. Puricase levels in blood were quantified using a validated colorimetric assay measuring the enzymatic activity of Puricase[®] (performed by Bio-Technology General - Israel).

Mortality: Each dog was examined for mortality and signs of pain and distress twice daily (a.m. and p.m.).

Clinical signs: Clinical signs were examined twice daily (a.m. and p.m.) for mortality and signs of pain and distress. On dosing days, clinical signs were examined at the time of dosing and approximately 2 hours after dosing. On non-dosing days and during the recovery period, animals were examined once. A modified Irwin's behavioral screening was performed prior to treatment and during weeks 12, 24, 39 and 51 (recovery animals) for all animals; and on day 74 prior to sacrifice of 3 male and 3 female dogs in the recovery/challenge group. Other observations included: changes in the skin, hair, eyes, and mucous membranes; respiratory, circulatory, autonomic and central nervous system functions; and somatomotor activity and behavior patterns.

Body weights: Body weights were recorded weekly, beginning one week prior to randomization, on day 1 (prior to dosing) and prior to scheduled necropsies.

Food consumption: Food consumption was recorded daily, beginning 1 week prior to randomization.

Ophthalmoscopy: Eyes of all animals were examined before the first dose and during weeks 12, 24, 39 and 51 (recovery). Using an indirect ophthalmoscope and a slit lamp

biomicroscope, the following ocular structures were examined: eyelids, conjunctiva, cornea, anterior chamber, iris, lens, vitreous body, and ocular fundus.

EKG (and heart rates): Electrocardiography recordings were performed once prior to treatment initiation and approximately 5 to 15 minutes post-dosing during weeks 12, 24, and 39. EKG was also performed on the recovery animals during week 51. The standard bipolar limb leads (I, II and III), augmented leads aVR, aVL, aVF, V1, V2, V3 and V4 were recorded. A heart rate correction of the QT interval (QTc) was calculated using a formula provided by (b) (4)

$$(\text{QTc} = \text{QT} - (0.87 \times \{60/\text{Heart Rate} - 1\}))$$

Hematology: Blood was collected prior to first dosing, during weeks 13 and 26 and before scheduled sacrifice (terminal and recovery). All animals were fasted overnight prior to the scheduled blood collection. Blood was collected from the jugular vein. The following parameters were examined:

Hematology Parameters		
Coagulation Parameters	White Blood Cell Parameters	Red Blood Cell Parameters
Activated partial thromboplastin time (APTT)	Differential leukocyte count (Absolute) <ul style="list-style-type: none"> - Neutrophil (NEUT) - Lymphocyte (LYM) - Monocyte (MONO) - Eosinophil (EOS) - Basophil (BASO) 	Erythrocyte count (RBC),
Prothrombin time (PT)	Leukocyte count (WBC)	Hemoglobin (HGB)
Platelet count (PLT)		Hematocrit (HCT)
		Mean corpuscular volume (MCV)
		Mean corpuscular hemoglobin (MCH)
		Mean corpuscular hemoglobin concentration (MCHC)
		Reticulocyte smear ^A
		Blood smear ^A

A: Prepared but not examined unless necessary to verify hematology results

Clinical chemistry: Blood was collected prior to first dosing, during weeks 13 and 26 and before scheduled sacrifice (terminal and recovery). All animals were fasted overnight prior to the scheduled blood collection. Blood was collected from the jugular vein. The following parameters were examined:

Serum Chemistry Parameters	
Alanine aminotransferase (ALT)	Inorganic Phosphorus (PHOS)
Albumin (ALB)	Potassium (K)
Aspartate aminotransferase (AST)	Sodium (Na)
Albumin/globulin ratio (AGR)	Sorbitol dehydrogenase
Alkaline phosphatase (ALP)	Total bilirubin (TBIL)
Calcium (Ca)	Urea Nitrogen
Chloride (Cl)	Total Protein
Cholesterol (CHOL)	Lactate dehydrogenase
Creatinine kinase (CREA)	Glucose (GLU)
Gamma glutamyl transferase (GGT)	Glucose (GLU)

Urinalysis: Urine, prior to collection of blood, was collected overnight from all surviving animals (fasted overnight). The following parameters were examined:

Urine Parameters	
Urine Chemistry	Urinalysis
Calcium	Appearance: Color
Chloride	Bilirubin
Nitrites	Blood
Sodium	Glucose
Potassium	Ketones
Phosphorus	Microscopy of sediment
	pH
	Protein
	Specific gravity
	Urobilinogen
	Leukocytes

Gross pathology: Animals were euthanized *in extremis* or at termination (week 39) or end of 12-week recovery period by exsanguination under barbiturate anesthesia. Terminal body weight was recorded. Macroscopic evaluation was performed. The external surface, all orifices, the cranial, pelvic, thoracic, and abdominal cavities, organs and tissues were examined.

Organ weights: Organ-to-body weight percentages and organ-to-brain weight ratios were calculated. The weights of the following organs were measured at necropsy:

Adrenals (2)	Prostrate
Brain	Salivary gland (mandibular (2))
Epididymis (2)	Spleen
Heart	Testis (2)
Kidneys (2)	Thyroid with parathyroid (2)
Liver with gallbladder (drained)	Uterus
Lung	
Ovary(2) with oviduct	
Pituitary gland	

Histopathology: The following tissues were collected from all animals at necropsy:

Dose: mg/kg →	0	0.4	1.5	10.0
Species	Dogs			
Adrenal (2)	X	X	X	X
Aorta	X	X	X	X
Bone (femur with bone marrow – articular surface of the distal end)	X	X	X	X
Brain	X	X	X	X
Cecum	X	X	X	X
Cervix	X	X	X	X
Colon	X	X	X	X
Duodenum	X	X	X	X
Epididymis (2) ^b	X	X	X	X
Esophagus	X	X	X	X
Eyes with optic nerves (2) ^a	X	X	X	X
Gall bladder with bile duct	X	X	X	X
Heart	X	X	X	X
Ileum	X	X	X	X
Injection site (all)	X	X	X	X
Jejunum	X	X	X	X
Kidney (2)	X	X	X	X
lacrimal gland	X	X	X	X
Lesions	X	X	X	X
Liver	X	X	X	X
Lungs with mainstem bronchi	X	X	X	X
Lymph nodes, mesenteric	X	X	X	X
Mammary Gland (females)	X	X	X	X
Ovary (2)	X	X	X	X
Pancreas	X	X	X	X
Pituitary gland	X	X	X	X
Prostate	X	X	X	X
Rectum	X	X	X	X
Salivary gland (mandibular -2)	X	X	X	X
Sciatic nerve	X	X	X	X
Skeletal muscle (thigh)	X	X	X	X
Skin	X	X	X	X
Spinal cord (cervical, thoracic & lumbar)	X	X	X	X
Spleen	X	X	X	X
Sternum with bone marrow ^c	X	X	X	X
Stomach	X	X	X	X
Testis (2) ^b	X	X	X	X
Thymus	X	X	X	X
Thyroid with parathyroid (2)	X	X	X	X
Tongue	X	X	X	X
Trachea	X	X	X	X
Urinary bladder	X	X	X	X
Uterus	X	X	X	X
Vagina	X	X	X	X

a: fixed in Davidson's solution
b: fixed in Bouin's solution
c: Bone marrow smears were prepared from the sternum of each animal.
Slides were prepared and held for possible future evaluation.

Tissues were fixed in 10% neutral-buffered formalin, with the exception of those marked. Tissues from all dogs at the primary and recovery necropsies were embedded in paraffin, stained with hematoxylin and eosin stain and examined microscopically.

Adequate Battery: yes (x), no ()—explain
Peer review: yes (x), no ()

Immunoglobulins and Complement Analysis: Blood samples (5 mL) were collected from the jugular vein of all animals (fasted) on day 1, during weeks 14 and 27 prior to dosing and during week 40 prior to the schedule sacrifice. The following parameters were measured: total IgA, IgE, IgG, IgM, C3, C4 and CH50 (performed by (b) (4)

Anti-Uricase, anti-Puricase[®] and anti-PEG IgG antibodies: Blood samples (5 mL) were collected from the jugular vein of all animals (fasted) on day 1, during weeks 14 and 27 prior to dosing and during week 40 prior to the schedule sacrifice. The following parameters were measured: anti-PEG IgG antibodies; anti-uricase, and anti-Puricase[®], anti-PEG IgG antibodies (performed by Bio-Technology General - Israel).

As depicted in the sponsor's tables 4 and 5 below, puricase accumulated in the plasma at all doses; puricase was still present in the plasma at measurable levels. Plasma levels of puricase reached steady state at about 12 weeks.

Results

Toxicokinetic: The TK data are summarized in the sponsor's tables below. Puricase was detected in the plasma of all dogs and plasma puricase concentrations increased with increasing dose in both male and female dogs. Puricase increased in a slightly greater than dose-proportional manner for both males and females between the LD and MD groups and greater than dose-proportional between the MD and HD groups. As depicted in the sponsor's tables 4 and 5, puricase accumulated in the plasma at all doses; measurable levels of puricase were still present one week after administration of the previous dose of puricase and plasma concentrations of puricase progressively increased over the course of the study. According to the sponsor, active plasma levels of puricase reached steady state concentrations at approximately 12 weeks. As indicated in the mean plasma concentrations table below, the highest values for the mean plasma active puricase concentrations were noted during week 24 for both males and females. At the end of the recovery period, plasma levels of puricase were not measurable in plasma of dogs from the 0.4 and 1.5 mg/kg/week groups. On the other hand, low but measurable levels were detected in the plasma of half the dogs from the 10 mg/kg dose group.

The average mean AUC values of puricase are shown in sponsor's table 8. The mean AUC values for puricase increased with dose but in a greater than dose-proportional manner. Exposure appears to be different between males and females in the low-dose group. At the 0.4 mg/kg dose, systemic exposure to puricase was 22.5% higher in the females than males.

Table 4. Mean Plasma Puricase Concentrations (µg/ml) in Male Dogs

Study Time	Puricase (mg/kg BW/week)							
	0		0.4		1.5		10	
	1 h Pre-dose	1 h Post-dose	1 h Pre-dose	1 h Post-dose	1 h Pre-dose	1 h Post-dose	1 h Pre-dose	1 h Post-dose
Day 1	<1.0	<1.6	<1.0	6.3	<1.0	26.5	<1.1	170.4
Week 2	<1.6	<1.3	<1.9	7.6	11.0	37.3	81.3	253.1
Week 3	<1.2	<1.5	<2.6	8.5	15.3	38.9	116.6	291.4
Week 4	<1.2	<2.0	<3.6	9.2	20.2	44.7	144.6	299.5
Week 12	<1.2	<1.5	<7.6	12.6	36.9	64.9	243.3	423.0
Week 24	<1.4	<1.4	<8.9	13.7	44.6	70.9	303.9	477.6
Week 39	<1.2	<1.4	<7.5	13.7	40.4	65.5	243.8	399.3
Week 40 ⁽¹⁾	<1.0	NA	<7.9	NA	39.1	NA	243.4	NA
Week 52 ⁽²⁾	<0.9	NA	<0.9	NA	<0.9	NA	<3.0	NA

(1) Prior to sacrifice of the non-recovery group. No injection given

(2) Prior to terminal sacrifice of the recovery group

NA, not applicable

Table 5. Mean Plasma Puricase Concentrations (µg/ml) in Female Dogs

Study Time	Puricase (mg/kg BW/week)							
	0		0.4		1.5		10	
	1 h Pre-dose	1 h Post-dose	1 h Pre-dose	1 h Post-dose	1 h Pre-dose	1 h Post-dose	1 h Pre-dose	1 h Post-dose
Day 1	<1.1	<1.2	<1.1	6.4	<1.1	26.6	<1.6	184.7
Week 2	<1.7	<1.4	<2.3	9.0	12.2	39.0	86.5	267.2
Week 3	<1.6	<1.5	<3.3	10.3	19.7	45.0	134.3	313.1
Week 4	<1.8	<2.2	<5.1	11.6	24.8	51.0	173.6	317.5
Week 12	<1.4	<1.1	10.6	16.3	36.4	65.8	249.1	445.2
Week 24	<1.9	<1.2	9.7	16.2	44.2	74.1	281.4	468.0
Week 39	<1.3	<1.4	9.4	15.5	31.7	55.7	222.0	378.3
Week 40 ⁽¹⁾	<0.9	NA	9.4	NA	31.0	NA	220.6	NA
Week 52 ⁽²⁾	<1.3	NA	<1.3	NA	<1.3	NA	<1.9	NA

(1) Prior to sacrifice of the non-recovery group. No injection given

(2) Prior to terminal sacrifice of the recovery group

NA, not applicable

Table 8. AUC for Active Plasma Puricase Concentration in EDTA-plasma Samples Obtained from Dose Groups 1-4

Puricase Dose Levels	AUC ($\mu\text{g}\cdot\text{Day}/\text{ml}$) (Animal Sex)		
	Male Dogs	Female Dogs	Male and Female Dogs Combined
Group 1 - PBS	< 465.5*	< 490.7*	< 485.5*
Group 2 - 0.4 mg/kg BW	3,038.3	3,721.5	3,379.9
Group 3 - 1.5 mg/kg BW	15,295.1	14,867.1	15,088.3
Group 4 - 10.0 mg/kg BW	99,899.4	98,104.7	99,002.1

*the numerical value given reflects the lower limit of quantitation obtained in each assay through the course of analyses

Mortality: No treatment-related deaths occurred; all animals survived until schedule study termination.

Clinical signs: No treatment-related clinical signs were observed. However, it should be pointed out that some transient clinical signs were observed in males and females in all treatment groups, including the controls. These clinical signs included: abnormal excretion (i.e, mucoid, liquid, discolored and nonformed feces), clear optic discharge and/or vomitus. These clinical signs were still evidence during the recovery period. Also, no treatment-related effects on behavior, autonomic and central function as measured by the Modified Irwin's Behavioral Test, were apparent.

Body weights: There were no statistically significant differences in group mean body weights or group mean body weight gains in males or females treated with puricase compared to vehicle treated control animals.

Food consumption: Food consumption was measured daily and weekly consumption relative to control was evaluated. Food consumption in the female was not affected by puricase. However, compared to controls food intake, significant difference ($p \leq 0.05$) was noted in males during days 57-63, 155-161, 211-217 and 218-224. Males treated with 0.4 mg/kg/week food intake was significantly reduced by 14%, 12%, and 18% during dosing days 57-63, 155-161 and 218-224, respectively. Males treated with 10 mg/kg/week showed a significant increase in food intake in by 18% during dosing days 211-217. These changes were not considered toxicologically relevant. Significant changes in food consumption are tabulated below:

Dosing Days	Sex	Dose Level: Puricase [®] (mg/kg/week)			
		Mean \pm SD	Percent change from control		
		0/0	0.4	1.5	10.0
57-63	M	355 \pm 14.6	↓14%*	↓10%	↑3%
155-161	M	339 \pm 17.1	↓12%*	↓4%	↑0.88%
211-217	M	280 \pm 24.3	↓3%	↑14%	↑18%*
218-224	M	299 \pm 48.2	↓18%*	↑2%	↑14%

*: Statistically significant when compared to control at $p \leq 0.05$

Ophthalmoscopy: No treatment-related changes were noted in the eye.

EKG: No evidence of puricase-induced cardiotoxicity was observed. There were no statistically significant differences in heart rate, Qt-or QTc intervals in males or females treated with puricase compared to the vehicle control treated animals during treatment weeks 12, 24 and 39 when measured 5 to 15 minutes after dosing. Also, no treatment-related effects on electrocardiograms were apparent during the recovery period.

Hematology: There were no statistically significant differences in the hematological parameters assessed in either the males or females during the treatment period with puricase compared to the vehicle treated animals. However statistically significant changes ($p \leq 0.05$) in hematology parameters was noted in females during week 52 compared to the vehicle control treated animals. These changes were not considered to be treatment-related.

Parameter	Study Week	Gender	Dose (mg/kg/day)			
			0	0.4	1.5	10.0
MCV (FL)	52	Females	64.2 ± 0.81	69.5 ± 1.17* (+8.3%)	68.0 ± 1.04* (+5.9%)	66.9 ± 1.42 (+4.2%)
MCH (PG)	52	Females	21.6 ± 0.32	22.9 ± 0.42* (+6.0%)	22.6 ± 0.38 (+4.6%)	22.2 ± 0.72 (+2.8%)

*: Statistically significant (at $p \leq 0.05$) differences compared to controls

Clinical chemistry: Occasional statistically significant ($p \leq 0.05$) changes in clinical chemistry parameters in puricase treated animals compared to the vehicle control treated animals were mainly limited to males. A slight decrease in total protein level was observed in LD males at weeks 13 and HD males at week 26 compared to vehicle control animals. There was no changes relative to control during week 40 and 52 (recovery). Minor treatment-related decrease in albumin was observed in the 0.4, 1.5 and 10.0 mg/kg/week treatment groups during weeks 26, 40 and 52 (recovery). Due to a lack of a dose-dependent pattern, these findings are considered to be of little toxicological significance. At week 40, sorbital dehydrogenase (SDH) levels were significantly lower in females the LD, MD and HD puricase groups compared to vehicle control.

Parameter	Study Week	Gender	Dose (mg/kg/day)			
			0	0.4	1.5	10.0
Total Protein (G/DL)	13	Males	5.9 ± 0.24	5.4 ± 0.43* (-8.5%)	5.9 ± 0.25 NC	5.6 ± 0.16 (-5.0%)
	26	Males	6.4 ± 0.12	6.1 ± 0.36 (-5.0%)	6.4 ± 0.39 NC	5.9 ± 0.33* (-8.0%)
Albumin (G/DL)	26	Males	3.4 ± 0.12	3.4 ± 0.15 NC	3.4 ± 0.09 NC	3.2 ± 0.14* (5.8%)
	40	Males	3.9 ± 0.12	3.5 ± 0.06* (-10.0%)	3.5 ± 0.06* (-10.0%)	3.5 ± 0.21* (-10.0%)
	52	Males	34.0 ± 2.1	29.0 ± 1.2* (-14.7%)	23 ± 1.5* (-14.7%)	24.0 ± 0.6* (-29.4%)
SDH (U/L)	40	Females	5.0 ± 1.0	3.0 ± 0.8* (66.7%)	3.0 ± 0.8* (66.7%)	3.0 ± 0.4* (66.7%)

*: Statistically significant (at $p \leq 0.05$) differences compared to controls

Urinalysis: There were no statistically significant changes in urine parameters in the Puricase[®]-treated animals compared to the vehicle treated animals.

Gross pathology: No drug-related changes were observed.

Organ weights: No drug-related changes on absolute and relative organ weight were observed in puricase-treated animals compared to control treated animals. Also, no treatment-related changes were noted in the absolute organ weight, organ weight relative-to-body weight and relative-to-brain weight at the end of the 12-week recovery period.

Histopathology: Histopathological evaluation was conducted on all study animals in both the treatment group and the recovery group. Treatment-induced vacuolation was the primary histopathology finding observed in dogs after 39-weeks of repeated intravenous administration of puricase compared to the vehicle control group. As depicted in the table below, vacuolation was identified in the adrenal cortex, duodenum, heart, jejunum, liver and spleen. Microscopic findings at the injection site were noted and consisted of inflammation, focal intimal proliferial regeneration, hemorrhage and medial regeneration. The sponsor's initial assessment of the vacuolation is summarized (*the reviewer paraphrase the sponsor's summary*) below:

Adrenal Cortex: Vacuolated cells were most prominent within the adrenal cortex of males and females in the high-dose group. The sponsor considered this finding to be treatment-related. No indication of recovery was noted; the vacuolated cells remained essentially unchanged following the recovery period.

Duodenum and Jejunum: Vacuolated/foamy cells were identified in the basal area of the lamina propria within the duodenum and jejunum of 2 males in the high-dose group. Vacuoles were present in the lamina propria of the jejunum of 2 females in the high-dose group. The sponsor presumed that these vacuolated cells were macrophages. The presence of the vacuolated cells remained essentially unchanged following the recovery period.

Liver (Hepatic Kupffer cells): Kupffer cells were vacuolated in all terminal sacrifice high-dose males and females. The severity score was rated minimal to slight. The appearance of the vacuoles was described as being clear and well defined, often pushing the nucleus to the periphery of the cell. One female in the low-dose group presented with vacuoles rated as slight. The status remained essentially unchanged following the recovery.

Heart: Minimal to slight vacuolization of intimal cells within the aortic outflow area of the heart was observed in 1 and 3 males in the 0.4 and 10 mg/kg/week groups, respectively. Vacuolated cells were not noted in any of the terminal-sacrifice females from any of the group. After the recovery period, vacuolated cells were observed in all males in the 10 mg/kg recovery group and one 10 mg/kg recovery female.

Spleen: Vacuoles were identified within the red pulp areas from all terminal sacrifice dogs of the 10 mg/kg/week and all but one (male) of the 1.5 mg/kg/week groups. The vacuoles were clear, round and variable in size. Congestion was also observed in the red pulp. The vacuoles were treatment-related but the sponsor stated there was no evidence for a direct causal relationship between treatment and congestion. *(The reviewer concurs with the sponsor.)*

Parameter	Period	Incidence of Microscopic Histological Changes							
		Total № of Animals with the Histological Finding (severity score) ^A							
		Males (mg/kg)				Females (mg/kg)			
		0.0	0.4	1.5	10.0	0.0	0.4	1.5	10.0
Number Examined →		3	3	3	3	3	3	3	3
Adrenal Cortex									
From Sponsor's pathologist									
Vacuolated Cells	Treatment	0	0	0	1 (1)	0	0	1 (2)	3 (1, 2, 3)
	Recovery	0	0	0	2 (2, 2)	0	0	0	3 (3, 2, 2)
Independent Pathologist									
Vacuolated Cortical Cells, Zona Fasciculata, Bilateral, Multifocal	Treatment	0	0	0	1 (1)	0	0	2 (1, 1)	3 (1, 1, 1)
	Recovery	0	0	0	3 (1, 2, 1)	1 (1)	3 (1,1,1)	2 (1,1)	3 (1,1,1)
Vacuolated Cortical Cells, Zona Fasciculata, Bilateral, Focal	Treatment	0	0	0	0	0	0	0	0
	Recovery	0	0	1 (1)	0 (3)	0	0	0	0
Vacuolated Cortical Cells Zona Glomerulus, Bilateral, Multifocal	Treatment	0	0	1 (2)	0	0	0	2 (1, 1)	0
	Recovery	0	0	0	2 (1,1)	0	0	0	1 (1)
Vacuolated Cortical Cells Zona Reticularis, Bilateral, Multifocal	Treatment	0	0	0	3 (1, 1, 1)	0	0	2 (1, 1)	3 (2, 1, 2)
	Recovery	0	0	0	3 (1, 1, 1)	0	1 (1)	2 (1, 1)	3 (2, 1, 2)
Medulla: Epithelial Cell, Vacuolation, Multifocal, Unilateral or bilateral	Treatment	0	0	0	0	0	1 (1)	3 (1, 1, 1)	2 (1, 1)
	Recovery	0	0	0	3 (1, 2, 1)	0	1 (1)	0	3 (1, 1, 1)
Medulla: Vacuolation, Dendritic Macrophage, Bilateral, Multifocal	Treatment					0	0	0	3 (2,1,1)
	Recovery	0	0	0	0	0	0	1 (1)	3 (1,1,1)
Heart									
Sponsor's Pathologist									
Intima vacuolation, great vessels	Treatment	0	1 (1)	0	3 (1, 1, 1)	0	0	0	0
	Recovery	0	0	0	3 (1, 2, 1)	0	0	0	1 (2)
Inflammation, chronic, intima of great vessels	Treatment	0	0	0	1 (1)	0	0	0	0
	Recovery	0	0	0	2 (2, 1)	0	0	0	0
Independent Pathologist									
Aorta: Endothelial Cells, Vacuolated, Focal	Treatment	0	0	0	3 (1,1,1)	0	0	0	0
	Recovery	0	0	0	1 (1)	0	0	0	1 (1)
Aorta: Endothelial Cells, Vacuolated, Multifocal	Treatment					0	0	0	0
	Recovery	0	0	0	1 (1)	0	0	0	0
Aorta: Macrophages, Vacuolated, Subendothelial, Focal	Treatment	0	0	0	1 (1)	0	0	0	0
	Recovery	0	0	0	2 (1,1)	0	0	0	1 (1)
Myocardial: Fatty Infiltration, Multifocal	Treatment	2 (1,1)	3 (1,1,1)	3 (1,1,1)	3 (1,1,1)	3 (1,1,1)	2 (1,1)	3 (1,1,1)	2 (1,1)
	Recovery	2 (1,1)	3 (1,1,1)	3 (1,1,1)	3 (1,2,2)	3 (1,1,1)	2 (1,1)	3 (1,1,1)	3 (1,1,1)
Duodenum									
Sponsor's Pathologist									
Vacuolated, lamina propria	Treatment	0	0	0	2 (1, 1)	0	0	0	0
	Recovery	0	0	0	3 (1, 3, 2)	0	0	0	3 (2, 2, 1)

Parameter	Period	Incidence of Microscopic Histological Changes							
		Total № of Animals with the Histological Finding (severity score) ^A							
		Males (mg/kg)				Females (mg/kg)			
		0.0	0.4	1.5	10.0	0.0	0.4	1.5	10.0
Independent Pathologist									
Vacuolated, lamina propria	Treatment	0	0	0	0	0	0	0	0
	Recovery	0	0	0	0	0	0	0	0
Jejunum									
Sponsor's Pathologist									
Vacuolated cells, lamina propria	Treatment	0	0	0	2 (1, 2)	0	0	0	2 (3, 2)
	Recovery	0	0	0	3 (2, 3, 3)	0	0	0	3 (2, 2, 2)
Independent Pathologist									
Vacuolated cells, lamina propria	Treatment	0	0	0	0	0	0	0	0
	Recovery	0	0	0	0	0	0	0	1 (1)
Liver									
Sponsor's Pathologist									
Vacuolated Kupffer cells	Treatment	0	0	0	3 (1, 2, 2)	0	1 (2)	0	3 (1, 2, 2)
	Recovery	0	0	0	3 (1, 2, 2)	0	0	0	3 (1, 1, 1)
Independent Pathologist									
Vacuolated Kupffer cells, multifocal	Treatment	0	0	0	3 (1, 1, 1)	0	0	0	3 (1, 2, 2)
	Recovery	0	0	0	3 (1, 1, 2)	0	0	0	3 (2, 1, 2)
Infiltrate, Lymphohistiocytic, Multifocal	Treatment	2 (1, 1)	1 (1)	3 (1, 1, 1)	3 (1, 1, 1)	3 (1, 1, 1)	2 (1, 1)	3 (1, 1, 1)	1 (1)
	Recovery	3 (1, 1, 1)	1 (1)	2 (1, 1)	3 (1, 1, 1)	2 (1, 1)	3 (1, 1, 1)	2 (1, 1, 1)	3 (1, 1, 1)
Spleen									
Sponsor's Pathologist									
Reticuloendothelial Cells, Vacuolation	Treatment	0	0	2 (1, 3)	3 (3, 4, 3)	0	0	3 (3, 2, 3)	3 (4, 3, 3)
	Recovery	0	1 (2)	2 (1, 3)	3 (3, 4, 3)	0	0	3 (3, 2, 3)	3 (3, 2, 3)
Congestion	Treatment	0	1 (3)	1 (3)	1 (3)	1 (3)	1 (3)	1 (4)	3 (3, 4, 4)
	Recovery	1 (3)	1 (3)	2 (1, 3)	2 (1, 3)	2 (3, 3)	0	3 (4, 3, 4)	2 (3, 3)
Independent Pathologist									
Macrophages, Vacuolation, Diffuse	Treatment	0	0	0	3 (3, 4, 4)	0	0	3 (2, 2, 2)	3 (4, 4, 4)
	Recovery	0	0	0	3 (2, 3, 3)	0	0	3 (2, 2, 2)	3 (3, 3, 4)
Macrophages, Vacuolation, Multifocal	Treatment	0	0	3 (2, 1, 1)	0	0	0	0	0
	Recovery	0	0	1 (2)	2 (1, 2)	0	0	0	0
Macrophages, Pigmented, Multifocal	Treatment	1 (1)	2 (1, 2)	1 (1)	1 (2)	2 (1, 1)	1 (1, 1)	3 (1, 2, 10)	3 (1, 1, 1)
	Recovery	3 (1, 1, 1)	2 (2, 1)	2 (1, 1)	2 (2, 2)	2 (1, 1)	2 (1, 2)	2 (1, 1)	3 (1, 1, 1)
Congestion, diffuse or multifocal	Treatment	2 (3, 2)	3 (3, 2, 1)	3 (3, 1, 2)	3 (1, 3, 1)	3 (1, 1, 1)	2 (1, 2)	3 (3, 2, 1)	3 (3, 3, 3)
	Recovery	1 (3)	1 (3)	2 (1, 3)	2 (1, 3)	2 (3, 3)	0	3 (4, 3, 4)	2 (3, 3)
Hyperplasia, Lymphoid, PALS	Treatment	0	2 (2, 2)	3 (3, 1, 2)	3 (3, 1, 2)	1 (1)	1 (2)	1 (2)	0
	Recovery	0	3 (1, 1, 2)	1 (1)	0	1 (1)	3 (1, 1, 2)	1 (2)	2 (2, 2)

A: Severity Score:

1 = Minimal: barely noticeable morphologic alteration in tissue that is very small and often equivocal

2 = Slight/Mild: Morphologic alteration is clearly present

3 = Moderate: Histological change is a prominent morphologic alteration in the tissue

4 = Moderately Severe: Histological change is a very prominent morphologic alteration that may greatly efface or obscure structures that are normally observed in the tissue

B: Histopathological analysis of Sponsor's pathologist

C: Histopathological analysis of independent pathologist

During the pre-BLA meeting held on April 17, 2008, the Agency raised concerns regarding the finding of vacuolation in multiple tissues in the 39-week repeat-dose toxicology study. The Agency requested that the sponsor include data to document the content of the vacuoles to support their claim that the vacuoles contain PEG and do not result in adverse effects to these tissues. To address the Agency concerns, the paraffin blocks of the above tissues were sent to (b) (4) for immunohistochemistry evaluation and the hemotoxylin and eosin (H&E) stained slides containing the adrenal gland, duodenum, jejunum, heart, liver and spleen were forwarded to (b) (4), an independent pathologist (b) (4) for evaluation and to provide a potential toxicological significance of the vacuoles in those tissues. His description of the histological findings of the H&E stained slides is highlighted below (*the reviewer paraphrased the sponsor's summary*) below:

Tissue	Histopathological Evaluation
Adrenal Cortex	<p>The adrenal glands changes were considered to be inconsequential.</p> <p>Location: In the adrenal glands of dogs dosed with 1.5 and 10 mg/kg, microscopic evaluation disclosed vacuolation predominately in cells of the zona fasciculata and zona reticularis with a few foci in the zona glomerulosa. A few affected animals showed cytoplasmic vacuolation in the epithelia; cells of the adrenal medulla.</p> <p>Appearance: The vacuoles in the cells of the adrenal cortex as well as the medullary epithelial cells were contiguous with other cytoplasmic content. Thus, the material in vacuoles was considered to most likely be lipids associated with steroid metabolism.</p> <p>Reversibility: <u>Vacuolation was not reversible.</u> Histological findings were similar in severity and incidence in the adrenal glands of dogs both at the end of the dosing period and at the end of the recovery period.</p> <p>Conclusion of independent pathologist: Vacuolation was considered to be a non-adverse adaptive response associated with exposure to the PEGylated test-article.</p>
Heart	<p>Intima in the aorta outflow area immediately distal to the aortic valve:</p> <ul style="list-style-type: none"> - Very minimal focal endothelial vacuolation, with and without endothelial infiltrated mononuclear cells or vacuolated macrophages were observed in all but one male (H42045) in the high-dose group both at the end of the dosing and the recovery period. Dog H42045 had a subendothelial macrophage infiltrate and vacuolated macrophage. - On female (H42070) in the 10 mg/kg dose group had minimal focal endothelial vacuolation with a few subendothelial mononuclear cells and vacuolated macrophages. These effects were considered very minimal, but were also considered test-article related. - Conclusion of independent pathologist: Due to the low cellularity of subendothelial mononuclear cell infiltrate and absence of intimal proliferation or a fibroblastic response, the observations in the aortic intimal changes are inconsequential. <p>Myocardium of the Heart:</p> <ul style="list-style-type: none"> - Vacuolation was associated with minimal infiltrates of unilocular adipose tissue cells (fatty infiltration). This effect was observed across all sex/dose groups including the control group at the end of the 39 weeks dosing and after the recovery periods.

Tissue	Histopathological Evaluation
	<p>- The minimal myocardial fatty infiltration, which was most frequently observed only in atrial or right ventricular muscle, was considered to be within normal limits and not associated with the administration of the test-article.</p>
Duodenum	<p>A peribasal histiocytic infiltrate was observed in the duodenum of males and females in the 10 mg/kg recovery group. The peribasal histiocytic infiltrate consisted of epithelioid macrophages that formed a linear aggregate at the base of the epithelial glands along the interface of the mucosa and submucosa.</p> <p>Conclusion of independent pathologist: The vacuolated macrophages and histiocytic responses observed are considered to be attributable to the PEGylated test article.</p>
Jejunum	<p>A peribasal histiocytic infiltrate was observed in the duodenum of males and females in the 10 mg/kg recovery group. The peribasal histiocytic infiltrate consisted of epithelioid macrophages that formed a linear aggregate at the base of the epithelial glands along the interface of the mucosa and submucosa.</p> <p>The vacuolated macrophages observed in the lamina propria of the jejunum of one female (H4070) in the 10 mg/kg recovery group were considered treatment-related.</p> <p>The vacuolar content of the macrophages in the intestine could not be further characterized.</p> <p>Conclusion of independent pathologist: The vacuolated macrophages and histiocytic responses observed were considered to be attributable to the PEGylated test-article.</p>
Liver	<p>Location: Vacuolation observed within the Kupffer cells along the sinusoidal borders of hepatocytes in the liver of the dogs treated with the 10 mg/kg dose of puricase was considered to be treatment-related.</p> <p>Appearance: Affected cells were globular with smoothly contoured plasma membranes. The cytoplasmic content of the vacuoles was clear.</p> <p>Reversibility: <u>Vacuolation was not reversible.</u> The numbers of dogs affected and the severity of the vacuolation were similar at the end of the 12-week recovery period to that at the end of the dosing period.</p> <p>Conclusion of independent pathologist: The vacuolation of Kupffer cells in the high-dose groups was considered to be a non-adverse adaptive response associated with exposure to the PEGylated test-article.</p>
Spleen	<p>Two treatment-related histopathological findings were noted:</p> <ul style="list-style-type: none"> - Vacuolated Macrophages (diffuse or multifocal). Vacuolated macrophages were observed in male and female administered puricase at the 1.5 and 10 mg/kg/week doses in both terminal and recovery groups. Predominately observed in the red pulp. - Periarteriolar Lymphoid Sheath Hyperplasia (PALS). PALS was noted with less consistency between the sexes and between dosing and recovery intervals. It was observed in all the high-dose males; it was not observed at the end of the recovery period. PALS was noted in one female in the control, low- and high-dose groups and in two females in the mid-dose group. At the end of the recovery period, it was observed in one female in the control and the three treatment groups. Due to PALS presence in the control group and no dose-dependent relationship, the pathologist suggests this may be a background finding in the spleen and not a treatment-related change.

Based on the above data, the target organs of toxicity included adrenal cortex, duodenum, jejunum, liver, spleen and heart. Vacuolation was observed in these tissues. Vacuolation is often associated with pegylated drugs. The presence of these vacuolated remained essentially unchanged following recovery period. The sponsor states that “since PEGylation is often used in pharmaceutical product formulation, the test-article related vacuolar effects in macrophages in the spleen, intestine and liver (Kupffer cells) as well as the adrenal medulla and the intima of the aorta are interpreted to be non-adverse effects related to the ingestion of the PEGylated protein. The vacuoles in the cytoplasm of macrophages in several sites most likely reflect the incorporation and isolation of the PEGylated test-article in phagosomes due to an inability of the macrophages to readily digest it.” Since the independent pathologist identified the presence of macrophages in the spleen, liver, duodenum and jejunum, the reviewer concurs that the vacuolation in these tissues most likely represent macrophages ingesting the PEGylated protein since there were no clinical pathology parameters changes associated with these findings to support toxicological relevance. However, due to the vacuoles still being presence after a 12-week recovery period, it is not known if prolonged accumulation of the indigestible material could eventually lead to toxicity and/or functional changes in these organs. The potential adverse effects of the vacuolation identified in the adrenal cortex and intima in the aorta outflow of the heart is still unknown and concerns the reviewer.

On February 27, 2009, the sponsor submitted unaudited data from the immunohistochemical analysis; the audited final immunohistochemistry report (b) (4) Study № IM1678) was submitted to the Agency on April 8, 2009.

The objective of the immunohistochemical evaluation was to determine if the cells that contained vacuoles were macrophages and to identify the contents of the vacuoles. Tissues showing vacuolization (i.e., vacuoles formation) at the end of the 39-week treatment period or 12-week recovery period were sent to (b) (4) for immunohistochemistry analysis. Sections of paraffin blocks containing sections of adrenal cortex, duodenum, heart (intimal cells), jejunum and liver (Kupffer cells) and spleen from 33 animals (table below identified the animals by their assigned number) were submitted for immunohistochemical analysis. Sections were stained for PEG (rabbit anti-PEG reagent (10 µg/mL)), and uricase (rabbit and anti-uricase reagent (5 µg/mL)), and replicate sections were stained for macrophage marker (MAC387 (10 µg/mL)) to ascertain if the vacuoles were being primarily formed in monocyte/macrophage lineage cells. For negative control antibody, paraffin sections were stained with 5 µg/mL or 10 µg/mL of rabbit anti-FITC for anti-uricase and anti-PEG, respectively. The negative control antibody for the macrophage staining was 10 µg/mL mouse IgG1κ

Dosing Group (mg/kg)	Animal Number			
	Main Study		Recovery Group	
	Males	Females	Males	Females
Control	H42024		H42027	H42052, H42053
0.4	H42031,	H42055	H42033, H42034, H42035	H42057, H42059
1.5	H42036, H42037	H42060, H42061, H42062	H42039, H42040	H42063, H42064, H42065
10	H42042, H42043, H2044	H42066, H42067, H42068	H42045, H42046, H42047	H42069, H42070, H42071

As depicted in the tables (reproduced from sponsor's tables) below, both the uricase and PEG portions of puricase were qualitatively identified in the cells and cytoplasmic vacuoles of the adrenal cortex, lamina propria of the duodenum and jejunum, heart (intimal cells, vascular walls of the aortic outflow tract), and liver (Kupffer cells) and spleen of dogs in the mid- and high-dose groups. The macrophage specific stain MAC387 demonstrated that the cells staining for the uricase and PEG in the liver, spleen and lamina propria of the duodenum and jejunum were macrophages. The cytoplasmic vacuoles which stained for uricase and PEG in the adrenal cortex and heart were not identified as being contained in macrophages.

Intravascular/Interstitial Proteinic Fluid. Intravascular and interstitial proteinic fluid from the adrenal gland, heart, liver, spleen and duodenum stained for uricase and PEG in all treatment groups at terminal sacrifice. As depicted in the table below, the intensity of the staining for uricase or PEG ranged from minimal to marked. The intensity increased with increasing dose. At the end of the recovery period, most dogs did not stain positive for the presence of uricase or PEG. Minimal staining of the proteinic fluid for uricase or PEG occurred occasionally in few male dogs in each treatment group; more organs in the high dose group stained positive for uricase or PEG. No organs from the recovery group females stained positive for PEG or uricase.

Male and Females Terminal Sacrifice: Intravascular/Interstitial Proteinic Fluid.

Immunohistochemical Scores: Intensity ¹ (Frequency) ²											
Intravascular//Interstitial Proteinic Fluid											
Dose (mg/kg)	Animal No	Adrenal		Heart		Liver		Spleen		Duodenum	
		Uricase	PEG								
Males											
0	H42024	Neg									
0.4	H42031	2+ (VR)	2+ (VR)	1-3+ (R-O)	1-3+ (O)	1-2+ (VR)	1-2+ (VR)	1-2+ (R-O)	Neg	1-2+ (R-O)	1-3+ (O)
1.5	H42036	1-3+ (O)	1-2+ (R-O)	1-3+ (O)	1-3+ (O)	1-3+ (O)	1-3+ (O)	1-4+ (O)	1-4+ (O)	NE	NE
	H42037	1-2+ (R-O)	1-2+ (R-O)	1-3+ (O)	1-3+ (O)	1-3+ (O)	1-3+ (O)	1-4+ (O)	1-3+ (R-O)	2-4+ (O-F)	1-3+ (O)
10.0	H42042	2-4+ (O)	1-3+ (O)	2-4+ (O-F)	2-4+ (O-F)	1-3+ (O)	1-3+ (O)	2-4+ (O-F)	1-3+ (R)	2-4+ (O-F)	2-4+ (O-F)
	H42043	2-4+ (O)	1-3+ (O)	2-4+ (O)	2-4+ (O)	2-4+ (O-F)	1-4+ (O-F)	1-4+ (F)	1-4+ (O-F)	2-4+ (O-F)	2-4+ (O-F)
	H42044	2-4+ (O)	1-4+ (O)	2-4+ (O-F)	2-4+ (O)	1-4+ (O-F)	1-3+ (O)	2-4+ (O-F)	1-3+ (R-O)	2-4+ (F)	2-4+ (O-F)
Females											
0.4	H42055	1-2+ (R)	1+ (VR)	1+ (R)	1+ (VR)	1-2+ (VR)	1+ (VR)	1+ (R)	Neg	NE	NE
1.5	H42060	1-2+ (R-O)	1-2+ (R-O)	1-2+ (R)	1-2+ (R)	1-2+ (R-O)	1-3+ (VR)	2+ (R)	Neg	1-3+ (R-O)	1-3+ (R-O)
	H42061	1-2+ (VR)	1-2+ (VR)	1-2+ (VR)	1+ (VR)	Neg	1-2+ (VR)	1-2+ (VR)	Neg	1-2+ (R-O)	1-3+ (O)
	H42062	1-2+ (R)	1-2+ (R)	1-3+ (R)	1-3+ (R-O)	1+ (VR)	1-2+ (VR)	1+ (VR)	1+ (VR)	NE	NE
1.0	H42066	1-4+ (O)	1-4+ (O)	2-4+ (O-F)	2-4+ (O-F)	2-4+ (O-F)	1-4+ (O)	2-4+ (O)	1-3+ (R-O)	NE	NE
	H42067	1-4+ (O)	1-4+ (O)	2-4+ (O-F)	2-4+ (O-F)	2-4+ (O-F)	1-3+ (O)	1-4+ (O)	1-4+ (O)	2-4+ (F)	2-4+ (F)
	H42068	1-4+ (O)	1-4+ (O)	2-4+ (O-F)	2-4+ (O-F)	1-4+ (O)	1-4+ (O)	1-4+ (R-O)	1-3+ (R)	2-4+ (F)	2-3+ (O)

1: Intensity: Neg = Negative; ± = equivocal; 1+ = minimal; 2+ = slight; 3+ = moderate; 4+ = marked (intense).

2: Frequency (% of cells or tissue elements): VR = very rare (<1% of cells of a particular cell type); R = rare (1-5% of cells of a particular cell type); R-O = rare to occasional (>5-25% of cells of a particular cell type); O = occasional (> 25-50% of cells of a particular cell type); O-F = occasional to frequent (>50-75% of cells of a particular cell type); F = frequent (>75-100% of cells of a particular cell type)
 NE: Not Evaluated
 *: Findings for jejunum generally similar to duodenum.

Liver. Macrophages (Kupffer cells) were identified in the liver in all groups of both genders at the end of the 39-week dosing and recovery periods. Consistent with the absence of vacuoles in the 0.4 and 1.5 mg/kg dose groups in both genders, the stain for uricase and PEG did not stain cells (i.e., vacuoles) for uricase and PEG in these groups. However, uricase and PEG was qualitatively identified in Kupffer cells in both males and females in the LD and MD groups. In the high-dose groups intracellular vacuoles stained for uricase and/or PEG; staining with an intensity ranging from minimal to marked. The percentage of cells staining for uricase and PEG ranged between 1% to 25% and 1-25%, respectively. Immunohistochemical analysis qualitatively identified uricase and PEG in Kupffer cells in all male treatment groups and the high-dose female group; staining with an intensity ranging from minimal to moderate. At the end of the recovery period, vacuoles in the liver of one male in the HD group stained slightly positive for uricase. The Kupffer cells in two males in the HD group stained for uricase. None of the vacuoles stained for PEG.

Males and Females Terminal Sacrifice: Liver

Immunohistochemical Scores: Intensity ¹ (Frequency) ²								
Liver								
		Vacuoles				Kupffer Cells		
Dose (mg/kg)	Animal No	H&E ³		Uricase	PEG	Uricase	PEG	MAC387
		(b) (4)	(b)					
Males								
0	H42024	None	None	Neg	Neg	Neg	Neg	3-4+ (freq)
0.4	H42031	None	None	Neg	Neg	1+ (very rare)	1-2+ (rare)	3-4+ (freq)
1.5	H42036	None	None	Neg	Neg	2+ (rare-occas)	2+ (rare-occas)	3-4+ (freq)
	H42037	None	None	Neg	Neg	2+ (rare-occas)	2+ (rare-occas)	3-4+ (freq)
10.0	H42042	Minimal	Minimal	1-2+ (very rare)	Neg	1-3+ (occas)	1-3+ (occas)	3-4+ (occas)
	H42043	Slight	Minimal	2-4+ (rare-occas)	2-4+ (rare-occas)	2-3+ (occas)	1-3+ (occas)	3-4+ (occas)
	H42044	Slight	Minimal	2-3+ (rare)	2+ (rare)	1-3+ (occas)	1-3+ (occas)	3-4+ (occas)
Females								
0.4	H42055	Slight	None	Neg	Neg	Neg	Neg	3-4+ (freq)
1.5	H42060	None	None	Neg	Neg	1-2+ (very rare)	Neg	3-4+ (occas-freq)
	H42061	None	None	Neg	Neg	Neg	Neg	3-4+ (occas-freq)
	H42062	None	None	Neg	Neg	Neg	Neg	3-4+ (occas-freq)
10.0	H42066	Slight	Minimal	2-3+ (rare)	1-2+ (rare)	1-3+ (occas)	1-3+ (occas)	3-4+ (occas-freq)

Immunohistochemical Scores: Intensity ¹ (Frequency) ²								
Liver								
		Vacuoles						
		H&E ³		Uricase	PEG	Kupffer Cells		
Dose (mg/kg)	Animal No	(b) (4)	(b)	Uricase	PEG	Uricase	PEG	MAC387
	H42067	Slight	Slight/Mild	1-2+ (very rare)	1-2+ (very rare)	1-3+ (occas)	1-2+ (rare-occas)	3-4+ (occas-freq)
	H42068	Slight	Slight/Mild	1-2+ (very rare)	1-2+ (very rare)	1-3+ (occas)	1-3+ (occas)	3-4+ (occas-freq)

1: Intensity: Neg = Negative; ± = equivocal; 1+ = minimal; 2+ = slight; 3+ = moderate; 4+ = marked (intense).
 2: Frequency (% of cells or tissue elements): very rare (<1% of cells of a particular cell type); rare (1-5% of cells of a particular cell type); rare-occas = rare to occasional (>5-25% of cells of a particular cell type); occas = occasional (> 25-50% of cells of a particular cell type); occas-freq = occasional to frequent (>50-75% of cells of a particular cell type);freq = frequent (>75-100% of cells of a particular cell type)
 3: H&E (hematoxylin & eosin): None = vacuoles not present.

Males and Females Recovery Sacrifice: Liver

Immunohistochemical Scores: Intensity ¹ (Frequency) ²								
Liver								
		Vacuoles						
		H&E ³		Uricase	PEG	Kupffer Cells		
Dose (mg/kg)	Animal No	(b) (4)	(b)	Uricase	PEG	Uricase	PEG	MAC387
Males								
0	H42027	None	None	Neg	Neg	Neg	Neg	3-4+ (freq)
0.4	H42033	None	None	Neg	Neg	Neg	Neg	3-4+ (freq)
	H42034	None	None	Neg	Neg	Neg	Neg	3-4+ (freq)
	H42035	None	None	Neg	Neg	Neg	Neg	3-4+ (freq)
1.5	H42039	None	None	Neg	Neg	Neg	Neg	3-4+ (occas-freq)
	H42040	None	None	Neg	Neg	Neg	Neg	3-4+ (occas)
10.0	H42045	Minimal	Minimal	Neg	Neg	Neg	Neg	3-4+ (occas)
	H42046	Slight	Minimal	Neg	Neg	2+ (rare)	Neg	3-4+ (occas-freq)
	H42047	Slight	Slight/Mild	1-2+ (very rare)	Neg	1-2+ (rare)	Neg	3-4+ (occas-freq)
Females								
0	H42052	None	None	Neg	Neg	Neg	Neg	3-4+ (freq)
	H42053	None	None	Neg	Neg	Neg	Neg	3-4+ (freq)
0.4	H42057	Slight	None	Neg	Neg	Neg	Neg	3-4+ (freq)
	H42059	None	Minimal	Neg	Neg	Neg	Neg	3-4+ (freq)
1.5	H42063	None	None	Neg	Neg	Neg	Neg	3-4+ (occas-freq)
	H42064	None	None	Neg	Neg	Neg	Neg	3-4+ (occas-freq)
	H42065	None	None	Neg	Neg	Neg	Neg	3-4+ (occas-freq)
10.0	H42069	Minimal	Slight/Mild	Neg	Neg	Neg	Neg	3-4+ (occas)
	H42070	Minimal	Slight/Mild	Neg	Neg	Neg	Neg	3-4+ (occas)
	H42071	Minimal	Minimal	Neg	Neg	Neg	Neg	3-4+ (occas-freq)

1: Intensity: Neg = Negative; ± = equivocal; 1+ = minimal; 2+ = slight; 3+ = moderate; 4+ = marked (intense).
2: Frequency (% of cells or tissue elements): very rare (<1% of cells of a particular cell type); rare (1-5% of cells of a particular cell type); rare-occas = rare to occasional (>5-25% of cells of a particular cell type); occas = occasional (> 25-50% of cells of a particular cell type); occas-freq = occasional to frequent (>50-75% of cells of a particular cell type);freq = frequent (>75-100% of cells of a particular cell type)
3: H&E (hematoxylin & eosin): None = vacuoles not present.

Spleen. Results from immunohistochemical analysis suggest that the vacuoles identified in H&E staining following 39-weeks of treatment with PEG-uricase possibility represented reticuloendothelial cells (i.e., monocytes/macrophages) engulfing the PEG-uricase. Monocytes and macrophages stained for uricase and/or PEG at terminal sacrifice in all treatment groups. Vacuoles scored as slight to moderate intensity severity after H&E staining in males and females in the 1.5 mg/kg dose group did not qualitatively stain for either uricase or PEG. However, monocytes/macrophages stained for both uricase and PEG; suggesting that the vacuoles identified in H&E staining were monocytes/macrophages containing the PEG-uricase. Immunohistochemical analysis qualitatively identified uricase in intracellular vacuoles that were scored as moderate to moderately severe after H&E staining in HD males. One to twenty-five percent of the vacuoles stained for uricase with minimal to moderate severity intensity. In one male in the HD group, <1% of the vacuoles stained for PEG with minimal to slight intensity. In contrast, to vacuoles of slight to moderate severity intensity being identified with H&E staining after the recovery period in mid- and high-dose males, no vacuoles in the spleen of mid-dose males stained positive for uricase or PEG. At the end of recovery, vacuoles in the spleen of one male in the HD group stained positive for uricase. Immunohistochemical analysis indicates that partial recovery is occurring; monocytes/macrophages stained for uricase and/or PEG in all treatment groups at the end of recovery. In the high dose male group 1 to 5 percent of the cells stained for uricase and PEG. The reviewer does not view this to be toxicologically significant but representing phagocytic activity.

Intracellular vacuoles scored as slight to moderate in severity after H&E staining in all females in the mid-dose groups did not stain for uricase or PEG. On the other hand, monocytes/macrophages stained for both uricase and PEG. Vacuoles in the spleen of one female in the 10 mg/kg dose group stained for uricase with minimal to moderate intensity. Monocytes/macrophages in the spleen stained for uricase and/or PEG in the mid- and high-dose groups. In contrast to vacuoles of minimal to moderate severity intensity being identified with H&E staining in mid- and high-dose females after the recovery period, no vacuoles in the spleen stained positive for uricase or PEG. Also, no monocytes/macrophages stained for uricase or PEG after the recovery period. Macrophages were identified in all treatment groups and control at the end of recovery period.

Males and Females Terminal Sacrifice: Spleen

Immunohistochemical Scores: Intensity ¹ (Frequency) ²								
Spleen								
Vacuoles								
H&E ³								
Dose (mg/kg)	Animal No	(b) (4)	(b)	Uricase	PEG	Monocyte/Macrophages		
						Uricase	PEG	MAC387
Males								
0	H42024	None	None	Neg	Neg	Neg	Neg	3-4+ (freq)
0.4	H42031	None	None	Neg	Neg	1-2+ (rare-occas)	1-2+ (rare)	3-4+ (freq)
1.5	H42036	Slight	Slight/Mild	Neg	Neg	2-4+ (rare-occas)	2-4+ (rare-occas)	3-4+ (freq)
	H42037	Moderate	Minimal	Neg	Neg	2-3+ (rare-occas)	2-3+ (very rare)	3-4+ (freq)
10.0	H42042	Moderate	Moderate	1-2+ (rare)	Neg	1-3+ (occas)	Neg	3-4+ (freq)
	H42043	Moderately Severe	Moderately Severe	1-3+ (rare)	Neg	2-4+ (occas-freq)	2-4+ (occas)	3-4+ (freq)
	H42044	Moderate	Moderately Severe	2-3+ (rare-occas)	1-2+ (very rare)	1-3+ (occas)	1-3+ (rare-occas)	3-4+ (freq)
Females								
0.4	H42055	None	None	Neg	Neg	1+ (rare)	Neg	3-4+ (freq)
1.5	H42060	Moderate	Slight/Mild	Neg	Neg	2-3+ (rare-occas)	Neg	3-4+ (freq)
	H42061	Slight	Slight/Mild	Neg	Neg	2+ (very rare)	Neg	3-4+ (freq)
	H42062	Slight	Slight/Mild	Neg	Neg	1+ (rare)	1+ (rare)	3-4+ (freq)
10.0	H42066	Moderately Severe	Moderately Severe	Neg	Neg	1-3+ (occas)	1-3+ (rare-occas)	3-4+ (freq)
	H42067	Moderate	Moderately Severe	1-3+ (rare)	Neg	2-4+ (rare-occas)	2-4+ (rare-occas)	3-4+ (freq)
	H42068	Moderate	Moderately Severe	Neg	Neg	1-3+ (rare-occas)	Neg	3-4+ (freq)

1: Intensity: Neg = Negative; ± = equivocal; 1+ = minimal; 2+ = slight; 3+ = moderate; 4+ = marked (intense).
 2: Frequency (% of cells or tissue elements): very rare (<1% of cells of a particular cell type); rare (1-5% of cells of a particular cell type); rare-occas = rare to occasional (>5-25% of cells of a particular cell type); occas = occasional (>25-50% of cells of a particular cell type); occas-freq = occasional to frequent (>50-75% of cells of a particular cell type);freq = frequent (>75-100% of cells of a particular cell type)
 3: H&E (hematoxylin & eosin): None = vacuoles not present.

Males and Females Recovery Sacrifice: Spleen

Immunohistochemical Scores: Intensity ¹ (Frequency) ²								
Spleen								
Vacuoles								
H&E ³								
Dose (mg/kg)	Animal No	(b) (4)	(b)	Uricase	PEG	Monocyte/Macrophages		
						Uricase	PEG	MAC387
Males								
0	H42027	None	None	Neg	Neg	Neg	Neg	3-4+ (freq)
0.4	H42033	None	None	Neg	Neg	1+ (very rare)	Neg	3-4+ (freq)
	H42034	None	None	Neg	Neg	1-2+ (very rare)	Neg	3-4+ (freq)
	H42035	Slight	Slight/Mild	Neg	Neg	1-2+ (rare)	1-2+ (rare)	3-4+ (freq)
1.5	H42039	Slight	Minimal	Neg	Neg	1+ (very rare)	1+ (very rare)	3-4+ (freq)
	H42040	Moderate	Slight/Mild	Neg	Neg	Neg	Neg	3-4+ (freq)

Immunohistochemical Scores: Intensity ¹ (Frequency) ²								
Spleen								
Vacuoles								
Dose (mg/kg)	Animal No	H&E ³		Uricase	PEG	Monocyte/Macrophages		
		(b) (4)	(b)			Uricase	PEG	MAC387
10.0	H42045	Moderate	Slight/Mild	Neg	Neg	1-2+ (rare)	1+ (very rare)	3-4+ (freq)
	H42046	Moderately Severe	Moderate	Neg	Neg	Neg	Neg	3-4+ (freq)
	H42047	Moderate	Moderate	1-2+ (very rare)	Neg	1-2+ (very rare)	1-2+ (very rare)	3-4+ (freq)
Females								
0	H42052	None	None	Neg	Neg	Neg	Neg	3-4+ (freq)
	H42053	None	None	Neg	Neg	Neg	Neg	3-4+ (freq)
0.4	H42057	None	None	Neg	Neg	Neg	Neg	3-4+ (freq)
	H42059	None	None	Neg	Neg	Neg	Neg	3-4+ (freq)
1.5	H42063	Moderate	Slight/Mild	Neg	Neg	Neg	Neg	3-4+ (freq)
	H42064	Slight	Slight/Mild	Neg	Neg	Neg	Neg	3-4+ (freq)
	H42065	Moderate	Slight/Mild	Neg	Neg	Neg	Neg	3-4+ (occas-freq)
10.0	H42069	Moderate	Moderate	Neg	Neg	Neg	Neg	3-4+ (freq)
	H42070	Slight	Moderate	Neg	Neg	Neg	Neg	3-4+ (freq)
	H42071	Moderate	Moderately Severe	Neg	Neg	Neg	Neg	3-4+ (freq)

1: Intensity: Neg = Negative; ± = equivocal; 1+ = minimal; 2+ = slight; 3+ = moderate; 4+ = marked (intense).
 2: Frequency (% of cells or tissue elements): very rare (<1% of cells of a particular cell type); rare (1-5% of cells of a particular cell type); rare-occas = rare to occasional (>5-25% of cells of a particular cell type); occas = occasional (> 25-50% of cells of a particular cell type); occas-freq = occasional to frequent (>50-75% of cells of a particular cell type);freq = frequent (>75-100% of cells of a particular cell type)
 3: H&E (hematoxylin & eosin): None = vacuoles not present.

Duodenum. The macrophage specific stain MAC387 stained macrophages in lamina propria of the duodenum. At the terminal sacrifice, vacuoles (5 to 25%) in two males administered the 10 mg/kg dose stained for both uricase and PEG with a slight to moderate intensity. Consistent with the results from the H&E staining, no vacuoles were present in the lamina propria of the duodenum of females in all treatment groups; however monocytes/macrophages stained for both uricase and PEG in two of the three females. Monocytes/macrophages stained for uricase and PEG in all three male dogs in the HD group. The monocytes/macrophages stained with an intensity ranging from minimal to moderate. At the end of recovery, vacuoles in the lamina propria of the duodenum of two males in the HD group stained positive for both uricase and PEG; one dog stained for uricase only. No vacuoles in the lamina propria of the duodenum of females stained positive for uricase or PEG after the recovery period.

Males and Females Terminal Sacrifice: Duodenum

Immunohistochemical Scores: Intensity ¹ (Frequency) ²								
Lamina Propria of Duodenum								
Vacuoles								
Dose (mg/kg)	Animal No	H&E ³		Uricase	PEG	Monocyte/Macrophages		MAC387
		(b) (4)	(b)			Uricase	PEG	
Males								
0	H42024	None	None	Neg	Neg	Neg	Neg	3-4+ (occas)
0.4	H42031	None	None	Neg	Neg	Neg	Neg	3-4+ (occas)
1.5	H42036	None	None	NE	NE	NE	NE	NE
	H42037	None	None	Neg	Neg	Neg	Neg	3-4+ (very rare)
10.0	H42042	Minimal	None	2-3+ (rare-occas)	2-3+ (rare-occas)	1-2+ (rare)	1-2+ (rare)	3-4+ (rare)
	H42043	None	None	Neg	Neg	1-2+ (rare)	1-2+ (rare)	3-4+ (rare)
	H42044	Slight	None	2-3+ (rare-occas)	1-2+ (rare-occas)	2-3+ (occas-freq)	1-2+ (rare)	3-4+ (occas)
Females								
0.4	H42055	None	None	NE	NE	NE	NE	NE
1.5	H42060	None	None	Neg	Neg	Neg	Neg	Neg
	H42061	None	None	Neg	Neg	1+ (rare)	Neg	3-4+ (occas)
	H42062	None	None	NE	NE	NE	NE	NE
10.0	H42066	None	None	NE	NR	NE	NE	NE
	H42067	None	None	Neg	Neg	2-3+ (occas)	1-2+ (occas)	3-4+ (occas-freq)
	H42068	None	None	Neg	Neg	1-2+ (rare-occas)	1-2+ (rare)	3-4+ (rare)

1: Intensity: Neg = Negative; ± = equivocal; 1+ = minimal; 2+ = slight; 3+ = moderate; 4+ = marked (intense).
 2: Frequency (% of cells or tissue elements): very rare (<1% of cells of a particular cell type); rare (1-5% of cells of a particular cell type); rare-occas = rare to occasional (>5-25% of cells of a particular cell type); occas = occasional (> 25-50% of cells of a particular cell type); occas-freq = occasional to frequent (>50-75% of cells of a particular cell type); freq = frequent (>75-100% of cells of a particular cell type)
 3: H&E (hematoxylin & eosin): None = vacuoles not present.

Males and Females Recovery Sacrifice: Duodenum

Immunohistochemical Scores: Intensity ¹ (Frequency) ²								
Lamina Propria of Duodenum								
Vacuoles								
Dose (mg/kg)	Animal No	H&E ³		Uricase	PEG	Monocyte/Macrophages		MAC387
		(b) (4)	(b)			Uricase	PEG	
Males								
0	H42027	None	None	Neg	Neg	Neg	Neg	3-4+ (occas)
0.4	H42033	None	None	Neg	Neg	Neg	Neg	3-4+ (rare)
	H42034	None	None	Neg	Neg	1+ (rare)	Neg	3-4+ (occas)
	H42035	None	None	Neg	Neg	Neg	Neg	3-4+ (very rare)
1.5	H42039	None	None	Neg	Neg	Neg	Neg	3-4+ (rare)
	H42040	None	None	Neg	Neg	Neg	Neg	3-4+ (very rare)
10.0	H42045	Minimal	None	1+ (rare)	Neg	1-2+ (rare-occas)	Neg	3-4+ (occas-freq)
	H42046	Moderate	None	±-1+ (very rare)	±-1+ (very rare)	1+ (rare)	1+ (very rare)	3-4+ (occas)
	H42047	Slight	None	±-1+	Neg	1+	Neg	3-4+

Immunohistochemical Scores: Intensity ¹ (Frequency) ²								
Lamina Propria of Duodenum								
Vacuoles								
Dose (mg/kg)	Animal No	H&E ³		Uricase	PEG	Monocyte/Macrophages		
		(b) (4)	(b)			Uricase	PEG	MAC387
				(very rare)		(rare)		(occas)
Females								
0	H42052	None	None	Neg	Neg	Neg	Neg	3-4+ (occas-freq)
	H42053	None	None	Neg	Neg	Neg	Neg	3-4+ (freq)
0.4	H42057	None	None	Neg	Neg	Neg	Neg	3-4+ (rare)
	H42059	None	None	Neg	Neg	Neg	Neg	3-4+ (rare)
1.5	H42063	None	None	Neg	Neg	Neg	Neg	3-4+ (occas)
	H42064	None	None	Neg	Neg	Neg	Neg	3-4+ (occas-freq)
	H42065	None	None	Neg	Neg	Neg	Neg	3-4+ (occas-freq)
10.0	H42069	Slight	None	Neg	Neg	Neg	Neg	3-4+ (rare)
	H42070	Slight	Minimal	Neg	Neg	Neg	Neg	3-4+ (very rare)
	H42071	Minimal	None	Neg	Neg	Neg	Neg	3-4+ (occas-freq)

1: Intensity: Neg = Negative; ± = equivocal; 1+ = minimal; 2+ = slight; 3+ = moderate; 4+ = marked (intense).
 2: Frequency (% of cells or tissue elements): very rare (<1% of cells of a particular cell type); rare (1-5% of cells of a particular cell type); rare-occas = rare to occasional (>5-25% of cells of a particular cell type); occas = occasional (> 25-50% of cells of a particular cell type); occas-freq = occasional to frequent (>50-75% of cells of a particular cell type);freq = frequent (>75-100% of cells of a particular cell type)
 3: H&E (hematoxylin & eosin): None = vacuoles not present.

Jejunum. Jejunum from males and females in the low- and mid-dose groups did not stain for uricase or PEG. The two males (H42042 and H42044), in the high-dose group, identified as having vacuoles in the lamina propria of the jejunum stained for uricase and PEG with slight to moderate intensity. Also, the two females (H42067 and H42068) identified as having vacuoles also stained for uricase and PEG but with a lesser intensity than the males; minimal intensity. Monocytes/macrophages stained for uricase and PEG in all three male dogs and two females in the HD group; suggesting that the observed vacuoles may be in the cytoplasm of monocytes/macrophages.

After the recovery period, vacuoles were noted in the lamina propria of the jejunum of one male (H42045) from the 10 mg/kg dose group; these <1% of the vacuoles stained minimally for uricase; based on the positive staining of monocyte/macrophages for uricase, the results indicate that the vacuoles were in these cells. No vacuole stained for PEG only. Equivocal results were observed in two female dogs (H42067 and H42068) in the HD groups also had vacuoles staining for both uricase and PEG; <1% of the vacuole stained minimally for uricase and PEG.

Males and Females Terminal Sacrifice: Jejunum

Immunohistochemical Scores: Intensity ¹ (Frequency) ²								
Lamina Propria of Jejunum								
Vacuoles								
Dose (mg/kg)	Animal No	H&E ³		Uricase	PEG	Monocyte/Macrophages		
		(b) (4)	(b)			Uricase	PEG	MAC387
Males								
0	H42024	None	None	Neg	Neg	Neg	Neg	3-4+ (occas)
0.4	H42031	None	None	Neg	Neg	Neg	Neg	3-4+ (rare)
1.5	H42036	None	None	Neg	Neg	1-2+ (very rare)	Neg	3-4+ (very rare)
	H42037	None	None	Neg	Neg	1-2+ (very rare)	1-2+ (very rare)	3-4+ (very rare)
10.0	H42042	Minimal	None	2-3+ (rare-occas)	2+ (rare)	1-2+ (rare)	1-2+ (rare)	3-4+ (rare)
	H42043	None	None	Neg	Neg	1-2+ (rare)	1-2+ (rare)	3-4+ (very rare)
	H42044	Slight	None	2-3+ (rare-occas)	1-2+ (rare-occas)	2-3+ (occas-freq)	1-2+ (occas-freq)	3-4+ (occas)
Females								
0.4	H42055	None	None	Neg	Neg	Neg	Neg	3-4+ (rare)
1.5	H42060	None	None	Neg	Neg	Neg	Neg	3-4+ (occas)
	H42061	None	None	Neg	Neg	Neg	1+ (rare)	3-4+ (rare-occas)
	H42062	None	None	Neg	Neg	1+ (very rare)	1+ (very rare)	3-4+ (occas-freq)
10.0	H42066	None	None	NE	NE	NE	NE	NE
	H42067	Moderate	None	±-1+ (very rare)	±-1+ (rare-occas)	1-2+ (very rare)	1-2+ (rare)	1-2+ (very rare)
	H42068	Slight	None	±-1+ (rare-occas)	±-1+ (rare-occas)	1-2+ (very rare)	1-2+ (rare)	3-4+ (rare)

1: Intensity: Neg = Negative; ± = equivocal; 1+ = minimal; 2+ = slight; 3+ = moderate; 4+ = marked (intense).
2: Frequency (% of cells or tissue elements): very rare (<1% of cells of a particular cell type); rare (1-5% of cells of a particular cell type); rare-occas = rare to occasional (>5-25% of cells of a particular cell type); occas = occasional (> 25-50% of cells of a particular cell type); occas-freq = occasional to frequent (>50-75% of cells of a particular cell type); freq = frequent (>75-100% of cells of a particular cell type)
3: H&E (hematoxylin & eosin): None = vacuoles not present.

Adrenal. No vacuoles stained for uricase or for PEG in the control, 0.4 and 1.5 mg/kg dose groups of either males or females. Immunohistochemical analysis qualitatively identified vacuoles in one male (H42042) in the HD group; less than 1% of the vacuoles stained minimally for both uricase and PEG. On the other hand, cortical cells in the zona reticularis and/or zona fasciculate stained for both uricase and PEG in all three male dogs. Also, three females at the 10 mg/kg dose level had less than 1% of vacuoles staining with a slight intensity for uricase only. Cortical cells in the zona reticularis and/or zona fasciculate stained for uricase only in all three female dogs. At the end of the recovery period, one female in the high-dose group had a rare vacuole scoring (1-5% of the cells) that stained with minimal to slightly intensity for uricase and PEG. None of the vacuoles stained for uricase or PEG at any dose in the males. All three female dogs in the HD groups stained for uricase while two females stained for PEG.

Males and Females Terminal Sacrifice: Adrenal

Immunohistochemical Scores: Intensity ¹ (Frequency) ²								
Adrenal								
		Vacuoles (Zona Reticularis and/or Zona Fasciculata)				Cortical Cells (Zona Reticularis and/or Zona Fasciculata)		
		H&E ³		Uricase	PEG	Uricase	PEG	MAC387
Dose (mg/kg)	Animal No	(b) (4)	(b) (4)					
Males								
0	H42024	None	None	Neg	Neg	Neg	Neg	Neg
0.4	H42031	None	None	Neg	Neg	Neg	Neg	Neg
1.5	H42036	None	None	Neg	Neg	Neg	Neg	Neg
	H42037	None	Slight/Mild	Neg	Neg	Neg	Neg	Neg
10.0	H42042	Slight	Minimal	1+ (very rare)	1+ (very rare)	2-3+ (rare)	2-3+ (rare)	Neg
	H42043	None	Minimal	Neg	Neg	2-3+ (rare)	2-3+ (rare)	Neg
	H42044	None	Minimal	Neg	Neg	2-3+ (rare)	2-3+ (rare)	Neg
Females								
0.4	H42055	None	None	Neg	Neg	Neg	Neg	Neg
1.5	H42060	Slight	Minimal	Neg	Neg	Neg	Neg	Neg
	H42061	None	Minimal	Neg	Neg	Neg	Neg	Neg
	H42062	None	Minimal	Neg	Neg	Neg	Neg	Neg
10.0	H42066	Slight	Slight/Mild	2+ (very rare)	Neg	2+ (very rare)	Neg	Neg
	H42067	Slight	Minimal	2+ (very rare)	Neg	2+ (very rare)	Neg	Neg
	H42068	Moderate	Slight/Mild	2+ (very rare)	Neg	2+ (very rare)	Neg	Neg

1: Intensity: Neg = Negative; ± = equivocal; 1+ = minimal; 2+ = slight; 3+ = moderate; 4+ = marked (intense).
2: Frequency (% of cells or tissue elements): very rare (<1% of cells of a particular cell type); rare (1-5% of cells of a particular cell type); rare-occas = rare to occasional (>5-25% of cells of a particular cell type); occas = occasional (> 25-50% of cells of a particular cell type); occas-freq = occasional to frequent (>50-75% of cells of a particular cell type);freq = frequent (>75-100% of cells of a particular cell type)
3: H&E (hematoxylin & eosin): None = vacuoles not present.

Heart. Consistent with the absence of vacuoles observed with the H&E stained sections in the 0.4 mg/kg dose group for both genders, the stain for uricase and PEG did not stain cells in the intimal lining of the aortic outflow tract of the heart for uricase or PEG. As depicted in the table below, no cytoplasmic vacuoles were visualized in H&E stained sections; however stain for uricase and PEG did stain cells in the intimal lining of the aortic outflow tract of the heart for uricase and/or PEG in both males and one female in the mid-dose group.

Cytoplasmic vacuoles identified with H&E staining in the HD male group stained for both uricase and PEG with slight to marked intensity. Imaging of the vacuoles identified in male No H42042M after H&E staining and stain for uricase and PEG are presented below (copied from the sponsor’s study report). Staining for uricase and PEG were scored with an intensity ranging from slight to marked in this male. For comparison purpose, imaging of the intima from a control dog is also shown below. Although vacuoles were not identified with H&E staining in females in the HD group, immunohistochemical analysis demonstrated vacuoles staining for both uricase and PEG with a staining intensity ranging from minimal to marked. The intimal lining of the aortic outflow of the heart stained for both uricase and PEG in all males and females in the HD group. At the end of recovery, no vacuoles or intimal tissues stained for PEG or uricase

in females. However, a small percentage of vacuoles (approx. 1-5% of the cells) stained for uricase, in two males in the HD group, with minimal intensity. The sponsor's photos of the vacuoles in the intimal lining of the aortic outflow from recovery male № H42046M are presented below.

Males and Females Terminal Sacrifice: Heart

Immunohistochemical Scores: Intensity ¹ (Frequency) ²								
Heart (Aortic Outflow Tract)								
Dose (mg/kg)	Animal №	Vacuoles				Intima, Subendothelium, Interstitium/Media		
		H&E ³	(b)	Uricase	PEG	Uricase	PEG	MAC387
Males								
0	H42024	None	None	Neg	Neg	Neg	Neg	Neg
0.4	H42031	Minimal	None	Neg	Neg	Neg	Neg	Neg
1.5	H42036	None	None	Neg	Neg	2-3+ (rare)	2-4+ (rare)	Neg
	H42037	None	None	Neg	Neg	2-3+ (rare)	Neg	Neg
10.0	H42042	Minimal	Minimal	2-4+ (occas)	2-4+ (occas)	2-4+ (rare-occas)	2-4+ (rare-occas)	Neg
	H42043	Minimal	Minimal	1-3+ (occas-freq)	1-3+ (occas-freq)	2-4+ (occas)	2-4+ (occas)	Neg
	H42044	Minimal	Minimal	1-3+ (occas-freq)	1-3+ (occas-freq)	2-4+ (rare-occas)	2-4+ (rare-occas)	Neg
Females								
0.4	H42055	None	None	Neg	Neg	Neg	Neg	Neg
1.5	H42060	None	None	Neg	Neg	Neg	Neg	Neg
	H42061	None	None	Neg	Neg	Neg	Neg	Neg
	H42062	None	None	Neg	Neg	1-3+ (very rare)	1-3+ (rare)	Neg
10.0	H42066	None	None	2-4+ (occas-freq)	2-4+ (occas-freq)	2-4+ (occas)	2-4+ (occas)	Neg
	H42067	None	None	1-3+ (occas-freq)	1-3+ (occas-freq)	1-3+ (occas)	1-3+ (occas)	Neg
	H42068	None	None	1-3+ (occas-freq)	1-3+ (occas-freq)	1-3+ (occas)	1-3+ (occas)	Neg

1: Intensity: Neg = Negative; ± = equivocal; 1+ = minimal; 2+ = slight; 3+ = moderate; 4+ = marked (intense).
2: Frequency (% of cells or tissue elements): very rare (<1% of cells of a particular cell type); rare (1-5% of cells of a particular cell type); rare-occas = rare to occasional (>5-25% of cells of a particular cell type); occas = occasional (> 25-50% of cells of a particular cell type); occas-freq = occasional to frequent (>50-75% of cells of a particular cell type); freq = frequent (>75-100% of cells of a particular cell type)
3: H&E (hematoxylin & eosin): None = vacuoles not present.

Terminal Sacrifice: Male № H42042M (HD group)

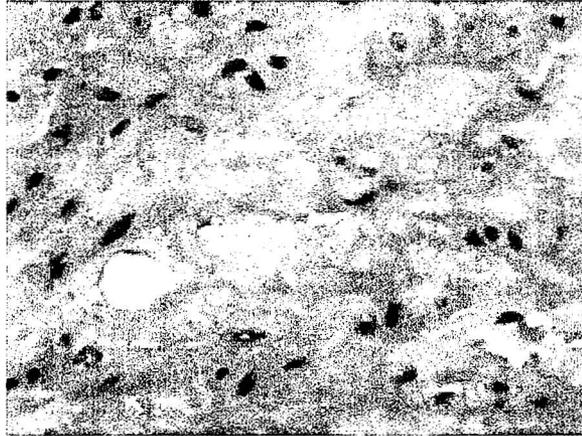


Figure 64. Vacuolated intima in aortic outflow tract in Group 4M H42042M (terminal sacrifice) heart, HE, 60X.



Figure 65. Detection reagent anti-uricase staining of intima and edges of vacuoles in Group 4M H42042M (terminal sacrifice) aortic outflow tract (heart), 60X.



Figure 66. Detection reagent anti-PEG staining of intima and edges of vacuoles in Group 4M H42042M (terminal sacrifice) aortic outflow tract (heart), 60X.

Terminal Sacrifice: Male №H42024M (Control)

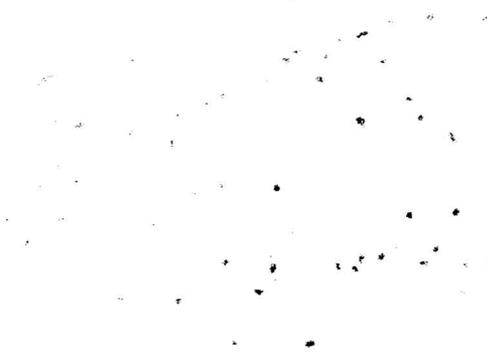


Figure 77. No staining of Group 1M H42024M (terminal sacrifice) aortic outflow tract (heart) by detection reagent anti-uricase, 60X.



Figure 78. No staining of Group 1M H42024M (terminal sacrifice) aortic outflow tract (heart) by detection reagent anti-PEG, minor diffuse background staining, 60X.

Males and Females Recovery Sacrifice: Heart

Immunohistochemical Scores: Intensity ¹ (Frequency) ²								
Heart (Aortic Outflow Tract)								
		Vacuoles						
		H&E ³		Intima, Subendothelium, Interstitium/Media				
Dose (mg/kg)	Animal No	(b) (4)	(b) (4)	Uricase	PEG	Uricase	PEG	MAC387
Males								
0	H42027	None	None	Neg	Neg	Neg	Neg	Neg
0.4	H42033	None	None	Neg	Neg	Neg	Neg	Neg
	H42034	None	None	Neg	Neg	Neg	Neg	Neg
	H42035	None	None	Neg	Neg	Neg	Neg	Neg
1.5	H42039	None	None	Neg	Neg	Neg	Neg	Neg
	H42040	None	None	Neg	Neg	Neg	Neg	Neg
10.0	H42045	Minimal	Minimal	1+ (rare)	Neg	1-2+ (rare)	Neg	Neg
	H42046	Slight	Minimal	±1+ (rare)	Neg	±1+ (rare-occas)	Neg	Neg
	H42047	Minimal	Minimal	Neg	Neg	Neg	Neg	Neg
Females								
0	H42052	None	None	Neg	Neg	Neg	Neg	Neg
	H42053	None	None	Neg	Neg	Neg	Neg	Neg
0.4	H42057	None	None	Neg	Neg	Neg	Neg	Neg
	H42059	None	None	Neg	Neg	Neg	Neg	Neg
1.5	H42063	None	None	Neg	Neg	Neg	Neg	Neg
	H42064	None	None	Neg	Neg	Neg	Neg	Neg
	H42065	None	None	Neg	Neg	Neg	Neg	Neg
10.0	H42069	None	None	Neg	Neg	Neg	Neg	Neg
	H42070	Slight	Minimal	Neg	Neg	Neg	Neg	Neg
	H42071	None	None	Neg	Neg	Neg	Neg	Neg

1: Intensity: Neg = Negative; ± = equivocal; 1+ = minimal; 2+ = slight; 3+ = moderate; 4+ = marked (intense).
2: Frequency (% of cells or tissue elements): very rare (<1% of cells of a particular cell type); rare (1-5% of cells of a particular cell type); rare-occas = rare to occasional (>5-25% of cells of a particular cell type); occas = occasional (> 25-50% of cells of a particular cell type); occas-freq = occasional to frequent (>50-75% of cells of a particular cell type); freq = frequent (>75-100% of cells of a particular cell type)
3: H&E (hematoxylin & eosin): None = vacuoles not present.

Recovery Sacrifice: Male № H42046M (HD Group)

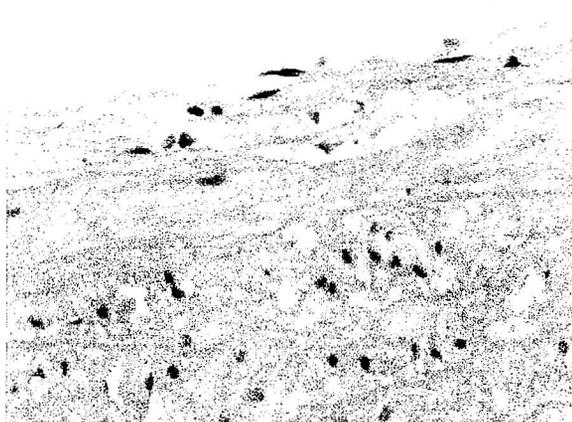


Figure 70. Vacuolated intima in aortic outflow tract in Group 4M H42046M (recovery sacrifice) heart. HE. 60X.



Figure 71. Detection reagent anti-uricase staining of intima and edges of vacuoles in Group 4M H42046M (recovery sacrifice) aortic outflow tract (heart), 60X.

Figure 72. No staining of vacuolated intima in Group 4M H42046M (recovery sacrifice) aortic outflow tract (heart) by detection reagent anti-PEG, minor diffuse background staining, 60X.

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Using the stain for macrophages, the results from the immunohistochemical analysis qualitatively identified the presence of macrophages in the liver, spleen and lamina propria of the duodenum and jejunum of both dosed and control dogs, and that the cytoplasmic vacuoles noted in the dosed dogs were in macrophages. However, sections of the adrenal cortex and heart did not stain positive for macrophages. The absence of positive staining for macrophages in the adrenal cortex and the vascular wall of the aortic outflow tract of the heart suggested that the vacuoles noted in these structures were present in the cytoplasm of cells. The independent pathologist, (b) (4), suggested that the vacuoles were within cytoplasm of cells, such as endothelial cells in the intima of the aortic outflow, having phagocytic function. The pathologist did not provide evidence to

support this statement. The primary function of endothelial cells in the intima of the aorta is that of a protective barrier. Most importantly, immunohistochemical staining demonstrated that most cells staining for uricase and PEG in the spleen, liver, duodenum and jejunum were macrophages. Thus, suggesting that the vacuoles identified in these tissues represented macrophages sequestering and digesting puricase. Based on this finding, the reviewer does not consider the presence of vacuoles in these tissues to be of toxicological relevance. However, the toxicological relevance of the vacuoles in the heart and adrenal gland is not known.

Immunoglobulins and Complement Analysis: No treatment-related effects were observed in any immunology parameter (IgA, IgE, IgG, IgM, C3, C4 and CH50) examined.

Anti-Uricase, anti-Puricase[®] and anti-PEG IgG antibodies: As depicted in the tables (reproduced from sponsor's submission) below, anti-uricase antibodies developed in approximately half of the animals in all puricase-treatment groups by week 27. The anti-uricase response was not dose-related or sex-related. Measurable amount of the anti-uricase antibody was not detected in 5/6 of the recovery animals after the 12-week recovery period (week 52). Anti-puricase antibodies developed in a few of the animals in each of the treatment groups. Seven out of twelve high-responder dogs showed cross-reactivity to uricase and puricase. The anti-uricase and anti-puricase antibodies lacked the ability to neutralize the enzymatic activity of puricase. No anti-m-PEG response was noted except in one dog (out of 12) in the high-dose group on week 27.

Summary of anti-uricase antibody response in plasma from male and female dogs (combined) receiving Puricase once weekly for 39-weeks.

Uricase Antibody Response № Positive Dogs/Total					
Dose Group (mg/kg)	Treatment Week				
	1	14	27	40	52
0	2/12	0/12	2/12	2/12	1/6
0.4	1/12	2/12	6/12	6/12	1/6
1.5	1/12	4/12	7/12	5/12	1/6
10.0	1/12	4/12	7/12	4/12	1/6

Summary of anti-mPEG antibody response in plasma from male and female dogs (combined) receiving Puricase once weekly for 39-weeks.

m-PEG Antibody Response № Positive Dogs/Total					
Dose Group (mg/kg)	Treatment Week				
	1	14	27	40	52
0	0/12	1/12	2/12	1/12	0/6
0.4	0/12	0/12	0/12	0/12	0/6
1.5	0/12	0/12	0/12	0/12	0/6
10.0	0/12	0/12	1/12	0/12	0/6

**Summary of anti-Puricase IgG antibody response in plasma from male
And female dogs (combined) receiving Puricase once weekly for 39-weeks.**

Dose Group (mg/kg)	Puricase Antibody № Positive Dogs/Total				
	Treatment Week				
	1	14	27	40	52
0	0/12	1/12	2/12	1/12	0/6
0.4	0/12	4/12	3/12	4/12	1/6
1.5	0/12	2/12	2/12	0/12	0/6
10.0	0/12	3/12	2/12	1/12	0/6

2.6.6.4 Genetic toxicology

Genotoxicity studies were not submitted with this application. Genotoxicity studies are not required for large biologic proteins that do not traverse the cell membrane. Hence, biologics are not expected to interact directly with DNA or other chromosomal material.

2.6.6.5 Carcinogenicity

Carcinogenicity studies were not submitted with this application. As per ICH S6, carcinogenicity assessment of biologic products is not always appropriate.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

There were no fertility and early embryonic development studies (Segment I) submitted with this application. These studies were not required to support the BLA submission due to previous agreement with the Agency in 2005. However, the current Agency policy is that the standard battery of reproduction and developmental toxicology studies should be conducted, when feasible. Therefore, the remaining studies in the standard battery should be completed as a post marketing requirement.

Embryofetal development

Study title: An Intravenous Study of the Effects of Puricase on Embryofetal Development in Rats.

Key study findings: Female rats treated with puricase from gestation day 6 through gestation day 15 in a definitive segment II study with the following findings:

1. No treatment-related mortalities or early abortions noted in the study.
2. No treatment-related clinical signs were noted in the study.
3. No treatment-related macroscopic changes were identified in the dams.

4. There were no significant malformations (external, soft tissue or skeletal) between treatment groups, indicating that puricase was not teratogenic under the conditions tested.
5. Overall, the results suggest that the NOAEL for maternal toxicity was 10 mg/kg/day based on the spleen effects (higher weight and microscopic finding of vacuolation) of 40 mg/kg. The 10 mg/kg/day dose was associated with a mean C_{max} value of 406.29 $\mu\text{g/mL}$ (GD 16) and a mean AUC_{0-24} value of 1,818.4 $\mu\text{g}\cdot\text{day/mL}$ (GD16).
6. No developmental toxicity was noted under the condition tested. Therefore, the NOAEL for developmental toxicity was 40.0 mg/kg.

Study no.:

(b) (4)
-441008

Volume #, and page #:

Electronic document

Conducting laboratory and location:

(b) (4)

Date of study initiation:

November 19, 2004

GLP compliance:

Yes

QA reports:

yes (x) no ()

Drug, lot #, and % purity:

Puricase[®], Lot № 36860011, purity was not included on the Certificate of Analysis; sponsor stated that it was assumed that the purity was 100%. *

* *The content was 11.4 mg/mL compared to a proposed limit range of 10.8-13.2 mg/mL*

Methods

Doses: 0, 5, 10 and 40 mg/kg

Dose selection: Doses were based on the results from the embryofetal range-finding study (Study Report № (b) (4)-441007) in which pregnant rats (n = 8/group) were intravenously administered puricase at dose levels of 0 (vehicle, phosphate buffered saline), 5, 10, 20, or 40 mg/kg/day on gestation days 6, 8, 10, 12, 14 and 16. There were no treatment-related deaths or overt clinical signs. Maternal mean body weight, body weight gains, net body weight gains and gravid uterine weights and food consumption throughout the dosing periods (GD 6-20) for all treatment groups were comparable to the control group values. Intrauterine growth and survival were unaffected at all dose levels. No treatment-related fetal malformations or developmental variations were noted. The spleen was identified as the target organ of toxicity. Dose-dependence increase in mean absolute spleen weights was observed; mean group spleen weight was 0.64, 0.69, 0.75, 0.78 and 1.0 grams for the 0, 10, 20 and 40 mg/kg/day groups, respectively. There were no treatment-related effects at the 5 mg/kg/day dose level. Relative to the control group, the percent differences were 7.8%, 17.2%, 21.9% and 56.3% for the 5, 10, 20 and 40 mg/kg/day groups, respectively. The increased spleen weight was statistically significantly in the 20 and 40 mg/kg/day groups ($p < 0.05$ and $p < 0.01$,

respectively). Treatment-related microscopic changes in the spleen consisted of vacuolation and extramedullary hematopoiesis. Vacuolation was observed in all dams in the high-dose group. Incidence of extramedullary hematopoiesis increased in a dose-dependent manner, correlating with the increased spleen weights. The severity of increased extramedullary hematopoiesis increased with dose in the 20 and 40 mg/kg/day groups. Toxicokinetic data indicated that exposure for active plasma Puricase (C_{max} and AUC) increased in a dose-proportional manner. AUC values were 9.4, 778.7, 1,614.9, 3,733.4 and 6,044.9 $\mu\text{g}\cdot\text{day}/\text{mL}$ for dosage levels of 0, 5, 10, 20 and 40 mg/kg, respectively. With the exception of one fetus (№ 62079-03) and three fetuses (№ 62117-05, № 62147-05 and № 62133-05) in the 10 and 40 mg/kg/day groups, respectively, plasma puricase levels were below the LOQ values. The sponsor stated that the puricase levels detected in these fetuses were most likely due to contamination from maternal blood. The reviewer concurs with the sponsor. Due to the large size of puricase, it is unlikely that the puricase measured was due to placental transfer. Species/strain: Rats/Crl:CD[®](SD)

Number/sex/group: See table below on group assignments

Group	Test Article	Dosage Level (mg/kg/day)	Dosage Concentration (mg/mL)	Dosage Volume (mL/kg/day)	Number of Females	
					Main Study	TK/Clinical Path. Satellite
1	Vehicle	0.0	0	5.0	25	6
2	Puricase	5.0	1	5.0	25	6
3	Puricase	10.0	2	5.0	25	6
4	Puricase	40.0	8	5.0	25	6

Route, formulation, volume, and infusion rate: Intravenous via the lateral caudal vein, dissolved in phosphate buffered saline, 5.0 mL/kg, administered slowly over a period of 30- to 60-second.

Satellite groups used for toxicokinetics: Yes, 6 animals/group.

Study design: Study drug was administered on gestation days 6, 8, 10, 12 and 16 for the embryofetal development phase. For the toxicokinetic/clinical pathology phase of the study, study drug was administered on gestation days 6, 8, 10, 12, 14, 16 and 18.

Parameters and endpoints evaluated:

Clinical signs: Animals were examined twice daily (once in the morning and once in the afternoon) for mortality and moribundity. On the first day of dosing, rats in the embryofetal development phase of the study were observed for signs of toxicity approximately 30-minutes after dosing, at approximately 1-hour intervals for the first 4 hours post-dosing and at the end of the work day. For the remainder of the study, the rats were observed for clinical signs 1-hour after dosing.

Body weights: Body weights were recorded on GD 0 (day in which there was evidence that mating was identified), and daily on GD 6 through GD 20 (main study animals for the main study animals). Body weights of the animals in the toxicokinetic/clinical pathology phase of the study were recorded on GD 0 and daily on GD 6 through 19 and on GD 19.

Food Consumption: Food consumption was recorded on GD 0 and daily on GD 6 through GD 20 for the main study animals. Food consumption was recorded on GD 0 and daily on GD 6 through GD 19 for animals in the toxicokinetic/clinical pathology phase of the study.

Clinical Chemistry. On gestation days 5 (the day prior to dosing) and 17 (last day of dosing), blood was collected from all maternal animals (non-fasted state). The following parameters were measured:

Serum Chemistry Parameters	
Calcium (Ca)	Potassium
Chloride (Cl)	Sodium
Creatinine	Sodium/Potassium ratio (Na/K ratio)
Glucose (GLU)	Urea Nitrogen
Inorganic Phosphorus (PHOS)	Uric Acid
Iron	Urea Nitrogen

Necropsy: Main Study - All surviving rats were euthanized by CO₂ on day 20 of gestation. Their major organs were examined. Any unusual gross findings were recorded. The spleen was removed, weighed and preserved in 10% neutral-buffered formalin and examined microscopically. Other maternal tissues were collected and preserved in 10% neutral buffered formalin for possible future histological evaluation as indicated by gross findings. The ovaries and uterus were removed and weighed. Uteri with no macroscopic evidence of implantation (i.e., that appeared nonpregnant) were placed in 10% ammonium sulfide solution to visualize early implantation loss. The number of corpora lutea on each ovary was counted and recorded.

TK/Clinical Pathology Satellite Study – On GD 19, blood was collected, the rats were weighed, observed and euthanized by CO₂. Pregnancy status was assessed.

Examination of Embryo and Fetuses:

Cesarean Section: The number of corpora lutea, total number of implantation sites, distribution of implantation sites, early and late resorptions, and number and location of all fetuses, dead and live fetuses was recorded. Each placenta was examined macroscopically.

Fetal Observations: Each fetus was weighed, sexed, and examined for external grossly (externally and oral cavity).

Visceral Examination: Visceral exams were performed on viable fetuses from each dam. Visceral examination included heart and major blood vessels dissection, soft tissue examination of the head and evaluation of kidney for renal papillae development.

Skeletal Examination: Skeletal examination was performed on viable fetus from each dam. Skeletal malformations and variations were assessed. The fetuses were placed in alcohol, and processed for skeletal examination. Following fixation in alcohol, each fetus was macerated in potassium hydroxide and stained in Alizarin Red S.

Blood Plasma: Fetal blood was collected via decapitation or from the umbilical vessels and pooled (per litter) on gestation day 19.

Toxicokinetic Analysis: To assess plasma level of Puricase, blood was collected prior to dosing and 1 hour post-dosing on gestation days 6 and 16. Blood samples were collected from the (3/time point) retro-orbital sinus of the maternal animals under isoflurane anesthesia.

Results

Embryofetal Development Phase (main study)

Mortality (dams): No treatment-related deaths or abortions occurred in the dams.

Clinical signs (dams): No treatment-related clinical signs were observed.

Body weight (dams): No treatment-related effects on body weight were noted. Mean body weights, and mean body weight gains of the dams in the puricase groups were comparable to that in the control group during gestation.

Food consumption (dams): Food consumption of the dams in the low dose (5.0 mg/kg/day) and high dose (40.0 mg/kg) groups were comparable to control food consumption during gestation. Increased food consumption was observed in the mid-dose (10.0 mg/kg/day) group. Dams in the 10.0 mg/kg/day group mean food consumption was statistically significantly higher than the control during gestation days 12-17 and 15-16. Relative to the control, at 10 mg/kg/day, the mean food consumption was statistically significantly increased by 5.6% and 6.8% during days 12-17 and 15-16, respectively. Because these significant changes were < 20% and not dose-related, these change were not considered treatment-related or toxicologically relevant. Statistically significant changes noted in food consumption are tabulated below.

Summary of significant changes in mean food consumption compared to control

Mean Food Consumption (g) ± SD (% change from control)				
Day Interval	Dose (mg/kg/day)			
	0	5	10	40
12-17	72	73	76 ± 4.7* (↑5.6%)	74
15-16	73	74	78 ± 5.5* (↑6.8%)	76

*: Significant different from control value, $p \leq 0.01$

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

Dams: Mean gravid uterine weight in the 5, 10 and 40 mg/kg puricase groups was comparable to that in the control group. Mean gravid uterine weights were 83.6 g, 79.4 g, 83.4 g and 78.2 g in the 0.0, 5, 10, and 40 mg/kg/day groups, respectively.

Maternal necropsy data. All animals in the puricase treatment groups survived to the scheduled sacrifice on gestation day 20. Macroscopic findings noted are summarized below. These gross findings are considered coincidental and not toxicologically relevant.

Summary of Macroscopic Findings

Macroscopic Finding	Incidence of Macroscopic Findings				
	No Examined→	Dose (mg/kg/day)			
		0	5.0	10.0	40.0
No Significant Changes Observed	25	25	25	25	
Nongravid -- Ammonium Sulfide Negative	21	22	23	21	
Placenta: Fused	3	0	2	1	
Amniotic Sac: Contents, Red Fluid	0	0	0	1	
Cervix: White Fluid Filled	0	1	0	0	
Uterus: White Fluid Filled	1	0	0	1	
Uterus: Contents, Thick Red	1	0	0	0	
Adrenal Glands Enlarged	0	0	0	1	
Kidney : Enlarged	0	1	0	0	
Kidneys: Firm	0	1	0	0	
Kidneys: Area(s), White	0	1	0	0	
Skin: Hair Loss	0	1	0	1	
Spleen: Enlarged	1	1	0	1	
	0	1	0	0	

Significant increase in absolute spleen weight was observed at the 40 mg/kg/day dose level. Compared to the control, the spleen weight was significantly (at $p \leq 0.01$) increased by 25%. As depicted in the table below, the mean spleen weight in the low- and mid-dose groups were comparable to the control group.

Spleen Mean Weight \pm SD			
Dose (mg/kg/day)			
0	5	10	40
0.72 \pm 0.107	0.72 \pm 0.194	0.72 \pm 0.90	0.90 \pm 0.133*
*: Significant different from control value, $p \leq 0.01$			

Maternal microscopic data. The spleen was identified as an organ of toxicity. Treatment-related changes were observed in the spleen of the dams in the embryo/fetal group. The table below summarizes the incidence and severity of the observed drug-related histopathological findings. Vacuolated macrophages were observed in all the dams in the high-dose group. This finding correlated with the significant increase (absolute) spleen weight in the high-dose group; relative to the control group a 25% increase in spleen weight was noted. An increase incidence of extramedullary hematopoiesis of the spleen was observed. The incidences of dams with extramedullary hematopoiesis were 0%, 20%, 32%, and 95% in the 0, 5, 10 and 40 mg/kg/day groups, respectively.

Incidence of Treatment-Related Microscopic Findings in the Spleen (25 females/group)				
Finding	Dose (mg/kg/day)			
	0	5	10	40

Extramedullary hematopoiesis	Total	0	5	8	23
	Minimal	NA	4	6	4
	Mild	NA	1	2	13
	Moderate	NA	0	0	6
Vacuolated macrophages	Total	0	0	0	25
	Minimal	NA	NA	NA	10
	Mild	NA	NA	NA	11
	Moderate	NA	NA	NA	4
NA: Not Applicable					

No historical control data was submitted for the microscopic observation made in the control groups of pregnant female rats. However based on (b) (4) historical control data available from non-pregnant, control rats (CrI:CD(SD), 10-13 weeks old) from 34 toxicology studies during the period of December, 1999 through May, 2005, the sponsor provided the following rationale for the extramedullary findings observed in the puricase-groups:

Extramedullary hematopoiesis observed in the low- and mid-dose groups. *“In the current study, the incidence of control group rats with extramedullary hematopoiesis was 0/25. For this reason alone, the incidences of rats with extramedullary hematopoiesis, i.e., 5/25 (20%), 8/25 (32%) and 23/25 (92%) in the 5, 10 and 40 mg/kg/day groups, respectively, were classified as being test article-related. However, the incidence (20%) of rats with extramedullary hematopoiesis in this study in the 5 mg/kg/day group was below the incidence (26.2%) of non-pregnant historical control rats at (b) (4). The incidence (32%) of rats with extramedullary hematopoiesis in this study in the 10 mg/kg/day group was only slightly above the incidence (26.2%) of the non-pregnant historical control rats at (b) (4). Given that the extramedullary hematopoiesis in the 5 and 10 mg/kg/day groups was primarily minimal in severity, the possibility exists that this is a normal background observation unrelated to the test article.”*

Extramedullary hematopoiesis observed in the high-dose groups. *“In the puricase 40 mg/kg/day group, the incidence of extramedullary hematopoiesis (92%) was higher than that observed in control rats. Unlike the minimal or mild severity of the extramedullary hematopoiesis observed in the 5 and 10 mg/kg/day groups, moderate extramedullary hematopoiesis was observed in the group that received 40 mg/kg/day. ... The toxicological significance, if any, of the test article-related extramedullary hematopoiesis in the 40 mg/kg/day group is unknown.”*

The reviewer does not concur with the sponsor conclusion that the extramedullary hematopoiesis observed in the 5 and 10 mg/kg/day dose levels are normal background observations. Under the study experimental conditions, extramedullary hematopoiesis was not observed in any of the concurrent control dams; and most importantly, the incidence of this finding was dose-related. Hence, this finding in these groups is

considered to be treatment-related. The reviewer does agree with the sponsor statement that the toxicological significance is unknown and warrant further evaluation.

Gestation Day 2 Laparohysterectomy Data. Data from cesarean section are presented in the table presented below. The number of dams pregnant was 22, 25, 23, and 24 in the 0.0, 1.0, 50.0, and 40.0 mg/kg/day dose groups, respectively. Intrauterine growth and survival were unaffected by puricase at dose levels of 5, 10 and 40 mg/kg/day. As depicted in the table below, there were no significance differences noted in the mean viable fetuses, % females, % males, dead fetuses, early resorptions, late resorptions, post-implantation losses, implantation sites, corpora lutea, pre-implantation losses or the number of gravid females. However, relative to the control, the mean male, mean female and mean combined male-female fetal body weight were statistically significantly lowered (5.4%, 5.7%, and 5.6%, respectively) in the 10 mg/kg/day dose group.

Summary of Cesarean Section Data

Parameter	Dose (mg/kg/day)			
	0.0	5.0	10.0	40.0
Total № of Dams	25	25	25	25
№ of Pregnant Dams	22	25	23	24
№ of Dams Not Pregnant	3	0	2	1
Pregnant/Total ♀ survived (%)	22/25 (55.0%)	25/25 (100%)	23/25 (92.0%)	24/25 (96%)
Total № Corpora Lutea (Mean ± S.D.)	374 (17.0 ± 2.31)	423 (16.9 ± 2.71)	386 (16.8 ± 2.02)	396 (16.5 ± 2.25)
Total № Implantation Site (Mean ± S.D.)	350 (15.9 ± 2.41)	398 (15.9 ± 1.93)	369 (16.0 ± 1.99)	368 (15.3 ± 2.44)
Pre-implantation Loss				
№ of ♀ Pre-implantation Loss (Mean ± S.D.)	24 (1.1 ± 1.19)	25 (1.0 ± 1.68)	17 (0.7 ± 1.51)	28 (1.29 ± 1.63)
% Pre-Implantation Loss	6.4%	5.1%	4.1%	6.9%
Post-implantation Loss				
№ of ♀ Post-implantation Loss (Mean ± S.D.)	14 (0.6 ± 0.79)	20 (0.8 ± 1.12)	18 (0.8 ± 0.9)	21 (0.9 ± 1.15)
% Post-Implantation Loss	4.0%	5.0%	4.9%	6.1%
№ of Live Fetuses (Mean ± S.D.)	336 (15.3 ± 2.43)	378 (15.1 ± 2.09)	351 (15.3 ± 2.09)	347 (14.5 ± 2.84)
% Viable Fetuses	96.1%	95.0%	95.1%	93.9%
Sex Ratio				
Total № ♂ (Mean ± S.D.)	169 (7.7 ± 2.97)	203 (8.1 ± 8.1)	187 (8.1 ± 2.24)	171 (7.1 ± 2.47)
Total № ♀ (Mean ± S.D.)	167 (7.6 ± 2.13)	174 (7.0 ± 2.41) ^A	164 (7.1 ± 1.96)	176 (7.3 ± 2.33)
% ♂: % ♀	49.3%:50.7%	54.2%:45.8% ^A	53.1%:46.9%	49.1%:50.9%
№ of Dead Fetuses (Mean ± S.D.)	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)
№ Early Resorptions (Mean ± S.D.)	14 (0.60 ± 0.79)	20 (0.8 ± 1.12)	18 (0.73 ± 1.49)	21 (0.9 ± 1.15)
№ Late Resorptions (Mean ± S.D.)	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)
Mean Body Wt. (g) of Live Fetuses (S.D.)				
Males	3.7 (0.24)	3.5 (0.37)*	3.7 (0.21)	3.6 (0.33)
Females	3.5 (0.24)	3.3 (0.36)*	3.5 (0.20)	3.4 (0.31)
Combined	3.6 (0.24)	3.4 (0.37)*	3.6 (0.20)	3.5 (0.31)

*: Significant different from control value, $p \leq 0.05$
A: Fetus No. 69508-09 had absent gonads, therefore, sex could not be determined; not included in calculations (arbitrarily assigned as a female in the raw data)

Offspring (malformations, variations, etc.):

Observed malformations and variations in fetuses of treated and control dams are summarized in the table below. There were no statistically significant increases in malformations or variations observed in litters of dams treated with puricase up to 40 mg/kg/day when compared with control litters upon external or visceral examination. However, there were a few isolated malformations that were considered to be spontaneous occurrences because a dose-dependent increase in incidence was not observed. Anal atresia was observed in one control fetus (№ 69581-08). Exencephaly and spina bifida was noted in one fetus in the 5 mg/kg/day group (№ 69508-09). This fetus also had gonads missing.

The only developmental variation observed in the study was hemorrhagic ring iris (unilateral) in one fetus (№ 69598-15) and renal papillae not fully developed in another fetus (№ 69609-11) in the high-dose group.

Skeletal malformation noted in the study was vertebral anomaly, fused cervical arches, costal cartilages and ribs) observed in one fetus (№ 69620-02) in the low-dose group. Skeletal developmental variations were noted in all treatment groups; consisting of ossification of cervical centrum № 1, 14 rudimentary ribs, unossified sternbra(e) № 5 and № 6 and reduced ossification of the 13th rib. Compared to control, there was a statistically significant increase (25.6%) occurrence of ossified 1st cervical centrum in the mid-dose group (87 fetuses from 20 litters). This skeletal developmental variation is not considered to be treatment-related because a similar increase was not observed at 40 mg/kg.

Summary of External, Visceral and Skeletal Examinations

Parameter	Dose (mg/kg/day)			
	0.0	5.0	10.0	40.0
№ of Litters Examined	22	25	23	24
№ Fetuses Examined	336	378	351	347
External Findings				
Anal atresia Total № Fetuses (% of litter)	1 (0.3%)	0 (0%)	0 (0%)	0 (0%)
Exencephaly with or without open eyelid Total № Fetuses (% of litter)	0 (0%)	1 (0.2%)	0 (0%)	0 (0%)
Spina bifida Total № Fetuses (% of litter)	0 (0%)	1 (0.2%)	0 (0%)	0 (0%)
Vertebral agenesis – anury Total № Fetuses (% of litter)	1 (0.3%)	0 (0%)	0 (0%)	0 (0%)
Total External Variations Percent per liter	0%	0%	0%	0%
Visceral Findings				
Gonads absent Total № Fetuses (% of litter)	0 (0%)	1 (0.2%)	0 (0%)	0 (0%)
Hemorrhagic ring around the iris Total № Fetuses	0	0	1	0
Renal papillae not fully developed	0	0	0	1
Total Soft Tissue Variations Percent per liter	0%	0%	0.3%	0.3%

Parameter	Dose (mg/kg/day)			
	0.0	5.0	10.0	40.0
№ of Litters Examined	22	25	23	24
№ Fetuses Examined	336	378	351	347
Skeletal Malformations and Developmental Variations				
Vertebral anomaly with or without associated rib anomaly Total № Fetuses (% of litter)	0 (0.0%)	1 (0.3%)	0 (0.0%)	0 (0.0%)
Cervical Centrum #1 Ossified Total № Fetuses (% of litter)	33 (10.5%)	76 (20.9%)	87 (25.6%)*	44 (11.9%)
14 th Rudimentary Rib (s) Total № Fetuses (% of litter)	9 (2.8%)	19 (4.8%)	20 (7.5%)	22 (5.8%)
Sternebra #5 and/or #6 Unossified Total № Fetuses (% of litter)	15 (4.6%)	28 (7.7%)	15 (4.5%)	19 (6.7%)
Reduced Ossification of the 13 th rib (s) Total № Fetuses (% of Litters)	7 (2.2%)	2 (0.5%)	2 (0.5%)	2 (0.5%)
Total Skeletal Variations Percent per liter	19.7%	34.8%	35.6%*	23.5%

*: Significant different from control value, $p \leq 0.05$

Toxicokinetics and Clinical Pathology Phase

Mortality (dams): Consistent with the results observed in the embryofetal development groups, no treatment-related deaths or abortions occurred in the dams. All females in survived to the scheduled euthanasia.

Clinical signs (dams): Consistent with the results observed in the embryofetal development groups, no treatment-related clinical signs were observed.

Body weight (dams): Consistent with the results observed in the embryofetal development groups, no treatment-related effects on body weight were noted. Mean body weights and mean body weight gains of the dams in the puricase groups were comparable to that in the control group during gestation.

Food consumption (dams): No treatment-related changes in food consumption were observed during gestation. Food consumption of the dams in the puricase groups was comparable to control food consumption during gestation.

Toxicokinetics: Toxicokinetic data for puricase activity and concentration is presented in the table below. The C_{max} and AUC_{0-24h} values indicate there was an increase in exposure with increasing dose. Plasma concentration of puricase increased in a dose-dependent manner. C_{max} was highest 1 hour after the last dose of puricase on gestation day (GD) 16. Mean plasma concentration \pm SEM was $189.12 \pm 25.71 \mu\text{g/mL}$, $406.29 \pm 82.98 \mu\text{g/mL}$ and $1,363.39 \pm 253.14 \mu\text{g/mL}$ following 5, 10 and 40 mg/kg/day of Puricase, respectively. On GD 6, the C_{max} did not increase in a dose-proportional manner (increased by 1.3-fold instead of 2-fold) from LD to MD. However, on GD 16, the C_{max} increased in a dose proportional manner from LD to MD. From MD to the HD (4-fold increase in dose), the C_{max} increased in a greater- (by 6.6-fold) and less- (by 3.4-fold) than proportional manner on GD 6 and GD 16, respectively. From LD to HD (8-fold

increase in dose), the C_{max} increased in a dose proportional manner and a less than (7.2-fold) dose-proportional manner on GD 6 and GD 16, respectively.

AUC_{0-24h} level of puricase increased with increased dosage in an approximate dose proportionate manner. Mean AUC_{0-24h} values following 0, 5, 10 and 40 mg/kg/day of puricase was < 9.3, 918.2, 1,818.4 and 7,091.7 $\mu\text{g}\cdot\text{day}/\text{mL}$, respectively. The sponsor reported that the circulating half-life of puricase was 2 days. This is in agreement with the half-life of 43.9 following 5 cycles of intravenous administration of puricase in rats (Report No 01-V359-2) determined from the decline of puricase activity after cessation of treatment.

Puricase Concentration and Activity in Maternal EDTA-Plasma Samples				
Gestation Day	Collection Interval	Dose (mg/kg/day)	Plasma Conc.($\mu\text{g}/\text{mL}$) Mean \pm SEM	Plasma Activity (U/mL) Mean \pm SEM
6	1 hour pre-dose	0	<0.92 \pm 0.00	<39.8 \pm 0.00
		5	<1.28 \pm 0.00	50.4 \pm 0.00
		10	<1.96 \pm 0.00	<78.2 \pm 0.0
		40	<1.10 \pm 0.00	<45.4 \pm 0.0
	1 hour post-dose	0	<0.92 \pm 0.00	<39.8 \pm 0.00
		5	82.64 \pm 25.10	3,097.6 \pm 918.2
		10	106.66 \pm 59.11	4,109.2 \pm 2,248.7
		40	699.34 \pm 78.23	26,032.9 \pm 2,853.6
16	1 hour pre-dose	0	<0.92 \pm 0.00	<39.8 \pm 0.00
		5	99.47 \pm 9.04	3,713.3 \pm 330.5
		10	253.75 \pm 2.37	9,687.7 \pm 89.1
		40	708.08 \pm 73.22	26,351.6 \pm 2,670.9
	1 hour post-dose	0	<0.92 \pm 0.00	<39.8 \pm 0.00
		5	189.12 \pm 25.71	7,048.3 \pm 940.3
		10	406.29 \pm 82.98	15,618.1 \pm 3,116.9
		40	1,363.39 \pm 253.14	50,778.2 \pm 9,233.8

Puricase did not transfer across the placental from the mother to the fetus. Puricase was not detected in fetal plasma samples from all treatment groups.

Clinical Chemistry. Statistically significant changes noted on gestation day 17 (after dosing) are summarized in the table below. Treatment-related decrease in uric acid levels was noted. Compared to GD 5 and control values, uric acid levels were decreased in all treatment groups of puricase. This observation is consistent with the pharmacological property of puricase and is not considered to be toxicologically significant. Mean urea nitrogen levels in the 40 mg/kg/day group were lower on GD 5 and GD 17. On GD 5, the urea nitrogen level was statistically lower (by 20%) than control at 40 mg/kg. Chloride value was statistically higher (by 2.0%) than control at 40 mg/kg on GD 17. Treatment-related decrease in uric acid was noted. Compared to GD 5 and control values, uric acid levels were decreased in all treatment groups of puricase. These statistically significant changes are considered incidental/not toxicologically significant.

Summary of significant clinical findings following intravenous administration of Puricase during gestation.

Parameter	Gestation Day	Mean \pm SD			
		Dose (mg/kg)			
		0	5	10	40
Uric Acid (mg/dL)	5	1.2 \pm 0.35	1.3 \pm 0.21	1.1 \pm 0.32	0.9 \pm 0.18
	17	1.5 \pm 0.53	0.0 \pm 0.0*	0.0 \pm 0.0	UR
Urea Nitrogen (mg/dL)	5	16.3 \pm 1.62	14.9 \pm 1.43	14.3 \pm 1.10	13.0 \pm 1.3*
	17	16.4 \pm 2.52	17.5 \pm 1.83	16.3 \pm 2.48	14.5 \pm 1.02
Chloride (mEq/L)	5	100 \pm 0.8	99 \pm 1.0	99 \pm 1.4	100 \pm 0.8
	17	96 \pm 0.5	97 \pm 1.2	96 \pm 0.8	98 \pm 1.0*

*: Significant different from control value, $p \leq 0.01$, using Dunnett's test
 UR: Below instrument range
 GD 5: day prior to dosing

Prenatal and postnatal development

Studies to evaluate the effects of puricase in pregnant/lactating females and on the development of the conceptus and offspring were not conducted.

2.6.6.7 Local tolerance

Local tolerance studies were not submitted with this application.

2.6.6.8 Special toxicology studies

Two studies were performed to assess the impact of pegloticase accumulation in vacuoles on macrophages function in the spleen.

Study title: The effect of repeated Puricase[®] administration on splenic macrophage vacuole formation and disappearance, and macrophage functionality.

Study No.: O1V378-2

Objective of the study: To obtain addition information on the kinetics of the induction of vacuoles after treatment with puricase and of their disappearance after cessation of treatment.

Method (copied from the sponsor's toxicology written summary): Female Sprague Dawley rats (4 - 6/ dose group) weighing 115 – 125 g were administered subcutaneous injections of pegloticase (lot#: 16860010) in PBS at doses of 0, 3.4, 10.2, and 34 mg/kg 3 times/week from Day 1 though Day 28 (total of 12 injections). The total number of rats used in the study was 86. The females were sacrificed on Days 4, 7, 14, 29, 44 and 58 postdosing, and the spleens were excised and processed for microscopic slide reading.

Spleen tissue from rats sacrificed on Study Days 4, 7, 14, and 29 were erroneously fixed in Bouin's solution rather than formalin. Because of false-positive findings (vacuoles) that were noted in some control rats, this portion of the study was repeated (01V378-4). Tissues from rats sacrificed from Study Days 44 and 58 were correctly fixed in formalin.

On Day 29, sections of the excised spleens were taken from the control and the pegloticase treated groups for evaluation in a macrophage functionality assay. Harvested splenocytes (macrophages) were incubated for 24 hours in the absence or presence of *E. coli* lipopolysaccharide (LPS). Secretion of TNF (Tumor Necrosis Factor), one indicator of splenic macrophage functionality, was measured as well as adherence to the tissue culture plates.

Key study findings:

- Repeated administration of puricase at a dose of 34 mg/kg resulted in the formation of vacuoles in splenic macrophages.
- The vacuoles were still evidence after cessation of treatment; 30 days post-dosing, vacuoles were still observed in the HD group.
- The repeated administration of puricase was associated with a decreased functionality of the spleen macrophages. As depicted in the figure below, results from the ex-vivo experiment clearly demonstrated that the high-dose of puricase decreased LPS-stimulated secretion of TNF for adherent macrophages.

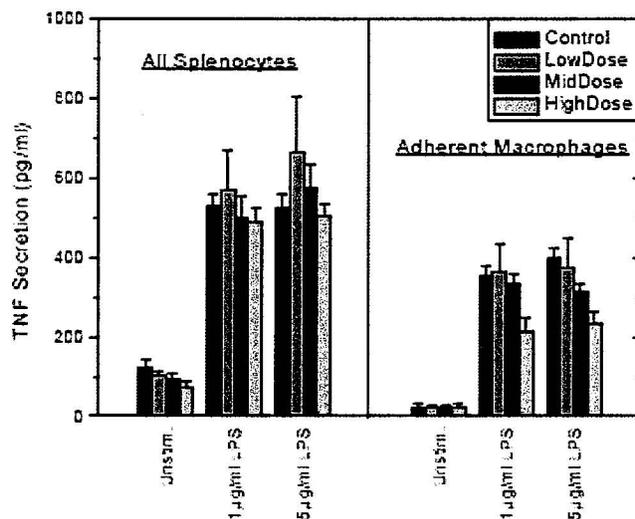


Figure 1. Endotoxin-Induced TNF Secretion *ex-vivo* from Total Splenocytes and from Adherent Macrophages

Study title: The effect of repeated Puricase[®] administration on splenic macrophage vacuole formation and disappearance, and macrophage functionality.

Study #: O1V378-4

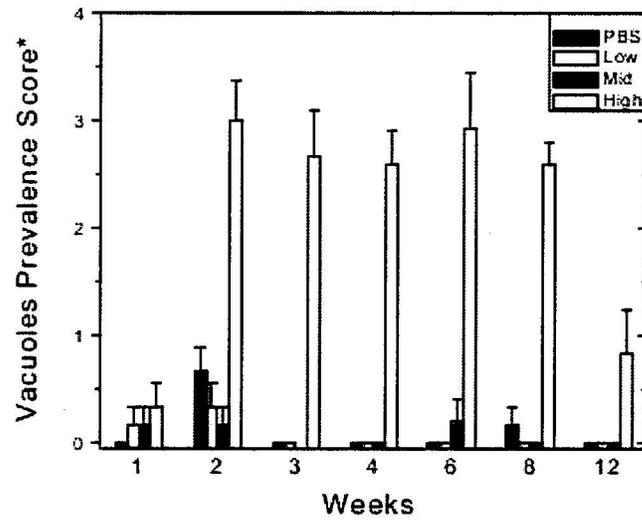
Objective of the study: To obtain addition information on the kinetics of the induction of vacuoles after treatment with puricase and of their disappearance after cessation of treatment.

Method (copied from the sponsor's toxicology written summary): Female Sprague Dawley (6/dose group) weighing 108 – 160 g were dosed subcutaneously with pegloticase (lot#: 16860010) in PBS at doses of 0, 3.4, 10.2 or 34 mg/kg 3 times/week for 4 weeks (total of 12 injection) followed by an 8 week recovery period. The total number of rats used in the study was 162. Rats were sacrificed at 1, 2, 3, 4, 6, 8, and 12 weeks after the first injection. The spleens were excised and processed in formalin for microscopic slide reading. Splenic macrophage vacuoles were scored based on the number of vacuoles per microscope field. On Day 29, portions of excised spleens were taken from control and pegloticase-treated groups for evaluation in an ex-vivo macrophage functionality assay. Harvested splenocytes (macrophages) were incubated for 24 hours in the absence or presence of *E. coli* LPS. Secretion of TNF, one indicator of splenic cell functionality, was measured as well as adherence to the tissue culture plates.

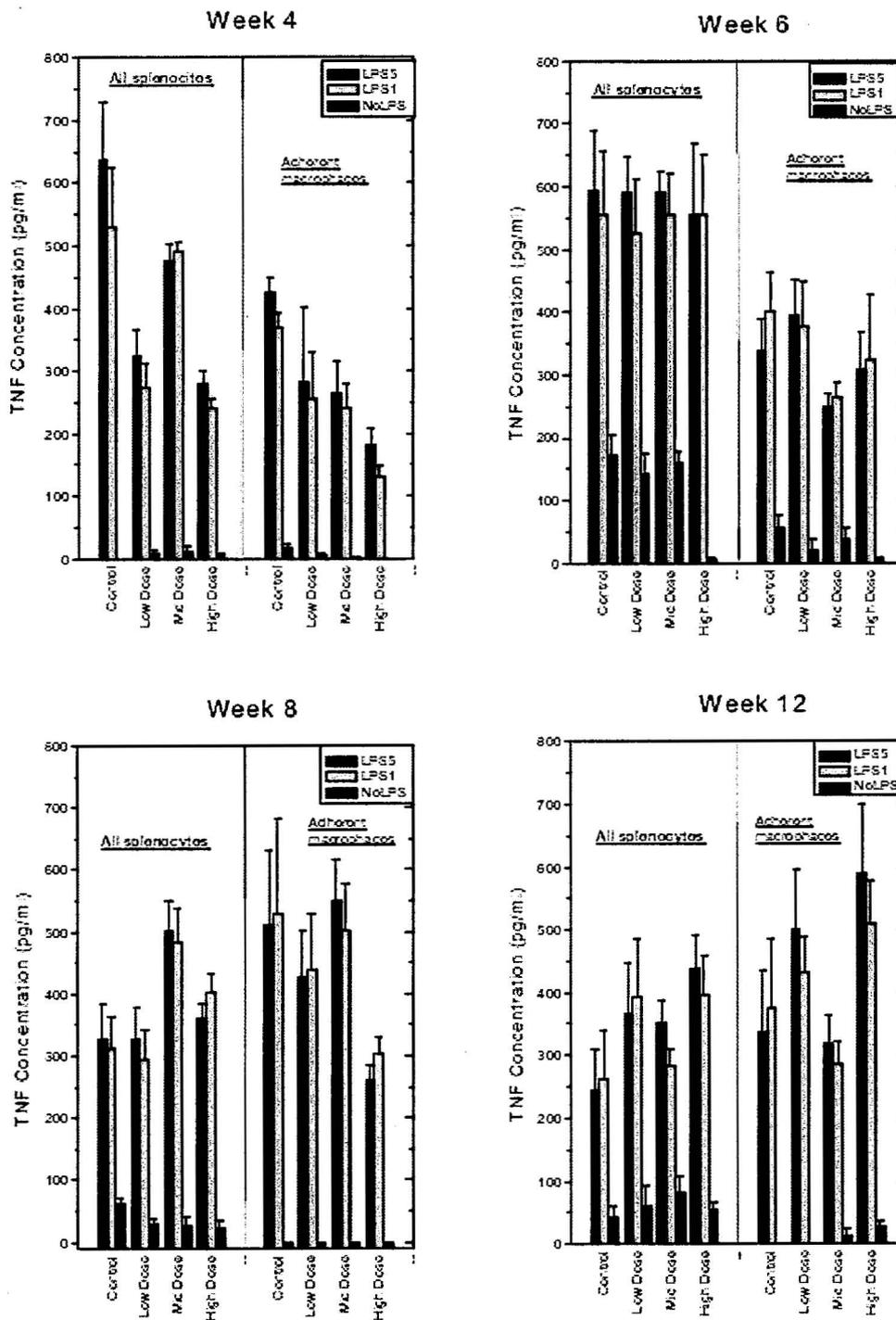
Key study findings:

- As depicted in the figure below, repeated administration of puricase resulted in formation of vacuoles in splenic macrophages. Vacuolization of spleen macrophages occurred after 2-weeks of treatment with the high-dose (34 mg/kg) of puricase only. At week 2, $\geq 1,000$ vacuoles per microscopic field were counted.
- At the end of the 8-week recovery period (week 12), the number of vacuoles counted had markedly diminished. Number of vacuoles counted ranged between 50-100 vacuoles per microscopic field.

Sponsor's Figure 1. Vacuoles formation and disappearance in spleen macrophages.



- The repeated administration of puricase was also associated with a decreased functionality of the spleen macrophages. A dose-dependent decrease in the TNF response to LPS challenge was observed in the adhered macrophages during week 4. As shown in the figure below, the decrease in the high-dose group was statistically significant ($p < 0.01$) relative to the control. Functionality returned within 2-weeks after cessation of treatments.



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Sponsor's Figure 2. Biological functionality of spleen cells and macrophages.

2.6.6.9 Discussion and Conclusions

See overall conclusion below.

2.6.6.10 Tables and Figures

N/A

2.6.7 TOXICOLOGY TABULATED SUMMARY

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusion: The Applicant has conducted the requested nonclinical studies, such that the risks for human use may be adequately judged, to support the clinical safety of intravenous pegloticase for the treatment of failure gout in patients at the proposed dose of 8 mg once every 2 weeks. The nonclinical studies were of sufficient duration and included adequate recovery period evaluations, and used the same, intravenous route of administration intended for clinical use for the general toxicology studies. The nonclinical study program was conducted in accordance with Agency recommendations provided in 2005 during product development; however, the development program is not in agreement with current ICH guidelines (Refer to the Guidance for Industry – Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals; ICH S6 (Jul 1997)).

Chronic and sub-chronic toxicology was investigated in dogs administered pegloticase by intravenous injection for 3 and 6 months. The 6-month chronic toxicology study is acceptable to support chronic clinical pegloticase treatment in accordance with the Guidance for Industry – Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals; ICH S6 (Jul 1997). The repeated administration of intravenous pegloticase for 3 months or 6 months was well-tolerated. The nonclinical safety issues relevant to clinical use identified in these studies in dogs include the formation of vacuoles in several organs. In the 3-month toxicity study, vacuolization was only noted in the splenic red pulp. Increasing dosing duration resulted in more organs presenting with vacuolization. In the 6-month repeat-dose toxicity, dose-dependent vacuolation was noted in the splenic red pulp, liver, zona reticularis and/or zona fasciculata of the adrenal gland, intimal lining of the aortic outflow tract of the heart, and lamina propria of the duodenum and jejunum. Immunohistochemical staining indicated that the vacuoles in the spleen, liver, duodenum and jejunum were located in the macrophages. Thus, suggesting that the vacuoles represented the normal physiological process; that is, the large pegylated substance is being absorbed from circulation by macrophages and digested. Due to the absence of associated clinical pathology or toxicity with the vacuoles in these organs, the presence of the vacuoles probably has no clinical relevance. It should be pointed out that the vacuoles persisted up to 12-weeks after cessation of treatment. One concern that arises is the potential clinical adverse events that may result from the long-term accumulation of these vacuoles. In studies assessing the effects of puricase-induced

splenic vacuolated macrophages, it was shown that the repeated administration of puricase was associated with a decrease in the functionality of the spleen macrophages. After cessation of puricase treatment, function recovered.

Using immunohistochemical staining, the cells containing vacuoles observed in the heart and adrenal gland were not identified as macrophages. The vacuoles observed in the adrenal gland were located in the zona reticularis and/or zona fasciculata. No pathologies or clinical pathologies were associated with these vacuoles. However, due to the high prevalence of cardiovascular diseases in the intended patient population, the potential accumulation of pegloticase in vacuoles in the endothelial cells may increase the incidence of the patient developing a cardiovascular event, such as atherosclerosis.

In conclusion, as per previous agreements with the sponsor, the nonclinical development program was adequate to support clinical safety of pegloticase. There were no effects on the QT interval in the electrocardiography assessments in dogs. Pegloticase clearance is not expected to be affected by renal impairment because of the large size of the molecule. There were no effects of age and/or sex suggested by the nonclinical study results. No drug-drug interactions are expected because pegloticase is not expected to interact with cytochrome P450 enzymes.

Unresolved toxicology issues (if any): Although previously agreed upon in 2005 based on policy at that time, the current nonclinical development program is deficient by current standards. Specifically, the full standard battery of reproductive toxicology studies has not been conducted. This previous agreement made with the sponsor will be honored; however, the remaining studies should be completed post-approval requirements.

The significance of the vacuolization in the heart/aortic outflow tissues and the adrenal gland is not known. There are no functional assessments of the endothelial cells in the heart following chronic exposure to pegloticase. Given the data to date, the longer duration of treatment appears to result in a greater number of tissues being affected. Given the known cardiovascular complications in gout patients, the potential impact of these endothelial vacuoles over time should be evaluated via further nonclinical studies if human data can not be obtained to adequately assess the potential long-term cardiovascular implications of this product in the hyperuricemic patient.

Recommendations: From the nonclinical pharmacology/toxicology perspective, based upon the information reviewed by this reviewer, this BLA may be approved. However, the following post-marketing studies should be required:

1. Conduct a fertility study in the rat model (Segment 1)
2. Conduct an embryo-fetal development study in the rabbit model (Segment 2)
3. Conduct a peri-natal and post-natal development study in the rat model (Segment 3)

4. Conduct a *in vitro* assessments of aortic endothelial cell function following co-culture with pegloticase.

Suggested labeling: See executive summary.

Signatures (optional):

Reviewer Signature BeLinda A. Hayes June 23, 2009

Supervisor Signature R. David Melton 6-23-09 Concurrence Yes No

APPENDIX/ATTACHMENTS

REFERENCES

Conover, C., Lejeune, L., Linberg R., Shum, K., and Shorr, R.G.L. (1996). Transitional vacuole formation following a bolus infusion of PEG-hemoglobin in the rat. *Art. Cells Blood Subs. And Immob. Biotech.* 24(6):599-611.

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Feig, D.I., Kang, D. and Johnson, R. (2008). Uric acid and cardiovascular risk. *N Engl J Med* 359: 1811-1821.

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Schumacher, H.R (2008). The pathogenesis of gout. *Cleve Clin J med* 75 Suppl 5:52-54.

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A
NEW NDA/BLA**

NDA Number: 125,293

Applicant: Savient
Pharmaceuticals

Stamp Date: October 31, 2008

Drug Name: Pegloticase

NDA/BLA Type: BLA

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

Filable

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

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A
NEW NDA/BLA**

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)			No, the sponsor has not submitted any toxicity studies to address the following product- and process-related impurities: (b) (4) <i>to be addressed</i>
11	Has the applicant addressed any abuse potential issues in the submission?			Not Applicable <i>during review</i>
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Non Applicable

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

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

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

Berinda A. Hayes Dec. 15, 2008
 Reviewing Pharmacologist Date

R. David Miller 12-15-2008
 Team Leader/Supervisor Date