CENTER FOR DRUG EVALUATION AND RESEARCH

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PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

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Indication:	For the long-term treatment of adult patients with Gaucher disease type 1
Applicant:	Genzyme Corporation
Review Division:	Division of Gastroenterology and Inborn Errors Products (DGIEP)
Reviewer:	Tamal K. Chakraborti, Ph.D.
Supervisor:	Sushanta K. Chakder, Ph.D.
Division Director:	Donna Griebel, MD
Project Manager:	Jessica M. Benjamin, MPH

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Reference ID: 3501244

previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 205494.

TABLE OF CONTENTS

1 EXECUTIVE SUMMARY 5

1.1 INTRODUCTION 5
1.2 BRIEF DISCUSSION OF NONCLINICAL FINDINGS 5
1.3 RECOMMENDATIONS 7

2 DRUG INFORMATION 11

2.1 DRUG 11
2.2 RELEVANT INDS, NDAS, BLAS AND DMFS 12
2.3 DRUG FORMULATION 12
2.4 COMMENTS ON NOVEL EXCIPIENTS 13
2.5 COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN 13
2.6 PROPOSED CLINICAL POPULATION AND DOSING REGIMEN 30
2.7 REGULATORY BACKGROUND 30

3 STUDIES SUBMITTED 31

3.1 STUDIES REVIEWED 31
3.2 STUDIES NOT REVIEWED 33
3.3 PREVIOUS REVIEWS REFERENCED 33

4 PHARMACOLOGY 33

4.1 PRIMARY PHARMACOLOGY 33
4.2 SECONDARY PHARMACOLOGY 52
4.3 SAFETY PHARMACOLOGY 54

5 PHARMACOKINETICS/ADME/TOXICOKINETICS 57

5.1 PK/ADME 64
5.2 TOXICOKINETICS 125

6 GENERAL TOXICOLOGY 126

6.1 SINGLE-DOSE TOXICITY 126
6.2 REPEAT-DOSE TOXICITY 128

7 GENETIC TOXICOLOGY 187

7.1 *IN VITRO* REVERSE MUTATION ASSAY IN BACTERIAL CELLS (AMES) 187
7.2 *IN VITRO* ASSAYS IN MAMMALIAN CELLS 189
7.3 *IN VIVO* CLASTOGENICITY ASSAY IN RODENT (MICRONUCLEUS ASSAY) 192
7.4 OTHER GENETIC TOXICITY STUDIES 196

8 CARCINOGENICITY 214

9 REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY 214

9.1 FERTILITY AND EARLY EMBRYONIC DEVELOPMENT 214
9.2 EMBRYONIC FETAL DEVELOPMENT 219
9.3 PRENATAL AND POSTNATAL DEVELOPMENT 237

Reference ID: 3501244

10 SPECIAL TOXICOLOGY STUDIES 249

11 INTEGRATED SUMMARY AND SAFETY EVALUATION 256

12 APPENDIX/ATTACHMENTS 262

1 Executive Summary

1.1 Introduction

Eliglustat (Cerdelga®) is a selective inhibitor of glucosylceramide synthase and resembles ceramide, the substrate for glucosylceramide synthase. Eliglustat is indicated for the long-term treatment of adult patients with Gaucher disease type 1 (GD1). Gaucher disease is caused by a deficiency of the lysosomal enzyme, glucocerebrosidase that results in the accumulation of its major natural substrate, glucosylceramide, especially in the liver, spleen, and bone marrow. The Applicant is seeking approval for eliglustat as a substrate reduction therapy (SRT) for GD1. The goal of the SRT is to reduce the rate of synthesis of glucosylceramide to match its impaired rate of catabolism in patients with GD1, thereby preventing glucosylceramide (GL-1) accumulation and alleviating clinical manifestations. Inhibition of glucosylceramide synthase by eliglustat results in a reduction of the accumulation of glucosylceramide, thereby allowing the patient's residual endogenous acid β-glucosidase levels to clear the substrate. This mechanism of action (MOA) differs from that of the enzyme replacement therapies (ERTs) commonly used to treat GD1 (augmenting acid-β-glucosidase activity).

1.2 Brief Discussion of Nonclinical Findings

Eliglustat was shown to inhibit (IC_{50} = 10 ng/mL) glucosylceramide synthase (GCS) in human K562 cells or human A375 cell-derived microsomes. In animal efficacy studies, eliglustat decreased GL-1 levels in peripheral tissues and plasma of normal rats and dogs following oral administration. In the D409V/null mouse model of GD1, eliglustat decreased the accumulation of GL-1 in tissues.

Eliglustat caused an inhibition of hERG channels expressed in HEK-293 cells with an IC_{50} value of 0.35 µg/mL, indicating a potential to cause QT prolongation. Eliglustat also inhibited sodium and calcium channels with IC_{50} values of 5.2 and 10.4 µg/mL, respectively. The above IC_{50} values for inhibition of hERG and sodium and calcium channels are about 8, 117, and 235 times greater, respectively, than the mean predicted (PK simulation) clinical C_{max} of 44.3 ng/mL. In isolated canine Purkinje fiber assay, eliglustat decreased action potential duration (APD_{60} and APD_{90}) at 1 and 10 µg/mL. In a single oral (gavage) dose conscious dog telemetry study at 1, 3, 10, 25, 50 and 80 mg/kg, eliglustat increased PR (50, 80 mg/kg) and QRS (10, 25, 50 and 80 mg/kg) intervals. In an IV (infusion) telemetry study in anesthetized dog at 1, 2.5 and 5 mg/kg, eliglustat increased PR, QRS and QT_c intervals at 2.5 and 5 mg/kg.

Chronic oral toxicology studies were conducted in rats (6-month) and dogs (1-year) at doses ranging from 5 to 50 mg/kg/day and 2 to 10 mg/kg/day, respectively. In the 6-month study in rats, there were no significant treatment-related histopathological findings in any organ or tissue. The no-observed-adverse-effect-level (NOAEL) was considered as 50 mg/kg/day.

Reference ID: 3501244

In an oral gavage study (GT-157-TX-48) in juvenile rats, animals were treated with Genz-112638 at 5, 15 and 25 mg/kg BID for 10 weeks starting at postnatal day 22 (PND 22) through day 91. Histopathological changes were seen in the lymph node (increased incidence/severity of lymphoid hyperplasia in the mandibular lymph nodes at 25 mg/kg BID) and eye (minimal grade unilateral neuropathy in the optic nerve of two animals at 25 mg/kg BID). The NOAEL was considered as 15 mg/kg BID.

A 28-day oral study (GT-157-TX-28) was conducted in male rats to evaluate specific effects of Genz-112638 on male reproductive organs and spermatogenesis. In this study male rats were treated at 15 or 50 mg/kg BID (30 or 100 mg/kg/day) for 4 weeks or at 100 mg/kg BID (200 mg/kg/day) for 2 weeks. At 200 mg/kg/day, sperm morphology remained affected until 14-weeks treatment-free recovery period. Microscopic observations at 200 mg/kg/day included germ cell necrosis in the testis, sloughed cells in the epididymis, acute inflammation in the coagulating glands, epithelial hyperplasia in the forestomach and severe atrophy of thymus and spleen. No effects on sperm count or motility were seen at 15 and 50 mg/kg BID doses. A follow-up 4-week oral study (GT-157-TX-31) was conducted at 36 mg/kg BID (72 mg/kg/day) in mature Cynomolgus monkeys to confirm the above effects of Genz-112638 on spermatogenesis in non-human primates. There were no significant treatment-related effects on any of the measured sperm parameters in this monkey study.

In a 52-week oral (gavage) study (GT-157-TX-23) in Beagle dogs, animals were treated at 2, 5 and 10 mg/kg/day. Histopathological changes were observed in the lung (peribronchiolar chronic inflammation/fibrosis at 10 mg/kg/day), heart (mesothelial hyperplasia in the atria of the heart was noted only in treated female animals), and testes (seminiferous epithelial degeneration and segmental hypoplasia in the testes were seen with a greater incidence in the treated groups). Histopathological changes observed in the lung e.g., peribronchiolar chronic inflammation/fibrosis was more prominent at 10 mg/kg/day group than in controls; however, there was no dose response, similar incidence in control and treated group and were also consistent with the common background. Mesolthelial hyperplasia in the heart was seen only in females and there was no dose response. Histopathological changes in the testes (seminiferous epithelial degeneration and segmental hypoplasia were seen with a greater incidence in the treated groups) were not dose-related and were also seen in control animals. Based on these, the NOAEL may be considered as 10 mg/kg/day.

Eliglustat was negative in the Ames test, chromosome aberration assay using human peripheral blood lymphocytes and oral *in vivo* mouse micronucleus test. In addition, Genz-399240, a human metabolite of eliglustat, was negative in the Ames test and chromosome aberration assay using human peripheral blood lymphocytes.

The reports of the 2-year oral carcinogenicity studies in mice (GT-157-TX-43) and rats (GT-157-TX-38) were reviewed by Dr. Sruthi King (pharmacology review of NDA 205494 dated April 15, 2014). In Sprague-Dawley rats, eliglustat tartrate was administered by oral gavage at doses up to 75 mg/kg/day in males (about 3.6 times the

recommended human daily dose of 100 mg BID, based on a body surface area basis) and 50 mg/kg/day in females (about 2.4 times the recommended human daily dose based on body surface area basis). In CD-1 mice, eliglustat tartrate was administered to males and females at up to 75 mg/kg/day (about 1.8 times the recommended human daily dose based on a body surface area basis) via dietary admixture. Eliglustat tartrate did not produce any treatment-related neoplasms in rats or mice.

In a fertility and early embryonic development study in rats, eliglustat increased pre-implantation loss at 30 (about 1.5 times the recommended human oral dose of 100 mg BID based on body surface area) and 100 mg/kg/day (about 5 times the recommended human oral dose based on body surface area). In an oral embryofetal development study in rats, animals were treated at 10, 30 and 120 mg/kg/day (about 0.5, 1.5 and 6 times the recommended human oral dose of 100 mg BID based on body surface area, respectively) from GD 6 to 17. Maternal toxicity (decreased body weight and food consumption) was observed at 120 mg/kg/day. At 120 mg/kg/day, there was an increase in the number of late resorptions, dead fetuses and mean post implantation loss. Fetal body weight was also reduced at 120 mg/kg/day. At 120 mg/kg/day, fetal visceral variations included dilated cerebral ventricles and fetal skeletal malformations included abnormal number of ribs or lumbar vertebra and fetal skeletal variations included poor bone ossification. In an oral embryofetal development study in New Zealand white rabbits, animals were treated at 10, 30 and 100 mg/kg/day (about 1, 3 and 10 times the recommended human oral dose of 100 mg BID based on body surface area, respectively) from GD 6 to 18. No significant treatment-related adverse effects on embryofetal development were observed in rabbits. In a pre and postnatal development study in rats, eliglustat did not appear to cause any significant adverse effects on pre and postnatal development at doses up to 100 mg/kg/day (about 5 times the recommended human oral dose of 100 mg BID based on body surface area).

The recommended human dose for eliglustat tartrate is 100 mg BID (free base: equivalent to 84 mg of eliglustat) or 200 mg/day (3.33 mg/kg/day). The mean clinical simulated C_{max} of 44.3 ng/mL and $AUC_{0\text{-}12h}$ of 307 ng.hr/mL were derived from PK simulations based on a PopPK model (Report No. SIM0124). The NOAEL in rats (50 mg/kg/day) in the 6-month toxicity study was approximately 2.4 times the recommended human dose of 100 mg BID based on body surface area. The exposure (AUC) at the NOAEL of 50 mg/kg/day in rats is approximately 8 to 12 times higher than the mean predicted $AUC_{0\text{-}12h}$ of 307 ng.hr/mL. The NOAEL in dogs (10 mg/kg/day) in the 12-month toxicity study was approximately 1.6 times the recommended human dose of 100 mg BID based on body surface area. The exposure (AUC) at the NOAEL of 10 mg/kg/day in dogs is approximately 10 to 15 times higher than the mean predicted $AUC_{0\text{-}12h}$ of 307 ng.hr/mL.

1.3 Recommendations

1.3.1 Approvability

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

Reference ID: 3501244

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The proposed draft labeling of Cerdelga® appears to conform to the specific requirements on content and format of labeling for human prescription drugs under 21CFR201.57. However, the Applicant should be asked to modify the label as recommended below.

8.1 Pregnancy

Applicant's Version:

(b) (4)

Evaluation: (b) (4)

Recommended Version:

(b) (4) **Pregnancy Category C**

Risk Summary

There are no adequate or well-controlled studies with CERDELGA in pregnant women. Animal reproduction study with eliglustat in rats produced a spectrum of anomalies at doses 6 times the recommended human dose. No fetal harm was observed with oral administration of eliglustat to pregnant rabbits at dose levels 10 times the recommended human dose. CERDELGA should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Animal Data

Reproduction studies have been performed in pregnant rats at oral doses up to 120 mg/kg/day (about 6 times the recommended human dose based on body surface area) and in pregnant rabbits at oral doses up to 100 mg/kg/day (about 10 times the recommended human dose based on body surface area). In rats, at 120 mg/kg/day

(about 6 times the recommended human dose based on body surface area), eliglustat increased the number of late resorptions, dead fetuses and post implantation loss, reduced fetal body weight, and caused fetal visceral variations (dilated cerebral ventricles), fetal skeletal variations (poor bone ossification) and fetal skeletal malformations (abnormal number of ribs or lumbar vertebra). Eliglustat did not cause fetal harm in rabbits at oral doses up to 100 mg/kg/day (about 10 times the recommended human dose based on body surface area). In a pre and postnatal development study in rats, eliglustat did not show any significant adverse effects on pre and postnatal development at doses up to 100 mg/kg/day (about 5 times the recommended human dose based on body surface area).

8.3 Nursing Mothers

Applicant's Version:

8.3 Nursing Mothers[16]

It is not known whether [redacted] (b) (4) in human milk. Because many drugs are [redacted] (b) (4) in human milk, [redacted] (b) (4) to a nursing woman.

Evaluation: The text should be modified as recommended below.

Recommended Version:

8.3 Nursing Mothers

It is not known whether CERDELGA is present in human milk. Because many drugs are present in human milk, [redacted] (b) (4)

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Applicant's Version:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

[redacted]

Evaluation: Carcinogenicity section should be modified as recommended below. Mutagenesis section should be modified to include the results of the mouse lymphoma assay. Impairment of fertility section should be modified to include the findings of the fertility and early embryonic development study in rats as recommended below.

Recommended Version:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis:

Carcinogenic potential of CERDELGA was assessed in 2-year carcinogenicity studies in rats and mice. In Sprague-Dawley rats, eliglustat [redacted] (b) (4) was administered by oral gavage at doses up to 75 mg/kg/day in males (about 3.6 times the recommended human daily dose of [redacted] (b) (4) mg BID, based on a body surface area basis) and 50 mg/kg/day in females (about 2.4 times the recommended human daily dose based on body surface area basis). In CD-1 mice, eliglustat tartrate was administered to males and females at up to 75 mg/kg/day (about 1.8 times the recommended human daily dose based on a body surface area basis) via dietary admixture. Eliglustat [redacted] (b) (4) did not produce any treatment-related neoplasms in rats or mice.

Mutagenesis:

Eliglustat was negative in the Ames test, chromosome aberration test in human peripheral blood lymphocytes, mouse lymphoma gene mutation assay and *in vivo* oral mouse micronucleus test.

Impairment of Fertility:

In a fertility and early embryonic development study in rats, eliglustat increased pre-implantation loss at 30 (about 1.5 times the recommended human oral dose based on body surface area) and 100 mg/kg/day (about 5 times the recommended human oral dose based on body surface area).

In mature male rats, eliglustat showed adverse effects on sperm morphology, testes (germ cell necrosis), and sloughed cells in the epididymis at 200 mg/kg/day (about 10 times the recommended human oral dose based on body surface area). Similar effects on sperm were not seen in mature Cynomolgus monkeys at 72 mg/kg/day (about 7 times the recommended human oral dose based on body surface area).

2 Drug Information

2.1 Drug

CAS Registry Number: 928659-70-5

Generic Name: Eliglustat

Code Name: Genz-112638

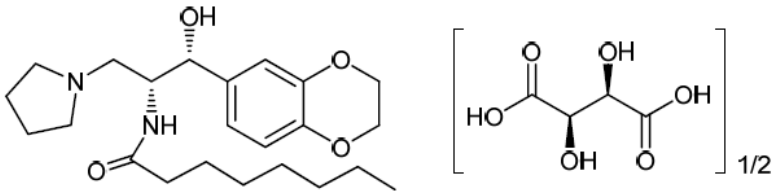
Chemical Name: N-((1R,2R)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-1-hydroxy-3-(pyrrolidin-1-yl)propan-2-yl)octanamide (2R,3R)-2,3-dihydroxysuccinate

Molecular Formula/Molecular Weight: $C_{23}H_{36}N_2O_4$ + ½ ($C_4H_6O_6$)/479.59

Reference ID: 3501244

Structure: The chemical structure of eliglustat tartrate is shown in the following Figure (from page 1 of Section 2.3.S.1).

Figure 1: Eliglustat Tartrate Chemical Structure



Pharmacologic Class: Glucosylceramide synthase inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 67589 (Genz-112638, Genzyme)

2.3 Drug Formulation

Eliglustat drug product (DP) is formulated as hard gelatin capsules. Each capsule contains 84 mg eliglustat free base (equivalent to 100 mg of eliglustat tartrate) and compendial excipients e.g., microcrystalline cellulose, lactose monohydrate, hypromellose and glyceryl behenate/ (b) (4) The components of the DP are shown in the table below (from page 1 of Section 2.3.P.1).

Table 1: Composition of Eliglustat Hard Capsules

Component	Reference to Quality Standard	Function	Amount (mg/capsule) [a]
Capsule Blend Composition			
Eliglustat[b]	In-house	drug substance	(b) (4)
Microcrystalline cellulose	NF / Ph. Eur.	(b) (4)	
Lactose monohydrate	NF / Ph. Eur.		
Hypromellose	USP / Ph. Eur.		
Glycerol behenate / (b) (4)	NF / Ph. Eur.		
Capsule			
Size 2 hard gelatin capsules (printed with black ink)[c]	In-house	encapsulation	1 capsule

[a] Target amounts provided. Refer to 2.3.P.3 Section 2 for the ranges for each excipient.
[b] Each capsule contains 84 mg of eliglustat (which is equivalent to 100 mg of eliglustat tartrate)
[c] Refer to Table 2 for the composition of the hard gelatin capsule. Refer to Table 3 for the composition of the black ink.

2.4 Comments on Novel Excipients

As mentioned above, eliglustat capsule contains compendial excipients e.g., microcrystalline cellulose, lactose monohydrate, hypromellose and glyceryl behenate/ (b) (4) From a nonclinical standpoint, there is no safety concern for the above excipients and are acceptable at the specified levels.

2.5 Comments on Impurities/Degradants of Concern

Drug Product (DP):

The following table (from page 2 of Section 2.3.P.5) shows the specifications of the drug product (DP).

Table 1: Eliglustat Hard Capsule Specifications

Test	Method	Acceptance Criteria (Release)	Acceptance Criteria (End of Shelf-Life)
Appearance (Contents)	Visual	White to off-white powder	White to off-white powder
Appearance (Capsule)	Visual	Pearl blue-green opaque cap and pearl white opaque body with 'GZ02' printed in black on the capsule	Pearl blue-green opaque cap and pearl white opaque body with 'GZ02' printed in black on the capsule
Identity	HPLC	The retention time of the principal peak in the sample chromatogram corresponds to the retention time of the principal peak in the reference chromatogram	NA[a]
	UV	Sample spectrum conforms to reference spectrum	NA[a]
Assay	HPLC	(b) (4) to (b) (4)% wt/wt	(b) (4) to (b) (4)% wt/wt
Degradation Products[b]	HPLC	*Specified identified degradation products*	*Specified identified degradation products*
		≤ (b) (4) wt/wt	≤ (b) (4), wt/wt
		≤ (b) (4) wt/wt	≤ (b) (4) wt/wt
		Unspecified degradation products	*Unspecified degradation products*
		No individual > (b) (4)%, wt/wt	No individual > (b) (4)%, wt/wt
		≤ (b) (4)% Total Degradation Products, wt/wt	≤ (b) (4)% Total Degradation Products, wt/wt
Uniformity of Dosage Units	USP <905> Ph. Eur. 2.9.40	Complies	NA[a]
Microbial Enumeration[c]	USP <61>, <62> Ph. Eur. 2.6.12, 2.6.13	Complies	Complies
Dissolution	USP <711> Ph. Eur. 2.9.3	Q = (b) (4)% in 30 minutes	Q = (b) (4)% in 30 minutes

[a] Not applicable as the method is not stability indicating
[b] Report any impurity above (b) (4)% wt/wt. Identify any impurity above (b) (4)% wt/wt
[c] Microbial Enumeration testing will be performed on one lot of drug product during each year of manufacture.

Seven degradation products have been identified and are listed in the following table (from page 11 of Section 2.3.P.5). The degradation pathways are shown in the following figure (from page 12 of Section 2.3.P.5). (b) (4) is the major degradant in the DP.

Table 6: Drug Product Degradation Products

Identifier	Description
[redacted]	[redacted]

(b) (4)

Figure 1: Degradation Pathways for Eliglustat Hard Capsules

(b) (4)

[redacted]

Based on the daily recommended dose for eliglustat (less than 1 g), the maximum content of (b) (4) and (b) (4) (specified identified degradation products) allowable for the DP was set at ≤ (b) (4)% (w/w) in accordance with the ICH Q3B guidance. As per the above guidance, the limit for unspecified degradants was set at ≤ (b) (4)% (w/w). The release specification limit for total degradation products was set at ≤ (b) (4)% (w/w), which accommodates the presence of the two specified and up to four unspecified degradation products at their maximum acceptance levels. The specifications for the degradants are below the qualification threshold of (b) (4)% and are acceptable from the nonclinical perspective.

Drug Substance (DS):

The specification of the DS is shown below (from page 1 of Section 3.2.S.4.1).

Table 1: Drug Substance Specifications

Test	Method	Acceptance Criteria (Release)	Acceptance Criteria (Stability)
Appearance	Visual	Powder	Powder
Colour	Visual	White to off-white	White to off-white
Identity	FTIR USP <197>, Ph Eur. 2.2.24	Sample spectrum conforms to reference spectrum	NA[a]
Identity	RP-HPLC	The retention time of the principal peak in the sample chromatogram corresponds to the retention time of the principal peak in the reference chromatogram	NA[a]
Thermal Analysis	DSC USP <891>, Ph. Eur. 2.2.34	Sample main thermal event conforms to reference main thermal event. Report other significant endo- or exothermic events	NA[a]
Impurities	RP-HPLC	*Specified identified impurities:*	*Specified identified impurities:*
		(b) (4)	
		Unspecified impurities[b]:	*Unspecified impurities[b]:*
		No individual greater than (b) (4) %, wt/wt	No individual greater than (b) (4) %, wt/wt
		≤ (b) (4) % Total Impurities, wt/wt	≤ (b) (4) % Total Impurities, wt/wt

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Page 1 of 2

Table 1: Drug Substance Specifications

Test	Method	Acceptance Criteria (Release)	Acceptance Criteria (Stability)
Chiral Analysis	Chiral HPLC	(b) (4)	
Assay	RP-HPLC		
Counter Ion	Ion Exclusion HPLC		
Residual solvents	Headspace GC, USP <621>, Ph. Eur. 2.2.28, 2.2.46		
Elemental Impurities	ICP-MS, USP <233> Ph. Eur. 2.2.58		
Residue on Ignition	Gravimetric, USP <281> Ph. Eur. 2.4.14		

[a] NA, method is not stability indicating
[b] Report any impurity above (b) (4)%; Identify any impurity above (b) (4)%

(b) (4) are potential impurities and/or degradants in the DS. The impurities which may be present in eliglustat tartrate DS are classified as follows: organic impurities, degradants and potential degradants, inorganic impurities (heavy metals, etc.) and

residual solvents. Identified organic impurities were also classified as specified or unspecified. The specified impurities have been observed during the manufacturing of (b) (4) Except for one impurity (b) (4) at ≤ (b) (4) % (w/w)], all specified organic impurities had a DS release specification of ≤ (b) (4) % (w/w). Unspecified identified organic impurities were potential impurities for which the chemical structure has been determined but that have not been observed in significant amounts in the DS. All unspecified organic impurities have a DS release specification of ≤ (b) (4) % (w/w).

Organic Impurities: Organic impurities in the DS arising from impurities in the (b) (4) are presented in the following table (from page 6-9 of Section 3.2.S.3.2).

Table 1: Overview of Eliglustat Tartrate Organic Impurities

Identifier	Structure	Type	Step formed in	Control Strategy
Resulting From (b) (4) **Impurities**				
(b) (4)				

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Page 6 of 51

Table 1: Overview of Eliglustat Tartrate Organic Impurities

Identifier	Structure	Type	Step formed in	Control Strategy
(b) (4)		(b) (4)		
Process Related Impurities				
(b) (4)				

Table 1: Overview of Eliglustat Tartrate Organic Impurities

Identifier	Structure	Type	Step formed in	Control Strategy
				(b) (4)
Unreacted Intermediates				
				(b) (4)
By Products				
				(b) (4)

Table 1: Overview of Eliglustat Tartrate Organic Impurities

Identifier	Structure	Type	Step formed in	Control Strategy
Potential Degradants in DS and DP				
(b) (4)				
(b) (4)				

The Applicant provided evaluations of potential genotoxic impurities (b) (4) in the DS. *In silico* (DEREK, Multicase version 2.3, and Leadscope version 1.328) analysis was done for all potential genotoxic impurities. As shown in the footnote of the above table, (b) (4) and (b) (4) were determined to have the potential to be genotoxic as a result of structural analysis; however, *in silico* analysis indicated these impurities are not genotoxic. (b) (4) was determined to have the potential to be genotoxic as a result of structural and *in silico* analysis. However, the results of the Ames assay (Report No. GT-157-TX-56) did not show any mutagenic activity. (b) (4) was determined to have the potential to be genotoxic as a result of structural and *in silico* analysis. However, the results of the Ames assay (Report No. GT-157-TX-55) did not show any mutagenic activity. Impurity (b) (4) was predicted to be negative for the genotoxic endpoint of concern per the draft ICH M7 guideline (i.e., bacterial mutagenicity) by the CDER computational

toxicology consult. In addition, [redacted] (b) (4) could be present in the DS at ≤ [redacted] (b) (4) ppm.

The specifications for elemental impurities in the DS met the acceptance criteria per the draft consensus ICH Guideline for Elemental Impurities Q3D (Current [redacted] (b) (4) version, July 26, 2013) and are acceptable from nonclinical standpoint. The specifications for the residual solvents in the DS are below the current ICH limits and are acceptable.

Overall, the specifications for all organic impurities in the DS at ≤ [redacted] (b) (4)% are below the qualification threshold of [redacted] (b) (4)% and are acceptable. However, impurity [redacted] (b) (4) specification of ≤ [redacted] (b) (4)% is above the qualification threshold of [redacted] (b) (4)%. The Applicant was asked to reduce the level of this impurity at or below the qualification threshold of [redacted] (b) (4)% or provide toxicological qualification of this impurity (Information Request letter dated January 8, 2014). In addition, the Applicant was also asked to provide toxicological qualification of [redacted] (b) (4) at ≤ [redacted] (b) (4) ppm in the drug substance (IR letter dated January 16, 2014). The Applicant provided toxicological qualification of impurity [redacted] (b) (4) at ≤ [redacted] (b) (4)% under SDN 016 dated January 27, 2014. In addition, in reference to the January 16, 2014 Information Request (IR) regarding CMC issues, the Applicant provided responses (SDN 017 dated February 7, 2014) to the outstanding CMC issues including toxicological qualification of [redacted] (b) (4) These are discussed below.

Toxicological Qualification of Impurity [redacted] (b) (4) at ≤ [redacted] (b) (4)% in the DS

The Applicant provided (SDN 016 dated January 27, 2014) toxicological qualification of impurity [redacted] (b) (4) at ≤ [redacted] (b) (4)% in the DS in response to the Agency's IR letter dated January 8, 2014. The impurity [redacted] (b) (4) was present in the DS at ≤ [redacted] (b) (4)%, which is above the ICH qualification threshold of [redacted] (b) (4)%. As mentioned above, the Applicant has conducted an Ames test with this impurity and was found to be negative (GT-157-TX-55).

In addition, eleven toxicology studies (as shown in the Table 1 from page 2 of the submission) were conducted with the Lot No. P0054 which contained the impurity [redacted] (b) (4) [redacted] (b) (4) at [redacted] (b) (4)% including the 52-week oral toxicity study in dogs (GT-157-TX-23) and 26-week oral toxicity study in rats (GT-157-TX-17).

Table 1* - Toxicology Studies Performed with Lot P0054

Batch No.	Purity (%)	Specified Impurities (%)	Study Number	Type of Study
P0054	(b) (4)		GT-157-TX-17	26-Week Toxicity Rat
			GT-157-TX-18	Preliminary Embryo-Fetal Development Rat
			GT-157-TX-19	Preliminary Embryo-Fetal Development Rabbit
			GT-157-TX-20	Embryo-Fetal Development Rat
			GT-157-TX-21	Embryo-Fetal Development Rabbit
			GT-157-TX-23	52-Week Toxicity Dog
			GT-157-TX-24	Fertility and Early Embryonic Rat
			GT-157-TX-25	2-Week Toxicity Mouse
			GT-157-TX-26	4-Day Toxicity Mouse
			GT-157-TX-27	2Week Toxicity Mouse
			GT-157-TX-28	4-Week Mature Rat

*please see Section 2.6.7 Table 5 and the corresponding written summaries in Section 2.6.6 for more information on these studies

The identified NOAELs for eliglustat in the chronic toxicity studies in rats and dogs were 50 and 10 mg/kg/day, respectively. Based on these, the NOAELs for (b) (4) in 6-month study in rats and 9-month study in dogs would be (b) (4) and (b) (4) mg/kg/day, respectively. The recommended dosage of eliglustat is 84 mg BID or 168 mg/day or 2.8 mg/kg/day. The estimated daily exposure to (b) (4) from the recommended dose of eliglustat would be about (b) (4) mg/day or (b) (4) mg/kg/day at ≤ (b) (4)%. The identified NOAELS for (b) (4) in rats (b) (4) mg/kg/day) and dogs (b) (4) mg/kg/day) provide adequate (about 18 and 4-fold, respectively) margins of safety over the estimated maximum exposure (b) (4) mg/kg/day) to (b) (4) from the drug product. Therefore, the level of (b) (4) in the DS at ≤ (b) (4)% is toxicologically qualified and is acceptable.

Toxicological Safety Assessment of (b) (4) in the DS

The Applicant provided toxicological qualification of (b) (4) under SDN 017 dated February 7, 2014 in reference to the Mid-Cycle Communication meeting held on January 9, 2014 between the Agency and Genzyme. Additional reference was made to the meeting minutes from this teleconference in which the ONDQA reviewers asked the Applicant to resolve several significant CMC issues including the toxicological qualification of (b) (4) as discussed above. Reference is also made to the January 16, 2014 Information Request (IR) reiterating these issues. The Applicant provided responses to the outstanding CMC issues including the (b) (4) qualification.

Due to operator safety concerns, (b) (4) The Applicant stated that as this reaction must be performed in (b) (4)

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The Applicant provided a comprehensive toxicological assessment of (b) (4) in this submission (DDN 017 dated February 7, 2014). (b) (4) were also included in this assessment. The toxicity of the potential (b) (4) was not assessed knowing that it is a (b) (4)

The levels of (b) (4) in the DS were provided in Table 1 (from page 10 of the Quality Information amendment of this submission) and levels of (b) (4) were provided in Table 2 (from page 14 of the Quality Information Amendment of this submission) below. The levels of these substances were found to be less than the limit of detection (LOD) of the respective assays for all batches tested.

Table 1: Determination of (b) (4) Levels by the Refined HPLC Analytical Method

Batch	(b) (4) (ppm) by Refined HPLC Analytical Method (LOD (b) (4) ppm)
P0054[a]	ND[c]
Q0858	ND[c]
Q1285	ND[c]
Q1902	ND[c]
S0825	ND[c]
S1517	ND[c]
S1553	ND[c]
T0476	ND[c]
T0509	ND[c]
T1136[b]	ND[c]
U0162	ND[c]
Y0688	ND[c]
Y0695	ND[c]
Y0700	ND[c]

[a] Batch used for rat and dog toxicology studies
[b] Batch used for carcinogenicity study
[c] ND = not detected, LOD = (b) (4) ppm

Table 2: Determination of (b) (4) Levels by GC-MS

Batch	(b) (4) (ppm) by New GC-MS (LOD (b) (4) ppm)	(b) (4) (ppm) by New GC-MS (LOD (b) (4) ppm)
P0054[a]	ND[c]	ND[c]
Q0858	ND[c]	ND[c]
Q1285	ND[c]	ND[c]
Q1902	ND[c]	ND[c]
S0825	ND[c]	ND[c]
S1517	ND[c]	ND[c]
S1553	ND[c]	ND[c]
T0476	ND[c]	ND[c]
T0509	ND[c]	ND[c]
T1136[b]	ND[c]	ND[c]
U0162	ND[c]	ND[c]
Y0688	ND[c]	ND[c]
Y0695	ND[c]	ND[c]
Y0700	ND[c]	ND[c]

[a] Batch used for rat and dog toxicology studies
[b] Batch used for carcinogenicity study
[c] ND = not detected, LOD = (b) (4) ppm

A tabular summary of the toxicity evaluation and its comparison to the worst case amount present in the DS based on the recommended dose of 84 mg BID of eliglustat (100 mg BID eliglustat tartrate) were provided in the following Table (from page 2 of the Toxicology Assessment of the Applicant's submission).

Table 1: Summary of Toxicological Assessment

	(b) (4)
Assay	
LOD	
LOQ	
Highest Level Detected in DS	
Acute (Single dose) LD 50	
NOEL (respiratory, cardiovascular, GI, hepatic renal)	
NOEL (neurological)	
NOEL (reproductive)	
Carcinogenic level	
Genotoxic level	
Worst case amount in DS (per day)	

NOEL: no observed effect level. Worst case values for any species.
NA: not available in public literature
[a] ppm is equivalent to µg/g
[b] Worst case in mice. No carcinogenicity observed in rats

A worst case model for (b) (4) and its potential by-products (b) (4) was calculated for final drug substance based upon the limit of quantitation (LOQ) of the assays. These calculations assumed the maximum theoretical exposure to these substances. Per the Applicant, review of the above theoretical exposure (concentration) against available published literature regarding the toxicities of these chemicals did not raise any significant safety concern. The estimated exposure to (b) (4) from the recommended dose of the drug product were calculated as follows (from page 4, 7 and 15 of the Toxicological Assessment of (b) (4) respectively) assuming LOQ as worse case.

Calculation of the Worst Case Amount of (b) (4) n a Dose (100 mg) of Eliglustat

Assumptions:

(b) (4)

Calculation of the Worst Case Amount of (b) (4) in a Dose (100 mg) of Eliglustat

Assumptions:

(b) (4)

Calculation of the Worst Case Amount of (b) (4) n a Dose (100 mg) of Eliglustat

Assumptions:

(b) (4)

The carcinogenic and mutagenic potential of both (b) (4) were also considered. With respect to (b) (4) it has been included in the Report on Carcinogens (Report on Carcinogens, 12' Edition, 2011, National Toxicology Program, Department of Health and Human Services) as reasonably anticipated to be a human carcinogen. In addition the calculations of worse case amounts of (b) (4) were found to be below those limits that have been shown to potentially be carcinogenic. Overall, as shown in the above Table 3, the worst case amount possible for each potential impurity was found to be well below the allowable limits from a toxicological perspective. Based upon the published information as well as the low levels of detectable impurity, the risks from exposure to (b) (4) from eliglustat tartrate DS was considered to be insignificant.

Overall, the Applicant provided a comprehensive toxicological safety assessment of the possible impurity in the DS, (b) (4), as requested by the Agency. Safety assessment of (b) (4) potential by-products of (b) (4) were also included in this assessment. The toxicity of the potential (b) (4) was not assessed knowing that it is a (b) (4) Assuming worse-case scenario (at LOQ of (b) (4) ppm), the estimated exposures to (b) (4) from the recommended dose of the drug product were calculated to be (b) (4) and (b) (4) µg/kg/day, respectively, or (b) (4) µg per day, respectively. No nonclinical toxicology information is available for (b) (4) in the literature and the Applicant did not provide any nonclinical information for (b) (4). However, (b) (4) is a (b) (4) (b) (4) and a type of (b) (4) composed of (b) (4) (b) (4) The possible carcinogenic and mutagenic potential of (b) (4) was assessed based on the information available for (b) (4) With respect to (b) (4) it has been included in the Report on Carcinogens (Report on Carcinogens, 12 Edition, 2011, National Toxicology Program, Department of Health and Human Services) as reasonably anticipated to be a human carcinogen. It is to be noted here that no (b) (4) was detected in any of the tested batches of the DS. Even in worst case scenario at LOQ of (b) (4) ppm, the above estimated exposure levels for (b) (4) (b) (4) are well below the allowable level of (b) (4) µg per day per the draft ICH M7 guidance [Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, February 6, 2013] and appear to be acceptable.

2.6 Proposed Clinical Population and Dosing Regimen

Cerdelga capsule is indicated for the long-term treatment of adult patients with Gaucher disease type 1 (GD1). The recommended dosage is 100 mg of eliglustat tartrate twice daily (BID).

2.7 Regulatory Background

The following are the major regulatory milestones:

- Type B Meeting (Pre-IND) held on December 15, 2003
- Type C Meeting held on July 17, 2008 [Regarding thorough QT (TQT) clinical study and clinical development program]
- Correspondence regarding Carcinogenicity Protocols (2008-2010)
- End of Phase 2 Meeting (Clinical) held on February 5, 2009
- End of Phase 2 Meeting (CMC) held on May 26, 2010
- Type C Meeting held on April 12, 2011 (Regarding clinical study enrollment challenges and alternate filing strategies)
- Type B Meeting (pre-NDA) held May 21, 2013

3 Studies Submitted

The following table shows the list of nonclinical studies submitted in this application.

3.1 Studies Reviewed

The following table shows the list of studies reviewed.

STUDY TITLE	REPORT NO.	PAGE
PHARMACOLOGY		
ABSORPTION		
In Vitro Evaluation of the Permeability of Genz-99067 using Caco-2 Cells	DMPK10-R047	
Permeability Determination of Eliglustat with Internal Permeability Standards Metoprolol Tartrate and Ranitidine HCl Using the In Situ Single Pass Rat Perfusion Assay	DMPK10-R054	
Pharmacokinetic Study of Genz-99067 Following a Single Intravenous and Oral Administration of Genz-112638 to Sprague-Dawley Rats	DMPK11-R025	
Pharmacokinetic Study of Genz-99067 Following a Single Intravenous and Oral Administration of Genz-112638 to Male and Female Beagle Dogs	DMPK11-R027	
Pharmacokinetic Study of Genz-99067 After a Single Intravenous and Oral Administration of Genz-112638 to Male and Female C57BL/6 Mice	DMPK11-R028	
Pharmacokinetic Study of Genz-99067 Following a Single Intravenous and Oral Administration of Genz-112638 to Male Cynomolgus Monkeys	DMPK11-R059	
DISTRIBUTION		
Quantitative Whole Body Autoradiography of Rats Following Oral Administration of 14C-Genz-112638	DMPK09-R001	
Determination of Red Blood Cell Partitioning of Genz-99067 in Rat, Dog and Human Whole Blood Using [14C]-Genz-112638	DMPK11-R030	
Protein Binding of Genz-99067 in Mouse, Rat, Dog, Monkey and Human Plasma Using Rapid Equil brium Dialysis (RED) and LC MS/MS	DMPK11-R031	
Pharmacokinetics, Tissue Distribution and Mass Balance Study in Sprague-Dawley Rats Following Single Administration of [^{14}C]Genz-112638 by Intravenous Injection and Oral Gavage	GT-157-PK-1	
Pharmacokinetics, Tissue Distribution and Mass Balance of Radioactivity in Sprague-Dawley Rats Following Single Oral Administration of 14C-Genz-112638 With and Without Pretreatment with Unlabeled Genz-112638	GT-157-PK-10	
Quantitative Tissue Distribution Using Whole-Body Autoradiography Following a Single, Oral Dose of [14C]Genz-99067 (125 mg/kg) to Cyclosporin A Pretreated and Untreated Male, CF-1 and Untreated PGP-Deficient Mice	GT-157-PK-17	
METABOLISM		
In Vitro Metabolic Profiles of [14C]Genz-99067 in Rat, Dog, Monkey and Human Liver Microsomes and Hepatocytes	DMPK10-R025	
Exposure of Genz–99067 and Its Metabolites in Rat Following Oral Administration of [14C]–Genz–112638	DMPK11-R019	
Exposure of Genz-99067 and Its Metabolites in Dog Following Oral Administration of [14C]-Genz-112638	DMPK11-R024	
Stability of Genz-99067 in Rat, Dog and Human Whole Blood	DMPK11-R029	
In Vitro Metabolic Stability of Genz-99067 in Mouse, Rat, Dog, Monkey and Human Liver Microsomes	DMPK11-R035	
In Vitro Metabolic Stability of Genz-99067 in Mouse, Rat, Dog, Monkey and Human Hepatocytes	DMPK11-R036	
In Vitro Metabolic Profiles of [14C]-Genz-99067 in Human Recombinant CYP2C19, CYP2D6 and CYP3A4 Enzymes	DMPK11-R043	
Metabolic Profiles of Genz-99067 in Rat Plasma Following Oral Administration of [14C]-Genz-112638/Amendment 1	DMPK11-R044a1	
Metabolic Profiles of Genz-99067 in Dog Plasma Following Oral Administration of [14C]-Genz-112638	DMPK11-R045	

Reference ID: 3501244

Pharmacokinetic Study of Genz-99067 and Its Ten Metabolites Following a Single Oral Administration of Genz-112638 to Male Cynomolgus Monkey	DMPK11-R092	
Pharmacokinetics of Genz-99067 and Its Three Metabolites, Genz-399207, Genz-399240 and Genz-682042, Following a Single Oral Administration of Genz-112638 to Female New Zealand White Rabbits	DMPK12-R003	
Genz-99067 Metabolic Pathway Elucidation Using Cryopreserved Human Hepatocytes Suspensions	DMPK12-R005	
Identification of Metabolites of Genz-99067 in Plasma and Urine from Preclinical Species and Comparison to Human/Amendment 1	DMPK13-R003a1	
EXCRETION		
Pharmacokinetics, Tissue Distribution and Mass Balance Study in Beagle Dogs Following Single Administration of [^{14}C]Genz 112638 by Intravenous Injection and Oral Gavage	GT-157-PK-2	
OTHER PHARMACOKINETIC STUDIES		
Pharmacokinetics of Genz-99067 Following a Single Oral Administration of Genz-112638 to Male and Female D409V/null Mice	DMPK11-R058	
Pharmacokinetic Study of Genz-399240 Following Subcutaneous Administration to Male Sprague-Dawley Rats	DMPK11-R087	
Pharmacokinetic Study of Genz-399240 Following a Single Intravenous or Oral Administration of Genz-399240 to Male Sprague-Dawley Rats	DMPK12-R004	
Prediction of the Pharmacokinetics of Genz-99067, the Free Base of Genz-112638, in Human from In Vitro and In Vivo Pharmacokinetic Data of Genz-99067 in Mouse, Rat, Dog, and Monkey	DMPK12-R062	
TOXICOLOGY		
Acute		
Dog		
Oral	GT-157-TX-4	
Rat		
Intravenous	GT-157-TX-39	
Oral	GT-157-TX-3	
Subacute/Subchronic/Chronic		
Mouse		
Oral, 2-Week	GT-157-TX-25*	
Oral, 4-Day	GT-157-TX-26	
Oral, 2-Week	GT-157-TX-27	
Oral, 13-Week	GT-157-TX-37	
Rat		
Oral, 28-Day	GT-157-TX-5	
4-Week, Oral	GT-157-TX-28	
Oral, 26-Week	GT-157-TX-17	
Dose-Ranging study, Juvenile rats, oral	GT-157-TX-47	
10-Week, juvenile rats, oral	GT-157-TX-48	
13-Week, metabolite, subcutaneous	GT-157-TX-61	
Dog		
28-Day, Oral	GT-157-TX-6	
13-Week, Oral	GT-157-TX-15	
52-Week, Oral	GT-157-TX-23	
GENOTOXICITY		
Ames test	GT-157-TX-1	
Chromosome aberration test	GT-157-TX-2	
Mouse oral bone marrow micronucleus test	GT-157-TX-16	
Ames test, Metabolite	GT-157-TX-60	
Chromosome aberration test, metabolite	GT-157-TX-62	
Ames test, impurity	GT-157-TX-55	
Ames test, impurity	GT-157-TX-66	
CARCINOGENICITY		
Mouse, Oral	GT-157-TX-43	
Rat, Oral	GT-157-TX-38	
REPRODUCTIVE TOXICITY		

Rat		
Fertility and early embryonic development	GT-157-TX-24	
Embryofetal development, preliminary	GT-157-TX-18	
Embryofetal development	GT-157-TX-20	
Pre and postnatal development	GT-157-TX-29	
Rabbit		
Embryofetal development, preliminary	GT-157-TX-19	
Embryofetal development	GT-157-TX-21	
Monkey		
4-Week, Oral	GT-157-TX-31	
SPECIAL TOXICOLOGY		
A Pilot Study to Compare the Behavioral Outcomes following Drug-Induced Peripheral Neuropathy in the Rat	GT-157-TX-22	
Review of Lymphoid Tissues from Six Studies Performed in the Dog or Rat with Genz-112638	GT-157-TX-46	

3.2 Studies Not Reviewed

Analytical method validation studies in Section 4.2.2.1 of this submission were not reviewed.

3.3 Previous Reviews Referenced

Pharmacology reviews of IND 67,589 are incorporated in the appropriate sections of this review.

4 Pharmacology

4.1 Primary Pharmacology

The following review is incorporated below from the pharmacology review of IND 67,589 dated July 30, 2004 by Dr. Dylan Yao of Division of Metabolism and Endocrinology Products (DMEP).

2.6.2.2 Primary pharmacodynamics

Mechanism of action: Genz-112638 acts as a [redacted] (b) (4) mimic of the glucosylceramide synthase [redacted] (b) (4) substrate and thus specifically inhibits glucosylceramide synthesis.

Drug activity related to proposed indication:

In vitro (non-GLP studies)**:**
Glucosylceramide synthase was inhibited by Genz-112638 in a dose-dependent manner in both dog kidney cell homogenates (IC50=115 nM) and whole human K562 cells (IC50=14 nM). The IC50 value for intact canine DH82 cells was 79 nM suggesting that Genz-112638 inhibits the canine and human forms of glucosylceramide synthase with similar potency.

In vivo (non-GLP studies):
It decreases glucosylceramide levels in various tissues from normal mouse (at 75, 125, 175 mg/kg QD for 56 days), rat (at 75, 250 or 500 mg/kg QD for 3 days) and dog (at 2.5, 5 and 12.5 mg/kg BID for 3 days) after oral gavage dosing suggesting that this compound was an inhibitor of glucosylceramide synthase when administered orally.

In male SD rats, significantly lower levels of glucosylceramide were observed in liver, kidney and spleen after oral gavage dosing of Genz-112638 at 37.5, 125, and 250 mg/kg, BID for three days. Significant depletion of glucosylceramide levels (~50% in the liver and kidney and ~20% in the spleen) were observed at the low dose of 37.5 mg/kg BID where no adverse effects were recorded.

Genz-112638 was given to a murine model of Fabry Disease (complete α-galactosidase knock-out) in 2 cohorts: monotherapy and maintenance therapy, GL3 levels were determined for testing the efficacy. In the monotherapy, Fabry mice were daily dosed by oral gavage at 0, 75, 125 or 175 mg/kg for 56 days. In the maintenance therapy, Fabry mice were treated with a single IV dose of α-galactosidase (1 mg/kg) prior to dosing with Genz-112638 for 56 consecutive days by oral gavage at 0, 75, 125 or 175 mg/kg. Genz-112638 was well tolerated in both study cohorts. In the monotherapy, the concentration of GL3 in the kidney was significantly lower in all treated groups compared to controls ($p<0.0001$). GL3 concentrations in liver were similar in all groups, indicating Genz-112638 was more effective in the kidney compared to the liver in the monotherapy cohort. In the maintenance cohort, Genz-112638 appeared to inhibit the re-accumulation of GL3 levels compared to controls in a dose related manner but the values were not statistically significant (large variability in the data set), and it seems more effective in the liver compared to the kidney in the maintenance therapy cohort.

In the Beagle dog, oral gavage dosing of Genz-112638 at 0, 2.5, 5 or 12.5 mg/kg BID for 3 days induced decreases of glucosylceramide levels in liver homogenates (52, 55 and 40% of vehicle levels, respectively), $p<0.01$ for all doses.

Evaluation of the Efficacy of Genz-112638 in Treating D409V/null Gaucher Mice (GT-157-EF-12)

The objective of this study was to examine the effects of repeated oral (gavage, 10 mL/kg) treatment of Genz-112638 on tissue glucosylceramide (GL-1) accumulation, histology and Gaucher cell formation in D409V/null Type 1 Gaucher Mice (male and female, n = 4/sex/dose). D409V/null mice are a heterozygous model of Type 1 Gaucher. Glucosylceramide in these mice begins to accumulate starting at three months of age and Gaucher cells begin to appear by four months of age. At year one, GL-1 in peripheral tissues is generally 16-fold above levels in wild type mice, and Gaucher cells are present in large numbers, particularly in the liver and lung. There is no accumulation of GL-1 in the brain, and the mice have a normal life span. D409V/wild type mice have normal levels of GL-1, do not form Gaucher cells, and have a normal life span.

In this study, Genz-112638 was administered once daily for 5 or 10 weeks starting at 7 month of age. Animals were dosed once each day for 3 days at 75 mg/kg, followed by 3 days at 100 mg/kg, and 3 days at 125 mg/kg, followed by 5 weeks or 10 weeks at the full dose of 150 mg/kg. Following the completion of dosing tissue samples were collected for histological and glycolipid analysis according to the study design shown in the table below. D409V/wild type controls (n =5) were included to determine the degree of lipid reduction compared to normal levels of GL-1. The study design is shown below (from page 5 of the report).

Reference ID: 3501244

Table 1: Study Design

Group #	Type of Mice	# of Mice	Gavage acclimation	Step-up Oral Doses	Full Oral Dose	Date of Sacrifice	Tissue Collection For GL-1 analysis *	Tissue Collection For histology
A	D409V / null	1 F 4 M	-	NA	NA	≤ 24 hours prior to start of compound dosing	Liver, Spleen, Lungs	Liver, Spleen, Lungs, Bone
B	D409V / wild type	2 F 3 M	-	NA	N/A	≤ 24 hours prior to start of compound dosing	Liver, Spleen, Lungs	Liver, Spleen, Lungs, Bone
C	D409V / null	4 F 4 M	1 week dosing with sterile water	75-100-125 mg/kg, 3 days each dose	150 mg/kg (full dose)	5 Weeks Post Full Dose	Liver, Spleen, Lungs	Liver, Spleen, Lungs, Bone
D	D409V / null	4 F 4 M	1 week dosing with sterile water	NA	0	5 Weeks Post Full Dose	Liver, Spleen, Lungs	Liver, Spleen, Lungs, Bone
E	D409V / null	4 F 4 M	1 week dosing with sterile water	75-100-125 mg/kg, 3 days each dose	150 mg/kg (full dose)	10 Weeks Post Full Dose	Liver, Spleen, Lungs	Liver, Spleen, Lungs, Bone
F	D409V / null	6 F 2 M	1 week dosing with sterile water	NA	0	10 Weeks Post Full Dose	Liver, Spleen, Lungs	Liver, Spleen, Lungs, Bone
G	D409V / wild type	4 F 1 M	1 week dosing with sterile water	NA	0	10 Weeks Post Full Dose	Liver, Spleen, Lungs	Liver, Spleen, Lungs, Bone

* Notes:

1. As listed in the study protocol kidney also was collected for biochemical analysis of GL-1. In the D409V/null mice kidney Gl-1 is highly variable and not informative for disease changes or treatment effects. Results are not shown in this report. Data is in notebook #14534 pages 130-131.

2. Bone marrow is listed for GL-1 analysis in the study protocol. However not enough sample was obtained for the analysis to be done.

There were no significant treatment-related clinical signs or effects on body weight. Both the number of Gaucher cells and accumulated GL-1 were reduced in the treated animals compared to the vehicle controls at 5 or 10 weeks following treatment. As compared to vehicle controls, GL-1 was reduced by 50-60% in the lung, 55-65% in the liver, and 45-50% in the spleen following treatment with Genz-112638 as shown in the following table (from page 10 of the report).

Table 2A
Levels of GL-1 in Liver Following 5 and 10 Weeks of Dosing with Genz-112638

		ng GL-1/mg tissue		
Group #	**Liver**	Average	std error	
A	9v/null naïve	67.04	9.66	
B	9v/wt naïve	16.96	2.14	
C	9v/null 112638 (5 weeks)	98.45	33.19	*p=0.0528*
D	9V/null vehicle (5 weeks)	226.54	28.90	
E	9v/null 112638 (10 weeks)	70.00	8.34	*p=0.0004*
F	9V/null vehicle (10 weeks)	197.91	22.32	
G	9v/wt naïve (10 weeks)	12.04	1.15	

Table 2B
Levels of GL-1 in Spleen Following 5 and 10 Weeks of Dosing with Genz-112638

		ng GL-1/mg tissue		
Group #	**Spleen**	average	std error	
A	9v/null naïve	286.48	37.53	
B	9v/wt naïve	61.25	6.25	
C	9v/null 112638 (5 weeks)	270.94	45.27	*p=0.0037*
D	9V/null vehicle (5 weeks)	490.13	65.82	
E	9v/null 112638 (10 weeks)	327.56	25.74	*p=0.0092*
F	9V/null vehicle (10 weeks)	541.64	51.71	
G	9v/wt naïve (10 weeks)	51.99	5.02	

Table 2C
Levels of GL-1 in Lung Following 5 and 10 Weeks of Dosing with Genz-112638

		ng GL-1/mg tissue		
Group #	**Lung**	average	std error	
A	9v/null naïve	360.18	78.53	
B	9v/wt naïve	22.43	7.48	
C	9v/null 112638 (5 weeks)	274.52	55.09	*p=0.0429*
D	9V/null vehicle (5 weeks)	489.19	54.53	
E	9v/null 112638 (10 weeks)	339.06	62.41	*p=0.0074*
F	9V/null vehicle (10 weeks)	665.32	32.70	
G	9v/wt naïve (10 weeks)	35.85	10.29	

The number of Gaucher cells in the liver was significantly reduced by the treatment compared to that of the control. Gaucher cell number was reduced by 60% at 5 weeks and 72% at 10 weeks following treatment with Genz-112638 when compared to control. Gaucher cells could not be quantitated in the lung and spleen; however Gaucher cells were identified more frequently in the vehicle control animals as compared to animals treated with Genz-112368. The data are shown in the following table (from page 12 of the report).

Figure 3: Quantitation of Gaucher Cells in Liver Following Dosing with Genz-112638

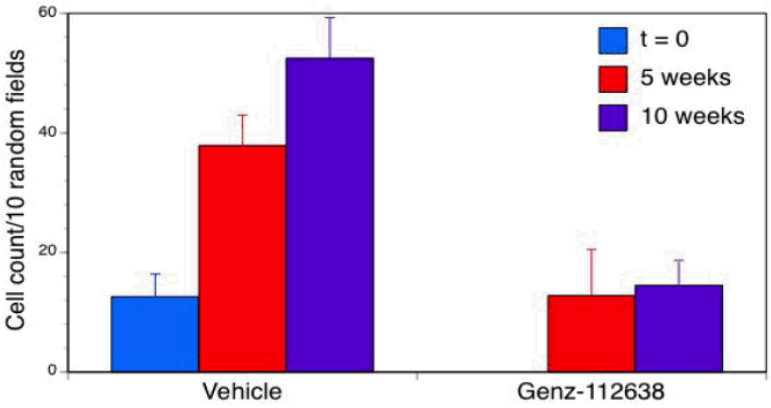


Table 4: Mean Gaucher Cell Count in Liver Following Dosing with Genz-112638

D409V/null Mice	Gaucher Cells		Std. error	
Study Day	Vehicle control	Genz-112638	Vehicle control	Genz-112638
0	13		4	
35	38	13	5	8
70	53	15	7	4

Plasma GL-1 levels were assessed at five week time point in Genz-112638 treated, vehicle control D409V/null Gaucher mice and D409V/WT controls**.** There was no significant difference in plasma GL-1 between D409V/null Gaucher mice and D409V/WT controls. Additionally there was no measurable reduction in the plasma GL-1 in D409V/null mice treated with Genz-112638 for five weeks compared to vehicle controls; treatment with Genz-112638 did not reduce plasma GL-1 to below the normal levels present in the D490V/null mice. The reasons for lack observable changes in the plasma GL-1 are not known. The data are shown in the following table (from page 11 of the report).

Table 3		Plasma GL-1	
	Dose group	average (ug/mL)	std error
A	D409V/null naïve - baseline	2.09	0.16
B	D409V/wt naïve - baseline	2.75	0.31
C	D409V/null - Genz-112638 5 wks	1.65	0.24
D	D409V/null - vehicle 5 wks	1.68	0.27

Additionally, lungs were processed for broncho-alveolar gavage fluid (BALF) collection. Neutrophils and multi-nucleated macrophages in BALF were increased several fold in the D409V/null compared to D409V/WT control mice. The increase in BALF neutrophil count in the D409V/null mice was slowed by treatment with Genz-112638 at both 5 and 10 weeks; however, levels were not normalized. There was no effect of treatment on macrophage and multinucleated (MN) macrophage counts. These values were variable and decreased in all groups at 5 and 10 weeks, thus a treatment effect could not be established. The following figure and table (from page 13 of the report) show the data.

Figure 4: Cell differentials in BALFs following Genz-112638 treatment

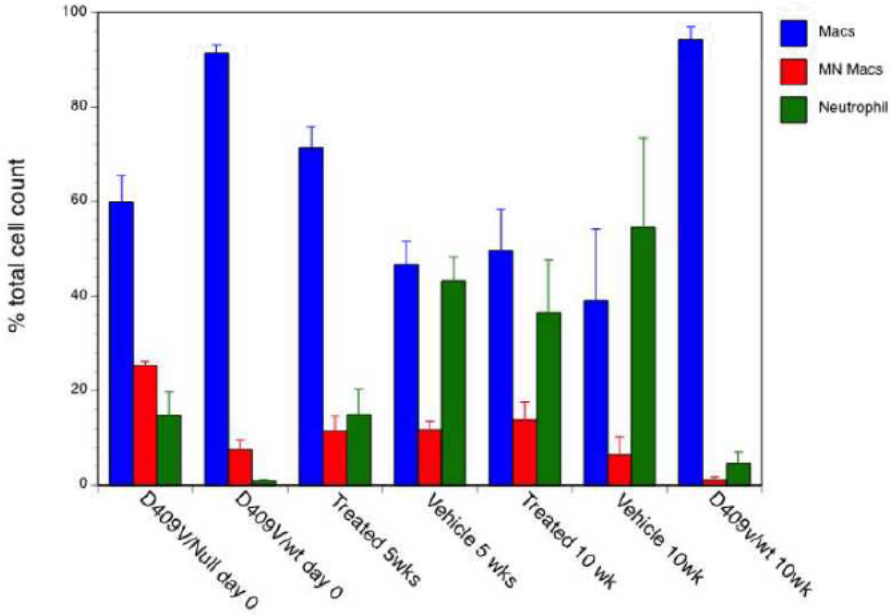


Table 5: Cell differentials in BALFs following Genz-112638 treatment

Treament Group	Dose Group Mean Values			Standard Error		
	Macs	MN Macs	Neutrophil	Macs	MN Macs	Neutrophil
D409V/Null day 0	59.80	25.36	14.84	5.70	0.86	4.84
D409V/wt day 0	91.49	7.55	0.96	1.74	1.99	0.26
Treated 5wks	71.35	11.51	14.99	4.44	3.08	5.40
Vehicle 5 wks	46.70	11.77	43.28	4.94	1.81	5.05
Treated 10 wk	49.63	13.88	36.50	8.71	3.73	11.10
Vehicle 10wk	39.00	6.50	54.50	15.18	3.75	18.91
D409v/wt 10wk	94.20	1.14	4.66	2.86	0.59	2.36

The Inhibition of GM1 Accumulation in Erythropoietic Cell Lines from Dog and Human in the Presence of Genz-112638 (GT-157-EF-2)

The objective of this study was to demonstrate the inhibition of glucosylceramide synthase in the dog and human erythropoietic cell preparations in the presence of Genz-112638. The inhibition of glycosphingolipid biosynthesis by Genz-99067 (active free base of Genz-112638) was determined by quantitating GM1 (monosialotetrahexosylganglioside) on human K-562 cells (erythroleukemic myeloid cell line) and on canine DH-82 cells (immortalized macrophage cell line). GM1 is a complex glycosphingolipid synthesized from glucosylceramide in four sequential enzymatic steps. In this study, K562 or DH82 cells were incubated at 37^0C for 3 days with Genz-99067 at final concentrations of 0.039 to 2500 nM (K562 cells) or 0.625 to 10000 nM

(DH82 cells). Assay buffer was used as the vehicle control. Following incubation, the cells were harvested and assayed for GM1 expression using the cholera toxin B subunit labeled with fluorescein isothiocyanate (FITC). The level of fluorescence was quantitated using fluorescence activated cell sorting (FACS).

Genz-99067 showed a dose related inhibition of GM1 synthesis in human K562 cells and in canine DH-82 cells. The IC_{50} values in the K562 cells were 24.0 and 33.1 nM for assays number 1 and 2, respectively. The IC_{50} values in the DH82 cells were 59.4 nM and 96.6 nM for assays number 1 and 2, respectively. There was no treatment-related effect on cell viability.

Evaluation of the Efficacy of Genz-112638 Delivered in Feed Compared to Delivered by Oral Gavage in Treating D409V/null Gaucher Mice (GT-157-EF-31)

The objective of this study was to compare efficacy and tolerability of Genz-112638 following treatment for 7 weeks, with dosing either once daily by oral gavage (10 mL/kg) at 150 mg/kg, or mixed in feed daily at 150, 300 or 450 mg/kg. Dosing was initiated starting at 10 weeks of age, at which time the D409V/null Gaucher mice have little measurable GL-1 (glucosylceramide) and no Gaucher cells. This study was designed to examine the effectiveness Genz-112368 monotherapy in preventing accumulation of GL-1 and formation of Gaucher cells. Additionally, the other objectives of this study were to understand the effects on tolerability and efficacy of the lower C_{max} and extended exposure when the drug was administered in feed as compared to by oral gavage.

In this study, 19 male and 11 female D409V/null mice were assigned to five treatment groups of 6 animals each as shown in the table below (from page 5 of the report). Three groups were administered Genz-112638 mixed in feed at 150, 300 or 450 mg/kg/day and a fourth group was administered diet alone as vehicle control. The fifth group was treated by oral gavage, at the maximum tolerated dose (MTD) of 150 mg/kg. All mice were treated for 7 weeks. Following the completion of the treatment tissue samples (liver, lung and spleen) were collected for histological and glycolipid analysis according to the study design. In addition, liver sections were labeled using a monoclonal anti-CD68 antibody in order to highlight Gaucher cells.

Table 1: Study Plan

Group #	Type of Mice	# of Mice	Gavage acclimation	Step-up Oral Doses	Full Oral Dose	Date of Sacrifice	Tissue Collection lipid analysis	Tissue Collection histology	Plasma Collection PK analysis
A	D409V / null	3 F 3 M	1 week dosing with sterile water	75-100-125 mg/kg, 3 days each dose (study days 1-7)	150 mg/kg once daily for 6 weeks	7 Weeks after the start of Oral Gavage Dosing (st udy day 1 –study day 49)	Liver, Lung, Spleen	Liver, Spleen, Lungs	2 hours post gavage Study days 7, 21, 35, 49
B	D409V / null	3 F 3 M	NA	NA	150 mg/kg /day	After 7 weeks of dosing (study day 49)	Liver, Lung, Spleen	Liver, Spleen, Lungs	~8 am Study days 7, 21, 35, 49
C	D409V / null	3 F 3 M	NA	NA	300 mg/kg /day	After 7 weeks of dosing (study day 49)	Liver, Lung, Spleen	Liver, Spleen, Lungs	~8 am Study days 7, 21, 35, 49
D	D409V / null	2 F 4 M	NA	NA	450 mg/kg /day	After 7 weeks of dosing (study day 49)	Liver, Lung, Spleen	Liver, Spleen, Lungs	~8 am Study days 7, 21, 35, 49
E	D409V / null	6 M	NA	NA	(Diet alone)	After 7 weeks of dosing (study day 49)	Liver, Lung, Spleen	Liver, Spleen, Lungs	~8 am Study days 7, 21, 35, 49

Note: brain and CSF collection were listed in study protocol in addition to the other tissues. However given this is a Gaucher Type 1 model, analysis of histology or GL-1 in brain will provide little useful information, so was not collected.

There was no significant treatment-related effect on clinical signs or body weight. CD68 staining in the liver was significantly lower in Groups A (150 mg/kg via oral gavage) and D (450 mg/kg in food) compared to the control Group E. CD68 staining in the liver was comparable in Groups A (150 mg/kg via gavage), B (150 mg/kg in food), and D (450 mg/kg in food). CD68 staining in the liver was significantly higher in Group C (300 mg/kg in food) compared to Group A (150 mg/kg via oral gavage). There were no statistical differences in CD68 staining in the liver between any of the groups with Genz-112638 delivered in the food.

In summary,

- There was a statistically significant reduction in CD68 staining in the liver following treatment at 150 mg/kg (oral gavage) and at 450 mg/kg of Genz-112638 (in food) compared to controls.
- There was no statistically significant difference in CD68 staining in the liver following administration of 150 mg/kg and 300 mg/kg of Genz-112638 via the food compared to untreated controls.

- There was a statistically significant reduction in CD68 staining in the liver at 150 mg/kg of Genz-112638 administered by oral gavage compared to 300 mg/kg administered in the food.
- The mid and high doses (300 and 450 mg/kg, respectively) of Genz-112638 administered in the food reduced GL-1 accumulation in the lung.
- Gaucher cells were observed in the spleens of the untreated control group (Group E) only.

In conclusion, Genz-112638 administered in food was effective at reducing GL-1 accumulation in D409V/null mice. However, higher doses were required to achieve a similar reduction in GL-1 accumulation in all tissues as achieved with oral gavage administration of Genz-112638.

Evaluation of the prevention of GL-1 accumulation in 10 week old D409V/null mice given Genz-112638 in feed or by oral gavage (GT-157-EF-33)

The objective of this study was to compare the effects of Genz-112638 on the levels of tissue GL-1 at the MTD by oral gavage (150 mg/kg/day) to the MTD by diet (450 mg/kg/day) in mice. This study included a genetically matched control with normal levels of GL-1, to enable comparison of GL-1 following treatment with Genz-112638 versus normal levels in tissues. In this study, Gaucher D409V/null mice (n = 6/group) of either sex (age ~10 weeks) were assigned to four groups (A-D) including a control group (E) of D409V/WT mice (that do not accumulate GL-1). Group A was sacrificed at t=0 (10 weeks old) to provide baseline tissue GL-1 levels. Group B received once a day oral gavage of 150 mg/kg Genz-112638 (10 mL/kg) for 8 weeks. Group C were fed with Genz-112638 as 0.225% in the diet for 8 weeks. Groups D (D409V/null) and E (D409V/WT) received no treatment. Groups B-E were sacrificed at day 56 and tissues (liver, lung, and spleen) were collected for GL-1 analysis and processed for histopathology. The following table (from page 3 of the report) shows the study design.

Table 1. Study design summary table

Group #	Mouse type	Treatment / duration	Animals Per Group	Animals Per Time Point	Comments
A	D409V/null	None / sacrificed at t=0	6	6	sacrifice at start of study
B	D409V/null	150 mg/kg Genz-112638 QD by oral gavage / 8 weeks	6	6	sacrifice at end of 8 weeks
C	D409V/null	450 mg/kg Genz-112638 dosed in food / 8 weeks	6	6	sacrifice at end of 8 weeks
D	D409V/null	No treatment / 8 weeks	6	6	sacrifice at end of 8 weeks
E	D409V/wt	No treatment / 8 weeks	6	6	sacrifice at end of 8 weeks

GL-1 levels in the D409V/null mice at 150 mg/kg/day by oral gavage were reduced by 14%, 50% and 36% in the liver, lung and spleen, respectively, as compared to vehicle controls. Significantly greater efficacy was observed at 450 mg/kg/day of Genz-112638 in the feed, with GL-1 normalized in the liver, lung and spleen following the 8 weeks of treatment. GL-1 was reduced by 71%, 73% and 73% in liver, lung and spleen, respectively, as compared to vehicle controls. In the liver, D409V/null mice treated with Genz-112638 either by oral gavage or in the food had a significantly lower area of CD68 staining compared to mice at t=0, indicating debulking was achieved with both routes of administration. However, quantitative analysis of GL-1 showed a significantly greater debulking of GL-1 at 450 mg/kg in the feed (73% from baseline) versus 150 mg/kg by oral gavage (16% from baseline). In the spleen and in the lung, Gaucher cells were unevenly distributed, thus the CD68 staining was not informative in these tissues.

<u>Combination therapy in D409V/null mice: IV dosing of Cerezyme followed by food dosing of Genz-112638 (GT-157-EF-35)</u>

The objective of this study was to determine if Genz-112638 can maintain the reduced levels of GL-1 achieved following Cerezyme (recombinant human glucocerebrosidase) administration. The secondary goal was to confirm Genz-112638 efficacy as monotherapy at 150 mg/kg/day oral dose, and compare that efficacy to the dose groups receiving Genz-112638 as maintenance therapy following Cerezyme.

In this study, Gaucher D409V/null mice of either sex (aged 4.5 to 6 months) were assigned to 10 groups (A-J). There were 4-6 mice/group. Groups A, C, D, G and H were treated with Cerezyme at single doses of 10 mg/kg (5 mL/kg) by injection (tail vein) on study days 1, 4, 8 and 11. Groups C and E were treated with Genz-112638 in the diet (*ad libitum*) for 5 weeks and Groups G and I were treated with Genz-112638 for 10 weeks starting at study day 12. Group B animals received the vehicle by tail-vein

injection, and Groups F and J were untreated. Groups A and B were euthanized at study day 16 to provide baseline tissue GL-1 levels post Cerezyme debulking (Group A) or vehicle control (Group B). Groups C-F were euthanized at study day 49 (5 weeks of Genz-112638 treatment for Groups C and E) and groups G-J were euthanized at study day 84 (10 weeks of Genz-112638 treatment for Groups G and I). At scheduled sacrifice time points, tissues (liver, lung, and spleen) were collected for GL-1 analysis and were processed for histopathology. Tissue sections were labeled with anti-CD68 monoclonal antibody to quantitate CD68 staining. CD68 is a general macrophage cell surface marker. Amount and intensity of staining correlates with the expansion of cell volume and surface area in Gaucher cells. The following table (from page 4 of the report) shows the study design.

TREATMENT

Study design summary table

Group #	Treatment	Duration	Animals Per Group	Animals Per Time Point	Comments
A	10mg/kg Cerezyme, tail vein injection	QD on study days 1, 4, 8, 11	4	4	Debulking baseline control, sacrifice at 2 weeks, study day 16
B	Vehicle, tail vein injection	QD on study days 1, 4, 8, 11	4	4	Untreated baseline control, sacrifice at 2 weeks, study day 16
C	10 mg/kg Cerezyme, tail vein injection 150 mg/kg/day Genz-112638 in feed	Cerezyme: QD on study days 1, 4, 8, 11 ; Genz-112638: starting on study day 12 for 5 weeks	6	6	Maintenance therapy dose group, sacrifice at 7 weeks, study day 49
D	10 mg/kg Cerezyme, tail vein injection	QD on study days 1, 4, 8, 11	5	5	Cerezyme-only dose group, sacrifice at 7 weeks, study day 49
E	150 mg/kg Genz-112638 in feed	Starting on day 12 for 5 weeks	5	5	Genz-112638 monotherapy, sacrifice at 7 weeks, study day 49
F	Untreated	Normal diet for 7 weeks	4	4	Age-matched control, sacrifice at 7 weeks, study day 49
G	10 mg/kg Cerezyme, tail vein injection 150 mg/kg/day Genz-112638 in feed	Cerezyme: QD on study days 1, 4, 8, 11 ; Genz-112638: starting on study day 12 for 10 weeks	6	6	Maintenance therapy dose group, sacrifice at 12 weeks, study day 84
H	10 mg/kg Cerezyme, tail vein injection	QD on study days 1, 4, 8, 11	5	5	Cerezyme-only dose group, sacrifice at 12 weeks, study day 84
I	150 mg/kg Genz-112638 in feed	Starting on day 12 for 10 weeks	5	5	Genz-112638 monotherapy, sacrifice at 12 weeks, study day 84
J	Untreated	Normal diet for 12 weeks	4	4	Age-matched control, sacrifice at 12 weeks, study day 84

Cerezyme treatment successfully reduced GL-1 levels to ~20% of the untreated animals in both liver and spleen, but did not clear lung GL-1 (group A versus B). In the liver and spleen, animals treated with Cerezyme with no further treatment showed significant re accumulation of GL-1 over 5 and 10 weeks (Groups D and H). Genz-112638 treatment subsequent to Cerezyme treatment prevented re-accumulation of GL-1 after 5 weeks of treatment in the liver and spleen (Group C). After 10 weeks of treatment, Genz-112638 delayed (but did not fully prevent) re-accumulation of GL-1 (Group G). Treatment with Genz-112638 alone decreased levels of GL-1 in the liver and spleen at both 5 and 10 weeks of dosing (Groups E and I) relative to untreated controls. In the lung, Cerezyme treatment did not reduce levels of GL-1 (Groups A versus B). However Genz-112638

(monotherapy) decreased GL-1 by 40-65% compared to controls (Groups E and I versus F and J).

Groups A and B, and G-J were evaluated for CD68 positive staining. CD68 results were provided for the liver. Due to low disease induction and uneven distribution of cells in the spleen and lung, CD68 staining was not informative in these tissues. In the liver, there were no statistically significant differences in mean percent area CD68 positive between treatment groups evaluated, which were considered most likely due to the low disease induction. However, there were following notable trends:

- Animals treated with Genz-112638 for 10 weeks, either as monotherapy (Group I) or following treatment with Cerezyme (Group G) showed low levels of CD68 staining compared to animals immediately following treatment with Cerezyme (Group A).
- Additionally, all animals treated with Genz-112638 had lower CD68 staining than untreated controls (Groups B and J)
- The degree of CD68 positive staining was similar in animals administered Cerezyme with no further treatment at 10 weeks and in the vehicle controls at baseline (Groups H and B).

In conclusion, treatment of Gaucher D409V/null mice with Cerezyme cleared most of the accumulated GL-1 from the liver and spleen but not in the lung. This was probably attributed to inaccessibility of circulating enzyme to alveolar macrophages, the site of the majority of lung storage. Treatment with Genz-112638 at 150 mg/kg/day in the diet was effective at maintaining or reducing tissue levels of GL-1 whether the mice were naïve to treatment or debulked of stored substrate through prior treatment with Cerezyme. Genz-112638 at 150 mg/kg/day was effective in preventing accumulation of GL-1. The liver histology results suggested that both Cerezyme and Genz-112638 can effectively reduce the amount of GL-1 storage in the Gaucher mouse liver.

<u>Evaluation of the Efficacy of Cerezyme Therapy Followed Genz-112638 Therapy in Treating the D409V/null Gaucher mice (GT-157-EF-41)</u>

This study examined the ability of Genz-112638 to maintain reduced levels of tissue GL-1, following debulking with Cerezyme in Gaucher mice (aged 2 to 3 months, n= 4-6/group). Genz-112638 treatment was started the day after Cerezyme treatment was terminated, and was administered in the diet at 150 mg/kg/day or 450 mg/kg/day. Two monotherapy dose groups also received 150 or 450 mg/kg/day Genz-112638 in the feed, with the goal of comparing Genz-112638 efficacy as monotherapy versus the same doses given after Cerezyme debulking. Cerezyme was administered as a single tail vein injection once daily, eight times over 2 weeks with at least 48 hours between doses (Groups A, C-E, I, K-M). Genz-112638 in the feed was provided freely (*ad libitum*) for 5 weeks (groups K & L) or 10 weeks (groups C & D), beginning the day following the last Cerezyme dose. Genz-112638 in the feed was provided freely (*ad libitum*) for 12 weeks beginning on study day 1 (groups F and G). The following table shows the study design (from page 5 of the report).

TREATMENT

Study design summary table

Group #	Treatment	Duration	Animals Per Group	Animals Per Time Point	Comments
A	10 mg/kg Cerezyme (CZ)	QD, 8 doses over two weeks	4	4	Initiate CZ dosing at same time as group C-E; Sacrifice 6 days after last dose
B	Untreated (naive)	Normal diet for 2 weeks	4	4	Sacrifice with group A
C	10 mg/kg Cerezyme + 150 mg/kg Genz-112638 in diet	CZ 8 doses over 2 weeks Genz-112638: Begin the day after last CZ dose; for 10 weeks	6	6	Sac 10 weeks after Genz-112638 dosing initiated
D	10 mg/kg Cerezyme + 450 mg/kg Genz-112638	CZ 8 doses over 2 weeks Genz-112638: Begin the day after last CZ dose; for 10 weeks	6	6	Sac 10 weeks Genz-112638 dosing initiated
E	10 mg/kg Cerezyme only	CZ 8 doses over 2 weeks; standard diet (no further treatment) for 10 weeks	5	5	CZ only control; sac 10 weeks after last CZ dose
F	Genz-112638 only, 150 mg/kg/day	Begin dosing at study day 1	5	5	Sac 12 weeks after dosing initiated
G	Genz-112638 only, 450 mg/kg/day	Begin dosing at study day 1	5	5	Sac 12 weeks after dosing initiated
H	Untreated	Standard diet for 12 weeks	5	5	Untreated control; Sacrifice with groups C – G
I	10 mg/kg Cerezyme	QD, 8 doses over two weeks;	4	4	Initiate CZ dosing at same time as group K-M; Sacrifice 6 days after last dose
J	Untreated	Standard diet for 2 weeks	4	4	Sacrifice with group I
K	10 mg/kg Cerezyme + 150 mg/kg Genz-112638	CZ 8 doses over 2 weeks Genz-112638: Begin the day after last CZ dose; for 5 weeks	6	6	Sac 5 weeks after Genz-112638 dosing initiated
L	10 mg/kg Cerezyme + 450 mg/kg Genz-112638	CZ 8 doses over 2 weeks Genz-112638: Begin the day after last CZ dose; for 5weeks	6	6	Sac 5 weeks after Genz-112638 dosing initiated
M	10 mg/kg Cerezyme only	QD, 8 doses over two weeks; standard diet (no further treatment) for 5 weeks	6	6	CZ only control; sac 5 weeks after last CZ dose
N	Untreated	Standard diet for 7 weeks	5	5	Untreated control; Sacrifice with groups K – M

Treatment of Gaucher D409V/null mice with Cerezyme reduced most of the accumulated GL-1 in the liver and spleen as observed in the previous study (GT-157-EF-35). Animals treated with Cerezyme with no further treatment showed a significant re-accumulation of GL-1. Both doses of Genz-112638 maintained the reduced levels of GL-1 from the Cerezyme treatment. The higher dose of Genz-112638 (450 mg/kg/day)

as maintenance therapy provided additional debulking at both 5 and 10 weeks. Monotherapy with 450 mg/kg of Genz-112638 provided debulking from baseline, and was equally effective with Cerezyme. As mentioned before, in the lungs, enzyme has poor distribution to alveolar macrophages, the site of the majority of lung storage. In this study, the eight doses of Cerezyme over two weeks was able to partially clear GL-1 in the lung, however Genz 112638 as monotherapy or post-Cerezyme appeared to be more effective.

4-Day Efficacy Study with Genz-112638 by Oral Administration in Rats for Exposure and Biomarkers (GT-157-EF-55)

The goal of this study is to examine *in vivo* activity and exposure of Genz-112638 in the plasma following oral dosing at or below the NOAEL for four days in male Sprague Dawley (SD) rats. In this study, male rats (n = 4/dose) were assigned to four groups of four animals per group. Test articles were administered by oral gavage (5 mL/kg) once daily for four days at 10, 25 and 50 mg/kg/day. Blood was collected at the following time points for plasma exposure and biomarker (GL1) analysis: 1 hour post dose 1 (study day 1), 24 hour post dose 1 (study day 2), 1 hour post dose 4 (study day 4), 2 hour post dose 4 (study day 4), 6 hour post dose 4 (study day 4), and 24 hour post dose 4 (study day 5).

Genz-112638 reduced plasma GL1 by a maximum of 30% relative to vehicle treated animals. The maximal effect was observed after four days of treatment at the 6 hour post dose at 50 mg/kg/day. Plasma levels of Genz-99067 were dose related, maximal at ~1 hour post dose, and cleared from the plasma by 24 hours post dose. The following table (from page 5 of the report) shows the change in the plasma GL1 levels.

Table 3. Change in plasma GL1 levels (% reduction vs. vehicle control)

Dose (mg/kg/day)	% reduction in plasma GL1 relative to vehicle control group					
	Day 1, 1 hr	Day 1, 24 hrs	Day 4, 1 hr	Day 4, 2 hrs	Day 4, 6 hrs	Day 4, 24 hrs
10	11	7	14	16	26	20
25	11	4	7	10	23	15
50	10	14	16	27	30	24

In Vitro Glucosylceramide Synthase Inhibition Testing of the Eliglustat Metabolites Genz-256222, Genz-258162, Genz-256416, Genz-258179, Genz-311752, Genz-120965, Genz-399207, Genz-399240, Genz-527862 and Genz-682042 (GT-157-EF-56)

The objective of this study was to define a concentration response relationship and IC_{50} determination for GL-1 synthase enzyme inhibition by ten Genz-112638 metabolites in microsomes from A375 human melanoma cells (lysed cell assay using HPLC) and in

intact B16 cells (intact cell assay using immunofluorescence for surface expression of GM3). The following ten metabolites were used:

Genz-120965 - AA-003, Genz-256222 - AA-001, Genz-256416 - AA-001, Genz-258179 - AA-003, Genz-399207 - AA-001, Genz-399240 - AA-001, Genz-527862 - AA-001, Genz-682042 - AA-001, Genz-258162 - AA-001, Genz-311752 - AA-004

Lysed cell assay: IC_{50} values for GL-1 synthase inhibition are shown in the following table (from page 5 of the report).

Table 1 IC50 for GL-1 Synthesis Inhibition in Human Cell Derived Microsomes

Metabolite Structure	Genz #	GL-1 Synthase IC50 (uM) data set #1	GL-1 Synthase IC50 (uM) data set #2:	Mean IC50 values
N-oxide	120965	7.99	10.90	9.45
amino	256222	6.00	7.73	6.87
7-keto	258162	1.21	0.963	1.09
5-carboxy	399240	>30	>30	>30
6-carboxy	399207	>30	>30	>30
7-hydroxyl	256416	1.50	1.23	1.35
6-keto	527862	1.86	1.70	1.78
6-hydroxyl	311752	2.73	3.09	2.92
5-hydroxyl	258179	2.13	2.14	2.14
4-carboxy	682042	>30	>30	>30

Intact cell assay: IC_{50} values for GL-1 synthase inhibition are shown in the following table (from page 5 of the report).

Table 2 IC50 for Inhibition of GM3 Expression on Intact B16 Cells

Metabolite Structure	Genz #	B16 cell-GM3 IC50 (uM) data set #1	B16 cell-GM3 IC50 (uM) data set #2	Mean IC50 values
6-hydroxyl	311752	1.43	1.57	1.54
5-hydroxyl	258179	2.06	2.6	2.47
N-oxide	120965	1.51	2.48	2.24
amino	256222	3.14	5.58	4.97
7-keto	258162	1.15	2.11	1.87
6-carboxy	399207	>10	>10	>10
5-carboxy	399240	>10	>10	>10
7-hydroxyl	256416	2.3	4.31	3.81
6-keto	527862	1.69	3.77	3.25
4-carboxy	682042	>10	>10	>10

In both human microsomes and intact B16 cells, ten chemically synthesized, confirmed metabolites of Genz-112638 appeared to have little or no pharmacological activity for GL-1 synthase inhibition. The IC_{50} values for the inhibition of GL-1 synthesis ranged from 1 to > 30 µM, the highest concentration tested. The IC_{50} values for GM3 synthesis inhibition ranged from 1.15 to > 10 µM, the highest concentration tested in this assay. The mean IC_{50} value for Genz-112638 was 19.6 nM for the inhibition of GL-1 synthesis and 57 nM for GM3 expression on B16 cells (GT-157-EF-59).

In Vitro Glucosylceramide Synthase Inhibition Testing of Eliglustat (Genz-112638) (GT-157-EF-59)

This study was conducted to determine the IC_{50} for glucosylceramide synthase (GCS) enzyme inhibition, in microsomes and in intact cells. Inhibition of NBD-C6-glucosylceramide formation was determined in A375 cell-derived microsomes (lysed cell assay) and inhibition of cell surface expression of GM3 was determined using intact B16 cells (intact cell assay).

The mean IC50 values in lysed cell assays are shown in the following table (from page 4 of the report).

Table 1: IC50 Values for GL-1 Synthesis Inhibition in Human Cell Derived Microsomes from Three Individual Assays

Genz #	GL-1 Synthase IC50 assay #1	GL-1 Synthase IC50 assay #2	GL-1 Synthase IC50 assay #3	GL-1 Synthase IC50 Mean value
112638 (eliglustat)	20.1 nM	19.8 nM	18.8 nM	19.6 nM

The mean IC_{50} values in intact cell assays are shown in the following table (from page 7 of the report).

Table 3: IC50 Values for Inhibition of GM3 Expression on B16 Cells from Three Individual Assays

Genz #	GM3-B16 cells IC50 assay #1	GM3-B16 cells IC50 assay #2	GM3-B16 cells IC50 assay #3	GM3-B16 cells IC50 Mean value
112638 (eliglustat)	82 nM	38 nM	50 nM	57 nM

Overall, the mean IC_{50} for GCS inhibition in human cell derived microsomes was determined to be 19.6 nM (8 ng/mL). The mean IC_{50} for the inhibition of GM3 expression on intact mouse B16 cells was 57 nM (23 ng/mL).

<u>The Efficacy of Genz-112638 as Determined by Decreased Glucosylceramide Levels in Livers from Beagle Dogs (GT-157-EF-7)</u>

This study was conducted to determine *in vivo* activity of Genz-112638 in normal Beagle dogs (n =3/sex/dose, 5-7 months old, male and female), following 28 days or 13 weeks of daily oral treatment. For the 28-day study, male and female dogs were randomized to four dose groups (0, 2.5, 5 and 12.5 mg/kg BID or 5, 10 and 25 mg/kg/day). Genz-112638 was administered daily by oral gavage for 28 days, administered as a twice daily dose (BID) approximately 6 hours apart. Two additional groups of male and female dogs in the vehicle control and high dose groups were followed for an additional 14 day recovery period. For the 13-week study, male and female dogs were randomized to 0, 2, 5 and 10 mg/kg/day dose groups. Genz-112638 was dosed daily by oral gavage for 13 weeks, administered as a once daily dose, approximately every 24 hours. Tissue samples from the liver were collected from a subset of male dogs from the 0 (vehicle control) and 10 mg/kg/day (high dose) groups at necropsy for GL-1 analysis.

For the 28-day study, liver glucosylceramide levels were reduced by 50%, 47% and 60% at 2.5, 5, and 12.5 mg/kg BID, respectively, compared to control. For the 13-week study, GL-1 synthesis in the liver was reduced by 32-55% compared to vehicle controls. Following the 14-day recovery period, glucosylceramide in the liver at 12.5 mg/kg BID was not different from vehicle control; indicating that the suppression of GL-1 synthesis by Genz-112638 was reversible after the removal of the inhibitor.

4.2 Secondary Pharmacology

Combination Screen Pharmacology Data Report (GT-157-EF-3) and *In Vitro* Pharmacology: High Throughput Profile Study of Four Compounds (GT-157-EF-60)

In this radioligand-binding study, Eliglustat was evaluated specifically for the identification of off-target activities. This assay included a broad range of 80 different receptors, transporters, and ion channels overexpressed in Chinese hamster ovary (CHO) cells or endogenously expressed in cells or tissues. At 10 μM, the highest concentration tested, eliglustat showed only minimal interaction with 71 components of the panel, but it did produce a significant (> 50%) inhibition of ligand binding to nine targets. These included the dopamine receptors D3 and D4.4, the serotonin receptors 5-HT1A, 5-HT2A, 5-HT2B, and 5-HT6, the mu opioid receptor, the non-specific sigma receptor, and the Ca^{2+} ion channel (L, verapamil site). Three major eliglustat metabolites (Genz-399207, Genz-399240, and Genz-682042) were also tested for the inhibition of specific radioligand binding for different receptors. There was no significant inhibition of any specific radioligand binding for any of these three metabolites up to 10 μM, the highest concentration tested.

In Vitro Pharmacology: Human 5HT2B Receptor Functional Assay (Agonist Effect) - Study of Genz-112638 (GT-157-EF-61)

The purpose of this study was to investigate the effects of Genz-112638 in the *in vitro* human 5-HT2B receptor functional assay for possible cardiac effects. Activation of the 5-HT2B receptors has been reported to be strongly associated with drug-induced valvular heart disease (Rothman RB and M Baumann, 2009, Expert Opin Drug Saf, 8:317-329). As determined by inositol-1-phosphate (IP1) release, eliglustat showed no specific activation of the 5HT2B receptor up to 100 μM, the highest concentration tested.

In Vitro Testing of Eliglustat-Imiglucerase Interaction by Glucocerebrosidase Activity Assay and (b) (4) Analysis (GT-157-EF-57)

The objective of this study was to examine the interaction of eliglustat and imiglucerase, a recombinant β-glucosidase (approved for the treatment of Gaucher disease). This study was conducted using the following two assays.

Quantitative assessment of imiglucerase activity in the presence of eliglustat, using the substrate 4-methylumbelliferyl-β-D-glucose: In this assay, eleven concentrations of eliglustat were tested, ranging from 0.001 μM to 37 μM. Eliglustat showed no inhibition of acid β-glucosidase activity through 12 μM (6 μg/mL). At 37 μM (18 μg/mL), minor inhibition was observed. The 12 uM and 37 uM eliglustat concentrations are about 300- and 900 times, respectively, the mean C_{max} of ~ 20 ng/mL observed at steady state in the phase 2 clinical trial.

Semi-quantitative assessment of physical interaction of imiglucerase and eliglustat using (b) (4) analysis: In this assay, eliglustat was tested at 100 uM concentration. Eliglustat bound very weakly both to the unmodified-imiglucerase surface and to the

CBE (conduritol β-epoxide) and MMTS (methyl methanethiolsulfonate)-active site blocked imiglucerase surfaces. These results indicated a weak interaction between eliglustat and imiglucerase.

Overall, in both assays eliglustat was shown not to interact with imiglucerase. Minor inhibition was observed at 37 uM, which is equal to 18,000 ng/mL of eliglustat (about 900-fold above the mean C_{max} of ~ 20 ng/mL observed at steady state in the phase 2 clinical trial). In the (b) (4) assay, only a weak physical interaction was observed with eliglustat and imiglucerase.

4.3 Safety Pharmacology

The following reviews are incorporated below from the pharmacology review of IND 67,589 dated July 30, 2004 by Dr. Dylan Yao (DMEP).

Neurological effects: Irwin Test: single dose p.o. @20, 100 and 400 mg/kg of Genz-112638 given to SD rats (6/g) resulted no significant gross behavioral or physiological changes observed during a 240 min postdose period compared to control.

Cardiovascular effects:

(1) In a very first study at 5, 15 and 25 mg/kg, I.V. infusion in dogs, ventricular fibrillation was noted at 15 mg/kg, since these doses were not tolerated, the study was terminated early and the data was not included in the submission. After that, an I.V., 2 min infusion of 1, 2.5 and 5 mg/kg was given to beagle dogs; it caused changes in heart rate and blood pressure parameters. Dose-related decreases in heart rate and increases in RR interval were observed following all three doses. 1 mg/kg caused a slight increase in

RR interval in the ECG, while significant changes in a number of ECG parameters, including increases in PR interval, QRS duration, QT, QTcF and QTcV intervals, were observed at doses of 2.5 (HED=83 mg) and 5 mg/kg. There were dose-related increases in atrio-ventricular (A-V) and intra-ventricular (I-V) conduction time measured at all three doses.

(2) In another study, effects of Genz-112638 on arterial blood pressure, heart rate, and lead II ECG in conscious dogs (telemetered) were assessed at single oral gavage doses of 1, 3, 10, 25, 50 and 80 mg/kg (6 male dogs/dose). Doses up to 80 mg/kg had no effect on blood pressure over 360 min following dosing. 80 mg/kg increases the heart rate and decreases RR interval 30-90 min postdose. PR interval prolongation was observed at 30-120 min following 50 mg/kg (HED=1667 mg) and at 60-120 min following 80 mg/kg dosing. QRS duration was prolonged at 60-90 min following 10 mg/kg (HED=333 mg), at 60-180 min following 25 mg/kg and at 30-360 min following 50 and 80 mg/kg dosing. These doses did not affect the QT, QTcF and QTcQ intervals. NOAEL=3 mg/kg (HED=1.67 mg/kg) for the CV effect in this single oral dose study. Refer to the tables and figures below for details (scanned from sponsor's data):

Table 5-12 Effects of Vehicle and Genz-112638 on QRS Duration in Conscious, Telemetered Male Beagle Dogs for the 1 and 3 mg/kg

Time (min)	QRS Duration (msec)		
	Vehicle 5 mL/kg	Genz-112638 1 mg/kg	Genz-112638 3 mg/kg
-30	41.3 ± 1.0	42.0 ± 1.8	40.3 ± 0.5
0	41.3 ± 1.2	39.3 ± 1.1	41.1 ± 0.8
30	41.3 ± 1.7	42.1 ± 1.6	41.6 ± 0.6
60	42.6 ± 1.5	42.0 ± 2.2	42.5 ± 1.0
90	41.7 ± 1.2	43.1 ± 2.0	42.3 ± 1.4
120	41.8 ± 1.7	42.4 ± 1.4	42.2 ± 1.4
180	41.0 ± 0.8	40.8 ± 0.9	41.8 ± 0.6
240	41.5 ± 1.7	40.5 ± 0.9	40.4 ± 1.5
300	41.4 ± 1.8	39.8 ± 1.0	41.9 ± 1.0
360	41.1 ± 1.9	40.2 ± 0.8	40.8 ± 1.3

The vehicle (reverse osmosis water) or Genz-112638 was administered orally, by gavage, at time 0. Data are mean ± s.e. m. of results obtained from 4 animals.

Table 5-13 Effects of Vehicle and Genz-112638 on QRS Duration in Conscious, Telemetered Male Beagle Dogs for the 10, 25, 50 and 80 mg/kg

Time (min)	QRS Duration (msec)				
	Vehicle 5 mL/kg	Genz-112638 10 mg/kg	Genz-112638 25 mg/kg	Genz-112638 50 mg/kg	Genz-112638 80 mg/kg
-30	38.9 ± 1.4	39.5 ± 1.4	39.5 ± 1.3	41.1 ± 2.0	40.5 ± 1.2
0	39.2 ± 1.6	38.9 ± 1.6	39.6 ± 1.6	40.0 ± 1.8	38.9 ± 1.4
30	41.3 ± 2.8	41.5 ± 0.8	42.0 ± 1.2	44.1 ± 1.6	48.8 ± 2.9**
60	39.2 ± 1.0	42.2 ± 0.7**	43.2 ± 1.2**	45.0 ± 1.1**	47.9 ± 1.5**
90	40.2 ± 1.4	42.5 ± 1.7	42.2 ± 1.7	45.5 ± 1.1**	50.9 ± 2.4**
120	40.3 ± 1.0	41.4 ± 1.1	43.5 ± 2.6*	46.0 ± 2.0**	47.9 ± 2.1**
180	39.8 ± 1.5	41.2 ± 1.2	42.0 ± 1.7	43.8 ± 2.0**	47.1 ± 2.3**
240	39.3 ± 1.1	39.8 ± 1.8	40.6 ± 1.5	43.7 ± 1.9**	44.6 ± 1.0**
300	39.2 ± 1.3	39.5 ± 1.1	40.5 ± 1.6	42.0 ± 1.4*	43.5 ± 1.2**
360	39.2 ± 1.6	40.7 ± 1.5	40.8 ± 1.3	41.8 ± 1.7	42.4 ± 1.8

The vehicle (RO water) or Genz-112638 were administered orally, by gavage, at time 0. Data are mean ± s.e. m. of results obtained from 4 animals.

* and ** denote $P < 0.05$ and $P < 0.01$ vs. vehicle, respectively.

Figure 5-19 Effect of Vehicle and Genz-112638 on QRS Duration in Conscious, Telemetered Male Beagle Dogs

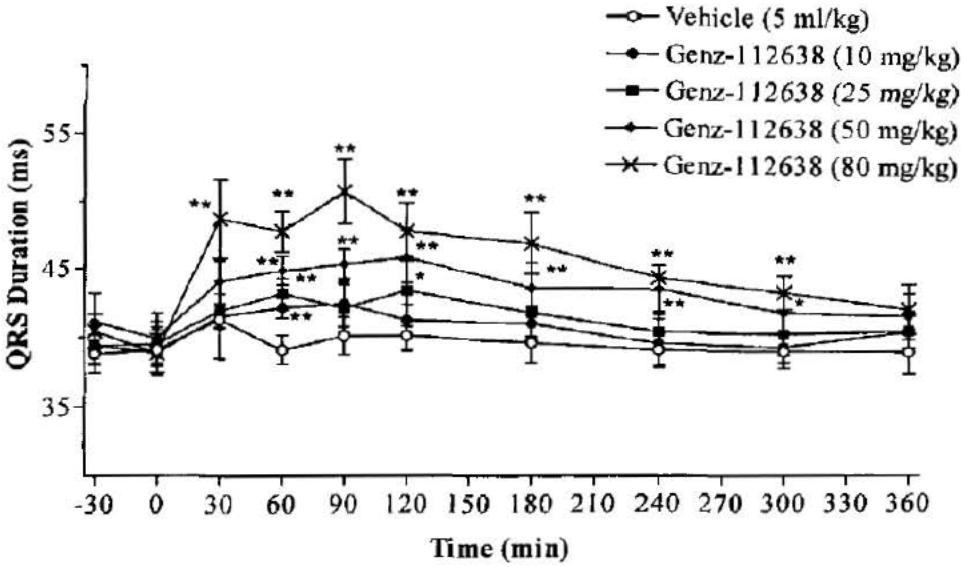
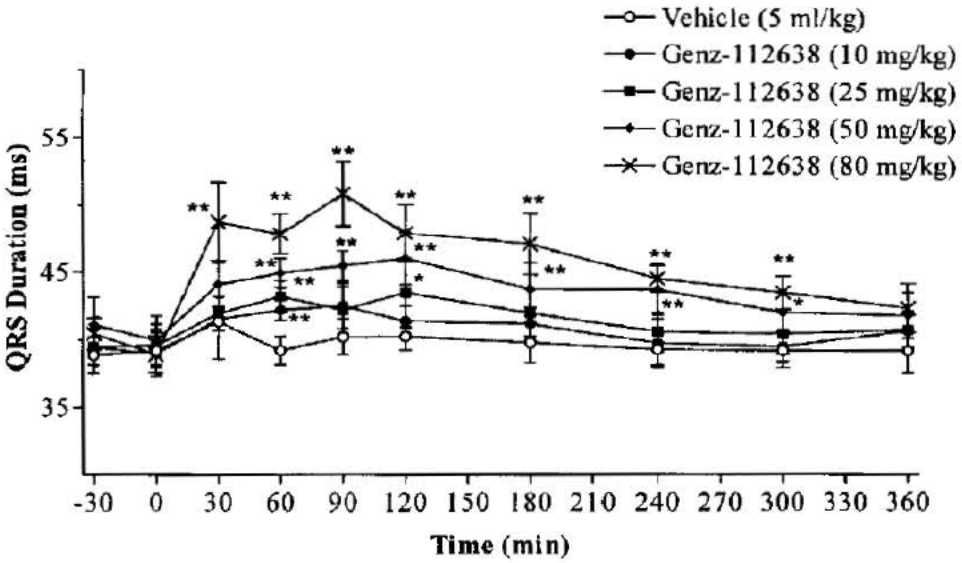


Figure 5-20 Effect of Vehicle and Genz-112638 on QRS Duration in Conscious, Telemetered Male Beagle Dogs



The vehicle or Genz-112638 were administered orally, by gavage, at time 0. Data are mean ± s.e. m. of results obtained from 4 animals. * and ** denote $P < 0.05$ and $P < 0.01$ vs. vehicle, respectively.

- HERG tail current: Genz-112638 inhibited HERG channels stably expressed in HEK293 cells in a concentration-dependent manner with IC25, IC50 and IC75 potency values of 0.11, 0.35 and 1.02 µg/mL, respectively.
- Cardiac action potential effects (beagle dog Purkinje fibers): 16 isolated dog Purkinje fibers (including controls) perfused with 0.03, 0.1, 0.3, 1, 10, 100 µg/mL Genz-112638 or vehicle (0.1% DMSO), there was a dose-related decrease in the action potential duration at doses of 0.3, 1, and 10 µg/mL. There was a total block of action potential at 100 µg/mL. There was a dose-related decrease in the up stroke amplitude of the action potential at concentrations of 1, 10 and 100 µg/mL.

Pulmonary effects: Respiratory rate and tidal volume: in conscious male rats, oral administration of Genz-112638 @ single dose of 20 or 100 mg/kg had no significant effect on the respiratory rate or tidal volume of conscious rats at 30 and 100 min postdose tested, however, @400 mg/kg, Genz-112638 significantly decreased respiration rate at 30 min postdose.

Renal effects: Single oral gavage doses of 20, 100 and 400 mg/kg given to SD rats (8 males/dose), urine samples were collected at 3, 6, and 24 hr postdose. At 100 mg/kg, an increase in urinary pH was seen 3-6 hr postdose; at 400 mg/kg, a significant decrease in potassium and chloride excretion (6-24 hr postdose) and an increase in urinary pH (0-3 and 3-6 hr postdose) were observed.

Gastrointestinal effects: GI motility: single dose p.o. at 20, 100, 400 mg/kg was given to SD rats, GI transit and gastric emptying were measured. LD 20 mg/kg has no effect on the endpoints. 100 and 400 mg/kg resulted in a profound inhibitory effect on GI transit and gastric emptying time.

Abuse liability: n/s

Effects of Genz-112638, Genz-399240 and Genz-399207 on Human Cardiac Sodium Channels Stably Expressed in HEK293 Cells (GT-157-TX-41)

This study was conducted to examine the effects of Genz 112638 and two metabolites, Genz-399240 and Genz-399207, on peak sodium current recorded from HEK293 cells (n = 4 cells/concentration) stably transfected with human cardiac sodium channel (hNav1.5) cDNA. The following concentrations were tested: Genz-112638 (0.3, 1, 3, 10 and 100 µg/mL), Genz-399240 (5 µg/mL) and Genz-399207 (30 µg/mL). Flecainide (100 µM) was used as positive control. Vehicle was dimethyl sulfoxide (DMSO, 0.1%).

Genz-112638 inhibited peak sodium currents, stimulated at a frequency of 1 Hz, in a concentration-dependent manner, with a statistically significant inhibition of peak current observed at concentrations of 1 µg/mL and above compared to control. The estimated IC_{25}, IC_{50} and IC_{75} values for Genz-112638 inhibition of peak sodium current were 1.8, 5.2 and 15 µg/mL, respectively. Genz-399240 produced a residual peak current of 90.2% when compared to control values. This equates to a decrease in peak current of 9.8 %. No statistically significant difference between the vehicle and Genz-399240

treated groups was observed. Genz-399207 produced a residual peak current of 85.4% when compared to control values. This equates to a decrease in peak current of 14.6%. No statistically significant difference between the vehicle and Genz-399207 treated groups was observed. Flecainide inhibited peak sodium current by 95.0%.

Effects of Genz-112638 on the Human Cav1.2 L-type Calcium Channels Expressed in Mammalian Cells (GT-157-TX-44)

The objective of this study was to examine the *in vitro* effects of Genz-112638 on L-type calcium current ($I_{ca,L}$) in Chinese hamster ovary (CHO) cells that over-express human Cav1.2 calcium channels. Genz-112638 inhibited hCav1.2 current by 30.2% at 10 μM (n = 3), 48.9% at 30 μM (n = 3) and 88.0% at 100 μM (n = 4). The IC_{50} for the inhibitory effect of Genz-112638 on hCav1.2 current was 24.8 μM. Nifedipine (0.1 μM), the positive control, inhibited hCav1.2 current (Mean, n = 2) by 73.8%.

Effects of Genz-399207 and Genz-399240 on the Human Cav1.2 L-type Calcium Channels Expressed in Mammalian Cells (GT-157-TX-45)

The objective of this study was to examine the *in vitro* effects of Genz-399207 and Genz-399240 (two metabolites of GENZ-112638) on L-type calcium current ($I_{ca,L}$) in CHO cells that over-express human Cav1.2 calcium channels. Genz-399207 inhibited hCav1.2 current by 0.5% at 10 μM (n = 3), 0.1% at 30 μM (n = 3) and 5.4% at 100 μM (n = 3). The IC_{50} for the inhibitory effect of Genz-399207 on hCav1.2 current was not calculated since the inhibition was less than 50%. Genz 399240 inhibited hCav1.2 current by 0.6% at 10 μM (n = 3), 0.8% at 30 μM (n = 4) and 12.3% at 100 μM (n = 3). The IC_{50} for the inhibitory effect of Genz-399240 on hCav1.2 current was not calculated since the inhibition was less than 50%. Nifedipine (0.1 μM), the positive control, inhibited hCav1.2 current (Mean, n = 2) by 79.3%.

Effect of Nine Eliglustat Metabolites on hERG Hill Current as Tested in Stably Transfected HEK293 cells (GT-157-TX-49)

The goal of this study was to determine the *in vitro* effects of nine eliglustat metabolites on mammalian cells stably expressing the hERG (human ether-à-go-go-related gene) cardiac potassium channel. For each metabolite, the concentration-response relationship was evaluated at multiple concentrations, to define potential concentration based effects on hERG tail current. The following table (from page 3 of the report) shows the concentration tested.

Table 1 Automated Patch Clamp Assay:

Test Article	Lot Number	Test concentrations (uM)	Molecular Weight
Genz-256222 (amino metabolite)	1	0.1, 0.3, 1.0	350
Genz-258162 (7-keto metabolite)	1	0.1, 0.3, 1.0 1.0, 10, 30	418
Genz-256416 (7-hydroxyl metabolite)	1, 2	0.1, 0.3, 1.0 1.0, 10, 30	420
Genz-258179 (5-hydroxyl metabolite)	1	0.1, 0.3, 1.0	420
Genz-311752 (6-hydroxyl metabolite)	1	0.1, 0.3, 1.0	420
Genz-120965 (N-oxide metabolite)	3	0.1, 0.3, 1.0	421
Genz-399207 (6-carboxy metabolite)	1	1.0, 10, 30	420
Genz-399240 (5-carboxy metabolite)	1	0.3, 1.0, 3.0	406
Genz-527862 (6-keto metabolite)	1	1.0, 10, 30	418

Table 2 Conventional Patch Clamp Assay:

Test Article	Lot Number	Test concentrations (uM)	Molecular Weight
Genz-256222 (amino metabolite)	2	1.0, 10, 30	350
Genz-258179 (5-hydroxyl metabolite)	2	1.0, 10, 30	420
Genz-311752 (6-hydroxyl metabolite)	2	1.0, 10, 30	420
Genz-120965 (N-oxide metabolite)	4	1.0, 10, 30	421

Only one metabolite, Genz-256222, demonstrated a significant concentration-dependent inhibition of hERG tail current, with IC_{50} of 5.1 uM. The other eight metabolites demonstrated minimal or no inhibition of hERG tail current in this assay. The following table (from page 5 of the report) shows the IC_{50} values.

Table 3 hERG IC50 Values for Eliglustat Metabolites

Test Article	hERG	Comments
Genz-256222 (amino metabolite)	IC_{50} = 5.1 µM (1.8 ug/ml)	
Genz-258162 (7-keto metabolite)	IC_{50} > 30 µM (>12.5 ug/ml)	≤ 20% inhibition of hERG tail current at the highest test concentration of 30 uM.
Genz-256416 (7-hydroxyl metabolite)	IC_{50} > 30 µM (>12.6 ug/ml)	≤ 20% inhibition of hERG tail current at the highest test concentration of 30 uM.
Genz-258179 (5-hydroxyl metabolite)	IC_{50} > 30 µM (>12.5 ug/ml)	≤ 20% inhibition of hERG tail current at the highest test concentration of 30 uM.
Genz-311752 (6-hydroxyl metabolite)	IC_{50} > 30 µM (>12.5 ug/ml)	≤ 20% inhibition of hERG tail current at the highest test concentration of 30 uM.
Genz-120965 (N-oxide metabolite)	IC_{50} > 30 µM (>12.6 ug/ml)	≤ 20% inhibition of hERG tail current at the highest test concentration of 30 uM.
Genz-399207 (6-carboxy metabolite)	Does not inhibit up to 30 uM (>12.6 ug/ml)	Highest concentration tested
Genz-399240 (5-carboxy metabolite)	Does not inhibit up to 3 uM (>1.2 ug/ml)	Test concentration for this assay limited by solubility in DMSO.
Genz-527862 (6-keto metabolite)	IC_{50} > 30 µM (>12.5 ug/ml)	≤ 20% inhibition of hERG tail current at the highest test concentration of 30 uM.

Effect of Genz-682042 on Cloned hERG Potassium Channels Expressed in Mammalian Cells (GT-157-TX-50)

The objective of this study was to examine the *in vitro* effects of Genz-682042 (metabolite) on the hERG potassium channel current (a surrogate for I_{Kr}, the rapidly activating and delayed rectifier cardiac potassium current). Two concentrations were tested: 10 and 300 µM. Genz-682042 did not cause significant inhibition of hERG current [1.3% (n = 3) at 10 µM and 2.0% at 300 µM (n = 3)]. The IC_{50} for the hERG inhibition was not determined but was estimated to be greater than 300 µM. The positive control, 90 nM cisapride, inhibited hERG current by (mean) 79.2% (n = 2).

Effects of Eight Metabolites of Genz-112638 on the Human Cav1.2 L-type Calcium Channels Expressed in Mammalian Cells (GT-157-TX-51)

The objective of this study was to determine the *in vitro* effects of the following (from page 7 of the report) eight metabolites of Genz-112638 on mammalian cells (CHO cells) stably expressing hCav1.2. Positive control was nifedipine (0.1 µM, n = 2). Metabolites were tested at three concentrations: 10, 30 and 100 µM.

4.2 Test Article

Test Article ID	Lot Number	Molecular Weight (g/mol)	Purity (%)
Genz-256416	Genz-256416-AA-003	420.54	91.61
Genz-258162	Genz-258162-AA-003	418.53	94.26
Genz-258179	Genz-258179-AA-003	420.54	98.12
Genz-120965	Genz-120965-20642	420.26	99.10
Genz-527862	Genz-527862-AA-004	418.53	94.45
Genz-682042	Genz-682042-AA-002	392.45	91.39
Genz-256222	Genz-256222-AQ-001	350.45	96.26
Genz-311752	Genz-311752-AA-004	420.54	97.97

The following table shows the results of this study.

Compound	% inhibition of hCav1.2 Current (Mean ± SEM, n = 3)			IC_{50} (Estimate)
	10 μM	30 μM	100 μM	
Genz-256416	1.2 ± 0.3		1.8 ± 0.4	> 100 μM
Genz-258162	2.3 ± 0.5		8.9 ± 1.0	> 100 μM
Genz-258179	1.7 ± 0.6		10.0 ± 0.7	> 100 μM
Genz-120965	1.6 ± 0.4		6.0 ± 1.4	> 100 μM
Genz-527862	1.5 ± 1.6		4.1 ± 2.0	> 100 μM
Genz-682042	0.2 ± 1.2		2.0 ± 1.1	> 100 μM
Genz-256222	3.6 ± 0.9*	24.5 ± 1.2	81.5 ± 1.9	> 50 μM
Genz-311752	2.8 ± 0.9*		5.4 ± 1.7	> 100 μM
Nifedipine (0.1 μM) inhibited hCav1.2 current by (mean) 85.0% (n = 2)				

*: n = 4

Overall, for all metabolites tested, the IC_{50} values were > 100 μM. Metabolite Genz-256222 had an IC_{50} of > 50 μM.

Effect of Eight Test Articles on Human Cardiac Sodium Channels (hNav1.5) Expressed in Mammalian Cells (GT-157-TX-52)

The objective of this study was to determine the *in vitro* effects of the following (from page 7 of the report) eight metabolites of Genz-112638 on mammalian cells (HEK293 cells) stably expressing hNav1.2. Positive control was lidocaine (2 mM, n = 2). Metabolites were tested at three concentrations: 9, 27 and 90 μM.

Test Article ID	Lot Number	Molecular Weight (g/mol)	Purity (%)
Genz-256416	Genz-256416-AA-003	420.54	91.61
Genz-258162	Genz-258162-AA-003	418.53	94.26
Genz-258179	Genz-258179-AA-003	420.54	98.12
Genz-120965	Genz-120965-20642	420.26	99.10
Genz-527862	Genz-527862-AA-004	418.53	94.45
Genz-682042	Genz-682042-AA-002	392.45	91.39
Genz-256222	Genz-256222-AQ-001	350.45	96.26
Genz-311752	Genz-311752-AA-004	420.54	97.97

The following table shows the results of this study.

Compound	% inhibition of hNav1.5 Current (Mean ± SEM, n = 3)			IC_{50} (Estimate)
	10 μM	30 μM	100 μM	
Genz-256416	0.4 ± 0.6		1.2 ± 0.3	> 100 μM
Genz-258162	0.0 ± 0.8		6.7 ± 1.3*	> 100 μM
Genz-258179	0.2 ± 0.2		4.3 ± 0.3	> 100 μM
Genz-120965	3.2 ± 0.4		9.4 ± 0.6	> 100 μM
Genz-527862	0.2 ± 0.4 (14 μM)		16.3 ± 1.3 (144 μM)	> 144 μM
Genz-682042	0.3 ± 0.7*		3.7 ± 1.1	> 100 μM
Genz-256222	3.9 ± 0.8 (9 μM)	30.9 ± 2.6 (27 μM)	90.1 ± 2.3 (90 μM)	37 μM
Genz-311752	0.6 ± 0.4*		5.5 ± 1.0	> 100 μM
Lidocaine (2 mM) inhibited hNav1.5 current by (mean) 85.0% (n = 2)				

*: n = 4

Overall, all the tested metabolites had IC50 values > 100 μM except Genz-256222, which had an IC_{50} of 37 μM.

Effect of Genz-399240 on Cloned hERG Potassium Channels Expressed in Mammalian Cells (GT-157-TX-54)

The objective of this study was to examine the *in vitro* effects of Genz-399240 on the hERG potassium channel current. Two concentrations were tested: 10 and 300 μM. Genz-399240 did not cause significant inhibition of hERG current [0.1% at 10 μM (n = 3) and 0.7% at 300 μM (n = 4)]. The IC_{50} for the hERG inhibition was not determined but was estimated to be greater than 300 μM. The positive control, 90 nM cisapride, inhibited hERG current by (mean) 84.0% (n = 2).

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption

In Vitro Evaluation of the Permeability of Genz-99067 using Caco-2 Cells (DMPK10-R047)

Methods: The objective of this study was to evaluate the permeability of Genz-99067 in vitro using the Caco-2 cell permeability model. Permeability of Genz-99067 was assessed at 12.5, 125 and 1250 µM. Labetalol and terbutaline were included in the assay at 10 µM concentrations as the internal high and low permeability standards, respectively.

Results: All tested concentrations of Genz-99067 exhibited higher permeability than the internal high permeability standard labetalol. The ratios of permeability of Genz-99067 to that of labetalol were 2.0, 1.9 and 1.6 at concentrations of 12.5, 125 and 1250 µM Genz-99067, respectively. These data indicates that Genz-99067 is a high permeability drug substance.

Permeability Determination of Eliglustat with Internal Permeability Standards Metoprolol Tartrate and Ranitidine HCl Using the In Situ Single Pass Rat Perfusion Assay (DMPK10-R054)

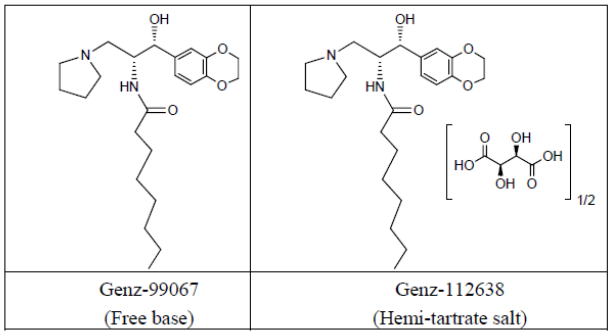
Methods: In this study, permeability of eliglustat was evaluated at three concentrations (6, 60, and 600 µg/mL) using in situ single pass perfusion assay. Eliglustat was co-perfused with the high permeability standard metoprolol tartrate at 68 µg/mL and the low permeability standard ranitidine hydrochloride at 35 µg/mL. Samples were collected as 10-minute fractions over a 90 minute period and analyzed for drug concentrations.

Results: At both the high and mid concentrations, eliglustat was demonstrated to be a high permeability drug in comparison to the high internal permeability standard metoprolol tartrate. At the low concentration, eliglustat permeability was less than that of metoprolol. The test to reference ratios (T/R) observed were 1.64, 1.50, and 0.19 at the high, mid, and low doses, respectively.

Pharmacokinetic Study of Genz-99067 Following a Single Intravenous and Oral Administration of Genz-112638 to Sprague-Dawley Rats (DMPK11-R025)

Methods: In this study, Sprague-Dawley rats were assigned to three treatment groups. Rats in group 1 (n = 6, 3/sex) received 1 mg/kg of Genz-112638, the hemi-tartrate salt form of Genz-99067 (salt correction factor of 0.844) as a single IV bolus dose. Rats in groups 2 (n = 8, 4/sex) and 3 (n = 8, 4/sex) received 3 and 10 mg/kg of Genz-112638, respectively, as a single oral dose by gavage. Serial blood samples were collected over 0 to 24 hours post dose, and urine was collected over 0 to 24 hours post IV dose. Concentrations of Genz-99067 in the plasma and urine were determined using liquid chromatography with tandem mass spectrometry (LC-MS/MS) method. The following figure (from page 20 of the report) shows the structures of Genz-99067 and Genz-112638.

Figure 1. Structures of Genz-99067 and Genz-112638



Results: Following IV administration, mean $AUC_{0-\alpha}$ and $t_{½}$ were 553 ng.hr/mL and 0.253 h, respectively. Mean plasma clearance (CL) was 27.0 mL/min/kg. The mean estimated volume of distribution at steady state was 429 mL/kg. Females had approximately 54% greater mean systemic exposure ($AUC_{0-\infty}$) than males (670 vs. 436 ng.hr/mL, respectively) and approximately 35% lower mean CL than males (21.4 vs. 32.7 mL/min/kg). No detectable levels of Genz-99067 were found in the urine of either sex. The following table (from page 16 of the report) shows the PK data following IV administration.

Table 4. Plasma and urinary pharmacokinetic parameters of Genz-99067 following after a single 0.844 mg/kg IV dose (administered as Genz-112638) in fasted male and female Sprague-Dawley rats

Gender	Rat ID	C_0	C_{last}	$t_{½}$	t_{last}	MRT	AUC_{last}	$AUC_{0-\infty}$	%Ext[a]	CL	CL_r	V_{ss}	V_z
		(ng/mL)		(h)			(ng•h/mL)			(mL/min/kg)		(mL/kg)	
Males	1	1730	7.20	0.307	2.00	0.288	499	502	0.636	28.0	0.00	484	746
	2	1390[b]	33.3	0.197	1.00	0.229	371	381	2.49	36.9	0.00	508	631
	3	1230	7.07	0.294	2.00	0.340	422	425	0.706	33.1	0.00	676	842
	Mean	1450	15.9	0.266	1.67	0.286	431	436	1.28	32.7	0.00	556	740
	SD	259	15.1	0.0600	0.577	0.0553	64.2	61.4	1.05	4.48	0.00	104	106
	%CV	17.8	95.3	22.5	34.6	19.4	14.9	14.1	82.3	13.7	-	18.8	14.3
Females	4	2750	6.67	0.272	2.00	0.258	756	759	0.345	18.5	0.00	286	436
	5	2310	33.0	0.173	1.00	0.204	542	550	1.50	25.6	0.00	313	383
	6	2280	6.17	0.275	2.00	0.253	699	701	0.349	20.1	0.00	304	478
	Mean	2450	15.3	0.240	1.67	0.238	666	670	0.731	21.4	0.00	301	432
	SD	264	15.3	0.0581	0.577	0.0297	111	108	0.664	3.70	0.00	13.4	47.5
	%CV	10.8	100	24.2	34.6	12.5	16.7	16.1	90.9	17.3	-	4.46	11.0
All	Mean	1950	15.6	0.253	1.67	0.262	548	553	1.00	27.0	0.00	429	586
	SD	595	13.6	0.0547	0.516	0.0475	152	151	0.841	7.20	0.00	155	184
	%CV	30.5	87.5	21.6	31.0	18.2	27.8	27.2	83.8	26.6	-	36.1	31.3

[a] % of $AUC_{0-\infty}$ due to extrapolation
[b] Determined by log-linear extrapolation of first 3 observed concentrations.
"-" Not applicable.

Following 3 mg/kg oral administration, mean C_{max} and t_{max} were 245 ng/mL and 0.271 hours, respectively. Mean AUC and $t_{½}$ values were 184 ng.hr/mL and 0.385 hours, respectively. In females, mean AUC and C_{max} values were approximately 108% higher (237 vs. 114 ng.hr/mL) and 250% higher (381 vs. 109 ng/mL), respectively, compared to males. The absolute bioavailability following a single oral administration of Genz-682452 was estimated to be 10.5%. The following table (from page 17 of the report) shows the PK data following 3 mg/kg oral administration.

Table 5. Plasma pharmacokinetic parameters of Genz-99067 following after a single 2.53 mg/kg PO dose (administered as Genz-112638) in fasted male and female Sprague-Dawley rats

Gender	Rat ID	C_{max}	C_{last}	t_{max}	$t_{½}$	t_{last}	MRT	AUC_{last}	$AUC_{0-\infty}$	%Ext[a]	%F[b]
		ng/mL		h				ng•h/mL			
Males	7	35.6	20.9	0.500	NC	1.00	NC	24.9	NC	NC	NC
	8	135	33.4	0.250	0.367	1.00	0.634	76.9	94.6	18.7	7.23
	9	112	8.13	0.167	0.460	2.00	0.712	88.4	93.7	5.76	7.17
	10	154	10.6	0.250	0.394	2.00	0.721	147	153	3.94	11.7
	Mean	109	18.3	0.292	0.407	1.50	0.689	84.3	114	9.47	8.70
	SD	52.0	11.5	0.144	0.0477	0.577	0.0476	50.1	34.0	8.06	2.60
	%CV	47.6	63.0	49.4	11.7	38.5	6.91	59.5	29.9	85.1	29.9
Females	11	550	6.30	0.250	0.277	2.00	0.454	311	313	0.805	15.6
	12	186	13.1	0.250	0.472	2.00	0.707	143	152	5.87	7.56
	13	405	7.75	0.250	0.315	2.00	0.512	227	230	1.53	11.4
	14	382	17.6	0.250	0.411	2.00	0.639	244	254	4.11	12.6
	Mean	381	11.2	0.250	0.369	2.00	0.578	231	237	3.08	11.8
	SD	150	5.18	0.000	0.0891	0.00	0.116	69.0	66.8	2.34	3.32
	%CV	39.3	46.3	0.000	24.1	0.00	20.1	29.8	28.1	76.0	28.1
All	Mean	245	14.7	0.271	0.385	1.75	0.625	158	184	5.82	10.5
	SD	178	9.09	0.0970	0.0717	0.463	0.105	96.3	83.5	6.00	3.24
	%CV	72.8	61.7	35.8	18.6	26.5	16.8	61.1	45.3	103	31.0

[a] % of $AUC_{0-\infty}$ due to extrapolation
[b] Individual %F calculated by ratio of dose-adjusted $AUC_{0-\infty}$ after PO dose to mean dose-adjusted $AUC_{0-\infty}$ after IV dose.
NC: Not calculated.

Following 10 mg/kg oral administration, C_{max} and t_{max} were 885 ng/mL and 0.469 hours, respectively. Mean AUC and $t_{½}$ were 924 ng.hr/mL and 0.479 hours, respectively. In females, mean AUC and C_{max} values were approximately 100% (1230 vs. 616 ng.hr/mL) and 76% higher (1130 vs. 643 ng/mL), respectively, compared to males. The absolute bioavailability following a single oral administration of Genz-682452 was estimated to be 16.3%. The following table (from page 18 of the report) shows the PK data following 10 mg/kg PO administration.

Table 6. Plasma pharmacokinetic parameters of Genz-99067 following after a single 8.44 mg/kg PO dose (administered a Genz-112638) in fasted male and female Sprague-Dawley rats

Gender	Rat ID	C_{max}	C_{last}	t_{max}	$t_{½}$	t_{last}	MRT	AUC_{last}	$AUC_{0-\infty}$	%Ext[a]	%F[b]
		ng/mL		h				ng•h/mL			
Males	15	455	38.5	0.500	0.412	2.00	0.789	480	503	4.56	11.5
	16	675	9.91	0.250	0.758	4.00	0.808	552	563	1.93	12.9
	17	706	33.7	0.500	0.340	2.00	0.676	651	667	2.48	15.3
	18	734	34.3	0.500	0.334	2.00	0.693	713	730	2.26	16.7
	Mean	643	29.1	0.438	0.461	2.50	0.741	599	616	2.80	14.1
	SD	127	13.0	0.125	0.201	1.00	0.0668	104	102	1.19	2.34
	%CV	19.8	44.6	28.6	43.7	40.0	9.01	17.3	16.6	42.4	16.6
Females	19	1450	9.98	0.500	0.476	4.00	0.838	1660	1660	0.412	24.8
	20	1320	95.4	0.500	0.386	2.00	0.785	1350	1400	3.79	20.9
	21	782	13.1	0.500	0.638	4.00	0.900	902	914	1.32	13.6
	22	955	5.33	0.500	0.488	4.00	0.776	950	954	0.393	14.2
	Mean	1130	31.0	0.500	0.497	3.50	0.825	1210	1230	1.48	18.4
	SD	311	43.1	0.000	0.104	1.00	0.0572	357	362	1.60	5.40
	%CV	27.6	139	0.000	21.0	28.6	6.94	29.4	29.4	108	29.4
All	Mean	885	30.0	0.469	0.479	3.00	0.783	907	924	2.14	16.3
	SD	340	29.5	0.000	0.150	1.07	0.0728	409	412	1.49	4.48
	%CV	38.4	98.2	0.000	31.3	35.6	9.30	45.1	44.6	69.4	27.6

[a] % of $AUC_{0-\infty}$ due to extrapolation
[b] Individual %F calculated by ratio of dose-adjusted $AUC_{0-\infty}$ after PO dose to mean dose-adjusted $AUC_{0-\infty}$ after IV dose.

<u>Pharmacokinetic Study of Genz-99067 Following a Single Intravenous and Oral Administration of Genz-112638 to Male and Female Beagle Dogs (DMPK11-R027)</u>

<u>Methods</u>: In this study, six Beagle dogs (3/sex) received a single 1 mg/kg IV bolus dose, followed by a single 3 mg/kg oral (PO) dose of Genz-112638, with treatments separated by a 7-day washout period. Serial blood samples were collected over 0 to 48 hours post dose after each treatment, and urine was collected over 0 to 48 hours post dose after the IV dose. Concentrations of Genz-99067 in the plasma and urine were determined by LC-MS/MS method.

Results: Following IV administration, mean $AUC_{0-\infty}$ and $t_{½}$ were 415 ng.hr/mL and 0.929 h, respectively. Mean plasma clearance (CL) was 34.8 mL/min/kg. The mean estimated volume of distribution at steady state (2380 mL/kg) was almost four-fold greater than total body water (0.6 L/kg), suggesting extensive extravascular tissue distribution. Males had approximately 24% greater mean systemic exposure (AUC) than females (459 vs. 371 ng.hr/mL, respectively) and approximately 19% lower mean CL than females (31.1 vs. 38.6 mL/min/kg). Mean renal clearance was approximately 1.15% (males) and 0.294% (females) of the mean systemic clearance. The PK parameters following IV administration is shown in the table (from page 16 of the report) below.

Table 4. Plasma and urinary pharmacokinetic parameters of Genz-99067 following a single 0.844 mg/kg IV dose (administered as Genz-112638) to fasted male and female beagle dogs

Gender	Dog ID[a]	C_0	C_{last}	$t_{½}$	t_{last}	MRT	AUC_{last}	$AUC_{0-\infty}$	%Ext[b]	CL	CL_r	% CLr/CL^c	V_{ss}	V_z
		(ng/mL)		(h)			(ng•h/mL)			(mL/min/kg)			(mL/kg)	
Male	1	419	6.99	1.32	6.00	1.55	380	393	3.38	35.7	0.631	1.77	3330	4080
	2	614	12.9	0.829	4.00	1.05	505	521	2.96	27.0	0.314	1.17	1700	1930
	3	858	14.1	0.937	4.00	1.16	443	462	4.13	30.4	0.162	0.531	2120	2470
	Mean	630	11.3	1.03	4.67	1.25	443	459	3.49	31.1	0.369	1.15	2380	2830
	SD	220	3.81	0.257	1.15	0.264	62.8	63.9	0.591	4.42	0.240	0.618	846	1120
	%CV	34.9	33.6	25.0	24.7	21.0	14.2	13.9	16.9	14.2	64.9	53.5	35.5	39.5
Female	4	509	15.5	1.06	4.00	1.34	356	380	6.23	37.0	0.298	0.804	2980	3390
	5	1210	33.1	0.508	2.00	0.596	406	430	5.63	32.6	0.0252	0.0773	1170	1430
	6	513	9.21	0.926	4.00	1.07	292	304	4.04	46.2	0.00	0.00	2970	3700
	Mean	745	19.3	0.830	3.33	1.00	351	371	5.30	38.6	0.108	0.294	2370	2840
	SD	405	12.4	0.287	1.15	0.378	57.2	63.4	1.13	6.90	0.165	0.444	1040	1230
	%CV	54.4	64.3	34.6	34.6	37.6	16.3	17.1	21.3	17.9	153	151	44.0	43.2
All	Mean	688	15.3	0.929	4.00	1.13	397	415	4.40	34.8	0.238	0.724	2380	2830
	SD	298	9.28	0.267	1.26	0.322	73.4	74.3	1.28	6.63	0.233	0.673	850	1050
	%CV	43.4	60.6	28.7	31.6	28.5	18.5	17.9	29.1	19.0	97.8	93.0	35.8	37.0

[a] Dogs received 1 mg/kg Genz-112638, equivalent to 0.844 mg/kg Genz-99067.
[b] % of $AUC_{0-\infty}$ due to extrapolation
[c] Percentage of total clearance due to renal clearance.

Following 3 mg/kg oral administration, mean C_{max} and t_{max} were 68.0 ng/mL and 0.445 hours, respectively. Mean AUC and $t_{½}$ were 131 ng.hr/mL and 1.06 hours, respectively. In males, mean $AUC_{0-\infty}$ and $t_{½}$ values were approximately 89% higher (171 vs. 90.6 ng.hr/mL) and approximately 88 % higher (1.39 vs. 0.741 hours), respectively, compared to females, however, C_{max} values were similar (66.2 versus 69.9 ng/mL) between males and females. The absolute bioavailability following a single oral administration of Genz-112638 was estimated to be 10.1% (12.3 % in males and 7.92% in females). The PK parameters following oral administration is shown in the table (from page 17 of the report) below.

Table 5. Plasma pharmacokinetic parameters of Genz-99067 following a single 2.53 mg/kg PO dose (administered as Genz-112638) to fasted male and female beagle dogs

Gender	Dog ID[a]	C_{max}	C_{last}	t_{max}	$t_{½}$	t_{last}	MRT	AUC_{last}	$AUC_{0-\infty}$	%Ext[b]	%F
		(ng/mL)		(h)				(ng•h/mL)			
Male	1	39.6	15.1	1.00	2.10	4.00	3.32	110	155	29.5	13.2
	2	113	12.2	0.500	0.948	4.00	1.60	241	258	6.47	16.5
	3	45.9	5.69	0.250	1.11	4.00	1.73	91.7	101	9.01	7.27
	Mean	66.2	11.0	0.583	1.39	4.00	2.22	148	171	15.0	12.3
	SD	40.7	4.82	0.382	0.627	0.00	0.960	81.7	79.8	12.6	4.68
	%CV	61.5	43.8	65.5	45.3	0.00	43.4	55.4	46.6	84.2	38.0
Female	4	77.6	17.9	0.250	0.885	2.00	1.34	77.6	100	22.7	8.82
	5	99.1	14.5	0.500	0.540	2.00	0.917	108	119	9.46	9.25
	6	32.9	8.70	0.167	0.798	2.00	1.25	42.1	52.1	19.2	5.71
	Mean	69.9	13.7	0.306	0.741	2.00	1.17	75.9	90.6	17.1	7.92
	SD	33.8	4.65	0.173	0.179	0.00	0.223	33.0	34.7	6.88	1.93
	%CV	48.3	34.0	56.7	24.2	0.00	19.1	43.5	38.3	40.2	24.4
All	Mean	68.0	12.3	0.445	1.06	3.00	1.69	112	131	16.1	10.1
	SD	33.5	4.49	0.306	0.543	0.00	0.847	68.2	70.6	9.17	4.01
	%CV	49.3	36.3	68.8	51.1	0.00	50.1	61.0	53.9	57.1	39.6

[a] Dogs received 3 mg/kg Genz-112638, equivalent to 2.53 mg/kg Genz-99067.
[b] % of $AUC_{0-\infty}$ due to extrapolation.

Pharmacokinetic Study of Genz-99067 After a Single Intravenous and Oral Administration of Genz-112638 to Male and Female C57BL/6 Mice (DMPK11-R028)

Methods: In this study, C57BL/6 mice were divided into 4 groups (n = 6/group). Mice in groups 1 (males) and 3 (females) received a single IV dose of 1 mg/kg (5 mL/kg) of Genz-112638 (equivalent to 0.843 mg/kg Genz-99067). Mice in groups 2 (males) and 4 (females) received a single oral 3 mg/kg dose (10 mL/kg) of Genz 112638 (equivalent to 2.53 mg/kg Genz-99067) by gavage. Serial blood samples were collected over 0 to 24 hours post dose, and plasma concentrations of Genz-99067 were determined by LC-MS/MS method.

Results: Following IV administration, mean $AUC_{0-\infty}$ was greater in males than females (146 and 52.5 ng.hr/mL, respectively); however, $t_{½}$ values were similar (0.546 and 0.557 hours, respectively). Plasma clearance in males was 96.0 mL/min/kg, whereas this value was greater in females (268 mL/min/kg). The mean steady-state volume of distribution in both males and females (2.07 and 9.44 L/kg, respectively) exceeded total body water (0.7 L/kg), suggesting extensive extravascular tissue distribution.

Following oral administration, in males, mean C_{max} and t_{max} were 115 ng/mL and 0.0833 hours, respectively. The $AUC_{0-\alpha}$ and $t_{½}$ were 20.5 ng.hr/mL and 0.214 hours, respectively. The mean oral bioavailability was 4.68%.

Pharmacokinetic Study of Genz-99067 Following a Single Intravenous and Oral Administration of Genz-112638 to Male Cynomolgus Monkeys (DMPK11-R059)

Methods: In this study, three male Cynomolgus monkeys received a single 1 mg/kg IV bolus dose, followed by a single 3 mg/kg oral (PO) dose of Genz-99067 (administered as Genz-112638, the hemi-tartrate salt form of Genz-99067), with treatments separated by a 7-day washout period. Serial blood samples were collected over 0 to 8 hours post dose, and the urine was collected over 0 to 8 hours post dose after the IV dose. Concentrations of Genz-99067 in the plasma and urine were determined by LC-MS/MS method.

Results: Following IV administration, mean $AUC_{0-\infty}$ and $t_{½}$ were 222 ng.hr/mL and 1.43 hours, respectively. Mean plasma clearance was 77.8 mL/min/kg. The mean steady-state volume of distribution was 5.13 L/kg, which is more than 8-fold greater than total body water (0.6 L/kg), suggesting extensive extravascular tissue distribution. Mean amount of Genz-99067 excreted in the urine was 15.6 µg (0.440% of the administered dose) and mean renal clearance was 0.443% of the mean systemic clearance. Following PO administration, mean observed C_{max} and t_{max} were 4.66 ng/mL and 0.250 hours, respectively. The mean $AUC_{0-\infty}$ and $t_{½}$ were 4.74 ng.hr/mL and 0.737 hours, respectively. The mean oral bioavailability was 0.803%. The following table (from page 15 and 16 of the report) show the PK data following IV and oral administration.

Table 4. Plasma and urinary pharmacokinetic parameters of Genz-99067 following after a single 1 mg/kg IV bolus dose of Genz-99067 (administered as Genz-112638) to fasted male cynomolgus monkeys

Treatment	Monkey ID	C_0	C_{last}	$t_{½}$	t_{last}	MRT	AUC_{last}	$AUC_{0-\infty}$	%Ext[a]	CL	CL_r	% CL_r/CL[b]	V_{ss}	V_z
		ng/mL		h			ng•h/mL		%	mL/min/kg			L/kg	
1.18 mg/kg Genz-112638 IV bolus dose[c]	3C21	243	0.557	1.27	8.00	1.15	268	269	0.381	62.0	0.174	0.281	4.29	6.84
	3C55	226	0.526	1.42	8.00	0.988	170	172	0.627	97.2	0.438	0.451	5.76	11.9
	3C7	281	1.06	1.59	8.00	1.20	223	225	1.08	74.1	0.443	0.598	5.33	10.2
	Mean	250	0.714	1.43	8.00	1.11	220	222	0.697	77.8	0.352	0.443	5.13	9.66
	SD	28.1	0.300	0.160	0.00	0.110	48.6	48.6	0.356	17.9	0.154	0.159	0.758	2.59
	%CV	11.2	42.0	11.2	0.00	9.93	22.1	21.9	51.1	23.0	43.7	35.8	14.8	26.8

[a] % of $AUC_{0-\infty}$ due to extrapolation
[b] Percentage of total clearance due to renal clearance.
[c] Equivalent to 1 mg/kg Genz-99067

Table 5. Plasma pharmacokinetic parameters of Genz-99067 following after a single 3 mg/kg PO gavage dose of Genz-99067 (administered as Genz-112638) to fasted male cynomolgus monkeys

Treatment	Monkey ID	C_{max}	C_{last}	t_{max}	$t_{½}$	t_{last}	MRT	AUC_{last}	$AUC_{0-\infty}$	%Ext[a]	%F
		ng/mL		h				ng•h/mL			
3.55 mg/kg Genz-112638 PO dose[d]	3C21	1.78	0.836	0.250	NC[b]	1.00	NC[b]	0.992	NC[b]	NC[b]	NC[b]
	3C55	5.20	0.730	0.250	0.718[c]	2.00	1.10	3.64	4.39	17.2	0.854
	3C7	6.99	0.710	0.250	0.757[c]	2.00	1.05	4.31	5.08	15.3	0.753
	Mean	4.66	0.759	0.250	0.737	1.67	1.07	2.98	4.74	16.2	0.803
	SD	2.65	0.0677	0.00	0.0281	0.577	0.0344	1.75	0.486	1.37	0.0714
	%CV	56.8	8.93	0.00	3.81	34.6	3.20	58.8	10.3	8.43	8.88

[a] % of $AUC_{0-\infty}$ due to extrapolation
[b] Not calculated due to poor estimate of $t_{½}$ (R^2=0.4)
[c] Calculated by log-linear regression of concentrations from t=0.5 to t=4 hours post-dose
[d] Equivalent to 3 mg/kg Genz-99067

Distribution:

Quantitative Whole Body Autoradiography of Rats Following Oral Administration of ^{14}C Genz-112638 (DMPK09-R001)

Methods: The purpose of this study was to assess the extent of absorption and distribution of ^{14}C-Genz-112638-derived radioactivity in rats following a single oral dose of ^{14}C-Genz-112638. In this study, quantitative whole-body autoradiographic (QWBA) techniques were used to determine the tissue distribution of radioactivity in male Long Evans (partially pigmented) rats following a single oral 50 mg/kg dose of ^{14}C-Genz-112638 (prepared with ^{14}C-Genz 99067, the free base form of ^{14}C-112638, specific activity = 48.455 µCi/mg). Carcasses were collected at 0.5, 2, 6, 12, 24, 48, 96, and 168 hours post dose. Radioactivity was measured in the blood, plasma, and protocol specified tissues, and radiation dosimetry parameters were calculated. The following structure shows the position of the radiolabel.

Radiolabeled Test Article

Test article:	^{14}C-Genz-99067[a]	Structure:
Molecular formula:	$C_{23}H_{36}N_2O_4$	
Molecular weight:	404.5	
Lot No.:	3497169A	
Specific activity:	48.455 µCi/mg	
Radiopurity:	98.7%	
Storage conditions:	Approximately -70°C	
a: Genz-112638 is the (b)(4)-tartaric acid salt of the freebase Genz-99067.		* signifies the position of the radiolabel

Results: Radioactivity was widely distributed to tissues at 0.5 hour post dose. The peak concentrations of radioactivity in the plasma (4710 ng-equivalents/g) and blood (3770 ng equivalents/g) were observed at 0.5 hour post dose; concentrations declined steadily to very low levels at 168 hours post dose. Most tissues had peak radioactivity concentrations at 0.5 hour post dose; however, in some tissues peak concentrations of radioactivity were measured at 2 hours post dose. The highest concentrations of radioactivity were found in the gastrointestinal tract contents, bile, urine, and liver. The highest peak tissue concentrations (excluding gastrointestinal tissues and contents), were observed in the liver, lung, adrenal gland, renal medulla, kidney, renal cortex, and spleen (range of 128000 to 21900 ng equivalents/g). The highest peak concentration of radioactivity in the bile (364000 ng equivalents/g) was observed at 0.5 hour post dose. The lowest measurable peak radioactivity concentrations were found in nasal turbinates and bone. Radioactivity in the central nervous system (CNS) e.g., cerebellum, cerebrum, medulla, olfactory lobe, and spinal cord were not measurable at any sampling time. Additionally, no radioactivity was measurable in the lens of the eye or abdominal fat at any sampling time. Low concentrations of radioactivity were detected in the testis through 6 hours post dose. Elimination was nearly complete from most tissues by 168 hours post dose; however, low concentrations of radioactivity remained in uveal tract of the eye, pigmented skin, liver, and eye. The ^{14}C-Genz-112638-derived radioactivity declined with half-life ($t_{1/2}$) values ranging from 1.24 hours (lung) to 312

hours (pigmented skin), while in most cases the $t_{1/2}$ values were less than 10 hours. The estimated $AUC_{0-\infty}$ for tissues ranged from 7370 ng-equivalent.hours/g (blood) to 2.22 mg-equivalent.hours/g (uveal tract).

The highest concentration in the pigmented skin was observed at 2 hours post dose (3290 ng-equivalents/g) and concentrations remained measurable through 168 hours post dose (1060 ng-equivalents/g). The highest concentration of radioactivity in the uveal tract (19100 ng-equivalents/g) was observed at 2 hours post dose and concentrations remained measurable through 168 hours post dose (3460 ng-equivalents/g). These data suggested that ^{14}C-Genz-112638-related radioactivity was selectively associated with melanin-containing tissues in the uveal tract and pigmented skin.

In pigmented male rats, uveal tract, pigmented skin, liver, eye, and pituitary gland were the five tissues expected to be exposed to the highest doses of radiation following a single oral administration of ^{14}C-Genz-112638. In humans, these tissues were estimated to be exposed to 29.2, 10.6, 5.15, 3.62, and 3.10 mRad (Rad = gray or absorbed dose), respectively. All other organs had lower radiation absorbed doses or absorbed doses that were incalculable. The total whole body effective dose was 3.25 mrem. This value was 923-fold below the FDA exposure limit of 3000 mrem after a single dose for human isotope studies. Based on the pharmacokinetic and dosimetry data, administration of a single oral 100-µCi dose of ^{14}C-Genz-112638 was not considered to represent a significant radiation exposure risk in humans.

Determination of Red Blood Cell Partitioning of Genz-99067 in Rat, Dog, and Human Whole Blood Using [^{14}C]-Genz-112638 (DMPK11-R030)

Methods: The partition coefficients of Genz-99067 between red blood cells (RBC) and plasma (K_{RBC}/Plasma) in the rat, dog and human were determined using [^{14}C]-Genz-112638 (specific activity: 24.90 mCi/mmmol) by liquid scintillation counting (LSC) method.

Results: [^{14}C]-Genz-112638 exhibited low RBC partitioning with K_{RBC}/Plasma of less than 2 in the rat, dog, and human whole blood. In male Sprague-Dawley rat whole blood, the mean K_{RBC}/Plasma was 0.714, 0.762, and 1.81 at 0.1, 1.0, and 10 µM of [^{14}C]-Genz-112638, respectively. In male Beagle dog whole blood, the mean K_{RBC}/Plasma was 0.867, 0.781, and 1.37 at 0.1, 1.0 and 10 µM of [^{14}C]-Genz-112638, respectively. In male human whole blood, the mean K_{RBC}/Plasma was 1.68 and 1.83 at 0.1 and 1.0 µM of [^{14}C]-Genz 112638, respectively. In female human whole blood, the mean K_{RBC}/Plasma was 1.83 and 1.86 at 0.1 and 1.0 µM of [^{14}C]-Genz 112638, respectively.

Protein Binding of Genz-99067 in Mouse, Rat, Dog, Monkey and Human Plasma Using Rapid Equilibrium Dialysis (RED) and LC-MS/MS (DMPK11-R031)

Methods: The extent of plasma protein binding of Genz-99067 was determined at 0.01, 0.1 and 1.0 µM concentrations in human plasma and at 0.1, 1.0 and 10 µM in the mouse, rat, dog, and monkey plasma using equilibrium dialysis and quantitated by LC-MS/MS.

Results: The mean percent bound of Genz-99067 to mouse plasma proteins was 98.9%, 98.7% and 95.3% at 0.1, 1.0 and 10 µM, respectively. The mean percent bound of Genz-99067 to rat plasma proteins was 99.0%, 97.2% and 79.7% at 0.1, 1.0 and 10 µM, respectively. The mean percent bound of Genz-99067 to dog plasma proteins was 98.2%, 97.4% and 91.5% at 0.1, 1.0 and 10 µM, respectively. The mean percent bound of Genz-99067 to monkey plasma proteins was 92.2%, 92.0% and 80.7% at 0.1, 1.0 and 10 µM, respectively. The mean percent bound of Genz-99067 to human plasma proteins was 82.9%, 79.5% and 76.4% at 0.01, 0.1 and 1.0 µM, respectively.

Pharmacokinetics, Tissue Distribution and Mass Balance Study in Sprague-Dawley Rats Following Single Administration of [^{14}C] Genz 112638 by Intravenous Injection and Oral Gavage (GT-157-PK-1)

The following review is incorporated below from the pharmacology review of IND 67589 dated July 30, 2004 by Dr. Dylan Yao (DMEP).

2.6.4.4 Distribution

Following I.V. injection to rats at 10 mg/kg, the radioactivity levels in most tissues were below quantifiable limits at 24 hours and thereafter. The only tissues which had quantifiable radioactivity levels (kidneys, liver, lungs, intestines/contents and testes) had levels representing 0.094 to 7.38% of the dose at 24 hours for intestines/contents and 0.014 to 0.74% for other tissues. Following oral gavage to rats at 100 mg/kg, in a similar manner to that noted for I.V. injection, most tissues had non-quantifiable levels of radioactivity at 24 hours and thereafter. Specifically, levels were quantifiable only in the stomach/intestines/contents (0.11 to 18.5% of dose at 24 hours) and kidneys, liver, lungs and testes (0.03 to 0.58%). For both routes, radioactivity levels decreased over time in all organs. In the dog I.V. and oral study, due to the complete excretion balance results, the radioactivity in individual tissue/organ was not analyzed (the animals were sacrificed on day 7).

Tissue distribution of [^{14}C] Genz-112638 (as ng-eq/gh or as % of dose) after a single IV injection (10 mg/kg) and a single oral administration (100 mg/kg) to male and female SD rats were summarized in the following scanned tables:

Table 17

MEAN (n=3) TOTAL RADIOACTIVITY IN TISSUE (ng-eq/g) FOLLOWING A SINGLE INTRAVENOUS INJECTION (10 mg/kg) OF [^{14}C] GENZ-112638 TO MALE SPRAGUE-DAWLEY RATS

Tissue	Sampling time							
	24 h		48 h		96 h		168 h	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Adrenals	nc	nc	nc	nc	nc	nc	nc	nc
Brain	nc	nc	nc	nc	nc	nc	nc	nc
Eyes	nc	nc	nc	nc	nc	nc	nc	nc
Femur	nc	nc	nc	nc	nc	nc	nc	nc
Heart	nc	nc	nc	nc	nc	nc	nc	nc
Kidneys	596	85	457	64	284	42	nc	nc
Liver	1307	630	312	107	569	274	299	22
Lymph nodes	nc	nc	nc	nc	nc	nc	nc	nc
Lungs	227	47	202	17	nc	nc	nc	nc
Muscle	nc	nc	nc	nc	nc	nc	nc	nc
Pancreas	nc	nc	nc	nc	nc	nc	nc	nc
Spleen	nc	nc	nc	nc	nc	nc	nc	nc
Skin	nc	nc	nc	nc	nc	nc	nc	nc
Stomach	nc	nc	nc	nc	nc	nc	nc	nc
Intestinal content	7329	7473	779	869	nc	nc	nc	nc
Small intestine	279	93	nc	nc	nc	nc	nc	nc
Large intestine	7102	4685	601	660	nc	nc	nc	nc
Spinal cord	nc	nc	nc	nc	nc	nc	nc	nc
Testes	244	29	93	83	nc	nc	nc	nc
Urinary bladder	nc	nc	nc	nc	nc	nc	nc	nc

nc: not calculated (more than 50% of individual values found BLQ)

Table 18

MEAN (n=3) TOTAL RADIOACTIVITY IN TISSUE (as % of dose) FOLLOWING A SINGLE INTRAVENOUS INJECTION (10 mg/kg) OF [^{14}C] GENZ-112638 TO MALE SPRAGUE-DAWLEY RATS

Tissue	Sampling time							
	24 h		48 h		96 h		168 h	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Adrenals	nc	nc	nc	nc	nc	nc	nc	nc
Brain	nc	nc	nc	nc	nc	nc	nc	nc
Eyes	nc	nc	nc	nc	nc	nc	nc	nc
Femur	nc	nc	nc	nc	nc	nc	nc	nc
Heart	nc	nc	nc	nc	nc	nc	nc	nc
Kidneys	0.06	0.01	0.05	0.01	0.03	0.01	nc	nc
Liver	0.74	0.36	0.19	0.05	0.24	0.10	0.21	0.02
Lymph nodes	nc	nc	nc	nc	nc	nc	nc	nc
Lungs	0.014	0.002	0.012	0.002	nc	nc	nc	nc
Muscle	nc	nc	nc	nc	nc	nc	nc	nc
Pancreas	nc	nc	nc	nc	nc	nc	nc	nc
Spleen	nc	nc	nc	nc	nc	nc	nc	nc
Skin	nc	nc	nc	nc	nc	nc	nc	nc
Stomach	nc	nc	nc	nc	nc	nc	nc	nc
Intestinal content	6.76	7.43	0.85	0.99	nc	nc	nc	nc
Small intestine	0.094	0.032	nc	nc	nc	nc	nc	nc
Large intestine	1.11	0.77	0.08	0.09	nc	nc	nc	nc
Spinal cord	nc	nc	nc	nc	nc	nc	nc	nc
Testes	0.028	0.002	0.011	0.010	nc	nc	nc	nc
Urinary bladder	nc	nc	nc	nc	nc	nc	nc	nc

nc: not calculated (more than 50% of individual values found BLQ)

Table 19

MEAN (n=3) TOTAL RADIOACTIVITY IN TISSUE (ng-eq/g) FOLLOWING A SINGLE INTRAVENOUS INJECTION (10 mg/kg) OF [^{14}C] GENZ-112638 TO FEMALE SPRAGUE-DAWLEY RATS

Tissue	Sampling time							
	24 h		48 h		96 h		168 h	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Adrenals	nc	nc	nc	nc	nc	nc	nc	nc
Brain	nc	nc	nc	nc	nc	nc	nc	nc
Eyes	nc	nc	nc	nc	nc	nc	nc	nc
Femur	nc	nc	nc	nc	nc	nc	nc	nc
Heart	nc	nc	nc	nc	nc	nc	nc	nc
Kidneys	373	51	298	46	210	23	nc	nc
Liver	770	176	285	250	434	47	240	27
Lymph nodes	nc	nc	nc	nc	nc	nc	nc	nc
Lungs	302	16	137	119	nc	nc	nc	nc
Muscle	nc	nc	nc	nc	nc	nc	nc	nc
Pancreas	nc	nc	nc	nc	nc	nc	nc	nc
Spleen	nc	nc	nc	nc	nc	nc	nc	nc
Skin	nc	nc	nc	nc	nc	nc	nc	nc
Stomach	nc	nc	nc	nc	nc	nc	nc	nc
Intestinal content	10912	2920	473	211	nc	nc	nc	nc
Small intestine	429	96	nc	nc	nc	nc	nc	nc
Large intestine	8541	2770	nc	nc	nc	nc	nc	nc
Spinal cord	nc	nc	nc	nc	nc	nc	nc	nc
Urinary bladder	nc	nc	nc	nc	nc	nc	nc	nc

nc: not calculated (more than 50% of individual values found BLQ)

Table 20

MEAN (n=3) TOTAL RADIOACTIVITY IN TISSUE (as % of dose) FOLLOWING A SINGLE INTRAVENOUS INJECTION (10 mg/kg) OF [^{14}C] GENZ-112638 TO FEMALE SPRAGUE-DAWLEY RATS

Tissue	Sampling time							
	24 h		48 h		96 h		168 h	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Adrenals	nc	nc	nc	nc	nc	nc	nc	nc
Brain	nc	nc	nc	nc	nc	nc	nc	nc
Eyes	nc	nc	nc	nc	nc	nc	nc	nc
Femur	nc	nc	nc	nc	nc	nc	nc	nc
Heart	nc	nc	nc	nc	nc	nc	nc	nc
Kidneys	0.036	0.004	0.032	0.003	0.023	0.003	nc	nc
Liver	0.39	0.05	0.16	0.14	0.25	0.03	0.16	0.02
Lymph nodes	nc	nc	nc	nc	nc	nc	nc	nc
Lungs	0.018	0.001	0.010	0.009	nc	nc	nc	nc
Muscle	nc	nc	nc	nc	nc	nc	nc	nc
Pancreas	nc	nc	nc	nc	nc	nc	nc	nc
Spleen	nc	nc	nc	nc	nc	nc	nc	nc
Skin	nc	nc	nc	nc	nc	nc	nc	nc
Stomach	nc	nc	nc	nc	nc	nc	nc	nc
Intestinal content	7.38	1.46	0.43	0.20	nc	nc	nc	nc
Small intestine	0.15	0.03	nc	nc	nc	nc	nc	nc
Large intestine	1.48	0.62	nc	nc	nc	nc	nc	nc
Spinal cord	nc	nc	nc	nc	nc	nc	nc	nc
Urinary bladder	nc	nc	nc	nc	nc	nc	nc	nc

nc: not calculated (more than 50% of individual values found BLQ)

Table 21

MEAN (n=3) TOTAL RADIOACTIVITY IN TISSUE (ng-eq/g) FOLLOWING A SINGLE ORAL ADMINISTRATION (100 mg/kg) OF [^{14}C] GENZ-112638 TO MALE SPRAGUE-DAWLEY RATS

Tissue	Sampling time							
	24 h		48 h		96 h		168 h	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Adrenals	nc	nc	nc	nc	nc	nc	nc	nc
Brain	nc	nc	nc	nc	nc	nc	nc	nc
Eyes	nc	nc	nc	nc	nc	nc	nc	nc
Femur	nc	nc	nc	nc	nc	nc	nc	nc
Heart	nc	nc	nc	nc	nc	nc	nc	nc
Kidneys	3266	668	2325	692	nc	nc	nc	nc
Liver	11184	1675	7148	1327	3383	434	1921	309
Lymph nodes	nc	nc	nc	nc	nc	nc	nc	nc
Lungs	1351	1171	nc	nc	nc	nc	553	497
Muscle	nc	nc	nc	nc	nc	nc	nc	nc
Pancreas	nc	nc	nc	nc	nc	nc	nc	nc
Spleen	nc	nc	nc	nc	nc	nc	nc	nc
Skin	nc	nc	nc	nc	nc	nc	nc	nc
Stomach	13997	3089	9782	1431	nc	nc	nc	nc
Spinal cord	nc	nc	nc	nc	nc	nc	nc	nc
Small intestine	11039	5449	nc	nc	nc	nc	nc	nc
Large intestine	80112	22985	18669	18061	nc	nc	nc	nc
Intestinal content	165900	48186	12377	16259	3393	2304	nc	nc
Testes	2254	455	863	752	788	688	820	136
Urinary bladder	nc	nc	nc	nc	nc	nc	nc	nc

nc: not calculated (more than 50% of individual values found BLQ)

Table 22

MEAN (n=3) TOTAL RADIOACTIVITY IN TISSUE (as % of dose) FOLLOWING A SINGLE ORAL ADMINISTRATION (100 mg/kg) OF [^{14}C] GENZ-112638 TO MALE SPRAGUE-DAWLEY RATS

Tissue	Sampling time							
	24 h		48 h		96 h		168 h	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Adrenals	nc	nc	nc	nc	nc	nc	nc	nc
Brain	nc	nc	nc	nc	nc	nc	nc	nc
Eyes	nc	nc	nc	nc	nc	nc	nc	nc
Femur	nc	nc	nc	nc	nc	nc	nc	nc
Heart	nc	nc	nc	nc	nc	nc	nc	nc
Kidneys	0.03	0.01	0.02	0.01	nc	nc	nc	nc
Liver	0.58	0.08	0.44	0.07	0.13	0.02	0.16	0.02
Lymph nodes	nc	nc	nc	nc	nc	nc	nc	nc
Lungs	0.007	0.006	nc	nc	nc	nc	0.005	0.005
Muscle	nc	nc	nc	nc	nc	nc	nc	nc
Pancreas	nc	nc	nc	nc	nc	nc	nc	nc
Spleen	nc	nc	nc	nc	nc	nc	nc	nc
Skin	nc	nc	nc	nc	nc	nc	nc	nc
Stomach	0.11	0.02	0.06	0.00	nc	nc	nc	nc
Spinal cord	nc	nc	nc	nc	nc	nc	nc	nc
Small intestine	0.29	0.13	nc	nc	nc	nc	nc	nc
Large intestine	1.10	0.33	0.16	0.13	nc	nc	nc	nc
Intestinal content	11.26	1.76	0.85	0.99	0.42	0.32	nc	nc
Testes	0.026	0.004	0.011	0.009	0.010	0.009	0.014	0.003
Urinary bladder	nc	nc	nc	nc	nc	nc	nc	nc

nc: not calculated (more than 50% of individual values found BLQ)

Table 23

MEAN (n=3) TOTAL RADIOACTIVITY IN TISSUE (ng-eq/g) FOLLOWING A SINGLE ORAL ADMINISTRATION (100 mg/kg) OF [^{14}C] GENZ-112638 TO FEMALE SPRAGUE-DAWLEY RATS

Tissue	Sampling time							
	24 h		48 h		96 h		168 h	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Adrenals	nc	nc	nc	nc	nc	nc	nc	nc
Brain	nc	nc	nc	nc	nc	nc	nc	nc
Eyes	nc	nc	nc	nc	nc	nc	nc	nc
Femur	nc	nc	nc	nc	nc	nc	nc	nc
Heart	nc	nc	nc	nc	nc	nc	nc	nc
Kidneys	nc	nc	nc	nc	nc	nc	nc	nc
Liver	8423	1061	5865	1158	2963	37	1486	437
Lymph nodes	nc	nc	nc	nc	nc	nc	nc	nc
Lungs	nc	nc	nc	nc	nc	nc	nc	nc
Muscle	nc	nc	nc	nc	nc	nc	nc	nc
Pancreas	nc	nc	nc	nc	nc	nc	nc	nc
Spleen	nc	nc	nc	nc	nc	nc	nc	nc
Skin	nc	nc	nc	nc	nc	nc	nc	nc
Stomach	21142	5152	11103	972	nc	nc	nc	nc
Spinal cord	nc	nc	nc	nc	nc	nc	nc	nc
Small intestine	16012	9980	nc	nc	nc	nc	nc	nc
Large intestine	187989	157248	8735	8511	nc	nc	nc	nc
Intestinal content	277978	87424	16875	9917	nc	nc	nc	nc
Bladder	nc	nc	nc	nc	nc	nc	nc	nc

nc: not calculated (more than 50% of individual values found BLQ)

Table 24

MEAN (n=3) TOTAL RADIOACTIVITY IN TISSUE (as % of dose) FOLLOWING A SINGLE ORAL ADMINISTRATION (100 mg/kg) OF [^{14}C] GENZ-112638 TO FEMALE SPRAGUE-DAWLEY RATS

Tissue	Sampling time							
	24 h		48 h		96 h		168 h	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Adrenals	nc	nc	nc	nc	nc	nc	nc	nc
Brain	nc	nc	nc	nc	nc	nc	nc	nc
Eyes	nc	nc	nc	nc	nc	nc	nc	nc
Femur	nc	nc	nc	nc	nc	nc	nc	nc
Heart	nc	nc	nc	nc	nc	nc	nc	nc
Kidneys	nc	nc	nc	nc	nc	nc	nc	nc
Liver	0.41	0.07	0.33	0.06	0.16	0.01	0.10	0.03
Lymph nodes	nc	nc	nc	nc	nc	nc	nc	nc
Lungs	nc	nc	nc	nc	nc	nc	nc	nc
Muscle	nc	nc	nc	nc	nc	nc	nc	nc
Pancreas	nc	nc	nc	nc	nc	nc	nc	nc
Spleen	nc	nc	nc	nc	nc	nc	nc	nc
Skin	nc	nc	nc	nc	nc	nc	nc	nc
Stomach	0.17	0.05	0.06	0.01	nc	nc	nc	nc
Spinal cord	nc	nc	nc	nc	nc	nc	nc	nc
Small intestine	0.58	0.34	nc	nc	nc	nc	nc	nc
Large intestine	3.19	2.99	0.16	0.07	nc	nc	nc	nc
Intestinal content	18.5	5.36	1.62	1.17	nc	nc	nc	nc
Bladder	nc	nc	nc	nc	nc	nc	nc	nc

nc: not calculated (more than 50% of individual values found BLQ)

Pharmacokinetics, Tissue Distribution and Mass Balance of Radioactivity in Sprague-Dawley Rats Following Single Oral Administration of ^{14}C-Genz-112638 with and without Pretreatment with Unlabeled Genz-112638 (GT-157-PK-10)

Methods: The purpose of this study was to determine the following parameters in male SD rats after a single oral administration of ^{14}C-Genz-112638 with and without pretreatment with unlabeled Genz-112638:

- Concentration and/or content of radioactivity in the whole blood, plasma [by liquid scintillation spectroscopy (LSC) and tissues (by quantitative whole-body autoradioluminography (QWBA) and LSC)
- Disposition kinetics (C_{max}, t_{max}, AUC, k and $t_{½}$) of total radioactivity in the whole blood, plasma and tissues
- Excretion of radioactivity: route of elimination and recovery of radioactivity up to 120 hours post dose
- Mass balance of radioactivity at 120 hours post dose.
- Distribution of radioactivity to the central nervous system (CNS)

The following table (from page 13 of the report) shows the study design.

The animals were assigned to study groups as follows:

Group No.	Dose Level (mg/kg)[d]	Dose Volume (mL/kg)	Animal Numbers
1[a]	100	5	1001-1018
2[b]	100	5	2001-2018
3[c]	100	5	3001-3005
4[a]	100	5	4001-4018
5[b]	100	5	5001-5018
6[c]	100	5	6001-6005

[a] Animals processed for Quantitative Whole-Body Autoradioluminography.
[b] Tissues collected for radioactivity determination by liquid scintillation spectroscopy.
[c] Mass balance groups.
[d] Animals of Groups 1, 2 and 3 were administered a single dose of ^{14}C-GENZ-112638 formulation. Animals of Groups 4, 5 and 6 were administered unlabelled formulation on Days 1 to 6 and ^{14}C-GENZ-112638 formulation on Day 7.

A total of 82 male SD rats were administered ^{14}C-Genz-112638 (specific activity = 59.2 mCi/mmmol, position of radiolabel was not provided) by oral gavage at a target dose level of 100 mg/kg (18 µCi/animal). Blood samples were collected from Group 1 and 4 animals at selected time points prior to euthanasia. Following blood collection, the animals were euthanized and radioactivity concentration in tissues was determined using QWBA. Groups 2 and 5 (3 animals per time point) were euthanized at selected time points and selected tissues were collected and radioactivity was determined using LSC. Urine, feces, carcass and cage rinses were collected from the Group 3 and 6 animals over designated intervals up to 120 h post dose. The radioactivity concentrations in these samples and in blood/plasma samples were measured by LSC.

Results:

Plasma and blood: Following oral administration of ^{14}C-GENZ-112638, the observed radioactivity concentrations in the blood were low, ranging from 0.4 to 3.3 µg eq/mL for Group 1 and 0.4 to 4.0 µg eq/mL for Group 4 (percent of dose below 1% at all the time points). The concentrations in the plasma ranged from 0.4 to 3.8 µg eq/mL for Group 1 and 0.5 to 4.3 µg eq/mL for Group 4. The mean C_{max} values were 3.82 µg eq/mL and 3.26 µg eq/mL for Group 1 and 4.54 µg eq/mL and 4.02 µg eq/mL for Group 4, respectively for the plasma and blood. The t_{max} for the plasma and blood was observed at 30 minutes or 1 hour post dose. The mean calculated areas under the radioactivity concentration *vs.* time curves ($AUC_{0\text{-}tlast}$) were 29.71 and 23.18 µg eq·hr/mL for Group 1 and 36.67 and 28.24 µg eq·hr/mL for Group 4, for the plasma and blood, respectively. The apparent terminal $t_{½}$ values reported for radioactivity in the plasma for Group 1 was 10.1 hours and for Group 4 the terminal $t_{½}$ in the plasma and blood were 8.63 and 8.48 hours, respectively. The mean blood to plasma ratios of radioactivity were similar for Groups 1 and 4 and ranged from 0.73 to 0.94. For all groups, the blood to plasma ratios generally remained constant, indicating that radioactivity was mostly eliminated at the same rate from both plasma and blood.

Tissues: In Group 1, 2, 4 and 5 animals, the highest tissue radioactivity concentration and AUC values were obtained from the gastrointestinal tract (GIT), notably the stomach and small and large intestine walls. High radioactivity concentrations were also found in the GIT contents. The kidney, liver, adrenal gland and urinary bladder wall also demonstrated relatively high concentrations of radioactivity. Limited exposure was observed in the brain, eye and spinal cord, for which the mean radioactivity concentrations were below the limit of quantification (LOQ) for a couple of time points for Groups 1 and 4. The radioactivity concentrations in the brain for Groups 2 and 5 (hemispheres, spinal cord, pituitary gland, mid-brain, cerebellum) indicated that most of the radioactivity found in the brain was associated with the pituitary gland. The percent of dose values for brain structures were 0 to 0.004% of the administered dose. The radioactivity concentrations ranged from 0 to 53.92 for pituitary gland and from 0 to 0.802 for other brain structures Group 2 (single dose) and 0 to 1.069 for other brain structures Group 5 (repeated dose). Radioactivity levels for Group 5 brain-related structures were generally higher than for Group 2 for earlier time points but for later time points (24 hours post-dose) radioactivity levels were higher for Group 2. For Group 5, no radioactivity was detected in any brain structures at 24 hours post dose but for Group 2, radioactivity was still detected in the pituitary gland and hemispheres at 24 hours post-dose. For Groups 1 and 4, the cerebro-spinal fluid (CSF) also had relatively high levels of radioactivity (with mean values ranging from below LOQ to 13.91 µg eq/g) as compared to the brain (with mean values below LOQ at all points). For Groups 1, 2, 4 and 5, the t_{max} of radioactivity for tissues occurred generally at 30 minutes to 1 hour post-dose.

In general, the tissue to plasma ratios for Groups 1 and 4 remained constant. The tissue to plasma ratios for animals administered a single radiolabeled dose (Group 1) were similar to animals administered repeated dosing with unlabeled drug followed by a single radiolabeled dose (Group 4).

Excretion of radioactivity: Radioactivity was mainly excreted *via* the feces, with approximately 78% and 83% recovered over 120 hours post-dose for Groups 3 and 6, respectively. Only 11% of the administered radioactivity was recovered in the urine for both Groups 3 and 6 and a further 0.5% to 0.6% of the radioactivity was recovered in cage washes. Most (at least 70%) of the radioactivity was excreted by 24 hours post-dose but excretion continued up to 120 hours post-dose giving a total mean recovery of 89.6% and 93.7% in the excreta from Groups 3 and 6, respectively.

In summary, the observed radioactivity concentrations in the blood and plasma were low. The blood to plasma ratios generally remained constant over the study interval. The highest tissue radioactivity concentrations were observed in the GIT, notably the stomach and small and large intestine walls. High radioactivity concentrations were also found in the GIT contents. Kidney, liver, adrenal gland and urinary bladder wall also demonstrated relatively high concentrations of radioactivity. Limited exposure was observed in the brain, eye and spinal cord for which the mean radioactivity concentrations were below the LOQ for a couple of time points for Groups 1 and 4. Radioactivity concentrations in the brain for Groups 2 and 5 (hemispheres, spinal cord, pituitary gland, mid-brain, cerebellum) animals indicated that most of the radioactivity found in the brain was associated with the pituitary gland. The percent of the administered dose values for the brain structures were low. Radioactivity levels for Group 5 brain-related structures were generally higher than for Group 2 for earlier time points but for later time points (e.g. 8 and 24 hours post-dose) radioactivity levels were higher for Group 2. For Group 5, no radioactivity was detected in any brain structures at 24 hours post-dose but, for Group 2, radioactivity was still detected in the pituitary gland and hemispheres at 24 hours post-dose. Excretion of radioactivity was mainly *via* the feces (with approximately 78% and 83% recovered over 120 hours post-dose for Group 3 and 6, respectively. Only 11% of the administered radioactivity was recovered in the urine over the same time period for both Groups 3 and 6.

Quantitative Tissue Distribution using Whole-Body Autoradiography following a Single, Oral Dose of [^{14}C]Genz-99067 (125 mg/kg) to Cyclosporin-A Pretreated and Untreated Male, CF-1 and untreated PGP-deficient mice (GT-157-PK-17)

Methods: The purpose of this study was to determine the tissue distribution following a single, oral (PO) administration of [^{14}C]Genz-99067 (125 mg/kg free base, specific activity = 20.07 mCi/mmol) to male CF-1 mice that are either treated or not treated with a PGP-inhibitor (cyclosporin-A) and to PGP-deficient, male mice using QWBA. Group 1 animals (six, male, CF-1 mice) were administered a single, PO administration of [^{14}C]Genz-99067 at 125 mg/kg. Group 2 (six, male, CF-1 mice) were administered a single, PO administration of a cyclosporine (50 mg/kg) at approximately 2 h before each was given a single, PO administration of [^{14}C]Genz-99067 at 125 mg/kg. Group 3 [six, male, PGP-Deficient mice (Crl:CF-1®*mdr1a*PGP)], were administered a single, PO administration of [^{14}C]-Genz-99067 at a target dose of 125 mg/kg. Two mice, per Group, per time point were euthanized and frozen at 0.5, 1, and 2 h post-dose. After euthanasia, a blood sample was collected for radioanalysis by LSC and the carcasses were frozen and embedded for QWAB.

Results: In all groups, the highest tissue radioactivity concentrations were observed in the gall bladder, kidneys, and urinary bladder. High radioactivity concentrations were also found in the stomach, small intestine, liver and kidney. Moderate radioactivity concentrations were found in lung, spleen, heart, adrenal, pituitary and prostate glands, pancreas, and bone marrow. Limited exposure was observed in the brain, eyes, testes, and spinal cord at all time points in normal mice, with mean radioactivity concentration close to the LOQ. In P-gp deficient mice, brain tissues showed an approximately 10-fold increase in radioactivity compared to normal mice, indicating that Genz-99067 is a substrate for the mouse P-gp transporter. The following table (from page 8 of the report) shows the concentrations of radioactivity in different tissues.

Table 3. Concentrations of [^{14}C]Genz-990067-Derived Radioactivity in Tissues of Male Mice After a Single Oral Dose at 125 mg/kg

Tissue	Concentration of (µg equivalents of Genz-990067 / g)																	
	Normal Mice						Normal Pre-Treated w/ CSA						PGP-KO Mice					
	0.5 h		1 h		2 h		0.5 h		1 h		2 h		0.5 h		1 h		2 h	
	An. 1	An. 2	An. 3	An. 4	An. 5	An. 6	An. 7	An. 8	An. 9	An. 10	An. 11	An. 12	An. 13	An. 14	An. 15	An. 16	An. 17	An. 18
Lg Intestine	23.78	15.57	41.58	13.45	14.31	11.78	12.99	17.22	23.33	33.05	9.17	32.09	23.42	40.34	31.41	17.88	76.79	30.65
Sm. Intestine	22.84	32.52	163.58	72.99	403.61	55.69	130.77	119.05	49.18	177.18	25.08	141.85	20.58	331.59	304.19	257.53	200.71	446.35
Stomach	94.77	66.99	83.68	169.25	63.07	59.16	139.54	28.52	52.35	128.02	22.99	94.66	63.00	248.00	158.46	34.02	85.80	58.56
Brain Cerebellum	1.20	1.54	2.20	0.83	1.00	0.81	1.07	1.39	1.26	2.42	0.73	1.64	9.51	26.55	21.71	7.49	21.02	18.44
Brain Cerebrum	1.35	1.44	1.98	BQL	0.90	0.90	0.71	1.32	1.11	1.78	0.62	1.60	7.87	26.26	22.09	7.14	20.92	19.60
Brain Medulla	1.54	1.45	1.57	0.28	0.93	0.85	0.59	1.33	1.18	1.73	0.65	1.34	8.96	23.79	21.98	7.10	20.93	17.19
Cerebral Spinal Fluid	2.28	1.95	1.94	0.84	2.42	1.31	1.06	1.33	1.31	1.51	1.67	2.85	2.88	2.24	1.82	1.64	5.61	2.28
Meninges	22.41	10.01	18.20	7.35	5.43	7.28	8.05	11.48	16.01	25.04	13.00	15.69	8.55	18.14	16.76	14.31	18.24	21.93
Spinal Cord	1.72	1.24	1.46	1.74	1.05	1.71	1.12	1.06	2.26	2.12	1.07	1.82	10.43	33.07	24.41	8.66	23.60	21.07
Skin	9.64	8.41	23.44	6.79	9.85	9.25	13.00	12.47	19.20	29.34	11.60	22.51	11.38	21.30	18.55	12.91	19.03	18.53
Adrenal Gland	18.03	28.42	35.65	11.38	17.24	8.84	16.27	29.26	23.27	35.92	11.29	39.81	27.67	72.16	42.27	21.41	42.14	26.71
Pituitary Gland	42.01	48.80	47.22	16.42	25.75	16.97	32.98	36.39	27.45	51.57	23.45	57.53	28.60	71.53	68.74	27.14	66.95	52.98
Bile(Gall Bladder)	1324	190.51	1873	1219	3489	2189	428.96	117.27	229.96	NP	150.58	1181	876.50	388.35	1105	383.46	1896	1556
Kidney	167.13	83.00	235.12	97.49	141.52	99.06	62.45	148.39	167.89	306.80	49.37	175.95	102.36	211.72	178.29	103.35	112.34	149.97
Liver	250.91	307.40	1365	138.37	176.38	124.17	122.42	196.08	128.57	227.40	54.73	184.03	266.18	496.84	383.07	199.25	313.50	277.02
Urinary Bladder	747.81	245.98	413.10	3499	4915*	2000	942.27	317.51	4003*	469.33	1250	1778	807.68	779.08	6224*	879.32	232.02	625.64
Eye (Lens)	BQL	BQL	0.66	0.31	0.47	0.42	0.30	0.96	0.72	0.51	BQL	0.33	0.57	0.64	0.97	0.78	1.71	1.55
Eye (Uveal)	3.98	1.40	27.88	9.15	3.64	0.71	2.64	3.11	13.93	23.15	10.18	27.94	3.54	10.11	5.06	13.05	24.81	8.55
Epididymis (entire)	16.04	18.04	15.20	9.24	9.77	12.35	13.51	19.47	18.49	NP	7.55	37.32	20.96	33.50	13.03	11.91	39.83	30.51
Epididymis (high)	29.92	30.91	26.02	16.35	15.96	22.01	21.63	33.89	32.52	NP	8.83	61.53	29.35	69.92	21.63	20.66	64.26	39.55
Epididymis (low)	8.82	9.94	10.64	6.90	8.54	5.92	9.01	12.92	12.96	NP	6.90	19.58	11.08	20.13	8.88	8.20	22.14	22.99
Prostate Gland	13.91	3.88	14.18	10.60	9.60	9.96	4.57	4.67	10.79	13.54	3.35	24.73	7.37	14.91	13.25	6.34	32.55	14.94
Testis	1.84	1.44	4.59	1.91	2.79	1.94	2.88	2.53	3.60	BQL	3.11	5.48	3.78	8.99	7.74	4.56	8.55	10.54
Lung	44.96	69.14	68.44	25.36	32.42	17.08	28.03	52.01	37.66	78.02	25.19	81.09	47.63	139.68	34.13	41.10	97.64	73.40
Pancreas	45.55	36.90	61.96	13.43	21.73	12.67	19.64	45.65	30.03	71.06	15.21	54.71	35.60	125.36	61.16	36.16	86.87	59.14
Bone	2.65	2.50	5.87	1.44	2.87	2.08	5.42	3.86	3.34	8.02	0.89	5.35	3.01	4.81	5.10	4.20	6.92	3.16
Heart	28.92	13.01	38.92	10.50	16.78	8.17	21.03	31.39	20.57	33.38	9.16	23.20	16.31	69.39	33.92	22.22	38.05	32.60
Skeletal Muscle	12.15	14.72	16.46	5.60	8.61	4.77	6.37	12.94	10.85	19.81	6.40	17.89	13.43	20.30	17.44	8.37	15.96	14.57

Table 3. cont. Concentrations of Radioactivity in Tissues of Male Mice After a Single Oral Dose of [^{14}C]Genz-990067 at 125 mg/kg

Tissue	Concentration of (µg equivalents of Genz-990067 / g)																	
	Normal Mice						Normal Pre-Treated w/ CSA						PGP-KO Mice					
	0.5 h		1 h		2 h		0.5 h		1 h		2 h		0.5 h		1 h		2 h	
	An. 1	An. 2	An. 3	An. 4	An. 5	An. 6	An. 7	An. 8	An. 9	An. 10	An. 11	An. 12	An. 13	An. 14	An. 15	An. 16	An. 17	An. 18
Blood (in heart)	17.67	12.78	34.77	13.04	15.34	10.47	19.64	22.43	31.24	31.35	13.11	33.71	24.34	23.13	27.56	19.55	20.36	27.05
Blood (LSC)	17.79	13.66	38.23	10.00	14.08	10.96	18.83	21.99	26.00	36.03	12.37	21.55	18.94	24.10	30.27	19.93	26.27	26.63
Plasma (LSC)	23.84	18.41	60.48	17.11	21.77	18.26	31.53	33.52	43.92	54.09	20.21	31.74	29.50	29.75	44.33	32.18	38.66	38.53
Bone Marrow	25.73	15.89	28.63	10.47	15.11	9.05	12.29	21.05	19.17	37.40	11.43	27.43	17.85	53.84	33.02	14.72	24.16	23.71
Lymph Nodes	18.70	14.89	34.23	8.10	12.40	8.53	9.00	18.41	17.41	33.10	12.03	37.49	16.65	35.78	NP	15.47	32.85	32.04
Spleen	41.07	30.25	36.50	10.96	22.63	11.29	23.07	38.93	30.01	46.32	11.10	45.99	21.58	62.19	43.02	24.70	37.49	32.44

NP = Not Present on section
Lower Limit of Quantitation = 0.298 ug equiv / g sample
Upper Limit of Quantitation = 3604 ug equiv / g sample
* AQL = Above upper limit of quantitation

Metabolism:

In Vitro Metabolic Profiles of [^{14}C]-Genz-99067 in Rat, Dog, Monkey and Human Liver Microsomes and Hepatocytes (DMPK10-R025)

Methods: The purpose of this study was to evaluate the *in vitro* metabolic profiles of [^{14}C]-Genz-99067 in the rat, dog, monkey and human liver microsomes and hepatocytes. In this study, [^{14}C]-Genz-99067 (5 µM, 20 mCi/mg) was incubated with the rat, dog, monkey and human liver microsomes and hepatocytes at 37^{0}C for 1 and 2 hour, respectively, prior to sample analysis. Incubations were terminated by the addition of acetontrile. Following centrifugation, the supernatant was concentrated, the remaining extract was centrifuged prior to LC-MS analyses.

Results: Forty two metabolites were identified *in vitro* following incubation of [^{14}C]-Genz-99067 in the rat, dog, monkey, and human liver microsomes and hepatocytes. Major metabolites were formed by oxidation of the octanoyl moiety, including 7-hydroxyl metabolite Genz-256416 and 6-hydroxyl metabolite Genz-311752, followed by oxidation of 2,3-dihydro-1,4-benzodioxane moiety across all species evaluated. Rat specific *in vitro* metabolites were detected in hepatocytes, which were formed via oxidative dealkylation of the 2,3-dihydro-1,4-benzodioxane moiety followed by glucuronidation and/or methylation. All metabolites detected in human liver microsomes and hepatocytes were observed in the rat, dog or monkey liver microsomal or hepatocyte. The following table (from page 14-21 of the report) provides a summary of all metabolites, their retention times and their percent abundance in the rat, dog, monkey, and human liver microsomes and hepatocytes.

Table 1. *In vitro* metabolic profiles of [^{14}C]-Genz-99067 in rat, dog, monkey, human liver microsomes and hepatocytes

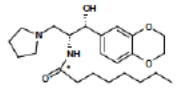
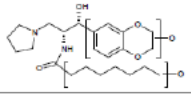
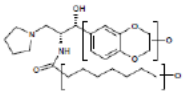
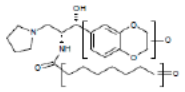
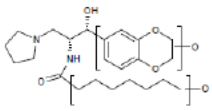
Compound	Structure	RT[a] (min)	Distribution of Radioactivity (%)							
			RLM	DLM	MkLM	HLM	RH	DH	MkH	HH
^{14}C-Genz-99067		(b) (4)	33.1	8.13	ND	44.0	ND	ND	ND	ND
M2			5.28	1.58	25.9	ND	ND	ND	3.78	2.45
M2 & M64	 M2  M64		NA	NA	NA	ND	ND	4.25	NA	NA
M3			1.34	ND	7.43	ND	ND	ND	ND	ND

Table 1. *In vitro* metabolic profiles of [^{14}C]-Genz-99067 in rat, dog, monkey, human liver microsomes and hepatocytes (continued)

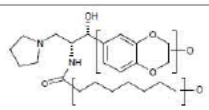
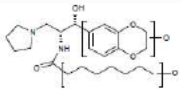
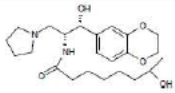
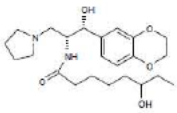
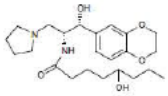
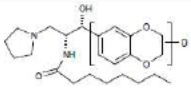
Compound	Structure	RT[a] (min)	Distribution of Radioactivity (%)							
			RLM	DLM	MkLM	HLM	RH	DH	MkH	HH
M4		(b) (4)	5.33	NA	NA	ND	1.36	1.78	ND	ND
M4 & M45	 M4/M45		NA	8.21	16.9	ND	NA	NA	ND	ND
Genz-256416 (M5)			20.9	59.9	26.3	31.3	9.30	44.2	26.0	34.9
Genz-311752 (M6)			ND	3.70	1.53	13.8	2.99	1.65	6.67	16.1
Genz-258179 (M7)			ND	ND	1.72	1.64	2.39	ND	ND	ND
M9			10.7	5.04	ND	3.18	ND	2.27	ND	ND

Table 1. *In vitro* metabolic profiles of [^{14}C]-Genz-99067 in rat, dog, monkey, human liver microsomes and hepatocytes (continued)

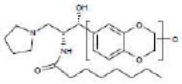
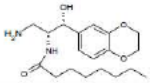
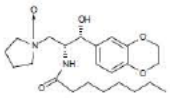
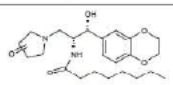
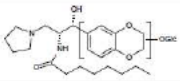
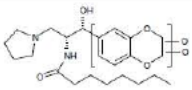
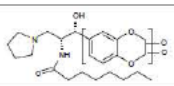
Compound	Structure	RT[a] (min)	Distribution of Radioactivity (%)							
			RLM	DLM	MkLM	HLM	RH	DH	MkH	HH
M10		(b) (4)	23.3	6.98	3.37	4.93	ND	ND	ND	ND
Genz-256222 (M11)			ND	ND	ND	ND	ND	2.00	ND	ND
Genz-120965 (M12)			ND	1.97	ND	ND	ND	ND	1.27	ND
M13			ND	NA	ND	1.14	ND	ND	ND	ND
M14			ND	ND	ND	ND	1.60	ND	ND	ND
M15			ND	ND	1.37	ND	ND	1.14	3.70	5.84
M16			ND	1.56	ND	ND	3.19	7.41	ND	ND

Table 1. *In vitro* metabolic profiles of [^{14}C]-Genz-99067 in rat, dog, monkey, human liver microsomes and hepatocytes (continued)

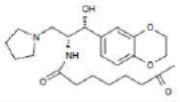
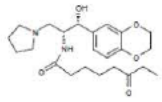
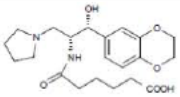
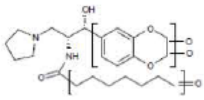
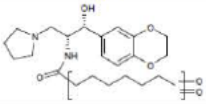
Compound	Structure	RT[a] (min)	Distribution of Radioactivity (%)							
			RLM	DLM	MkLM	HLM	RH	DH	MkH	HH
Genz-258162 (M17)		(b) (4)	ND	2.91	3.31	ND	ND	22.5	13.6	12.9
Genz-527862 (M18)			ND	ND	2.71	ND	ND	ND	2.80	4.27
Genz-399240 (M24) & M54	 M24  M54		ND	ND	ND	ND	NA	NA	8.28	2.76
M26			ND	ND	ND	ND	ND	3.59	7.16	4.43
M32	Solvent front peak (structure unknown)		ND	ND	ND	ND	6.61	2.07	12.2	5.87

Table 1. *In vitro* metabolic profiles of [^{14}C]-Genz-99067 in rat, dog, monkey, human liver microsomes and hepatocytes (continued)

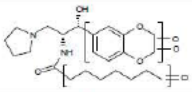
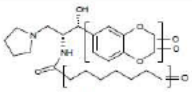
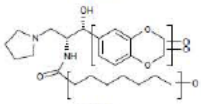
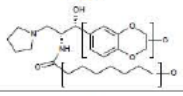
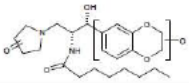
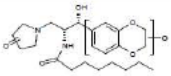
Compound	Structure	RT[a] (min)	Distribution of Radioactivity (%)							
			RLM	DLM	MkLM	HLM	RH	DH	MkH	HH
M37		(b) (4)	ND	ND	ND	ND	1.73	ND	ND	ND
M39 & M55	 M39  M55		ND	ND	ND	ND	6.84	2.33	7.31	1.26
M46			ND	ND	1.90	ND	ND	ND	ND	ND
M47			ND	ND	1.82	ND	ND	ND	ND	ND
M48			ND	ND	1.49	ND	ND	ND	ND	ND

Table 1. ***In vitro*** **metabolic profiles of [^{14}C]-Genz-99067 in rat, dog, monkey, human liver microsomes and hepatocytes (continued)**

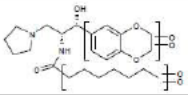
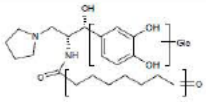
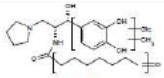
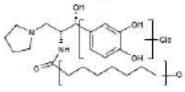
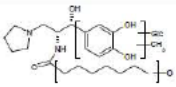
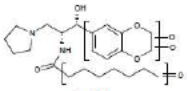
Compound	Structure	RT[a] (min)	Distribution of Radioactivity (%)							
			RLM	DLM	MkLM	HLM	RH	DH	MkH	HH
		(b) (4)								
M49			ND	ND	ND	ND	1.99	ND	ND	ND
M50			ND	ND	ND	ND	5.12	ND	ND	ND
M51 & M52	 M51  M52		ND	ND	ND	ND	21.1	ND	ND	ND
M53 & M54	 M53  M54		ND	ND	ND	ND	13.5	NA	NA	NA

Table 1. ***In vitro*** **metabolic profiles of [^{14}C]-Genz-99067 in rat, dog, monkey, human liver microsomes and hepatocytes (continued)**

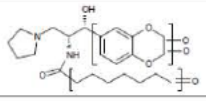
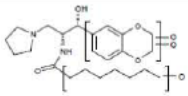
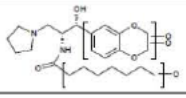
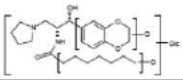
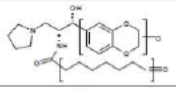
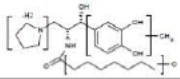
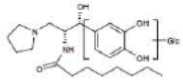
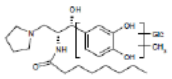
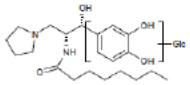
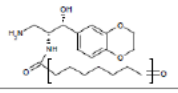
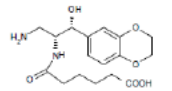
Compound	Structure	RT[a] (min)	Distribution of Radioactivity (%)							
			RLM	DLM	MkLM	HLM	RH	DH	MkH	HH
		(b) (4)								
M54			ND	ND	ND	ND	NA	1.61	NA	NA
M56			ND	ND	ND	ND	5.32	ND	1.36	ND
M57			ND	ND	ND	ND	1.79	ND	ND	ND
M58			ND	ND	ND	ND	2.19	ND	ND	ND
M59			ND	ND	4.22	ND	1.63	3.19	4.59	7.18
M60			ND	ND	ND	ND	1.76	ND	ND	ND
M61			ND	ND	ND	ND	3.46	ND	ND	ND

Table 1. *In vitro* metabolic profiles of [^{14}C]-Genz-99067 in rat, dog, monkey, human liver microsomes and hepatocytes (continued)

Compound	Structure	RT[a] (min)	Distribution of Radioactivity (%)							
			RLM	DLM	MkLM	HLM	RH	DH	MkH	HH
M62		(b) (4)	ND	ND	ND	ND	3.92	ND	ND	ND
M63			ND	ND	ND	ND	2.23	ND	ND	ND
M65			ND	ND	ND	ND	ND	ND	1.36	ND
M71			ND	ND	ND	ND	ND	ND	NA	1.95
Total	-	-	100	100	100	100	100	100	100	100

[a]: RT (Retention time) was based on radio-LC chromatograms
RLM = Rat liver microsomes; DLM = Dog liver microsomes; MkLM = Monkey liver microsomes; HLM = Human liver microsomes; RH = Rat hepatocytes; DH = Dog hepatocytes; MkH = Monkey hepatocytes; HH = Human hepatocytes
"-": not applicable
NA: metabolite detected but no quantifiable percent of radioactivity

Exposure of Genz-99067 and Its Metabolites in Rat Following Oral Administration of [^{14}C]-Genz-112638 (DMPK11-R019)

Methods: This study was conducted to evaluate exposure of Genz-99067 (free base of Genz-112638) and its metabolites in the rat plasma following repeated oral administration of Genz-112638. In this study, two groups (n = 25/sex) of SD rats were treated with Genz-112638 at 50 mg/kg once daily for 13 consecutive days, followed on Day 14 by a single 50 mg/kg of [^{14}C]-Genz-112638 (specific activity = 24.06 mCi/mmol or 49.97 µCi/mg) via oral gavage. Following the [^{14}C]-Genz-112638 dose, blood was collected at various time points for analysis. Four additional untreated animals were used to collect control plasma.

Results: The following ten metabolites were identified and quantified in the rat plasma: Genz-256416, Genz-311752, Genz-258179, Genz-258162, Genz-527862, Genz-399207, Genz-399240, Genz-682042, Genz-120965, and Genz-256222. The mean AUC_{0-t} of Genz-99067 was 2890 ng.hr/mL and 5480 ng.hr/mL in male and female rats, respectively. The mean AUC_{0-t} of eight quantifiable metabolites was 507 ng.hr/mL and 319 ng.hr/mL for Genz-256416, 258 ng.hr/mL and 68.1 ng.hr/mL for Genz-311752, 186 ng.hr/mL and 35.9 ng.hr/mL for Genz-258179, 206 ng.hr/mL and 77.6 ng.hr/mL for Genz-258162, 38.9 ng.hr/mL and 11.4 ng.hr/mL for Genz-527862, 11.8 ng.hr/mL and 13.6 ng.hr/mL for Genz-399240, 160 ng.hr/mL and 192 ng.hr/mL for Genz-256222, and 6.97 ng.hr/mL and 13.8 ng.hr/mL for Genz-120965 in male and female rats, respectively. Overall, the results of the study indicated that there were apparent gender differences in plasma exposures of Genz-99067 and its metabolites. The following table (from page 764 of the report) shows the PK parameters of Genz-99067 and its metabolites.

Compound Identification	Gender	C_{max}		t_{max}	AUC_{0-t}		Ratio of AUC_{0-t} (R_{AUC}) Male/Female
		Mean	SE	Mean	Mean	SE	
		(ng/mL)		(h)	(ng•h/mL)		
Genz-99067 (Parent)	Male	1050	47.1	0.500	2890	172	0.528
	Female	1680	246	0.500	5480	278	
Genz-256416	Male	256	17.5	0.500	507	27.6	1.59
	Female	145	9.37	0.500	319	15.0	
Genz-311752	Male	185	20.3	0.500	258	17.9	3.80
	Female	41.2	5.02	0.500	68.1	4.00	
Genz-258179	Male	127	18.2	0.500	186	15.6	5.17
	Female	20.5	3.48	0.500	35.9	2.00	
Genz-258162	Male	104	8.91	0.500	206	14.7	2.65
	Female	43.5	4.82	0.500	77.6	7.42	
Genz-527862	Male	26.3	1.89	0.500	38.9	3.99	3.43
	Female	5.87	0.933	0.500	11.4	0.773	
Genz-399240	Male	10.4	0.655	1.00	11.8	0.756	0.870
	Female	6.20	1.27	1.00	13.6	1.30	
Genz-256222	Male	50.7	5.69	0.500	160	10.3	0.834
	Female	49.5	5.72	0.500	192	23.3	
Genz-120965	Male	5.33	0.788	0.500	6.97	0.758	0.504
	Female	6.75	1.09	0.500	13.8	0.940	

In summary, Genz-99067, parent compound, exhibited the highest plasma exposure compared to its metabolites, with mean AUC_{0-t} of 2890 ng.h/mL and 5480 ng.h/mL in male and female rats, respectively. Eight of the ten metabolites, namely Genz-256416, Genz-311752, Genz-258179, Genz-258162, Genz-527862, Genz-399207, Genz-399240, Genz 682042, Genz-256222 and Genz-120965, were quantifiable. Two metabolites, Genz- 399207 and Genz-682042, were below the LOQ in all collected plasma samples. There were apparent gender differences in the plasma exposures of Genz-99067 and its metabolites in rats. The mean AUC_{0-t} of Genz-256416, Genz-311752, Genz-258179, Genz-258162, and Genz-527862 was 1.59-fold, 3.80-fold, 5.17-fold, 2.65-fold, and 3.43-fold greater in male rats than in female rats, respectively. The mean AUC_{0-t} of Genz-399240 and Genz-256222 were approximately equal in both genders. The mean AUC_{0-t} of Genz-99067 and Genz-120965 in male rats were approximately half of that observed in female rats.

<u>Exposure of Genz-99067 and Its Metabolites in Dog Following Oral Administration of [^{14}C]-Genz-112638 (DMPK11-R024)</u>

Methods: This study was conducted to evaluate exposure of Genz-99067 (free base of Genz-112638) and its metabolites in the dog plasma following repeated oral administration of Genz-112638. Two groups, consisting of 4 male or 4 female Beagle dogs were treated with Genz-112638 at 10 mg/kg for 13 consecutive days, followed on Day 14 by a single 10 mg/kg [^{14}C]-Genz-112638 (specific activity = 24.9 mCi/mmol or 51.71 µCi/mg) dose (20 µCi/kg) via oral gavage (5 mL/kg). Blood was collected at various time points for analysis. One additional pre-dose animal per gender was used as control.

Results: The following ten metabolites were identified and quantified in the dog plasma: Genz 256416, Genz-311752, Genz-258179, Genz-258162, Genz-527862, Genz-399207, Genz-399240, Genz-682042, Genz-120965, and Genz-256222. Mean peak plasma concentrations of [^{14}C]-Genz-112638/g were observed at 1 h postdose (3.402 µg equiv), while the female peaked at 0.5 h post-dose (4.361 µg eq). Plasma concentrations declined significantly by the 24 h time point and the levels remained detectable in both genders. The mean AUC_{0-t} of Genz-99067 was 2680 ng.hr/mL and 1090 ng.hr/mL in male and female dogs, respectively. Overall, the results of the study indicated that there were apparent gender differences in plasma exposures of Genz-99067 and its metabolites. The following table (from page 730 of the report) shows the PK parameters.

Table 12 Pharmacokinetic parameters (Mean±SD) of Genz-99067 and its metabolites on Day 14 following 14-day oral administration of 10 mg/kg QD dose to Beagle dogs

Compound Identification	PK parameter	Male						Female						Ratio of AUC_{0-t} (R_{AUC}) Male/Female
		Dog 1	Dog 2	Dog 3	Dog 4	Mean	SD	Dog 5	Dog 7	Dog 8	Dog 10	Mean	SD	
Genz-99067	t_{max} (h)	0.500	0.500	0.500	0.500	0.500	0.00	0.500	0.500	0.500	0.500	0.500	0.00	2.45
	C_{max} (ng/mL)	441	1520	889	288	785	553	447	768	1230	503	737	357	
	AUC_{0-t} (ng•h/mL)	774	5780	3520	628	2680	2460	758	890	1970	752	1090	589	
Genz-256416	t_{max} (h)	0.250	0.500	1.00	0.250	0.500	0.354	0.500	0.250	0.250	0.500	0.375	0.144	2.20
	C_{max} (ng/mL)	434	559	458	370	455	78.5	481	377	508	386	438	66.3	
	AUC_{0-t} (ng•h/mL)	1030	3200	2340	1040	1900	1060	1100	575	1070	710	864	262	
Genz-311752	t_{max} (h)	0.250	0.500	1.00	0.250	0.500	0.354	0.500	0.250	0.250	0.500	0.375	0.144	1.97
	C_{max} (ng/mL)	104	106	92.7	74.0	94.2	14.7	113	114	126	113	117	6.35	
	AUC_{0-t} (ng•h/mL)	248	682	556	226	428	227	250	167	254	199	218	41.9	
Genz-258179	t_{max} (h)	0.500	2.00	1.00	0.250	0.938	0.774	0.500	0.250	0.250	0.500	0.375	0.144	1.66
	C_{max} (ng/mL)	20.6	22.1	20.0	15.7	19.6	2.75	21.6	32.7	27.1	28.8	27.6	4.61	
	AUC_{0-t} (ng•h/mL)	54.7	111	130	55.0	87.8	38.8	51.5	48.1	60.5	50.8	52.7	5.41	
Genz-258162	t_{max} (h)	0.500	2.00	2.00	0.500	1.25	0.866	0.500	0.500	0.500	0.500	0.500	0.00	1.30
	C_{max} (ng/mL)	641	351	313	459	441	147	674	693	790	566	681	91.8	
	AUC_{0-t} (ng•h/mL)	1970	3150	2460	1750	2330	621	1970	1310	2460	1440	1790	528	
Genz-527862	t_{max} (h)	0.500	2.00	2.00	1.00	1.38	0.750	0.500	0.500	0.500	0.500	0.500	0.00	1.62
	C_{max} (ng/mL)	28.7	25.2	23.9	25.6	25.9	2.03	31.5	40.5	40.2	30.8	35.8	5.32	
	AUC_{0-t} (ng•h/mL)	89.8	223	185	87.3	146	68.6	89.3	73.7	120	78.3	90.2	20.7	
Genz-399207	t_{max} (h)	2.00	2.00	2.00	NA	2.00	0.00	2.00	NA	2.00	2.00	2.00	0.00	0.816
	C_{max} (ng/mL)	4.10	1.00	1.04	0.00	1.54	1.78	3.22	0.00	2.77	2.67	2.17	1.46	
	AUC_{0-t} (ng•h/mL)	17.3	2.60	4.11	0.00	6.01	7.74	13.1	0.00	9.83	6.51	7.37	5.61	

Table 12 Pharmacokinetic parameters (Mean±SD) of Genz-99067 and its metabolites on Day 14 following 14-day oral administration of 10 mg/kg QD dose to Beagle dogs (continued)

Analyte	Gender	Male						Female						Ratio of AUC_{0-t} (RAUC) Male/Female
	Animal ID	Dog 1	Dog 2	Dog 3	Dog 4	Mean	SD	Dog 5	Dog 7	Dog 8	Dog 10	Mean	SD	
Genz-399240	t_{max} (h)	2.00	NA	NA	NA	2.00	0.00	2.00	2.00	2.00	2.00	2.00	0.00	NA
	C_{max} (ng/mL)	0.950	0.00	0.00	0.00	0.238	0.475	0.875	0.770	0.747	0.812	0.801	0.0562	
	AUC_{0-t} (ng•h/mL)	0.00[a]	0.00	0.00	0.00	0.00	NA	2.24	0.00	0.00	0.00	0.559	1.12	
Genz-256222	t_{max} (h)	1.00	4.00	2.00	1.00	2.00	1.41	1.00	0.500	0.500	2.00	1.00	0.707	1.48
	C_{max} (ng/mL)	28.8	43.8	47.4	31.7	37.9	9.06	35.6	47.7	43.8	40.7	42.0	5.11	
	AUC_{0-t} (ng•h/mL)	277	629	621	325	463	188	282	282	370	317	313	41.5	
Genz-120965	t_{max} (h)	0.250	0.500	0.500	0.250	0.375	0.144	0.500	0.500	0.250	0.500	0.438	0.125	2.33
	C_{max} (ng/mL)	26.0	67.1	27.3	18.3	34.7	22.0	28.5	22.1	34.2	21.0	26.5	6.13	
	AUC_{0-t} (ng•h/mL)	29.4	159	62.6	22.5	68.3	62.8	34.4	19.5	43.0	20.4	29.3	11.4	

[a]: AUC is not calculated with less than 3 quantifiable time points, and is reported as zero.

Stability of Genz-99067 in Rat, Dog, and Human Whole Blood (DMPK11-R029)

Methods: The stability of Genz-99067 in male rat, male dog, male and female human whole blood was determined by measuring the percent remaining after 4 hours incubation at 37^0C using LC-MS/MS. Three concentrations of Genz-99067 were tested (0.1, 1.0 and 10 µM).

Results: In male SD rat whole blood, the mean percentage of remaining Genz-99067 was 99.1%, 98.4%, and 95.4% at 0.1, 1.0, and 10 µM, respectively. In male Beagle dog whole blood, the mean percentage of remaining Genz 99067 was 102%, 104%, and 102% at 0.1, 1.0, and 10 µM, respectively. In male human whole blood, the mean percentage of remaining Genz 99067 was 99.1%, 95.0%, and 93.8% at 0.01, 0.1, and 1.0 µM, respectively. In female human whole blood, the mean percentage of remaining Genz 99067 was 92.3%, 94.9%, and 97.0% at 0.01, 0.1, and 1.0 µM, respectively. Overall, the results indicated that Genz-99067 was stable in the rat, dog, and human whole blood for up to 4 hours at 37^0C. The following table (from page 11 of the report) shows the results.

Table 1. Summary of whole blood stability of Genz-99067 in rat, dog, and human whole blood

Species	Gender	Compound	Concentration (μM)	Percent remaining (%) (Inter-lot Mean ± SD, n=3)		
				0 hour	1 hour	4 hours
Rat	Male	Genz-99067	0.1	100	97.3 ± 2.97	99.1 ± 1.74
			1.0	100	101 ± 1.62	98.4 ± 0.555
			10	100	99.0 ± 3.90	95.4 ± 3.81
		Genz-644442 (negative control)	10	100	106 ± 6.16	99.4 ± 7.22
		Enalapril (positive control)	10	100	12.6 ± 1.30	0.0928 ± 0.0387
Dog	Male	Genz-99067	0.1	100	101 ± 3.70	102 ± 4.93
			1.0	100	101 ± 1.84	104 ± 0.315
			10	100	101 ± 3.23	102 ± 3.16
		Enalapril (negative control)	10	100	101 ± 3.16	105 ± 5.24
		Genz-650035 (positive control)	10	100	78.7 ± 3.51	48.5 ± 10.1
Human	Male	Genz-99067	0.01	100	102 ± 5.65	99.1 ± 4.50
			0.1	100	100 ± 2.79	95.0 ± 2.23
			1.0	100	96.2 ± 1.85	93.8 ± 1.67
		Enalapril (negative control)	10	100	100 ± 1.20	100 ± 1.72
		Procaine (positive control)	10	100	0.145 ± 0.0110	0.0721 ± 0.0360
	Female	Genz-99067	0.01	100	98.7 ± 5.56	92.3 ± 6.89
			0.1	100	100 ± 1.47	94.9 ± 1.01
			1.0	100	99.1 ± 3.07	97.0 ± 4.50
		Enalapril (negative control)	10	100	100 ± 3.23	100 ± 2.49
		Procaine (positive control)	10	100	0.150 ± 0.0323	0.0681±0.0211

In Vitro Metabolic Stability of Genz-99067 in Mouse, Rat, Dog, Monkey, and Human Liver Microsomes (DMPK11-R035)

Methods: Genz-99067 (free base of tartrate salt Genz-112638) was incubated with mouse, rat, dog, monkey, and human liver microsomes and the resulting concentration-time course of Genz-99067 depletion in the presence of NADPH was used to calculate the *in vitro* intrinsic clearance of Genz-99067. Three concentrations of Genz-99067 were tested (0.05, 0.2 and 1.0 μM).

Results: The intrinsic hepatic clearance of Genz-99067 in the mouse liver microsomes was 1870, 1550, and 617 μL/min/mg protein at 0.05, 0.2, and 1.0 μM of Genz-99067, respectively. The intrinsic hepatic clearance of Genz-99067 in the rat liver microsomes was 2200, 913 and 257 μL/min/mg protein at 0.05, 0.2, and 1.0 μM of Genz-99067, respectively. The intrinsic hepatic clearance of Genz-99067 in the dog liver microsomes was 1180, 1240, and 829 μL/min/mg protein at 0.05, 0.2, and 1.0 μM of Genz-99067, respectively. The intrinsic hepatic clearance of Genz-99067 in the monkey liver microsomes was 5020, 4030, and 1052 μL/min/mg protein at 0.05, 0.2, and 1.0 μM of Genz-99067, respectively. The intrinsic hepatic clearance of Genz-99067 in the human liver microsomes was 99.5, 136, and 122 μL/min/mg protein at 0.05, 0.2, and 1.0 μM of Genz-99067, respectively. The extrapolated *in vivo* hepatic metabolic blood clearance calculated from *in vitro* values of Genz-99067 in all species tested are summarized in the following table (from page 20 of the report).

Table 5. Summary of hepatic metabolic clearance extrapolated from *in vitro* intrinsic clearance of Genz-99067 in mouse, rat, dog, monkey, and human liver microsomes

Species	Liver Microsomal Protein Concentration (mg/mL)	Genz-99067 Nominal Incubation Concentration (µM)	Extrapolated *In Vivo* Hepatic Metabolic Clearance (mL/min/kg) (Mean ± SD, n=3)
Mouse	0.05	0.05	36.7 ± 0.143
		0.2	32.8 ±0.464
		1.0	16.6 ± 0.441
Rat	0.1	0.05	33.6 ±0.322
		0.2	23.1 ± 1.18
		1.0	8.65 ±0.0977
Dog	0.02	0.05	20.0 ±0.252
		0.2	20.2 ± 0.0197
		1.0	17.8 ± 0.454
Monkey	0.02	0.05	38.6 ± 0.165
		0.2	38.1 ± 0.163
		1.0	27.4 ± 0.529
Human	0.2	0.05	9.57 ± 0.0274
		0.2	11.0 ± 0.151
		1.0	10.6 ± 0.0244

Overall, Genz-99067 exhibited NADPH-dependent metabolism in the mouse, rat, dog, monkey, and human liver microsomes over the tested concentration range (0.05 to 1.0 µM). The extrapolated *in vivo* hepatic metabolic clearance was generally drug concentration independent in the human and dog but appeared to be concentration-dependent in the rat, mouse, and monkey with the hepatic clearance lower at 1.0 µM than that at 0.2 and 0.05 µM.

In Vitro Metabolic Stability of Genz-99067 in Mouse, Rat, Dog, Monkey, and Human Hepatocytes (DMPK11-R036)

Methods: Genz-99067 (free base of tartrate salt Genz-112638) was incubated with mouse, rat, dog, monkey, and human hepatocytes, respectively, and the resulting concentration-time course of Genz-99067 depletion was used to calculate the *in vitro* intrinsic clearance of Genz-99067. Three concentrations of Genz-99067 were tested (0.05, 0.2, and 1.0 µM).

Results: The intrinsic hepatic metabolic clearance of Genz-99067 in the mouse hepatocytes was 608, 674, and 436 µL/min/million cells at 0.05, 0.2, and 1.0 µM of Genz- 99067, respectively. The intrinsic hepatic metabolic clearance of Genz-99067 in rat hepatocytes was 531, 299, and 121 µL/min/ million cells at 0.05, 0.2, and 1.0 µM of Genz-99067, respectively. The intrinsic hepatic metabolic clearance of Genz-99067 in dog hepatocytes was 119, 130, and 89.7 µL/min/ million cells at 0.05, 0.2, and 1.0 µM of Genz-99067, respectively. The intrinsic hepatic metabolic clearance of Genz-99067 in monkey hepatocytes was 451, 432, and 159 µL/min/million cells at 0.05, 0.2, and 1.0 µM of Genz-99067, respectively. The intrinsic hepatic metabolic clearance of Genz 99067 in human hepatocytes was 49.9, 49.0, and 30.1 µL/min/million cells at 0.05, 0.2, and 1.0 µM of Genz-99067, respectively. The *in vitro-in vivo* extrapolated hepatic

metabolic clearance values of Genz-99067 in all species tested are summarized in the following table (from page 20 of the report).

Table 5. Summary of hepatic metabolic clearance extrapolated from *in vitro* intrinsic clearance of Genz-99067 in mouse, rat, dog, monkey, and human hepatocytes

Species	Hepatocyte Concentration (million cells/mL)	Genz-99067 Nominal Incubation Concentration (µM)	Extrapolated *In Vivo* Hepatic Metabolic Clearance (mL/min/kg) (Mean ± SD, n=3)
Mouse	0.1	0.05	33.0 ± 1.88
		0.2	35.2 ± 0.473
		1.0	26.4 ± 1.62
Rat	0.1	0.05	27.0 ± 1.14
		0.2	19.3 ± 1.86
		1.0	9.87 ± 1.24
Dog	0.1	0.05	11.3 ± 0.353
		0.2	11.9 ± 0.562
		1.0	9.30 ± 0.781
Monkey	0.1	0.05	41.7 ± 0.0662
		0.2	41.3 ± 0.0741
		1.0	35.9 ± 0.329
Human	0.5	0.05	10.1 ± 0.486
		0.2	10.0 ± 0.670
		1.0	7.59 ± 0.364

In summary, Genz-99067 exhibited metabolism in the mouse, rat, dog, monkey, and human hepatocytes over the concentration range from 0.05 to 1.0 μM. The extrapolated *in vivo* hepatic metabolic clearance was generally drug concentration-independent in the human, monkey, dog, and mouse but appeared to be concentration-dependent in the rat with the hepatic clearance lower at 1.0 μM than that at 0.05 and 0.2 μM.

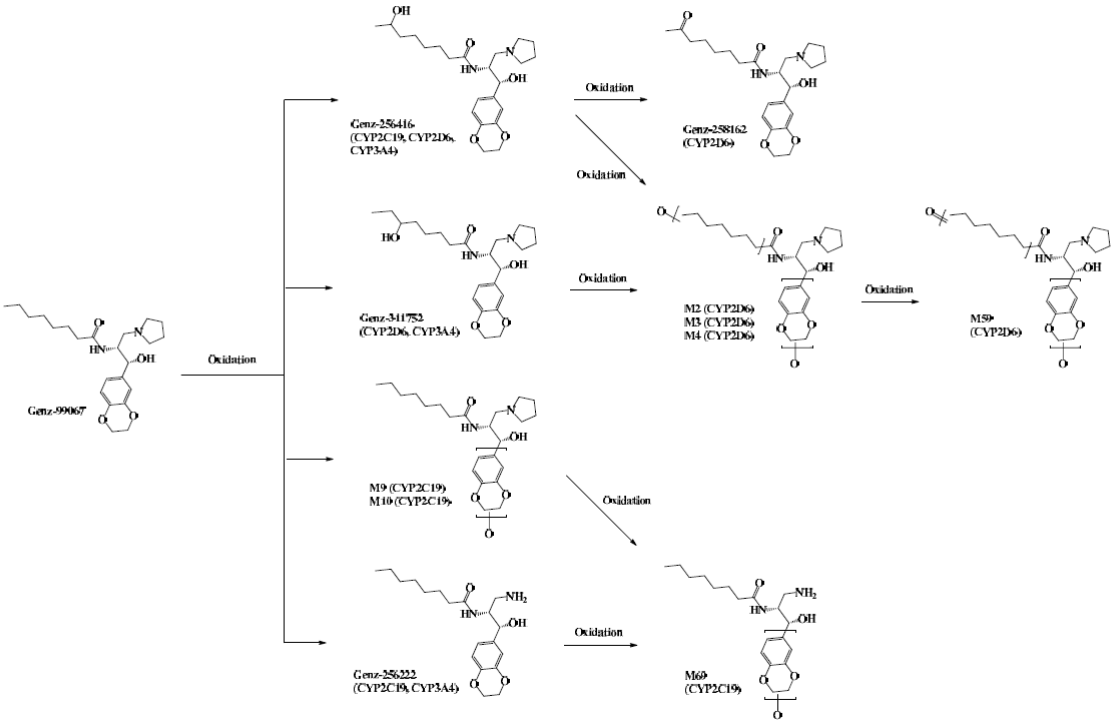
In Vitro Metabolic Profiles of [^{14}C]-Genz-99067 in Human Recombinant CYP2C19, CYP2D6, and CYP3A4 Enzymes (DMPK11-R043)

Methods: The objective of this study was to evaluate *in vitro* metabolic profiles of [^{14}C] Genz-99067 in human recombinant CYP2C19, CYP2D6 and CYP3A4 enzymes. [^{14}C]Genz-112638 (5 μM, specific activity = 24.90 mCi/mmol) was incubated with human recombinant CYP2C19, CYP2D6 and CYP3A4 at 37^{0}C for 1 hour. Incubations without NADPH regeneration system served as negative controls. The putative structures of the metabolites were determined or proposed on the basis of HPLC-MS analyses and comparison with available authentic reference standards for the putative metabolites.

Results: Eleven metabolites were identified *in vitro* following the incubation of [^{14}C]-Genz 112638 in human recombinant CYP2C19, CYP2D6 and CYP3A4 enzymes. Genz-99067 was completely metabolized in CYP2D6, while 80% of parent drug remained following CYP2C19 and CYP3A4 incubations. In negative control studies, no metabolites were observed in recombinant enzyme incubations without the NADPH regeneration system, demonstrating metabolism of Genz-99067 in human recombinant CYP2C19, CYP2D6, and CYP3A4 enzymes was NADPH-dependent. The proposed *in vitro* metabolic pathway for Genz-99067 is shown in the following figure (from page 16 of the report).

Reference ID: 3501244

Figure 1. Proposed *in vitro* metabolic pathways of [^{14}C]-Genz-99067 in human recombinant CYP2D6, CYP 2C19, and CYP3A4 enzymes



Oxidation on the octanoyl moiety resulted in the formation of 7-hydroxyl metabolite Genz-256416, 6-hydroxyl metabolite Genz-311752, and 7-ketone metabolite Genz 258162. The M2, M3, M4, and M59 metabolites were formed by oxidations on both octanoyl and 1,4 benzodioxane moieties. The M9 and M10 metabolites were proposed as the mono-oxidation product of 1,4-benzodioxane moiety, while the M11 and M69 metabolites were derived from oxidative dealkylation of pyrrolidine moiety.

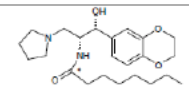
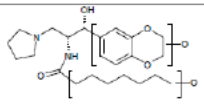
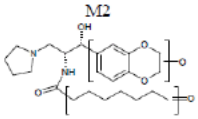
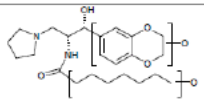
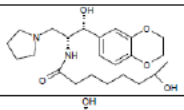
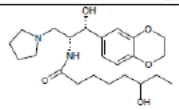
In summary, eleven metabolites were identified *in vitro* following the incubation of [^{14}C]-Genz 112638 in human recombinant CYP2C19, CYP2D6 and CYP3A4 enzymes. Genz-99067 was completely metabolized by CYP2D6. Major metabolites were formed by oxidation of the octanoyl moiety, including 7-hydroxyl metabolite Genz-256416, 6-hydroxyl metabolite Genz-311752, and 7-ketone metabolite Genz-258162, or by oxidation of both octanoyl and 2,3-dihydro-1,4-benzodioxane moieties, including M2 and M4. Approximately 80% of the parent drug remained following CYP2C19 and CYP3A4 incubations. Amino metabolite Genz-256222 was the major metabolite formed by CYP2C19, and Genz-311752 was the major metabolite formed by CYP3A4.

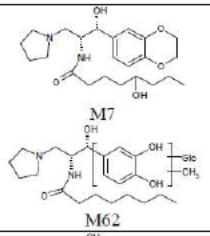
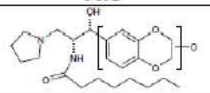
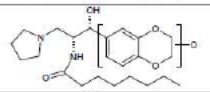
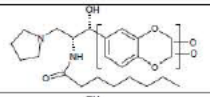
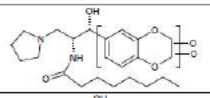
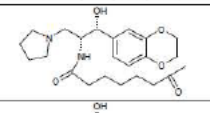
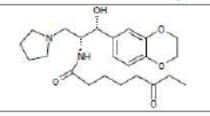
<u>Metabolic Profiles of Genz-99067 in Rat Plasma Following Oral Administration of [^{14}C]-Genz-112638 (DMPK11-R044A1)</u>

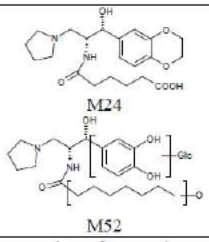
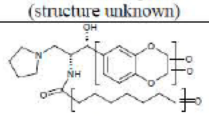
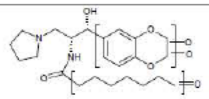
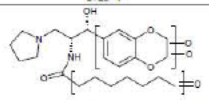
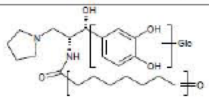
Methods: This study was conducted in Sprague Dawley rats following 14-day once a day repeated dosing of Genz-112638 at 50 mg/kg via oral gavage with the last dose on Day 14 containing 100 µCi/kg of [^{14}C]-Genz-112638. On Day 14, blood samples were collected at pre-dose, 1 hour, 4 hours and 8 hours post administration of [^{14}C]-Genz-112638 for biotransformation study.

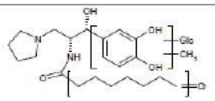
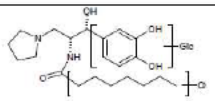
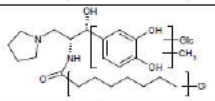
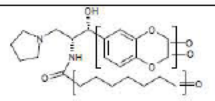
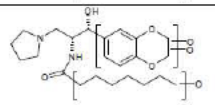
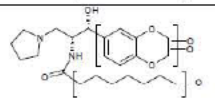
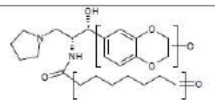
Results: A total of 28 metabolites of Genz-99067 were quantified (>1% of total radioactive response) in the rat plasma. Major metabolic pathways of Genz-99067 in the rat plasma were oxidations on the n-octanoyl moiety, mainly the formation of hydroxyl and ketone metabolites, and multiple O-glucuronide conjugations, following the oxidation, ring opening and dealkylation of 2,3-dihydro-1,4-benzodioxane. The following table (from page 14-18 of the report) shows all metabolites, their retention times and their abundance in rat plasma.

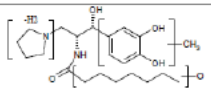
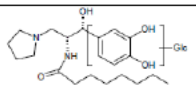
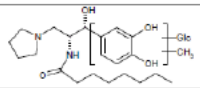
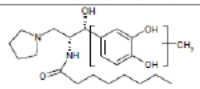
Table 1. Metabolic profiles of [^{14}C]-Genz-99067 in rat plasma

Compound	Proposed Structure	Retention Time[a] (minute:second)	Radioactive Peak ID	Distribution of Radioactivity (%)			
				Male		Female	
				1 h post-dose	4 h post-dose	1 h post-dose	4 h post-dose
^{14}C-Genz-99067		(b) (4)	26	24.8	31.6	41.5	43.5
M2 & M64	 M2  M64		13	2.64	ND	1.19	ND
M4			14	3.16	1.30	1.82	1.28
Genz-256416 (M5)			17	7.08	2.59	3.92	2.72
Genz-311752 (M6)			18	3.04	1.30	ND	ND

Compound	Proposed Structure	Retention Time[a] (minute:second)	Radioactive Peak ID	Distribution of Radioactivity (%)			
				Male		Female	
				1 h post-dose	4 h post-dose	1 h post-dose	4 h post-dose
Genz-258179 (M7) & M62	 M7 M62	(b) (4)	20	3.24	2.59	1.47	NA
M9			24	1.21	ND	1.68	ND
M10			25	3.76	2.33	6.86	3.84
M15			21	1.36	1.55	2.03	1.92
M16			22	1.11	ND	1.82	1.44
Genz-258162 (M17)			15	3.89	ND	1.19	ND
Genz-527862 (M18)			16	1.78	ND	ND	ND

Compound	Proposed Structure	Retention Time[a] (minute:second)	Radioactive Peak ID	Distribution of Radioactivity (%)			
				Male		Female	
				1 h post-dose	4 h post-dose	1 h post-dose	4 h post-dose
Genz-399240 (M24) & M52	 M24 M52	(b) (4)	4	2.40	NA	3.15	NA
M32	Solvent front peak (structure unknown)		1	3.84	8.29	2.94	2.24
M37			7	1.76	NA	NA	NA
M37 & M54	 M54		7 for M37 6 for M54	NA	4.15	3.01	2.56
M39			9	1.45	1.55	ND	ND
M50			2	1.10	ND	1.05	ND

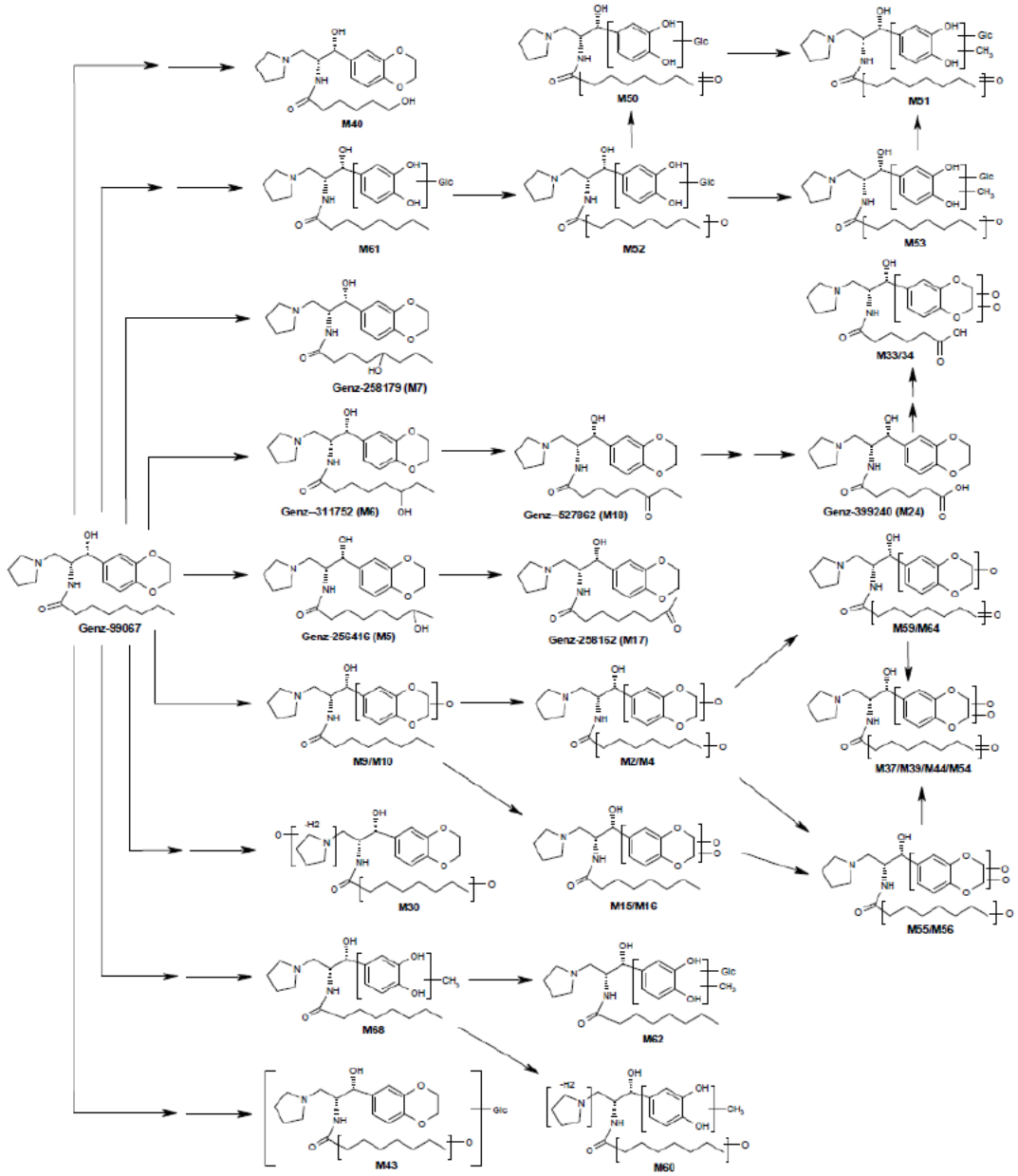
Compound	Proposed Structure	Retention Time[a] (minute:second)	Radioactive Peak ID	Distribution of Radioactivity (%)			
				Male		Female	
				1 h post-dose	4 h post-dose	1 h post-dose	4 h post-dose
M51		(b) (4)	3	1.44	2.07	1.19	1.44
M52			4	NA	2.07	NA	3.52
M53			5	1.09	ND	ND	ND
M54			6	1.29	NA	NA	NA
M55			8	1.76	1.04	ND	1.76
M56			10	1.55	1.30	ND	ND
M59			11	2.49	1.30	1.47	1.44

Compound	Proposed Structure	Retention Time[a] (minute:second)	Radioactive Peak ID	Distribution of Radioactivity (%)			
				Male		Female	
				1 h post-dose	4 h post-dose	1 h post-dose	4 h post-dose
M60		(b) (4)	12	ND	1.55	ND	ND
M61			19	1.41	2.33	2.66	4.80
M62			20	NA	NA	NA	1.28
M68			23	1.32	ND	ND	ND
Total	NA	NA	NA	80.0	68.9	78.9	73.8

[a]: Retention time was based on radio-LC chromatograms.

The proposed *in vivo* metabolic pathway of Genz-99067 in the rat plasma is shown in the following figure (from page 45 of the report) including six additional minor metabolites, which were less than 1% by radioprofiling and were identified in a separate study.

Figure 26. Proposed metabolic pathway of Genz-99067 in rat plasma



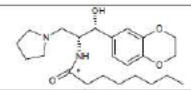
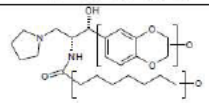
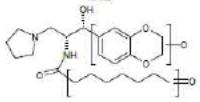
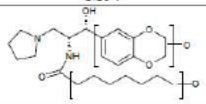
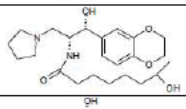
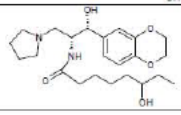
Note: M30, M33, M34, M40, M43, and M44 were detected and identified by LC/MS only [2].

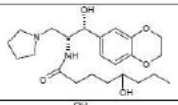
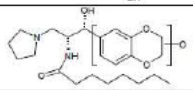
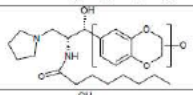
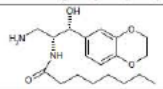
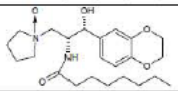
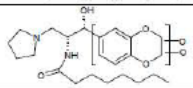
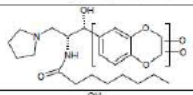
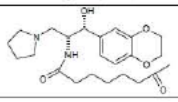
<u>Metabolic Profiles of Genz-99067 in Dog Plasma Following Oral Administration of [^{14}C]-Genz-112638 (DMPK11-R045)</u>

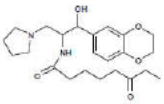
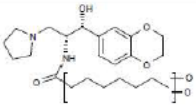
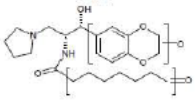
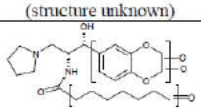
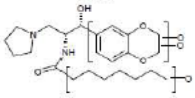
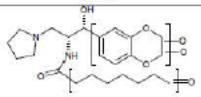
<u>Methods</u>: This study was conducted in male and female Beagle dogs (n = 4/sex) following 14-day once daily repeated dosing of Genz-112638 at 10 mg/kg via oral gavage with the last dose on Day 14 containing 20 µCi/kg [^{14}C]-Genz-112638. On Day 14, blood samples were collected at pre-dose, 1 hour, 4 hours and 8 hours post administration of [^{14}C]-Genz-112638 for biotransformation study.

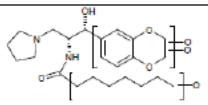
Results: A total of 21 metabolites of Genz-99067 were detected in the dog plasma. Oxidation on the n-octanoyl moiety, mainly the formation of 7-hydroxyl metabolite M5 and 7-ketone metabolite M17, was the major metabolic pathway of Genz-99067 in the dog plasma. The following tables (from pages 12-15 of the report) show the all metabolites, their retention times, and their abundance in the dog plasma.

Table 1. Metabolic profiles of [^{14}C]-Genz-99067 in dog plasma

Compound	Proposed Structure	Retention Time[a] (minute:second)	Radioactive Peak ID	Distribution of Radioactivity (%)			
				Male		Female	
				1 h post-dose	4 h post-dose	1 h post-dose	4 h post-dose
^{14}C-Genz-99067		(b) (4)	18	22.2	16.3	16.2	3.18
M2 & M64	 M2  M64		6	2.44	1.58	3.05	ND
M4			7	1.59	ND	1.89	ND
Genz-256416 (M5)			10	14.4	12.2	9.23	5.57
Genz-311752 (M6)			11	3.19	2.54	3.38	2.12

Compound	Proposed Structure	Retention Time[a] (minute:second)	Radioactive Peak ID	Distribution of Radioactivity (%)			
				Male		Female	
				1 h post-dose	4 h post-dose	1 h post-dose	4 h post-dose
Genz-258179 (M7)		(b) (4)	12	1.41	1.11	ND	ND
M9			15	2.25	2.38	2.64	2.92
M10			16	1.50	ND	1.15	ND
Genz-256222 (M11)			17	1.03	2.22	1.15	2.92
Genz-120965 (M12)			19	1.31	1.43	2.06	ND
M15			13	1.13	ND	1.24	ND
M16			14	2.44	ND	4.04	ND
Genz-258162 (M17)			8	16.3	16.8	19.7	15.4

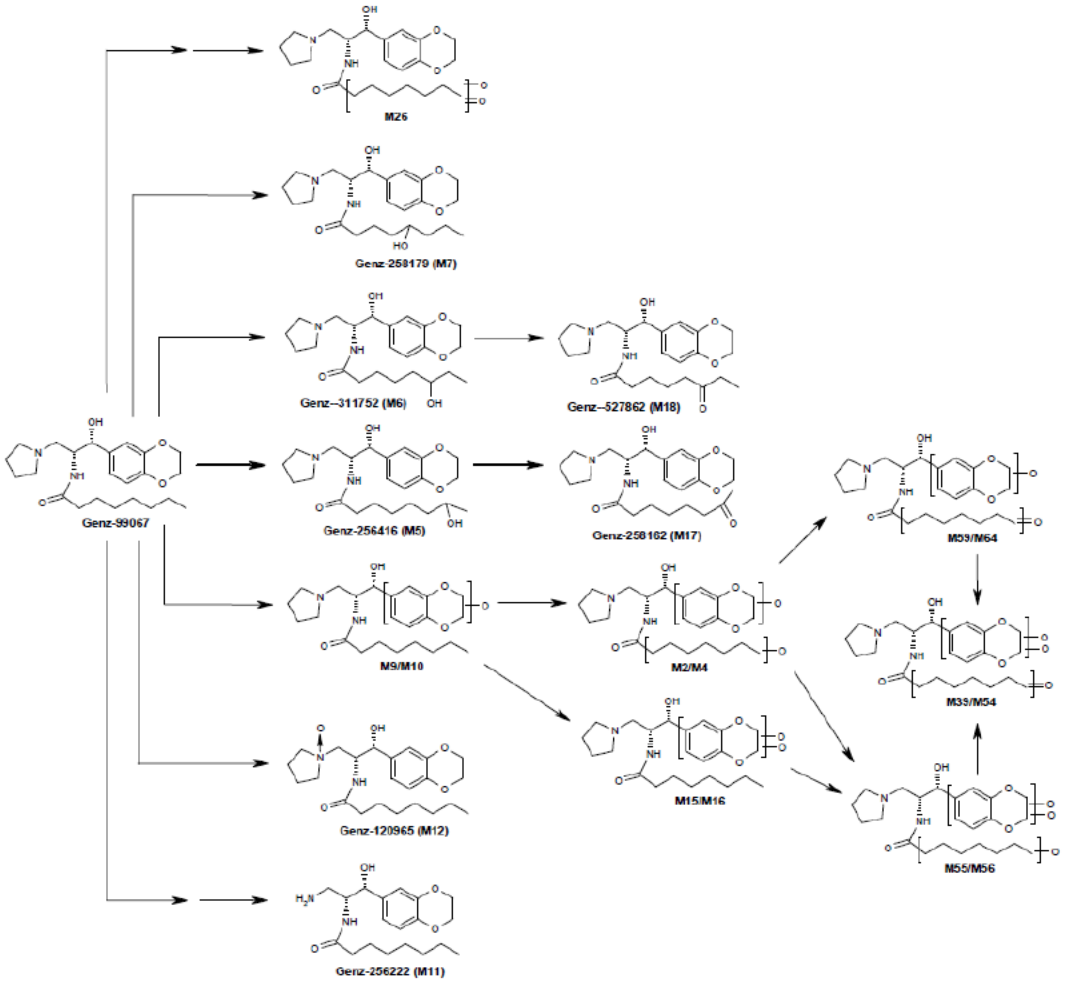
Compound	Proposed Structure	Retention Time[a] (minute:second)	Radioactive Peak ID	Distribution of Radioactivity (%)			
				Male		Female	
				1 h post-dose	4 h post-dose	1 h post-dose	4 h post-dose
Genz-527862 (M18)		(b) (4)	9	1.22	1.74	ND	ND
M26 & M59	 M26  M59		5	5.35	3.49	5.11	3.45
M32	Solvent front peak (structure unknown)		1	ND	2.85	4.86	22.8
M39 & M55	 M39  M55		3	1.78	3.49	1.24	2.39
M54			2	2.06	2.85	1.24	1.59

Compound	Proposed Structure	Retention Time[a] (minute:second)	Radioactive Peak ID	Distribution of Radioactivity (%)			
				Male		Female	
				1 h post-dose	4 h post-dose	1 h post-dose	4 h post-dose
M56		(b) (4)	4	1.13	3.01	ND	1.33
Total	NA	NA	NA	82.7	74.0	78.2	63.7

[a]: Retention time was based on Radio-LC chromatograms

The proposed *in vivo* metabolic pathway of Genz-99067 in the dog plasma is shown in the following figure (from page 36 of the report).

Figure 20. Proposed *in vivo* metabolic pathway of Genz-99067 in dog plasma



Pharmacokinetic Study of Genz-99067, and Its Ten Metabolites Following a Single Oral Administration of Genz-112638 to Male Cynomolgus Monkey (DMPK11-R092)

Methods: Four male Cynomolgus monkeys (n = 3/dose) were treated at a single 30 mg/kg dose of Genz-99067 (administered as Genz-112638, the hemi-tartrate salt form of Genz-99067) via oral gavage (3 mL/kg). Serial blood samples were collected over 0 to 24 hours post-dose. Plasma concentrations of Genz-99067 and its ten metabolites (Genz-256416, Genz-311752, Genz-258179, Genz-258162, Genz-527862, Genz-399207, Genz-399240, Genz-682042, Genz-256222 and Genz-120965) were determined by LC-MS/MS methods.

Results: The mean C_{max} of Genz-99067 was 64.9 ng/mL at 2.17 hour (t_{max}). The mean $AUC_{0-\infty}$ of Genz-99067 was 329 ng.hr/mL. The PK parameters for Genz-99067 are shown in the table below (from page 17 of the report).

Table 12. Pharmacokinetic parameters of Genz-99067 following a single 30 mg/kg PO dose of Genz-99067 (administrated as Genz-112638) to fasted male Cynomolgus monkeys

Animal ID	C_{max}	C_{last}	T_{max}	$t_{1/2}$	t_{last}	MRT	AUC_{last}	$AUC_{0-\infty}$	%Ext
	(ng/mL)		(h)				(ng•h/mL)		%
1	28.0	2.16	4.00	2.28	12.00	(b) (4)	151	158	4.48
2	52.6	1.02	2.00	2.03	12.00		159	162	1.84
3[a]	81.6	3.89	2.00	1.23[b]	12.00		318	325[b]	2.13[b]
3A	114	4.27	0.500	1.72	12.00		657	668	1.59
Mean	64.9	2.48	2.17	2.01	12.0		322	329	2.64
SD	44.3	1.65	1.76	0.279	0.00		290	293	1.60
%CV	68.3	66.4	81.0	13.9	0.00		89.9	89.0	60.8

[a]: Animal 3 was not included in statistics due to emesis
[b]: calculation based on extrapolation from time points at 4, 6 and 8 hours post dose

The PK parameters of the ten metabolites of Genz-99067 are shown in the following tables (from pages 18-20 of the report).

Table 13. Pharmacokinetic parameters of the ten metabolites of Genz-99067 following a single 30 mg/kg PO dose of Genz-99067 (administrated as Genz-112638) to fasted male Cynomolgus monkeys

Compound ID	Subject	C_{max}	C_{last}	t_{max}	$t_{1/2}$	t_{last}	MRT	AUC_{last}	$AUC_{0-\infty}$	%Ext
		(ng/mL)		(h)				(ng•h/mL)		(%)
Genz-256416	1	31.6	1.21	4.00	5.68	24.0	(b) (4)	218	227	4.36
	2	67.0	0.672	2.00	5.77	24.0		221	226	2.47
	3[a]	21.9	1.58	2.00	3.06	12.0		82.9	89.9	7.75
	3A	96.5	0.812	0.500	5.46	24.0		272	278	2.30
	Mean	65.0	0.898	2.17	5.63	24.0		237	244	3.04
	SD	32.5	0.279	1.76	0.163	0.00		30.3	29.5	1.14
	CV%	50.0	31.1	81.0	2.89	0.00		12.8	12.1	37.5
Genz-311752	1	13.7	1.57	4.00	4.05	12.0		74.5	83.7	11.0
	2	19.4	0.763	2.00	3.31	12.0		58.7	62.3	5.85
	3[a]	15.2	0.74	2.00	2.98	12.0		49.4	52.5	6.05
	3A	94.2	0.548	0.50	5.37	24.0		203	208	2.05
	Mean	42.4	0.96	2.17	4.25	16.0		112	118	6.29
	SD	44.9	0.54	1.76	1.05	6.93		79.4	78.5	4.48
	CV%	106	56.1	81.0	24.6	43.3		70.8	66.6	71.2
Genz-258179	1	1.83	0.502	4.00	ND	8.00		8.24	ND	ND
	2	2.63	0.985	2.00	ND	4.00		5.54	ND	ND
	3[a]	2.29	0.551	2.00	ND	6.00		5.91	ND	ND
	3A	21.2	0.591	0.500	2.64[b]	12.0		45.3	47.5[b]	4.74[b]
	Mean	8.55	0.693	2.17	2.64	8.00		19.7	47.5	4.74
	SD	11.0	0.257	1.76	NA	4.00		22.2	NA	NA
	CV%	128	37.1	81.0	NA	50.0		113	NA	NA
Genz-258162	1	49.7	0.612	4.00	4.33	24.0		232	236	1.62
	2	49.1	1.36	2.00	2.58	12.0		147	152	3.33
	3[a]	57.2	1.65	2.00	2.52	12.0		163	169	3.54
	3A	214	0.934	0.500	4.40	24.0		626	632	0.938
	Mean	104	0.969	2.17	3.77	20.0		335	340	1.96
	SD	95.0	0.375	1.76	1.03	6.93		256	257	1.23
	CV%	91.1	38.7	81.0	27.4	34.6		76.4	75.5	62.8
Genz-527862	1	4.04	0.777	4.00	ND	8.00		14.5	ND	ND
	2	3.37	1.41	2.00	ND	4.00		6.47	ND	ND
	3[a]	5.46	0.862	2.00	ND	6.00		13.6	ND	ND
	3A	25.3	0.583	0.500	2.37	12.0		59.4	61.4	3.25
	Mean	10.9	0.923	2.17	2.37	8.00		26.8	61.4	3.25
	SD	12.5	0.432	1.76	NA	4.00		28.5	NA	NA
	CV%	114	46.8	81.0	NA	50.0		106	NA	NA

[a]: Animal 3 was not included in statistics due to emesis
[b]: calculation based on extrapolation from time points at 4, 6 and 12 hours post dose

Table 13. Pharmacokinetic parameters of the ten metabolites of Genz-99067 following a single 30 mg/kg PO dose of Genz-99067 (administrated as Genz-112638) to fasted male Cynomolgus monkeys (continued)

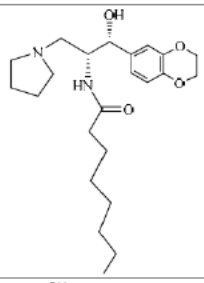
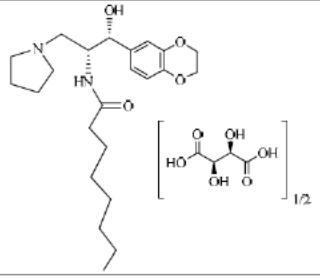
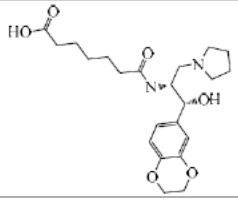
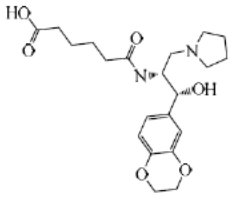
Compound ID	Subject	C_{max}	C_{last}	t_{max}	$t_{1/2}$	t_{last}	MRT	AUC_{last}	$AUC_{0-\infty}$	%Ext
		(ng/mL)		(h)				(ng•h/mL)		(%)
Genz-399207	1	25.6	1.81	4.00	ND	6.00	(b) (4)	73.2	ND	ND
	2	20.9	0.620	2.00	ND	6.00		40.4	ND	ND
	3[a]	13.7	1.65	2.00	ND	4.00		25.0	ND	ND
	3A	16.9	0.738	1.00	1.10	6.00		38.8	40.0	2.92
	Mean	21.1	1.06	2.33	1.10	6.00		50.8	40.0	2.92
	SD	4.35	0.656	1.53	NA	0.00		19.4	NA	NA
	CV%	20.6	62.1	65.5	NA	0.00		38.2	NA	NA
Genz-399240	1	280	0.677	4.00	1.43[b]	24.0		982	984[b]	0.142[b]
	2	208	1.89	2.00	1.18[c]	12.0		513	516[c]	0.623[c]
	3[a]	90.7	0.894	2.00	0.858	8.00		210	212	0.523
	3A	190	1.17	1.00	1.74	12.0		506	509	0.577
	Mean	226	1.25	2.33	1.45	16.0		667	670	0.447
	SD	47.6	0.610	1.53	0.281	6.93		273	272	0.265
	CV%	21.1	49.0	65.5	19.4	43.3		40.9	40.6	59.3
Genz-682042	1	1.93	1.93	4.00	ND	4.00		3.69	ND	ND
	2	1.06	1.06	2.00	ND	2.00		0.530	ND	ND
	3[a]	0.615	0.615	2.00	ND	2.00		0.308	ND	ND
	3A	1.74	0.733	1.00	ND	4.00		3.61	ND	ND
	Mean	1.58	1.24	2.33	NA	3.33		2.61	NA	NA
	SD	0.457	0.619	1.53	NA	1.15		1.80	NA	NA
	CV%	29.0	49.9	65.5	NA	34.6		69.0	NA	NA
Genz-256222	1	2.53	0.594	4.00	ND	8.00		10.9	ND	ND
	2	4.84	0.578	2.00	2.56	8.00		14.6	16.7	12.8
	3[a]	2.99	0.507	2.00	2.53	8.00		10.0	11.8	15.6
	3A	16.9	0.943	0.500	3.39	12.0		51.1	55.7	8.27
	Mean	8.09	0.705	2.17	2.97	9.33		25.5	36.2	10.5
	SD	7.72	0.206	1.76	0.588	2.31		22.2	27.6	3.17
	CV%	95.4	29.3	81.0	19.8	24.7		87.0	76.2	30.2
Genz-120965	1	0.530	0.530	2.00	ND	2.0		0.265	ND	ND
	2	0.991	0.991	2.00	ND	2.0		0.496	ND	ND
	3[a]	0.557	0.557	2.00	ND	2.0		0.279	ND	ND
	3A	2.80	1.41	0.500	ND	2.0		2.88	ND	ND
	Mean	1.44	0.977	1.50	NA	2.00		1.21	NA	NA
	SD	1.20	0.440	0.866	NA	0.000		1.45	NA	NA
	CV%	83.3	45.1	57.7	NA	0.0		119	NA	NA

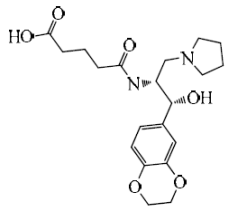
[a]: Animal 3 was not included in statistics due to emesis
[b]: calculation based on extrapolation from time points at 6, 8 and 12 hours post dose
[c]: calculation based on extrapolation from time points at 4, 6 and 8 hours post dose

Pharmacokinetic Study of Genz-99067, and Its Three Metabolites, Genz-399207, Genz-399240 and Genz-682042, Following a Single Oral Administration of Genz-112638 to Female New Zealand White Rabbits (DMPK12-R003)

Methods: Four female New Zealand white rabbits were treated at single 25.3 mg/kg dose of Genz-99067 (administered as Genz-112638, the hemi-tartrate salt form of Genz-99067 at 30 mg/kg) via oral gavage. Serial blood samples were collected over 0 to 24 hours after each dose, and plasma concentrations of Genz-99067, and its three metabolites, Genz-399207, Genz-399240 and Genz-682042, were determined by LC-MS/MS methods. The structures of the parent compound and three metabolites are shown in the figure (from page 14 of the report) below.

Figure 1. Structures of Genz-99067, Genz-112638 and three metabolites, Genz-399207, Genz-399240 and Genz-682042

Compound Name	Structure
Genz-99067 (Free base)	
Genz-112638 (Hemi-tartrate salt)	
Genz-399207	
Genz-399240	

Genz-682042	

Results: The mean C_{max} of Genz-99067 was 88.5 ng/mL at t_{max} of 0.708 hour. The mean $AUC_{0-\infty}$ of Genz-99067 was 128 ng.hr/mL, and the $t_{½}$ was 3.90 hour. The mean C_{max} of three metabolites were 6.56 ng/mL, 173 ng/mL, and 6.68 ng/mL for Genz 399207, Genz-399240, and Genz-682042, respectively. The mean t_{max} of Genz-399207, Genz-399240, and Genz-682042 was 1.00 hour. The mean $AUC_{0-\infty}$ of Genz-399240 was 372 ng.hr/mL. The mean AUC_{0-last} was 7.51 ng.hr/mL and 11.9 ng.hr/mL for Genz 399207 and Genz-682042, respectively. The PK parameters are shown in the table (from page 12 of the report) below.

Table 2. Pharmacokinetic parameters of Genz-99067 and its metabolites, Genz-399207, Genz-399240 and Genz-682042 following a single 25.3 mg/kg oral dose of Genz-99067 (administered as Genz-112638) to fasted female New Zealand White rabbits

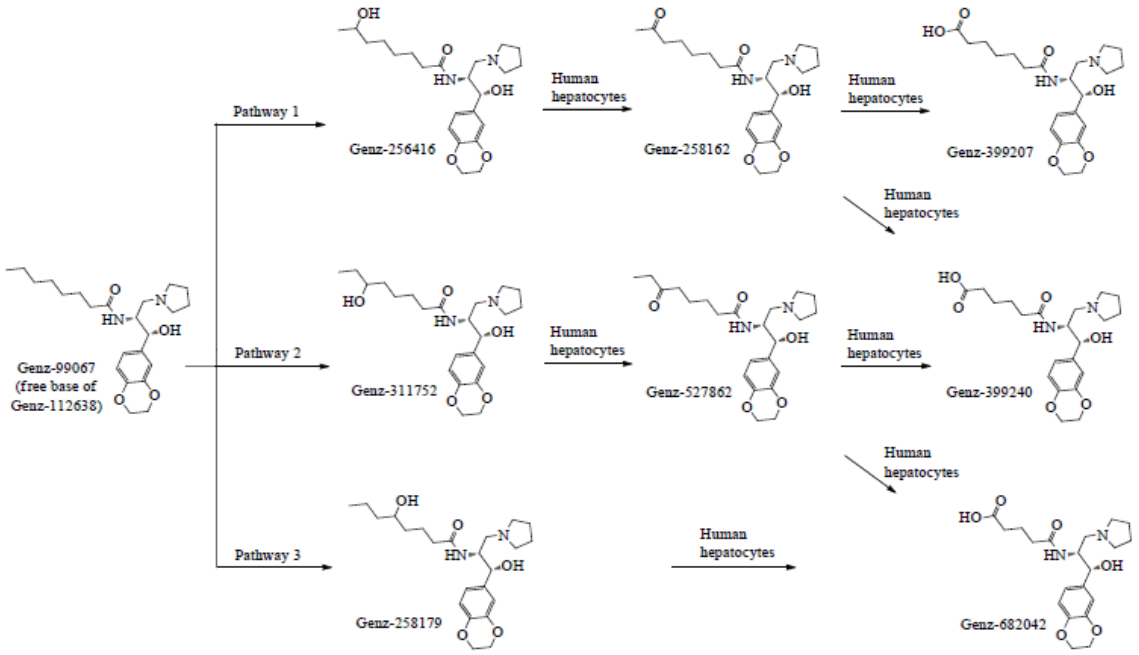
Compound ID	Subject	C_{max}	C_{last}	t_{max}	$t_{1/2}$	t_{last}	MRT	AUC_{last}	$AUC_{0-\infty}$	%Ext
		(ng/mL)		(h)				(ng•h/mL)		(%)
Genz-99067	1	17.5	0.890	0.250	2.43	8.00	(b) (4)	34.3	37.5	8.32
	2	18.4	2.02	2.00	ND	24.0		91.9	ND	ND
	3	13.9	1.19	0.500	ND	24.0		66.3	ND	ND
	4	304	0.502	0.083	5.37	24.0		215	219	1.78
	Mean	88.5	1.15	0.708	3.90	20.0		102	128	5.05
	SD	144	0.64	0.878	2.08	8.00		78.8	128	4.62
	CV%	162	56.0	124	53.4	40.0		77.4	100	91.5
Genz-399207	1	3.57	2.67	1.00	ND	2.00		4.34	ND	ND
	2	9.57	3.36	1.00	ND	2.00		10.3	ND	ND
	3	7.13	3.92	1.00	ND	2.00		8.66	ND	ND
	4	5.97	2.01	1.00	ND	2.00		6.80	ND	ND
	Mean	6.56	2.99	1.00	NA	2.00		7.51	NA	NA
	SD	2.49	0.830	0.00	NA	0.00		2.55	NA	NA
	CV%	38.0	27.7	0.00	NA	0.00		33.9	NA	NA
Genz-399240	1	96	0.647	1.00	7.11	24.0		295	302	2.20
	2	282	1.29	1.00	ND	24.0		483	ND	ND
	3	193	1.63	1.00	10.1	24.0		525	549	4.33
	4	120	0.561	1.00	6.66	24.0		260	266	2.03
	Mean	173	1.03	1.00	7.96	24.0		391	372	2.85
	SD	83.7	0.515	0.00	1.88	0.00		133	154	1.28
	CV%	48.5	49.9	0.00	23.6	0.00		33.9	41.4	45.0
Genz-682042	1	3.71	0.646	1.00	ND	4.00		8.09	ND	ND
	2	11	0.501	1.00	ND	4.00		19.3	ND	ND
	3	6.45	0.865	1.00	ND	4.00		13.9	ND	ND
	4	5.57	2.12	1.00	ND	2.00		6.30	ND	ND
	Mean	6.68	1.03	1.00	NA	3.50		11.9	NA	NA
	SD	3.10	0.740	0.00	NA	1.00		5.89	NA	NA
	CV%	46.3	71.6	0.00	NA	28.6		49.5	NA	NA

Genz-99067 Metabolic Pathway Elucidation Using Cryopreserved Human Hepatocyte Suspensions (DMPK12-R005)

Methods: In this study, the metabolic pathways of Genz-99067 were elucidated using cryopreserved human hepatocyte suspensions. Hydroxyl metabolites, Genz-256416, Genz-311752 and Genz-258179, or ketone metabolites, Genz-258162 and Genz-527862 (each at a final concentration of 1 μM) were incubated individually in cryopreserved human hepatocytes suspensions. Incubation was conducted at 37^0C for 2 hours in triplicate for each metabolite.

Results: Genz-99067 underwent sequential oxidation of octanoyl moiety via three distinct metabolic pathways in human hepatocytes. In the first metabolic pathway, 7-hydroxyl metabolite Genz-256416 was metabolized to 7-ketone metabolite Genz-258162, followed by 7-carboxy metabolite Genz-399207 and 6-carboxy metabolite Genz-399240. In a second metabolic pathway, 6-hydroxyl metabolite Genz-311752 was metabolized to 6-ketone metabolite Genz-527862, followed by 6-carboxy metabolite Genz-399240 and 5-carboxy metabolite Genz-682042. In a third metabolic pathway, 5-hydroxyl metabolite Genz-258179 was metabolized to 5-carboxy metabolite Genz-682042. The proposed metabolic pathway is shown below (from page 24 of the report).

Figure 6. Proposed metabolic pathways of oxidation of Genz-99067 octanoyl moiety



Identification of Metabolites of Genz-99067 in Plasma and Urine from Preclinical Species and Comparison to Human (DMPK13-R003A1)

Methods: In this study, detection and identification of additional metabolites of Genz-99067 were conducted using plasma samples from rats, rabbits, monkeys, and humans, and urine samples from rats and monkeys. Unlabeled Genz-112638 and radiolabeled [^{14}C]-112638 were used in the following (from page 13 of the report) animal and human studies.

Table 1. Study sample information

Study Sample[1]	Plasma				Urine	
Sample Reference [1-6]	DMPK11-R019	DMPK12-R003	DMPK11-R092	GZGD02107	DMPK11-R025	DMPK11-R059
Species	Rat	Rabbit	Monkey	Human	Rat	Monkey
Strain	Sprague Dawley	New Zealand White	Cynomolgus	N/A	Sprague Dawley	Cynomolgus
Test Article	Genz-112638 and [^{14}C]-Genz-112638	Genz-112638	Genz-112638	Genz-112638 and [^{14}C]-Genz-112638	Genz-112638	Genz-112638
Number of Animals (Subjects)/Time Point	5	4	3	8	3	3
Gender	Male	Female	Male	Male	Male	Male
Method of Administration	Oral	Oral	Oral	Oral	IV	IV
Dose (expressed as free base)	42.2 mg/kg QD for 14 days with the last dose on Day 14 containing 84.4 µCi/kg of [^{14}C]-Genz-112638	25.3 mg/kg	30 mg/kg	84.4 mg/subject	0.844 mg/kg	1 mg/kg
^{14}C-Dose (expressed as free base)	84.4 µCi/kg	-	-	100 µCi/subject	-	-
Sampling Time Post-dose	1h and 4h post [^{14}C]-Genz-112638 dosing	1h, 4h	1h, 4h	1h, 4h	0 to 24h (urine plus 20 mL cage rinse)[2]	0 to 4h, 4 to 8h

1. Samples from previous *in vivo* studies in animals and clinical studies in human were used in this study.
2. Rat urine samples and cage wash were combined during sample collection.
"-": Not applicable

Results: Seven metabolites (M30, M33, M34, M35, M40, M43, and M44) identified and quantified in the human plasma were detected in the rabbit and monkey plasma. Except M35, all the other six metabolites were detected in the rat plasma. In addition, M36, which was not quantifiable (<1%), but detected and identified in the human plasma, was also detected in the rabbit and monkey plasma. Four metabolites (M72, M73, M74, and M75) found in the human urine were also detected in the monkey urine, but not in the rat urine. The following table (from page 14-17 of the report) shows the structures of the metabolites.

Table 2. Structures of Genz-99067 and its putative metabolites

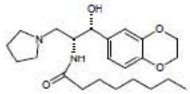
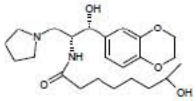
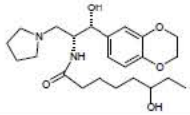
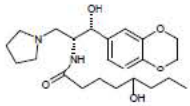
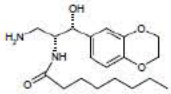
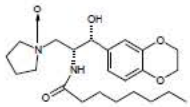
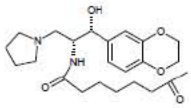
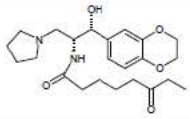
Sample type	Compound	RT (min)	Structure
		(b) (4)	
Parent standard	Genz-99067		
Metabolite standards	Genz-256416 (M5)		
	Genz-311752 (M6)		
	Genz-258179 (M7)		
	Genz-256222 (M11)		
	Genz-120965 (M12)		
	Genz-258162 (M17)		
	Genz-527862 (M18)		

Table 2. Structures of Genz-99067 and its putative metabolites (continued)

Sample type	Compound	RT (min)	Structure
		(b) (4)	
Metabolite standards	Genz-399240 (M24)		
	Genz-399207 (M25)		
	Genz-682042 (M31)		
Plasma	M30		
	M33		
	M34		
	M35		
	M36		

Table 2. Structures of Genz-99067 and its putative metabolites (continued)

Sample type	Compound	RT (min)	Structure
		(b) (4)	
Plasma	M40		
	M43		
	M44		
Urine	M72		
	M73		
	M74		
	M75		

Excretion:

Pharmacokinetics, Tissue Distribution and Mass Balance Study in Beagle Dogs following Single Administration of [^{14}C] Genz-112638 by Intravenous Injection and Oral Gavage (GT-157-PK-2)

Methods: The objective of this study was to examine the blood and plasma pharmacokinetics (PK), tissue distribution and mass balance of total radioactivity in Beagle dogs following single administration of [^{14}C] GENZ-112638 (specific activity = 44.80 mCi/mmol) by oral gavage and IV injection. In addition, the unchanged drug levels in the plasma were determined. Three male and three female dogs were assigned to a single group and were treated once by IV injection and then once by oral gavage with the radiolabeled test item as shown in the following study design (from page 9 of the report):

Treatment and route	Day	Animal number	Dose-level (mg/kg)	Radioactive dose-level (MBq/kg)	Dosage volume (mL/kg)
First Intravenous (iv)	1	3 males 3 females	2.5	0.925	2
Second Oral (po)	15	3 males 3 females	25	1.85	5

The test item was administered as a solution in normal saline for both the routes. Blood samples were collected from each animal at the following time-points: IV route: 0, 0.083, 0.25, 0.5, 1, 2, 4, 6, 10, 24, 48, 72, 120 and 168 hours, post-dose; oral route: 0, 0.25, 0.5, 1, 2, 3, 4, 6, 10, 24, 48, 72, 120 and 168 hours, post-dose. In addition, for each administration, excreta were collected in fractions of 24 hours, pre-dose, and then over a 168-hour period. Following the last blood sampling after gavage, the animals were sacrificed and selected tissues were dissected out and weighed. Blood, plasma, excreta, brain and bile samples were analyzed for total radioactivity by LSC. Plasma samples were analyzed for unchanged drug by LC-MS/MS. Selected urine and plasma samples were analyzed for metabolic pattern by radio HPLC.

Results:

Pharmacokinetics (Plasma): Following IV bolus injection at 2.5 mg/kg, the mean plasma unchanged drug and total radioactivity levels decreased in a biphasic manner from 0.083 hour post-dose (1715/859 ng/mL and 2572/1907 ng-eq/g, respectively) at 4 or 6 hours for the unchanged drug or at 168 hours for radioactivity. The blood radioactivity profiles were similar to that of the plasma, however, maximal radioactivity levels were lower (1677/1333 ng-eq/g) than that in the plasma.

Following oral gavage at 25 mg/kg, the mean plasma unchanged drug and total radioactivity levels increased to a C_{max} (3173/1891 ng/mL and 9117/9157 ng-eq/g, respectively) at 0.5-1 hours, followed by a decrease at 10 hours for the unchanged drug or at 168 hours for radioactivity. Again, the blood radioactivity profiles were similar to that in the plasma, however, with lower C_{max} for radioactivity (7294/8158 ng-eq/g) than in the plasma. There was no evidence of enterohepatic recycling or sex differences.

The PK parameters for plasma (P), unchanged drug (UD), and plasma and blood (B) radioactivity (RA) are shown in the following table (from page 10 of the report).

Route & dose (mg/kg)	Matrix & analysis	Sex	C_{max}[1] (ng/mL) or (ng-eq/g)	t_{max} (h)	AUC_{0-t} (ng*h/mL) or (ng-eq*h/g)	$AUC_{0-\infty}$ (ng*h/mL) or (ng-eq*h/g)	$t_{1/2z}$ (h)	CL (mL/min/kg)	V_z (L/kg)	F%[2]
iv, 2.5	P/UD	male	1715	-	1775	1904	1.3	25.42	2.60	-
	P/RA		2572	-	9619	11800	101.5	-	-	-
	B/RA		1677	-	5638	6315	19.4	-	-	-
iv, 2.5	P/UD	female	859	-	1105	1135	1.1	39.76	3.88	-
	P/RA		1907	-	8653	11425	122.7	-	-	-
	B/RA		1333	-	5026	5758	20.6	-	-	-
po, 25	P/UD	male	3173	0.667	9279	9477	1.6	-	-	49.8
	P/RA		9117	0.500	62636	71046	76.3	-	-	60.2
	B/RA		7294	0.667	41209	45248	14.2	-	-	71.7
po, 25	P/UD	female	1891	0.500	5376	5478	1.5	-	-	48.3
	P/RA		9157	0.667	63692	72740	67.8	-	-	63.7
	B/RA		8158	0.667	44488	49816	22.5	-	-	86.5

1: concentration at 0.083 hour time-points for intravenous route.
2: F % of the oral dosage form was calculated using $AUC_{0-\infty}$.
-: not calculated/applicable.

The low volume of distribution (Vz) indicated that the test article was not highly distributed to tissues.

Excretion balance: The mean total cumulative excretion (CE) of the administered radioactive dose over the 168-hour periods was as follows (from page 10 of the report). Most (> 74%) of the recovered radioactivity in the excreta was eliminated within the first 24 hours post-dose for both the routes and sexes.

Route & dose (mg/kg)	Sex	CE in urine (%)	CE in feces (%)	CE in cage wash (%)	Vomit (%)	Total CE in excreta (%)
iv, 2.5	male	25.6	63.9	1.94	-	91.4
	female	25.0	66.5	2.57	-	94.1
po, 25	male	30.0	49.7	3.55	8.49	91.7
	female	25.6	56.5	3.36	7.26	90.3

-: not applicable.

Metabolic patterns in plasma and urine: A single small metabolite peak in addition to unchanged drug was found in the plasma after oral dose at 30 minutes post-dose. No metabolites were found in the plasma following IV dose. Three to five peaks (two to four metabolites and unchanged drug) were found in the urine samples collected at 0-8 and 8-24 hours post-IV and oral dosing. Unchanged drug for males/females represented 14.3/8.14% after IV injection and 34.3/6.29% after oral gavage in the 0-8 hour fraction; the proportions of unchanged drug were generally lower or negligible in the later fraction (8-24 hours).

Tissue levels: The brain radioactivity levels were non-quantifiable at 168 hours post gavage dose. In the bile, radioactivity levels were quantifiable in males (1078 ng-eq/g) and females (807 ng-eq/g).

Other Pharmacokinetic studies:

Pharmacokinetics of Genz-99067 Following a Single Oral Administration of Genz-112638 to Male and Female D409V/null Mice (DMPK11-R058)

Methods: In this study, each of six D409V/null mice (n = 3/sex) received a single 150 mg/kg dose of Genz-112638 by oral gavage. Seven serial blood samples were collected over 0 to 8 hours post-dose and concentrations of Genz-99067 in the plasma were determined by LC-MS/MS.

Results: Following oral administration of 150 mg/kg Genz-112638 (equivalent to 127 mg/kg Genz-99067) in male mice, mean C_{max} and t_{max} were 1450 ng/mL and 0.667 hours, respectively; and mean $AUC_{0-\infty}$ and $t_{½}$, z (volume of distribution) were 4230 ng.hr/mL and 0.975 hours, respectively. In female mice, mean C_{max} and t_{max} were 1420 ng/mL and 0.250 hours, respectively; and mean $AUC_{0-\infty}$ and $t_{½}$, z were 2600 ng.hr/mL and 1.16 hours, respectively. Males generally had greater systemic exposure to Genz-99067 than females, with higher mean $AUC_{0-\infty}$ (4230 versus 2600 ng.hr/mL). Male mice exhibited a slower rate of absorption than females, with mean observed t_{max} values of 0.667 and 0.250 hours, respectively. The PK parameters are shown below (from page 7 of the report).

Analyte	Gender	C_{max}	C_{last}	t_{max}	$t_{½, z}$	t_{last}	MRT	AUC_{last}	$AUC_{0-\infty}$
		ng/mL		h				ng·h/mL	
Genz-99067	Male	1450	7.43	0.667	0.975	8.00	2.01	4220	4230
	Female	1420	16.5	0.250	1.16	7.67	1.56	2570	2600

Pharmacokinetic Study of Genz-399240 Following Subcutaneous Administration to Male Sprague-Dawley Rats (DMPK11-R087)

Methods: In this study, three escalating doses (1, 5, and 25 mg/kg) of Genz-399240 were each administered subcutaneously (SC) to three male Sprague Dawley rats, and blood samples were collected over 0 to 48 hours post-dose. Concentrations of Genz-399240 in the plasma were determined by LC-MS/MS method.

Results: At 1 mg/kg, the mean C_{max} and t_{max} values were 716 ng/mL and 0.250 h, respectively. The mean $AUC_{0-\infty}$ and ($t_{½}$) were 685 ng.hr/mL and 0.530 h, respectively. At 5 mg/kg, the mean C_{max} and t_{max} were 2240 ng/mL and 0.250 h, respectively. The mean $AUC_{0-\infty}$ and $t_{½}$ were 2500 ng.hr/mL and 0.650 h, respectively. At 25 mg/kg, the mean C_{max} and t_{max} were 13400 ng/mL and 0.194 h, respectively. The mean $AUC_{0-\infty}$ and $t_{½}$ were 13400 ng.hr/mL and 2.01 h, respectively. The following table (from page 12 of the report) shows the PK data.

Table 2. Pharmacokinetic parameters of Genz-399240 following a single 1, 5 and 25 mg/kg subcutaneous administration of Genz-399240 to non-fasted male Sprague-Dawley rats

Dose/Route	Animal Number	C_{max} (ng/mL)	C_{last} (ng/mL)	t_{max} (h)	$t_{½,z}$ (h)	t_{last} (h)	MRT (b) (4)	AUC_{last} (ng•h/mL)	$AUC_{0-\infty}$ (ng•h/mL)	AUC_{extrap}[a] (%)
1 mg/kg single SC dose	862	729	2.39	0.250	0.443	4.00		667	668	0.229
	863	866	0.640	0.250	0.578	6.00		647	647	0.0824
	864	553	0.645	0.250	0.569	6.00		740	741	0.0714
	Mean	716	1.23	0.250	0.530	5.33		685	685	0.128
	SD	157	1.01	0.00	0.0755	1.15		49.0	49.3	0.0880
	%CV	21.9	82.4	0.0	14.2	21.7		7.1	7.2	69.0
5 mg/kg single SC dose	865	2510	0.730	0.250	0.641	8.00		2930	2930	0.0230
	866	1720	0.698	0.250	0.622	8.00		2400	2400	0.0261
	867	2500	0.772	0.250	0.688	8.00		2180	2180	0.0351
	Mean	2240	0.733	0.250	0.650	8.00		2500	2500	0.0281
	SD	453	0.0371	0.00	0.0340	0.00		386	386	0.00629
	%CV	20.2	5.1	0.0	5.2	0.0		15.4	15.4	22.4
25 mg/kg single SC dose	868	13300	1.01	0.0830	1.90	12.0		11800	11800	0.0234
	869	16700	1.65	0.250	1.92	12.0		16100	16100	0.0284
	870	10100	0.992	0.250	2.20	12.0		12300	12300	0.0255
	Mean	13400	1.22	0.194	2.01	12.0		13400	13400	0.0258
	SD	3300	0.375	0.0964	0.168	0.00		2350	2350	0.00251
	%CV	24.7	30.8	49.6	8.4	0.0		17.5	17.5	9.7

[a]: % of $AUC_{0-\infty}$ due to extrapolation

<u>Pharmacokinetic Study of Genz-399240 Following a Single Intravenous or Oral Administration of Genz-399240 to Male Sprague-Dawley Rats (DMPK12-R004)</u>

Methods: A single dose of Genz-399240 (metabolite of Genz-112638) was administered to male Sprague Dawley rats via IV bolus (IV, n = 2) at 3 mg/kg, or oral gavage (n= 3/dose) at 10 mg/kg or 30 mg/kg. Serial blood samples were collected over 0 to 24 hours after each dose, and plasma concentrations of Genz-399240 were determined by LC-MS/MS.

Results: Following IV administration of 3 mg/kg of Genz-399240, the mean initial concentration (C_0) was 14700 ng/mL, and the mean $AUC_{0-\infty}$ was 1590 ng.hr/mL. The mean plasma clearance (CL) was 31.7 mL/min/kg. The mean estimated volume of distribution at steady state (V_{ss}) was 0.337 L/kg. The mean terminal half-life ($t_{½}$) was 1.01 hours. The renal clearance (CL_r) was 4.08 mL/min/kg.

Following oral administration of 10 mg/kg Genz-399240, the mean C_{max} and t_{max} were 19.4 ng/mL and 1.50 hours, respectively. The mean $AUC_{0-\infty}$ and $t_{½}$ were 109 ng.hr/mL and 9.71 hours, respectively. The mean oral bioavailability was 1.44%. Following oral administration of 30 mg/kg Genz-399240, the mean C_{max}, t_{max}, $AUC_{0-\infty}$, and estimated $t_{½}$ were 63.9 ng/mL, 0.583 hours, 297 ng.hr/mL, and 5.08 hours, respectively. The mean oral bioavailability was 1.64%.

The PK parameters are shown in the following table (from page 14 of the report).

Table 3Pharmacokinetic parameters of Genz-399240 following a single 3 mg/kg IV dose of Genz-399240 to fasted male Sprague-Dawley rats

Dose/Route	Rat ID	C_0	C_{max}	C_{last}	t_{max}	$t_{1/2}$	t_{last}	MRT	AUC_{last}	$AUC_{0-\infty}$	%Ext	CL	CL_T	$\%CL_T$	V_{ss}	V_z	%F
		(ng/mL)				(h)			(ng•h/mL)			(mL/min/kg)			(mL/kg)		
3 mg/kg IV bolus	461	14900	NA	1.19	NA	0.887 a)	4.00	(b) (4)	1740	1740	0.0873	28.7	4.12	14.4	330	2200	NA
	462	14600	NA	1.01	NA	1.13 a)	4.00		1440	1440	0.114	34.7	4.04	11.6	343	3390	NA
	Mean	14700	NA	1.10	NA	1.01	4.00		1590	1590	0.101	31.7	4.08	13.0	337	2800	NA
	SD	218	NA	0.127	NA	0.171	0.00		214	214	0.0190	4.26	0.0607	1.94	9.31	840	NA
	CV%	1.48	NA	11.6	NA	17.0	0.00		13.5	13.4	18.8	13.4	1.49	14.9	2.77	30.1	NA
10 mg/kg PO dose	463	NA	14.0	1.55	2.00	17.5	24.0		88.3	127	30.7	NA	NA	NA	NA	NA	1.66
	464	NA	31.8	1.42	0.500	8.25 b)	8.00		71.4	88.3	19.1	NA	NA	NA	NA	NA	1.35
	465	NA	12.5	8.70	2.00	3.36 c)	8.00		69.1	111	37.9	NA	NA	NA	NA	NA	1.30
	Mean	NA	19.4	3.89	1.50	9.71	13.3		76.2	109	29.3	NA	NA	NA	NA	NA	1.44
	SD	NA	10.7	4.17	0.866	7.19	9.24		10.5	19.7	9.46	NA	NA	NA	NA	NA	0.197
	CV%	NA	55.2	107	57.7	74.1	69.3		13.7	18.1	32.4	NA	NA	NA	NA	NA	13.7
30 mg/kg PO dose	466	NA	69.8	10.2	0.500	6.91 c)	8.00		221	323	31.5	NA	NA	NA	NA	NA	1.39
	467	NA	42.9	0.561	1.00	4.61	24.0		226	230	1.62	NA	NA	NA	NA	NA	1.42
	468	NA	79.0	0.512	0.250	3.72	24.0		336	338	0.811	NA	NA	NA	NA	NA	2.11
	Mean	NA	63.9	3.76	0.583	5.08	18.7		261	297	11.3	NA	NA	NA	NA	NA	1.64
	SD	NA	18.8	5.58	0.382	1.65	9.24		64.7	58.8	17.5	NA	NA	NA	NA	NA	0.406
	CV%	NA	29.4	148	65.5	32.5	49.5		24.8	19.8	155	NA	NA	NA	NA	NA	24.8

a): calculation based on extrapolation from time points at 2 and 4 hours post dose
b): calculation based on extrapolation from time points at 6 and 8 hours post dose
c): calculation based on extrapolation from time points at 4 and 6 hours post dose

<u>Prediction of the Pharmacokinetics of Genz-99067, the Free Base of Genz-112638, in Human from *In Vitro* and *In Vivo* Pharmacokinetic Data of Genz-99067 in Mouse, Rat, Dog, and Monkey (DMPK12-R062)</u>

<u>Methods</u>: Human pharmacokinetics (PK) of Genz-99067 (free base of the tartrate salt Genz-112638) was predicted from *in vitro* ADME data of Genz-99067 obtained in the mouse, rat, dog, monkey, and human and *in vivo* preclinical PK data of Genz-99067 obtained in the mouse, rat, dog, and monkey. The mean human oral PK concentration-time curve of Genz-99067 was predicted based on oral PK parameters in the preclinical species using inputs from multiple methodologies. The mean non-compartmental PK parameters of Genz-99067 in human were predicted by standard non-compartment PK analysis of the predicted mean human PK profile.

Results: Genz-99067 was predicted to exhibit high systemic clearance (26.9 mL/min/kg), large volume of distribution at terminal phase (14.4 L/kg), and short terminal half-life (6.15 hours) in human. Following a single oral administration, Genz 99067 was predicted to have low oral bioavailability (8.97%) with t_{max} of 1.67 hours in human. The predicted PK parameters were consistent (i.e., within 2-fold) with the values of the corresponding parameters observed in the clinical study GZGD02107. Genz-99067 was predicted to exhibit low systemic accumulation (R_c =1.37) and a moderate steady state peak-to-trough ratio of 3.27.

The mean human plasma pharmacokinetic parameters of Genz-99067 are summarized in the table below (from page 25-27 of the report).

Table 4. Summary of the predicted human PK parameters of Genz-99067 used in the generation of composite single dose human plasma concentration-time profile

Parameter	Prediction Method Number[a,b]	Method Description	Predicted Value
$CL_{p,human}$ (mL/min/kg)	CPM01	single species allometric scaling from mouse unbound plasma clearance	26.9
	CPM02	single species allometric scaling from rat unbound plasma clearance	26.9
	CPM03	single species allometric scaling from dog unbound plasma clearance	26.9
	CPM04	single species allometric scaling from monkey unbound plasma clearance	26.9
	CPM05	multiple species allometry based on total plasma clearance	21.2
	CPM06	multiple species allometry based on unbound plasma clearance	26.9
	CPM07	scaling from in vitro intrinsic clearance in human liver microsomes	12.9
$VD_{ss,human}$ (L/kg)	VPM01	single species allometric scaling from mouse unbound steady state volume of distribution	31.9
	VPM02	single species allometric scaling from rat unbound steady state volume of distribution	5.47
	VPM03	single species allometric scaling from dog unbound steady state volume of distribution	20.6
	VPM04	single species allometric scaling from monkey unbound steady state volume of distribution	12.3
	VPM05	multiple species allometry based on total steady state	3.73

Parameter	Prediction Method Number[a,b]	Method Description	Predicted Value
		volume of distribution	
	VPM06	multiple species allometry based on unbound steady state volume of distribution	11.6
	VPM07	Oie-Tozer equation	8.21
$F_{a,human}F_{g,human}$	F2PM01	scaling by IVIVE from *in vitro* intrinsic clearance in mouse liver microsomes	0.0701
	F2PM02	scaling by IVIVE from *in vitro* intrinsic clearance in rat liver microsomes	0.161
	F2PM03	scaling by IVIVE from *in vitro* intrinsic clearance in dog liver microsomes	0.348
	F2PM04	scaling by IVIVE from *in vitro* intrinsic clearance in monkey liver microsomes	0.0691
k_a (h^{-1})	RPM01	average of the first order oral absorption rate constants determined in multiple animal species	1.76

a: Predicted by various methodologies as described in Section 3
b: CPM = human clearance prediction method; VPM = human volume of distribution prediction method; F2PM = human (FaFg) value prediction method; RPM = human oral absorption rate constant k_a prediction method.

Table 5. Summary of predicted mean non-compartment human PK parameters of Genz-99067 after single or once-every-12 hours repeated oral administration of 84.4 mg/84.8 kg of Genz-99067 (eliglustat 100 mg/84.8 kg)

Parameter	Value	
Dose (mg/84.8 kg)	84.4	
CL_p	26.9 mL/min/kg	137 L/h
VD_z	14.4 L/kg	1220 L
$t_{½,z}$ (h)	6.15	
T_{max} (h)	1.67	
C_{max} (ng/mL)	5.50	
F_{oral} (%)	8.97	
$C_{ss,max}$ (ng/mL)	7.43	
$C_{ss,min}$ (ng/mL)	2.27	
Peak-to-trough ratio	3.27	
$AUC_{ss,0-\tau}$ (ng·h/mL)	55.4	
$AUC_{0-\tau}$ (ng·h/mL)	40.4	
R_c	1.37	
$t_{½,eff}$ (h)	6.37	

5.2 Toxicokinetics

Toxicokinetics is discussed in toxicity studies.

6 General Toxicology

6.1 Single-Dose Toxicity

Acute Toxicity Study in Rats (GT-157-TX-3) Dogs (GT-157-TX-4) by Oral Route

The following review is incorporated below from the pharmacology review of IND 67,589 dated March 18, 2004 and July 30, 2004 by Dr. Dylan Yao (DMEP).

Single dose studies:

SD rats, single oral dose (study # GT-157-TX-3), dose range finding study:
Methods: Genz-112638 was given to 5 groups of rats (3/s/g) as single oral doses of 200, 400, 800, 900, and 1000 mg/kg, and observed for 10 days.
Results:
Mortality:
@ 1000 mg/kg, 1/3 M found dead on day 3 and 1/3 F was moribund sacrificed on day 6.
@ 900 mg/kg, 1/3 F was euthanized on day 2.
@ 800 mg/kg, 1/3 M and 1/3 F were moribund sacrificed.
Clinical sings: Doses at ≥400 mg/kg induced salivation, wet fur, labored and/or abnormal sneezing and breathing, decreased fecal output and/or abnormal distension. @ 200 mg/kg, recurring breathing abnormalities and decreased feces output were noted. Body weight loss at 200 g/kg was apparent.
NOAEL<200 mg/kg

Beagle dogs (study # GT-157-TX-4), dose range finding study:
Methods: oral (capsule) dosing
Group 1 (1M, 1F): single dose, dose escalating: 50, 100, 35, 25 mg/kg
Group 2 (1M, 1F): maximum tolerated dose 25 mg/kg, single dose
Group 3 (2M, 2F): repeat dose 25 mg/kg/d for 10 days
Results:
No mortality was observed.

Emesis was observed at doses of 25, 35, 50 and 100 mg/kg.
Body weight effect was noted at single dose of 25 mg/kg.
NOAEL<25 mg/kg

2.6.6.2 Single-dose toxicity

Single dose toxicity was assessed in SD rats and Beagle dogs.

Rat study:

SD rats (3/s/g) were given a single dose of Genz-112638 at 400, 800, 900, or 1000 mg/kg by oral gavage. The endpoints observed include survival and clinical signs.
After administration with single dose of 400, 800, 900 or 1000 mg/kg, animals showed signs of salivation, wet fur, areas of fur staining, thin fur, labored and/or abnormal sneezing and breathing, decreased fecal output and/or abdominal distention.

Dog study:

Beagle dogs (1M, 1F) were orally fed by gavage with escalating single dose of Genz-112638 at 25, 35, 50 and 100 mg/kg; another 1M and 1F dogs were fed maximum tolerated dose 25 mg. Emesis was observed at doses of 25, 35, 50 and 100 mg/kg. A decrease in body weight (↓ 1% M and ↓ 4% F) was noted for animals administered a single, maximum tolerated dose 25 mg followed by a 14-day observation period.

Single Dose Intravenous Infusion Toxicity Study with a 14-Day Recovery Period in Sprague-Dawley Rats (GT-157-TX-39)

Methods: This study (GLP-complied, initiated on November 12, 2008, LAB Research Inc., Laval, Quebec, Canada) was conducted to evaluate the toxicity of Genz-112638 (Lot No. S1517, purity = 100%) following a single IV infusion to Sprague-Dawley (SD) rats (n = 5/sex/dose) with a 14-day recovery period. The test article and vehicle (0.9% sodium chloride for injection, USP) were administered by a single 1-hour IV infusion. After dosing on Day 1, the animals were observed for 14 days, following which they were euthanized on Day 15. All animals were subjected to a necropsy examination. The study design is shown in the following table (from page 8 of the report).

Treatment Groups	Dose Level (mg/kg)	Dose Conc. (mg/mL)	Dose Volume (mL/kg)	Dose Rate (mL/kg/hr)	Number of Animals	
					Males	Females
1. Control[a]	0	0	2	2	5	5
2. Low Dose	3	1.5	2	2	5	5
3. Mid Dose	10	5	2	2	5	5
4. High Dose	20	10	2	2	5	5

[a] The Group 1 animals received the control article, 0.9% Sodium Chloride for Injection, USP.

Parameters examined included mortality, clinical condition, body weight, food consumption, hematology, clinical chemistry, coagulation, urinalysis, macroscopic examination and organ weights.

Results: There was no mortality. The maximum nonlethal dose was 20 mg/kg. There were no significant treatment-related effects on clinical signs, body weights, food consumption, clinical pathology parameters (hematology, coagulation, clinical chemistry and urinalysis), organ weights, and gross necropsy.

6.2 Repeat-Dose Toxicity

Mouse

Oral Palatability Study by Dietary Admixture in Mice (Study No. TSR 30377; Report No. GT-157-TX-25)

4-Day Oral Palatability Study by Dietary Admixture in Mice (Study No. 30724 TSR; Report No. GT-157-TX-26)

Study Title: 2-Week Oral Palatability Study by Dietary Admixture in Mice ((Study No. 30593 TSS; Report No. GT-157-TX-27)

13-Week Oral (Diet) Toxicology Study in CD-1 Mice (GT-157-TX-37/459308)

The reviews of the above studies are incorporated below from the pharmacology review of IND 67,589 dated May 28, 2009 by Dr. Tamal K. Chakraborti (DGIEP).

Reference ID: 3501244

Study Title: Oral Palatability Study (Study No. TSR 30377; Report No. GT-157-TX-25) by Dietary Admixture in Mice

Methods: In this study, three groups (n = 5/sex/group) of CD-1 mice received Genz-112638, daily in powdered diet, at concentrations of 1, 3 and 5% in the feed. An additional group of five males and five females received the untreated food under the same experimental conditions and acted as a control group. The dietary admixtures were supplied *ad libitum*. Following the mortality observed at the beginning of the treatment period, surviving animals given 3% and 5% Genz-112638 were prematurely sacrificed on Days 4 and 5, respectively, and all other surviving animals (Groups 1 and 2) were sacrificed on Day 8. The animals were checked twice a day for mortality and morbidity. Clinical signs were recorded at least once a day. Body weight was recorded once at pretest, on Day 1 and then every 2 days until the end of the study. Food consumption was recorded every 2 days. At sacrifice, the animals were subjected to a full macroscopic examination.

Results:

5% Genz-112368 in the Feed: One male and one female were prematurely sacrificed on Day 3 or 4 and two males were found dead on Day 4. Clinical signs in moribund animals included thin appearance, prostration, dyspnea, piloerection, coldness to the touch and half closed eyes were observed as well as a marked body weight loss. No abnormal necropsy findings were noted. Following these events, it was decided to prematurely sacrifice the remaining animals from this group on Day 4, as they showed coldness to the touch, prostration, half closed eyes, piloerection and dyspnea.

3% Genz-112638 in the Feed: One female was found dead on Day 4 and all other animals from the same group were prematurely killed on Day 4 or 5, as they were in poor clinical conditions (thin appearance, prostration, hypoactivity, hunched posture, coldness to the touch and half closed eyes). At necropsy, blackish contents/deposits were observed in the stomach of three males and three females, in the ileum of one male, in the jejunum of one male and one female. Irregular color of the liver was noted in another female.

1% Genz-112638 in Feed: One male and one female were found dead on Day 8 and showed thin appearance, hunched posture, hypoactivity, moderate tremors and soiled urogenital region from Day 7. Two males and one female were prematurely sacrificed on Day 5 or 6, as similar clinical signs were previously noted. At necropsy, blackish contents/deposits were observed in the stomach, jejunum and/or cecum of four decedent animals.

Study Title: 4-Day Oral Palatability Study by Dietary Admixture (Study No. 30724 TSR; Report No. GT-157-TX-26) in Mice

Methods: In this study, four groups (n = 2/sex/group) of CD-1 mice received Genz-112638, daily in powdered diet, at 100, 250, 500 and 1000 mg/kg/day for 4 days. The dietary admixtures were supplied *ad libitum*. The animals were checked twice a day for mortality and morbidity. Clinical signs were recorded at least once a day. Body weight was recorded once before allocation of the animals into groups, on the first day of treatment and then every 2 days until the end of the study. Food consumption was recorded every 2 days. On completion of the treatment period, the animals were sacrificed for full macroscopic examination.

Results: There was no mortality or significant clinical signs. Mean body weight loss was observed in females at 100 mg/kg/day and in both males and females at 250, 500 and 1000 mg/kg/day, whereas the mean body weight of males at 100 mg/kg/day was not affected. Increased food consumption was observed with increased dose level. There was no significant necropsy finding. Based on these results, the dose-levels of 0, 50, 150 and 450 mg/kg/day were selected for a further 2-week palatability study in mice.

Study Title: 2-Week Oral Palatability Study (Study No. 30593 TSS; Report No. GT-157-TX-27) by Dietary Admixture in Mice

Methods: In this study, CD-1 mice received Genz-112638 at 61, 178 or 769 mg/kg/day (males) and 60, 215 or 646 mg/kg/day (females). An additional group of five males and five females received untreated food under the same experimental conditions and acted as a control group. The dietary admixtures were supplied *ad libitum*. Satellite animals (four males and four females in each treated group, and two males and two females in the control group) were added for toxicokinetics (TK). The animals were checked twice a day for mortality and morbidity. Clinical signs were recorded at least once a day. Body weight was recorded at pretest, on Day 1 of treatment and then on Days 3, 6, 8, 11 and 14. Food consumption was recorded on Days 1, 3, 6, 8 and 11. Blood samples were collected from satellite animals on Days 2 and 14 at designated time-points for TK analyses. The main animals were sacrificed on Day 15 for a full macroscopic examination. The satellite animals were sacrificed after the last blood sampling and were not subjected to necropsy.

Results: Treatment-related adverse effects were observed in both sexes at the high dose. These consisted of thin appearance, piloerection, soiled abdomen and/or urogenital region, hunched posture and/or pallor of extremities. These resulted in some premature sacrifices during the second week of the study, weight loss in males during the entire treatment period and in females from Day 1-11. No target organs were identified. The MTD was considered as 178 mg/kg/day in males and 215 mg/kg/day in females.

Study Title: 13-Week Oral (Diet) Toxicology Study in CD-1 Mice

Key Study Findings:

- In a 13-week oral (diet) toxicology study in CD-1 mice, animals were treated with Genz-112638 at 50, 150 and 350 mg/kg/day.
- There was no treatment-related mortality or clinical signs.
- The mean body weight gains (Day 0-91) in males were 89%, 79.8% and 21.7% of control at 50, 150 and 350 mg/kg/day, respectively. The mean body weight gains (Day 0-91) in females were 80%, 74% and 56% of control at 50, 150 and 350 mg/kg/day, respectively.
- Food consumption was decreased at all doses in both sexes.
- There were no significant treatment-related effects on hematology, serum chemistry, gross pathology, or histopathology.
- At 350 mg/kg/day, liver and adrenal gland weights were increased in males. At 150 mg/kg/day, liver weight was increased in males.
- Liver and adrenal could be the target organ.

Report No./Study No.: GT-157-TX-37/459308

Volume #, and Page #: Vol. 2, Page 75

Conducting Laboratory and Location: Not provided

Date of Study Initiation: Not provided

Date of Study Completion: Not provided

GLP Compliance: A statement of compliance was not included.

QA Report: yes ( ) no (X).

Drug, Lot #, and % Purity: Genz-112638. Lot and purity data not provided.

Formulation/Vehicle: Not provided

Methods: The sponsor did not provide details of the method.

Dosing:

Species/Strain: CD-1 mice

No./Sex/Group: 10/sex/group

Satellite Groups Used for Toxicokinetics: None

Age: Not provided

Weight: Not provided

Doses in Administered Units: 50, 150, and 350 mg/kg/day

Route, Form, and Volume: Oral (diet)

Study Design: Not provided

Observations and Times: Details were not provided

Results:

Mortality: There was no treatment-related mortality.

Clinical Signs: There were no significant treatment-related clinical signs.

Body Weights: The mean initial (Day 0) and final (Day 91) body weight of the control males was 34.4 and 48.7 g, respectively. The mean initial (Day 0) and final (Day 91) body weight of control females was 24.6 and 32.8 g, respectively. In males, final body weights were 99.8%, 94.9% and 81.5% of control at 50, 150 and 350 mg/kg/day, respectively. In females, final body weights were 95%, 93% and 92.4% of control at 50, 150, 350 mg/kg/day, respectively. The mean body weight gains (Day 0-91) in males were 89%, 79.8% and 21.7% of control at 50, 150 and 350 mg/kg/day, respectively (control = 14.3 g). The mean body weight gains (Day 0-91) in females were 80%, 74% and 56% of control at 50, 150 and 350 mg/kg/day, respectively (control = 8.5 g). The body weight data is shown below.

Mean Body Weight (g) of Males

Treatment (mg/kg/day)	Initial (Day 1)	Final (Day 91)	Weight Gain (g)	% of Control
0	34.4	48.7	14.3	100
50	35.9	48.6	12.7	89
150	34.8	46.1	11.4	79.8
300	36.6	39.7	3.1	21.7

Mean Body Weight (g) of Females

Treatment (mg/kg/day)	Initial (Day 1)	Final (Day 91)	Weight Gain (g)	% of Control
0	24.6	32.8	8.5	100
50	24.4	31.2	6.8	80
150	24.2	30.5	6.3	74
300	25.6	30.3	4.8	56

Food Consumption: The mean initial (Day 0) and final (Day 91) food consumption in control males were 6.7 and 6.2 g/animal/day, respectively. The mean initial and final food consumption in control females were 4.3 and 6.8 g/animal/day, respectively. The final food consumption in males were 98.4%, 100% and 90.3% of control at 50, 150 and 350 mg/kg/day, respectively. The final food consumption in females were 84%, 97% and 73.5% of control at 50, 150 and 350 mg/kg/day, respectively.

Organ Weights: In males, liver weight (absolute: 103%, 107% and 101% of control at 50, 150 and 350 mg/kg/day, respectively; relative: 102%, 112% and 123% of control at 50, 150 and 350 mg/kg/day, respectively) was increased at all doses compared to control. Also in males treated at 350 mg/kg/day, both absolute (107%, 98% and 139% of control at 50, 150 and 350 mg/kg/day, respectively) and relative adrenal gland weight (106%, 103% and 167% of control at 50, 150 and 350 mg/kg/day, respectively) was higher compared to the control. Significant increases in the brain, heart and testes weight were also noted in male animals treated at 350 mg/kg/day. The organ weight changes are shown in the following Table.

ORGAN	GROUP							
	0 mg/kg/day		50 mg/kg/day		150 mg/kg/day		350 mg/kg/day	
	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
LIVER								
Absolute (g)	2.33	1.69	2.40 (103%)*	1.66 (98%)	2.49 (107%)	1.66 (98%)	2.36 (101%)	1.56 (92%)
Relative (% BW)	5.04	5.38	5.13 (102%)	5.59 (104%)	5.67 (112%)	5.73 (107%)	6.18 (123%)	5.46 (101%)
ADRENAL								
Absolute (g)	0.0044	0.0095	0.0047 (107%)	0.0101 (106%)	0.0043 (98%)	0.0104 (109%)	0.0061 (139%)	0.0090 (95%)
Relative (%BW)	0.00962	0.03084	0.01021 (106%)	0.03414 (111%)	0.00995 (103%)	0.03721 (120%)	0.01607 (167%)	0.03233 (105%)
BRAIN								
Absolute (g)	0.49	0.50	0.51 (104%)	0.50 (100%)	0.51 (104%)	0.48 (96%)	0.50 (102%)	0.48 (96%)
Relative (% BW)	1.071	1.635	1.093 (102%)	1.686 (103%)	1.163 (108%)	1.690 (103%)	1.305 (122%)	1.694 (103%)
HEART								
Absolute (g)	0.23	0.16	0.24 (104%)	0.16 (100%)	0.23 (100%)	0.16 (100%)	0.22 (96%)	0.15 (94%0
Relative (% BW)	0.508	0.530	0.520 (102%)	0.550 (104%)	0.525 (103%)	0.544 (103%)	0.582 (115%)	0.531 (100%)
TESTES								
Absolute (g)	0.27	_	0.25 (92%0	_	0.26 (96%)	_	0.28 (104%)	_
Relative (%BW)	0.588	_	0.548 (93%)	_	0.601 (102%)	_	0.725 (123%)	_

*: Values in parentheses are % of control

Hematology: There were no significant treatment-related changes.

Serum Chemistry: No significant treatment-related changes were observed.

Gross Pathology: No treatment-related gross lesions were observed.

Histopathology: There were no significant treatment-related histopathology changes in any organ/tissue in either sex.

Toxicokinetics: Results were not provided. The sponsor stated that plasma levels of Genz-112638 ranged from 21 to 515 ng/mL at 350 mg/kg/day, 5 to 112 ng/mL at 150 mg/kg/day, and below the detection to 20 ng/mL at 50 mg/kg/day group.

Summary: In a 13-week oral (diet) toxicology study (b) (4) Study No. 459308; GT-157-TX-37) in CD-1 mice, animals received Genz-112638 at 50, 150 and 350 mg/kg/day. There was no treatment-related mortality or clinical signs. The mean body weight gains (Day 0-91) in males were 89%, 79.8% and 21.7% of control at 50, 150 and 350 mg/kg/day, respectively. The mean body weight gains (Day 0-91) in females were 80%, 74% and 56% of control at 50, 150 and 350 mg/kg/day, respectively. Food consumption was decreased at the high dose in males and at the low and high dose in females. There were no significant treatment-related effects on hematology, serum chemistry, gross pathology, or histopathology. At 350 mg/kg/day, liver and adrenal gland weights were increased in males. At 150 mg/kg/day, liver weight was increased in males.

Rat

A 28-Day Oral Gavage Toxicity Study with Genz-112638 (With a 14-Day Recovery) in Albino rat (GT-157-TX-5)

The review of the above study is incorporated below from the pharmacology review of IND 67,589 dated July 30, 2004 by Dr. Dylan Yao (DMEP).

Study title: A 28-day oral gavage toxicity study with Genz-112638 (with a 14-day recovery) in Albino rat

Key study findings:

- Rats were well tolerated with oral doses up to 100 mg/kg/day (50 mg/kg, b.i.d.).
- Body weight gain decrement was seen in MD and HD females (↓ 18 and 22%), but no effect in males.
- Moderate increases of ALT (↑ 63 and 50%, M/F) and K (↑ 10 and 10%, M/F) in HD males and females were recorded.
- There were no histopath findings correlated with observations in the HD animals.
- Linear TK with accumulation on Day 28.
- NOAEL=10 mg/kg/day.

Study no.: GT-157-TX-5
Volume #, and page #: Vol 2.11, page 123
Conducting laboratory and location: (b) (4) (b) (4)
Date of study initiation: May 8, 2003
GLP compliance: y
QA report: yes (x) no ( )
Drug, lot #, and % purity: Genz-112638, lot #909246, purity 99%

Methods

Doses: 0, 10, 30, 100 mg/kg/day (0, 5, 15, 50 mg/kg b.i.d.)
Species/strain: rat/SD
Number/sex/group or time point (main study): 10/s/g
Route, formulation, volume, and infusion rate: oral gavage with weekly prepared solution formulation (water added to white to off-white powder) at dose volume of 5 mL/kg, twice daily with 6 hrs apart.
Satellite groups used for toxicokinetics or recovery: 10/s/g (TK) and 5/s/g (recovery) with same dose levels treated concurrently
Age: ~6 weeks of age at the start of dosing
Weight: 167 to 215 g for males and 139 to 172 g for females
Sampling times: terminated and necropsied on Day 28
Unique study design or methodology: n/a

Observation and Times:

Clinical signs: twice daily
Body weights: once pretest and then weekly
Food consumption: once pretreatment and then weekly
Ophthalmoscopy: once pretreatment and once at the end of Week 4
EKG: n/a
Hematoloy: collected on Day 28 and the end of recovery period
Clinical chemistry: collected on Day 28 and the end of recovery period
Urinalysis: collected on Day 28 and the end of recovery period
Gross pathology: at necropsy
Organ weights: see histopath table
Histopathology: C and HD animals were processed for full histopath examination while LD and MD groups were checked for tissues showing treatment related findings and gross lesions only.

Adequate Battery: yes (x), no ( )
Peer review: n/s

Results:

Mortality: no deaths during the treatment and recovery period

Clinical signs: salivation in most of the animals at HD commenced on Day 1 till the end of the treatment, occasionally seen in LD and MD groups.

Body weights: dose-related decrease in body weight gain was observed in females at all doses, and HD F gained similar weight during the recovery period compared to controls; weight effect was unremarkable in males.

Dose (m/k/d)	0		10		30		100	
	M	F	M	F	M	F	M	F
Wt gain @wk 4 (g)	171.9 -	88.5 -	172.5 ↓ 0%	76.8 ↓ 13%	161.8 ↓ 6%	72.6 ↓ 18%*	167.8 ↓ 2%	69.2 ↓ 22%**

*, $p<0.05$; **, $p<0.01$

Food consumption: no effect in all males and LD and MD females; a slightly non-ss lower FC was noted in HD females.

Ophthalmoscopy: unremarkable

Hematology: ss ↑ PLT counts (25%) and PT (7%) in HD F

Clinical chemistry: changes were noted in males and females at HD 100 mg/kg/day, and they were reversed after recovery period:

ALT ss ↑ 63 and 50%, HD, M & F;
K ss ↑ 10 and 10%, HD M & F;
Cl non-ss ↑ 3 and 2.2%, HD M & F;
PHOS non-ss ↑ 16 and 16%, HD M & F;
Na non-ss ↑ 1 and 1.5%, HD M & F.

Urinalysis: unremarkable

Gross pathology: no findings

Organ weights: unremarkable

Histopathology: no findings

Toxicokinetics: On Days 1 and 28, blood samples were collected at 15 and 30 min, and 1, 2, 4, and 6 hrs postdose. Plasma concentrations of Genz-99067 (the free-base form of Genz-112638) were assayed, the lower limit of quantification was 5 ng/mL. Terminal $t_{1/2}$ value ranged from 0.740 to 2.09 hrs, with no apparent relationship to dose level or gender. Tmax was ~0.5 hr postdose. Exposure increased with dose, and was higher on Day 28 than Day 1 suggesting accumulation.

Day 1

Gender	Group No.	Dose Level (mg/kg/day)	Dose Level (mg/kg/bid)	C_{max} ng/mL	$AUC_{0\text{-tlast}}$ ng•h/mL	Dose Proportionality	C_{max} Ratio	$AUC_{0\text{-tlast}}$ Ratio
Males	2	10	5	12.3	NC	1	1	NC
	3	30	15	44.1	98.2	3	3.59	1
	4	100	50	467	789	10	38.1	8.03
Females	2	10	5	16.0	24.6	1	1	1
	3	30	15	72.3	162	3	4.51	6.59
	4	100	50	454	1134	10	28.3	46.0

Day 28

Gender	Group No.	Dose Level (mg/kg/day)	Dose Level (mg/kg/bid)	C_{max} ng/mL	$AUC_{0\text{-tlast}}$ ng•h/mL	Dose Proportionality	C_{max} Ratio	$AUC_{0\text{-tlast}}$ Ratio
Males	2	10	5	24.6	32.5	1	1	1
	3	30	15	168	173	3	6.86	5.34
	4	100	50	722	1394	10	29.4	42.9
Females	2	10	5	232	320	1	1	1
	3	30	15	49.4	89.8	3	0.213	0.281
	4	100	50	780	1972	10	3.36	6.16

NC Not Calculated.

Other: n/a

28-Day Oral Toxicology Study Report in Mature Male Rats with Twice Daily Dosing (32267 TSR; GT-157-TX-28)

The review of the above study is incorporated below from the pharmacology review of IND 67,589 dated December 18, 2008 by Dr. Tamal K. Chakraborti (DGIEP).

Study Title: 28-Day Toxicology Study in Mature Male Rats with Twice Daily Dosing
(b) (4) study No. 32267 TSR, Report No: GT-157-TX-28)

The objective of this study was to evaluate specific effects of Genz-112638 on male reproductive organs and spermatogenesis. This study is not relevant for the dose selection of the proposed carcinogenicity study and was not reviewed in detail. However, a brief summary of findings is presented here. In a 28-day oral toxicology study (Study No. 32267 TSR, Report No. GT-157-TX-28) in male rats with a 9-week recovery period, animals were administered Genz-112638 by oral gavage twice daily at 15 or 50 mg/kg BID (30 or 100 mg/kg/day) for 4 weeks or at 100 mg/kg BID (200 mg/kg/day) for 2 weeks. At 200 mg/kg/day, mortality was observed and the dosing was terminated after 2 weeks of treatment due to severe toxicity (two animals found dead and three sacrificed prematurely), marked body weight losses, reduced food consumption and clinical signs. Electrolytes and liver enzymes as well as sperm motility and morphology were affected at 200 mg/kg/day. Sperm morphology remained affected until 14-weeks treatment-free recovery period. Gross pathology examinations revealed grey foci on the forestomach mucosa, small thymus and spleen. Microscopic examinations revealed germ cell necrosis in the testis, sloughed cells in the epididymis, acute inflammation in the coagulating glands, epithelial

hyperplasia in the forestomach and severe atrophy of thymus and spleen. After 3-weeks treatment-free recovery period, there was a decrease in the number of elongated spermatids along with sloughed cells in the epididymis. Epithelial cell hyperplasia in the coagulating glands was still present. After 6-weeks treatment-free recovery period, two rats still had lesions in the testis and epididymis, and three rats still had hyperplasia of the epithelium of the coagulating glands, but all effects showed reversibility and were not observed after 9-weeks treatment-free period. At 100 mg/kg/day, a lower mean body weight gain, lower food consumption and signs of ptyalism were observed. In addition, WBC, RBC counts and hemoglobin levels were affected as were electrolytes and liver enzymes. There were no significant treatment-related effects on sperm counts, motility or morphology. Microscopic examinations revealed bilateral atrophy of the coagulating glands. After 3-weeks treatment-free recovery period, atrophy of the coagulating glands was still present and sloughed cells in the epididymis were also observed. No abnormalities were observed after 6-weeks treatment-free recovery period. At 30 mg/kg/day, there were no effects on body weight gain or food consumption and no clinical signs. Hemoglobin concentration, eosinophil counts and other serum chemistry parameters were affected but returned to control levels at 3-weeks recovery period. There were no significant treatment-related effects on sperm counts, motility or morphology. There were no effects on organ weights, and no significant macroscopic or microscopic abnormalities were observed. The dose-level of 200 mg/kg/day exceeded the MTD and the dose level of 100 mg/kg/day resulted in lower body weight gain and food consumption. Complete reversibility was observed at both dose-levels for all effects after 9-weeks treatment-free recovery period. The 100 mg/kg/day appeared to be a tolerated dose.

26-Week Oral Gavage Study in Rats with a 8-Week Recovery Period (GT-157-TX-17)

The review of the above study is incorporated below from the pharmacology review of IND 67,589 dated December 18, 2008 by Dr. Tamal K. Chakraborti (DGIEP).

Study Title: 26-Week Oral Gavage Study in Rats with a 8-Week Recovery Period

Key Study Findings: In a 26-week oral (gavage) toxicology study in SD rats, animals were treated with Genz-112638 at 5, 15 and 50 mg/kg/day. A total of three animals died (one male and one female at 50 mg/kg/day and one male at 15 mg/kg/day) and deaths of males were attributed to gavage accident. The cause of death of one female was not determined. The sponsor stated that the death of this female did not appear to be treatment-related. However, the sponsor did not explain why this death of the female rat was not considered treatment-related. There were no significant treatment-related effects on body weights or food consumption, hematology, serum chemistry, urinalysis, gross pathology, organ weights or histopathology. The target organ could not be identified in the absence of any significant histopathology findings in any organ or tissue. The MTD appeared to be higher than 50 mg/kg/day in the absence of any reduction of body weight gain or histopathology findings.

Report No./Study No.: GT-157-TX-17/27126

Volume #, and Page #: Volume 2, 162

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: February 20, 2004

Date of Study Completion: January 20, 2006

GLP Compliance: A statement of compliance was included.

QA Report: yes (X) no ( ).

Drug, Lot #, and % Purity: Genz-112638, Lot No. P0054, 99.8%

Formulation/Vehicle: Purified water

Methods:

Dosing:

Species/Strain: SD rats

No./Sex/Group: 14/sex/group

Satellite Groups Used for Toxicokinetics: Yes (6/sex/dose)

Age: 6 weeks old

Weight: Males: 201-244 g; Females: 149-196 g

Doses in Administered Units: 5, 15, and 50 mg/kg/day

Route, Form, and Volume: Oral gavage, solution, and 5 mL/kg

Study Design: The study design is shown in the following Table (from page 17 of the study report).

Group		Number of animals	Dose-level (mg/kg/day)	Animal numbers
1	Recovery	6 males	0	E21901 to E21906
		6 females		E22101 to E22106
	Principal	14 males		E21907 to E21920
		14 females		E22107 to E22120
2	Principal	14 males	5	E21921 to E21934
		14 females		E22121 to E22134
	Satellite	6 males		E21935 to E21940
		6 females		E22135 to E22140
3	Principal	14 males	15	E21941 to E21954
		14 females		E22141 to E22154
	Satellite	6 males		E21955 to E21960
		6 females		E22155 to E22160
4	Recovery	6 males	50	E21961 to E21966
		6 females		E22161 to E22166
	Principal	14 males		E21967 to E21980
		14 females		E22167 to E22180
	Satellite	6 males		E21981 to E21986
		6 females		E22181 to E22186

Observations and Times:

Clinical Signs: Clinical signs were observed once daily.

Mortality: Mortality was observed twice daily.

Body Weights: Body weights were recorded once a week during first 13 weeks and then once every 4 weeks.

Food Consumption: Food consumption was recorded once a week during first 13 weeks and then once every 4 weeks.

Ophthalmoscopy: Ophthalmological examinations were performed on 10 animals/sex/dose at the beginning of the treatment period and on 10 animals/sex from the control and high-dose groups on one occasion at the end of the treatment period.

Hematology: Hematology was conducted at necropsy.

Clinical Chemistry: Serum chemistry was conducted at necropsy.

Urinalysis: Urinalysis was performed for 10 animals/sex from each group at the end of the treatment period. These examinations were not carried out at the end of the treatment-free period since no treatment-related changes were observed at the end of the treatment period.

Gross Pathology: Gross pathology was conducted at necropsy.

Organs Weighed: The following organs were weighed from all main study animals as listed in the following Table (from page 26 of the study report).

TISSUE PROCEDURE TABLE

Organs	Organ weights	Preservation of tissues	Microscopic examination
Macroscopic lesions		X	X
Adrenals	X	X	X
Aorta		X	X
Brain (including medulla/pons cerebellar and cerebral cortex)	X	X	X
Cecum		X	X
Colon		X	X
Duodenum		X	X
Epididymides	X	X	X
Esophagus		X	X
Eyes with Harderian glands		X	X
Femoral bone with articulation		X	X
Heart	X	X	X
Ileum (with Peyers patches)		X	X
Jejunum		X	X
Kidneys	X	X	X
Larynx		X	X
Liver	X	X	X
Lungs with bronchi		X	X
Lymph nodes (mandibular and mesenteric)		X	X
Mammary gland area		X	X
Optic nerves		X	X
Ovaries (with oviducts)	X	X	X
Pancreas		X	X
Pituitary gland		X	X
Prostate		X	X
Rectum		X	X
Salivary glands (sublingual and submandibular)		X	X
Sciatic nerve		X	X
Seminal vesicles		X	X
Skeletal muscle		X	X
Skin		X	X
Spinal cord (cervical, thoracic and lumbar)		X	X
Spleen	X	X	X
Sternum with bone marrow		X	X
Stomach with forestomach		X	X
Testes	X	X	X
Thymus	X	X	X
Thyroids with parathyroids	X	X	X
Tongue		X	X
Trachea		X	X
Ureters		X	X
Urinary bladder		X	X
Uterus (horns and cervix)		X	X
Vagina		X	X

Histopathology: All tissues shown in the above Table (from page 27 of the study report) were examined for histopathology from the control and the high dose group.

Toxicokinetics: Blood samples were collected from TK animals on Day 1 and at the end of the treatment period at 0.25, 0.5 and 4 hours post-dose.

Results:

Mortality: One male at 50 mg/kg/day (animal No. E2l974) was found dead on Day 119 (Week 17). Ptyalism was observed from Day 8. At the macroscopic post-mortem examination, foamy contents were found in the trachea and the lungs, which were reddish in color. The mesenteric lymph nodes were reddish in color and the mandibular lymph nodes were enlarged. The colon was distended with gas and the right kidney showed dilated pelvis. In addition, cryptorchidism of both testes was observed. At microscopic examination, slight alveolar edema was noted. According to the sponsor, the cause of death was accidental, probably due to gavage accident. One male at 15 mg/kg/day (animal No. E21948) was found dead on Day 141 (Week 21). Areas of hair loss on its forelimbs were observed from Week 9. At the macroscopic post-mortem examination, the major findings consisted of enlarged liver, reddish coloration of the thymus and alopecia on the right foreleg. At microscopic examination, slight hemorrhage of the spinal cord was observed. The cause of death was attributed to gavage accident. One female at 50 mg/kg/day (animal no. E22l77) was found dead on Day 171 (Week 25). Ptyalism was observed from Week 2. No macroscopic findings were observed at necropsy. At microscopic examination, the major findings consisted of slightly vacuolated cortical cells in the adrenals and moderate lipoid tissue in the femoral bone. The cause of death of this animal was not determined. However, the sponsor stated that the death of this female did not appear to be treatment-related. Overall, as per the sponsor, two deaths (males) were attributed to gavage accident and the cause of death for the other female although unknown but was considered not related to the treatment.

Clinical Signs: During the treatment period, ptyalism was observed in all animals at 50 mg/kg/day, and chromodacryorrhea was noted in one high dose male in Weeks 4 to 9. The sponsor stated that since ptyalism was generally noted in rats when the test article is administered by gavage, it was not considered to be a treatment-related adverse effect.

Body Weights: The mean initial (Week 1) and final (Week 26) body weight of the control males was 224 and 593 g, respectively. The mean initial and final body weight of control females was 171 and 349 g, respectively. In males, final body weights were 103, 105 and 104% of control at 5, 15 and 50 mg/kg/day, respectively. In females, final body weights were 99%, 94% and 98% of control at 50, 250, 1000 mg/kg/day, respectively. Overall, there were no significant treatment-related effects.

During the treatment period, when compared to controls, a slightly higher mean body weight gain was observed in treated males, without a dose-relationship. A slightly lower mean body weight gain was noted in treated females during the period of weeks 14 to 26, without a dose-relationship and especially in weeks 22 to 26. Overall, during the study, similar mean body weight gains were noted in control and treated animals. During the treatment-free period, the mean body weight gain, in previously

treated males, was similar to that of the controls, whereas it was moderately higher than controls in previously treated females. This effect on the body weight was observed with an opposite trend in both sexes, was slight and thus was not considered to be related to treatment with the test item. The body weight gain data is shown in the following Table (from page 32 of the study report).

Mean body weight gain (expressed in g)

Sex	Male				Female			
Dose-level (mg/kg/day)	0	5	15	50	0	5	15	50
Treatment period								
. Weeks 1 to 14	+301	+319	+325	+324	+141	+142	+135	+140
% compared to controls		*+6*	*+8*	*+8*		*0*	*-4*	*-1*
. Weeks 14 to 26	+68	+69	+73	+70	+37	+31	+24	+30
% compared to controls		*+1*	*+7*	*+3*		*-16*	*-35*	*-19*
. Weeks 1 to 26	+369	+388	+398	+394	+178	+173	+159	+170
% compared to controls		*+5*	*+8*	*+7*		*-3*	*-11*	*-4*
Treatment-free period								
. Weeks 26 to 34	+39			+42	+20			+43
% compared to controls				*+8*				*+115*

Food Consumption: The mean initial (Week 1) and final (Week 25) food consumption in control males were 25 and 24.6 g/animal/day, respectively. The mean initial and final food consumption in control females were 16.8 and 18.3 g/animal/day, respectively. There were no significant meaningful treatment-related changes.

Ophthalmology: No significant treatment-related changes were observed.

Organ Weights: No significant treatment-related changes were observed.

Hematology: There were no significant and consistent treatment-related changes.

Serum Chemistry: No significant treatment-related changes were observed.

Urinalysis: No significant treatment-related changes were observed.

Gross Pathology: No treatment-related gross lesions were noted in any animal of either sex in any dose group during necropsy.

Histopathology: There were no significant treatment-related histopathology changes in any organ/tissue in either sex.

Toxicokinetics: The Cmax reached at 0.25-0.5 hours. The systemic exposure (as measured by the Cmax and AUC_{0-t}) increased with the dose-level in a supra-linear manner on Day 1 and in

Week 26. There appears to be drug accumulation upon repeated dosing, as the exposure in Week 26 was 1.6-5.4 and 1.6-3.6-fold higher than that on Day 1, using both Cmax and AUC, respectively. There was an apparent gender difference in exposure at Week 26, as the systemic exposure was higher in females than that in males at mid- and high dose. The following Tables (from page 35 and 36 of the study report) show the TK data on Day 1 and Week 26.

Table 1a: Toxicokinetic parameters of Genz-112638 on day 1 after oral administration to Sprague-Dawley rats

Sampling period	Kinetic parameter	Unit	Group/dose-level (mg/kg/day) and sex					
			2/5		3/15		4/50	
			Male	Female	Male	Female	Male	Female
Day 1	C_{max}	(ng/mL)	28.3	37.9	250	147	635	1054
	t_{max}	(h)	0.5	0.5	0.5	0.5	0.5	0.25
	$AUC_{(0-t)}$	(ng*h/mL)	22.4	22.9	271	282	1067	1754
	Ratio C_{max}/dose		5.7	7.6	16.7	9.8	12.7	21.1
	Ratio $AUC_{(0-t)}$/dose		4.5	4.6	18.1	18.8	21.3	35.1

Table 1b: Toxicokinetic parameters of Genz-112638 in week 26 after oral administration to Sprague-Dawley rats

Sampling period	Kinetic parameter	Unit	Group/dose-level (mg/kg/day) and sex					
			2/5		3/15		4/50	
			Male	Female	Male	Female	Male	Female
Week 26	C_{max}	(ng/mL)	62.9	140	402	790	1268	1850
	t_{max}	(h)	0.25	0.25	0.5	0.25	0.5	0.25
	$AUC_{(0-t)}$	(ng*h/mL)	39.4	54.7	431	1023	2424	3815
	Ratio C_{max}/dose		12.6	28	26.8	52.7	25.4	37
	Ratio $AUC_{(0-t)}$/dose		7.9	10.9	28.7	68.2	48.5	76.3

Summary: In a 26-week oral (gavage) toxicology study in SD rats, animals were treated with Genz-112638 at 5, 15 and 50 mg/kg/day. A total of three animals died (one male and one female at 50 mg/kg/day and one male at 15 mg/kg/day) and deaths of males were attributed to gavage accident. The cause of death of one female was not determined. The sponsor stated that the death of this female did not appear to be treatment-related. There were no significant treatment-related effects on body weights or food consumption, hematology, serum chemistry, urinalysis,

gross pathology, organ weights or histopathology. The target organ could not be identified in the absence of any significant histopathology findings in any organ or tissue. The MTD appeared to be higher than 50 mg/kg/day in the absence of any significant treatment-related toxicity or histopathology findings.

Study title: Oral (Gavage) Administration 10-Week Toxicity Study in the Juvenile Rat with an Assessment of Fertility

Study no.:	GT-157-TX-48
Study report location:	EDR, Section 4.2.3.5.4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 30, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Genz-112638, S1553, 99.85%

Key Study Findings:

- In an oral gavage study in juvenile rats, animals were treated with Genz-112638 at 5, 15 and 25 mg/kg BID for 10 weeks commencing at postnatal day 22 (PND 22).
- Mortalities were observed at all doses including control and deaths were mainly attributed to gavage errors.
- Gross pathology findings included large mandibular lymph nodes with increased incidence at 25 mg/kg BID in females.
- In females, liver weights were increased at the high dose.
- Histopathological changes were seen in the lymph node (increased incidence/severity of lymphoid hyperplasia in the mandibular lymph nodes at 25 mg/kg BID) and eye (minimal grade unilateral neuropathy in the optic nerve of two animals at 25 mg/kg BID).
- The target organ could be the lymph node and eye based on the histopathology findings.
- The NOAEL was considered as 15 mg/kg BID.

Methods:

Doses:	5, 15, 25 mg/kg BID or 10, 30 and 50 mg/kg/day
Frequency of dosing:	BID
Route of administration:	Oral (Gavage)
Dose volume:	5 mL/kg
Formulation/Vehicle:	Deionized water
Species/Strain:	SD rats
Number/Sex/Group:	10/sex/dose
Age:	PND 21
Weight:	Male: 57.2 g; Female: 54.3 g
Satellite groups:	Toxicokinetics, Recovery
Study design:	Study design is shown below
Deviation from study protocol:	There were no major deviations from the protocol. Minor deviations did not affect the integrity or outcome of the study.

The following tables show the animal numbers (from page 18 of the report) and study design (from page 16 of the report).

Group number	Animal identification numbers							
	Toxicity Treatment		Treatment-free		Fertility		Toxicokinetics#	
	Male	Female	Male	Female	Male	Female	Male	Female
1	1-10	61-70	11-20	71-80	121-140	201-220	-	-
2	21-30	81-90	-	-	141-160	221-240	281-283	290-292
3	31-40	91-100	-	-	161-180	241-260	284-286	293-295
4	41-50	101-110	51-60	111-120	181-200	261-280	287-289	296-298

for toxicokinetic purposes only, no other experimental observation data from these animals has been reported

Group number	Group description	Dose level (mg/kg/BID)	Number of animals							
			Toxicity Treatment		Treatment-free		Fertility		Toxicokinetics	
			Male	Female	Male	Female	Male	Female	Male	Female
1	Control	0	10	10	10	10	20	20	-	-
2	Low	5	10	10	-	-	20	20	3	3
3	Intermediate	15	10	10	-	-	20	20	3	3
4	High I	25	10	10	10	10	20	20	3	3

Basis of dose selection: Doses were selected based on the results of a 28-day oral (gavage) dose range-finding study (GT-157-TX-47) in juvenile rats (n = 10/sex/dose, age = PND 22) at 15, 30 and 50 mg/kg BID (10 mL/kg). Test article was administered

from PND 22 to PND 49. Two animals at 30 mg/kg BID and five animals at 50 mg/kg BID died immediately after dosing on PND 22 or 23 and were replaced. One female at 30 mg/kg BID and one female at 50 mg/kg BID died after convulsive episodes immediately after dosing on PND 33 or 35 and two other animals at 50 mg/kg BID were found dead after dosing on PND 27 or 28. There were no macroscopic findings to indicate cause of death. In surviving animals, there were no significant treatment-related effects on hematology, serum chemistry, and organ weights. Histopathology was not conducted. Results of toxicokinetics showed exposure at all doses with values being similar to those seen in adult animals and above the mean exposure at steady state in Phase 2 (145 ng.hr/mL) clinical trial. Based on the results of this dose ranging study, the doses for the current study were selected at 5, 15 and 25 mg/kg BID.

Observations:

Mortality: All animals were examined twice daily for mortality.

Clinical Signs: All animals were examined twice daily for clinical signs.

Body Weights: Body weight was recorded on PND 14, 21 and at pre-dose on PND 22 and then twice weekly until necropsy.

Food Consumption: Food consumption was recorded twice weekly.

Long bone maturation: The long bones of all toxicity and recovery animals were measured weekly from PND 21.

Sexual maturation: Vaginal opening and balano-preputial separation (all males) were assessed daily from PND 30 and PND 40, respectively, until development was complete.

Functional observational battery (FOB): During Week 6, all toxicity animals were observed and evaluated for motor activity (control and high dose), learning and behavior (maze test, control and high dose) and for the following (from page 20 of the report) parameters:

posture convulsion
activity excessiv e vocalisation
gait arousal upon opening cage
tremor

Each toxicity animal was removed from its cage and observed for:

ease of removal lacrimation
ease of handling lacrimation type
excessive vocalisation salivation
tremor respiration
convulsion piloerection
palpebral closure appearance of fur
exophthalmus other

Sensory function: Auditory startle responses of all control and high dose toxicity animals were assessed on PND 57 (Week 6).

Hematology: Hematology was conducted at Week 10.

Clinical Chemistry: Clinical chemistry was conducted at Week 10.

Urinalysis: Urinalysis was conducted at Week 10.

Immune function analysis**:** Blood samples were collected for the assessment of lymphocyte subsets by flow cytometry using the following markers: CD3 (T-cells), CD4 (T-helper cells), CD8 (T-suppressor cells), CD20 (B-cells), and CD161(NK cells).

GL-1 biomarker: Blood samples were collected at Week 10 for analysis of GL-1.

Gross Pathology: Gross pathology was conducted at necropsy.

Organ Weights: The following (from page 27-28 of the report) organs were weighed.

adrenals (E) (W)	optic nerves (E)
animal identification	ovaries (E) (F) (W)
aorta (E)	oviducts (E)
bone marrow smear (femur)[1]	pancreas (E)
Brain[5] (E) (W)	Peyer's patch (E)
caecum (E)	pituitary (E) (F) (W)
colon (E)	popliteal lymph nodes (E)
duodenum (E)	prostate (E) (F) (W)
eyes[2]	rectum (E)
femur with bone marrow and stifle joint (E)	salivary glands – mandibular, sublingual, parotid
gross lesions (E)	sciatic nerves[5] (E)
Harderian glands[3]	seminal vesicles (E) (F) (W)
head (not processed)	skin and subcutaneous tissue
heart (E) (W)	spinal cord cervical[5] (E)
ileum (E)	spinal cord lumbar[5] (E)
jejunum (E)	spinal cord thoracic[5] (E)

kidney (E) (W)	spleen (E) (W)
lacrimal glands[3]	sternum with bone marrow (E)
larynx stomach	(E)
liver (E) (W)	testes and epididymides[4] (E) (F) (W)
lungs with mainstem bronchi and bronchioles (E)	thymus (E) (W)
mammary	thyroids with parathyroids (E) (W)
mandibular lymph nodes (E)	tongue
mesenteric lymph nodes (E)	trachea (E)
muscle (quadriceps)	trachea bifurcation
nares	urinary bladder (E)
nasal cavity	ureters (E)
nasopharynx	uterus including cervix (E) (F) (W)
oesophagus (E)	vagina (E) (F)
Zy	mbal glands[3]

Fixative = 10% neutral buffered formalin except where indicated
1 tissue taken into methanol
2 tissue taken into Davidson's fixative
3 preserved with head (*in situ*)
4 tissue taken into Bouin's fixative and processed to at least the block stage
5 additional paraffin sections of brain, cervical, thoracic and lumbar regions of the spinal cord and of the sciatic nerve were cut at a nominal thickness of 5um and stained with Luxol Fast Blue for myelin. All sections were evaluated by the study pathologist
(E) all tissues processed and examined microscopically
(F) tissues preserved fertility animals only
Bone tissue designated for histopathological examination was decalcified using Kristenson's fluid

Histopathology: The tissues listed in the above table from the control and high dose main study and recovery animals were examined for histopathology.

Seminology: Sperm counts and sperm viability were assessed from one epididymis from the each fertility male from the control and high dose groups.

Special Evaluation (Fertility): At PND 91 (Week 11), one male was paired with one female from the same group for up to 15 days. Fertility females were sacrificed on Day 13 of gestation (GD 13) and were examined macroscopically. The ovaries and uteri were removed and examined for the following: macroscopy, pregnancy status, number of corpora lutea, number of implantations, live fetuses, resorptions (early and late) and dead fetuses.

Toxicokinetics: Blood samples were collected from TK animals at necropsy on PND 22 at 1 hour post-dose.

Dosing Solution Analysis: Dosing formulations were analyzed on Weeks 1 and 10 for the analysis of concentrations. The target range for the preparation of the formulations was 90 to 110% of nominal.

Results:

Mortality: There were eight deaths in total during the study. One Male (# 36) at 15 mg/kg BID was found dead on PND 88. There were no clinical observations and

necropsy and histopathology did not reveal the cause of death. One female (# 87) at 5 mg/kg BID was found dead on PND 30. There were no adverse clinical observations recorded prior to death. The cause of death could not be determined. However, necropsy showed a clear cyst with surrounding black area in the left lung lobe. Per the Applicant, due to the isolated incidences of these deaths and lack of any dose relationship or marked toxicity at any dose-level, above deaths were considered incidental. Male numbers 55 (toxicity) and 183 (fertility) at 25 mg/kg BID were found dead on PND 32. In addition female number 272 (fertility) at the high dose was found dead on PND 35. Necropsy findings for all three animals included abnormal contents in the trachea. Deaths were considered to be due to gavage error. One control fertility male (# 130) was sacrificed on PND 50 due to poor condition following clinical observations of hunched, thin, uncoordinated movements, pale and a swollen abdomen. Necropsy showed clear fluid in the abdomen, which suggested accidental dosing trauma. Female number 247 (fertility) at 15 mg/kg BID was found dead approximately 15 minutes post-dose on PND 23. Male number 60 (treatment-free) at 25 mg/kg BID was found dead approximately 30 minutes post-dose on PND 25. Due to the time of death, these were considered likely to be due to accidental dosing trauma. Both these animals were replaced. The following table shows the mortality.

Animal #	Dose (mg/kg BID)	Sex	Day of Death /Sacrificed	Cause
36	15	M	PND 88	Unknown
87	5	F	PND 30	Unknown
55	25	M	PND 32	Likely gavage error
183	25	M	PND 32	Likely gavage error
272	25	F	PND 35	Likely gavage error
130	0	M	PND 50	Likely gavage error
247	15	F	PND 23	Likely gavage error
60	25	M	PND 25	Likely gavage error

Clinical Signs: There were no significant treatment-related clinical signs in surviving animals.

Body Weights: The mean initial (PND 22) and final (PND 92) weights of control males were 57.2 and 475.9 g, respectively. The mean initial (PND 22) and final (PND 92) weights of control females were 54.3 and 278.5 g, respectively. There were no significant treatment related effects.

Food Consumption: The mean initial (Week 1) and final (Week 10) food consumption of control males was 12.5 and 29.1 g/animal/day, respectively. The mean initial (Week 1)

Reference ID: 3501244

and final (Week 10) food consumption of control females was 12.4 and 20.3 g/animal/day, respectively. There were no significant treatment related effects.

Long bone maturation: There were no significant treatment related effects.

Sexual maturation: There were no significant treatment related effects.

Functional observational battery (FOB): There were no significant treatment related effects on FOB parameters.

Sensory function: There were no significant treatment related effects.

Hematology: In females, there was a slight increase in mean red blood cell (RBC) count at 15 or 25 mg/kg BID. In females at 25 mg/kg BID, there was also a slight increase in mean white blood cell (WBC) count. In males at 15 or 25 mg/kg BID, there was a slight decrease in mean platelet volume and a significant decrease in platelet distribution width at 25 mg/kg BID, when compared to the control.

Clinical Chemistry: There was a significant decrease in potassium concentration in males and females at 25 mg/kg BID compared to control. In both sexes at 15 and 25 mg/kg BID, there was an increase in mean glucose concentration compared to controls. In males at 25 mg/kg BID, there was a significant increase in urea and creatinine concentration compared to the control. In males at 15 and 25 mg/kg BID, there was also a significant increase in inorganic phosphorous concentration compared to the controls.

Urinalysis: In males at 15 and 25 mg/kg BID, there was a significant increase in mean urine volume and a slight decrease in specific gravity when compared to the respective controls.

Immune function analysis**:** There were no significant treatment-related effects on the immune system.

GL-1 biomarker: The following table (from page 806 of the report) shows the mean values of GL-1. Females had higher GL-1 levels than that of males. There was no apparent treatment-related decrease in GL-1 in either sex. The Applicant stated that the high degree of biological variation in the plasma GL-1 in this study likely obscured a dose related lowering of GL-1 in the plasma.

Table 2. Mean, STDEV and CV% for the male and female rat groups

		GL1 MEAN	STDEV	CV%
Male	control	0.96	0.26	27%
	5 mg/kgBID	0.89	0.26	30%
	15 mg/kgBID	0.92	0.32	35%
	25 mg/kgBID	0.91	0.26	29%
Female	control	2.83	0.69	24%
	5 mg/kgBID	2.91	0.65	23%
	15 mg/kgBID	3.56	1.11	31%
	25 mg/kgBID	3.16	0.71	23%

Gross Pathology: In females, large mandibular lymph nodes were observed with increased incidence at 25 mg/kg BID.

Organ Weights: In females, there was a significant increase in mean liver weight at 25 mg/kg BID compared to the control. At the end of the recovery period, female liver weight at 25 mg/kg BID was comparable to the control.

Histopathology: In the mandibular lymph nodes, there was an increased incidence/severity in lymphoid hyperplasia at 25 mg/kg BID. Lymphoid hyperplasia was characterized by increased numbers of enlarged germinal centers with plasmacytosis in the medullary cords. Additionally, histiocytic foci were recorded in the mandibular lymph node in treated females and males with increased incidence. These were characterized by discrete aggregates of plump eosinophilic macrophages. The following table (from page 37 of the report) shows the microscopic findings.

Group incidence of selected microscopic findings

		Ma les				Females			
		1M	2M	3M	4M	1F	2F	3F	4F
Tissue and finding	Level (mg/kg/BID)	0	5	15	25	0	5	15	25
Mandibular node	No. examined:	10	10	9	10	10	9	10	10
Lymphoid hyperplasia	Grade -	1	0	1	1	6	4	3	0
	1	3	3	3	2	3	5	3	3
	2	3	4	2	3	1	0	4	4
	3	3	2	3	2	0	0	0	3
	4	0	1	0	2	0	0	0	0
Histiocytic foci	Grade -	8	7	7	4	10	8	9	7
	1	2	3	2	6	0	1	1	3

Key: "-" = finding not present, 1 = minimal, 2 = slight, 3 = moderate, 4 = moderately severe

In addition, a minimal grade unilateral neuropathy was observed in the optic nerve of two animals at 25 mg/kg BID (Animal Numbers 50M and 111F). Neuropathy was

characterized by irregular loss of cells in the inner nuclear layer with retinal folding, disorganization/disruption of normal architecture with irregular hypercellularity and a phagocytic reaction (very prominent in Animal Number 50M). In Animal Number 111F, there was also a significant reduction in the number of nerve fibers. Per the Applicant, these findings were unilateral and were not considered treatment related. However, since these were seen at the high dose in both sexes and these were not seen in any control animals, relation to the treatment may not be ruled out.

Seminology: There were no significant treatment related effects.

Special Evaluation (Fertility): In all groups, the median pre-coital time was 2.0 to 3.0 days and there were no adverse effects of treatment on the mating, fertility or fecundity indices. There was no significant adverse effect of treatment on the mean number of implantations, pre- or post-implantation loss or the number of live embryos. The mean number of corpora lutea in all treated groups was less (15.9, 16.5 and 15.5 at low, mid and high dose, respectively, control = 17.4) than the control, with statistical significance at 5 and 25 mg/kg BID. However, the mean number of corpora lutea in the control group was unusually high and was above the background range seen in this laboratory. Due to the lack of any dose-relationship and high control incidences, relation to the treatment is not clear. The following table (from page 132 of the report) shows the caesarian data.

Test article	Control		Genz-112638	
Group	1	2	3	4
Level (mg/kg/BID)	0	5	15	25

	Group 1	Group 2	Group 3	Group 4	Statistics
Number of females with live embryos on Day 13 gestation	20	20	20	19	
Mean number of corpora lutea per female	17.4	15.9*	16.5	15.5**	J
Mean number of implantations per female	15.4	14.3	15.2	14.2	J
Pre-implantation loss:					
mean%	11.0	10.3	8.8	9.1	
number of dams affected	12	12	10	13	F+
Early intrauterine deaths:					
mean number	0.7	0.3	0.7	0.4	
number of dams affected	12	5	9	7	F+
Late intrauterine deaths:					
mean number	0.0	0.0	0.0	0.0	
number of dams affected	0	0	0	0	X
Dead embryos:					
mean number	0.0	0.0	0.0	0.0	
number of dams affected	0	0	0	0	X
Post-implantation loss:					
mean%	4.3	1.9	4.7	2.8	
number of dams affected	12	5	9	7	F+
Mean number of embryos per female	14.8	14.0	14.5	13.8	J

F+ = Cochran-Armitage and Fisher's Exact (upper tail)
J = Kruskal-Wallis, Terpstra-Jonckheere, Wilcoxon
X = not analysed

* P<0.05
** P<0.01
*** P<0.001

Toxicokinetics: Plasma concentrations of Genz-99067 at 1 hour post-dose on PND 22 were greater with increasing dose level. No other details were provided.

Dosing Solution Analysis: Dosing solution analysis results were within the range with the exception of one replicate from Group 3 (86%) in Week 1. This, however, was stated not to have affected the outcome or integrity of the study and the third sample analyzed was within the target range (96%), giving a mean result of 92%.

Summary: In an oral gavage study in juvenile rats, animals were treated with Genz-112638 at 5, 15 and 25 mg/kg BID for 10 weeks commencing at PND 22. Mortalities were observed at all doses including control and deaths were mainly attributed to gavage errors. A minimal grade unilateral neuropathy was observed in the optic nerve of two animals at 25 mg/kg BID. Per the Applicant, these findings were unilateral and were not considered treatment related. Histopathological changes were seen in the lymph node (increased incidence/severity of lymphoid hyperplasia in the mandibular lymph nodes at 25 mg/kg BID) and eye (minimal grade unilateral neuropathy in the optic nerve of two animals at 25 mg/kg BID). The target organ could be the lymph node and eye based on the histopathology findings. The NOAEL was considered as 15 mg/kg BID.

Study title: 13-Week Toxicity Study with Genz-399240-AA (Metabolite) by the Subcutaneous Route in Rats followed by a 4-Week Treatment-Free Recovery Period

Study no.:	GT-157-TX-61
Study report location:	EDR, Section 4.2.3.7.5
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 19, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Genz-399240-AA, Batch No. 007, 99%

Key Study Findings:

- In a 13-week subcutaneous toxicity study with Genz-399240-AA (major human metabolite of Genz-112638 exceeding 10% of the drug exposure) in rats, animals were treated daily at 1, 3 and 6 mg/kg/day.
- Gross pathology findings included injection site reactions (thickening and red discoloration) at mid and high doses, which mostly recovered at the end of the recovery period.
- Histopathological changes were seen at the injection sites (hemorrhage, edema, degeneration/necrosis of the subcutis and mononuclear or mixed inflammatory cell infiltrates followed by fibroplasia/fibrosis), kidney (tubular hyaline droplets in males) and skeletal muscles (minimal mixed inflammatory cell infiltrates in males). At the end of the 4-week treatment-free recovery period, there were no differences at injection sites between treated and control rats; which suggested

complete recovery. The kidney changes (hyaline droplet) did not appear to be treatment-related, as this is a common finding in male rats. The skeletal muscle findings were considered most likely to be secondary to inflammation observed at the injection sites and therefore were not considered toxicologically significant.

- The NOAEL was considered to be 6 mg/kg/day.

Methods:

Doses:	1, 3, 6 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Subcutaneous
Dose volume:	5 mL/kg
Formulation/Vehicle:	50 mM phosphate buffered saline, pH 7.4
Species/Strain:	SD rats
Number/Sex/Group:	10-16/sex/dose
Age:	6 weeks
Weight:	Male: 211 g; Female: 162 g
Satellite groups:	Toxicokinetics (TK)
Study design:	Study design is shown below
Deviation from study protocol:	As per the Applicant, the protocol deviations were considered not to have compromised the validity or integrity of the study.

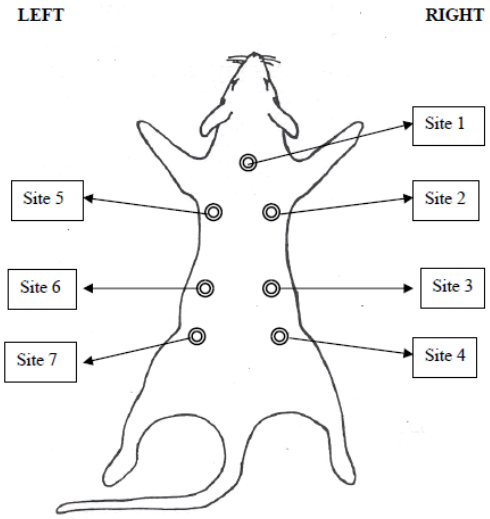
The following table shows the study design (from page 19 of the report). At the end of the treatment period, the principal animals in each group were sacrificed, except for the first six Group 1 and 4 animals per sex, which were kept for a 4-week treatment-free period.

The treatment groups for the present study are detailed in the following table:

Group	Treatment	Number and identity of animals		Dose-level (mg/kg/day)	Concentration (mg/mL)
1	Vehicle	Main	16 males X26881 to X26896 16 females X27001 to X27016	0	0
		Satellite	4 males X26897 to X26900 4 females X27017 to X27020		
2	Test item	Main	10 males X26901 to X26910 10 females X27021 to X27030	1	0.2
		Satellite	4 males X26911 to X26914 4 females X27031 to X27034		
3	Test item	Main	10 males X26915 to X26924 10 females X27035 to X27044	3	0.6
		Satellite	4 males X26925 to X26928 4 females X27045 to X27048		
4	Test item	Main	16 males X26929 to X26944 16 females X27049 to X27064	6	1.2
		Satellite	4 males X26945 to X26948 4 females X27065 to X27068		

The test article was administered at seven injection sites as per the following (from page 21 of the report) schedule.

The scheme below summarizes the different injection sites.



Injection site identification and dose day sequence

Treatment Site	Treatment days
Site 1	Days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85
Site 2	Days 2, 9, 16, 23, 30, 37, 44, 51, 58, 65, 72, 79, 86
Site 3	Days 3, 10, 17, 24, 31, 38, 45, 52, 59, 66, 73, 80, 87
Site 4	Days 4, 11, 18, 25, 32, 39, 46, 53, 60, 67, 74, 81, 88
Site 5	Days 5, 12, 19, 26, 33, 40, 47, 54, 61, 68, 75, 82, 89
Site 6	Days 6, 13, 20, 27, 34, 41, 48, 55, 62, 69, 76, 83, 90
Site 7	Days 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91

Basis of dose selection: Doses were selected based on the results of a single dose SC study (DMPK11-R087) in rats at 1, 5, 25 mg/kg. There were no significant treatment-related clinical signs or mortality. Since, there was no toxicity or mortality up to single SC dose of 25 mg/kg, it appears that higher than 6 mg/kg/day could have been tested in the current study.

Observations:

Mortality: All animals were examined once daily for mortality.

Clinical Signs: All animals were examined once daily for clinical signs.

Body Weights: Body weight was recorded on a weekly basis.

Food Consumption: Food consumption was recorded once weekly.

Ophthalmoscopy: Ophthalmological examinations were performed for all animals at pre-dose and for the control and high-dose animals on one occasion at the end of the treatment period.

Hematology: Hematology was conducted at the end of the treatment period.

Clinical Chemistry: Clinical chemistry was conducted at the end of the treatment period.

Urinalysis: Urinalysis was conducted at the end of the treatment period.

Bone marrow: Two bone marrow smears were prepared from the femoral bone of each animal at necropsy.

Gross Pathology: Gross pathology was conducted at necropsy.

Organ Weights: The following (from page 29 of the report) organs were weighed.

Organs	Organ weights	Preservation of tissues	Microscopic examination
Macroscopic lesions		X	X
Adrenals	X	X	X
Aorta		X	X
Brain (including medulla/pons cerebellar and cerebral cortex)	X	X	X
Cecum		X	X
Colon		X	X
Duodenum		X	X
Epididymides	X	X	X
Esophagus		X	X
Eyes with Harderian glands		X	X
Femoral bone with articulation		X	X
Gut-associated lymphoid tissue (GALT)		X	X
Heart	X	X	X
Ileum		X	X
Injection site 1[a]		X	X
All injection sites		X	
Jejunum		X	X
Kidneys	X	X	X
Larynx		X	X
Liver	X	X	X
Lungs with bronchi		X	X
Lymph nodes (mandibular and mesenteric)		X	X
Mammary gland area		X	X
Optic nerves		X	X
Ovaries (with oviducts)	X	X	X
Pancreas		X	X
Pituitary gland		X	X
Prostate		X	X
Rectum		X	X
Salivary glands (sublingual and submandibular)		X	X
Sciatic nerve		X	X
Seminal vesicles		X	X
Skeletal muscle		X	X
Skin		X	X
Spinal cord (cervical, thoracic and lumbar)		X	X
Spleen	X	X	X
Sternum with bone marrow		X	X
Stomach with forestomach		X	X
Testes	X	X	X
Thymus	X	X	X
Thyroids with parathyroids	X	X	X
Tongue		X	X
Trachea		X	X
Ureters		X	
Urinary bladder		X	X
Uterus (horns and cervix)		X	X
Vagina		X	X

[a]: only injection site one was examined microscopically.

Histopathology: The tissues listed in the above table from the control and high dose main study and recovery animals were examined for histopathology.

Toxicokinetics: Blood samples were collected on day 1, week 4 and week 13 from satellite animals from all groups (groups 1 to 4), at 0.25, 0.5, 1, 3, 6 and 24 hours post-dose.

Dosing Solution Analysis: Test item concentrations in the administered dose formulations were analyzed in weeks 1, 4, 8 and 13.

Results:

Mortality: One principal Group 1 female was found dead on day 71 (week 11), with no clinical signs observed prior to death. This death was considered accidental.

Clinical Signs: There were no significant treatment-related clinical signs.

Body Weights: The mean initial (Day 1) and final (Day 91) weights of control males were 203 and 495 g, respectively. The mean initial (Day 1) and final (Day 91) weights of control females were 161 and 289 g, respectively. There were no significant treatment related effects.

Food Consumption: The mean initial (Day 1-7) and final (Day 85-91) food consumption of control males was 26.6 and 28.1 g/animal/day, respectively. The mean initial (Day 1-7) and final (Day 85-91) food consumption of control females was 19.0 and 20.9 g/animal/day, respectively. There were no significant treatment related effects.

Ophthalmoscopy: There were no significant treatment related effects.

Hematology: There were no significant treatment related effects.

Clinical Chemistry: There were no significant treatment related effects.

Urinalysis: There were no significant treatment related effects.

Gross Pathology: Red discoloration and thickening were observed at the injection sites. Thickening was observed at higher incidence in treated males, more specifically at the injection sites 5 and 6, on Day 89 or 90, respectively. This change was not seen in females (possibly as a result of lower injection volumes). The injection site macroscopic findings are shown in the table (from page 43 of the report) below.

Reference ID: 3501244

Incidence selected macroscopic findings at the injection sites

Sex	Male				Female			
Dose-level (mg/kg/day)	0	1	3	6	0	1	3	6
Number examined	10	10	10	10	9	10	10	10
Injection site 1								
Injection site 2								
Thickening	-	-	1	-	-	-	-	-
Injection site 3								
Injection site 4								
Red discoloration	-	-	1	-	-	-	-	-
Injection site 5								
Red discoloration	-	-	1	1	-	-	-	-
Thickening	-	-	**2**	**2**	-	-	-	-
Injection site 6								
Red discoloration	1	3		3	-	2	-	3
Thickening	-	**1**	**4**	**5**	-	-	-	-
Injection site 7								
Red discoloration	4	2	6	5	4	2	3	5
Thickening	-	-	1	-	-	1	-	-

In bold: considered to be Genz-399240-AA-related.

Organ Weights: Higher mean absolute and relative-to-body liver weights were observed in males at 6 mg/kg/day. Although there were no microscopic correlates, this weight difference was attributed to the treatment. The following table (from page 42 of the report) shows the liver weight data. There were no significant treatment related effects in recovery animals.

Liver weight differences when compared to control groups (expressed in %)

Sex	Male			Female		
Group	2	3	4	2	3	4
Dose-level (mg/kg/day)	1	3	6	1	3	6
Exam. animals / Num. of animals	10/10	10/10	10/10	10/10	10/10	10/10
Body weight	+6	-5	+5	+2	0	+4
- Liver						
. absolute	+11	+1	+15*	+2	-1	0
. relative	+5	+7	+10**	0	-1	-3

Statistically significant from controls: *: $p<0.05$, **: $p<0.01$.

Histopathology: In males, minimal mixed inflammatory cell infiltrates were observed around the skeletal muscle in 4 of 10 males at 6 mg/kg/day (0 of 10 in control male). This finding was considered to be secondary, most likely related to inflammation observed at the injection sites and therefore was not considered to be toxicologically significant. In addition, tubular hyaline droplets (grade 1) in the kidney were seen at a marginally higher incidence in males at 6 mg/kg/day (5 of 10) than in controls (2 of 10).

This is a common finding in male rats and a relationship to the treatment was unclear. Histopathological changes were also seen at the injection site (hemorrhage, edema, degeneration/necrosis of the subcutis and mononuclear or mixed inflammatory cell infiltrates followed by fibroplasia/fibrosis) at higher incidence and/or severity in males treated at 1, 3 or 6 mg/kg/day, especially at injection sites 5, 6 or 7. The following table (from page 44 of the report) shows the injection site changes at the injection site 1.

Incidence and severity of fibroplasia/fibrosis at injection site 1

Sex	Male				Female			
Dose-level (mg/kg/day)	0	1	3	6	0	1	3	6
Number examined	10	10	10	10	9	10	10	10
Mononuclear inflammatory cell infiltrates (injection site 1)								
grade 1	4	5	4	4	1	4	4	3
grade 2	1	3	4	4	-	-	-	1
grade 3	1	-	-	-	-	-	-	-
Total number of affected rats	6	8	8	8	1	4	4	4
Fibroplasia/fibrosis (injection site 1)								
grade 1	5	6	7	5	2	3	2	7
grade 2	1	-	1	2	-	-	1	-
grade 3	-	-	-	1	-	-	-	-
Total number of affected rats	*6*	*6*	*8*	*8*	*2*	*3*	*3*	*7*

-: not observed.

Toxicokinetics: The plasma samples analyzed from control animals were generally below the LOQ (< 0.500 ng/mL) for detection of Genz-399240-AA, except for a few samples (20 of 144 samples) for which Genz-399240-AA levels ranged between 0.507 and 3.74 ng/mL (one sample level was 11.7 ng/mL). Per the Applicant, these levels corresponded to less than 10% of the maximum concentrations calculated at the low dose levels and were therefore considered as trace levels only and without any impact on the scientific outcome of the study. The reason/source of Genz-399240-AA in control samples was not explained. The maximum plasma concentration was reached at 0.25 or 0.5 hour after injection in all animals. Mean C_{max}, AUC_{0-t} and $t_{½}$ values are shown in the table below (from page 40 of the report).

Mean toxicokinetic parameters following Genz-399240-AA administration:

Sampling Period	Group / Dose-level (mg/kg/day) TK	units	2 / 1 M	2 / 1 F	3 / 3 M	3 / 3 F	4 / 6 M	4 / 6 F
Day 1	t_{max}	h	0.25 [0.25 – 0.5]	0.375 [0.25 – 0.5]	0.25 [0.25 – 0.25]	0.25 [0.25 – 0.25]	0.25 [0.25 – 0.5]	0.25 [0.25 – 0.5]
	C_{max}	ng/mL	669 ± 146 (22)	678 ± 191 (28)	1968 ± 199 (10)	1910 ± 221 (12)	4173 ± 166 (4)	3840 ± 132 (3)
	AUC_{0-t}	ng.h/mL	590 ± 146 (25)	566 ± 47 (8)	1629 ± 206 (13)	1904 ± 74 (4)	3703 ± 418 (11)	3782 ± 148 (4)
	app $t_{1/2}$	h	0.325 ± na (na)	0.492 ± 0.075 (15)	0.491 ± na (na)	0.521 ± 0.011 (2)	0.546 ± na (na)	0.521 ± 0.008 (1)
Week 4	t_{max}	h	0.375 [0.25 – 0.5]	0.375 [0.25 – 0.5]	0.25 [0.25 – 0.25]	0.375 [0.25 – 0.5]	0.25 [0.25 – 0.25]	0.25 [0.25 – 0.25]
	C_{max}	ng/mL	530 ± 62 (12)	538 ± 113 (21)	1715 ± 156 (9)	1885 ± 215 (11)	4088 ± 197 (5)	4313 ± 615 (14)
	AUC_{0-t}	ng.h/mL	604 ± 136 (22)	497 ± 76 (15)	1650 ± 128 (8)	1950 ± 59 (3)	3724 ± 157 (4)	4552 ± 512 (11)
	app $t_{1/2}$	h	0.630 ± 0.137 (22)	0.558 ± 0.187 34	0.526 ± 0.006 (1)	0.539 ± 0.025 (5)	0.566 ± 0.058 (10)	0.569 ± 0.051 (9)

M: male; F: female. na: not applicable.
app $t_{1/2}$: apparent terminal half-life.
Inter-animal variability: 0-20% : slight; 20-50%: moderate; >50%: marked.

Dosing Solution Analysis: The test item concentrations in the dosing formulations analyzed in weeks 1, 4, 8 and 13 remained within an acceptable range of -7.1% to +0.5% when compared to the nominal values.

Summary: In a 13-week subcutaneous toxicity study with Genz-399240-AA (metabolite of Genz- Genz-112638) in rats, animals were treated daily at 1, 3 and 6 mg/kg/day. Histopathological changes were seen at the injection sites (hemorrhage, edema, degeneration/necrosis of the subcutis and mononuclear or mixed inflammatory cell infiltrates followed by fibroplasia/fibrosis), kidney (tubular hyaline droplets in males) and skeletal muscles (minimal mixed inflammatory cell infiltrates in males). At the end of the 4-week treatment-free recovery period, injection site reactions were recovered. The kidney changes (hyaline droplet) did not appear to be treatment-related, as this is a common finding in male rats. The skeletal muscle findings were considered most likely to be secondary to inflammation observed at the injection sites and therefore were not considered toxicologically significant. The NOAEL was considered to be 6 mg/kg/day.

Dog

Study title: A 28-day oral gavage toxicity study with Genz-112638 (with a 14-day recovery) in the Beagle dog (GT-157-TX-6)

The review of the above study is incorporated below from the pharmacology review of IND 67,589 dated July 30, 2004 by Dr. Dylan Yao (DMEP).

Study title: A 28-day oral gavage toxicity study with Genz-112638 (with a 14-day recovery) in the Beagle dog

Key study findings:
There were no treatment related findings on clinical signs, body weights, and clinical pathology parameters. No EKG findings at the dose levels tested, although safety pharmacology studies in the dog have shown that a single oral dosing at ⩾10 mg induced QRS interval prolongation and other changes. Histologically, atrophy of lymphoid organs/tissues (thymus, lymph nodes and gut lymphoid tissues) in 10 and 25 mg/kg/day treated dogs was the major finding and was reversed after 2-week recovery. The exposure levels were highly variable over dose levels, but generally increases following dose with exception of MD males. NOAEL=5 mg/kg/day based on histopath findings.

Study no.: GT-157-TX-6
Volume #, and page #: Vol 2.13, and page 50
Conducting laboratory and location: (b) (4) (b) (4)
Date of study initiation: May 8, 2003
GLP compliance: y
QA report: yes ( ) no (X)
Drug, lot #, and % purity: Genz-112638, lot #909246, and purity 100%

Methods

Doses: 0, 5, 10, 25 mg/kg/day (0, 2.5, 5, 12.5 mg/kg b.i.d.)
Species/strain: dog/Beagle
Number/sex/group or time point (main study): 3/s/g
Route, formulation, volume, and infusion rate: oral gavage with weekly prepared solution formulation, twice daily dosed at 5 mL/kg/dose, ~6 hrs apart
Satellite groups used for toxicokinetics or recovery: 2/s in C and HD groups for recovery
Age: ~5 to 7 months old
Weight: 6.5 to 9.5 kg for males and 6.1 to 7.8 kg for females
Sampling times: necropsied on Day 28
Unique study design or methodology: n/s

Observation and Times:

Clinical signs: twice daily
Body weights: once pretest and then weekly
Food consumption: once daily
Ophthalmoscopy: once pretest and once during Week 4
EKG: performed during Weeks 2 and 4
Hematology: collected once pretest and at Weeks 4 and 6 (recovery animals)
Clinical chemistry: collected once pretest and at Weeks 4 and 6 (recovery animals)
Urinalysis: overnight urine samples were collected pretest and at Weeks 4 and 6 (recovery animals)
Gross pathology: at necropsy
Organ weights: see histopath table
Histopathology: all animals were processed for histopath examination
Adequate Battery: yes (X), no ( )
Peer review: n/s

Results:

Mortality: no deaths

Clinical signs: no treatment related clinical signs

Body weights: unremarkable

Food consumption: unremarkable

Ophthalmoscopy: no findings

EKG: no findings

Hematology: unremarkable

Clinical chemistry: unremarkable

Urinalysis: no findings

Gross pathology: unremarkable

Organ weights: ss ↓ thymus weight (↓ 48% absolute wt and ↓ 43% relative to body wt) in HD F; the thymus weight was not reversed after recovery (↓ 42% and 52% absolute and relative wt, respectively)

Histopathology: findings were in females only, lymphoid atrophy was observed in the thymus, lymph nodes and gut associated lymphoid tissue in 1 female dog at 10 mg/kg/day dose and all female dogs in the 25 mg/kg/day group. Strangely, the HD males showed some lymphoid atrophy at the end of recovery period, which was not seen in the main study.

Main study: findings seen in females only

Doses, mg/kg/d	**0**	**5**	**10**	**25**
No animals examined	**3**	**3**	**3**	**3**
Lymphoid atrophy in gut				
Cecum	0	0	0	2
Duodenum	0	0	0	1
Ileum	0	0	1	3
Jejunum	0	0	0	3
Lymphoid atrophy, Lymph nodes				
Mandibular	0	0	0	1
Mesenteric	0	0	1	3
Lymphoid atrophy, Thymus	0	0	0	2

Recovery:

Doses, mg/kg/d	**0**		**25**	
	M	**F**	**M**	**F**
No animals examined	2	2	2	2
Lymphoid atrophy, gut				
Duodenum	0	0	1	0
Jejunum	0	0	1	0
Lymphoid atrophy, lymph nodes				
Mandibular	0	0	1	0
Mesenteric	0	0	1	0
Lymphoid atrophy, Thymus	0	0	1	0

Toxicokinetics: On Days 1 and 28, blood samples were collected at 15 and 30 min, and 1, 2, 4 and 6 hrs post-the first daily dose. Plasma concentration of Genz-99076 (the free-base form of Genz-112638) were assayed and the lower limit of quantification was 5 ng/mL. The exposure values were highly variable between individual animals of each group (up to ~24x difference) at each time point. Terminal $t_{1/2}$ ranged from 0.579 to 1.22 hrs. Tmax was observed at 30 min postdose. Cmax and AUC was proportional following dose except MD males. Accumulation was noted on Day 28.

Day 1

Gender	Group No.	Dose Level (mg/kg/day)	Dose Level (mg/kg/dose)	C_{max} ng/mL	AUC_{0-inf} ng•h/mL	Dose Proportionality	C_{max} Ratio	AUC_{0-inf} Ratio
Males	2	5	2.5	166	306	1	1	1
	3	10	5	67.4	105	2	0.407	0.343
	4	25	12.5	343	792	5	2.07	2.58
Females	2	5	2.5	17.6	47.4	1	1	1
	3	10	5	74.4	114	2	4.22	2.40
	4	25	12.5	437	953	5	24.8	20.1

Day 28

Gender	Group No.	Dose Level (mg/kg/day)	Dose Level (mg/kg/dose)	C_{max} ng/mL	AUC_{0-inf} ng•h/mL	Dose Proportionality	C_{max} Ratio	AUC_{0-inf} Ratio
Males	2	5	2.5	213	255	1	1	1
	3	10	5	105	177	2	0.495	0.696
	4	25	12.5	516	1235	5	2.43	4.85
Females	2	5	2.5	30.2	75.2	1	1	1
	3	10	5	85.4	172	2	2.83	2.28
	4	25	12.5	722	1493	5	23.9	19.9

Other: n/a

Histopathology inventory:

Study	GT-157-TX-5	GT-157-TX-6
Species	Rat	Dog
Adrenals	X*	X*
Aorta	X	X
Bone Marrow smear	X	X
Bone (femur)		
Brain	X*	X*
Cecum	X	X
Cervix	X	
Colon	X	X
Duodenum	X	X
Epididymis	X	X
Esophagus	X	X
Eye	X	X
Fallopian tube		
Gall bladder	X	X
Gross lesions	X	X
Harderian gland		
Heart	X*	X*
Ileum	X	X
Injection site		
Jejunum	X	X
Kidneys	X*	X*
Lachrymal gland		X
Larynx		
Liver	X*	X*
Lungs	X*	X*
Lymph nodes, cervical		
Lymph nodes mandibular	X	X
Lymph nodes, mesenteric	X	X
Mammary Gland	X	X
Nasal cavity		
Optic nerves	X	X
Ovaries	X*	X*
Pancreas	X	X
Parathyroid		
Peripheral nerve		
Pharynx		
Pituitary	X*	X*
Prostate	X*	X
Rectum	X	X
Salivary gland	X	X
Sciatic nerve	X	X
Seminal vesicles	X	
Skeletal muscle	X	X
Skin	X	X
Spinal cord	X	X
Spleen	X*	X*
Sternum		X
Stomach	X	X
Testes	X*	X*
Thymus	X*	X*
Thyroid	X*	X*
Tongue	X	X
Trachea	X	X
Urinary bladder	X	X
Uterus	X*	X*
Vagina	X	X

X, histopathology performed
*, organ weight obtained

Study title: 13-Week Toxicity Study with Genz-112638 by Oral (Gavage) in Beagle Dogs followed by a 4-Week Treatment-Free Recovery Period

Study no.:	GT-157-TX-15
Study report location:	EDR, Section 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 19, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Genz-112638, Batch No. 909246, 100%

Key Study Findings:

- In a 13-week oral (gavage) toxicity study in Beagle dogs, animals were administered Genz-112638 at 2, 5 or 10 mg/kg/day.
- Histopathological changes were seen in the thymus (lymphoid depletion) at all doses including the control. However, the severity was highest at the high dose in both sexes.
- The target organ appeared to be the thymus based on the above effects.
- Based on the adverse effect on thymus weight and size with associated lymphoid depletion (highest severity) at 10 mg/kg/day, the NOAEL appeared to be 5 mg/kg/day.

Methods:

Doses:	2, 5, 10 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Subcutaneous
Dose volume:	5 mL/kg
Formulation/Vehicle:	Purified water
Species/Strain:	Beagle dogs
Number/Sex/Group:	4-6/sex/dose
Age:	6 months
Mean Weight (Range):	Male: 8.6 kg (7.3-9.7 kg) Female: 7.2 (6.5-8.2 kg)
Satellite groups:	None
Study design:	Study design is shown below
Deviation from study protocol:	As per the Applicant, the protocol deviations were considered not to have compromised the validity or integrity of the study.

The following table shows the study design (from page 17 of the report).

Group	Number of animals	Dose-level (mg/kg/day)	Animal numbers
1	6 males 6 females	0	D51001 to D51006 D51151 to D51156
2	4 males 4 females	2	D51007 to D51010 D51157 to D51160
3	4 males 4 females	5	D51011 to D51014 D51161 to D51164
4	6 males 6 females	10	D51015 to D51020 D51165 to D51170

Basis of dose selection: The dose-levels were selected based on the results of the above 28-day toxicity study (GT-157-TX-6) in dogs with a 14-day recovery period at 0, 2.5, 5 or 12.5 mg/kg/dose BID (5, 10 and 25 mg/kg/day).

Observations:

Mortality: All animals were examined twice daily for mortality.

Clinical Signs: All animals were examined once daily for clinical signs.

Body Weights: Body weight was recorded on a weekly basis.

Food Consumption: Food consumption was recorded daily.

Electrocardiography (ECG): Electrocardiographic examinations were performed on all animals before the beginning of the treatment period and at least 2 hours after dosing on one occasion in weeks 6 and 13 using standard leads I, II and III.

Ophthalmoscopy: Ophthalmological examinations were performed on all animals at pre-dose and in Week 13.

Hematology: Hematology was conducted at the pre-dose, Week 6, the end of the treatment period and at recovery.

Clinical Chemistry: Clinical chemistry was conducted at pre-dose, Week 6, the end of the treatment period and at recovery.

Urinalysis: Urinalysis was conducted at pre-dose, the end of the treatment period and at recovery.

Bone marrow: Bone marrow samples were collected at the pre-dose, the end of the treatment period and at recovery.

Immunophenotyping: At pre-dose, in week 6 and at the end of the treatment and recovery periods, blood was collected from all animals for immunofluorescent staining for lymphocyte subset as shown in the following table (from page 21 of the report).

Antibody combination	Lymphocyte subset evaluated
CD3-PE / CD4-FITC	T-Helper cells (CD3+ / CD4+)
CD3-PE / CD8-FITC	T-Suppressor cells (CD3+ / CD8+)
CD5-FITC / Anti Canine B cells-PE	CD5+ / Anti Canine B Cells -: pan T cells CD5- / Anti Canine B Cells +: pan B cells

Gross Pathology: Gross pathology was conducted at necropsy.

Organ Weights: The following (from page 26 of the report) organs were weighed.

Organs	Organ weights	Preservation of tissues	Microscopic examination
Macroscopic lesions		X	X
Adrenals	X	X	X
Aorta		X	X
Brain (including medulla/pons cerebellar and cerebral cortex)	X	X	X
Cecum		X	X
Colon		X	X
Duodenum		X	X
Epididymides	X	X	X
Esophagus		X	X
Eyes		X	X
Femoral bone with articulation		X	
Gall bladder		X	X
Heart	X	X	X
Ileum		X	X
Jejunum (with Peyers patches)	X	X	X
Kidneys	X	X	X
Larynx		X	X
Liver	X	X	X
Lungs with bronchi		X	X
Lymph nodes (mandibular and mesenteric)	X	X	X
Mammary gland area		X	X
Optic nerve		X	X
Ovaries	X	X	X
Oviducts		X	X
Pancreas		X	X
Pituitary gland		X	X
Prostate		X	X
Rectum		X	X
Salivary glands (parotid and submandibular)		X	X
Sciatic nerve		X	X
Skeletal muscle		X	X
Skin		X	X
Spinal cord (cervical, thoracic and lumbar)		X	X
Spleen	X	X	X
Sternum with bone marrow		X	X
Stomach		X	X
Testes	X	X	X
Thymus	X	X	X
Thyroids with parathyroids	X	X	X
Tongue		X	X
Trachea		X	X
Ureters		X	X
Urinary bladder		X	X
Uterus (horns and cervix)		X	X
Vagina		X	X

Histopathology: The tissues listed in the above table from all animals from the control and high dose groups were examined for histopathology.

Toxicokinetics: Blood samples were collected on day 1, in week 6 and at the end of the treatment at 0.25, 0.5, 1, 2, 4 and 6 hours post-dose.

Dosing Solution Analysis: The concentration and stability of the dosing solutions were checked. The concentration of samples, from each control and test item dosage form prepared for use in weeks 1, 4, 6, 8 and 13, was determined. Two dosage forms were prepared at 0.4 and 2 mg/mL of Genz-112638. To evaluate the stability, each dosage form was sampled and analyzed after 0 (just after preparation), 4 and 9 days storage at 4^0C.

Results:

Mortality: There were no mortalities.

Clinical Signs: Clinical signs included hyperactivity, excessive salivation, and slight to moderate erythema (ear), vomiting, and emaciation. These signs were seen at all doses including control and toxicological significance is not clear.

Body Weights: The mean initial (Week 1) and final (Week 13) weights of control males were 8.4 and 9.5 kg, respectively. The mean initial (Week 1) and final (Week 13) weights of control females were 7.5 and 8.6 kg, respectively. There were no significant treatment related effects.

Food Consumption: Quantitative food consumption data were not provided. Per the Applicant, there were no significant treatment related effects.

Ophthalmoscopy: Hypoplasia of the optic disc was noted during pre-dose and in week 13 in one male (No.D51010) at 2 mg/kg/day. As this abnormality was already present during pre-dose in this animal, this effect was not considered treatment-related. There were no other significant treatment related effects.

Electrocardiography (ECG): There were no significant treatment related effects.

Hematology: At the high dose in Week 12 following changes were observed:

- Slightly higher fibrinogen level in males (2.28 *vs.* 1.90 g/L in controls); when compared to pre-dose values, no change was noted (2.61 g/L in pre-dose)
- Slightly higher leucocyte count [11.30 *vs.* 8.21 Giga ($10e^9$)/L in controls] in females, associated with slightly higher neutrophil count (7.31 *vs.* 4.99 Giga/L in controls); when compared to pre-dose values, no change was noted (leucocyte: 12.57 Giga/L, neutrophil: 8.08 Giga/L in pre-dose).

Reference ID: 3501244

These changes were not considered to be of toxicological significance since all these changes were slight, not relevant when compared to the pre-dose values, and all the individuals remained within the range of the historical background data. There were no other significant treatment related effects.

Clinical Chemistry: The following changes were observed at Week 12:

- Slightly higher chloride level in males at 10 mg/kg/day (110.4 *vs.* 108.3 mmol/L in controls and 108.1 mmol/L in pre-dose)
- Slightly higher gamma-glutamyl transferase (GGT) activity in females at 2 mg/kg/day (5 *vs.* 2 IU/L in controls and 6 IU/L in pre-dose).

Since all these differences from controls were slight and with all the individuals within the range of the historical background data, they were not considered to be of toxicological significance. There were no other significant treatment related effects.

Urinalysis: There were no significant treatment related effects.

Bone marrow: There were no significant treatment related effects.

Immunophenotyping: There were no significant treatment related effects.

Gross Pathology: Thymus size was reduced in 1 of 4 females at 5 mg/kg/day and 1 of 4 males and 2 of 4 females at 10 mg/kg/day. This correlated with a slight to moderate lymphoid depletion in microscopic examination. In addition, one female (No.D51165) at 10 mg/kg/day had reduced size and brownish color of the right ovary, reduced size, irregular color and presence of a serous cyst in the left ovary and reduced size of both uterine horns. These findings correlated with dysgenesis of the ovaries and uterus at microscopic examination and were thus considered to be without toxicological importance.

Organ Weights: The following table (from page 39 of the report) shows the organ weight changes.

Sex	Male				Female			
Group	1	2	3	4	1	2	3	4
Dose-level (mg/kg/day)	0	2	5	10	0	2	5	10
Body weight	-	-2	-5	-3	-	-23**	-9	-13
- *Thymus*								
. absolute	-	+9	+3	-1	-	-27	-5	-32
. relative	-	+11	+6	+1	-	-8	+4	-22
- *Jejunum*								
. absolute	-	-28	-10	-16	-	+5	+26	+5
. relative	-	-28	-6	-14	-	+37	+38	+24
- *Mesenteric lymph nodes*								
. absolute	-	+5	-4	+1	-	-4	-1	+3
. relative	-	+5	-1	+2	-	+25	+9	+19
- *Mandibular lymph nodes*								
. absolute	-	+9	+6	-1	-	-21	-2	-5
. relative	-	+11	+10	+1	-	+6	+9	+8
- *Ileum*								
. absolute	-	-12	-17	-23*	-	-	-	-
. relative	-	-9	-13	-21	-	-	-	-

Statistically significant *: $p<0.05$, **:$p<0.01$.

In females, lower mean thymus weights were observed in 1 of 4, 1 of 4 and 3 of 4 animals at 2, 5 and 10 mg/kg/day, respectively. As it was associated with a higher mean severity of lymphoid depletion, a relationship to treatment could not be ruled out. The weight changes observed in the jejunum, mesenteric lymph nodes and mandibular lymph nodes were not dose-related and/or without the same trend in both sexes. These differences did not appear to be of toxicological significance. In males, lower mean weights of the ileum were observed in 3 of 4, 3 of 4 and 4 of 4 animals at 2, 5 and 10 mg/kg/day respectively. As there was no relevant histopathological finding, it was considered to be without toxicological significance. These were not seen in recovery animals.

Histopathology: Histopathological changes were seen in the thymus (minimal to moderate lymphoid depletion) of treated and control animals. However, the severity was highest at the high dose in both sexes. The following table (from page 41 of the report) shows lymphoid depletion in the thymus.

Lymphoid depletion in the thymus at the end of the treatment period

	Male				Female			
Group	1	2	3	4	1	2	3	4
Dose-level (mg/kg/day)	0	2	5	10	0	2	5	10
Grade 1	1	2	1	-	2	1	3	-
Grade 2	3	2	3	4	2	2	1	1
Grade 3	-	-	-	-	-	1	-	2
Total affected	4	4	4	4	4	4	4	3
Mean severity	*1.8*	*1.5*	*1.8*	*2.0*	*1.5*	*2.0*	*1.3*	*2.7*

Toxicokinetics: Mean plasma levels of Genz 112638 increased after dosing on Day 1, to reach a mean C_{max} at 0.36 to 0.63 hour (t_{max}) post-dosing. The exposure (C_{max} and AUC_{0-t}) increased with the dose in a dose proportional manner in both sexes, except at 2 mg/kg/day. There appeared to be a gender difference in exposure as exposure in males was generally higher than that in females. The TK parameters are shown in the following table (from page 38 of the report).

Toxicokinetic parameters of Genz-112638 on day 1, in weeks 6 and 13 after oral administration to beagle dogs

Sampling period	Kinetic parameter	Unit	Group, dose-level (mg/kg/day), sex					
			2		3		4	
			2		5		10	
			M	F	M	F	M	F
Day 1	C_{max}	(ng/mL)	433	161	1262	522	2078	1497
	t_{max}	(h)	0.63	0.44	0.38	0.63	0.42	0.50
	$AUC_{(0-t)}$	(ng*h/mL)	891	132	1687	832	3504	2510
	Ratio C_{max}/dose		216	80	252	104	208	150
	Ratio AUC/dose		445	66	337	166	350	251
Week 6	C_{max}	(ng/mL)	440	125	1120	917	2015	2061
	t_{max}	(h)	0.63	0.50	0.38	0.50	0.58	0.50
	$AUC_{(0-t)}$	(ng*h/mL)	930	97.1	2070	1991	4039	4061
	Ratio C_{max}/dose		220	62	224	183	202	206
	Ratio AUC/dose		465	49	414	398	404	406
Week 13	C_{max}	(ng/mL)	351	201	970	685	1954	1769
	t_{max}	(h)	0.63	0.38	0.38	0.50	0.50	0.46
	$AUC_{(0-t)}$	(ng*h/mL)	848	120	1637	1179	3932	3309
	Ratio C_{max}/dose		175	100	194	137	195	177
	Ratio AUC/dose		424	60	327	236	393	331

M: male, F: female.

Dosing Solution Analysis:

Stability: The results of the analyses demonstrated a satisfactory stability of the dosage forms (0.4 and 2 mg/mL) obtained over a 9-day period (4^0C, protected from light).

Concentration: None of the measured concentrations deviated from the nominal values by more than ± 5%.

Summary: In a 13-week oral (gavage) toxicity study in Beagle dogs, animals were administered Genz-112638 at 2, 5 or 10 mg/kg/day. Histopathological changes were seen in the thymus (lymphoid depletion) at all doses including the control. However, the severity was highest at the high dose in both sexes. Based on the adverse effect on thymus weight and size with associated lymphoid depletion (highest severity) at 10 mg/kg/day, the NOAEL appeared to be 5 mg/kg/day. The target organ appeared to be the thymus based on the above effects.

Study Title: 52-Wee Oral (gavage) Toxicity Study in Beagle Dogs with an 8-Week Recovery Period (GT-157-TX-23)

The review of the above study is incorporated below from the pharmacology review of IND 67,589 dated June 29, 2006 by Dr. Tamal K. Chakraborti (DGIEP).

52-Week Study by Oral Route (Gavage) in Beagle Dogs Followed by an 8-Week Treatment Free Period

Study No.: GT-157-TX-23

Volume and Page: Vol. 1, page 1

Testing Laboratory: (b) (4)

Dates of Initiation and Completion of Study: July 13, 2004 and September 19, 2005

GLP & QAU Requirements: The statement of compliance with GLP regulations was not signed. The QAU statement was also not signed. This report should not be considered as final reports in the absence of signed GLP and QAU statements and may be considered as draft report.

Species and Strain: Beagle dogs (approximately 8 months old) with body weight range of 7.1 to 10.6 kg in males and 6.9 to 9.6 kg in females.

Drug, Batch and Purity: Genz-112638, BR-05-089-P0054, and 99.8%

Vehicle: Purified water

Methods: In this study, groups of four or six male and four or six female beagle dogs were treated with Genz-l 12638 by oral gavage at 2, 5 or 10 mg/kg/day (5 ml/kg) for 52 weeks. The dose-levels were selected based on the results of previous 28-day and 13-week toxicity studies in Beagle dogs. In the 28-day study with a 14-day recovery period, oral (gavage) administration of Genz-112638 at 5, 10 or 25 mg/kg/day (administered twice daily at doses of 2.5, 5 and 12.5 mg/kg/dosing) resulted in lymphoid atrophy at 10 and 25 mg/kg/day. This finding was still present at the end of the 2-week recovery period at the 25 mg/kg/day. The NOAEL was considered to be 5 mg/kg/day. In the 13-week toxicity study, the animals were treated at 2, 5 or 10 mg/kg/day, followed by a 4-week treatment-free period. Genz-112638 appeared to be

tolerated and did not induce any significant treatment-related changes at any dose-levels, however, it affected the body weight gain at all dose-levels. At 10 mg/kg, there was a decrease in the thymus weight and size, correlating with depletion at microscopic examination. These changes were no longer observed after the 4-week treatment-free period. Consequently, the NOAEL was considered to be 5 mg/kg/day. Based on these results, the highest dose for the current study was selected as 10 mg/kg/day.

The following table (from page 17 of the study report) shows the study design.

The treatment groups and dose-levels are detailed in the following table:

Group	Number of animals		Dose-level (mg/kg/day)	Animal number	
	Principal	Recovery		Principal	Recovery
1	4 males 4 females	2 males 2 females	0	F50601 to F50604 F50651 to F50654	F50605 and F50606 F50655 and F50656
2	4 males 4 females	-	2	F50607 to F50610 F50657 to F50660	-
3	4 males 4 females	-	5	F50611 to F50614 F50661 to F50664	-
4	4 males 4 females	2 males 2 females	10	F50615 to F50618 F50665 to F50668	F50619 and F50620 F50669 and F50670

-: not applicable

An additional control group (six males and six females) received the vehicle (purified water) under the same experimental conditions. At the end of the treatment period, the last two males and females from groups 1 and 4 were kept for an 8-week treatment-free recovery period. Blood samples for toxicokinetics were taken from all study animals on day 1 and in weeks 26 and 52 at the following times: 0, 0.5, 1, 3 and 6 hours postdose. The animals were examined daily for clinical signs and mortality. Body weight and food consumption were recorded once during pre-dose and then on a weekly basis. Electrocardiography (ECG) and ophthalmological examinations were performed before the beginning of the treatment period, at least 2 hours after dosing in week 26 and at the end of the treatment and treatment-free periods. Hematological, blood biochemical and urinary investigations were performed before the beginning of the treatment period, in weeks 12 and 25, and at the end of the treatment and recovery periods. At the end of the treatment or recovery period, the animals were sacrificed and a complete macroscopic examination was performed. Selected organs were weighed and designated tissues were examined microscopically. The following table (from page 25 of the study report) shows the organs and tissues which were weighed and examined histopathologically.

TISSUE PROCEDURE TABLE

Organs	Organ weights	Preservation of tissues	Microscopic examination
Macroscopic lesions		X	X
Adrenals	X	X	X
Aorta		X	X
Brain (including medulla/pons cerebellar and cerebral cortex)	X	X	X
Cecum		X	X
Colon		X	X
Duodenum		X	X
Epididymides	X	X	X
Esophagus		X	X
Eyes		X	X
Femoral bone with articulation		X	
Gall bladder		X	X
Heart	X	X	X
Ileum		X	X
Jejunum* (with Peyer's patches)	X	X	X
Kidneys	X	X	X
Larynx		X	X
Liver	X	X	X
Lungs with bronchi		X	X
Lymph nodes (mandibular and mesenteric)	X	X	X
Mammary gland area		X	X
Optic nerve		X	X
Ovaries	X	X	X
Oviducts		X	X
Pancreas		X	X
Pituitary gland		X	X
Prostate		X	X
Rectum		X	X
Salivary glands (parotid and submandibular)		X	X
Sciatic nerve		X	X
Skeletal muscle		X	X
Skin		X	X
Spinal cord (cervical, thoracic and lumbar)		X	X
Spleen	X	X	X
Sternum with bone marrow		X	X
Stomach		X	X
Testes	X	X	X
Thymus	X	X	X
Thyroids with parathyroids	X	X	X
Tongue		X	X
Trachea		X	X
Ureters		X	X
Urinary bladder		X	X
Uterus (horns and cervix)		X	X
Vagina		X	X

*: 40 cm of jejunum were measured and weighed.

Results:

Clinical Signs: Clinical signs included ptyalism, hyperactivity, abnormal vocalization, vomiting, ventral recumbancy and abdominal breathing across all doses. Some of these clinical signs (lateral and ventral recumbency, abdominal breathing, hyperactivity, and vomiting) were also observed in control animals.

Mortality: There were no unscheduled deaths.

Body Weight: The mean initial and final body weights of control males were 8.5 and 10.7 kg, respectively. The mean initial and final body weight of control females were 7.9 and 10.3 kg, respectively. There were no significant treatment-related changes.

Food Consumption: The food consumption data was not provided. However, the sponsor stated that there were no significant treatment-related changes.

Hematology: The following statistically significant differences were noted between controls and males at 10 mg/kg/day in Week 12: higher red blood cell count (7.14 vs. 6.14 G/L), hemoglobin concentration (16.9 vs. 14.5 g/dL) and packed cell volume (0.49 vs. 0.42). In Week 25 and 51, no differences from controls were noted in any hematological parameters. There were no significant treatment-related effects on hematology parameters during the recovery period.

Serum Chemistry: There were no significant treatment-related changes.

Urinalysis: There were no significant treatment-related changes.

Toxicokinetics: Mean plasma levels of Genz-112638 increased rapidly after treatment on Day 1, to reach a mean Cmax at 0.5 to 1.0 hours (Tmax) post-dosing. The Cmax and AUC_{0-t} increased with doses in a dose proportional manner for both sexes, except at 2 mg/kg/day and in females at 5 mg/kg/day which showed low exposure. At 2 mg/kg/day, a marked gender effect was noted since the exposure was approximately 2-fold higher in the males than in the females. At 5 mg/kg/day, the exposure was 10-fold higher in males than in females. No time effect was evident in any dose group. The TK parameters are shown in the following table (from page 34 of the study report).

Reference ID: 3501244

Toxicokinetic parameters of Genz-112638 on day 1 and in weeks 26 and 52 after oral administration to beagle dogs

Sampling period	Kinetic parameter	Unit	Group, dose-level (mg/kg/day), sex					
			2		3		4	
			2		5		10	
			M	F	M	F	M	F
Day 1	C_{max}	(ng/mL)	196	92.6	816	80.2	1239	823
	t_{max}	(h)	0.6	0.5	0.6	0.6	0.6	0.6
	$AUC_{(0-t)}$	(ng*h/mL)	324	156	1483	138	2662	1413
	Ratio C_{max}/dose		98	46	163	16	124	82
	Ratio AUC/dose		162	78	297	28	266	141
Week 26	C_{max}	(ng/mL)	322	193	1063	122	2527	920
	t_{max}	(h)	0.6	0.5	0.5	0.6	0.5	0.8
	$AUC_{(0-t)}$	(ng*h/mL)	766	404	2326	234	5224	1891
	Ratio C_{max}/dose		161	97	213	24	253	92
	Ratio AUC/dose		383	202	465	47	522	189
Week 52	C_{max}	(ng/mL)	220	141	942	102	1606	1633
	t_{max}	(h)	0.5	0.5	0.8	0.8	0.7	1.0
	$AUC_{(0-t)}$	(ng*h/mL)	531	248	2297	203	4460	3115
	Ratio C_{max}/dose		110	71	188	20	161	163
	Ratio AUC/dose		266	124	459	41	446	312

M: male, F: female.

Ophthalmoscopy: There were no treatment-related effects.

Electrocardiography: Negative T-wave and increased R or S wave amplitude were noted throughout the treatment period with the same incidence and severity in control and treated animals. Thus, these changes were considered to be of no toxicological significance.

Organ Weight: There were no treatment-related effects.

Gross Pathology: There were no significant treatment-related findings.

Histopathology: Treatment-related histopathological changes were observed in the lung (peribronchiolar chronic inflammation/fibrosis at 10 mg/kg/day), heart (mesothelial hyperplasia in the atria of the heart was noted only in treated female animals), and testes (seminiferous epithelial degeneration and segmental hypoplasia in the testes were seen with a greater incidence in the treated groups). The following table shows the histopathological findings in the lung, heart and testes.

Organs	Sex (n = 4/sex)	Dose (mg/kg/day)			
		0	2	5	10
LUNG					
Focal pneumonitis	Male	0	0	1	1
	Female	-	-	-	-
Perivascular lymphocytic cuffing	Male	0	0	0	4
	Female	0	2	1	1
Focal interstitial fibrosis	Male	0	0	0	1
	Female	1	0	0	0
Peribronchiolar chronic inflammation/fibrosis	Male	0	0	0	1
	Female	1	0	0	1
Bronchiolar/interstitial mineralization	Male	1	0	0	0
	Female	0	0	2	0
Granulomas	Male	1	0	1	0
	Female	-	-	-	-
HEART					
Cystic glands	Male	0	0	0	1
	Female	0	2	1	1
Localized mesothelial hyperplasia	Male	-	-	-	-
	Female	0	1	1	1
TESTES					
Semniferous epithelial degeneration (left)	Male	1	1	0	2
	Female				
Semniferous epithelial degeneration (right)	Male	1	2	1	3
	Female				
Segmental hypoplasia (left)	Male	0	0	1	1
	Female				

-: Not observed

In a 52-week oral (gavage) study in Beagle dogs, animals were treated at 2, 5 and 10 mg/kg/day. The target organ of toxicity appeared to be the lung (peribronchiolar chronic inflammation/fibrosis at 10 mg/kg/day), heart (mesothelial hyperplasia in the atria of the heart was noted only in treated female animals), and testes (seminiferous epithelial degeneration and segmental hypoplasia in the testes were seen with a greater incidence in the treated groups). The NOAEL could not be determined as treatment-related findings were observed at all dose levels. However, 10 mg/kg/day appeared to be a tolerated dose.

Addendum: Histopathological changes observed in the lung e.g., peribronchiolar chronic inflammation/fibrosis was more prominent at 10 mg/kg/day group than in controls; however, there was no dose response, similar incidence in control and treated group and were also consistent with the common background. Mesolthelial hyperplasia in the heart was seen only in females and there was no dose response. Histopathological changes in the testes (seminiferous epithelial degeneration and segmental hypoplasia were seen with a greater incidence in the treated groups) were not dose-related and were also seen in control animals. Based on these, the NOAEL may be considered as 10 mg/kg/day.

7 Genetic Toxicology

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

Ames Test with Genz-112638 (GT-157-TX-1)

The review of the above study is incorporated below from the pharmacology review of IND 67,589 dated July 30, 2004 by Dr. Dylan Yao (DMEP).

Study title: Bacterial reverse mutation assay

Key findings: The study is valid. The results of the Ames assay indicated that, under the conditions of this study, Genz-112638 did not cause a positive mutagenic response in either the presence or absence of S9 activation system.

Study no.: GT-157-TX-1

Volume #, and page #: Vol 2.9, and page 101
Conducting laboratory and location: (b) (4) (b) (4)
Date of study initiation: April 21, 2003
GLP compliance: y
QA reports: yes (x) no ( )
Drug, lot #, and % purity: Genz-112638, lot #909246, and purity 99%

Methods

Strains/species/cell line: *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537and *Escherichia coli* tester strain WP2 *uvr*A

Doses used in definitive study: 50, 150, 500, 1500 and 5000 µg per plate

Basis of dose selection: Dose-range finding study was performed with 8 dose levels of the test article at 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 µg/plate to decide the level of cytotoxicity (study B1).

Negative controls: Sterile distilled water

Positive controls: See the Table below

Positive Control	Concentration (µg/plate)
2-aminoanthracene (b) (4) Lot No. (b) (4) Exp. Date 14-Sep-2005 CAS No (b) (4) Purity 97.4%	1.0
	10
2-nitrofluorene (b) (4) Lot No. (b) (4) Exp. Date 08-Mar-2006 CAS No. (b) (4) Purity 99.9%	1.0
Sodium azide (b) (4) Lot No. (b) (4) Exp. Date 05-Jan-2004 CAS No. (b) (4) Purity 99.8%	1.0
9-aminoacridine (b) (4) Lot No. (b) (4) Exp. Date 08-Nov-2004 CAS No. (b) (4) Purity >97%	75
Methyl methanesulfonate (b) (4) Lot No. (b) (4) Exp. Date 23-Jul-2004 CAS No. (b) (4) Purity 99.9%	1,000

Incubation and sampling times: Plates were incubated for ~48 to 72 hrs at 37±2°C and then counted

Results

Study validity: Triple plates at each dose level of the test compound as well as negative and positive controls were incubated with either the presence or absence of rat liver S9 in the confirmatory assay (study B2); revertant colonies were counted either entirely by automated colony counter or entirely by hand unless the plate exhibited toxicity; criteria for positive results were valid, which were based on a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article; data sets for tester strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than 3-times the mean vehicle control value; data sets for TA98, TA100 and WP2 *uvr*A were judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than 2-times the mean vehicle control value. No contaminant colonies were observed on the sterility plates for the test article dilutions and

the S9 mixes. Toxicity was observed beginning at 1500 or at 5000 µg/plate, the maximum dose plated in the confirmatory assay was 5000 µg/plate. The assay is deemed valid.

Study outcome: The results of confirmatory mutation assay (study B2) were summarized in the table below (scanned from the sponsor's data):

Test Article Id : Genz-112638
Study Number : (b) (4) Experiment No : B2

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	13 ± 4	126 ± 7	10 ± 3	6 ± 1	12 ± 1
50	10 ± 2	140 ± 12	12 ± 3	6 ± 2	10 ± 1
150	12 ± 1	134 ± 6	11 ± 1	4 ± 1	13 ± 3
500	11 ± 1	135 ± 18	11 ± 3	7 ± 2	12 ± 1
1500	13 ± 1	154 ± 5	10 ± 1	8 ± 1	12 ± 1
5000	0 ± 0	37 ± 15	0 ± 0	0 ± 0	0 ± 0
Positive	141 ± 16	572 ± 134	360 ± 17	1256 ± 171	118 ± 5

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	16 ± 3	154 ± 15	13 ± 3	7 ± 2	12 ± 2
50	17 ± 4	153 ± 10	11 ± 5	4 ± 1	10 ± 3
150	17 ± 1	162 ± 14	11 ± 1	6 ± 2	13 ± 3
500	16 ± 3	159 ± 7	11 ± 4	5 ± 1	12 ± 0
1500	18 ± 4	170 ± 7	10 ± 1	8 ± 0	13 ± 1
5000	7 ± 7	103 ± 7	1 ± 1	2 ± 4	0 ± 0
Positive	467 ± 153	693 ± 9	141 ± 15	70 ± 9	712 ± 50

Vehicle = Vehicle Control
Positive = Positive Control
Plating aliquot: 100 µL

There were no positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. It was concluded that Genz-112638 did not cause a positive mutagenic response under the assay conditions.

7.2 *In Vitro* Assays in Mammalian Cells

In Vitro Mammalian Chromosome Aberration Test (GT-157-TX-2)

The review of the above study is incorporated below from the pharmacology review of IND 67,589 dated July 30, 2004 by Dr. Dylan Yao (DMEP).

Study title: *In vitro* mammalian chromosome aberration test

Key findings: The assay is deemed valid. Genz-112638 did not show significant increase in either structural or numerical chromosome aberration in HPBL cultures.

Study no.: GT-157-TX-2

Volume #, and page #: Vol 2.9, and page 180
Conducting laboratory and location: (b) (4)

Date of study initiation: April 17, 2003

GLP compliance: y
QA reports: yes (x) no ( )
Drug, lot #, and % purity: Genz-112638, lot #909246, and purity 99%

Methods

Strains/species/cell line: Human peripheral blood lymphocytes (HPBL)

Doses used in definitive study:

Treatment Condition	Treatment Time	Recovery Time	Dose Levels (µg/mL)
Non-activated	4 hr	16 hr	3.13, 6.25, 12.5, 25, 50, 60, 80
	20 hr	0 hr	1.56, 3.13, 6.25, 12.5, 25, 50, 60
S9 activated	4 hr	16 hr	3.13, 6.25, 12.5, 25, 50, 60, 80

Basis of dose selection: Selection of dose levels was based on toxicity. Substantial toxicity (at least 50% reduction in mitotic index relative to the solvent control) was observed at doses ⩾ 47.95 µg/mL in the non-activated and S9 activated 4 hr exposure groups. Substantial toxicity was observed at dose level 14.385 µg/mL in the non-activated 20 hour exposure group. Based on these findings, the doses chosen for the chromosome aberration assay ranged from 3.13 to 80 µg/mL for both the non-activated and the S9 activated 4 hr exposure groups, and from 1.56 to 60 µg/mL for the non-activated 20 hr exposure group.

Negative controls: Solvent vehicle (water) for the test article

Positive controls: MMC, 0.3 and 0.6 µg/mL, in the non-activated study; CP, 20 and 40 µg/mL, in the S9 activated study.

Incubation and sampling times: The cells were treated for 4 and 20 hrs in the non-activated groups and for 4 hrs in the S9 activated group. All cells were harvested 20 hrs after treatment initiation.

Results

Study validity: The dose selection was based on cytotoxicity. Double flasks on each dose concentration were used. A positive result was determined if the percentage of cells with chromosome aberrations was statistically significantly increased relative to the negative control. The study is valid.

Study outcome: The positive and negative controls fulfilled the requirement for a valid test. The percentage of cells with structural or numerical aberrations in the test article-treated groups was not significantly increased above that of negative control at any dose level. The results of the assay are summarized in the following table (scanned from the sponsor's data):

Treatment (µg/mL)	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored	Aberrations Per Cell (Mean +/- SD)	Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
Water	-	4	9.0	200	0.000 ±0.000	0.0	0.0
Genz-112638							
12.5	-	4	7.5	200	0.000 ±0.000	0.0	0.0
25	-	4	7.1	200	0.000 ±0.000	0.0	0.0
50	-	4	4.3	200	0.000 ±0.000	0.0	0.0
MMC, 0.6	-	4	7.8	200	0.115 ±0.335	0.0	11.0**
Water	+	4	8.5	200	0.000 ±0.000	0.0	0.0
Genz-112638							
12.5	+	4	8.2	200	0.000 ±0.000	0.0	0.0
25	+	4	7.1	200	0.000 ±0.000	0.0	0.0
50	+	4	4.0	200	0.000 ±0.000	0.0	0.0
CP, 20	+	4	7.7	200	0.100 ±0.317	0.0	9.5**
Water	-	20	8.2	200	0.000 ±0.000	0.0	0.0
Genz-112638							
6.25	-	20	7.0	200	0.000 ±0.000	0.0	0.0
12.5	-	20	7.6	200	0.000 ±0.000	0.5	0.0
25	-	20	3.7	200	0.000 ±0.000	0.0	0.0
MMC, 0.3	-	20	7.7	200	0.115 ±0.377	0.0	9.5**

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

Bone Marrow Micronucleus Test by Oral Route in Mice (GT-157-TX-16)

The review of the above study is incorporated below from the pharmacology review of IND 67,589 dated June 29, 2006 by Dr. Tamal K. Chakraborti (DMEP).

Study Title: *In Vivo* Bone Marrow Micronucleus Test by Oral Route in Mice

Key Findings: Negative

Study No.: GT-157-TX-16

Volume #, and Page #: Vol. 1, page 1
Conducting Laboratory and Location: (b) (4)
Date of Study Initiation: January 24, 2004
GLP Compliance: A statement of compliance was included.

QA Reports: yes (X) no ( )
Drug, Lot #, and % Purity: Genz-112638, Lot No. 909246, and 100%

Methods:

Strains/species: Swiss mice

Doses used in definitive study: 68.75, 137.5 and 275 mg/kg/day (10 ml/kg) for 2 days (two treatments 24 hours apart)

Basis of dose selection: In order to determine the highest dose-level, several preliminary tests were performed on groups of six animals (n =3/sex/dose) at 200, 275, 350, 500 and 2000 mg/kg/day (administered twice). The interval between each administration was 24 hours. At 2000 mg/kg/day, 2 of 3 males and 3 of 3 females died immediately after the first administration, the surviving animal showed hypoactivity and piloerection. At 500 mg/kg/day, 1 of 3 males and 1 of 3 females died during the 2 hours following the first treatment, surviving animals showed hypoactivity and piloerection. At 350 mg/kg/day, 1 of 3 females died immediately after the first treatment and 1 of 2 surviving females died immediately after the second treatment. Hypoactivity, dyspnea and piloerection were noted in males as well as in the surviving female. At 275 mg/kg/day, no mortality was induced in treated animals (2 males and 3 females) and piloerection was noted in all treated animals following the second treatment. At 200 mg/kg/day, no mortality and no clinical signs were noted following both treatments. Consequently, 275 mg/kg/day was selected as the top dose-level for the main test. The two other selected dose-levels were 68.75 and 137.5 mg/kg/day.

Negative controls: Water for injection

Positive controls: Cyclophosphamide (CPA), oral, 50 mg/kg, one treatment. The study design is shown below (from page 11 of the study report).

Treatment	Animals per group		Target dose-level mg/kg/day	Sampling time after the last treatment
Vehicle	Principal	5 males 5 females	0	24 h
Low dose-level	Principal	5 males 5 females	68.75	24 h
Intermediate dose-level	Principal	5 males 5 females	137.5	24 h
High dose-level	Principal	8 males (1) 8 females (1)	275	24 h
Positive control CPA	Principal	5 males 5 females	50	24 h

(1) Since no mortality occurred, only the five surviving animals of each sex were subjected to bone marrow analysis. The supplementary animals were humanely killed and bone marrow smears were not prepared.

Incubation and sampling times: The animals of the treated and vehicle control groups were sacrificed 24 hours after the last treatment and the animals of the positive control group were sacrificed 24 hours after the single treatment. For each animal, the number of the micronucleated polychromatic erythrocytes (MPE) was counted in 2000 polychromatic erythrocytes. The polychromatic (PE) and normochromatic (NE) erythrocyte ratio was established by scoring a total of 1000 erythrocytes (PE + NE).

Results:

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Two slides were examined per animal. A result to be considered positive, a statistically significant increase in the frequency of MPE must be demonstrated when compared to the concurrent vehicle control group. Reference to historical data, or other considerations of biological relevance was also taken into account in the evaluation of data obtained.

Study outcome: Negative. The positive control produced positive results. The following table shows the results of test.

Table 3: Results of the cytogenetic test: data summary

Group	Doses (mg/kg/day)	MPE/1000PE mean	(sd)		PE/NE ratio mean	(sd)	Time of sacrifice after the last administration
Males							
Vehicle	-	0.4	(0.7)		0.2	(0.1)	
Test item	68.75	1.3	(1.3)		0.3	(0.1)	24 h
	137.5	1.0	(0.6)		0.4	(0.1)	
	275	0.5	(0.4)		0.4	(0.1)	
Cyclophosphamide	50 mg/kg	24.0	(6.2)	***	0.8	(0.3)	
Females							
Vehicle	-	1.4	(1.0)		0.6	(0.1)	
Test item	68.75	0.6	(0.5)		0.6	(0.1)	
	137.5	0.6	(0.5)		0.9	(0.4)	24 h
	275	0.4	(0.4)		0.6	(0.2)	
Cyclophosphamide	50 mg/kg	17.0	(5.4)	***	0.9	(0.2)	

Five animals per group

Route: Oral
- vehicle and test item: two administrations separated by a 24-hour interval
- cyclophosphamide: one administration

Vehicle: water for injections

MPE: Micronucleated Polychromatic Erythrocytes
PE: Polychromatic Erythrocytes
NE: Normochromatic Erythrocytes
sd: Standard Deviation

Statistical tests used : *** $p < 0.001$
The 2 x 2 contingency table for MPE (except for the lowest dose group of males where the Mann-Withney test was used)
Student's "t" test for PE/NE ratio

7.4 Other Genetic Toxicity Studies

Study title: Ames Test with Genz-399240-AA (Metabolite)

Study no.:	GT-157-TX-60
Study report location:	EDR 4.2.3.7.5
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 3, 2012
Date of study completion:	May 2, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Genz-339240-AA, Batch No. 007, 99%

Key Study Findings: Negative

Methods: Plate incorporation method

Strains:	*Salmonella typhimurium*: TA 1535, TA 1537, TA 98, TA 100 and TA 102
Concentrations in definitive study:	312.5, 625, 1250, 2500 and 5000 µg/plate
Basis of concentration selection:	Preliminary assay at 10, 100, 500, 1000, 2500 and 5000 µg/plate
Negative control:	0.9% NaCl
Positive control:	Shown below (from page 9 of the report)
Formulation/Vehicle:	0.9% NaCl
Incubation & sampling time:	48-72 hours

Positive control: The following table (from page 9 of the report) shows the positive controls.

2.1.1.3 Positive controls

Six known mutagens, dissolved in dimethylsulfoxide (except for Mitomycin C which was dissolved in distilled water), were used to check the sensitivity of the test system:

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

Study Validity: This study was considered valid if the following criteria were met.

- The number of revertants in the vehicle controls is consistent with the historical data of the testing facility
- The number of revertants in the positive controls is higher than that of the vehicle controls [at least 2-fold increase (for the TA 98, TA 100 and TA 102 strains) and at least 3-fold increase (for the TA 1535 and TA 1537 strains)] and is consistent with the historical data of the testing facility

Evaluation criteria: A 2-fold increase (for the TA 98, TA 100 and TA 102 strains) or 3-fold increase (for the TA 1535 and TA 1537 strains) in the number of revertants

compared with the vehicle controls, for any strain, with evidence of a dose-relationship was considered as a positive result.

Results: Genz-339240-AA did not increase the number of revertants, either with or without S9 mix, in any of the five strains under the conditions of the study. The following (from page 19-20 of the report) table shows the results of the confirmatory test.

Table 2. First experiment
Direct plate incorporation method

- revertant colony numbers per plate using strains :- TA 1535, TA 1537, TA 98

Strain	Compound	Dose level per plate	S-9 mix	Mean revertant colony counts	SD	Ratio treated / solvent	Individual revertant colony counts
TA 1535	0.9% NaCl		-	14	7		10, 11, 22
	TEST ITEM	312.5 µg	-	25	3	1.7	22, 28, 24
		625 µg	-	12	6	0.9	17, 6, 14
		1250 µg	-	18	1	1.3	17, 18, 19
		2500 µg	-	10	2	0.7	11, 7, 11
		5000 µg	-	14	4	1.0	10, 13, 18
	NAN3	1 µg	-	662	49	46.2	607, 703, 675
	0.9% NaCl		+	17	6		14, 14, 24
	TEST ITEM	312.5 µg	+	15	3	0.9	13, 14, 19
		625 µg	+	22	6	1.2	29, 17, 19
		1250 µg	+	15	4	0.8	17, 10, 17
		2500 µg	+	13	2	0.7	14, 11, 13
		5000 µg	+	20	16	1.2	38, 7, 16
	2AM	2 µg	+	498	61	28.7	464, 462, 569
TA 1537	0.9% NaCl		-	7	1		8, 7, 7
	TEST ITEM	312.5 µg	-	9	3	1.2	5, 10, 11
		625 µg	-	8	4	1.1	12, 8, 5
		1250 µg	-	7	2	0.9	7, 5, 8
		2500 µg	-	9	5	1.3	14, 4, 10
		5000 µg	-	11	3	1.5	8, 14, 10
	9 AA	50 µg	-	367	87	50.0	461, 289, 351
	0.9% NaCl		+	6	2		5, 4, 8
	TEST ITEM	312.5 µg	+	7	3	1.3	11, 5, 6
		625 µg	+	6	1	1.1	6, 6, 7
		1250 µg	+	7	1	1.2	6, 6, 8
		2500 µg	+	7	3	1.3	5, 10, 7
		5000 µg	+	9	1	1.6	10, 10, 8
	2AM	2 µg	+	64	8	11.4	69, 69, 55
TA 98	0.9% NaCl		-	20	3		23, 18, 19
	TEST ITEM	312.5 µg	-	29	5	1.5	24, 34, 30
		625 µg	-	18	2	0.9	17, 20, 16
		1250 µg	-	18	5	0.9	17, 13, 23
		2500 µg	-	19	12	1.0	23, 29, 6
		5000 µg	-	20	13	1.0	8, 18, 34
	2NF	0.5 µg	-	151	28	7.5	171, 162, 119
	0.9% NaCl		+	26	4		31, 23, 25
	TEST ITEM	312.5 µg	+	25	13	1.0	19, 17, 40
		625 µg	+	27	9	1.0	18, 35, 28
		1250 µg	+	28	4	1.1	25, 28, 32
		2500 µg	+	30	10	1.2	42, 25, 24
		5000 µg	+	18	9	0.7	23, 24, 8
	2AM	2 µg	+	722	35	27.4	708, 761, 696

SD Standard deviation
\- Absence of S-9
\+ Presence of S-9

TEST ITEM: Genz-399240-AA

Table 2 (continued)

- revertant colony numbers per plate using strains :- TA 100 and TA 102

Strain	Compound	Dose level per plate	S-9 mix	Mean revertant colony counts	SD	Ratio treated / solvent	Individual revertant colony counts
TA 100	0.9% NaCl		-	122	9		119, 114, 132
	TEST ITEM	312.5 µg	-	138	16	1.1	128, 129, 156
		625 µg	-	137	17	1.1	156, 133, 122
		1250 µg	-	135	9	1.1	145, 127, 132
		2500 µg	-	143	31	1.2	178, 122, 128
		5000 µg	-	158	31	1.3	127, 189, 158
	NAN3	1 µg	-	639	73	5.3	668, 693, 556
	0.9% NaCl		+	106	6		107, 111, 99
	TEST ITEM	312.5 µg	+	113	11	1.1	123, 116, 101
		625 µg	+	95	3	0.9	96, 92, 97
		1250 µg	+	104	6	1.0	109, 97, 105
		2500 µg	+	101	19	1.0	96, 84, 122
		5000 µg	+	108	10	1.0	101, 120, 103
	BAP	5 µg	+	394	37	3.7	418, 351, 412
TA 102	0.9% NaCl		-	456	101		569, 375, 423
	TEST ITEM	312.5 µg	-	532	91	1.2	464, 496, 635
		625 µg	-	528	43	1.2	495, 576, 513
		1250 µg	-	430	21	0.9	453, 413, 424
		2500 µg	-	461	70	1.0	537, 400, 447
		5000 µg	-	442	16	1.0	438, 429, 460
	MMC	0.5 µg	-	2162	119	4.7	2222, 2025, 2240
	0.9% NaCl		+	533	95		592, 584, 423
	TEST ITEM	312.5 µg	+	447	53	0.8	490, 464, 388
		625 µg	+	590	94	1.1	632, 656, 483
		1250 µg	+	515	46	1.0	465, 556, 525
		2500 µg	+	453	132	0.8	601, 408, 349
		5000 µg	+	558	143	1.0	435, 525, 715
	2AM	10 µg	+	2833	431	5.3	2535, 3327, 2637

SD Standard deviation
- Absence of S-9
+ Presence of S-9

TEST ITEM: Genz-399240-AA

Study title: I*n Vitro* Mammalian Chromosome Aberration Test with Genz-399240-AA (Metabolite) in Cultured Human Lymphocytes

Study no.:	GT-157-TX-62
Study report location:	EDR, Section: 4.2.3.7.5
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 18, 2012
Date of study completion:	October 19, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Genz-399240-AA, Batch No. 007, 99%

Key Study Findings: Negative

Methods:

Cell line:	Human peripheral blood lymphocytes (HPBL)
Concentrations in definitive study:	• 3.91, 7.81, 15.6, 31.3, 62.5, 125, 250 and 500 µg/mL for the first experiment, both with and without S9 mix • 3.91, 7.81, 15.6, 31.3, 62.5, 125, 250 and 500 µg/mL for the second experiment without S9 mix
Basis of concentration selection:	Cytotoxicity
Negative control:	0.9% NaCl
Positive control:	• Without S9 mix: Mitomycin C (MMC), used at final concentrations of 2 and 3 µg/mL for the 3-hour treatment and at 0.2 and 0.3 µg/mL for the continuous treatment • With S9 mix: Cyclophosphamide (CPA), used at final concentrations of 12.5 and 25 µg/mL
Formulation/Vehicle:	0.9% NaCl
Incubation & sampling time:	First experiment: 3 h, harvest = 20 h Second experiment: 20 h, harvest = 20 h

Study Validity: This study is considered valid since the following criteria were met.

- The frequency of cells with structural chromosome aberration in the vehicle controls (≤ 5%) was consistent with the historical data.
- The frequency of cells with structural chromosome aberration in the positive controls was significantly higher than that of the vehicle controls and consistent with the historical data.

Criteria for positive results: A test item was considered positive for inducing chromosomal aberrations if a statistically significant increase in the frequency of cells with structural chromosome aberration was observed with evidence of a dose-relationship.

Criteria for a negative response: A test item was considered negative for inducing chromosomal aberrations if no significant increase is observed in the number of cells with chromosomal aberrations for any of the dose-levels analyzed, at any treatment durations. Reference to historical data or other considerations of biological relevance were also taken into account in the evaluation of the findings.

Results: Under the experimental conditions of this study, Genz-399240-AA did not induce chromosome aberrations in cultured human lymphocytes, in the presence or the absence of S9. The following (from pages 20, 22, and 24 of the report) tables show the results.

Table 2. First experiment without S9 mix: chromosome aberration (3-hour treatment, 20-hour harvest)

Doses µg/mL	Slide Nb.	Nb. of cells scored	NA	Structural chromosome aberrations (type and number)									Cells with structural chromosome aberrations			
				G	Chromatid		Chromosome		MA	PU	Total +G	Total -G	Nb. +G	Frequency (%) +G	Nb. -G	Frequency (%) -G
					D	Exch	D	Exch								
0	38 C1	100	2	0	0	0	1	0	0	0	**4**	**2**	1	**2.0**	1	**1.0**
	12 C2	100	2	2	1	0	0	0	0	0			3		1	
125	6 C1	100	1	0	0	0	0	0	0	0	**0**	**0**	0	**0.0**	0	**0.0**
	14 C2	100	0	0	0	0	0	0	0	0			0		0	
250	26 C1	100	0	0	1	0	0	0	0	0	**2**	**2**	1	**1.0**	1	**1.0**
	40 C2	100	1	0	1	0	0	0	0	0			1		1	
500	22 C1	100	0	0	0	0	0	0	0	0	**1**	**1**	0	**0.5**	0	**0.5**
	7 C2	100	1	0	0	0	1	0	0	0			1		1	
MMC 3 µg/mL	33 C1	50	0	0	27	13	3	0	0	0	**76**	**71**	23	**41.0**	23	**39.0**
	8 C2	50	0	5	14	1	13	0	0	0			18		16	***

Nb.: number
NA: numerical aberrations, G: gap, D: deletion, Exch: exchange, MA: multiple aberrations, PU: pulverization.
C1: culture 1 (Male)
C2: culture 2 (Male)
0: vehicle control (0.9% NaCl)
MMC: Mitomycin C
Statistical analysis: χ^2 test *** : $p < 0.001$ (performed only for cells with structural aberrations excluding gaps)

Table 4. Second experiment without S9 mix: chromosome aberration (20-hour treatment; 20-hour harvest)

Doses µg/mL	Slide Nb.	Nb. of cells scored	NA	Structural chromosome aberrations (type and number)									Cells with structural chromosome aberrations			
				G	Chromatid		Chromosome		MA	PU	Total +G	Total -G	Nb. +G	Frequency (%) +G	Nb. -G	Frequency (%) -G
					D	Exch	D	Exch								
0	58 C1	100	0	1	0	0	2	0	0	0	**4**	**3**	3	**2.0**	2	**1.5**
	46 C2	100	0	0	1	0	0	0	0	0			1		1	
125	64 C1	100	0	0	1	0	1	0	0	0	**2**	**2**	2	**1.0**	2	**1.0**
	53 C2	100	1	0	0	0	0	0	0	0			0		0	
250	54 C1	100	0	0	2	0	0	0	0	0	**3**	**3**	2	**1.5**	2	**1.5**
	65 C2	100	1	0	0	0	1	0	0	0			1		1	
500	66 C1	100	1	1	0	0	0	0	0	0	**4**	**2**	1	**2.0**	0	**1.0**
	56 C2	100	0	1	2	0	0	0	0	0			3		2	
MMC	45 C1	100	0	1	7	0	0	0	0	0	**17**	**13**	8	**7.5**	7	**6.5**
0.2 µg/mL	57 C2	100	0	3	1	1	4	0	0	0			7		6	*

Nb.: number
NA: numerical aberrations, G: gap, D: deletion, Exch: exchange, MA: multiple aberrations, PU: pulverization.
C1: culture 1 (Female)
C2: culture 2 (Female)
0: vehicle control (0.9% NaCl)
MMC: Mitomycin C
Statistical analysis: χ^2 test *: $p < 0.05$ (performed only for cells with structural aberrations excluding gaps)

Table 6. First experiment with S9 mix: chromosome aberration
(3-hour treatment; 20-hour harvest)

Doses µg/mL	Slide Nb.	Nb. of cells scored	NA	Structural chromosome aberrations (type and number) G	Chromatid D	Chromatid Exch	Chromosome D	Chromosome Exch	MA	PU	Total +G	Total -G	Cells with structural chromosome aberrations Nb. +G	Frequency (%) +G	Nb. -G	Frequency (%) -G
0	20 C1	100	0	0	0	0	0	0	0	0	**1**	**0**	0	**0.0**	0	**0.0**
	4 C2	100	0	1	0	0	0	0	0	0			0		0	
125	41 C1	100	0	0	1	0	1	0	0	0	**4**	**3**	2	**2.0**	2	**1.5**
	42 C2	100	0	1	1	0	0	0	0	0			2		1	
250	15 C1	100	0	1	1	0	0	0	0	0	**3**	**1**	2	**1.5**	1	**0.5**
	25 C2	100	0	1	0	0	0	0	0	0			1		0	
500	44 C1	100	0	0	0	0	2	0	0	0	**3**	**3**	2	**1.5**	2	**1.5**
	43 C2	100	1	0	1	0	0	0	0	0			1		1	
CPA 12.5 µg/mL	21 C1	50	0	3	13	2	0	0	0	0	**38**	**33**	11	**24.0**	11	**23.0**
	3 C2	50	0	2	14	1	3	0	0	0			13		12	***

Nb.: number
NA: numerical aberrations, G: gap, D: deletion, Exch: exchange, MA: multiple aberrations, PU: pulverization.
C1: culture 1 (Male)
C2: culture 2 (Male)
0: vehicle control (0.9% NaCl)
CPA: Cyclophosphamide
Statistical analysis: χ^2 test ***: $p < 0.001$ (performed only for cells with structural aberrations excluding gaps)

Study title: Ames Test with (b) (4) **(Impurity)**

Study no.: GT-157-TX-55
Study report location: EDR 4.2.3.7.6
Conducting laboratory and location: (b) (4)
Date of study initiation: April 7, 2011
Date of study completion: September 6, 2011
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: (b) (4) Batch No. 004, 99.45%

Key Study Findings: Negative

Methods: Plate incorporation (first test), pre-incubation (second study, +S9)

Strains:	*Salmonella typhimurium*: TA 1535, TA 1537, TA 98, TA 100 and TA 102
Concentrations in definitive study:	312.5, 625, 1250, 2500 and 5000 µg/plate
Basis of concentration selection:	Preliminary assay at 10, 100, 500, 1000, 2500 and 5000 µg/plate
Negative control:	Water
Positive control:	Shown below (from page 12 of the report)
Formulation/Vehicle:	Water
Incubation & sampling time:	48-72 hours

Positive control: The following table (from page 12 of the report) shows the positive controls.

2.1.4 Positive controls

Six known mutagens, dissolved in dimethylsulfoxide (except for Mitomycin C which was dissolved in distilled water), were used to check the sensitivity of the test system:

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

Study Validity: This study was considered valid if the following criteria were met.

- The number of revertants in the vehicle controls is consistent with the historical data of the testing facility
- The number of revertants in the positive controls is higher than that of the vehicle controls (at least 2-fold increase (for the TA 98, TA 100 and TA 102 strains) and at least 3-fold increase (for the TA 1535 and TA 1537 strains)) and is consistent with the historical data of the testing facility.

Evaluation criteria: A 2-fold increase (for the TA 98, TA 100 and TA 102 strains) or 3-fold increase (for the TA 1535 and TA 1537 strains) in the number of revertants compared with the vehicle controls, in any strain, with evidence of a dose-relationship was considered as a positive result.

Results: (b) (4) did not increase the number of revertants, either with or without S9 mix, for any of the five strains under the conditions of the study. The following (from page 19-22 of the report) table shows the results.

Table 2. First experiment
Direct plate incorporation method

- revertant colony numbers per plate using strains :- TA 1535, TA 1537, TA 98

Strain	Compound	Dose level per plate	S-9 mix	Mean revertant colony counts	SD	Ratio treated / solvent	Individual revertant colony counts
TA 1535	WATER FOR INJECTIONS		-	15	2		17, 13, 16
	TEST ITEM	312.5 µg	-	13	2	0.8	14, 13, 11
		625 µg	-	13	5	0.8	17, 13, 8
		1250 µg	-	11	4	0.7	14, 12, 7
		2500 µg	-	14	2	0.9	14, 16, 12
		5000 µg	-	9	4	0.6	12, 11, 5
	NAN3	1 µg	-	726	29	47.3	707, 759, 711
	WATER FOR INJECTIONS		+	20	2		20, 19, 22
	TEST ITEM	312.5 µg	+	17	1	0.8	18, 16, 16
		625 µg	+	16	1	0.8	17, 16, 16
		1250 µg	+	13	4	0.7	18, 10, 12
		2500 µg	+	20	5	1.0	24, 14, 22
		5000 µg	+	16	3	0.8	13, 18, 18
	2AM	2 µg	+	330	52	16.2	390, 303, 297
TA 1537	WATER FOR INJECTIONS		-	7	4		4, 6, 11
	TEST ITEM	312.5 µg	-	5	4	0.8	4, 2, 10
		625 µg	-	4	3	0.6	7, 4, 1
		1250 µg	-	10	3	1.4	13, 7, 10
		2500 µg	-	9	5	1.3	14, 8, 5
		5000 µg	-	6	2	0.8	4, 6, 7
	9 AA	50 µg	-	204	33	29.1	181, 241, 189
	WATER FOR INJECTIONS		+	6	1		6, 6, 7
	TEST ITEM	312.5 µg	+	8	3	1.3	7, 6, 12
		625 µg	+	9	2	1.4	7, 10, 10
		1250 µg	+	8	4	1.2	4, 7, 12
		2500 µg	+	6	2	1.0	8, 4, 7
		5000 µg	+	9	3	1.4	10, 5, 11
	2AM	2 µg	+	134	19	21.2	128, 155, 119
TA 98	WATER FOR INJECTIONS		-	20	3		23, 19, 18
	TEST ITEM	312.5 µg	-	21	8	1.1	20, 30, 14
		625 µg	-	20	11	1.0	14, 32, 13
		1250 µg	-	19	9	0.9	17, 11, 28
		2500 µg	-	15	4	0.7	10, 17, 17
		5000 µg	-	24	10	1.2	32, 28, 13
	2NF	0.5 µg	-	124	30	6.2	138, 89, 144
	WATER FOR INJECTIONS		+	26	6		20, 25, 32
	TEST ITEM	312.5 µg	+	28	2	1.1	26, 29, 29
		625 µg	+	26	6	1.0	20, 31, 26
		1250 µg	+	25	5	1.0	20, 30, 24
		2500 µg	+	33	4	1.3	30, 37, 31
		5000 µg	+	38	7	1.5	35, 46, 32
	2AM	2 µg	+	1282	19	50.0	1280, 1265, 1302

SD Standard deviation
- Absence of S-9
+ Presence of S-9

TEST ITEM: (b) (4)

Table 2 (continued)

- revertant colony numbers per plate using strains :- TA 100 and TA 102

Strain	Compound	Dose level per plate	S-9 mix	Mean revertant colony counts	SD	Ratio treated / solvent	Individual revertant colony counts
TA 100	WATER FOR INJECTIONS		-	129	9		126, 139, 122
	TEST ITEM	312.5 µg	-	124	13	1.0	120, 139, 114
		625 µg	-	128	10	1.0	135, 117, 133
		1250 µg	-	118	10	0.9	115, 110, 129
		2500 µg	-	130	10	1.0	141, 123, 125
		5000 µg	-	126	4	1.0	128, 129, 122
	NAN3	1 µg	-	591	98	4.6	704, 529, 540
	WATER FOR INJECTIONS		+	150	17		169, 138, 143
	TEST ITEM	312.5 µg	+	180	21	1.2	190, 194, 156
		625 µg	+	185	19	1.2	171, 206, 178
		1250 µg	+	187	14	1.2	178, 180, 204
		2500 µg	+	201	5	1.3	201, 206, 196
		5000 µg	+	162	5	1.1	165, 165, 156
	BAP	5 µg	+	659	66	4.4	721, 668, 589
TA 102	WATER FOR INJECTIONS		-	330	31		296, 338, 356
	TEST ITEM	312.5 µg	-	317	10	1.0	316, 308, 327
		625 µg	-	394	25	1.2	390, 420, 371
		1250 µg	-	373	33	1.1	388, 335, 396
		2500 µg	-	375	13	1.1	384, 361, 381
		5000 µg	-	367	12	1.1	356, 366, 380
	MMC	0.5 µg	-	2043	212	6.2	1806, 2213, 2110
	WATER FOR INJECTIONS		+	372	20		375, 351, 390
	TEST ITEM	312.5 µg	+	486	12	1.3	496, 489, 472
		625 µg	+	483	73	1.3	412, 478, 558
		1250 µg	+	527	31	1.4	492, 540, 549
		2500 µg	+	447	125	1.2	501, 537, 304
		5000 µg	+	388	5	1.0	389, 393, 383
	2AM	10 µg	+	4603	648	12.4	5124, 4807, 3878

SD Standard deviation
- Absence of S-9
+ Presence of S-9
TEST ITEM: (b) (4)

Table 3. Second experiment
Direct plate incorporation method (without S9 mix) and pre-incubation method (with S9 mix)

- revertant colony numbers per plate using strains :- TA 1535, TA 1537, TA 98

Strain	Compound	Dose level per plate	S-9 mix	Mean revertant colony counts	SD	Ratio treated / solvent	Individual revertant colony counts
TA 1535	WATER FOR INJECTIONS		-	12	11		7, 5, 25
	TEST ITEM	312.5 µg	-	11	2	0.9	10, 13, 10
		625 µg	-	12	7	1.0	20, 11, 6
		1250 µg	-	15	5	1.2	17, 10, 19
		2500 µg	-	14	5	1.1	16, 8, 17
		5000 µg	-	13	3	1.1	11, 12, 16
	NAN3	1 µg	-	712	19	57.7	693, 731, 711
	WATER FOR INJECTIONS		+	10	2		8, 11, 12
	TEST ITEM	312.5 µg	+	15	3	1.4	14, 18, 12
		625 µg	+	15	7	1.5	13, 23, 10
		1250 µg	+	19	4	1.9	19, 16, 23
		2500 µg	+	13	6	1.3	13, 19, 8
		5000 µg	+	15	6	1.5	18, 8, 20
	2AM	2 µg	+	202	12	19.5	213, 190, 202
TA 1537	WATER FOR INJECTIONS		-	6	2		4, 7, 7
	TEST ITEM	312.5 µg	-	4	2	0.7	6, 2, 4
		625 µg	-	6	3	1.0	4, 10, 4
		1250 µg	-	6	5	0.9	11, 2, 4
		2500 µg	-	6	2	0.9	7, 6, 4
		5000 µg	-	7	3	1.2	5, 10, 7
	9 AA	50 µg	-	403	63	67.2	416, 335, 459
	WATER FOR INJECTIONS		+	10	3		8, 14, 8
	TEST ITEM	312.5 µg	+	7	2	0.7	6, 10, 6
		625 µg	+	8	3	0.8	7, 11, 6
		1250 µg	+	11	5	1.1	16, 6, 10
		2500 µg	+	10	2	1.0	8, 10, 12
		5000 µg	+	10	3	1.0	10, 7, 13
	2AM	2 µg	+	187	46	18.7	201, 224, 135
TA 98	WATER FOR INJECTIONS		-	19	3		22, 16, 19
	TEST ITEM	312.5 µg	-	16	6	0.9	13, 23, 13
		625 µg	-	20	2	1.1	19, 20, 22
		1250 µg	-	20	3	1.1	20, 23, 18
		2500 µg	-	20	3	1.0	23, 17, 19
		5000 µg	-	16	2	0.9	17, 18, 14
	2NF	0.5 µg	-	242	34	12.8	281, 218, 228
	WATER FOR INJECTIONS		+	33	3		30, 34, 36
	TEST ITEM	312.5 µg	+	35	4	1.1	30, 38, 37
		625 µg	+	36	1	1.1	36, 36, 35
		1250 µg	+	35	6	1.0	31, 41, 32
		2500 µg	+	32	4	1.0	34, 35, 28
		5000 µg	+	30	5	0.9	24, 31, 34
	2AM	2 µg	+	1930	68	57.9	1855, 1946, 1988

SD Standard deviation
\- Absence of S-9
\+ Presence of S-9

TEST ITEM: (b) (4)

Table 3 (continued)

- revertant colony numbers per plate using strains :- TA 100 and TA 102

Strain	Compound	Dose level per plate	S-9 mix	Mean revertant colony counts	SD	Ratio treated / solvent	Individual revertant colony counts
TA 100	WATER FOR INJECTIONS		-	89	11		80, 102, 86
	TEST ITEM	312.5 µg	-	92	16	1.0	86, 110, 80
		625 µg	-	96	5	1.1	95, 91, 101
		1250 µg	-	96	6	1.1	96, 101, 90
		2500 µg	-	105	10	1.2	116, 96, 103
		5000 µg	-	112	9	1.3	103, 113, 121
	NAN3	1 µg	-	519	42	5.8	519, 561, 478
	WATER FOR INJECTIONS		+	133	16		152, 123, 125
	TEST ITEM	312.5 µg	+	134	9	1.0	132, 144, 126
		625 µg	+	125	13	0.9	119, 117, 140
		1250 µg	+	107	6	0.8	102, 114, 105
		2500 µg	+	149	26	1.1	169, 120, 157
		5000 µg	+	140	6	1.1	137, 147, 137
	BAP	5 µg	+	663	35	5.0	623, 686, 679
TA 102	WATER FOR INJECTIONS		-	324	12		311, 334, 328
	TEST ITEM	312.5 µg	-	295	42	0.9	323, 315, 246
		625 µg	-	314	42	1.0	272, 356, 313
		1250 µg	-	309	10	1.0	315, 315, 298
		2500 µg	-	336	14	1.0	350, 323, 334
		5000 µg	-	311	35	1.0	351, 298, 285
	MMC	0.5 µg	-	2417	80	7.5	2350, 2394, 2506
	WATER FOR INJECTIONS		+	460	13		447, 459, 473
	TEST ITEM	312.5 µg	+	465	28	1.0	469, 435, 491
		625 µg	+	495	28	1.1	464, 501, 519
		1250 µg	+	489	21	1.1	510, 487, 469
		2500 µg	+	523	57	1.1	462, 575, 531
		5000 µg	+	522	19	1.1	537, 527, 501
	2AM	10 µg	+	1919	574	4.2	2537, 1402, 1817

SD Standard deviation
- Absence of S-9
+ Presence of S-9
TEST ITEM: (b) (4)

Study title: Ames Test with [redacted] (b) (4) (Impurity)

Study no.:	GT-157-TX-56
Study report location:	EDR 4.2.3.7.6
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	October 19, 2011
Date of study completion:	November 15, 2011
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	[redacted] (b) (4) Batch No. [redacted] (b) (4) purity data not provided

Key Study Findings: Negative

Methods: Plate incorporation (first test), pre-incubation (second study, +S9)

Strains:	*Salmonella typhimurium*: TA 98 as well as a mixture of Salmonella strains TA7001-7006 (miniaturized Ames II test)
Concentrations in definitive study:	10, 30, 100, 300, 1000 and 3000 µg/plate
Basis of concentration selection:	Not provided
Negative control:	Water
Positive control:	Shown below (from page 2 of the report)
Formulation/Vehicle:	Water
Incubation & sampling time:	48-72 hours

Positive control: The following (from page 1 of the report) shows the positive controls.

Positive control:	-S9-Mix: 625/1200 ng/mL 4-Nitroquinoline N-oxide / 2-Nitrofluorene +S9-Mix: 10 µg/mL 2-Aminoanthracene

Study Validity: The applicant did not provide the criteria for a valid study. Please see the comments below.

Evaluation criteria: Not provided

Results: [redacted] (b) (4) did not increase the number of revertants, either with or without S9 mix, in TA98 under the conditions of the study. The following (from page 1-2 of the report) table shows the results.

(b) (4)

Report No.:	All2531

BACTERIAL REVERSE MUTATION TEST **(AMES II)**

Project Number:	GT-157-TX-56
Test substance:	(b) (4)
Batch number:	
Start of study:	19-Oct-2011
End of evaluation:	28-Oct-2011
Reference ID:	GENZ Study ID: GT-157-TX-56

Mutagenicity **Result:**

General negative	TA98 S9+ negative	TA98 S9- negative
	Mixed S9+ negative	Mixed S9- negative

Comment: **not mutagenic**

Toxicity: **Toxic without S9-mix in both strains at 300 µg/mL and above. With S9-mix in Mixed strains at 3000 µg/mL and in TA98 at 1000 µg/mL and above**

Tables:

Concentration in µg/mL	30% S9-mix	MIXED STRAINS TA7001 - 7006 series of Salmonella his⁻ mutant tester strains				
		No. of positive wells				
		Plate 1	Plate 2	Mean value	Standard deviation	Ratio *
0	-	0	0	0.0	0.0	**1.0**
10	-	0	0	0.0	0.0	**n.a.**
30	-	1	0	0.5	0.7	**n.a.**
100	-	1	2	1.5	0.7	**n.a.**
300**	-	0	0	0.0	0.0	**n.a.**
1000**	-	0	0	0.0	0.0	**n.a.**
3000#	-	0	0	0.0	0.0	**n.a.**
Positive Control	-	48	48	48.0	0.0	**n.a.**

Concentration in µg/mL	30% S9-mix	MIXED STRAINS TA7001 - 7006 series of Salmonella his⁻ mutant tester strains				
		No. of positive wells				
		Plate 1	Plate 2	Mean value	Standard deviation	Ratio *
0	+	0	1	0.5	0.7	**1.0**
10	+	1	0	0.5	0.7	**1.0**
30	+	0	1	0.5	0.7	**1.0**
100	+	2	2	2.0	0.0	**4.0**
300	+	1	4	2.5	2.1	**5.0**
1000	+	1	2	1.5	0.7	**3.0**
3000**	+	0	0	0.0	0.0	**0.0**
Positive Control	+	48	46	47.0	1.4	**94.0**

0 µg/mL = negative control: ultrapure water

Positive control: -S9-Mix: 625/1200 ng/mL 4-Nitroquinoline N-oxide / 2-Nitrofluorene
+S9-Mix: 10 µg/mL 2-Aminoanthracene

* = based on negative control

** = low bacterial background

= bubble-formation

n.a. = not applicable

(b) (4)

(b) (4)

End of evaluation:	28-Oct-2011
Reference ID:	GENZ Study ID: GT-157-TX-56

Tables:

Concentration in µg/mL	30% S9-mix	TA98 Salmonella typhimurium strain hisD3052 rfa uvrB +R				
		No. of positive wells		Mean value	Standard deviation	Ratio *
		Plate 1	Plate 2			
0	-	1	1	1.0	0.0	**1.0**
10	-	1	1	1.0	0.0	**1.0**
30	-	1	1	1.0	0.0	**1.0**
100	-	0	1	0.5	0.7	**0.5**
300**	-	0	0	0.0	0.0	**0.0**
1000#	-	0	0	0.0	0.0	**0.0**
3000#	-	0	0	0.0	0.0	**0.0**
Positive Control	-	42	40	41.0	1.4	**41.0**

Concentration in µg/mL	30% S9-mix	TA98 Salmonella typhimurium strain hisD3052 rfa uvrB +R				
		No. of positive wells		Mean value	Standard deviation	Ratio *
		Plate 1	Plate 2			
0	+	1	1	1.0	0.0	**1.0**
10	+	0	2	1.0	1.4	**1.0**
30	+	3	0	1.5	2.1	**1.5**
100	+	1	1	1.0	0.0	**1.0**
300	+	1	0	0.5	0.7	**0.5**
1000**	+	1	2	1.5	0.7	**1.5**
3000#	+	0	0	0.0	0.0	**0.0**
Positive Control	+	46	48	47.0	1.4	**47.0**

0 µg/mL = negative control: ultrapure water

Positive control: -S9-Mix: 625/1200 ng/mL 4-Nitroquinoline N-oxide / 2-Nitrofluorene
+S9-Mix: 10 µg/mL 2-Aminoanthracene

* = based on negative control

** = low bacterial background

= bubble-formation

Comments: This study was conducted using Salmonella tester strain TA98 as well as a mixture of Salmonella strains TA7001-7006 (miniaturized Ames II test). The Applicant did not provide a full report of this study and provide a justification for this Ames II test. The Applicant provided a two-page summary. The CDER Genetic toxicology subcommittee was consulted (dated January 8, 2014) seeking the Subcommittee's opinion and advice on whether the Ames test conducted by the Applicant was adequate to conclude that this impurity is not genotoxic. The Committee agreed that (b) (4) appears to be non-genotoxic. However, the Committee noted that the Applicant did not provide any rationale for not conducting a standard Ames assay. The Committee also mentioned the lack of detail provided with the report (e.g., methodology, etc.).

8 Carcinogenicity

Carcinogenicity study reports (mouse: GT-157-TX-43; rat: GT-157-TX-38) were reviewed (pharmacology review of NDA 205494 dated April 15, 2014) by Dr. Sruthi King (DGIEP). Please refer to the above review of Dr. King.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Study of Fertility and Early Embryonic Development to Implantation by Oral Route (Gavage) in Rats

Study no.:	GT-157-TX-24
Study report location:	EDR Section 4.2.3.5.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 30, 2004
Date of completion:	February 27, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Genz-112638, Batch # P0054, 99.8%

Key Study Findings:

- In a fertility and early embryonic development study in male and female rats, animal were treated with Genz-112638 from prior to and during mating and until implantation (females) or sacrifice (males) at 10, 30 and 100 mg/kg/day by oral gavage.
- Pre-implantation losses were increased at 30 and 100 mg/kg/day (statistically significant) compared to control.

Methods:

Doses:	10, 30, 100 mg/kg/day
Frequency of dosing:	Once a day, 7 days a week Males: 29 days before mating and during the mating period (until sacrifice) Females: 15 days before mating, during the mating period, during pregnancy until day 7 post coitum
Dose volume:	5 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	Water
Species/Strain:	Sprague Dawley rats
Number/Sex/Group:	24/sex/dose
Satellite groups:	None
Study design:	Shown in the table (from page 14 of the report)
Deviation from study protocol:	Protocol deviations were considered not to have compromised the validity or integrity of the study.

The following table (from page 14 of the report) shows the study design.

The treatment groups are detailed in the following table:

Group	Number of animals	Dose-level (mg/kg/day)	Animal numbers
1	24 males 24 females	0	G26331 to G26354 G26471 to G26494
2	24 males 24 females	10	G26355 to G26378 G26495 to G26518
3	24 males 24 females	30	G26379 to G26402 G26519 to G26542
4	24 males 24 females	100	G26403 to G26426 G26543 to G26566

Basis of dose selection: The doses for the current study were selected based on the results of the embryofetal development study (GT-157-TX-20) in rats at 10, 30 and 120 mg/kg/day from days 6 to 17 *post-coitum*. Maternal toxicity [lower body weight gain (days 6-18 *p.c.*, -30%; reduced food consumption (ranged between -12% and -21%) were observed at 120 mg/kg/day. The numbers of late resorptions and dead fetuses and, the post-implantation loss were increased in comparison to the control group values. The fetal body weight was significantly reduced (-18%) at the high dose. In addition, in a 28-day toxicity study (GT-157-TX-5) in rats, animals were treated at 10, 30 and 100 mg/kg/day. In females, a slight reduction in body weight gain was observed at 30 and 100 mg/kg/day. Minor changes in several hematological and clinical chemistry

parameters were noted, however, no macroscopic or microscopic changes or effects on organ weights were observed. Based on the above, the following doses were selected for the current study: 10, 30 and 100 mg/kg/day.

Observations and Results:

Mortality: Mortality was observed twice daily. There were no mortalities.

Clinical Signs: Clinical signs were observed once daily. All males at 100 mg/kg/day had ptyalism from the beginning of the dosing period until the end of the treatment. At 30 mg/kg/day in males, 23 animals had ptyalism from the middle of the dosing period until near the end. In females at 100 mg/kg/day, majority of animals had ptyalism from the second week of dosing until the end of the treatment period (Day 7 *post-coitum*).

Body Weight: The body weight of males was recorded on Day 1, then twice a week until sacrifice. The body weight of females was recorded on Day 1, then twice a week until mated and on days 0, 4, 8, 11 and 15 *post-coitum*.

The mean initial (Day 1) and final (Day 50) body weights of control males were 305 and 463 g, respectively. In the premating period, the mean initial (Day 1) and final (Day 15) body weights of control females were 230 and 250 g, respectively. In the pregnancy period, the mean initial (Day 0) and final (Day 15) body weight of control females were 256 and 337 g, respectively. There were no significant treatment-related effects in either sex.

Food Consumption: Food consumption for males was recorded once a week. In females, food consumption was recorded as follows:

- During the pre-mating period: weekly from the first day of treatment
- During pregnancy: over the periods Day 0 to Day 8 and Day 8 to Day 15 *post-coitum*

The mean initial (Day 1-8) and final (Day 22-29) food consumption in control males were 28 and 26 g/animal/day, respectively. In the premating period, the mean initial (Day 1-8) and final (Day 8-15) food consumption of control females were 18 and 18 g/animal/day, respectively. In the pregnancy period, the mean initial (Day 0-8) and final (Day 8-15) food consumption of control females were 24 and 26 g/animal/day, respectively. There were no significant treatment-related effects in either sex.

Toxicokinetics: Not performed

Dosing Solution Analysis: Dosing formulations containing 0.4 and 33.3 mg/mL of test item were analyzed for stability. The dosage forms were found to be stable for nine days at +4°C. During the treatment period, the test item concentrations were determined in samples taken from each control and test item dosage form prepared for use on the first day of treatment at Week 4 and during the last week of treatment. The nominal and actual concentrations of the test item in the administered dosage forms analyzed were

Reference ID: 3501244

satisfactory as the deviations from nominal concentrations remained within an acceptable range of ± 10%.

Necropsy: A macroscopic examination of the principal thoracic and abdominal organs (especially the reproductive organs) was performed on all animals. There were no significant necropsy findings in either sex.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.): On day 15 *post-coitum* the mated females were sacrificed and a hysterectomy performed. The ovaries and uterus of the females were examined to determine the following: number of *corpora lutea*, number and distribution of dead and live concepti (embryos), number and distribution of early and late resorptions, number and distribution of implantation sites, early resorptions, and late resorptions.

Pre-implantation losses were increased at 30 and 100 mg/kg/day (statistically significant) compared to control. The following table (from page 47 of the report) shows the hysterectomy data.

HYSTERECTOMY DATA (Summary table)

Dose: (mg/kg/day)		0	10	30	100
Pregnant Females Alive at Term	N	23	23	24	24
with all Dead Concepti	N	0	0	0	0
with Live Concepti	N	23	23	24	24
Corpora Lutea	TOTAL	355	366	372	369
No. per animal	MEAN	15.4 d	15.9	15.5	15.4
	S.D.	1.6	2.0	1.7	2.9
Implantation Sites	TOTAL	346	357	354	340
No. per animal	MEAN	15.0 d	15.5	14.8	14.2
	S.D.	1.7	2.2	3.1	4.0
Preimplantation Loss	TOTAL	9 f	9	18	29**
	%	2.5	2.5	4.8	7.9
Concepti	N	325	339	339	313
No. per animal	MEAN	14.1 d	14.7	14.1	13.0
	S.D.	2.0	2.5	3.4	4.3
Alive	%	100.0	100.0	100.0	100.0
Dead	%	0.0	0.0	0.0	0.0
Live Concepti	N	325 f	339	339	313
% of implantation sites		93.9	95.0	95.8	92.1
No. per animal	MEAN	14.1 d	14.7	14.1	13.0
	S.D.	2.0	2.5	3.4	4.3
Dead Concepti	N	0 f	0	0	0
% of implantation sites		0.0	0.0	0.0	0.0
No. per animal	MEAN	0.0	0.0	0.0	0.0
	S.D.	0.0	0.0	0.0	0.0
Postimplantation Loss	TOTAL	21 f	18	15	27
% of implantation sites		6.1	5.0	4.2	7.9
No. per animal	MEAN	0.9 d	0.8	0.6	1.1
	S.D.	1.3	1.0	1.3	2.3

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test ** = p<0.01

Summary: In a fertility and early embryonic development study in male and female rats, animal were treated with Genz-112638 from prior to and during mating and until implantation (females) or sacrifice (males) at 10, 30 and 100 mg/kg/day by oral gavage. Pre-implantation losses were increased at 30 and 100 mg/kg/day (statistically significant) compared to control.

9.2 Embryonic Fetal Development

Study title: Study for Effects on Embryo-Fetal Development by Oral Route (Gavage) in Rats

Study no.:	GT-157-TX-20
Study report location:	EDR Section 4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 14, 2004
Date of study completion:	February 17, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Genz-112638, Batch No. P0054, 99.8%

Key Study Findings:

- In an oral embryofetal development study with Genz-112638 in rats, animals were treated at 10, 30 and 120 mg/kg/day from GD 6 to GD 17.
- Maternal toxicity (decreased body weight and food consumption) was observed at 120 mg/kg/day.
- At 120 mg/kg/day, there was an increase in the number of late resorptions, dead fetuses and post implantation loss. The fetal body weight was also reduced at 120 mg/kg/day.
- At 120 mg/kg/day, fetal visceral variations included dilated cerebral ventricles and fetal skeletal malformations included abnormal number of ribs or lumbar vertebra and fetal skeletal variations included poor bone ossification.
- At 30 and 10 mg/kg/day, no litter parameters at cesarean section or any findings at fetal examination were indicative of treatment-related effects.

Methods:

Doses:	10, 30, 120 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	5 mL/kg
Route of administration:	Oral (Gavage)
Formulation/Vehicle:	Purified water
Species/Strain:	SD rats
Number/Sex/Group:	24/dose
Satellite groups:	None
Study design:	Shown in the table (from page 13 of the report) below
Deviation from study protocol:	Protocol deviations were not considered to have compromised the validity or integrity of the study.

The following table (from page 13 of the report) shows the study design.

The treatment groups are detailed in the following table:

Group	Number of mated females	Dose-level (mg/kg/day)	Animal numbers
1	24*	0	E27121 to E27144
2	24*	10	E27145 to E27168
3	24*	30	E27169 to E27192
4	24*	120	E27193 to E27216

*: the first 20 pregnant females were taken into consideration for fetal examinations.

Basis of dose selection: The doses were selected based on the results of a dose ranging embryofetal development study (GT-157-TX-18) in rats at 10, 30 and 100 mg/kg/day. In this study, three groups of seven mated female Sprague-Dawley rats were treated with Genz-112638 (Batch No. P0054) by daily oral gavage at 10, 30 or 100 mg/kg/day (5 mL/kg) from GD 6 to GD 17. Control group (seven females) received purified water. Clinical signs and mortality were checked daily. Body weight and food consumption were recorded at designated intervals. On GD 20, the dams were sacrificed and subjected to a macroscopic examination. The gravid uterus was weighed. The fetuses were removed by hysterectomy. The following litter parameters were recorded: number of corpora lutea, implantation sites, early and late resorptions, dead and live fetuses. The fetuses were weighed, sexed and submitted to external examination.

There were no mortalities in this study. At 10 and 30 mg/kg/day, there were no significant treatment-related clinical signs or effects on body weight and food consumption. There were no treatment-related necropsy findings. There were no significant treatment related effects on litter parameters. At 100 mg/kg/day, body weight gain (-14%) was reduced in dams associated with a slightly low fetal weight (-10%).

Based on the results of this study, 10, 30 and 120 mg/kg/day doses were selected for the current embryofetal development study.

Observations:

Mortality: Mortality was observed twice a day.

Clinical Signs: Clinical signs were observed on a daily basis.

Body Weight: Body weight was recorded on GD 2, 4, 6, 9, 12, 15, 18 and 20.

Food Consumption: Food consumption was recorded for the following intervals: GD 2-4, 4-6, 6-9, 9-12, 12-15, 15-18 and 18-20.

Toxicokinetics: Not conducted

Dosing solution analysis: Concentration and stability of the dosage formulations were analyzed at the start of the treatment. During the treatment period, the concentration of dosage forms prepared for use in the study was checked.

Necropsy: Principal thoracic and abdominal organs were collected on GD 20.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.): On GD 20, animals were sacrificed and ovaries and uteri were examined for the following: number of corpora lutea, number and distribution of dead and live fetuses, number and distribution of early and late resorptions, number and distribution of uterine scars, number and distribution of implantation sites, implantation sites, early and late resorptions, etc.

Offspring (Malformations, Variations, etc.): Fetuses were examined for external, visceral and skeletal changes.

Results:

Mortality: There were no mortalities.

Clinical Signs: At 120 mg/kg/day, 19 females had ptyalism (more often during the second half of the dosing period). There were no other significant treatment related clinical signs.

Body Weight: The mean initial (Day 2) and final (Day 20) body weights were 236 and 389 g, respectively. At 120 mg/kg/day, body weight gain was lower than controls (GD 6 to 12, -17%). The body weight gain during the whole dosing period (GD 6-18) was also significantly lower than controls. The mean body weights were also lower compared to the control group at the high dose. At 30 and 10 mg/kg/day, body weight was not affected by treatment. The following table (from page 21 of the report) shows the body weight changes.

Body weight gain and net body weight change (g)

Dose-level (mg/kg/day)	0	10	30	120
Days 6 to 9 *p.c.*	13	16 (+23%)	16 (+23%)	11 (-15%)
Days 6 to 12 *p.c.*	36	39 (+8%)	39 (+8%)	30* (-17%)
Days 9 to 12 *p.c.*	23	22 (-4%)	23 (0%)	19 (-17%)
Days 12 to 15 *p.c.*	19	20 (+5%)	21 (+11%)	12** (-37%)
Days 15 to18 *p.c.*	39	39 (0%)	42 (+7%)	24*** (-38%)
Days 18 to 20 *p.c.*	31	32 (+3%)	33 (+6%)	25** (-19%)
Days 6 to18 *p.c.* (dosing period)	94	97 (+3%)	101 (+7%)	66*** (-30%)
Net body weight change	56	59 (+5%)	59 (+5%)	42** (-25%)

Net body weight change: (body weight on day 20 *p.c.* minus body weight on day 6 *p.c.*) minus gravid uterine weight.
*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$.
p.c.: *post-coitum*.

Food Consumption: Mean initial (Day 2-4) and final (Day 18-20) food consumptions of control animals were 22 and 30 g/animal/day, respectively. At 120 mg/kg/day, mean food consumption was significantly lower than the control group values. At 30 and 10 mg/kg/day, mean food consumption was not affected by treatment. The food consumption data are shown in the following table (from page 22 of the report).

Food consumption (g/day)

Dose-level (mg/kg/day)	0	10	30	120
Days 6 to 9 *p.c.*	25	26 (+4%)	26 (+4%)	22** (-12%)
Days 9 to12 *p.c.*	26	27 (+4%)	27 (+4%)	23*** (-12%)
Days 12 to 15 *p.c.*	28	29 (+4%)	29 (+4%)	24*** (-14%)
Days 15 to18 *p.c.*	29	30 (+3%)	30 (+3%)	23*** (-21%)

**: p<0.01, ***: p<0.001.

Dosing solution analysis: Results of the dosing solution analyses demonstrated satisfactory stability of the dosage form examined (33.33 mg/mL) over a 9-day period at +4°C. Throughout the study, nominal and actual concentrations of the test item in the administered dosage forms were satisfactory as the deviations from nominal concentration remained in an acceptable range of ± 10%.

Necropsy: At 120 mg/kg/day, mean uterine weight was significantly lower (-29%) than the control group value. At 30 and 10 mg/kg/day, the gravid uterine weight was similar to the control group value. At scheduled necropsy, four females at 120 mg/kg/day had uterine horns with small placenta and one case of enlarged placenta. In one female, this finding was associated with brownish content in the vagina. Brownish content in the uterus and/or vagina was also observed in two other females at 120 mg/kg/day group. Small placentae were considered to be treatment related as they were limited to the high-dose only.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.):

The number of corpora lutea was similar in treated and control groups. At 120 mg/kg/day, when compared to the control group value, the number of late resorptions and the mean post implantation loss were significantly higher and the number of dead fetuses was slightly higher and the mean number of live fetuses was slightly lower. The mean fetal body weight was markedly lower than that in the control group (20% reduction in both sexes). These findings were considered to be related to the treatment. At 30 and 10 mg/kg/day, all the litter parameters were similar to the control group values. In addition, the percentage of male fetuses was similar among the groups and the fetal body weight was unaffected by treatment at low and mid dose. The following (from pages 36-37 of the report) tables show the cesarean data.

HYSTERECTOMY DATA (Summary table)

Dose: (mg/kg/day)		0	10	30	120
Pregnant Females Alive at Term	N	18	21	22	22
with Total Resorptions	N	0	0	0	0
with all Dead Fetuses	N	0	0	0	0
with Live Fetuses	N	18	21	22	22
Corpora Lutea	TOTAL	266	297	325	310
No. per animal	MEAN	14.8 d	14.1	14.8	14.1
	S.D.	1.7	3.1	2.7	1.7
Implantation Sites	TOTAL	244	276	308	293
No. per animal	MEAN	13.6 d	13.1	14.0	13.3
	S.D.	2.5	2.7	1.9	2.3
Preimplantation Loss	TOTAL	22 f	21	17	17
	%	8.3	7.1	5.2	5.5
Fetuses	N	220	265	288	229
No. per animal	MEAN	12.2 d	12.6	13.1	10.4
	S.D.	3.2	2.7	1.9	3.2
Alive	%	100.0	100.0	100.0	97.8
Dead	%	0.0	0.0	0.0	2.2
Live Fetuses	N	220 f	265**	288	224#
% of implantation sites		90.2	96.0	93.5	76.5
No. per animal	MEAN	12.2 d	12.6	13.1	10.2
	S.D.	3.2	2.7	1.9	3.2
Dead Fetuses	N	0 f	0	0	5
% of implantation sites		0.0	0.0	0.0	1.7
No. per animal	MEAN	0.0 d	0.0	0.0	0.2**
	S.D.	0.0	0.0	0.0	0.4

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test ** = p<0.01 # = p<0.001

HYSTERECTOMY DATA (Summary table)

Dose: (mg/kg/day)		0	10	30	120
Resorptions + Scars	N	24 f	11**	20	64#
% of implantation sites		9.8	4.0	6.5	21.8
No. per animal	MEAN	1.3 d	0.5	0.9	2.9**
	S.D.	1.7	0.7	0.8	2.3
Implant Scars	N	0 f	0	0	0
% of implantation sites		0.0	0.0	0.0	0.0
No. per animal	MEAN	0.0	0.0	0.0	0.0
	S.D.	0.0	0.0	0.0	0.0
Resorptions: early	N	24 f	11**	20	31
% of implantation sites		9.8	4.0	6.5	10.6
No. per animal	MEAN	1.3 d	0.5	0.9	1.4
	S.D.	1.7	0.7	0.8	1.4
Resorptions: late	N	0 f	0	0	33#
% of implantation sites		0.0	0.0	0.0	11.3
No. per animal	MEAN	0.0 d	0.0	0.0	1.5#
	S.D.	0.0	0.0	0.0	1.7
Postimplantation Loss	TOTAL	24 f	11**	20	69#
% of implantation sites		9.8	4.0	6.5	23.5
No. per animal	MEAN	1.3 d	0.5	0.9	3.1**
	S.D.	1.7	0.7	0.8	2.3
Male Fetuses	N	105 f	136	156	117
	%	47.7	51.3	54.2	52.2
Female Fetuses	N	115 f	129	132	107
	%	52.3	48.7	45.8	47.8
Fetal Body Weight (g)	MEAN	3.43 d	3.50	3.67	2.80#
	S.D.	0.41	0.36	0.23	0.32
Male Fetuses	MEAN	3.52 d	3.58	3.77	2.83#
	S.D.	0.39	0.40	0.23	0.36
Female Fetuses	MEAN	3.38 d	3.40	3.55	2.71#
	S.D.	0.41	0.36	0.25	0.26

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test ** = p<0.01 # = p<0.001

Offspring (Malformations, Variations, etc.):

External changes: At 120 mg/kg/day, two fetuses from the same litter showed external malformations: anasarca and ectrodactyly in one fetus (E27201/06, male) and gastroschisis in one fetus (E27201/04, male). These findings were not considered to be treatment-related as they concerned only a single litter and as malformations were also noted in one fetus from the controls (E27129/10) showed multiple malformation (cleft palate and cleft lips, and meningocele).

Visceral changes: No visceral malformations were noted in any fetuses at 120 mg/kg/day. The two fetuses with visceral malformations were distributed in the control and 30 mg/kg/day groups. In the absence of any dose related increase

and the low incidence and the distribution (including in the control group), these findings were considered to be spontaneous findings. At 120 mg/kg/day, 10 fetuses distributed in seven litters presented dilated cerebral ventricles as visceral variations. This finding was considered to be a variation because of the degree of the dilation (a severe dilatation was considered as a malformation) and it was considered to be treatment-related. The different other variations were distributed among the groups with no apparent treatment or no dose-related increase.

Skeletal changes:

Skeletal malformations: At 120 mg/kg/day, one fetus each in two litters showed one missing vertebrae and 25 pre-sacral vertebrae in consequence. In addition, at 120 mg/kg/day, two fetuses in one single litter had 12 ribs only (instead of 13) but the number of presacral vertebrae remained unchanged. As both findings concerned the vertebral column affecting four fetuses in three litters, the relation to the treatment could not be ruled out. At 10 mg/kg/day, two fetuses in one single litter had 25 pre-sacral vertebrae with one missing lumbar vertebra. As this finding concerned one litter only in the treated groups and was not present at 30 mg/kg/day, a relation to the treatment was ruled out.

Skeletal variations: Skeletal variations at 120 mg/kg/day included incomplete ossification of thoracic centrum, unossified and incomplete ossification of caudal centrum, incomplete ossification of 1st to 4th sternebra, unossified 1st to 4th, 5th, and 6th sternebra and unossified 4th metacarpals. The following (from page 25 of the report) table shows the skeletal variations.

Summary of notable skeletal variations (%)

Dose-level (mg/kg/day)	0	10	30	120
Incomplete ossification of thoracic centrum				
. mean number of fetuses affected/litter	7.1	10.7	9.8	20.3
. number of litters	[4]	[8]	[9]	[14]**
Unossified caudal centrum				
. mean number of fetuses affected/litter	6.3	7.2	2.5	34.1 ***
. number of litters	[4]	[2]	[3]	[15]**
Incomplete ossification of caudal centrum				
. mean number of fetuses affected/litter	10.6	5.4	7.8	40.6 ***
. number of litters	[6]	[3]	[4]	[19]***
Incomplete ossification of 1st to 4 th sternebra				
. mean number of fetuses affected/litter	28.0	36.3	27.8	76.7 ***
. number of litters	[13]	[15]	[18]	[19]
Unossified 1st to 4 th sternebra				
. mean number of fetuses affected/litter	1.6	2.8	1.5	18.5 ***
. number of litters	[1]	[2]	[2]	[12]***
Unossified 5 th sternebra				
. mean number of fetuses affected/litter	40.4	38.1	24.7	73.1 **
. number of litters	[14]	[15]	[14]	[19]
Unossified 6 th sternebra				
. mean number of fetuses affected/litter	3.8	3.5	1.5	20.2 **
. number of litters	[2]	[2]	[2]	[10]*
Unossified 4th metacarpal(s)				
. mean number of fetuses affected/litter	0.8	3.3	1.3	18.0 **
. number of litters	[1]	[1]	[1]	[9]**

*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$.

Summary: In an oral embryofetal development study in rats, animals were treated with Gez-112638 at 10, 30 and 120 mg/kg/day from GD 6 to GD 17. Maternal toxicity (decrease body weight and food consumption) was observed at 120 mg/kg/day. At 120

mg/kg/day, there was an increase in the number of late resorptions, dead fetuses and post implantation loss. Fetal body weight was also reduced at 120 mg/kg/day. At 120 mg/kg/day, fetal visceral variations included dilated cerebral ventricles and fetal skeletal malformations included abnormal number of ribs or lumbar vertebra and fetal skeletal variations included poor bone ossification. At 30 and 10 mg/kg/day, no litter parameters at cesarean section or any findings at fetal examination were indicative of treatment-related effects.

Study title: Study for Effects on Embryo-Fetal Development by Oral Route (Gavage) in Rabbits

Study no.:	GT-157-TX-21
Study report location:	EDR Section 4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 13, 2004
Date of study completion:	February 17, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Genz-112638, Batch No. P0054, 99.8%

Key Study Findings:

- In an oral embryofetal development study in New Zealand white rabbits, animals were treated at 10, 30 and 100 mg/kg/day from GD 6 to GD 18.
- Eliglustat did not appear to cause significant adverse effects on embryofetal development up to 100 mg/kg/day in this study.

Methods:

Doses:	10, 30, 100 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	3 mL/kg
Route of administration:	Oral (Gavage)
Formulation/Vehicle:	Purified water
Species/Strain:	New Zealand white rabbits
Number/Sex/Group:	20/dose
Satellite groups:	4/sex/dose (toxicokinetics)
Study design:	Shown in the table (from page 14 of the report) below
Deviation from study protocol:	Protocol deviations were not considered to have compromised the validity or integrity of the study.

The following table (from page 14 of the report) shows the study design.

The treatment groups are detailed in the following table:

Group	Number of mated females	Dose-level (mg/kg/day)	Animal numbers
1	20* + 4**	0	E30991 to E31010 + E31011 to E31014
2	20* + 4**	10	E31015 to E31034 + E31035 to E31038
3	20* + 4**	30	E31039 to E31058 + E31059 to E31062
4	20* + 4**	100	E31063 to E31082 + E31083 to E31086

*: only the first 16 pregnant females were taken into consideration for fetal examinations.
**: satellite animals.

Basis of dose selection: Doses were selected based on the results of a dose ranging embryofetal development study (GT-157-TX-19) in rabbits at 10, 30 and 100 mg/kg/day. In this study, three groups of six mated female New Zealand white rabbits were treated with Genz-112638 (Batch No. P0054) by daily oral gavage at 10, 30 or 100 mg/kg/day (3 mL/kg) from GD 6 to GD 18. Control group (six females) received purified water. Clinical signs and mortality were checked daily. Body weight and food consumption were recorded at designated intervals. On GD 29, the dams were sacrificed and subjected to a macroscopic examination. Gravid uterus was weighed. Following litter parameters were recorded: number of corpora lutea, implantation sites, early and late resorptions, dead and live fetuses. Fetuses were weighed, sexed and examined for external changes.

There were no mortalities in this study. At 30 and 100 mg/kg/day, clinical signs included loud breathing in one and three animals, respectively. Body weight and food consumption were reduced at 100 mg/kg/day. None of the necropsy findings were considered treatment related and litter parameters were similar in all groups. There were no significant treatment-related fetal external variations or malformations.

Based on the results of this study, 10, 30 and 100 mg/kg/day were selected for the embryofetal development study.

Observations:

Mortality: Mortality was observed twice a day.

Clinical Signs: Clinical signs were observed on a daily basis.

Body Weight: Body weight was recorded on GD 2, 4, 5, 6, 9, 12, 15, 19, 24 and 29.

Food Consumption: Food consumption was recorded for the following intervals: GD 2-4, 4-5, 5-6, 6-9, 9-12, 12-15, 15-19, 19-24 and 24-29.

Toxicokinetics: Plasma levels of the test item were determined in the satellite females at the beginning and the end of the treatment period. Blood samples were collected on GD 6 and 18 at the following time-points: 0.5, 1, 2, 4, 8 and 24 hours post-dose.

Dosing solution analysis: Before the start of treatment, the formulations were analyzed for concentration and stability. During the treatment period, concentration of dosage forms prepared for use in the study was checked.

Necropsy: Principal thoracic and abdominal organs were collected on GD 29.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.): On GD 29, animals were sacrificed and ovaries and uterus were examined for the following: number of corpora lutea, number and distribution of dead and live fetuses, number and distribution of early and late resorptions, number and distribution of uterine scars, number and distribution of implantation sites, implantation sites, early and late resorptions, etc.

Offspring (Malformations, Variations, etc.): Fetuses were examined for external, visceral and skeletal changes.

Results:

Mortality: At 100 mg/kg/day, three females (E31077, E31078 and E31065) were found dead on GD 11, 17 and 18, respectively. No clinical signs were recorded before death. In two females, foamy contents were noted in the trachea and the lungs. A brownish deposit, blackish foci and/or reddish area were observed in the stomach mucosa of the deceased females. The intestine was distended with gas in 2 of 3 above females. These deaths were attributed to gavage error (foamy contents in the trachea and the lungs) and were not considered to be treatment-related.

Clinical Signs: Loud breathing was transiently recorded in 3, 4, 2 and 4 of 20 females at 0, 10, 30 and 100 mg/kg/day, respectively. This was not considered treatment related as it was also observed in the control group.

Body Weight: The mean initial (Day 2) and final (Day 29) body weights were 3.414 and 3.877 kg, respectively. The final body weights were 101, 102, 103% of control at 10, 30 and 100 mg/kg/day, respectively. There were no significant treatment-related changes on body weight. However, mean body weight gain was reduced (83% of control gain, control = 242 g) at the high dose as shown in the following table (from page 23 of the report).

Reference ID: 3501244

Mean body weight gain values (grams)

Dose-level (mg/kg/day)	0	10	30	100
Days 6 to 9	26	39	32	-6
Days 6 to 12	85	101	92	42
Days 6 to 15	154	197	186	136
Days 6 to 19 (dosing period)	242	270	279	202

Food Consumption: The mean initial (Day 6-9) and final (Day 15-19) food consumption of control animals were 178 and 191 g/animal/day, respectively. Final food consumption values were 105, 111 and 98% of control at 10, 30 and 100 mg/kg/day, respectively. The food consumption data are shown in the following table (from page 24 of the report).

Mean food consumption values (grams/day)

Dose-level (mg/kg/day)	0	10	30	100
Days 6 to 9	178	193	193	166
Days 9 to 12	178	197	190	170
Days 12 to 15	168	180	186	164
Days 15 to 19	191	200	212	188

Dosing solution analysis: Results demonstrated satisfactory stability of the dosage form examined (33.33 mg/mL) over a 9-day period at +4°C. Throughout the study, the nominal and actual concentrations of the test item in the administered dosage forms were considered satisfactory as the deviations from nominal concentration remained in an acceptable range of ± 10%.

Necropsy: There were no significant treatment related necropsy findings.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.):

There were no significant treatment-related effects on the number of implantation sites, pre- and post-implantation loss, number of resorptions and dead fetuses. The percentage of male fetuses was similar among the groups. The mean fetal body weight was unaffected by the treatment. The following (from pages 41-42) tables show the cesarean data.

HYSTERECTOMY DATA (Summary table)

Dose: (mg/kg/day)		0	10	30	100
Pregnant Females Alive at Term	N	18	16	17	15
with Total Resorptions	N	0	0	0	0
with all Dead Fetuses	N	0	0	0	0
with Live Fetuses	N	18	16	17	15
Corpora Lutea	TOTAL	198	175	203	173
No. per animal	MEAN	11.0 d	10.9	11.9	11.5
	S.D.	1.8	1.4	1.8	1.8
Implantation Sites	TOTAL	173	144	164	149
No. per animal	MEAN	9.6 d	9.0	9.6	9.9
	S.D.	2.4	1.7	1.4	2.2
Preimplantation Loss	TOTAL	25 f	31	39	24
	%	12.6	17.7	19.2	13.9
Fetuses	N	166	138	158	141
No. per animal	MEAN	9.2 d	8.6	9.3	9.4
	S.D.	2.4	1.6	1.4	2.2
Alive	%	98.2	100.0	100.0	99.3
Dead	%	1.8	0.0	0.0	0.7
Live Fetuses	N	163 f	138	158	140
% of implantation sites		94.2	95.8	96.3	94.0
No. per animal	MEAN	9.1 d	8.6	9.3	9.3
	S.D.	2.3	1.6	1.4	2.3
Dead Fetuses	N	3 f	0	0	1
% of implantation sites		1.7	0.0	0.0	0.7
No. per animal	MEAN	0.2 d	0.0	0.0	0.1
	S.D.	0.4	0.0	0.0	0.3

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test

HYSTERECTOMY DATA (Summary table)

Dose: (mg/kg/day)		0	10	30	100
Resorptions + Scars	N	7 f	6	6	8
% of implantation sites		4.0	4.2	3.7	5.4
No. per animal	MEAN	0.4 d	0.4	0.4	0.5
	S.D.	0.6	0.7	0.5	0.9
Implant Scars	N	0 f	0	0	0
% of implantation sites		0.0	0.0	0.0	0.0
No. per animal	MEAN	0.0	0.0	0.0	0.0
	S.D.	0.0	0.0	0.0	0.0
Resorptions: early	N	5 f	3	3	4
% of implantation sites		2.9	2.1	1.8	2.7
No. per animal	MEAN	0.3 d	0.2	0.2	0.3
	S.D.	0.6	0.5	0.4	0.5
Resorptions: late	N	2 f	3	3	4
% of implantation sites		1.2	2.1	1.8	2.7
No. per animal	MEAN	0.1 d	0.2	0.2	0.3
	S.D.	0.3	0.5	0.4	0.7
Postimplantation Loss	TOTAL	10 f	6	6	9
% of implantation sites		5.8	4.2	3.7	6.0
No. per animal	MEAN	0.6 d	0.4	0.4	0.6
	S.D.	0.7	0.7	0.5	1.1
Male Fetuses	N	90 f	75	79	84
	%	55.2	54.3	50.0	60.0
Female Fetuses	N	73 f	63	79	56
	%	44.8	45.7	50.0	40.0
Fetal Body Weight (g)	MEAN	40.9 d	40.3	42.1	39.2
	S.D.	4.7	3.6	3.5	3.0
Male Fetuses	MEAN	41.8 d	40.6	42.6	39.2
	S.D.	4.5	4.5	3.9	3.8
Female Fetuses	MEAN	40.2 d	40.1	41.7	38.7
	S.D.	5.1	4.0	4.2	3.2

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test

Offspring (Malformations, Variations, etc.):

External changes: There were no significant treatment related fetal external changes.

Visceral changes: Whitish foci were observed in the liver of 5 of 138, 3 of 150 and 4 of 140 fetuses from 4, 3 and 2 litters at 10, 30 and 100 mg/kg/day, respectively. Although there was no dose-response and low incidence, a relation to the treatment could not be ruled out as this was not observed in the control animals. The few other findings (hemorrhagic eye, dilated cerebral ventricle, small gall bladder, enlarged stomach, enlarged testis, enlarged and hemorrhagic ovary, translucent testis, hemorrhagic testis, short innominate artery, absence of

brachiocephalic trunk, reddish foci on the thymus) were low in incidence and commonly recorded in fetuses of this strain and age. Thus they were not considered to be treatment related.

Skeletal changes:

Skeletal malformations: Although malformations related to sternebra (fused sternebrae, split sternebrae, hemisternebrae) concerned three fetuses from three different litters at the high dose-level, no relation to treatment could be established because of the low litter incidence and the presence of this finding in the control group at the same incidence. The following (page 55 of the report) table shows the above skeletal malformations.

Dose: (mg/kg/day)		0	10	30	100
Litters Evaluated	N	16	16	16	15
Fetuses Evaluated	N	145	138	150	140
STERNEBRA					
Litter Incidence	N	3	0	0	3
Fetal Incidence	N	3	0	0	3
FUSED STERNEBRAE					
Fetal Incidence	N	3 f	0	0	3
	%	2.1	0.0	0.0	2.1
Litter Incidence	N	3 f	0	0	3
	%	18.8	0.0	0.0	20.0
Affected Fetuses/Litter	MEAN%	2.8 d	0.0	0.0	2.4
	S.D.	6.8	0.0	0.0	5.0
SPLITTED STERNEBRA(E)					
Fetal Incidence	N	1 f	0	0	1
	%	0.7	0.0	0.0	0.7
Litter Incidence	N	1 f	0	0	1
	%	6.3	0.0	0.0	6.7
Affected Fetuses/Litter	MEAN%	0.7 d	0.0	0.0	0.8
	S.D.	2.8	0.0	0.0	3.2
HEMISTERNEBRA(E)					
Fetal Incidence	N	0 f	0	0	1
	%	0.0	0.0	0.0	0.7
Litter Incidence	N	0 f	0	0	1
	%	0.0	0.0	0.0	6.7
Affected Fetuses/Litter	MEAN%	0.0 d	0.0	0.0	0.8
	S.D.	0.0	0.0	0.0	3.2

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test

Skeletal variations: There were following two significant skeletal variations:

- Incomplete ossification of the 5th sternebra was significantly higher at 10 and 100 mg/kg/day when compared to the control. The incidence of this finding was not clearly dose-related and within (b) (4) historical control data and it was not considered to be treatment related. The following (from page 60 of the

report) table shows the incidences of incomplete ossification of the 5th sternebra.

Dose: (mg/kg/day)		0	10	30	100
Litters Evaluated	N	16	16	16	15
Fetuses Evaluated	N	145	138	150	140
THORACIC VERTEBRA(E): ENLARGED ARCH					
Fetal Incidence	N	0 f	0	1	0
	%	0.0	0.0	0.7	0.0
Litter Incidence	N	0 f	0	1	0
	%	0.0	0.0	6.3	0.0
Affected Fetuses/Litter	MEAN%	0.0 d	0.0	0.6	0.0
	S.D.	0.0	0.0	2.3	0.0
THORACIC VERTEBRA(E): UNOSSIFIED HEMI-VERTEBRA(E)					
Fetal Incidence	N	0 f	0	1	0
	%	0.0	0.0	0.7	0.0
Litter Incidence	N	0 f	0	1	0
	%	0.0	0.0	6.3	0.0
Affected Fetuses/Litter	MEAN%	0.0 d	0.0	0.7	0.0
	S.D.	0.0	0.0	2.8	0.0
STERNEBRA					
Litter Incidence	N	14	13	12	15
Fetal Incidence	N	37	52	42	57
INCOMPLETE OSSIFICATION OF 5th STERNEBRA					
Fetal Incidence	N	29 f	50**	41	53**
	%	20.0	36.2	27.3	37.9
Litter Incidence	N	12 f	13	12	14
	%	75.0	81.3	75.0	93.3
Affected Fetuses/Litter	MEAN%	20.5 d	35.2	27.1	36.5
	S.D.	24.8	27.2	22.4	21.5

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test ** = p<0.01

- Unossified interparietal showed statistical significance at 100 mg/kg/day (18 *vs.* 3 in control) when it was expressed as a total number of affected fetuses (18 *vs.* 3 in control). The incidence of this finding was also higher than controls but not statistically significant when it was expressed as a mean percentage per litter and based on the number of litters affected. As these values were not statistically significant when compared to the control group values, and as the incidence was dose-related (similar number of affected fetuses in the control and the 30 mg/kg/day group) and did not correlate with any other relevant delay in ossification, this single finding was considered to be fortuitous. The following table (from page 58 of the report) shows the incidences of unossified interparietal variations.

Dose: (mg/kg/day)		0	10	30	100
Litters Evaluated	N	16	16	16	15
Fetuses Evaluated	N	145	138	150	140
HEAD-SKULL					
Litter Incidence	N	5	8	5	9
Fetal Incidence	N	8	20	6	30
INCOMPLETE OSSIFICATION OF INTERPARIETAL					
Fetal Incidence	N	5 f	9	3	12
	%	3.4	6.5	2.0	8.6
Litter Incidence	N	5 f	6	3	6
	%	31.3	37.5	18.8	40.0
Affected Fetuses/Litter	MEAN%	3.1 d	5.9	2.0	7.7
	S.D.	4.9	9.0	4.2	12.5
UNOSSIFIED INTERPARIETAL					
Fetal Incidence	N	3 f	9	3	18#
	%	2.1	6.5	2.0	12.9
Litter Incidence	N	2 f	3	3	6
	%	12.5	18.8	18.8	40.0
Affected Fetuses/Litter	MEAN%	1.9 d	5.7	2.1	13.4
	S.D.	5.8	13.7	4.5	22.1
ENLARGED FONTANEL					
Fetal Incidence	N	0 f	1	0	0
	%	0.0	0.7	0.0	0.0
Litter Incidence	N	0 f	1	0	0
	%	0.0	6.3	0.0	0.0
Affected Fetuses/Litter	MEAN%	0.0 d	0.6	0.0	0.0
	S.D.	0.0	2.5	0.0	0.0

Statistical key: d-ANOVA + Dunnett-test f-Fishers exact test # = p<0.001

Toxicokinetics: On GD 6 and GD 18, test item was not quantifiable in control animal plasma samples except in the sample at 24 hours post-dose on GD 18. Per the applicant, this happened due to contamination. The Applicant stated that this isolated contaminated sample did not have any consequence for the integrity and validity of the study. At 10 mg/kg/day, plasma levels of the drug were just above the LOQ (5 ng/mL) at the 1 hour time-point on GD 6 and at 0.5 and 1 hours post-dose on GD 18 and was below the LOQ in all other samples. At 30 mg/kg/day, the C_{max} was 39.2 ng/mL on GD 6 and 52.2 ng/mL on GD 18. At 100 mg/kg/day, the C_{max} value was 144 ng/mL on GD 6 and 304 ng/mL on GD 18. Mean plasma levels of Genz-112638 increased from 0.5 hours to 1 hour post-dose on GD 6 and 18. Systemic exposure (C_{max} and AUC_{0-t}) on both GD 6 and 18 increased with the dose. In addition, exposure (C_{max} and AUC_{0-t}) on GD 18 was higher than GD 6. The following table (from page 27 of the report) shows the TK data.

Dose-level (mg/kg/day)	10		30		100	
Day of sampling (*p.c.*)	Day 6	Day 18	Day 6	Day 18	Day 6	Day 18
C_{max} (ng/mL)	6.23	13.7	39.2	52.2	144	304
T_{max} (h)	1	0.5	2	1	0.5	0.5
. AUC (ng.h/mL)	1.56	9.41	46.5	137	696	1163
. Ratio C_{max}/dose	0.623	1.37	1.31	1.74	1.44	3.04
. Ratio AUC/dose	0.156	0.941	1.55	4.567	6.96	11.63

Summary: In an oral embryofetal development study in New Zealand white rabbits, animals were treated at 10, 30 and 100 mg/kg/day from GD 6 to 18. No significant treatment-related adverse effects on embryofetal development were observed in this study.

9.3 Prenatal and Postnatal Development

Study title: Study for Effects on Pre- and Post-Natal Development by the Oral Route (Gavage) in Rats

Study no.:	GT-157-TX-29
Study report location:	EDR Section 4.2.3.5.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 11, 2007
Date of study completion:	August 31, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Genz-112638, Batch Nos. Q0858 (purity = 99.99%) and S1517 (purity = 100%)

Key Study Findings:

- In a pre- and post-natal development study in rats, animals were treated at 10, 30 and 100 mg/kg/day by oral gavage.

F_0 Generation:

- Two females at 100 mg/kg/day and two females at 30 mg/kg/day and one female at 10 mg/kg/day were sacrificed prematurely. Clinical signs at 100 mg/kg/day included ptyalism (hypersalivation) during gestation and lactation.
- Body weight (7% reduction compared to control) and food consumption (4-14% reduction compared to control) were reduced at 100 mg/kg/day.

- The mean duration of gestation was longer at 100 mg/kg/day (22.0 days *vs.* 21.5 days in control) compared to control.
- At 100 mg/kg/day, animals had a lower (9.8 vs. 12.0 in control) number of delivered pups per litter than that in the control and the mean post implantation loss was higher (25.5% vs. 9.4% in control) at 100 mg/kg/day than that in the control group.

F_1 Generation:

- Body weights were reduced by approximately 10% in both sexes at 100 mg/kg/day. There were no body weight changes at 10 and 30 mg/kg/day.
- Balanopreputial separation was achieved slightly earlier (50.6-50.9 days vs. 52.4 days in control) in all male groups treated with Genz-112638.
- There were no significant treatment related effects on learning and memory and spontaneous locomotor activity.
- The mean numbers of *corpora lutea* and implantations were lower at 100 mg/kg/day (15.9 vs. 17.3 in control and 14.3 vs. 16.1 in control, respectively) and the mean pre implantation loss was higher (9.9 vs. 6.9 in control) when compared to the controls.
- Statistically significantly lower absolute testes and epididymides weights were observed in F_1 males at 100 mg/kg/day. In addition, small epididymides and testes were observed in gross necropsy at 10 and 100 mg/kg/day. These findings (small epididymides and testes) do not appear to be treatment-related in the absence of dose response.
- Eliglustat did not appear to cause any significant adverse effects on pre and postnatal development up to 100 mg/kg/day in this study.

Methods: The objective of this study was to evaluate the potential toxic effects of Genz-112638 on the pregnant/lactating female (F_0 generation) and on development of the conceptus and the offspring through sexual maturity (F_1 generation). The test item was administered daily by oral route (gavage) to pregnant female rats from implantation through weaning [GD 6 to Day 21 *post-partum* (pp)]. Principal dams and the pups were sacrificed on day 22 pp and a macroscopic examination of the principal thoracic and abdominal organs was performed. The numbers of implantation scars were recorded for the dams. On the day of culling (Day 4 pp), one male pup from each principal litter was retained for possible further examination.

F1 generation: Clinical signs, body weight and food consumption were recorded for the F1 generation. The F1 pups were tested for auditory function, pupil constriction, learning and memory, spontaneous locomotor activity, testes descent and the day of sexual maturity (preputial separation or vaginal opening). At 12 and 14 weeks of age, F1 pups were paired for mating. The F1 generation females were sacrificed on day 15 *post-coitum* or at least 1 week after the end of the pairing period if no evidence of mating had been observed. Males were sacrificed after the female hysterectomies had been completed. Macroscopic examination of the principal thoracic and abdominal organs was performed for all animals and, for pregnant females, the numbers of *corpora lutea* and implantations, classified as dead and live concepti, early and late resorptions or implantation scars, were recorded. In addition, microscopic examination was performed only on the reproductive organs of the F1 generation males (epididymides, testes, seminal vesicles, coagulating glands and prostate) and on the macroscopic lesions.

Doses:	10, 30, 100 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	5 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	Purified water
Species/Strain:	Sprague Dawley female rats
Number/Sex/Group:	24/dose
Satellite groups:	2-6/dose for toxicokinetics (TK)
Study design:	Shown in the table (from page) below
Deviation from study protocol:	

The study design is shown below (from page 21 of the report).

Group	Number of animals		Dose-level (mg/kg/day)	Animal numbers	
	Principal	Satellite		Principal	Satellite
1	24 females	2 females	0	Q20111 to Q20134	Q20135 and Q20136
2	24 females	6 females	10	Q20141 to Q20164	Q20165 to Q20170
3	24 females	6 females	30	Q20171 to Q20194	Q20195 to Q20200
4	24 females	6 females	100	Q20201 to Q20224	Q20225 to Q20230

Basis of dose selection: The doses were selected based on the results of the fertility study (GT-157-TX-24) at 10, 30 and 100 mg/kg/day in rats. In this study, there were no treatment related premature deaths, clinical signs and there no effect on body weights, body weight gain or food consumption. Based on these, the above doses were selected for the current study.

Observations and Results:

F_0 Dams:

Mortality: Mortality was observed twice a day.

100 mg/kg/day: Two females (Q20201 and Q20213) were prematurely sacrificed on day 25 *post-coitum* because of no delivery. Female Q20213 was not pregnant while female Q20201 had total resorptions. Female Q20209 was prematurely sacrificed on day 1 *post-partum* because of dead litter. No maternal clinical signs were observed prior to the death of the litter to explain the mortality. Macroscopic examination revealed distended stomach with red contents. The cause of the death was not evident.

30 mg/kg/day: Female Q20176 was found dead on day 21 *post-coitum*. Clinical signs on the day of death of this animal included hypotonia, dyspnea and lacrimation. At necropsy, red lungs were observed. The cause of the death was not determined. Female Q20174 was found dead on day 1 *post-partum* with no apparent clinical signs prior to death. There were no necropsy findings in this animal.

10 mg/kg/day: Female Q20163 delivered one pup which was found cannibalized on day 1 *post-partum* and this animal was sacrificed. On day 0 *post-partum*, this animal had pallor of the eyes and extremities. At macroscopic examination, the cause of the moribundity could not be established.

Clinical signs: Clinical signs were observed twice a day.

Hypersalivation (ptyalism) was observed in 16 of 24 females during gestation and 20 of 22 females during lactation at 100 mg/kg/day. This started around mid- to late-gestation and generally lasted until the end of lactation. Four females at 100 mg/kg/day also had loud breathing for a maximum of 3 days, during the first half of the lactation period. No treatment-related clinical signs were observed at 10 or 30 mg/kg/day.

Body weight: The body weight was recorded on days 2, 6, 9, 12, 15, 20 *post-coitum* and on days 1, 4, 7, 10, 14, 18 and 21 *post-partum* and prior to premature sacrifice.

The mean initial (Day 6 pp) and final (Day 20 pp) body weights of control animals were 261 and 378 g, respectively. The final body weight was reduced (93% of control) at 100 mg/kg/day. At 100 mg/kg/day, a statistically significantly lower mean body weight gain was observed from day 12 until day 20 *post-coitum*. There were no significant treatment related effects on mean body weight or body weight gain at 10 or 30 mg/kg/day. The following table (from page 38 of the report) shows the body weight data.

Dose-level (mg/kg/day)	0	10	30	100
Gestation				
Day 6 *post-coitum*	261	260	261	262
Day 20 *post-coitum*	378	382	384	351*
Days 12 - 15 *post-coitum*	+20	+18	+21	+15*
Days 15 - 20 *post-coitum*	+63	+67	+68	+41#
Days 6-20 *post-coitum*	+117	+122	+124	+89#
Lactation				
Day 1 *post-partum*	295	296	297	278
Day 21 *post-partum*	324	328	326	324
Days 1-21 *post-partum*	+29	+32	+29	+46#

*: $p<0.05$; #: $p<0.001$.

Food consumption: Food consumption was recorded for the following intervals: Days 2-6, 6-9, 9-12, 12-15 and 15-20 *post-coitum* and days 1-7, 7-14 and 14-21 *post-partum.*

The mean initial (Day 2-6) and final (Day 15-20) food consumption in control animals were 24 and 29 g/animal/day, respectively. At 100 mg/kg/day, mean food consumption was reduced (4-14% reduction) compared to controls throughout the gestation and lactation periods. There were no significant treatment related effects on mean food consumption at 10 or 30 mg/kg/day.

Uterine content: The numbers of implantation scars were recorded for the dams.

The mean duration of gestation was longer at 100 mg/kg/day (22.0 days *vs.* 21.5 days) compared to control. No effects were observed at 10 or 30 mg/kg/day.

The mean numbers of implantation sites were comparable with the controls for all treated groups. However, at 100 mg/kg/day, animals had a lower (9.8 vs. 12.0 in control) number of delivered pups per litter than the controls and the mean post implantation loss was higher (25.5% vs. 9.4% in control) at 100 mg/kg/day than in the control group. The following table (from page 39 of the report) shows the uterine data.

Mean number of implantations sites, delivered pups and post-implantation loss

Dose-level (mg/kg/day)	0	10	30	100
Mean number of implantation sites	13.2	13.3	13.8	13.2
Mean number of pups delivered	12.0	11.5	13.0	9.8
Mean post-implantation loss (%) (calculated manually)	9.4	16.8	5.1	25.5
Standard deviation	8.4	21.6	5.3	18.7

Necropsy: The principal dams and the non-selected pups were sacrificed on day 22 *post-partum* and a macroscopic examination of the principal thoracic and abdominal organs was performed.

There were no significant treatment related necropsy findings.

Toxicokinetics: Blood samples for TK analysis were collected from all groups on day 6 and day 17 *post-coitum* at 0.5, 1 and 4 hours post-dose and at pre-dose on day 17 *post-coitum* only. Two satellite females were allocated to the control group and blood samples were collected at 0.5 and 1 hour post-dose on days 6 and 17 *post-coitum*. All satellite females were sacrificed after blood sampling on day 17 *post-coitum*.

Plasma exposure to Genz-99067, as measured by C_{max} and $AUC_{0\text{-}4h}$, on both day 6 and day 17 *post-coitum* increased in a greater than dose-proportional manner between 10 and 30 mg/kg/day. Between 30 and 100 mg/kg/day, both C_{max} and $AUC_{0\text{-}4h}$ increased in a less then dose-proportional manner on day 6 *post-coitum* and in an approximately dose-proportional manner on day 17 *post-coitum*. The TK parameters are shown in the following table (from page 36 of the report).

Selected toxicokinetic parameters of Genz-99067

Group	2	3	4
Dose-level of Genz-112638 (mg/kg/day)	10	30	100
Day 6 *post-coitum*			
C_{max} (ng/mL)	126	629	1197
t_{max} (h)	0.5	0.5	0.5
$AUC_{(0-4h)}$ (ng.h/mL)	303	1380	3014
Day 17 *post-coitum*			
C_{max} (ng/mL)	140	602	1823
t_{max} (h)	0.5	0.5	0.5
$AUC_{(0-4h)}$ (ng.h/mL)	277	1228	4817

Dosing solution analysis: Stability of the dosing formulation containing 0.4 and 33.3 mg/mL of test item was analyzed. During the course of the treatment period, the concentration of the test item in solutions was determined. The concentrations of the test article were determined in samples taken from each dosage form (including the control) during the first and the last weeks of treatment of the principal animals. In addition, concentrations of the test article were also determined in samples taken from each dosage form (including the control) for the satellite animals. The acceptance criterion for the concentration of test article was set at nominal value ± 10%.

The results of the analyses of dosing solutions demonstrated satisfactory stability of the dosage forms analyzed (0.4 and 33.3mg/mL) over a 11-day storage period at +4°C. In addition, the measured concentrations of the test item in the vehicle remained within an acceptable range of variation (-4.5% to 3.4%) compared to the nominal value. The test concentrations in the administered dosage forms analyzed during the first and last weeks of treatment remained within an acceptable range of (1.5% to +6.5%) of variation compared to the nominal values. The test concentrations in the administered dosage forms analyzed during the first and last weeks of treatment on satellites remained within an acceptable range of (-0.5% to +3.5%) of variation compared to the nominal values.

F_1 Generation

Survival: One male (Q20021) at 10 mg/kg/day died accidentally on day 24 during the water T-maze testing. This was not related to treatment. One female (Q20245) from the control group was sacrificed after delivering, although no evidence of mating had been observed.

Clinical signs: There were no significant treatment related clinical signs.

Body weight: The mean initial (Day 1) and final (Day 99) body weight of control males were 61 and 570 g, respectively. The mean initial (Day 1) and final (Day 71) body weights of control females were 59 and 282 g, respectively. The final body weights of treated males were 100%, 100% and 89% of control at low, mid and high dose, respectively. The final body weights of treated females were 101%, 98% and 90% of control at low, mid and high dose, respectively. Overall, body weights were reduced by approximately 10% in both sexes at 100 mg/kg/day. There were no changes at 10 and 30 mg/kg/day.

Food consumption: The mean initial (Day 1-8) and final (Day 64-71) of food consumption of control males were 15 and 32 g/animal/day, respectively. The mean initial (Day 1) and final (Day 71) food consumption of control females were 15 and 22 g/animal/day, respectively. Final food consumption was not affected by the treatment in either sex.

Physical development: There were no effects of treatment on the mean age at testes descent. However, balanopreputial separation was achieved slightly earlier (50.6-50.9 days vs. 52.4 days in control) in all treated males. The following table (from page 121 of the report) shows the balaopreputial separation data.

F1 GENERATION
SUMMARY OF CLEAVAGE OF THE BALANOPREPUTIAL GLAND

Sex: male

	Dose-level (mg/kg/day)			
	0	10	30	100
n	20	19	20	20
Mean age of appearance	52.4	50.9	50.6	50.6
SD	4.9	4.4	1.9	2.1
Body weight (grams)	309.9	292.8	305.4	272.0

This was not considered treatment-related as there was no dose response and the mean body weights were slightly lower than the controls. The mean age at appearance of vaginal opening was similar among all the groups, including the controls, although the mean body weight at 100 mg/kg/day was lower. All animals were positive for pupil constriction reflex and acoustic startle response at 4 weeks of age.

Neurological assessment: There were no significant treatment related effects on learning and memory (maze test) and spontaneous locomotor activity.

Reproduction: The mean number of days taken to mate was one day longer at 30 or 100 mg/kg/day than that of the controls and was not considered treatment related due to lack of a dose-response. The fertility indices were 90% or greater and the gestation indices were 100% for all animals treated with Genz-112638. The mean numbers of *corpora lutea* and implantations were lower at 100 mg/kg/day (15.9 vs. 17.3 in control and 14.3 vs. 16.1 in control, respectively) and the mean pre implantation loss was higher (9.9 vs. 6.9 in control) when compared with the controls. In the control group, one

female had total resorptions. The mean number of live concepti was higher in treated animals compared to control and the post-implantation loss was higher in control than the groups-treated with Genz-112638. The numbers of early resorptions was higher in the control group compared to the Genz-112638 treated animals. The numbers of late resorptions in the control was comparable to the Genz-112638 treated animals (except at 30 mg/kg/day, lower than control).The following table (from page 139-140 of the report) shows the hysterectomy data.

F1 **GENERATION**

HYSTERECTOMY DATA (Summary table)

Dose (mg/kg/day)		0	10	30	100
Pregnant Females Alive at Term	N	18	18	18	19
with Total Resorptions	N	1	0	0	0
with all Dead Concepti	N	0	0	0	0
with Live Concepti	N	17	18	18	19
Corpora Lutea	TOTAL	311	309	295	302
No. per animal	MEAN	17.3 d	17.2	16.4	15.9
	S.D.	2.3	2.4	2.2	2.4
Implantation Sites	TOTAL	289	299	285	272
No. per animal	MEAN	16.1 d	16.6	15.8	14.3
	S.D.	2.4	2.2	2.5	2.5
Preimplantation Loss	MEAN%	6.9 d	3.0	3.7	9.9
	S.D.	7.6	4.6	6.1	9.6
Concepti	TOTAL	248	281	276	254
No. per animal	MEAN	13.8 d	15.6	15.3	13.4
	S.D.	4.4	2.2	2.3	2.6
Live Concepti	TOTAL	248 f	281**	275#	254**
	MEAN%	85.9 d	94.1	96.8	93.5
	S.D.	23.5	6.8	5.8	9.8
Dead Concepti	TOTAL	0 f	0	1	0
	MEAN%	0.0 d	0.0	0.3	0.0
	S.D.	0.0	0.0	1.3	0.0

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test ** = p<0.01 # = p<0.001

F1

GENERATION
HYSTERECTOMY DATA (Summary table)

Dose (mg/kg/day)		0	10	30	100
Resorptions + Scars	TOTAL	41 f	18**	9#	18**
% of implantation sites		14.2	6.0	3.2	6.6
No. per animal	MEAN	2.3 d	1.0	0.5	0.9
	S.D.	4.0	1.2	0.9	1.5
Implant Scars	TOTAL	17 f	0#	0#	0#
% of implantation sites		5.9	0.0	0.0	0.0
No. per animal	MEAN	0.9 d	0.0	0.0	0.0
	S.D.	4.0	0.0	0.0	0.0
Resorptions: early	TOTAL	16 f	9	5*	10
% of implantation sites		5.5	3.0	1.8	3.7
No. per animal	MEAN	0.9 d	0.5	0.3	0.5
	S.D.	1.6	0.9	0.6	1.4
Resorptions: late	TOTAL	8 f	9	4	8
% of implantation sites		2.8	3.0	1.4	2.9
No. per animal	MEAN	0.4 d	0.5	0.2	0.4
	S.D.	0.7	1.0	0.5	0.7
Postimplantation Loss	MEAN%	14.1 d	5.9	3.2	6.5
	S.D.	23.5	6.8	5.8	9.8
Dams Affected	N	13 f	10	6*	9
	%	72.2	55.6	33.3	47.4

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test * = p<0.05 ** = p<0.01 # = p<0.001

Macroscopy: Statistically significantly lower absolute testes and epididymides weights were noted in F1 males at 100 mg/kg/day. These weight changes are shown in the following table (from page 43 of the report).

Principal differences (expressed in %) noted between treated and control animals in the absolute and relative organ weights

Sex	Male		
Group	2	3	4
Dose-level (mg/kg/day)	10	30	100
Body weight	-2	+2	-8*
- Epididymides			
. absolute	-8	+1	-13**
. relative	-5	+1	-4
- Prostate			
. absolute	-7	-5	-11
. relative	-4	-6	-2
- Testes			
. absolute	-5	0	-13**
. relative	-3	-1	-4

Statistically significant from controls: *:p<0.05, **: p<0.01,
The significance concerned the organ weights values and not the percentages.

The Applicant stated that these differences were mainly due to the contribution of a single animal with spontaneous seminiferous tubule atrophy which may also occur spontaneously in controls, a relationship to treatment was considered unlikely. Per the Applicant, the minimally lower absolute prostate weights were considered not to be treatment-related. They were considered to be most likely due to the decreased final body weight.

In males, small epididymides and testes were observed at 10 and 100 mg/kg/day. These findings (small epididymides and testes) do not appear to be treatment-related in the absence of dose response. The following table (from page 44 of the report) shows the data.

Incidences of main macroscopic findings

Group	1	2	3	4
Number of animals per group	20	20	20	20
Small testis(es)	0	1	0	1
Small epididymis(ides)	0	2	0	1

<u>Histopathology</u>: Occasional unilateral or bilateral, moderate to marked atrophy of seminiferous tubules was noted in occasional control and treated rats. This finding generally correlated with organ weights and macroscopic findings. Since this was observed in controls and the incidence was low, it was considered to be sporadic and

unrelated to treatment. The following table (from page 44 of the report) shows the main microscopic findings.

Incidences of main microscopic findings

Group	1	2	3	4
Number of animals per group	20	20	20	20
Atrophy of seminiferous tubules	1	2	0	1

F2 Generation:

The Applicant did not provide the F_2 generation data.

Summary: In a pre- and post-natal development study in rats, animal were treated at 10, 30 and 100 mg/kg/day by oral gavage.

F_0 Generation: Two females at 100 mg/kg/day and two females at 30 mg/kg/day and one female at 10 mg/kg/day were sacrificed prematurely. Clinical signs at 100 mg/kg/day included ptyalism (hypersalivation) during gestation and lactation. Body weight (7% reduction compared to control) and food consumption (4-14% reduction compared to control) were reduced at 100 mg/kg/day. The mean duration of gestation was longer at 100 mg/kg/day (22.0 days *vs.* 21.5 days) compared to control. At 100 mg/kg/day, animals had a lower (9.8 vs. 12.0 in control) number of delivered pups per litter than the controls and the mean post implantation loss was higher (25.5% vs. 9.4% in control) at 100 mg/kg/day than in the control group.

F1 Generation: Body weights were reduced by approximately 10% in both sexes at 100 mg/kg/day. There were no changes at 10 and 30 mg/kg/day. Food consumption was not affected by the treatment in either sex. Balanopreputial separation was achieved slightly earlier (50.6-50.9 days vs. 52.4 days in control) in all treated males. This was not considered treatment-related as there was no dose response and the mean body weights were slightly lower than the controls. There were no significant treatment related effects on learning and memory and spontaneous locomotor activity. The mean numbers of *corpora lutea* and implantations were lower at 100 mg/kg/day (15.9 vs. 17.3 in control and 14.3 vs. 16.1 in control, respectively) and the mean pre implantation loss was higher (9.9 vs. 6.9 in control) when compared with the controls. Statistically significantly lower absolute testes and epididymides weights were noted in F_1 males at 100 mg/kg/day. In males, small epididymides and testes were observed in gross necropsy at 10 and 100 mg/kg/day. These findings (small epididymides and testes) do not appear to be treatment-related in the absence of dose response.
Overall, in this pre and postnatal development study in rats, eliglustat did not appear to cause any significant adverse effects on pre and postnatal development up to 100 mg/kg/day.

10 Special Toxicology Studies

4-Week Study by Twice Daily Oral Administration to Mature Cynomolgus Monkeys followed by a Treatment-Free Period (Report No. GT-157-TX-31)

Methods: This is an exploratory study and was not conducted in compliance with Good Laboratory Practice (GLP) regulations. Impaired spermatogenesis was observed in rodents. The objective of this study was to confirm this effect in non-human primates and to evaluate the potential adverse effects of Genz-112638 on male reproductive organs and on sperm production in mature male (n = 4, mature) monkeys. In this study, each animal received an oral (gavage) daily dose of 72 mg/kg/day (36 mg/kg BID, 6 hours apart, 1 mL/kg) for 4 weeks. After the 28 days of dosing, animals remained on study for an additional 12 to 29 weeks to permit evaluations through one or more spermatogenesis cycles. Sperm samples were collected and analyzed approximately every 14 days throughout the study, including the post-dosing period. Blood samples for toxicokinetics and GL1 analysis were collected on Days 1 and 28 at 1, 2, 3, 4, 5 and 6 hours after the first daily dose. Each animal was checked for mortality and morbidity at least twice a day during the treatment and treatment-free periods. Clinical signs were observed on a daily basis. Semen was collected twice a week for the following sperm analysis: motility (M), morphology (MN), live sperm (LS), sperm concentration (SC), and total live sperm (TLS).

Results: There were no significant treatment-related effects on any of the measured sperm parameters. The following table (from page 13-14 of the report) shows the results of sperm analysis.

Appendix 1

Individual Monkey Spermatogenesis Data

Monkey	timept	Colldt	Sperm volume	mor_ normal	motility	live_ sperm	sperm_ conc	total_live sperm
120-694-MF	Baseline	4/10/2007	0.5	85	65	0	1628	692
120-694-MF	Baseline	4/27/2007	0.5	95	70	99	1710	846
120-694-MF	Baseline	5/11/2007	1	99	99	99	1520	1505
120-694-MF	Baseline	5/25/2007	1	99	80	80	390	312
120-694-MF	Baseline	6/8/2007	0.7	99	95	95	2000	1330
120-694-MF	Baseline	6/22/2007	0.8	90	70	70	2050	1148
120-694-MF	Baseline	7/6/2007	0.5	99	99	99	340	168
120-694-MF	Baseline	7/20/2007	0.5	99	85	85	2140	910
120-694-MF	Baseline	8/3/2007	0.5	99	90	90	1050	473
120-694-MF	Baseline	8/17/2007	0.5	98	99	99	980	485
120-694-MF	Baseline	9/4/2007	0.5	99	98	98	400	196
120-694-MF	Baseline	10/19/2007	0.5	98	99	99	940	931
120-694-MF	Genz_trt	11/16/2007	0.5	99	80	80	402	161
120-694-MF	Genz_trt	11/30/2007	0.5	99	60	75	1370	514
120-694-MF	Post_trt	12/14/2007	0.5	99	90	90	640	461
120-694-MF	Post_trt	12/28/2007	0.5	99	80	80	1940	776
120-694-MF	Post_trt	1/11/2008	0.5	99	90	95	410	390
120-694-MF	Post_trt	2/8/2008	0.5	99	95	98	3550	2738
120-694-MF	Post_trt	2/22/2008	0.3	99	99	99	2730	811
120-694-MF	Post_trt	3/18/2008	0.2	99	85	85	5200	884
120-694-MF	Post_trt	4/4/2008	0.8	99	95	95	1870	1421
120-694-MF	Post_trt	4/18/2008	0.5	99	50	50	3380	845
64Y	Baseline	11/30/2007	0.8	99	10	20	3500	560
64Y	Baseline	12/14/2007	1	99	80	80	1760	1408
64Y	Baseline	12/28/2007	0.6	99	30	60	1200	432

64Y	Genz_trt	1/11/2008	1	99	90	95	1000	950
64Y	Genz_trt	1/25/2008	0.5	99	99	99	1625	804
64Y	Post_trt	2/8/2008	1	99	99	99	2450	2426
64Y	Post_trt	2/22/2008	1	99	70	70	1100	770
64Y	Post_trt	3/18/2008	0.9	99	95	95	2200	1881
64Y	Post_trt	4/4/2008	0.9	99	80	80	690	497
64Y	Post_trt	4/18/2008	1	99	99	99	1200	1188
64Y	Post_trt	5/16/2008	0.3	98	90	90	2680	724
64Y	Post_trt	6/13/2008	0.5	99	95	95	2430	1154
64Y	Post_trt	6/27/2008	0.8	99	70	60	2450	1176
64Y	Post_trt	7/25/2008	0.4	99	95	95	4730	1798
64Y	Post_trt	8/15/2008	0.4	99	95	95	4260	1619
64Y	Post_trt	8/29/2008	0.3	99	95	99	5240	1556
3C52	Baseline	2/26/2008	0.2	99	95	95	3940	749
3C52	Baseline	3/21/2008	0.3	99	80	80	3015	724
3C52	Baseline	6/27/2008	0.1	99	98	98	3,800	372
3C52	Baseline	7/25/2008	0.2	99	80	90	1250	225
3C52	Baseline	8/15/2008	0.7	99	95	95	730	485
3C52	Baseline	8/29/2008	0.8	99	80	99	1910	1513
3C52	Baseline	9/16/2008	0.3	99	90	95	3700	1055
3C52	Baseline	10/3/2008	0.7	99	95	99	2050	1421
3C52	Genz_trt	10/21/2008	0.4	99	95	95	2340	889
3C52	Post_trt	11/11/2008	0.3	99	60	95	2120	604
3C52	Post_trt	12/5/2008	0.6	99	80	95	1230	701
3C52	Post_trt	12/23/2008	0.5	99	60	85	1170	497
3C52	Post_trt	1/6/2009	0.9	99	90	95	1820	1556
3C52	Post_trt	1/27/2009	0.5	99	85	99	1970	975
3C32	Baseline	2/26/2008	0.3	99	40	60	2550	459
3C32	Baseline	3/21/2008	0.3	99	70	70	11	3
3C32	Baseline	6/13/2008	0.2	99	99	99	5070	1004
3C32	Baseline	6/27/2008	0.1	99	97	97	3100	301
3C32	Baseline	7/25/2008	0.2	99	20	95	2900	551
3C32	Baseline	8/15/2008	0.2	99	40	60	2730	328
3C32	Baseline	8/29/2008	0.3	99	55	90	1160	313
3C32	Baseline	9/16/2008	0.2	99	60	70	2440	342
3C32	Baseline	10/3/2008	0.6	99	70	99	1310	724
3C32	Genz_trt	10/21/2008	0.6	99	95	95	3120	1778
3C32	Post_trt	11/11/2008	0.2	99	80	99	790	156
3C32	Post_trt	12/5/2008	0.6	99	60	80	1500	720
3C32	Post_trt	12/23/2008	0.3	99	80	85	2610	666
3C32	Post_trt	1/6/2009	0.6	99	60	85	1370	699
3C32	Post_trt	1/27/2009	0.3	99	80	95	1210	345

The C_{max} of Genz-112368 on Day 1 ranged from 8.5 to 346 ng/mL and C_{max} on Day 28 ranged from 2.4 to 1050 ng/mL. The AUC on Day 1 ranged from 22 to 1150 ng.hr/mL and the AUC on Day 28 ranged from 5.5 to 3730 ng.hr/mL. The following tables (from page 22 of the report) show the TK data.

Genz-112638 (Plasma) Treatment PO Study Day 1

Parameter	Units	Subject 120-665	Subject 120-694	Subject 32424	Subject 3C32	Subject 3C52	Subject 64y	Mean	S.D.	%CV
Original Dose	mg/kg	60	60	60	60	60	60			
Tmax	Hours	3.00	2.00	2.00	1.00	1.00	3.00	2.00	0.894	44.7
Cmax	ng/mL	102	32.2	558	45.0	8.52	346	182	222	121.9
AUC	ng*Hours/mL	292	74.6	1750	117	22.1	1150	568	715	126.0

Genz-112638 (Plasma) Treatment PO Study Day 28

Parameter	Units	Subject 120-694	Subject 3C32	Subject 3C52	Subject 64y	Mean	S.D.	%CV
Original Dose	mg/kg	60	60	60	60			
Tmax	Hours	2.00	2.00	1.00	4.00	2.25	1.26	55.9
Cmax	ng/mL	23.2	4.52	2.41	1050	270	520	192.6
AUC	ng*Hours/mL	63.4	14.2	5.54	3730	953	1850	194.2

On Day 28, GL1 levels were reduced for all four animals. GL1 for individual animals were reduced by 37-85% at 28 days (1 hour post dose) when compared to the Day 1.

Overall, sperm morphology was not affected by the treatment. However, there were significant variations in sperm counts and motility between animals and over time. It was concluded that there were no significant treatment related effects on any measured sperm parameters.

A Pilot Study to Compare the Behavioral Outcomes following Drug-Induced Peripheral Neuropathy in the Rat (Report No. GT-157-TX-22)

Methods: The objective of this pilot study was to determine the behavioral endpoints for evaluating the potential effects of Genz-112638 to induce a peripheral neuropathy in male SD rats (n = 6/group). The effects of Genz-112638 were compared to Taxol (paclitaxel), which is known to induce peripheral neuropathies that result in both sensory and motor dysfunction. In this study, Genz-112638 was administered at 75 mg/kg, BID (150 mg/kg/day) by oral gavage for 4 days. The dose was then reduced to 50 mg/kg BID (100 mg/kg/day) for 35 days. Taxol was administered by intraperitoneal (IP) injection at 16 mg/kg on Day 10, 17, 24 and 31. In this study, the following behavioral parameters were examined: thermal paw stimulation for pain sensitivity, open field test for activity (locomotion and vertical rearing), foot fault test, and force plate analysis of spontaneous tremor. In addition, the dorsal ganglia of the lumbar vertebra, and the sciatic nerve and hind paw dermis were collected for histopathological evaluations to assess the degree and amount of axonal disruption caused by each drug treatment. The following table (from page 11 of the report) shows the study design. Negative control animals were treated with distilled water for 35 days.

4.7 Table 1 – Treatment Groups

Group No.	Treatment	Dose
1	Vehicle Only	na
2	Taxol injection	*16 mg/kg I.p.
3	Genz-112638	**50 mg/kg BID

* -- The original dose was 32 mg/kg but was reduced on 6/07/04.
** - The original dose was 75 mg/kg but was reduced on 6/01/04.

Animals were observed for clinical signs on daily basis. Body weights were recorded prior to randomization, on Day 0, and on the behavioral testing days 7, 14, 21, 28 and then again at necropsy. Necropsy was conducted on Day 35.

Results: Nine animals were found dead. Four animals from Group 2 (Taxol 32 mg/kg) were found dead in the first week following the initial dose. Taxol dose was reduced to 16 mg/kg and no further mortality was seen. Five animals from Group 3 (Genz-112638) died during the course of the study. A necropsy was performed on the initial two dead animals from this group, which were treated at higher dose (75 mg/kg BID) and most of the internal organs appeared normal. The lungs for both of these animals (#7590 & 7583) appeared mottled, which correlated with observed wheezing.

Thermal paw test did not reveal any Genz-112638 related effects on paw withdrawal latencies in response to thermal stimulation compared to the negative control. Taxol reduced this response compared to control and Genz-112638. The tremor test (force plate tremor test) did not register any tremors for any animals in this study at any time points. Activity measurements in the open field test revealed significant reductions in the amount of horizontal movement in the Genz-112638 group as compared to the control group. This effect was apparent as early as Day 7, and it occurred at each testing period throughout the study. There were no significant differences for the other groups. The analysis for the foot fault test examined the number of times a foot slip occurred in any paw for each group as a function of overall movement during the test (quadrants crossed). The results indicated significant differences for both treatment and time. Further comparisons revealed an overall treatment difference for Taxol vs. controls and for Genz-112638 vs. controls as well as a time difference for the Taxol group on Day 28 and Genz-112638 vs. their respective baseline data. Histopathological examinations did not reveal any treatment-related axonal disruption in the dorsal root ganglion, right sciatic nerve, or hindpaw dermis in any of the animals. Thus, there were no significant differences among any of the groups.

Overall, the results of this pilot study indicated that Genz-112638 did not show peripheral neuropathy in the thermal paw stimulation test and did not induce tremor (although a positive control for tremor was not used). However, Genz-112638 reduced locomotor activity.

Review of Lymphoid Tissues from Six Studies Performed in the Dog or Rat with Geng-112638 (Report No. GT-157-TX-46)

Methods: The objective of this independent review was to describe and compare the findings on lymphoid tissues observed in six studies in rats and dogs with Genz-112638. The following table (from page 5 of the report) shows the details of these six studies.

Table A. Title and reference numbers of the studies

Project No.: GT-157-TX-5	
Title	A 28-day oral gavage toxi city study with Genz-112638 (with a 14-day recovery) in albino rat.
Facility/study number	(b) (4)/ Project No. 800159.
Project No.: GT-157-TX-6	
Title	A 28-day oral gavage toxi city study with Genz-112638 (with a 14-day recovery) in the beagle dog.
Facility/study number	(b) (4)/Project No.: 800160.
Project No.: GT-157-TX-15	
Title	13-week toxicity study by oral route (gavage) in beagle dogs followed by a 4-week treatment-free period.
Facility/study number	(b) (4)/*Study No. 26628 TCC.*
Project No.: GT-157-TX-17	
Title	26-week toxicity study by oral route (gavag e) in rats followed by an 8-week treatment-free period.
Facility/Study Number	(b) (4)/*Study No. 27126 TCR.*
Project No.: GT-157-TX-23	
Title	52-week toxicity study by oral route (gavage) in beagle dogs followed by an 8-week treatment-free period.
Facility/Study Number	(b) (4)/*Study No. 27127 TCC.*
Project No.: GT-157-TX-28	
Title	4-week toxicity study by twice daily oral (gavage) administration to m ature rats followed by a treatm ent-free period.
Facility/Study Number	(b) (4)/*Study No. 32267 TSR.*

Rat Studies: Four studies were conducted in Sprague Dawley (SD) rats. Two 4-week studies were conducted in rats. In study GT-157-TX-5, four groups of male and female Sprague-Dawley rats (approximately 28 days old) were treated with Genz-112638 at 0, 5, 15 or 50 mg/kg BID. In study GT-157-TX-28, three groups of male Sprague-Dawley rats (12 weeks old) were treated at 0, 15 or 50 mg/kg BID for 29 days and one group was treated at 100 mg/kg BID for 16 days. The rats from this high-dose group were euthanized prematurely due to poor health conditions. In a 26-week toxicity study by oral route (gavage) followed by an 8-week treatment-free period (GT-157-TX-17), four groups of male and female Sprague-Dawley rats (approximately 7 weeks old) were respectively treated at 0, 5, 15 or 50 mg/kg/day.

Dog Studies: Two studies were conducted in dogs. In study GT-157-TX-6, four groups of male and female dogs were respectively treated at 0, 2.5, 5 and 12.5 mg/kg BID for 28 days with a 14-day recovery period. In study GT-157-TX-15, dogs (n = 4/sex/dose) were treated for 13-week followed by a 4-week recovery period at 0, 2, 5 and 10 mg/kg/day. In study, GT-157-TX-23, dogs (n = 4/sex/dose) were treated with Genz-112638 for 52 weeks followed by an 8-week recovery period at 0, 2, 5 and 10 mg/kg/day.

In order to compare the findings from the six studies, a review of organ weights, macroscopic data and microscopic examination of lymphoid organs was performed (Kuper C.F. et al., 2000, Histopathologic Approaches to Detect Changes Indicative of Immunotoxicity, Toxicol Pathol, 28:454-66). The following (from page 6 of the report) table shows the tissues examined.

TISSUE PROCEDURE TABLE

Organs	Organ Weights*	Microscopic Examination*
Peyer's patches (ileum)		X
Lymph nodes (mandibular and mesenteric)		X
Spleen X		X
Bone marrow (sternum)		X
Thymus X		X

*: the organ weights were not available in project GT-157-TX-28. In addition, only the lymphoid tissues with macroscopic abnormalities were sampled in this study which focused on the male reproductive tract.

Results: Gross macroscopic findings were observed in the thymus and spleen of male rats at 100 mg/kg BID for 2 weeks. In this analysis, the organs with macroscopic abnormalities were collected and examined histologically. The changes in the thymus (5 of 10 male rats) included a decrease of the size and cellularity of the cortex and medulla, with decreased cortex/medulla ratio. In the spleen, there was a slight decrease in size and/or cellularity of the periarteriolar lymphoid sheaths in 2 of 3 rats. In the dogs, atrophic changes in the thymus, characterized by minimal to moderate decrease in the cortex size, were noted in female dogs at 12.5 mg/kg BID for 4 weeks and in females at 10 mg/kg/day for 13 weeks. These findings correlated with lower thymus weights. However, peripheral lymphocyte counts were unaffected. In addition, atrophic findings

were also seen in the lymph nodes and gut associated lymphoid tissue (GALT) in females at 12.5 mg/kg BID for 4 weeks, which consisted of minimal to slight decrease in germinal center development of mesenteric or mandibular lymph nodes. The findings in the GALT were less prominent than in controls. These changes in females were not present at the end of the treatment-free period, indicating that these were reversible.

Per the review pathologist, these findings (seen in the thymuses, lymph nodes and GALT) were related to the treatment with Genz-112638, although they were of low magnitude and were questionable in males. There were some discrepancies noted between the 5 mg/kg BID for 4 weeks and the 10 mg/kg/day in the 13- and 52-week dog studies. While some changes were seen in the thymus in the 13-week study, no significant differences between treated and control animals were noted in the 4- and 52-week studies. In addition, the changes seen in the lymph nodes and GALT of female dogs at 12.5 mg/kg/day for 4 weeks were not observed at lower doses in the 4-, 13-, or 52-week studies. It was mentioned that the effects due to the stress or to the normal involution of the thymus (mainly in the dogs) over time could have contributed to these effects (Greaves P, 2007, In: Histopathology of Preclinical Toxicity Studies, Hemopoietic and Lymphatic Systems, 4, 125-127; Gruver AL and Sempowski GD, 2008, Cytokines, Leptin, and Stress-Induced Thymic Atrophy, Journal of Leukoc Biol, 84:915-23). From a morphological standpoint the changes seen in the thymus of the dogs and rats were similar. These changes consisted of a decrease of the size of the cortex and/or medulla, along with a decreased cortex/medulla ratio. This finding was observed in rats at 100 mg/kg BID for 2 weeks. These changes in dogs were less prominent than that in rats, seen in females at 10 mg/kg/day or 12.5 mg/kg BID, respectively, for 13 weeks or 4 weeks.

11 Integrated Summary and Safety Evaluation

Eliglustat (Cerdelga®) is a glucosylceramide synthase (GCS) inhibitor that resembles ceramide, the substrate for GCS. Eliglustat is indicated for the long-term treatment of adult patients with Gaucher disease type 1 (GD1) and is being developed as a substrate reduction therapy (SRT) for GD1. The recommended dosage is 100 mg capsule twice daily (BID).

Eliglustat (Genz-112638) has been evaluated in a comprehensive program of nonclinical studies which included pharmacology, ADME (absorption, distribution, metabolism and excretion), acute toxicology (rat, dog), and repeated dose toxicology (4, 13 and 26-week studies in rats and 4, 13 and 52-week studies in dog), juvenile toxicology (10-week study in juvenile rats), genotoxicity (Ames test, chromosome aberration assay, mouse bone marrow micronucleus test), carcinogenicity (2-year carcinogenicity studies in mice and rats), reproductive toxicity (fertility and early embryonic development in rats, embryofetal development in rats and rabbits and pre and post-natal development in rats) and special toxicology. In addition, studies were conducted to qualify one of the metabolites of eliglustat that was identified as a disproportionate metabolite in humans, and two impurities that elicited structural alerts *in silico* were evaluated for genotoxicity.

In vitro pharmacology studies revealed the potency (IC_{50} = 10 ng/mL) for the inhibition of GCS in human K562 cells or human A375 cell-derived microsomes. No inhibition of off-target enzymes such as glycosidases (up to 37 µM) and disaccharases were observed. In animal efficacy studies (*in vivo*), eliglustat decreased GL-1 levels in peripheral tissues and plasma in normal rats and dogs following oral administration. In the D409V/null mouse model of GD1, eliglustat decreased the accumulation of GL-1 in tissues. In older GD1 mice with already established disease, eliglustat significantly reduced both the number of enlarged, activated, macrophages typical of Gaucher disease ("Gaucher cells") (Kolter T and K Sandhoff, 2006, Sphingolipid Metabolism Diseases, *Biochim Biophys Acta*, 1758:2057-2079) and the accumulated GL-1 in peripheral tissues and plasma. Potential secondary pharmacodynamic (PD) effects of eliglustat were examined (*in vitro*) in screening assays. In these screening assays, eliglustat showed binding to dopamine (D3, D4), serotonin (5HT1A, 5-HT2A, 5-HT2B and 5-HT6), mu opioid receptors and calcium channels with IC_{50} values of 10 µM. This concentration (10 µM) is approximately 100 times greater than the mean predicted clinical C_{max} of 44.3 ng/mL (~100 nM).

Several safety pharmacology studies were conducted with eliglustat by oral route, which included central nervous system (CNS, rat), pulmonary (rat), renal (rat), gastrointestinal (rat) and *in vitro* and *in vivo* (dog) cardiovascular safety pharmacology studies. Eliglustat did not cause any significant CNS effects in rats when tested at 20,100 and 400 mg/kg. In pulmonary safety pharmacology study in rats at 20, 100 and 400 mg/kg, eliglustat caused about 27% decrease in respiration rate at 400 mg/kg. In renal safety pharmacology study in rats at 20, 100, and 400 mg/kg, eliglustat increased urinary pH (100 and 400 mg/kg) and decreased potassium and chloride excretion at 400 mg/kg. In gastrointestinal safety pharmacology study in rats at 20, 100 and 400 mg/kg, eliglustat decreased gastrointestinal (GI) transit and GI emptying at 100 and 400 mg/kg. Eliglustat caused inhibition of hERG channel expressed in HEK-293 cells with an IC_{50} value of 0.35 µg/mL, indicating a potential to cause QT prolongation. Eliglustat inhibited sodium and calcium channel with IC_{50} values of 5.2 and 10.4 µg/mL, respectively. These values are about 8, 117, and 235 times greater, respectively, than the mean predicted (PK simulation) clinical C_{max} of 44.3 ng/mL. In isolated canine Purkinje fiber assay, eliglustat decreased action potential duration (APD_{60} and APD_{90}) at 1 and 10 µg/mL. In a single oral (gavage) dose conscious dog telemetry study at 1, 3, 10, 25, 50 and 80 mg/kg, eliglustat increased PR (50, 80 mg/kg) and QRS (10, 25, 50 and 80 mg/kg) intervals. In an IV (infusion) telemetry study in anesthetized dog at 1, 2.5 and 5 mg/kg, eliglustat increased PR, QRS and QTc intervals at 2.5 and 5 mg/kg.

The ADME of eliglustat have been evaluated *in vivo* in several species used for toxicology studies including mouse, rat, rabbit, dog and monkey, and *in vitro* in mouse, rat, dog, monkey and human. The pharmacokinetic properties of eliglustat were generally consistent across species e.g., mouse, rat, dog and monkey. Terminal half-lives in male mice, male rats, male dogs and male monkeys were 0.546, 0.266, 1.03 and 1.43 hour, respectively. Following a single oral dose at 3 mg/kg dose, eliglustat was rapidly absorbed with a t_{max} of within 1 hour across all species tested. Oral bioavailability in male mice, male rats, male dogs and male monkeys were 4.68%, 8.70%, 12.3%, and

0.803%, respectively. Eliglustat exhibited moderate-to-high plasma protein binding across nonclinical species and human, with mouse ≈ rat ≈ dog (97.2 to 99.0%) > monkey (92.0 to 92.2%) > human (76.4 to 79.5%). *In vivo* tissue distribution profiles were comparable across nonclinical species. Following a single oral administration of [^{14}C]-Genz-99067 to mice at 125 mg/kg, or [^{14}C] Genz-112638 to rats at 50 or 100 mg/kg, radioactivity was rapidly and extensively distributed to tissues and decreased over time, with maximal concentrations were observed at approximately 0.5 to 1 hour. High concentration of radioactivity was observed in the gall bladder, kidneys, liver, urinary bladder, and intestine, and moderate concentration of radioactivity observed in lung, spleen, heart, adrenal, pituitary and prostate glands, and bone marrow. Negligible or limited exposure was observed in the brain, eyes, testes and spinal cord. However, a 10-fold increase in radioactivity was observed in the brain tissue of P-glycoprotein (P-gp) deficient mice, compared to normal mice. Eliglustat was extensively metabolized in the rat, dog, monkey, and human. Metabolism of eliglustat occurred on the octanoyl, 2,3-dihydro-1,4 benzodioxane, and pyrrolidine moieties of the parent molecule. Major metabolic pathways of eliglustat included sequential oxidations of the octanoyl moiety. The primary metabolites included the 7-hydroxyl metabolite Genz 256416 by cytochrome P450 (CYP450)2D6, CYP3A4 and CYP2C19, the 6-hydroxyl metabolite Genz-311752 by CYP2D6 and CYP3A4, the 5-hydroxyl metabolite Genz 258179 by CYP3A4, and the amine metabolite Genz-256222 by CYP3A4 and CYP2C19. In human hepatocytes, primary hydroxyl metabolites, Genz-256416 and Genz-311752, were metabolized to secondary ketone metabolites, Genz-258162 and Genz-527862, respectively, by CYP2D6 and CYP3A4, and further metabolized to acid metabolites Genz-399207, Genz-399240 and Genz-682042 in human hepatocytes. *In vitro* metabolite profiles were consistent across rat, dog, monkey and human liver microsomes and hepatocytes. All metabolites detected in human liver microsomes and hepatocytes were observed *in vitro* in at least one of the nonclinical species tested. The 7-hydroxyl metabolite Genz-256416 was the major metabolite of eliglustat in the rat, dog, monkey, and human liver microsomes and hepatocytes. *In vivo* metabolite profiles were similar for the primary hydroxyl metabolites and secondary ketone metabolites, but different for acid-containing metabolites in rat, dog, rabbit, monkey, and human. All circulating metabolites identified in the clinical study (GZGD02107) by were also observed in preclinical species tested. The only major human circulating metabolite exceeding 10% of total drug related exposure in human, Genz-399240, was low or below detectable levels in the rat and dog plasma, but at appreciable levels in the rabbit and monkey plasma. In rats, following a single 10 mg/kg IV dose of [^{14}C]-eliglustat, 95 to 97% of the total radioactivity was recovered within 168 hours with approximately 81 to 82% of total radioactivity eliminated in the feces, and approximately 11 to 14% of total radioactivity in the urine. The majority of the radioactivity was excreted in the feces and urine within 24 to 48 hours. In dogs, following a single 2.5 mg/kg IV dose of [^{14}C] eliglustat, 91 to 94% of the total radioactivity was recovered within 168 hours and radioactivity was primarily eliminated in the feces (64% to 67% of total radioactivity), with 25% of total radioactivity eliminated in the urine. In dogs, following a single 25 mg/kg oral dose of [^{14}C] eliglustat, 90 to 92% of the total radioactivity was recovered after 168 hours with 50% to 57% of total radioactivity eliminated in the feces, and 26% to 30% of total radioactivity eliminated in the urine.

Acute toxicology studies were conducted by intravenous (IV infusion, rat) and oral (rat and dog) routes at doses ranging from 3-20 mg/kg (IV, rats) and 200 to 1000 mg/kg (oral, gavage) in rats and 25 to 100 mg/kg (oral, capsule) in dogs. The maximum nonlethal doses in rats were 20 mg/kg and 400 mg/kg by IV and oral route, respectively. In rats, clinical sings observed at doses ≥400 mg/kg included salivation, wet fur, labored and/or abnormal sneezing and breathing, decreased fecal output and/or abnormal distension. At 200 mg/kg, recurring breathing abnormalities and decreased feces output were noted. The maximum nonlethal dose in dogs was 100 mg/kg by oral route. In dogs, emesis was observed at all doses.

Chronic oral toxicology studies were conducted in rats (6-month) and dogs (1-year) at doses ranging from 5 to 50 mg/kg/day and 2 to 10 mg/kg/day, respectively. In the 26-weeek study in rats, there were no significant treatment-related histopathological findings in any organ or tissue. The NOAEL was considered as 50 mg/kg/day.

In an oral gavage study in juvenile rats, animals were treated with Genz-112638 at 5, 15 and 25 mg/kg BID for 10 weeks commencing at PND 22 through Day 91. Mortalities were observed at all doses including control and were deaths were mainly considered due to gavage errors. Gross pathology findings included large mandibular lymph nodes with increased incidence at 25 mg/kg BID in females. In females, liver weights were increased at the high dose. Histopathological changes were seen in the lymph node (increased incidence/severity of lymphoid hyperplasia in the mandibular lymph nodes at 25 mg/kg BID) and eye (minimal grade unilateral neuropathy in the optic nerve of two animals at 25 mg/kg BID). The target organ could be the lymph node and eye based on the histopathology findings. The NOAEL was considered as 15 mg/kg BID.

A 28-day oral study was conducted in male rats to evaluate specific effects of Genz-112638 on male reproductive organs and spermatogenesis at 15 or 50 mg/kg BID (30 or 100 mg/kg/day) for 4 weeks or at 100 mg/kg BID (200 mg/kg/day) for 2 weeks. Mortality was observed at 200 mg/kg/day, and the dosing was terminated after 2 weeks of treatment due to severe toxicity. Electrolytes and liver enzymes as well as sperm motility and morphology were affected at 200 mg/kg/day. At 200 mg/kg/day, sperm morphology remained affected until 14-weeks treatment-free recovery period. Gross pathology examinations revealed grey foci on the forestomach mucosa, small thymus and spleen. Microscopic examinations revealed germ cell necrosis in the testis, sloughed cells in the epididymis, acute inflammation in the coagulating glands, epithelial hyperplasia in the forestomach and severe atrophy of thymus and spleen. No effects on sperm count or motility were seen at 50 and 15 mg/kg BID doses. A follow-up 4-week oral study (GT-157-TX-31) was conducted at 36 mg/kg BID (72 mg/kg/day) in mature Cynomolgus monkeys to confirm the above effects of Genz-112638 on spermatogenesis in non-human primates. There were no significant treatment-related effects on any of the measured sperm parameters in this monkey study.

In 28-study in dogs, histological changes included atrophy of lymphoid organs/tissues (thymus, lymph nodes and gut lymphoid tissues) at 10 and 25 mg/kg/day, which was reversed after 2-week recovery. In a 13-week oral (gavage) toxicity study in Beagle dogs, animals were administered Genz-112638 at 2, 5 or 10 mg/kg/day. Histopathological changes were seen in the thymus (lymphoid depletion) of treated and control animals. However, the severity of lymphoid depletion was highest at the high dose. In a 52-week

Reference ID: 3501244

oral (gavage) study in Beagle dogs, animals were treated at 2, 5 and 10 mg/kg/day. Histopathological changes were observed in the lung (peribronchiolar chronic inflammation/fibrosis at 10 mg/kg/day), heart (mesothelial hyperplasia in the atria of the heart was noted only in treated female animals), and testes (seminiferous epithelial degeneration and segmental hypoplasia in the testes were seen with a greater incidence in the treated groups). Histopathological changes observed in the lung, heart and testes did not appear to be treatment related as the incidences were not dose related and were also seen in control animals. The NOAEL appeared to be 10 mg/kg/day.

Eliglustat was negative in the Ames test, chromosome aberration assay using human peripheral blood lymphocytes and oral *in vivo* mouse micronucleus test. In addition, Genz-399240, a human metabolite of eliglustat, was negative in the Ames test and chromosome aberration assay using human peripheral blood lymphocytes. Two impurities were tested for potential genotoxicity. Impurity (b) (4) was negative in the Ames test and impurity (b) (4) was negative in the Ames II test.

The reports of the 2-year oral carcinogenicity studies in mice (GT-157-TX-43) and rats (GT-157-TX-38) were reviewed by Dr. Sruthi King (pharmacology review of NDA 205494 dated April 15, 2014). In Sprague-Dawley rats, eliglustat tartrate was administered by oral gavage at doses up to 75 mg/kg/day in males (about 3.6 times the recommended human daily dose of 100 mg BID, based on a body surface area basis) and 50 mg/kg/day in females (about 2.4 times the recommended human daily dose based on body surface area basis). In CD-1 mice, eliglustat tartrate was administered to males and females at up to 75 mg/kg/day (about 1.8 times the recommended human daily dose based on a body surface area basis) via dietary admixture. Eliglustat tartrate did not produce any treatment-related neoplasms in rats or mice.

In an oral fertility and early embryonic development study in male and female rats at 10, 30 and 100 mg/kg/day, eliglustat increased pre-implantation loss at 30 and 100 mg/kg/day (statistically significant). In an oral embryofetal development study in rats, animals were treated at 10, 30 and 120 mg/kg/day from GD 6 to GD 17. Maternal toxicity (decrease body weight and food consumption) was observed at 120 mg/kg/day. At 120 mg/kg/day, there was an increase in the number of late resorptions, dead fetuses and post implantation loss. Fetal body weight was also reduced at 120 mg/kg/day. At 120 mg/kg/day, fetal visceral variations included dilated cerebral ventricles and fetal skeletal malformations included abnormal number of ribs or lumbar vertebra and fetal skeletal variations included poor bone ossification. In an oral embryofetal development study in New Zealand white rabbits, animals were treated at 10, 30 and 100 mg/kg/day from GD 6 to GD 18. No significant treatment-related adverse effects on embryofetal development were observed in this study. In a pre and postnatal development study in rats, eliglustat did not appear to cause any significant adverse effects on pre and postnatal development up to 100 mg/kg/day.

Evaluations of potential genotoxic impurities (b) (4) in eliglustat tartrate were also provided in the submission.

In silico (DEREK, Multicase, and Leadscope) analysis was done for all potential genotoxic impurities. [redacted] (b) (4) were determined to have the potential to be genotoxic as a result of structural analysis; however, *in silico* analysis indicated these impurities were not genotoxic. [redacted] (b) (4) were determined to have the potential to be genotoxic as a result of structural and *in silico* analysis. However, the results of the Ames assay did not show any mutagenic activity for both of these impurities.

The Applicant provided toxicological qualification of impurity [redacted] (b) (4) at ≤ (b) (4) % in the drug substance (DS) in response to the Agency's information request. The impurity [redacted] (b) (4) was present in the DS at ≤ (b) (4) %, which is above the ICH qualification threshold of (b) (4) %. The Applicant provided (SDN 016 dated January 27, 2014) toxicological qualification of the above impurity. As mentioned above, Ames test with this impurity was also conducted and was found to be negative. The estimated daily exposure to [redacted] (b) (4) at ≤ (b) (4) % from the recommended dose (84 mg BID) of eliglustat would be about [redacted] (b) (4) mg/day or [redacted] (b) (4) mg/kg/day. The identified NOAELS for [redacted] (b) (4) in rats ([redacted] (b) (4) mg/kg/day) and dogs [redacted] (b) (4) mg/kg/day) provide adequate (about 18 and 4-fold, respectively) margin of safety over the estimated maximum exposure [redacted] (b) (4) mg/kg/day) to [redacted] (b) (4) from the drug product. Therefore, the level of [redacted] (b) (4) in the DS at ≤ (b) (4) % is toxicologically qualified and appears to be acceptable.

The Applicant also provided a comprehensive toxicological safety assessment of the potential impurity, [redacted] (b) (4) in the DS as requested by the Agency. Safety assessment of [redacted] (b) (4) potential by-products of [redacted] (b) (4) were also included in this assessment. The toxicity of the potential [redacted] (b) (4) was not assessed knowing that it is a [redacted] (b) (4) ssuming worse-case scenario (at LOQ of (b) (4) ppm), the estimated exposures to [redacted] (b) (4) from the recommended dose of the drug product were calculated to be [redacted] (b) (4) and [redacted] (b) (4) µg/kg/day, respectively, or [redacted] (b) (4) and [redacted] (b) (4) µg per day, respectively. No nonclinical toxicology information is available for [redacted] (b) (4) in the literature and the Applicant did not provide any nonclinical information specifically for [redacted] (b) (4) Therefore, no comments could be made regarding the safety of [redacted] (b) (4) in the absence of any nonclinical information. However, [redacted] (b) (4) is a [redacted] (b) (4) and a type of [redacted] (b) (4) [redacted] (b) (4) of [redacted] (b) (4) The possible carcinogenic and mutagenic potential of [redacted] (b) (4) was assessed based on the information available for [redacted] (b) (4) With respect to [redacted] (b) (4) it has been included in the Report on Carcinogens (Report on Carcinogens, 12 Edition, 2011, National Toxicology Program, Department of Health and Human Services) as reasonably anticipated to be a human carcinogen. It is to be noted that no [redacted] (b) (4) was detected in any of the tested batches of the DS. This is a theoretical risk. Even in worst case scenario at LOQ of [redacted] (b) (4) ppm, the above estimated exposure levels for [redacted] (b) (4) are well below the allowable level of 1.5 µg per day per the draft ICH M7 guidance [Assessment

and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, February 6, 2013] and appear to be acceptable.

Overall, the recommended human dose for eliglustat is 100 mg BID or 200 mg/day (3.33 mg/kg/day). The NOAELs and associated exposures measured in the chronic toxicology studies in rats and dogs are shown in the following Table (from page 58 of Section 2.4).

Table 12: NO(A)ELs and Associated Exposure Parameters

Study	Sex	NO(A)EL (mg/kg)	Day	C_{max} (ng/mL)	AUC_{last} (ng*h/mL)
6-month rat (GT-157-TX-17)	M	50	184	1329	2341
	F			1521	3787
3-month dog (GT-157-TX-15)	M	5	90	970	1637
	F			685	1179
12-month dog (GT-157-TX-23)	M	10	364	1620	4489
	F			1637	3130

The mean clinical simulated C_{max} of 44.3 ng/mL and $AUC_{0\text{-}12h}$ of 307 ng.hr/mL were derived from PK simulations based on a PopPK model (Report No. SIM0124). The NOAEL in rats (50 mg/kg/day) in the 6-month toxicity study was approximately 2.4 times the above recommended human dose of 100 mg BID based on body surface area. The exposure (AUC) at the NOAEL of 50 mg/kg/day in rats is approximately 8-12 times higher than the mean predicted AUC of 307 ng.hr/mL. The NOAEL in dogs (10 mg/kg/day) in the 12-month toxicity study was approximately 1.6 times the above recommended human dose of 100 mg BID based on body surface area. The exposure (AUC) at the NOAEL of 10 mg/kg/day in dogs is approximately 10-15 times higher than the mean predicted AUC of 307 ng.hr/mL.

In conclusion, this NDA contains adequate nonclinical studies and meets the guidelines and satisfies the criteria for marketing authorization of eliglustat. From a nonclinical perspective, this application is recommended for approval for its proposed use as indicated in the label.

12 Appendix/Attachments

None

Reference ID: 3501244

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

/s/

TAMAL K CHAKRABORTI
05/05/2014

SUSHANTA K CHAKDER
05/05/2014

Comments on N205494 Eliglusat

From A. Jacobs

Date 4/28/14

1. I concur that there are no pharm/tox approvability issues.

2. I concur with the division's recommendation for the pregnancy category to be C (b) (4)

3. I concur with proposed changes to pharm-tox sections of labeling.

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

/s/

ABIGAIL C JACOBS
04/28/2014

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	205494
Supporting document/s:	000
Applicant's letter date:	September 19, 2013
CDER stamp date:	September 20, 2013
Product:	Eliglustat Tartrate
Indication:	Long term treatment of adult patients with Gaucher disease type 1
Applicant:	Genzyme Corporation Cambridge, MA
Review Division:	Division of Gastroenterology and Inborn Error Products
Reviewer:	Sruthi Tallapragada King, PhD
Supervisor/Team Leader:	Sushanta Chakder, PhD
Division Director:	Donna Griebel, MD
Project Manager:	Jessica Benjamin, MPH

Template Version: September 1, 2010

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Any information or data necessary for approval of NDA 205494 that Genzyme Corporation does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 205494.

Reference ID: 3489781

TABLE OF CONTENTS

1 EXECUTIVE SUMMARY 6

1.1 INTRODUCTION 6
1.2 BRIEF DISCUSSION OF NONCLINICAL FINDINGS 6
1.3 RECOMMENDATIONS 9

2 DRUG INFORMATION 10

2.1 DRUG 10
2.2 RELEVANT INDS, NDAS, BLAS AND DMFS 10
2.3 DRUG FORMULATION 10
2.4-2.7 COMMENTS ON NOVEL EXCIPIENTS, COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN, PROPOSED CLINICAL POPULATION AND DOSING REGIMEN, REGULATORY BACKGROUND 10

3 STUDIES SUBMITTED 11

3.1 STUDIES REVIEWED 11
3.2 STUDIES NOT REVIEWED 11
3.3 PREVIOUS REVIEWS REFERENCED 11

4-7 PHARMACOLOGY, PHARMACOKINETICS/ADME/TOXICOKINETICS (TK), GENERAL TOXICOLOGY 11

8 CARCINOGENICITY 11

9-10 REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY, SPECIAL TOXICOLOGY STUDIES 46

11 INTEGRATED SUMMARY AND SAFETY EVALUATION 47

12 APPENDIX/ATTACHMENTS 52

Reference ID: 3489781

Table of Tables

Table 1: Summary of body weight gain and mortality rates after dietary administration of eliglustat tartrate for 105/106 weeks in CD-1 mice 13

Table 2: Onset of tumors in CD-1 mice receiving eliglustat tartrate for 105/106 weeks as a dietary admixture 14

Table 3: Cumulative mortalities in CD-1 mice receiving eliglustat tartrate for 105/106 weeks as a dietary admixture 16

Table 4: Intercurrent mortality comparison in CD-1 mice receiving eliglustat tartrate for 105/106 weeks as a dietary admixture, taken from draft review of Dr. Min Min; 4A (Males), 4B (Females) 18

Table 5: Incidences and most common causes of mortality in CD-1 mice receiving eliglustat tartrate for 105/106 weeks as a dietary admixture (applicant's table 18

Table 6: Summary of clinical signs in CD-1 mice receiving eliglustat tartrate for 105/106 weeks as a dietary admixture (applicant's table) 19

Table 7: Mean body weight (g) and body weight change (g) in CD-1 mice receiving eliglustat tartrate for 105/106 weeks as a dietary admixture (applicant's table), 7A (Males), 7B (Females) 20

Table 8: List of organs/tissues from treated CD-1 harvested and processed for histopathology 24

Table 9: Summary of overall tumor data in CD-1 mice receiving eliglustat tartrate for 105/106 weeks as a dietary admixture (applicant's table) 25

Table 10: Incidence of selected neoplasms in CD-1 mice receiving eliglustat tartrate for 105/106 weeks as a dietary admixture, as compared to (b) (4) and Lit Data 26

Table 11: Incidence and severity of hepatocellular hypertrophy in CD-1 mice receiving eliglustat tartrate for 105/106 weeks as a dietary admixture (applicant's table) 27

Table 12: Plasma levels of glucosylceramide (GL-1) after dietary administration of eliglustat tartrate for 13 weeks in in CD-1 mice 27

Table 13: Onset of tumors in SD rats treated with eliglustat tartrate for 2 years via oral gavage 29

Table 14: Tumor types in SD rats with p-values ≤ 0.05 for dose response relationship or pair-wise comparisons (combined controls, low, medium and high dose groups), taken from draft review of Dr. Min Min 31

Reference ID: 3489781

Table 15: Summary of body weight gain and mortality rates in SD rats treated with eliglustat tartrate for 2 years via oral gavage 32

Table 16: Cumulative mortalities in SD rats treated with eliglustat tartrate for 2 years via oral gavage 34

Table 17: Intercurrent mortality comparison in SD rats treated with eliglustat tartrate for 2 years via oral gavage, taken from draft review of Dr. Min Min; 17A (Males), 17B (Females) 36

Table 18: Incidences and most common causes of mortality in SD rats treated with eliglustat tartrate for 2 years via oral gavage (applicant's table) 37

Table 19: Mean body weight (g) and body weight change (g) in SD rats treated with eliglustat tartrate for 2 years via oral gavage (applicant's table); 19A (Males), 19B (Females) 38

Table 20: Incidence of palpable masses in SD rats treated with eliglustat tartrate for 2 years via oral gavage 41

Table 21: List of organs/tissues from treated SD rats harvested and processed for histopathology 42

Table 22: Summary of overall tumor data in SD rats treated with eliglustat tartrate for 2 years via oral gavage (applicant's table) 43

Table 23: Incidence of selected neoplasms in SD rats treated with eliglustat tartrate for 2 years via oral gavage (applicant's table), as compared to (b) (4) and Lit Data 43

Table 24: List of major non-neoplastic findings in SD rats treated with eliglustat tartrate for 2 years via oral gavage (applicant's table) 45

Table 25: Toxicokinetics of eliglustat tartrate in SD rats on Day 1 and Week 13 of treatment 46

Reference ID: 3489781

Table of Figures

Figure 1: Kaplan-Meier plot of survival in CD-1 mice receiving eliglustat tartrate for 105/106 weeks as a dietary admixture (applicant's figures); 1A (Males), 1B (Females) 16

Figure 2: Kaplan-Meier plot of survival in SD rats treated with eliglustat tartrate for 2 years via oral gavage (applicant's figures); 2A (Males), 2B (Females) 34

Reference ID: 3489781

1 Executive Summary

1.1 Introduction

Eliglustat tartrate is the active pharmacological ingredient in Cerdelga, which is being developed as a substrate reduction therapy (SRT) for Gaucher Disease, a rare lysosomal storage disorder resulting from a deficiency of the enzyme glucocerebrocidase (also known as acid β-glucosidase). Acid β-glucosidase is the rate-limiting enzyme in the hydrolysis of glucosylceramide (GL-1) to glucose and ceramide. Deficiency in acid β-glucosidase causes accumulation of GL-1, particularly in lysosomes and macrophages. Increased GL-1 accumulation in macrophages causes them to appear "lipid-engorged" and infiltrate the organs of the reticuloendothelial system (spleen, liver, bone marrow, and to some extent in the lungs), producing organomegaly. Therefore, Gaucher Disease is a chronologically debilitating, multisystem disease that is characterized by hepatosplenomegaly and hypersplenism, which produce hematological abnormalities (anemia, thrombocytopenia) and bone disease (bone marrow infection, low bone mineral density), and to a lesser extent lung disease (interstitial lung disease, pulmonary hypertension). Disease presentation can occur at any age and clinical severity can range from asymptomatic to severely debilitating. It is an orphan disease and is estimated to occur in 1 in 100,000 births. Current treatment options for Gaucher patients are enzyme replacement therapy (ERT) and SRT. Approved ERT products (such as Ceredase®, Cerezyme®, VPRIV™, and Elelyso™) are recombinant human enzymes which have been shown to effectively decrease GL-1 accumulation and improve symptoms of Gaucher patients. The current approved SRT for Gaucher disease is Zavesca™; however this product is only approved in adults for whom ERT is not a viable option. The currently approved ERTs require intravenous (IV) administration for the remainder of the patient's life, while the only approved oral formulation, the SRT, is limited for use in adults only who do not respond to ERT.

In this application, the applicant is proposing an oral formulation of SRT, eliglustat at the proposed dosage of 84 mg to be taken twice a day (equivalent to 100 mg eliglustat tartrate taken BID). The target population for eliglustat tartrate is CYP2D6 Intermediate Metabolizers and Extensive Metabolizers (b) (4).

1.2 Brief Discussion of Nonclinical Findings

For details on the nonclinical safety assessment (pharmacology, pharmacokinetics, and toxicology (general toxicology, genetic toxicology, reproductive toxicology)), please refer to the review of Dr. Tamal Chakraborti.

The carcinogenicity studies conducted in support of approval of eliglustat tartrate are reviewed here. Two, 2-year rodent carcinogenicity studies were conducted: oral gavage study in Sprague-Dawley rats and dietary study in CD-1 mice. Dose selection for both studies was based on previous agreement with the CDER Executive Carcinogenicity Assessment Committee (ECAC). The highest doses tested were 75 mg/kg/day in mice (both sexes) and 75 mg/kg/day and 50 mg/kg/day in male and female rats, respectively.

Reference ID: 3489781

Studies in both species failed to demonstrate any carcinogenic potential of eliglustat tartrate at up to the highest doses tested in either sex.

Mouse study:
In CD-1 mice (60/sex/dose), eliglustat tartrate was administered at 10, 25, and 75 mg/kg/day, by dietary admixture for 105/106 weeks and was not carcinogenic in either sex at all doses tested. Control animals (2 groups of 60/sex each) were fed the powdered, maintenance diet. There was no treatment-related effect on tumor latency in either sex. Poly-3 trend tests revealed an increase in incidence of 3 rare tumor types at the HD in males and females: benign cortical adenoma (2/60 in high dose (HD) males, as compared to 0/120 controls), benign pheochromocytoma (2/60 in HD females, as compared to 0/120 controls), and malignant skin fibrosarcoma (2/58 in HD females, as compared to 0/120 controls). The incidence of these tumors fell within the range of those observed in control animals (historical data from the testing laboratory and/or published data from the literature), and therefore were considered to be unrelated to treatment by the applicant. Additional analyses conducted by FDA statistical reviewer of specified tumor combinations revealed that the following tumor types had p values ≤ 0.05 for dose relationships: Adrenal gland (Adenoma/Cortex), Liver (Hemangiosarcoma), Lungs (Adenocarcinoma+Adenoma) in Males and Adrenal Gland (pheochromocytoma) and Skin (fibrosarcoma) in Females). When pair-wise relationships were evaluated, the following had p values ≤ 0.05: Lungs (Adenocarcinoma and Adenocarcinoma + Adenoma in MD males) and Uterus (Endometrial Sarcoma in low dose (LD) females). However, none of the incidences of tumors in either sex was considered to have a positive dose response relationship or a significant increase in tumor incidence from pair-wise comparisons.

There was an 11% increase in the mortality rate of HD males and 2% decrease in HD females, as compared to combined controls; however, a treatment-related effect was not apparent in the incidence or cause of mortality in either sex. Mid dose (MD) and HD males showed a slight increase in clinical signs of poor health, such as thin appearance, hunched posture, abdominal breathing, and an abscess in the urogenital region, leading to unscheduled sacrifice. A dose-dependent effect on body weight was not apparent. Overall, a decrease in body weight gain in either sex did not exceed 10%, as compared to combined controls. LD and HD females had ~12% and ~2.5% increase in body weight gain, respectively, as compared to combined controls, while LD and HD males had ~4% and 3% increase, respectively, as compared to combined controls. TK analysis was not conducted in mice, since plasma levels of the test article were below the lower limit of quantitation (5 ng/mL). However, measurement of plasma GL-1 levels revealed a decrease in levels with treatment, as compared to control, although a dose-response was not apparent. The predicted human exposure (AUC) at doses of 50-150 mg BID was 307 ng·h/mL.

There was a statistically significant, treatment-related increase in the incidence and severity (Grade 3 or 4, particularly at higher doses) of hepatocellular hypertrophy in males (MD and HD) and females (all doses), as compared to controls. The hypertrophy

Reference ID: 3489781

was not associated with necrosis or hepatocellular degeneration and may be related to CYP450 induction in mice.

Rat study:
In SD rats (50 sex/dose), eliglustat tartrate was administered by oral gavage at 10, 25, and 75 mg/kg/day in males for 105 weeks and at 5, 15, and 50 mg/kg/day in females for 103 weeks. Two groups of 50 rats/sex were assigned as Control animals and received vehicle via oral gavage (drinking water). The test article was not carcinogenic in either sex at any of the doses tested. A dose-dependent effect on tumor latency was not apparent. Malignant tumors appeared earlier in test article treated males (as early as 18 weeks after treatment initiation at the LD). Females treated at the HD of test article had mammary gland adenocarcinomas starting at 38 weeks, as compared to 76 weeks in Group 2 control females.

A poly-3 test was conducted and revealed an increase in incidence of several tumors: granulocytic leukemia in 2/50 LD males, odontoma in 1/50 MD males, and mammary gland adenomas in females (1/50, 2/50, and 2/50 at LD, MD, and HD, respectively), as compared to 0/100 controls/sex. However, the incidence of tumors in males and females fell within the range of those observed in control animals from historical data in the testing laboratory and published literature. Therefore, the increased incidence of these tumors was considered to be unrelated to treatment with test article in either sex. Additional analyses conducted by FDA statistical reviewer revealed a $p \leq 0.05$ for dose response relationship for skin fibroma in males only. However, the incidence of this tumor did not have a positive dose response relationship or reveal a statistically significant increase in tumor incidence in the pair-wise comparisons (combined control vs. individual treatment group).

There was no statistically significant, treatment-related effect on survival in either sex. There was a >22% and >8% decrease in body weight gain in HD males and females, respectively, as compared to combined controls. The mortality rate in HD males was 7% lower than Combined Controls. In females, the mortality rate was similar between combined controls and HD females, while it was at least 15% higher in LD and MD females. TK analysis revealed that the exposure to test article was ~3.6x and 2.6x higher in HD males and females, respectively, than the predicted human exposure of 307 ng·h/mL at clinical doses of 50-150 mg BID.

There was an increased incidence and severity of non-neoplastic findings in the lungs of HD males, as compared to combined controls: granulomata (32/49, as compared to 10/100 controls), chronic bronchio-aveolar inflammation (7/49, as compared to 1/100 controls), and bronchiectasis (5/49, as compared to 1/100 controls). These findings may correlate with adverse clinical signs observed in HD males, such as loud breathing, ptyalism, and dyspnea. There were several changes observed in the genital tract of males, with slightly higher incidences in the HD group. Swollen spermatids in the testes (1/50, 2/50, and 4/50 at LD, MD, and HD, respectively, as compared to 0/100 in controls) and inflammation in the coagulating glands (1/50 at LD and MD and 4/50 at HD, as compared to 0/100 in controls) were observed, but with low severity.

Reference ID: 3489781

Inflammation in the coagulating glands was observed in a preliminary 28-day toxicity study at doses up to 100 mg/kg/day; however, these effects were reversed after a 9-week treatment-free period.

In females, there were no findings in the lungs similar to those in males. There was a higher incidence of squamous metaplasia in the endometrium (3/50 at MD and 13/50 at HD, as compared to 6/100 controls). No such findings were observed in the 26-week repeat-dose toxicity study at doses up to 50 mg/kg/day

Overall, administration of eliglustat tartrate to CD-1 mice (via dietary admixture) and SD rats (via oral gavage) for 2 years failed to produce any treatment-related, statistically significant increased incidences of neoplasms by CDER criteria. Eliglustat tartrate was not carcinogenic in either mice or rats in 2-year oral studies at doses up to 75 mg/kg/day (mice) and 75 mg/kg/day and 50 mg/kg/day in male and female rats, respectively.

1.3 Recommendations

1.3.1 Approvability

The results of the carcinogenicity studies in rats and mice will not preclude marketing approval of Eliglustat Tartrate at the proposed dose for the proposed indication.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Applicant's Version:

Carcinogenesis: (b) (4)

Recommended Version:

Carcinogenesis:

Carcinogenic potential of CERDELGA was assessed in 2-year carcinogenicity studies in rats and mice. In Sprague-Dawley rats, eliglustat tartrate was administered by oral gavage at doses up to 75 mg/kg/day in males (about 3.6 times the recommended human daily dose of 100 mg BID, based on a body surface area) and 50 mg/kg/day in

Reference ID: 3489781

females (about 2.4 times the recommended human daily dose based on body surface area). In CD-1 mice, eliglustat tartrate was administered to males and females at up to 75 mg/kg/day (about 1.8 times the recommended human daily dose based on a body surface area) via dietary admixture. Eliglustat tartrate did not produce any treatment-related neoplasms in rats or mice.

2 Drug Information

2.1 Drug

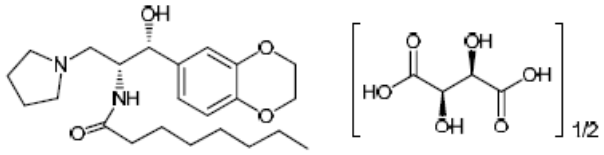
Generic Name: Eliglustat (Eliglustat tartrate)

Code Name: Genz-112638 (Genzyme), (b) (4) (Sanofi), (b) (4) (Corden Pharma)

Chemical Name: Octanamide, *N*-[(1*R*,2*R*)-2-(2,3-dihydro-1,4-benzodioxin-6- yl)-2-hydroxy-1-(1-pyrrolidinylmethyl)ethyl]-, (2*R*,3*R*)-2,3- dihydroxybutanedioate (2:1)

Molecular Formula/Molecular Weight

Structure or Biochemical Description



Pharmacologic Class: Glucosylceramide synthase inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 67589

2.3 Drug Formulation

The drug product is formulated as hard gelatin capsules of 84 mg each (containing 100 mg eliglustat tartrate) for oral administration, to be taken twice a day.

2.4-2.7 Comments on Novel Excipients, Comments on Impurities/Degradants of Concern, Proposed Clinical Population and Dosing Regimen, Regulatory Background

Please refer to nonclinical review of this NDA by Dr. Tamal Chakraborti for details.

Reference ID: 3489781

3 Studies Submitted

3.1 Studies Reviewed

GT-157-TX-38: Carcinogenicity Study by Oral Route (Gavage) in Rats
GT-157-TX-43: Dietary Carcinogenicity Study in Mice

3.2 Studies Not Reviewed

Please refer to the nonclinical review of Dr. Tamal Chakraborti for a list of the pharmacology and toxicology studies submitted in support of this NDA. These studies are not reviewed here.

3.3 Previous Reviews Referenced

IND 67589: December 18, 2009 (SDN 060, 062) and May 28, 2009 (SDN 070, 074) by Dr. Tamal Chakraborti

4-7 Pharmacology, Pharmacokinetics/ADME/Toxicokinetics (TK), General Toxicology

A review of the pharmacology, pharmacokinetics/ADME/TK, general toxicology, and genetic toxicology studies can be found in the nonclinical review of Dr. Tamal Chakraborti. Please refer to his review for detailed information.

8 Carcinogenicity

Study title: Dietary Carcinogenicity Study in Mice

Study no.:	28253 TCS (GT-157-TX-43)
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 6, 2009 (date of first dose)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Genz-112638, Lot # T1136, Purity 99.72%
CAC concurrence:	Yes (Executive CAC Recommendation on May 27, 2009)

Key Study Findings

Reference ID: 3489781

Eliglustat tartrate did not produce any treatment-related statistically significant increased incidences of neoplasms, per CDER criteria, when administered as a dietary admixture to CD-1 mice at doses up to 75 mg/kg/day in either sex.

There was no treatment-related effect on tumor latency in either sex. Statistical analysis by the applicant (Conventional Peto test and Exact Peto test) failed to reveal any statistically significant trends in tumor incidence. Poly-3 test statistics revealed an increase in incidence of 3 rare tumor types at the HD in males and females: benign cortical adenoma (2/60 HD males, as compared to 0/120 controls), benign pheochromocytoma (2/60 HD females, as compared to 0/120 controls), and malignant skin fibrosarcoma (2/58 HD females, as compared to 0/120 controls). The incidence of these tumors fell within the range of those observed in control animals, based on historical data from the testing laboratory and published data from the literature, and therefore were considered to be unrelated to treatment by the applicant. Additional analyses conducted by FDA statistical reviewer of specified tumor combinations revealed that the following tumor types had p values ≤ 0.05 for dose relationships: Adrenal gland (Adenoma/Cortex), Liver (Hemangiosarcoma), Lungs (Adenocarcinoma+Adenoma) in Males and Adrenal Gland (pheochromocytoma) and Skin (fibrosarcoma) in Females). When pair-wise relationships were evaluated, the following had p values ≤ 0.05: Lungs (Adenocarcinoma and Adenocarcinoma + Adenoma in MD males) and Uterus (Endometrial Sarcoma in LD females). However, none of the incidences of tumors in either sex was considered to have a positive dose response relationship or an increase in tumor incidence from pair-wise comparisons.

Adequacy of Carcinogenicity Study

Dose selection was based on recommendations from the Executive CAC (May 27, 2009)[1]. The rationale for dose selection was based on results from a 13-week preliminary dietary toxicity study in CD-1 mice (the strain used in the pivotal carcinogenicity study). In the preliminary study, the mice received with 50, 150, and 350 mg/kg/day eliglustat tartrate as a dietary admixture. There were no treatment-related deaths or changes in clinical signs, clinical chemistry or hematology parameters in either sex. There was a decrease in body weight (high dose only) and increased weight of the liver (high and mid dose) and adrenal gland (high dose only) in males. Changes in organ weights in males did not correlate with histopathological findings. In females, there was a decrease in food consumption at the high dose and low dose. TK analysis demonstrated that animals were exposed to drug at all doses. Based on reduced body weight gain, the Division and CDER Executive Carcinogenicity Assessment Committee (ECAC) concluded that the maximum tolerated dose (MTD) was 75 mg/kg/day and should be set as the high dose for the pivotal carcinogenicity study.

[1] Please refer to the nonclinical review of the SPA for the 2-year dietary study protocol by Dr. Tamal Chakraborti for details on the study results (SDN 070 and 074, Dated May 28, 2009) and ECAC recommendations in Appendix #1A.

Reference ID: 3489781

The applicant conducted oral palatability studies of 4 days, 7 days, and 2 weeks in duration. The MTD from the 2-week palatability study was determined to be 178 mg/kg/day in males and 215 mg/kg/day in females, based on the observation of adverse clinical signs (thin appearance, piloerection, soiled abdomen and/or urogenital region, hunched posture) and weight loss in both sexes. No target organs of toxicity were identified.

The target doses achieved in the study were within acceptable ranges. Therefore, based on applicant's dose selection, confirmation of chemical/vehicle stability and achieved dosages, the study is adequate and acceptable.

The table below summarizes the body weight and mortality rates between combined controls (Group 1 and Group 2 vehicle-treated animals combined) and individual dose groups.

Table 1: Summary of body weight gain and mortality rates after dietary administration of eliglustat tartrate for 105/106 weeks in CD-1 mice

Body weight (% difference in mean body weight gain from combined controls			Mortality Rates at the end of the experiment		
Dose	Male	Female	Dose	Male	Female
			Comb, Ctrl	51%	65%
LD	4.09	11.73	LD	55%	54%
MD	-1.17	-9.26	MD	65%	66%
HD	2.93	2.47	HD	62%	63%

There was < 10% decrease in body weight gain in either sex, as compared to combined controls, and a clear dose-response relationship was not apparent. MD females had a 9.3% decrease in body weight gain, while LD and HD females had ~12% and ~2.5% increase, respectively, in body weight gain, as compared to combined controls. In males, body weight gain was ~4% and 3% in LD and HD males, respectively, while MD males had ~1.2% decrease in body weight gain, as compared to combined controls. There was an 11% increase in the mortality rate of HD males and 2% decrease in HD females, as compared to combined controls. Clinical signs of poor health were observed with a slight increased incidence in MD and HD males, as compared to dual controls. Based on these findings, it is unclear whether the maximum tolerated dose (MTD) was reached. The applicant selected doses for the carcinogenicity study based on recommendations from ECAC; however, the high dose (75 mg/kg/day), which was postulated to be the MTD, was not previously tested in the dose-ranging studies.

Appropriateness of Test Models

The [redacted] (b) (4) mouse is an acceptable test model for the carcinogenicity study.

Evaluation of Tumor Findings

Reference ID: 3489781

There was no apparent treatment-related effect on tumor latency. The table below summarizes the onset of tumors in males and females. The following doses were used: 0, 0, 10, 25, and 75 mg/kg/day and are noted as Groups 1-5, respectively.

Table 2: Onset of tumors in CD-1 mice receiving eliglustat tartrate for 105/106 weeks as a dietary admixture

	Males			Females		
Group	Week of onset	Organ	Corresponding neoplasm	Week of onset	Organ	Corresponding neoplasm
1 (Ctrl)	73	Systemic	Malignant lymphoma	51	Systemic	Malignant lymphoma
2 (Ctrl)	66	Lung	Adenocarcinoma	40	Systemic	Malignant lymphoma
3 (Low Dose)	56	Systemic	Malignant lymphoma	64	Colon	Adenocarcinoma
4 (Mid Dose)	63	Lung	Adenocarcinoma	48	Mammary	Adenocarcinoma
5 (High Dose)	48	Systemic	Malignant lymphoma	52	Systemic	Malignant lymphoma

Conventional Peto test and Exact Peto test failed to reveal any statistically significant trends in tumor incidence. Trend statistics using Poly-3 test revealed an increase in incidence of 3 rare tumor types at the HD in males and females: benign cortical adenoma (2/60 HD males, as compared to 0/120 controls), benign pheochromocytoma (2/60 HD females, as compared to 0/120 controls), and malignant skin fibrosarcoma (2/58 HD females, as compared to 0/120 controls). The applicant concluded that the incidence of these rare tumors fell within the range of those observed in control animals from historical data in the testing laboratory (b) (4) and published literature (See Table 11 Below); therefore, the increased incidence of these tumors was considered to be unrelated to treatment with test article.

In addition, the statistical reviewer Dr. Min Min combined the following tumor types (taken from Dr. Min's draft review) for additional analysis, based on this nonclinical reviewer's request:

Mouse:

- Colon adenocarcinoma and adenoma for male mice only.
- Duodenum adenocarcinoma and adenoma for male mice only.
- Liver hepatocellular adenoma and carcinoma.
- Lung bronchio-alveolar adenoma and carcinoma for male mice only.
- Mammary gland adenocarcinoma and adenoma plus squamous cell carcinoma for female mice only.
- Ovaries tubulostromal adenoma and benign granulose cell tumor plus mixed sex cord and stromal tumor for female mice only.
- Prostate adenocarcinoma and adenoma for male rats only.
- Skin squamous cell carcinoma and papilloma plus benign hair follicle tumor for female mice only.
- Uterus endometrial stromal polyp and sarcoma for female mice only.

The results from these additional analyses showed that the following tumor types had p values ≤ 0.05 for dose response relationships or pair-wise comparisons for Combined Controls and each of the dose groups (Low Dose (L), Mid Dose (M), and High Dose (H))

Reference ID: 3489781

are shown in the table below from Dr. Min's draft review. However, none of the incidences of tumors in either sex was considered to have a positive dose response relationship. Likewise, none of the pair-wise comparisons (combined control vs. individual treatment group) revealed an increase in tumor incidence.

Methods

Doses: 0, 10, 25, 75 mg/kg/day (targeted dose levels)

Frequency of dosing: Free access to food (treated or untreated diet) for 105/106 weeks

Dose volume: Mean food consumption per day 5.7 g/day (males), 7.2 g/day (females)

Route of administration: Dietary (because of short plasma half-life in the mouse)

Formulation/Vehicle: SAFE A04 C P2.5 powdered maintenance diet (Batch #s: 90107, 90414, 09229, 10180, 10203, 10258, 11137, 09336), distributed weekly

Basis of dose selection: Previous 13-week dietary toxicity study in CD-1 mice

Species/Strain: (b) (4)

Number/Sex/Group:

Group	Dose	Notation	Principal	Satellite 1	Satellite 2
1	0	Ctrl	60/sex	6/sex	5/sex
2	0	Ctrl	60/sex	6/sex	5/sex
3	10	Low Dose (LD)	60/sex	20/sex	5/sex
4	25	Mid Dose (MD)	60/sex	20/sex	5/sex
5	75	High Dose (HD)	60/sex	20/sex	5/sex

Age: 6 weeks

Animal housing: Individual polycarbonate cages

Paradigm for dietary restriction: None

Dual control employed: Yes

Interim sacrifice: Yes at 13 weeks for GL-1 levels or toxicokinetics analysis

Satellite groups: Yes, Satellite 1 (Toxicokinetics analysis) and Satellite 2 (GL-1 level assessment)

Deviation from study protocol: None which affected the integrity of the study

Observations and Results

Mortality

Animals were checked twice daily for signs of morbidity and mortality during the treatment period. Moribund animals were sacrificed and a blood smear was prepared from moribund Principal animals only.

Reference ID: 3489781

Results: The table below summarizes the cumulative mortalities observed over the course of study. The number of deaths shown during each interval represents the number of animals found dead + the number of animals sacrificed prematurely.

Table 3: Cumulative mortalities in CD-1 mice receiving eliglustat tartrate for 105/106 weeks as a dietary admixture

	Males (N= 60/group)					Females (N = 60/group)				
Group	1	2	3	4	5	1	2	3	4	5
Dose (mg/kg/day)	0	0	10	25	75	0	0	10	25	75
Weeks 1-52	2	3	5	7	5	7	4	4	3	1
Weeks 53-78	8	8	12	18	11	14	15	9	13	8
Weeks 70-105	30	34	33	38	37	42	36	31	39	40
Total deaths (Weeks 1-105)	30	34	33	38	37	42	36	31	39	40
Total survivors at terminal sacrifice	30	26	27	22	23	18	24	29	21	20
Survival rate	50%	43%	45%	37%	38%	30%	40%	48%	35%	33%

Overall, the cumulative survival rate in both sexes was similar to controls. There was a slight decrease in survival rate in MD and HD males, as compared to both Group 1 and 2 controls. There were no statistically significant differences in Kaplan Meier survival curves of test article-treated animals (either sex), when compared to controls (either as dual controls (i.e. Group 1 and Group 2 separately) or combined controls (i.e. Group 1 + Group 2 controls)). Kaplan Meier curves are shown in the applicant's figures (applicant's Figures 1 and 2) below.

Figure 1: Kaplan-Meier plot of survival in CD-1 mice receiving eliglustat tartrate for 105/106 weeks as a dietary admixture (applicant's figures); 1A (Males), 1B (Females)

Reference ID: 3489781

Figure 1A:

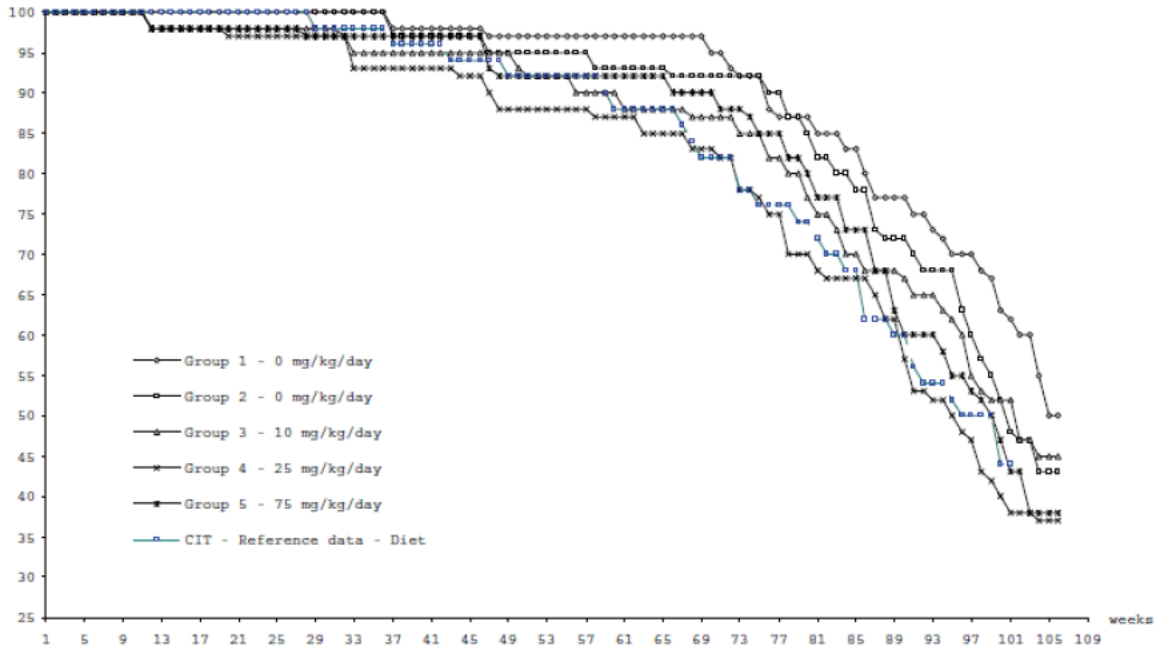
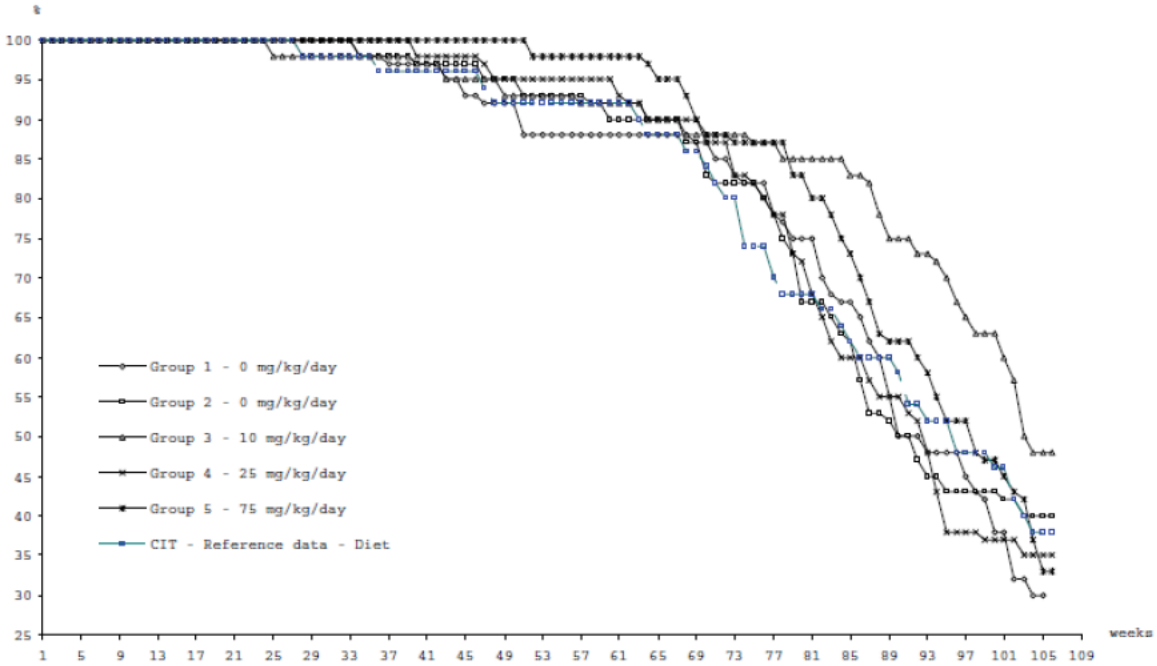


Figure 1B:



Independent analysis of survival data by FDA statistical reviewer Dr. Min Min was in agreement with the applicant's findings that there were no statistical differences in survival between test article-treated animals (both sexes) and each control group (Intercurrent mortality shown in tables below from Dr. Min's draft review). However,

when controls were combined, Dr. Min identified statistically significant pair-wise difference in survival between low dose females and controls ($p<0.05$), which may be related to different dates of starting the terminal sacrifice.

Table 4: Intercurrent mortality comparison in CD-1 mice receiving eliglustat tartrate for 105/106 weeks as a dietary admixture, taken from draft review of Dr. Min Min; 4A (Males), 4B (Females)

Table 4A:

Test	P-Value (across four groups)	P-Value (combined controls vs low)	P-Value (combined controls vs medium)	P-Value (combined controls vs high)
Dose Response	0.2354	0.6587	0.0617	0.2454
Homogeneity	0.1329	0.4905	0.0223	0.1518

Table 4B:

Test	P-Value (across four groups)	P-Value (combined controls vs low)	P-Value (combined controls vs medium)	P-Value (combined controls vs high)
Dose Response	0.8673	0.0433	0.9764	0.5010
Homogeneity	0.2302	0.0610	0.9183	0.5917

A treatment-related effect was not apparent in the incidence or cause of mortality in either sex. The incidences of cardiovascular lesions (atrial thrombosis/dilation) and carcinoma/adenoma was slightly higher in MD and HD, as compared to controls. In females, the incidences of skin lesion (ulcer) and malignant lymphoma were slightly higher in test article-treated females, as compared to Group 1 or Group 2 controls. The applicant's table below lists the incidence and most common causes of mortality.

Table 5: Incidences and most common causes of mortality in CD-1 mice receiving eliglustat tartrate for 105/106 weeks as a dietary admixture (applicant's table)

Incidences and most common causes of mortality

Sex	Male					Female				
Groups	1	2	3	4	5	1	2	3	4	5
Dose-level (mg/kg/day)	0	0	10	25	75	0	0	10	25	75
Number examined	30	34	33	38	37	42	36	31	39	40
Skin lesion	7	5	11	7	9	8	4	2	7	2
Genito-urinary lesion	6	2	2	4	1	4	9	5	4	3
Cardiovascular lesion	5	5	4	10	8	2	-	2	3	1
Amyloidosis	1	5	-	-	-	3	6	4	4	6
Tumor										
. malignant lymphoma	5	3	4	1	4	14	4	10	10	12
. histiocytic sarcoma	-	1	1	1	-	2	4	1	5	4
. carcinoma/adenoma	4	6	3	10	8	2	5	3	3	5
. sarcoma	-	1	-	1	3	5	0	3	0	4

-: not observed.

Clinical Signs

Animals were checked at least once a day during the treatment period. Principal animals were palpated for masses every two weeks after 6 months of treatment. The time of onset, location, size, appearance, and progression of palpable masses was recorded.

Results: Clinical signs in males included signs of poor health, such as thin appearance, hunched posture, abdominal breathing, and an abscess in the urogenital region, leading to unscheduled sacrifice. There was a slightly increased incidence of clinical signs with dose in males, particularly in Group 4 and 5, as compared to controls. Clinical signs in females were similar to controls, with the exception of abscess in the urogenital region, observed in 2 females each at the MD and HD, as compared to controls. These findings are summarized in the applicant's table below.

Table 6: Summary of clinical signs in CD-1 mice receiving eliglustat tartrate for 105/106 weeks as a dietary admixture (applicant's table)

Group (daily dose mg/kg)	Group 1 (0)		Group 2 (0)		Group 3 (10)		Group 4 (25)		Group 5 (75)	
Gender	M	F	M:	F	M	F	M	F	M	F
Number of Principal Animals	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**
Clinical Observations (%)	-	-	-	-	-	-	-	-	-	-
Thin Appearance	30	37	35	35	35	22	38	32	42	23
Hunched Posture	37	48	42	42	40	37	43	47	48	37
Coldness to the touch	7	13	10	13	7	10	12	7	8	5
Hard Abdomen	8	12	13	10	7	10	15	3	3	13
Abdominal breathing	15	15	17	15	10	10	15	18	22	10
Increase in size abdomen	17	32	15	33	5	37	25	30	12	42
Abscess urogenital region	8	0	10	0	13	0	17	2	15	2

Body Weights

For Main Study and Satellite 1 animals, body weight was recorded prior to allocation into treatment groups, twice after allocation, on Day 1 of treatment, once a week during the first 13 weeks of the treatment period, once every 4 weeks thereafter until the end of the study, and on the day of study termination. For Satellite 2 animals, body weight was measured before allocation into treatment groups, twice after allocation, on Day 1 of treatment and once a week during the 13-week treatment period.

Results: There were no statistically significant, treatment-related changes in body weight over the entire dosing period, as compared to controls. There was a 9.3% decrease in body weight gain in MD females, while LD and HD females had ~12% and ~2.5% increase, respectively, in body weight gain, as compared to combined controls. In males, body weight gain was ~4% and 3% in LD and HD males, respectively, while MD males had ~1.2% decrease in body weight gain, as compared to combined controls. Therefore, a dose response relationship was not apparent. There was an 11% increase in the mortality rate of HD males and 2% decrease in HD females, as compared to combined controls. Based on these findings, it is unclear whether the MTD was reached. The applicant's tables below summarize the changes in body weight.

Reference ID: 3489781

Table 7: Mean body weight (g) and body weight change (g) in CD-1 mice receiving eliglustat tartrate for 105/106 weeks as a dietary admixture (applicant's table), 7A (Males), 7B (Females)

Table 7A:

Sex			Male		
Study group	1	2	3	4	5
Dose-level (mg/kg/day)	0	0	10	25	75
Body weight					
Week 1	32.6	32.3	32.6 (-0.0%) [+0.9%]	32.8 (+0.6%) [+1.5%]	32.1 (-1.5%) [-0.6%]
Week 13	41.2	41.2	43.0 (+4.4%) [+4.4%]	43.2* (+4.9%) [+4.9%]	41.3 (+0.0%) [+0.0%]
Week 26	45.6	45.9	47.6 (+4.4%) [+3.7%]	47.5 (+4.2%) [+3.5%]	45.6 (+0.0%) [-1.0%]
Week 54	48.5	49.0	50.2 (+3.5%) [+2.4%]	50.8 (+4.7%) [+3.7%]	48.1 (-0.8%) [-1.8%]
Week 78	50.4	49.8	51.4 (+2.0%) [+3.2%]	51.3 (+1.8%) [+3.0%]	48.3 (-4.2%) [-3.0%]
Week 104	49.3	49.9	50.7 (+2.8%) [+1.6%]	49.3 (+0.0%) [-1.2%]	49.3 (+0.0%) [-1.2%]
Body weight change					
Weeks 1 to 13	+8.7	+8.9	+10.4* (+19.5%) [+16.9%]	+10.4* (+19.5%) [+16.9%]	+9.2 (+5.8%) [+3.4%]
Weeks 13 to 26	+4.3	+4.7	+4.6 (+7.0%) [-2.1%]	+4.2 (-2.3%) [-10.6%]	+4.3 (+0.0%) [-8.5%]
Weeks 26 to 54	+2.7	+3.3	+2.5 (-7.4%) [-24.2%]	+3.5 (+29.6%) [+6.1%]	+2.5 (-7.4%) [-24.2%]
Weeks 54 to 104	+0.9	+0.8	+1.1 (+22.2%) [+37.5%]	+0.9 (+0.0%) [+12.5%]	+2.1 (+133.3%) [+162.5%]
Weeks 1 to 104	+16.4	+17.6	+18.5 (+12.8%) [+5.1%]	+17.3 (+5.5%) [-1.7%]	+17.6 (+7.3%) [+0.0%]

Statistical significance compared to control group 1: *: $p<0.05$.
Difference from control group 1 in brackets and difference from control group 2 in square brackets.

Reference ID: 3489781

Table 7B:

Mean body weight (g) and body weight change (g) in principal females

Sex	Female				
Study group	1	2	3	4	5
Dose-level (mg/kg/day)	0	0	10	25	75
Body weight					
Week 1	24.6	25.0	24.7 (+0.4%) [-1.2%]	24.9 (+1.2%) [-0.4%]	24.6 (+0.0%) [-1.6%]
Week 13	31.7	32.0	31.8 (+0.3%) [-0.6%]	32.0 (+0.9%) [+0.0%]	32.5 (+2.5%) [+1.6%]
Week 26	34.6	35.2	35.7 (+3.2%) [+1.4%]	34.7 (+0.3%) [-1.4%]	36.4 (+5.2%) [+3.4%]
Week 54	37.9	37.8	38.4 (+1.3) [+1.6%]	38.2 (+0.8%) [+1.1%]	39.7 (+4.8%) [+5.0%]
Week 78	39.8	38.8	41.6 (+4.5%) [+7.2%]	40.8 (+2.5%) [+5.2%]	41.7 (+4.8%) [+7.5%]
Week 104	39.8	41.2	42.1 (+5.8%) [+2.2%]	39.4 (-1.0%) [-4.4%]	41.6 (+4.5%) [+1.0%]
Body weight change					
Weeks 1 to 13	+7.1	+7.0	+7.1 (+0.0%) [+1.4%]	+7.1 (+0.0%) [+1.4%]	+8.0 (+12.7%) [+14.3%]
Weeks 13 to 26	+2.9	+3.2	+4.0* (+37.9%) [+25%]	+2.8 (-3.4%) [-12.5%]	+3.9 (+34.5%) [+21.9%]
Weeks 26 to 54	+3.0	+2.6	+2.7 (-10.0%) [+3.8%]	+3.4 (+13.3%) [+30.8%]	+3.2 (+6.7%) [+23.1%]
Weeks 54 to 104	+2.0	+3.1	+3.8 (+90%) [+22.6%]	+2.4 (+20.0%) [-22.6%]	+1.2 (-40.0%) [-61.3%]
Weeks 1 to 104	+15.0	+16.1	+17.5 (+16.7%) [+8.7%]	+14.3 (-4.7%) [-11.2%]	+17.2 (+14.7%) [+6.8%]

Statistical significance compared to control group 1: *: $p<0.05$.
Difference from control group 1 in brackets and difference from control group 2 in square brackets.

Feed Consumption

For Main Study animals, feed consumption was recorded twice before treatment initiation (during Week -2 and Week -1), after allocation into treatment groups, once a week during the first 13 weeks of treatment and once every 4 weeks until the end of the study. For Satellite 2 animals, food consumption was recorded at the same intervals as Main Study animals for the first 13 weeks of treatment.

Results: There were no statistically significant changes in food consumption over the entire dosing period, as compared to controls. There was a statistically significant increase in food consumption in LD males during Week 13 (5.3%, $p<0.05$ as compared to Group 1 controls) and in MD males during Week 25 (9.8%, $p<0.01$ as compared to Group 1 controls).

Ophthalmology

Ophthalmological examinations were conducted on the first 10 surviving animals/sex in Groups 1 and 5 during Weeks 26/27, 52, 78, and at the end of treatment. If the fundus was not visible at the end of treatment of the examined animals, 5 additional animals/group/sex were examined.

Results: There were occasional ophthalmological findings in treated males and females; however, it is unclear that the findings were treatment-related. The findings included invisible fundus (4/sex/control group, 4 HD males, and 5 HD females) and mass in vitreous body (5 HD males and 1 control female).

Hematology

Blood smears were prepared on moribund animals before sacrifice. A differential white blood cell count was determined in Control (Group 1) and high dose animals (Group 5). At necropsy, blood samples were collected from the first 10 surviving animals/sex/group and analyzed for the following parameters: erythrocytes (RBC), hemoglobin (HB), mean cell volume (MCV), packed cell volume (PCV), mean cell hemoglobin concentration (MCHC), mean cell hemoglobin (MCH), thrombocytes, platelets (PLT), leucocytes (WBC), differential white blood cell count with morphology (neutrophils(N), eosinophils (E), basophils (B), lymphocytes and large unstained cells (L + LUC), monocytes (M)), and reticulocytes (RTC).

Results: There was a decrease in PLT at the HD in males, as compared to Group 1 (16%) and Group 2 (8.4%) controls at the end of the treatment period; however, the decreases were not statistically significant. There were no statistically significant, treatment-related changes in males or females over the course of the treatment period.

Clinical Chemistry

At necropsy, blood samples were collected from the first 10 surviving animals/sex/group and analyzed for the following parameters: sodium (Na+), potassium (K+), chloride (Cl-

Reference ID: 3489781

), calcium (Ca++), inorganic phosphorous (I.PHOS), glucose (GLUC), urea (UREA), creatinine (CREAT), total bilirubin (TOT.BIL), total proteins (PROT), albumin (ALB), albumin/globulin ratio (A/G), total cholesterol (CHOL), triglycerides (TRIG), alkaline phosphatase (ALP), aspartate aminotransferase (ASAT), and alanine aminotransferase (ALAT).

Results: There were no significant treatment-related changes in serum biochemistry.

Gross Pathology

Principal animals were sacrificed at the end of the treatment period. Satellite 1 animals were sacrificed at the end of blood sampling for toxicokinetics, and Satellite 2 animals were sacrificed for GL-1 analysis. Moribund animals were sacrificed and examined to identify the cause of death.

Results: There were no treatment-related changes in gross pathology.

Histopathology

The following organs/tissues were harvested and processed for histopathology (shown in applicant's table below).

Reference ID: 3489781

Table 8: List of organs/tissues from treated CD-1 harvested and processed for histopathology

TISSUE PROCEDURE TABLE

Organs	Preservation of tissues	Microscopic examination
Macroscopic lesions	X	X
Adrenals	X	X
Aorta	X	X
Brain (including medulla/pons cerebellar and cerebral cortex)	X	X
Cecum	X	X
Clitoral gland	X	X
Colon	X	X
Duodenum	X	X
Epididymides	X	X
Esophagus	X	X
Eyes (with optic nerves and harderian glands)	X	X
Femoral bone with articulation	X	X
Gall bladder	X	X
Heart	X	X
Ileum (with Peyers patches)	X	X
Jejunum	X	X
Kidneys	X	X
Larynx	X	X
Liver	X	X
Lungs with bronchi	X	X
Lymph nodes (mandibular and mesenteric)	X	X
Mammary gland area (females)	X	X
Mammary gland area (males)	X	
Nasal cavity (with nasopharynx and paranasal sinus)	X	
Ovaries (with oviducts)	X	X
Pancreas	X	X
Pituitary gland	X	X
Preputial gland	X	X
Prostate	X	X
Rectum	X	
Salivary glands (sublingual and submandibular)	X	X
Sciatic nerve	X	X
Seminal vesicles	X	X
Skeletal muscle	X	X
Skin	X	X
Spinal cord (cervical, thoracic and lumbar)	X	X
Spleen	X	X
Sternum with bone marrow	X	
Stomach with forestomach	X	X
Testes	X	X
Thymus	X	X
Thyroids with parathyroids	X	X
Tongue	X	X
Trachea	X	X
Urinary bladder	X	X
Uterus (horns and cervix)	X	X
Vagina	X	X
Zymbal gland (with external ear)	X	

Peer Review: Yes

Neoplastic: The number of animals with neoplasms and number of malignant neoplasms was slightly higher in females, as compared to males. The number of

primary neoplasms was higher in HD females (67 total) as compared to Group 1 and Group 2 females (58 and 60 each, respectively). There were 8 HD males (N = 60) with metastases. There were no statistically significant differences in tumor findings between test article-treated and control animals, in either sex. The applicant's table below summarizes the tumor findings in each sex.

Table 9: Summary of overall tumor data in CD-1 mice receiving eliglustat tartrate for 105/106 weeks as a dietary admixture (applicant's table)

Overall tumor data

Sex	Male					Female				
Group	1	2	3	4	5	1	2	3	4	5
Dose-level (mg/kg/day)	0	0	10	25	75	0	0	10	25	75
Total number of animals	60	60	60	60	60	60	60	60	60	60
. number of animals with neoplasms	40	37	33	37	38	41	41	42	42	46
. number of animals with more than one primary neoplasm	22	15	10	19	19	16	11	15	12	15
. number of animals with metastases	2	4	0	2	8	4	2	2	2	4
. number of primary neoplasms	67	52	49	63	63	58	60	62	56	67
. number of benign neoplasms	41	25	24	33	30	20	25	22	20	28
. number of malignant neoplasms	26	27	25	30	33	38	35	40	36	39

Conventional Peto test and Exact Peto test failed to reveal any statistically significant trends in tumor incidence. The applicant pooled hemangiosarcomas and hemangiomas from all organs and no statistically significant findings were made.

Trend test statistics using Poly-3 test revealed an increase in incidence of 3 tumor types at the HD in males and females: benign cortical adenoma (2/60 HD males, as compared to 0/120 controls), benign pheochromocytoma (2/60 HD females, as compared to 0/120 controls), and malignant skin fibrosarcoma (2/58 HD females, as compared to 0/120 controls). The table below summarizes these findings.

Table 10: Incidence of selected neoplasms in CD-1 mice receiving eliglustat tartrate for 105/106 weeks as a dietary admixture, as compared to (b) (4) and Lit Data

Males	Organ/System Tumor	Ctrl*	LD	MD	HD	(b) (4) Ctrl@	Lit Data#
	Adrenal glands/Cortical adenoma (incidence shown as # tumors/total # animals per group)	0/120	1/60	0/58	2/60	13/178	33/2896
	Percentage (%)	0	1.7	0	3.3	7.3	1.1
	Range$					0-16.7	0-7.1
Females	Adrenal glands/Pheochromocytoma	0/120	0/60	0/60	2/60	0/179	11/3167
	Percentage (%)	0	0	0	3.3	0	0.3
	Range$						0-5.0
	Skin/Fibrosarcoma	0/120	0/60	0/60	2/58	3/179	21/3232
	Percentage (%)	0	0	0	3.4	1.7	0.6
	Range$					0-4.0	0-6.7

*Groups 1 and 2 controls combined (N = 120)
@ (b) (4) Ctrl data: Data from 2003-2010 (three carcinogenicity studies)
#Lit data: Spontaneous neoplastic lesions in (b) (4) CD-1(ICR) mice from 18 month-2 year carcinogenicity studies (b) (4)
$Range: Range of % values from control groups from (b) (4) data and Lit data

When compared to (b) (4) control data and LIT data, the increased incidence of the identified rare tumors (in HD males and HD females) was within previously observed ranges. Therefore, the applicant concluded that the increased incidence of tumors was not related to treatment with test article, rather were spontaneous occurrences.

Statistical analysis by FDA reviewer Dr. Min is showed in Table 3. The following tumor types had p values ≤ 0.05 for dose relationships: Adrenal gland (Adenoma/Cortex), Liver (Hemangiosarcoma), Lungs (Adenocarcinoma+Adenoma) in Males and Adrenal Gland (pheochromocytoma) and Skin (fibrosarcoma) in Females). When pair-wise comparisons were evaluated, the following had p values ≤ 0.05: Lungs (Adenocarcinoma and Adenocarcinoma+Adenoma in MD males) and Uterus (Endometrial Sarcoma in LD females). However, a positive dose-response relationship or increase in tumor incidence was not apparent.

Non Neoplastic: There was a statistically significant, treatment-related increase in the incidence and severity of hepatocellular hypertrophy in males and females, as compared to controls. Males in the MD and HD were affected, whereas females at LD, MD, and HD were affected. Grade 3 severity (moderate) was observed in 2 and 4 MD and HD males, respectively. The hypertrophy was not observed with any necrosis or hepatocellular degeneration and may be related to CYP450 induction in mice. The applicant's table below summarizes the liver findings.

Table 11: Incidence and severity of hepatocellular hypertrophy in CD-1 mice receiving eliglustat tartrate for 105/106 weeks as a dietary admixture (applicant's table)

Incidence and severity of hepatocellular hypertrophy

Sex	Male					Female				
Groups	1	2	3	4	5	1	2	3	4	5
Dose-level (mg/kg/day)	0	0	10	25	75	0	0	10	25	75
Number examined										
Hepatocellular hypertrophy	10	4	11	19**	29**	3	5	11*	17**	25**
. grade 1	7	3	10	11	14	3	3	8	13	14
. grade 2	3	1	1	6	11	0	1	3	4	11
. grade 3	0	0	0	2	4	0	1	0	0	0

*: p<0.05; **: p<0.0005.

Toxicokinetics (TK)

TK of test article was assessed in satellite animals (the first 18 animals/sex/group in Groups 3, 4, and 5). Sampling from Control animals were taken on Day 7 or Day 89/90. The first 2 animals/sex in Group 1 and the first 9 animals/sex in Groups 3, 4, and 5 in the satellite animals were sampled for TK measurements. Test article-treated animals (3/sex/group) were sampled at three time points: 0.25, 0.5, and 1 h post-dose. Control group animals were sampled once at 0.5 h post dose.

Results: TK analysis was not conducted, since plasma levels of the test article were below the lower limit of quantitation (5 ng/mL).

Plasma levels of Glucosylceramide (GL-1)

Plasma GL-1 levels were determined in samples taken from Satellite 2 animals during Week 13. Analysis was conducted under non-GLP conditions.

Results: Plasma levels decreased in test article-treated animals, as compared to control, suggesting systemic exposure of drug. However, a clear dose response was not apparent. The results are shown in the applicant's table below.

Table 12: Plasma levels of glucosylceramide (GL-1) after dietary administration of eliglustat tartrate for 13 weeks in in CD-1 mice

Group (Daily dose mg/kg): Satellite 2 animals	Group 6 (0)		Group 7 (10)		Group 8 (25)		Group 9 (75)	
GL-1 Plasma Levels (Non-GLP Results: satellite 2 animals)	M	F	M	F	M	F	M	F
Mean Values	1.49	2.45	0.99	1.88	1.17	2.30	0.87	2.03
Standard Deviation	0.16	0.44	0.26	0.51	0.10	0.17	0.34	0.48

Reference ID: 3489781

Dosing Solution Analysis

The concentration of control or test article in the dietary admixture was determined monthly (during Weeks 1, 4, 8, 13), followed by once every 3 months (during Weeks 27, 41, 52, 65, 78, 92, 99, 104). A concentration was deemed acceptable if it was within 20% of the nominal concentration (i.e. nominal concentration ± 20%.

Results: The doses of control and test article-containing samples were within acceptable ranges at each sampling time.

Study title: Carcinogenicity Study by Oral Route (Gavage) in Rats

Study no.:	GT-157-TX-38
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 29, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Genz-112638, Lot # T1136, 99.4-100.8%
CAC concurrence:	Yes

Key Study Findings

Eliglustat tartrate did not produce any treatment-related neoplasms at statistically significant increased incidences, per CDER criteria, when administered by oral gavage to SD rats (50/sex/dose) at 10, 25, and 75 mg/kg/day in males for 105 weeks and at 5, 15, and 50 mg/kg/day in females for 103 weeks.

A dose-dependent effect on tumor latency was not apparent. Malignant tumors appeared earliest in test article treated males, as compared to test article-treated females and control animals. Poly-3 test statistics revealed an increase in incidence of several tumors: granulocytic leukemia in 2/50 LD males, odontoma in 1/50 MD males, and mammary gland adenomas in females (1/50, 2/50, and 2/50 at LD, MD, and HD, respectively), as compared to 0/100 controls/sex. However, the incidence of tumors in males and females fell within the range of those observed in control animals from historical data in the testing laboratory and published literature. Therefore, the increased incidence of these tumors was considered to be unrelated to treatment with test article in either sex. Additional analyses conducted by FDA statistical reviewer revealed a $p \leq 0.05$ for dose response relationship for skin fibroma in males only. However, the incidence of this tumor did not have a positive dose response relationship or reveal a statistically significant increase in tumor incidence in the pair-wise comparisons (combined control vs. individual treatment group).

Reference ID: 3489781

Adequacy of Carcinogenicity Study:

The carcinogenicity study was adequate and acceptable.

Dose selection was based on findings from a 28-day study in Sprague-Dawley rats, with concurrence from the CDER ECAC (Please refer to Dr. Tamal Chakraborti's nonclinical review of the Special Protocol Assessment for study design details and ECAC recommendations (SDN 060 and 062 dated December 18, 2008)[2]. Briefly, the ECAC recommended doses of 10, 25, and 75 mg/kg/day for males, based on mortality observed at 200 mg/kg/day (administered as 100 mg/kg BID) in a 28-day oral toxicity study with 9-week recovery period. In females, the ECAC agreed with the applicant's selection of 5, 15, and 50 mg/kg/day based on AUC comparisons (about 30-fold higher than human AUC at 100 mg BID dosing).

Therefore, based on applicant's dose selection, confirmation of chemical/vehicle stability and achieved dosages, the study is adequate and acceptable.

Appropriateness of Test Models

The Sprague-Dawley rat is an acceptable model for the 2-year oral carcinogenicity study.

Evaluation of Tumor Findings

The table below shows the week of onset of the first malignant neoplasm. The following doses were used in Males: Vehicle Control (0 mg/kg/day, Ctrl), 10 mg/kg/day (Low Dose, LD), 25 mg/kg/day (Mid Dose, MD), and 75 mg/kg/day (High Dose, HD). In females, Groups 1 and 2 were Vehicle Controls (0 mg/kg/day, Ctrl), 5, 15, and 50 mg/kg/day were the Low Dose (LD), Mid dose (MD) and High Dose (HD), respectively. The first malignant neoplasm was observed in LD males during Week 18 (leukemia) and in HD females during Week 38 (adenocarcinoma). A dose-related effect on tumor onset or type of tumor was not apparent.

Table 13: Onset of tumors in SD rats treated with eliglustat tartrate for 2 years via oral gavage

Males			
Group Dose (mg/kg/day)	Week of onset	Organ	Corresponding neoplasm
1 (0, Ctrl)	73	Brain	Astrocytoma
2 (0, Ctrl)	91	Bone	Osteosarcoma
3 (10, LD)	18	Hemolympho-reticular system	Leukemia
4 (25, MD)	43	Mammary gland	Adenocarcinoma
5 (75, HD)	45	Subcutaneous tissue	Sarcoma

[2] Please refer to the nonclinical review of the SPA for the 2-year oral gavage study protocol by Dr. Tamal Chakraborti for details on the study results (SDN 060 and 062, Dated December 18, 2008) and ECAC recommendations in Appendix #1B.

Reference ID: 3489781

Females			
Group/ Dose (mg/kg/day)	Week of onset	Organ	Corresponding neoplasm
1 (0, Ctrl)	65	Thymus	Thymoma
2 (0, Ctrl)	76	Mammary gland area	Adenocarcinoma
3 (5, LD)	64	Mammary gland area	Adenocarcinoma
4 (15, MD)	69	Mammary gland area	Adenocarcinoma
5 (50, HD)	38	Mammary gland area	Adenocarcinoma

The applicant performed trend test statistics (Conventional Peto test) on neoplastic findings. Mammary tumors were pooled. However, a statistically significant, treatment-related trend in tumor incidence was not revealed.

Trend statistics using Poly-3 test revealed an increase in incidence of several tumors ($p < 0.005$ for common tumors and $p < 0.025$ for rare tumors (tumors not found in control animals)). The following rare tumors were identified in males: granulocytic leukemia in 2/50 animals, as compared to 0/100 controls, and odontoma in 1/50 males, as compared to 0/100 controls). The applicant concluded that the incidence of these rare tumors in males fell within the range of those observed in control animals from historical data in the testing laboratory (b) (4) and published literature (See Table 24 below); therefore, the increased incidence of these tumors was considered to be unrelated to treatment with test article in males.

In females, there was an increase in incidence of rare, benign mammary gland adenomas at the MD and HD ($p = 0.018$). The type of tumor was observed in less than 1% of control females. There was also an increase in the incidence of fibroadenomas and fibromas. However, the incidence of all three of these tumor types (adenoma, fibroadenoma, and fibroma) was within the range of historical control data from (b) (4) and published literature. The applicant did not combine mammary gland adenomas, fibroadenomas and fibromas, since several animals had multiple mammary neoplasm and the incidences of these tumor types were similar amongst dose groups.

Independent analysis of the tumor findings was conducted by FDA statistical reviewer Dr. Min, who combined the following tumor types for additional analyses, based on this nonclinical reviewer's request:

Reference ID: 3489781

Rat:

- Adrenal medullas benign and malignant pheochromocytoma.
- Kidney tubule adenoma and carcinoma for male rats only.
- Liver hepatocellular adenoma and carcinoma.
- Lung bronchio-alveolar adenoma and carcinoma for male rats only.
- Mammary gland adenocarcinoma and adenocarcinoma arising in fibroden for female rats only.
- Mammary gland fibroadenoma and adenoma plus fibroma for female rats only.
- Pancreas acinar cell adenocarcinoma and adenoma plus islet cell adenoma.
- Pituitary gland pars distalis and intermedia adenoma.
- Prostate adenocarcinoma and adenoma for male rats only.
- Skin benign and malignant basal cell tumor for male rats only.
- Skin squamous cell papilloma and carcinoma plus keratoacanthoma for male rats only.
- Thyroid follicular cell b-adenoma and carcinoma
- Thyroid c-cell b-adenoma and carcinoma.

The results from these additional analyses showed a $p \leq 0.05$ for dose response relationship for Combined Controls for skin fibroma in males only, as shown in the table below from Dr. Min's review. Pair-wise comparisons for Combined Controls and each of the dose groups (Low Dose (L), Mid Dose (M), and High Dose (H)) are also shown in the table below. However, none of the incidences of tumors in either sex was considered to have a positive dose response relationship. Likewise, none of the pair-wise comparisons (combined control vs. individual treatment group) revealed an increase in tumor incidence.

Table 14: Tumor types in SD rats with p-values ≤ 0.05 for dose response relationship or pair-wise comparisons (combined controls, low, medium and high dose groups), taken from draft review of Dr. Min Min

	Organ Name	Tumor Name	0 mg Cont N=102	10 mg Low N=55	25 mg Med N=53	75 mg High N=51	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
Male										
	SKIN	FIBROMA	2	1	2	4	0.029	0.711	0.371	0.086
			[69]	[30]	[30]	[31]	.	.	.	.
.	.									

The table below summarizes the body weight and mortality rates between combined controls (Group 1 and Group 2 vehicle-treated animals combined) and individual dose groups.

Table 15: Summary of body weight gain and mortality rates in SD rats treated with eliglustat tartrate for 2 years via oral gavage

Body weight (% difference in mean body weight gain from combined controls			Mortality Rates at the end of the experiment		
Dose	Male	Female	Dose	Male	Female
			Comb. Ctrl	58%	56%
LD	-4.55	-4.32	LD	60%	72%
MD	-4.75	8.95	MD	62%	71%
HD	-22.8	-8.95	HD	51%	56%

There was a > 22% and > 8% decrease in body weight gain in HD males and HD females, respectively, as compared to combined controls. The mortality rate in HD males was 7% lower than Combined Controls. In females, the mortality rate was similar between combined controls and HD females, while it was ≥15% higher in LD and MD females. Based on these findings, the MTD was reached in both males and likely reached in females in the rat study.

Reference ID: 3489781

Methods

Doses:

Mg/kg/day	Group #				
Sex	1 (Ctrl)	2 (Ctrl)	3 (LD)	4 (MD)	5 (HD)
Male (105 weeks)	0	0	10	25	75
Female (103 weeks)	0	0	5	15	50

Frequency of dosing: Once daily
Dose volume: 5 mL/kg/day
Route of administration: Oral gavage
Formulation/Vehicle: Solution in Vehicle/Drinking water
Basis of dose selection: 28-day toxicity studies (GT-157-TX-28 and GT-157-TX-5), 26-week toxicity study (GT-157-TX-17); results are summarized below:

Study	Study design	Results
GT-157-TX-28	30 or 100 mg/kg/day for 4 weeks or 200 mg/kg/day for 2 weeks via oral gavage in adult males only	• MTD < 100 mg/kg/bid • NOAEL (systemic effects) = 30 mg/kg/day • NOEL (effects on spermatogenesis and male reproductive organs) = 30 mg/kg/day
GT-157-TX-5	10, 30, 100 mg/kg/day via oral gavage in adult males and females	• NOAEL = 30 mg/kg/day
GT-157-TX-17	5, 15, 50 mg/kg/day for 26 weeks followed by an 8-week treatment-free period in adult males and females	• NOAEL = 50 mg/kg/day • NOEL = 15 mg/kg/day

Species/Strain: Rat/Sprague-Dawley Crl CD® (SD) IGS BR
Number/Sex/Group: Principal: 50/sex/group
Satellite: 4/sex/group (Group 1 control only), 10/sex/group (Groups 3-5), 0/sex/group (Group 2 control)
Age: 6 weeks old at initiation of treatment
Males: 176-238 g body weight
Females: 135-187 g body weight
Animal housing: 2 rats/sex/group/cage
Paradigm for dietary restriction: No diet restriction
Dual control employed: Yes (2 vehicle control groups)

Reference ID: 3489781

Interim sacrifice: No (satellite animals were not sacrificed after Week 13 TK sampling)
Satellite groups: Yes (for TK analysis)
Deviation from study protocol: None which affected the results of the study

Observations and Results

Mortality

Mortality and morbidity were checked twice daily during the treatment period.

Results: The table below summarizes the cumulative mortalities observed over the course of study. The number of deaths shown during each interval represents the number of animals found dead + the number of animals sacrificed prematurely.

Table 16: Cumulative mortalities in SD rats treated with eliglustat tartrate for 2 years via oral gavage

	Males (N= 50/group)					Females (N = 50/group)				
Group	1	2	3	4	5	1	2	3	4	5
Dose (mg/kg/day)	0	0	10	25	75	0	0	5	15	50
Weeks 1-52	2	3	5	5	4	0	1	1	1	4
Weeks 53-78	8	6	7	12	5	8	7	12	11	10
Weeks 79-103	22	17	16	13	17	18	21	22	23	11
Total deaths (Weeks 1-103*)						26	29	35	35	25
Total deaths (Weeks 1-105**)	32	26	28	30	26					
Total survivors at terminal sacrifice	18	24	22	20	24	24	21	15	15	25
Survival rate	36%	48%	44%	40%	48%	48%	42%	30%	30%	50%

*Survival in females at Week 103
**Survival in males at Week 105

There were no statistically significant changes in survival rate (unscheduled deaths) between control and test article-treatment in either sex, when compared to dual controls and combined controls for each sex (p = 0.2241 (Males), p = 0.5465 (Females)). There were no statistically significant differences in survival curves between combined controls and treatment groups for either sex (p = 0.7851 (Males), p = 0.1644 (Females)). The survival curves are shown in the applicant's figures below.

Figure 2: Kaplan-Meier plot of survival in SD rats treated with eliglustat tartrate for 2 years via oral gavage (applicant's figures); 2A (Males), 2B (Females)

Reference ID: 3489781

Figure 2A:

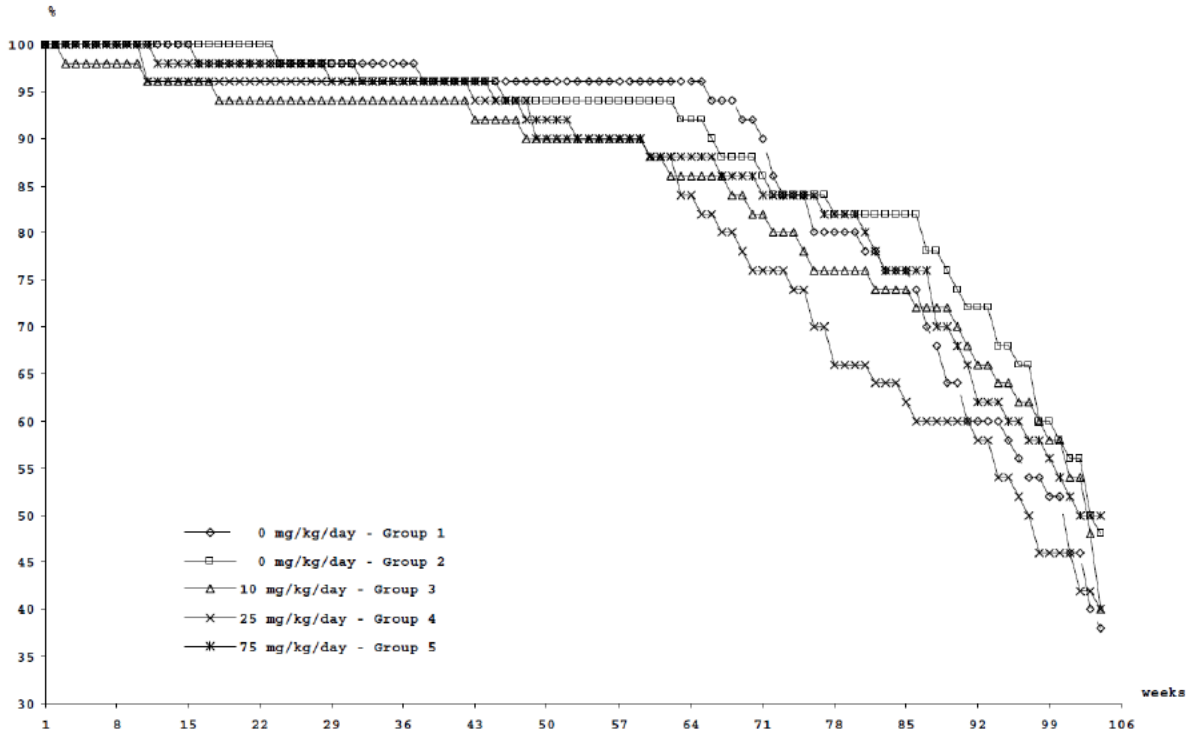
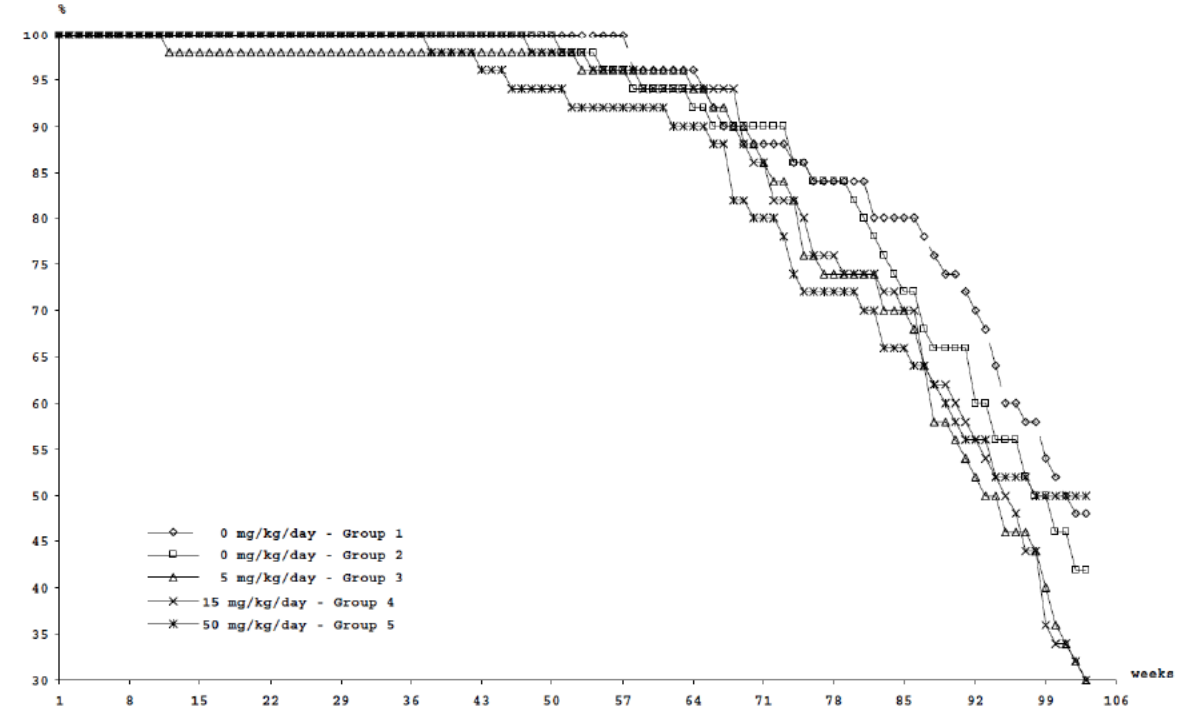


Figure 2B:



Independent analysis of survival by FDA statistical reviewer Dr. Min was in agreement with the applicant's findings that there were no statistical differences in survival between test article-treated animals (both sexes) when compared to dual controls or combined controls. The intercurrent mortality is shown in the tables below (from Dr. Min's draft review).

Table 17: Intercurrent mortality comparison in SD rats treated with eliglustat tartrate for 2 years via oral gavage, taken from draft review of Dr. Min Min; 17A (Males), 17B (Females)

Table 17A: Males

Test	P-Value (across four groups)	P-Value (combined controls vs low)	P-Value (combined controls vs medium)	P-Value (combined controls vs high)
Dose Response	0.7335	0.7945	0.4459	0.7499
Homogeneity	0.6534	0.7883	0.3508	0.5987

Table 17B: Females

Test	P-Value (across four groups)	P-Value (combined controls vs low)	P-Value (combined controls vs medium)	P-Value (combined controls vs high)
Dose Response	0.9528	0.0826	0.1584	0.7448
Homogeneity	0.0636	0.0714	0.0489	0.6578

Pituitary neoplasia and mammary neoplasia were the most common cause of mortality in males (pituitary only) and females (pituitary and mammary). The incidence of pituitary neoplasia in males and females was similar across dose groups, including controls; however, the number of HD animals with this finding was slightly lower than the remaining dose groups. Mammary neoplasia was observed in 1 male/dose at the MD and HD only and in 12, 10, and 9 females at the LD, MD, and HD, as compared to 5 females in each control group. Of these, only 1, 1, 1, and 2 of the deaths in Ctrl Group 1, Ctrl Group 2, LD, MD, and HD males, respectively, which were considered to be related to treatment. In females, there was 1 death each in Ctrl Group 1 and HD which was considered to be related to dosing. However, the sponsor did not provide an explanation for this conclusion. The applicant's table below summarizes the incidence and most common cause of mortality in males and females.

Table 18: Incidences and most common causes of mortality in SD rats treated with eliglustat tartrate for 2 years via oral gavage (applicant's table)

Incidences and most common causes of mortality

Sex	Male					Female				
Group	1	2	3	4	5	1	2	3	4	5
Dose-level (mg/kg/day)	0	0	10	25	75	0	0	5	15	50
Number examined	32	26	28	30	26	26	29	35	35	25
Pituitary neoplasia	10	11	10	9	5	18	19	21	22	10
Mammary neoplasia	-	-	-	1	1	5	5	12	10	9
Dosing-related deaths	1	1	1	2	1	1	-	-	-	1

-: not observed.

Clinical Signs

Clinical signs were monitored once daily and detailed clinical examinations were made once a week until the end of the study. If an animal lost teeth, it was isolated until the new tooth was visible and given powdered diet. Clinical signs were monitored daily.

Results: There was a dose-dependent increase in ptyalism in males (4-6% in controls, as compared to 40-76% at the MD and HD. There was also an increase in dyspnea (62%, as compared to controls) and loud breathing (70%, as compared to controls) in HD males. Females also experienced loud breathing and dyspnea, but there was no apparent dose response (~50% in all dose groups). There were no other treatment-related clinical signs.

Body Weights

Body weights for Principal females and satellite animals (both sexes) were recorded once before group allocation, prior to blank gavage on dosing treatment Day 1, once a week during the first 14 weeks of treatment, once every 4 weeks until the end of the study, and at study termination. For Principal males, body weight was recorded before group allocation, before blank gavage, on Day 1 of treatment, once a week during the first 26 weeks of treatment, and once every two weeks from Weeks 26-86, once every 4 weeks until the end of the study, and at study termination.

Results: A statistically significant decrease in body weight was observed in high dose males, as compared to Group 1 controls, beginning in Week 12 of treatment. Over the course of the entire treatment period, there was a 26.9% and 20.9% change in body weight in high dose males, as compared to Group 1 and Group 2 controls, respectively. There were no statistically significant, treatment-related changes in body weight in females. The applicant's table below summarizes the changes in body weight over the course of the treatment period. Mean body weight (g) and percent body weight change from control Groups 1 and 2 are shown in parentheses and brackets, respectively. Statistical significance is shown as *$p < 0.05$ or **$p < 0.01$, as compared to Group 1 control.

Reference ID: 3489781

Table 19: Mean body weight (g) and body weight change (g) in SD rats treated with eliglustat tartrate for 2 years via oral gavage (applicant's table); 19A (Males), 19B (Females)

Table 19A:

Sex Male					
Study group	1	2	3	4	5
Number at study start	50	50	50	50	50
Dose-level (mg/kg/day)	0 (control)	0 (control)	10 25		75
Body weight					
Week 1	209	207	207 (-1.0%) [0.0%]	208 (-0.5%) [+0.5%]	204 (-2.4%) [-1.4%]
Week 12	490	492	487 (-0.6%) [-1.0%]	481 (-1.8%) [-2.2%]	467* (-4.7%) [-5.1%]
Week 14	508	510	502 (-1.2%) [-1.6%]	497 (-2.2%) [-2.5%]	478** (-5.9%) [-6.3%]
Week 26	593	583	572 (-3.5%) [-1.9%]	576 (-2.9%) [-1.2%]	541** (-8.8%) [-7.2%]
Week 54	679	677	669 (-1.5%) [-1.2%]	668 (-1.6%) [-1.3%]	599** (-11.8%) [-11.5%]
Week 104	724	686	682 (-5.8%) [-0.6%]	695 (-4.0%) [1.3%]	578** (-20.2%) [-15.7%]
Body weight change					
Weeks 1 to 13	+289	+295	+288 (-0.3%) [-2.4%]	+281 (-2.8%) [-4.7%]	+268* (-7.3%) [-9.2%]
Weeks 13 to 26	+93	+79**	+76** (-18.3%) [-3.8%]	+87 (-6.5%) [+10.1%]	+69** (-25.8%) [-12.7%]
Weeks 26 to 54	+86	+94	+98 (+14.0%) [+4.3%]	+91 (+5.8%) [-3.2%]	+59** (-31.4%) [-37.2%]
Weeks 54 to 104	+55	+11	+21 (-61.8%) [+90.9%]	+38 (-30.9%) [+245.5%]	+1* (-98.2%) [-90.9%]
Weeks 1 to 104	+517	+478	+475 (-8.1%) [-0.6%]	+487 (-5.8%) [1.9%]	+378** (-26.9%) [-20.9%]

Statistical significance compared to group 1 control: *: p<0.05, **: p<0.01

Table 19B:

Mean body weight (g) and body weight change (g)

Sex Fem			ale		
Study group	1	2	3	4	5
Number at start	50	50	50	50	50
Dose-level (mg/kg/day)	0 (control)	0 (control)	5 15		50
Body weight					
Week 1	161	161	162 (+0.6%) [+0.6%]	159 (-1.2%) [-1.2%]	161 (+0.0%) [+0.0%]
Week 13	300	295	298 (-0.7%) [+1.0%]	296 (-1.3%) [+0.3%]	299 (-0.3%) [+1.4%]
Week 26	339	334	339 (0.0%) [+1.5%]	336 (-0.9%) [+0.6%]	333 (-1.8%) [-0.3%]
Week 54	410	407	421 (+2.7%) [+3.4%]	409 (-0.2%) [+0.5%]	395 (-3.7%) [-2.9%]
Week 103	485	485	455 (-6.2%) [-6.2%]	511 (+5.4%) [+5.4%]	453 (-6.6%) [-6.6%]
Body weight change					
Weeks 1 to 13	+140	+134	+136 (-2.9%) [+1.5%]	+137 (-2.1%) [+2.2%]	+139 (-0.7%) [+3.7%]
Weeks 13 to 26	+39	+39	+42 (+7.7%) [+7.7%]	+40 (+2.6%) [+2.6%]	+33 (-15.4%) [-15.4%]
Weeks 26 to 54	+71	+73	+82 (+15.5%) [+12.3%]	+72 (+1.4%) [-1.4%]	+62 (-12.7%) [-15.1%]
Weeks 54 to 103	+87	+83	+81 (-6.9%) [-2.4%]	+97 (+11.5%) [+16.9%]	+72 (-17.2%) [-13.3%]
Weeks 1 to 103	+325	+325	+297 (-8.6%) [-8.6%]	+357 (+9.8%) [+9.8%]	+292 (-10.2%) [-10.2%]

Difference from group 1 control in brackets and difference from group 2 control in square bracket.

Feed Consumption

For Principal females, food consumption (per cage) was recorded once a week for the first 13 weeks of treatment and once every 4 weeks thereafter until the end of treatment. For Principal males, food consumption (per cage) was recorded once a week during the first 25 weeks of treatment, once every 2 weeks from Weeks 25-85, and once every 4 weeks until the end of the study. Food consumption was calculated per animal per day.

Results: There were no statistically significant, treatment-related changes in food consumption in either sex, as compared to control (shown in the applicant's table below).

Ophthalmology

Ophthalmological examination was performed on the first 10 surviving Principal animals/sex/group in Groups 1 (Vehicle control) and Group 5 (high dose) during Weeks 26, 52, 78 and at completion of treatment.

Results: There were no treatment-related effects in the eye.

Hematology

Blood smears were prepared on moribund animals before sacrifice. A differential white blood cell count was determined in Control (Group 1) and high dose animals (Group 5). At necropsy, blood samples were collected from the first 10 surviving animals/sex/group and analyzed for the following parameters: erythrocytes (RBC), hemoglobin (HB), mean cell volume (MCV), packed cell volume (PCV), mean cell hemoglobin concentration (MCHC), mean cell hemoglobin (MCH), thrombocytes (PLT), leucocytes (WBC), differential white blood cell count with morphology (neutrophils(N), eosinophils (E), basophils (B), lymphocytes and large unstained cells (L + LUC), monocytes (M)), and reticulocytes (RTC).

Results: There were no treatment-related effects on hematological parameters.

Clinical Chemistry

At necropsy, blood samples were collected from the first 10 surviving animals/sex/group and analyzed for the following parameters: sodium (Na+), potassium (K+), chloride (Cl-), calcium (Ca++), inorganic phosphorous (I.PHOS), glucose (GLUC), urea (UREA), creatinine (CREAT), total bilirubin (TOT.BIL), total proteins (PROT), albumin (ALB), albumin/globulin ratio (A/G), total cholesterol (CHOL), triglycerides (TRIG), alkaline phosphatase (ALP), aspartate aminotransferase (ASAT), and alanine aminotransferase (ALAT).

Results: There was a 12.5% and 20.56% increase ($p < 0.05$, as compared to Group 1 and Group 2 controls, respectively) in IPHOS levels and 69.8% and 80% increase ($p < 0.01$, as compared to Group 1 and Group 2 controls, respectively) in ALAT activity.

Reference ID: 3489781

Gross Pathology

Animals (Principal and Satellite) which survived until scheduled termination were sacrificed by exsanguination after intraperitoneal pentobarbital injection. Principal animals which survived until scheduled necropsy underwent a complete post-mortem examination which included external surfaces, all orifices, the cranial, thoracic, abdominal and pelvic cavities, organs and tissues associated with each cavity, external surface of the brain and neck and associated organs. Satellite animals which died prematurely were subjected to complete macroscopic examination to identify a cause of death.

Results: The table below summarizes the incidence of palpable masses (PM) and those which were confirmed malignant.

Table 20: Incidence of palpable masses in SD rats treated with eliglustat tartrate for 2 years via oral gavage

Group	Males (N = 50)			Females (N = 50)		
Group	# animals with PM	# PM	# PM confirmed malignant	# animals with PM	# PM	# PM confirmed malignant
1 (Ctrl)	42	69	12	48	100	15
2 (Ctrl)	44	81	12	50	110	15
3 (Low Dose)	39	68	13	48	115	20
4 (Mid Dose)	42	73	13	48	102	16
5 (High Dose)	37	57	8	46	92	18

There was an increased incidence of enlarged pituitary in Groups 1-4 males (9-10 animals, N = 26-30/group) which were deceased and in 1-7 males in the same dose groups which survived until termination. The incidence of enlarged pituitary was lower in high dose males. This finding was not observed in females. There were no other significant treatment-related gross macroscopic findings.

Histopathology

The following organs were harvested and preserved from all Principal animals (shown in applicant's table below):

Reference ID: 3489781

Table 21: List of organs/tissues from treated SD rats harvested and processed for histopathology

Table 4. **TISSUE PROCEDURE TABLE**

Organs	Preservation of tissues	Microscopic examination
Macroscopic lesions	X X	
Adrenals X		X
Aorta X		X
Brain (including medulla/pons cerebellar and cerebral cortex)	X X	
Cecum X		X
Clitoral gland	X	X
Colon X		X
Duodenum X		X
Epididymides X		X
Esophagus X		X
Eyes (with optic nerves and harderian glands) X		X
Femoral bone with articulation	X	X
Heart X		X
Ileum (with Peyers patches) X		X
Jejunum X		X
Kidneys X		X
Larynx X		X
Liver X		X
Lungs with bronchi	X	X
Lymph nodes (mandibular and mesenteric) X		X
Mammary gland area (females) X		X
Mammary gland area (males) X		
Nasal cavity (with nasopharynx and paranasal sinus) X		
Ovaries (with oviducts) X		X
Pancreas X		X
Pituitary gland	X	X
Preputial gland	X	X
Prostate X		X
Rectum X		
Salivary glands (sublingual and submandibular) X		X
Sciatic nerve	X	X
Seminal vesicles	X	X
Skeletal muscle	X	X
Skin X		X
Spinal cord (cervical, thoracic and lumbar) X		X
Spleen X		X
Sternum with bone marrow	X	
Stomach with forestomach	X	X
Testes X		X
Thymus X		X
Thyroids with parathyroids	X	X
Tongue X		X
Trachea X		X
Urinary bladder	X	X
Uterus (horns and cervix) X		X
Vagina X		X
Zymbal gland (with external ear) X		

Peer Review: Yes (on at least 10% of histological slides from each group and on all slides from identified target organs and tumors).

Neoplastic: The applicant's table below summarizes the overall tumor findings (including malignant and benign neoplasms). The number of animals with neoplasms was slightly lower in males, as compared to females; however, the percentage of animals of each sex (N = 50) with neoplasms was similar between males and females. The total number of animals with metastases were 4 males (one each in Group 1 and 2 Ctrls and two at the MD) and 4 females (one Group 1 Ctrl, two at MD and one at HD).

Table 22: Summary of overall tumor data in SD rats treated with eliglustat tartrate for 2 years via oral gavage (applicant's table)

Overall tumor data

Sex	Male					Female				
Group	1	2	3	4	5	1	2	3	4	5
Dose-level (mg/kg/day)	0	0	10	25	75	0	0	5	15	50
Total number of animals	50	50	50	50	50	50	50	50	50	50
. number of animals with neoplasms	42	44	39	42	37	48	50	48	48	46
. number of animals with more than one primary neoplasm	19	23	19	20	14	29	34	38	33	31
. number of animals with metastases	1	1	0	2	0	0	1	0	2	1
. number of primary neoplasms	69	81	68	73	57	100	110	115	102	92
. number of benign neoplasms	55	83	67	91	52	90	60	86	46	74
. number of malignant neoplasms	14	17	14	19	16	25	13	16	11	18

Poly-3 test statistics revealed higher incidences of several rare tumors (< 1% incidence in Controls). In males, there was a higher incidence of granulocytic leukemia at the LD (10 mg/kg/day) in 2 of 50 males, as compared to 0/100 males in the controls. Odontoma was observed in 1 of 50 MD (25 mg/kg/day) males, as compared to 0/100 control males. The incidences of these rare tumors in males were similar to testing laboratory (b) (4) historical control data and published literature (Lit). Furthermore, there was no dose-relationship in the incidence of either tumor. In females, there was an increase in incidence of mammary gland adenomas at the MD and HD (15 and 50 mg/kg/day). There was also an increase in the incidence of fibroadenomas and fibromas. However, the incidence of all three of these tumor types was within the range of historical control data (from (b) (4) and LIT data. The applicant did not combine these tumors because several neoplasms were observed in various dose groups. The table below summarizes the incidence of neoplastic lesions (shown as % of animals with tumor) in each sex and compares the findings to (b) (4) Ctrl[3]) historical controls and published data (Lit data)[4].

Table 23: Incidence of selected neoplasms in SD rats treated with eliglustat tartrate for 2 years via oral gavage (applicant's table), as compared to (b) (4) and Lit Data

Males	Organ/System Tumor	Ctrl N = 100	LD N = 50	MD N = 50	HD N = 50	(b) (4) Ctrl	Lit Data
	Hemolymphoreticular Leukemia, granulocytic	0	4	2	0	0-2	0.56-2.85

[3] Control data for Sprague-Dawley rats from (b) (4) from carcinogenicity studies (1990-2008)

[4] Compilation of spontaneous neoplastic lesions and survival in Sprague-Dawley rats from control Groups, (b) (4)

	Teeth Odontoma	0	0	2	0	--	1.67
Females	Mammary gland Adenocarcinoma	16	30	22	18	18-31.6	8.57-58.33
	Adenocarcinoma arising in fibroadenoma	5	8	2	2	0-10	
	Fibroadenoma	38	40	40	40	32.7-52.6	13.33-62.31
	Fibroma	2	0	0	2		
	Adenoma	0	2	4	4	1.3-2	1.14-32

Statistical analysis by FDA reviewer Dr. Min is shown in Table 15. Fibroma in skin (Males only) showed a p-value ≤ 0.05 for a dose-response relationship (p = 0.029, as compared to combined controls) and at the high dose only for a pair-wise relationship (p = 0.086, as compared to combined controls). However, a positive dose-response relationship or increase in tumor incidence was not apparent.

Non Neoplastic: The organs with significant findings were the lungs, uterus, testes, and coagulating glands. Granulomata in the lungs, which consisted of multinucleated giant cell and macrophage aggregates, were observed in males, with a higher incidence and severity at the HD. There were a total of 3/49, 2/49, and 32/49 males affected with granulomata in the LD, MD, and HD, respectively, as compared to 10/100 in the controls (combined Groups 1 and 2). There was also a higher incidence of and severity of chronic bronchio-alveolar inflammation in HD males (7/49 HD males, as compared to 1/100 controls). Bronchiectasis was observed only in HD males (5/49 males) and was considered to be secondary to inflammation-induced mechanical bronchial obstruction, as shown in the applicant's table below. These findings may correlate with adverse clinical signs observed in HD males, such as loud breathing, ptyalism, and dyspnea. There were several changes observed in the genital tract of males, with slightly higher incidences in the HD group. Swollen spermatids in the testes (1/50, 2/50, and 4/50 at LD, MD, and HD, respectively, as compared to 0/100 in controls) and inflammation in the coagulating glands (1/50 at LD and MD and 4/50 at HD, as compared to 0/100 in controls) were observed, but with low severity. Inflammation in the coagulating glands was observed in a preliminary 28-day toxicity study at doses up to 100 mg/kg/day; however, these effects were reversed after a 9-week treatment-free period. In females, there was a higher incidence of endometrial squamous metaplasia at the HD of 50 mg/kg/day: 13/50 affected females, as compared to 3/50 and 6/100 affected females in the MD and Control groups. Despite the higher incidence at the HD, the severity of endometrial squamous metaplasia was generally minimal. No such findings were made in the 26-week repeat-dose toxicity study at doses up to 50 mg/kg/day. The applicant's table below summarizes the non-neoplastic findings in males and females.

Reference ID: 3489781

Table 24: List of major non-neoplastic findings in SD rats treated with eliglustat tartrate for 2 years via oral gavage (applicant's table)

Daily Dose (mg/kg)	0	0	0	0	10	5	25	15	75	50
Number of Principal Animals	**M: 50**	**F: 50**	**M: 50**	**F: 50**	**M: 50**	**F: 50**	**M: 50**	**F: 50**	**M: 50**	**F: 50**
- number of benign neoplasms	55	90	83	60	67	86	91	46	52	74
- number of malignant neoplasms	14	25	17	13	14	16	19	11	16	18
Non-neoplastic findings	-	-	-	-	-	-	-	-	-	-
Lungs[a]	-	-	-	-	-	-	-	-	-	-
- Granulomata	-	-	-	-	-	-	-	-	-	-
- Grade 1	3	-	4	-	2	-	2	-	9	-
- Grade 2	-	-	2	-	-	-	-	-	9	-
- Grade 3	-	-	1	-	1	-	-	-	13	-
- Grade 4	-	-	-	-	-	-	-	-	1	-
- Number of affected rats	3	-	7	-	3	-	2	-	32	-
- Inflammation; bronchio-alveolar, chronic	-	-	-	-	-	-	-	-	-	-
- Grade 2	-	N/A	-	N/A	-	N/A	-	N/A	5	N/A
- Grade 3	-	N/A	1	N/A	-	N/A	-	N/A	2	N/A
- Number of affected rats	-	-	1	-	-	-	-	-	7	-
- Bronchiectasis		-	-	-	-	-	-	-	-	-
- Grade 1	-	N/A	-	N/A	-	N/A	-	N/A	3	N/A
- Grade 3	-	N/A	-	N/A	-	N/A	-	N/A	1	N/A
- Grade 4	-	N/A	-	N/A	-	N/A	-	N/A	1	N/A
- Number of affected rats	-	-	-	-	-	-	-	-	5	-
Uterus	-	-	-	-	-	-	-	-	-	-
- Squamous metaplasia; endometrium	-	-	-	-	-	-	-	-	-	-
- Grade 1	N/A	2	N/A	3	N/A	-	N/A	3	N/A	12
- Grade 2	N/A	-	N/A	-	N/A	-	N/A	-	N/A	1
- Grade 3	N/A	-	N/A	1	N/A	-	N/A	-	N/A	-
- Number of affected rats	-	2	-	4	-	-	-	3	-	13
Testes	-	-	-	-	-	-	-	-	-	-
- Swollen spermatids	-	-	-	-	-	-	-	-	-	-
- Grade 1	-	N/A	-	N/A	-	N/A	1	N/A	2	N/A
- Grade 2	-	N/A	-	N/A	-	N/A	1	N/A	-	N/A
- Grade 3	-	N/A	-	N/A	1	N/A	-	N/A	1	N/A
- Grade 4	-	N/A	-	N/A	-	N/A	-	N/A	1	N/A
- Number of affected rats	-	-	-	-	1	-	2	-	4	-
Coagulating glands	-	-	-	-	-	-	-	-	-	-
- Inflammation	-	-	-	-	-	-	-	-	-	-
- Grade 1	-	N/A	-	N/A	-	N/A	-	N/A	1	N/A
- Grade 3	-	N/A	-	N/A	1	N/A	1	N/A	3	N/A
- Number of affected rats	-	-	-	-	1	-	1	-	4	-

Toxicokinetics (TK)

The first 2 animals/sex in Group 1 and the first 9 animals/sex in Groups 3, 4, and 5 in the satellite animals were sampled for TK measurements. Test article-treated animals (3/sex/group) were sampled at three timepoints: 0.25, 0.5, and 1 h post-dose. Control group animals were sampled once at 0.5 h post dose. Animals were sampled on Day 1 and during Week 13 of the study.

Reference ID: 3489781

Results: The toxicokinetic parameters are shown in the table below.

Table 25: Toxicokinetics of eliglustat tartrate in SD rats on Day 1 and Week 13 of treatment

		Males (N = 10, satellite animals)			Females (N = 10, satellite animals)		
Sampling Time	Dose (mg/kg/day)	10	25	75	5	15	50
Day 1	C_{max} (ng/mL)	50.9	136	872	26.7	118	782
	T_{max} (h)	0.5	0.5	0.25	0.5	0.25	0.5
	AUC_{0-t} (ng·h/mL)	31.8	100	532	18.5	84.6	516
	$C_{max/dose}$	5.1	5.4	11.6	5.3	7.8	15.6
	$AUC_{0-t/dose}$	3.2	4.0	7.1	3.7	5.6	10.3
Week 13	C_{max} (ng/mL)	187	620	1935	143	703	1296
	T_{max} (h)	0.5	0.5	0.25	0.25	0.25	0.5
	AUC_{0-t} (ng·h/mL)	129	486	1135	75.9	415	825
	$C_{max/dose}$	18.7	24.8	25.8	28.5	46.9	25.9
	$AUC_{0-t/dose}$	12.9	19.4	15.1	15.2	27.6	16.5

TK analysis demonstrated that study animals were systemically exposed to test article. Exposure to test article (C_{max} and AUC) increased in a greater than dose-proportional manner in both sexes at both sampling times. Accumulation of drug (2-5x) was observed during Week 13, as compared to exposure on Day 1.

Dosing Solution Analysis

Samples (each control and each test article dose) were checked for concentration in Week 1 and then once every 3 months. The measured concentration was the nominal concentration ± 10%.

Results: Dosing solution analysis revealed that the test article concentrations were within the acceptable range of -6.1% to +6.1 %, which fell within the acceptance criteria (± 10%).

9-10 Reproductive and Developmental Toxicology, Special Toxicology Studies

A review of the reproductive and developmental toxicology studies and special toxicology studies (if any) can be found in the nonclinical review of Dr. Tamal Chakraborti. Please refer to his review for detailed information.

Reference ID: 3489781

11 Integrated Summary and Safety Evaluation

Gaucher Disease is a rare lysosomal storage disorder resulting from a deficiency of the enzyme glucocerebrocidase (also known as acid β-glucosidase), which is the rate-limiting enzyme in the hydrolysis of glucosylceramide (GL-1) to glucose and ceramide. Deficiency in acid β-glucosidase causes accumulation of GL-1, particularly in lysosomes and macrophages. Lipid engorged macrophages infiltrate the organs of the reticuloendothelial system (spleen, liver, bone marrow, and to some extent in the lungs), producing organomegaly. Therefore, Gaucher Disease is a chronologically debilitating, multisystem disease that is characterized by hepatosplenomegaly and hypersplenism, which produce hematological abnormalities (anemia, thrombocytopenia) and bone disease (bone marrow infection, low bone mineral density), and to a lesser extent lung disease (interstitial lung disease, pulmonary hypertension). Disease presentation can occur at any age and clinical severity can range from asymptomatic to severely debilitating. Current treatment options for Gaucher patients are enzyme replacement therapy (ERT) and substrate reduction therapy (SRT). Approved ERT products (such as Ceredase®, Cerezyme®, VPRIV™, and Elelyso™) are recombinant human enzymes which have been shown to effectively decrease GL-1 accumulation and improve symptoms of Gaucher patients; however, these products require life-long intravenous (IV) administration. The only approved SRT for Gaucher disease at this time is Zavesca™, which is taken orally; however this product is only approved in adults for whom ERT is not a viable option. In the current submission, the applicant is seeking approval of an oral formulation of Eliglustat tartrate as a SRT, with a proposed dosage is 84 mg to be taken twice a day. Eliglustat tartrate was shown to be a potent and specific inhibitor of GL-1 synthesis in vitro and in vivo (normal and Gaucher disease type 1 mice). The target population for eliglustat tartrate is CYP2D6 Intermediate Metabolizers and Extensive Metabolizers (b) (4)

To support marketing approval of this product, the applicant has submitted an extensive nonclinical safety package, including pharmacology, pharmacokinetics, repeat-dose toxicology, reproductive and developmental toxicology, juvenile animal toxicology, immunotoxicology, and genetic toxicology studies. For a complete review of the nonclinical safety studies, please refer to the review of Dr. Tamal Chakraborti.

Eliglustat was nongenotoxic in vitro (Ames test, chromosomal aberration test in human peripheral blood lymphocytes) and in vivo (mouse micronucleus assay). The 2-year oral carcinogenicity studies in the mouse and rat were reviewed here in detail.

<u>Mouse study:</u>
In CD-1 mice (60/sex/dose), eliglustat tartrate was administered at 10, 25, and 75 mg/kg/day, by dietary admixture for 105/106 weeks and was not carcinogenic in either sex at all doses tested, when compared to controls (2 groups of 60/sex each fed the powdered, maintenance diet). There was no treatment-related effect on tumor latency in either sex. Poly-3 trend tests revealed an increase in incidence of 3 rare tumor types at the high dose (HD) in males and females: benign cortical adenoma (2/60 HD males, as compared to 0/120 controls), benign pheochromocytoma (2/60 HD females, as compared to 0/120 controls), and malignant skin fibrosarcoma (2/58 HD females, as

Reference ID: 3489781

compared to 0/120 controls). However, the incidence of these tumors fell within the range of those observed in control animals (historical data from the testing laboratory and/or published data from the literature), and therefore these were considered to be unrelated to treatment by the applicant. Additional analyses conducted by FDA statistical reviewer were in agreement with the applicant's conclusions. Statistical analysis of specific tumor combinations revealed that the following tumor types had p values ≤ 0.05 for dose relationships: Adrenal gland (Adenoma/Cortex), Liver (Hemangiosarcoma), Lungs (Adenocarcinoma+Adenoma) in Males and Adrenal Gland (pheochromocytoma) and Skin (fibrosarcoma) in Females). When pair-wise relationships were evaluated, the following had p values ≤ 0.05: Lungs (Adenocarcinoma and Adenocarcinoma + Adenoma in mid dose (MD) males) and Uterus (Endometrial Sarcoma in low dose (LD) females). However, none of the incidences of tumors in either sex was considered to have a positive dose response relationship or an increase in tumor incidence from pair-wise comparisons.

There was a statistically significant, treatment-related increase in the incidence and severity (Grade 3 or 4, particularly at higher doses) of hepatocellular hypertrophy in males (MD and HD) and females (all doses), as compared to controls. The hypertrophy was not associated with necrosis or hepatocellular degeneration and maybe related to CYP450 induction in mice.

Rat study:
In SD rats (50 sex/dose), eliglustat tartrate was administered by oral gavage at 10, 25, and 75 mg/kg/day in males for 105 weeks and at 5, 15, and 50 mg/kg/day in females for 103 weeks and was shown to be non-carcinogenic at the doses tested, when compared to vehicle controls (two groups of 50 rats/sex which received drinking water). A dose-dependent effect on tumor latency was not apparent. However, malignant tumors appeared earlier in test article-treated males (as early as 18 weeks after treatment initiation at the LD) and females at the high dose had mammary gland adenocarcinomas starting at 38 weeks, as compared to 76 weeks in control females. Poly-3 trend tests revealed an increase in incidence of several tumors: granulocytic leukemia in 2/50 LD males, odontoma in 1/50 MD males, and mammary gland adenomas in females (1/50, 2/50, and 2/50 at LD, MD, and HD, respectively), as compared to 0/100 controls/sex. However, the incidence of tumors in males and females fell within the range of those observed in control animals from historical data in the testing laboratory and published literature, and therefore, the findings were considered to be unrelated to treatment with test article in either sex. Additional analyses conducted by FDA statistical reviewer were in agreement with the applicant's conclusions. Statistical analysis of tumor combinations revealed a p ≤ 0.05 for dose response relationship for skin fibroma in males only. However, the incidence of this tumor did not have a positive dose response relationship or reveal an increase in tumor incidence in the pair-wise comparisons (combined control vs. individual treatment group).

There was an increased incidence and severity of the following non-neoplastic findings in the lungs of HD males, as compared to combined controls: granulomata (32/49, as

Reference ID: 3489781

compared to 10/100 controls), chronic bronchio-aveolar inflammation (7/49, as compared to 1/100 controls), and bronchiectasis (5/49, as compared to 1/100 controls). These findings may correlate with adverse clinical signs observed in HD males, such as loud breathing, ptyalism, and dyspnea. There were several changes observed in the genital tract of males, with slightly higher incidences in the HD group. Swollen spermatids in the testes (1/50, 2/50, and 4/50 at LD, MD, and HD, respectively, as compared to 0/100 in controls) and inflammation in the coagulating glands (1/50 at LD and MD and 4/50 at HD, as compared to 0/100 in controls) were observed, but with low severity. In females, there was a higher incidence of squamous metaplasia in the endometrium (3/50 at MD and 13/50 at HD, as compared to 6/100 controls).

Overall, administration of eliglustat tartrate to CD-1 mice (via dietary admixture) and SD rats (via oral gavage) for 2 years failed to produce any treatment-related neoplasms at statistically significant increased incidences by CDER criteria. Eliglustat tartrate was not carcinogenic in either mice or rats in 2-year oral studies at doses up to 75 mg/kg/day (mice) and 75 mg/kg/day and 50 mg/kg/day in male and female rats, respectively.

Reference ID: 3489781

Executive CAC
Date of Meeting: April 8, 2014

Committee: Abigail Jacobs, Ph.D., OND-IO, Acting Chair
Paul Brown, Ph.D., OND IO, Member
Tim McGovern, Ph.D., OND IO, Alternate Member
Sushanta Chakder, Ph.D. , Supervisor
Sruthi Tallapragada King, Ph.D., Presenting Reviewer

Author of Draft: Sruthi Tallapragada King, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA # 205494
Drug Name: Eliglustat
Sponsor: Genzyme Corporation, Cambridge MA

Background:

Gaucher Disease is a rare lysosomal storage disorder resulting from a deficiency of the enzyme glucocerebrocidase, which is the rate-limiting enzyme in the hydrolysis of glucosylceramide (GL-1) to glucose and ceramide. The Applicant is seeking marketing approval of eliglustat tartrate as a substrate reduction therapy (SRT) for adult patients with type 1 Gaucher disease who are CYP2D6 Intermediate and Extensive Metabolizers. Eliglustat tartrate was shown to be a potent inhibitor of GL-1 synthesis in vitro and in vivo. The goal of treatment with this SRT is to reduce GL-1 accumulation in multiple organs, thereby alleviating the clinical manifestations of Gaucher disease. The applicant conducted 2-year carcinogenicity studies in CD-1 mice and Sprague-Dawley rats with eliglustat tartrate to assess its carcinogenic potential.

Mouse Carcinogenicity Study

In the 2-year carcinogenicity study in CD-1 mice (60/sex/dose), eliglustat tartrate was administered to mice (60/sex/dose) at 10, 25, and 75 mg/kg/day, by dietary admixture for 105/106 weeks. Control animals (2 groups of 60/sex each) were fed the powdered, maintenance diet. The high dose used in the mouse carcinogenicity study was based on the MTD derived from a 13-week dietary toxicity study in mice, and the protocol was based on previous concurrence with the CDER Exec CAC. No neoplasms occurred at statistically significant increased incidences by CDER criteria.

Reference ID: 3489781

Rat Carcinogenicity Study

In the 2-year rat carcinogenicity study, eliglustat tartrate was administered by oral gavage to SD rats (50/sex/dose) at 10, 25, and 75 mg/kg/day in males for 105 weeks and at 5, 15, and 50 mg/kg/day in females for 103 weeks. Two groups of 50 rats/sex were assigned as Controls and received the vehicle (drinking water, treated by reverse osmosis) via oral gavage. The dose levels for the the 2-year rat carcinogenicity study were based on the MTD in males and AUC in females, and the protocol was based on previous concurrence with the CDER Exec CAC. No neoplasms occurred at statistically significant increased incidences by CDER criteria.

Executive CAC Recommendations and Conclusions:

Mouse:

- The Committee concurred that the study was acceptable, noting prior Exec CAC concurrence with the protocol.
- The Committee concurred that there were no drug-related neoplastic findings in male and female mice at any of the doses tested.

Rat:

- The Committee concurred that the study was acceptable, noting prior Exec CAC concurrence with the protocol.
- The Committee concurred that there were no drug-related neoplasms in in male and female rats at any of the doses tested.

Abigail Jacobs, Ph.D.
Acting Chair, Executive CAC

cc:\

Division of Gastroenterology and Inborn Error Products/Division File, [DGIEP]
Sushanta Chakder, Ph.D./Supervisory Pharmacologist, [DGIEP]
Sruthi Tallapragada King, Ph.D./Reviewer, [DGIEP]
Jessica Benjamin, MPH/RPM, [DGIEP]
/ASeifried, OND-IO

12 Appendix/Attachments

Appendix 1A: Minutes from the CDER ECAC meeting: Mouse carcinogenicity study: special protocol assessment

Appendix 1B: Minutes from the ECAC meeting: Rat carcinogenicity study: special protocol assessment

Appendix 2: Non-neoplastic lesions in male and female CD-1 mice treated with eliglustat tartrate for 2 years

Appendix 3: Neoplastic lesions in male and female CD-1 mice treated with eliglustat tartrate for 2 years

Appendix 4: Non-neoplastic lesions in male and female SD rats treated with eliglustat tartrate for 2 years

Appendix 5: Neoplastic lesions in male and female SD rats treated with eliglustat tartrate for 2 years

Reference ID: 3489781

Appendix 1A: Minutes from the CDER ECAC meeting: Mouse carcinogenicity study: special protocol assessment

APPENDIX-1

Food and Drug Administration
Center for Drug Evaluation and Research
Office of New Drugs

FACSIMILE TRANSMITTAL SHEET

DATE: May 27, 2009

To: Lauren Sykes	**From:** Adele Seifried
Company: Genzyme Corporation	OND IO
Fax number: (617) 768-6417	**Fax number:** 301-796-9855
Phone number: (617) 768-6917	**Phone number:** 301-796-0535

Subject: Response to Carcinogenicity Special Protocol Assessment Request - Final CAC Report – IND 67,589

Total no. of pages including cover: 4

Comments:

Document to be mailed: ☐ YES ☑ NO

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Executive CAC
Date of Meeting: May 27, 2009

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Karen Davis Bruno, Ph.D., DMEP, Alternate Member
Sushanta K. Chakder, Ph.D., DGP, Supervisory Pharmacologist
Tamal Chakraborti, Ph.D., DGP, Presenting Reviewer

Author of Draft: Tamal Chakraborti, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

The committee did not address the sponsor's proposed statistical evaluation for the carcinogen bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following the CDER/CBER Guidance for Industry, Providing Regulatory Submission in Electronic Format- Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (June 2008) and the associated Study Data Specifications document.

IND #: 67,589
Drug Name: Genz-112638
Sponsor: Genzyme Corporation, Cambridge, MA

Background: Genz-112638 is ceramide analog inhibitor of glucosylceramide synthase indicated for the treatment of Type 1 Gaucher disease. The drug is currently under Phase 2 clinical trial.

Mouse Dose Selection

The sponsor has proposed to conduct a 2-year oral (dietary admixture) carcinogenicity study in CD-1 mice at 0, 0, 30, 90 and 150 mg/kg/day. The sponsor's dose selection was based on the maximum tolerated dose (MTD).

In a 13-week oral (diet) toxicology study (b) (4) Study No. 459308; GT-157-TX-37) in CD-1 mice, animals received Genz-112638 at 50, 150 and 350 mg/kg/day. There was no treatment-related mortality or clinical signs. The mean body weight gains (Day 0-91) in males were 89%, 79.8% and 21.7% of control at 50, 150 and 350 mg/kg/day, respectively. The mean body weight gains (Day 0-91) in females were 80%, 74% and 56% of control at 50, 150 and 350 mg/kg/day, respectively. Food consumption was decreased at the high dose in males and at the low and high dose in females. There were no significant treatment-related effects on hematology, serum chemistry, gross pathology, or histopathology. At 350 mg/kg/day, liver and adrenal gland weights were increased in males. At 150 mg/kg/day, liver weight was increased in males.

IND 67,589 SDN 70 and SDN 74

Executive CAC Recommendations and Conclusions: The committee recommended doses of 0, 0, 10, 25, and 75 mg/kg/day in both sexes based on the MTD (reduction in body weight gain).

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\
/Division File, DGP
/SChakder, DGP
/TChakraborti, DGP
/RPM/CStark/DGP
/ASeifried, OND IO

Appendix 1B : Minutes from the ECAC meeting: Rat carcinogenicity study: special protocol assessment

Food and Drug Administration
Center for Drug Evaluation and Research
Office of New Drugs

FACSIMILE TRANSMITTAL SHEET

DATE: 12/9/08

To: Lauren Sykes **Senior Regulatory Affairs Associate**	**From: Sam Habet, RP.h., Ph.D.** **Senior Clinical Pharmacologist and Science Policy Analyst (Detail)** **OND**
Company: Genzyme Corporation	OND IO E-mail: sayed.alhabet@fda.hhs.gov
Fax number: 617-768-6417	**Fax number:** 301-796-9855
Phone number: 617-768-6917	**Phone number:** 301-796-1496

Subject: Response to Carcinogenicity Special Protocol Assessment Request – Exec- CAC Minutes - IND **67,589 (date of submission October 10 (# 60) and November 7, 2008 (#62))**

Total no. of pages including cover: 4 + 1 (signature page) = 5 pages

Comments:

Document to be mailed: ❑ **YES** ☑ **NO**

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26

Executive CAC

Date of Meeting: **December 9, 2008**

Committee: David Jacobson-Kram, Ph.D., D.A.B.T., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Sushanta K. Chakder, Ph.D., DGP, Supervisory Pharmacologist and Alternate Member
Tamal Chakraborti, Ph.D., DGP, Presenting Reviewer

Coordinator: Sam Habet, R.Ph., Ph.D., OND IO, Senior Clinical Pharmacologist/ Science Policy Analyst (Detail)

Author of Draft: Tamal Chakraborti, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

IND #:	67,589
Drug Name:	Genz-112638
Date of Submission:	October 10, 2008, Serial # 060 and November 7, 2008, serial number 062
Sponsor:	Genzyme Corporation, Cambridge, MA

Background: Genz-112638 is ceramide analog inhibitor of glucosylceramide synthase indicated for the treatment of Type 1 Gaucher disease. The drug is currently under Phase 2 clinical trial. The clinical treatment schedule for Genz-112638 may be once or twice daily administration. In the on-going Phase 2 clinical trial (GZGD00304) for Type 1 Gaucher disease, patients have received doses ranging from 50 to 100 mg BID.

Rat Carcinogenicity Study Protocol

The sponsor has proposed to conduct a 2-year oral (gavage) carcinogenicity study in SD rats at 0, 0, 5, 15 and 50 mg/kg/day by gavage in water (once a day dosing). The sponsor's dose selection was based on its assessment of a maximum tolerated dose (MTD).

In a 28-day oral (gavage) toxicology study (Study No. 800159, Report No. GT-157-TX-5) in SD rats, animals were administered Genz-112638 at 10, 30 and 100 mg/kg/day (5, 15 and 50 mg/kg BID). In this study, at 100 mg/kg/day, clinical signs included gastrointestinal (GI) irritation, and excessive salivation, along with lower body weight gain at 30 and 100 mg/kg/day in females only. Reversible changes in several hematological and serum chemistry parameters were observed at 100 mg/kg/day. There were no significant treatment-related macroscopic or microscopic changes.

In a 28-day oral (gavage) toxicology study (Study No. 32267 TSR, Report No. GT-157-TX-28) in male rats with a 9-week recovery period, animals were administered Genz-112638 at 30 and 100 mg/kg/day (15 or 50 mg/kg BID) for 4 weeks or at 200 mg/kg/day (100 mg/kg BID) for 2 weeks. The objective of this study was to evaluate specific effects of Genz-112638 on male reproductive organs and spermatogenesis. Mortality was observed at 100 mg/kg BID or 200 mg/kg/day, and the dosing was terminated after 2 weeks of treatment due to severe toxicity (marked body weight losses, reduced food consumption and clinical signs). Electrolytes and liver enzymes as well as sperm motility and morphology were affected at 200 mg/kg/day. Gross pathology examinations revealed grey foci on the forestomach mucosa, small thymus and spleen. Microscopic examinations revealed germ cell necrosis in the testis, sloughed cells in the epididymis, acute inflammation in the coagulating glands, epithelial hyperplasia in the forestomach and severe atrophy of thymus and spleen. At 100 mg/kg/day, a lower mean body weight gain, lower food consumption and signs of ptyalism were observed. In addition, WBC, RBC counts and hemoglobin levels were affected as were electrolytes and liver enzymes. There were no significant treatment-related effects on sperm counts, motility or morphology. Microscopic examinations revealed bilateral atrophy of the coagulating glands. At 30 mg/kg/day, there were no effects on body weight gain or food consumption and no clinical signs.

In a 26-week oral (gavage) toxicology study in SD rats, animals were treated with Genz-112638 at 5, 15 and 50 mg/kg/day. A total of three animals died (one male and one female at 50 mg/kg/day and one male at 15 mg/kg/day) and deaths of males were attributed to gavage accident. The cause of death of one female was not determined. However, the sponsor stated that the death of this female did not appear to be treatment-related. There were no significant treatment-related effects on body weights or food consumption, hematology, serum chemistry, urinalysis, gross pathology, organ weights or histopathology. The target organ could not be identified in the absence of any significant histopathology findings in any organ or tissue. The MTD appeared to be higher than 50 mg/kg/day in the absence of any significant treatment-related toxicity or histopathology findings.

Executive CAC Recommendations and Conclusions:

1. The Committee concurred with the proposed doses of 0, 0, 5, 15 and 50 mg/kg/day for female rats based on AUC comparisons (the exposure at the high dose being about 30-fold higher than human AUC of 128 ng.hr/mL at 100 mg BID).

2. Based on the observed mortality at 200 mg/kg/day in the 28-day study (Study No. 32267 TSR), the Committee recommended the high, mid and low doses at 75, 25 and 10 mg/kg/day, respectively, for male rats.

IND 67,589 SDN 60 and SDN 62

David Jacobson-Kram, Ph.D. D.A.B.T.
Chair, Executive CAC

cc:\
/Division File, DGP
/SChakder, DGP
/TChakraborti, DGP
/RPM/CStark/DGP
/DJacobson-Kram, OND IO
/AJacobs, OND IO
/PBrown, OND IO
/SHabet, OND IO

Appendix 2: Non-neoplastic lesions in male and female mice (Applicant's table from Study# GT-157-TX-43)

Table: 2

NUMBER OF ANIMALS WITH NON-NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. DEATHS

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
ABDOMINAL CAVITY :	-	-	1	-	-
- Fibrin :	-	-	1	-	-
ADIPOSE TISSUE :	3	3	-	-	1
- Hyperplasia; br.fat :	3	2	-	-	-
- Inflammation :	-	1	-	-	1
ADRENAL GLANDS :	60	60	60	58	60
- Hyperpl.; subcapsul.:	37	25	29	30	36
- Hyperpl.; cortical :	2	3	4	1	8
- Hyperpl.foc.medulla :	-	1	4	-	1
- Hypertr.cortic.cell :	25	20	17	15	22
- Hypertr.foc.medulla :	-	-	-	2	-
- Atrophy; cortex :	1	4	1	-	1
- Pigment; corticomed.:	2	2	5	4	5
- Angiectasis :	2	1	-	-	-
- Congestion :	1	1	1	2	-
- Amyloid :	4	10	4	3	5
- Hemopoiesis :	3	-	1	2	4
- Inflammation :	4	2	7	6	2
- Access.cortic.tissue:	2	1	6	6	4
- Vacuolation; cortex :	1	-	1	-	-
- Mineralization :	-	-	-	1	-
- Metaplasia; osseous :	1	-	-	-	-
AORTA :	60	60	60	60	59
- Inflamm.; adj. tis. :	10	10	8	19	6
- Inflammation; wall :	-	1	1	1	-
- Hemorrhage :	-	2	1	-	-
- Mineralization :	1	-	1	-	-
- Dilation :	-	-	1	-	-
AXILLARY LYMPH NODES :	5	5	11	3	6
- Inc. plasma cell nbr:	3	4	9	3	3
- Inc. follicle devlpt:	-	1	3	2	-
- Sin. red blood cells:	-	-	2	-	-
- Hemangiectasis :	-	-	-	1	-
- Hemopoiesis :	-	-	-	-	2
BILE DUCT EXTRAHEP. :	-	-	-	1	-
- Inflammation :	-	-	-	1	-
- Dilated Lumen :	-	-	-	1	-

Reference ID: 3489781

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
BONE :	-	1	-	1	1
- Inflammation :	-	-	-	1	1
BONE MARROW :	60	60	60	59	60
- Hyperplasia; angiom.:	-	1	-	-	-
- Inc. myeloid cells :	32	26	34	30	33
- Inc. erythroid cells:	2	4	1	6	8
- Pigment :	-	-	-	1	-
BRAIN :	60	60	59	58	60
- Inflammat.;vascular :	-	1	-	-	-
- Inflamm.;ventricle :	-	-	-	-	1
- Mineralization :	6	3	4	8	8
CECUM :	59	60	60	60	60
- Hyperplasia;gland :	1	-	-	1	-
- Edema :	-	-	-	-	1
- Amyloid :	-	1	-	1	1
- Inflammation :	-	2	1	-	1
- Pigment :	1	2	2	-	6
- Diverticulum :	-	-	-	1	-
CERVICAL LYMPH NODES :	1	1	-	-	1
- Inc. plasma cell nbr:	-	1	-	-	-
COLON :	59	60	60	60	60
- Amyloid :	-	1	1	1	-
- Inflammation :	-	-	-	-	1
DUODENUM :	60	58	60	58	60
- Hyperplasia; avill. :	8	6	10	8	4
- Hyperplasia; crypt :	1	-	-	1	-
- Hyperplasia; Brun.g.:	3	-	1	1	1
- Atrophy;villous :	-	-	-	-	1
- Degen./necrosis :	1	1	-	-	-
- Amyloid :	8	9	8	7	12
- Dilation; gland :	9	6	8	11	4
- Inflammat.;adj.tiss.:	3	3	1	-	2
- Hemorrhage :	-	1	-	-	-
- Infiltr.; mono.cells:	-	-	-	-	2
- Infiltr.; eosino. :	1	-	-	-	-

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
EARS :	-	4	5	5	3
- Ulcer :	-	1	3	5	3
- Sero-cellular crust :	-	3	3	5	3
- Inflammation :	-	3	3	5	3
- Hemorrhage/congest. :	-	1	-	-	-
- Acanthosis :	-	-	1	-	-
- Hyperkeratosis :	-	-	2	1	-
- Chondropathy :	-	1	-	-	-
EPIDIDYMIDES :	60	60	60	59	59
- Hyperplasia;tubule :	1	-	-	-	1
- Reduced sperm cont. :	37	33	25	29	28
- Dilation; tub. lumen:	2	-	1	1	1
- Sloughing; germ cell:	3	6	3	2	5
- Inflammat;vascular :	-	1	-	-	-
- Infiltr.; mono.cells:	11	7	3	-	5
- Infiltr.; granuloc. :	2	-	-	1	2
- Inflammat.;adj.fat :	-	-	-	1	2
- Granuloma; spermatic:	1	-	2	1	-
- Stasis; sperm :	-	-	2	3	-
- Amyloid :	-	2	-	-	-
- Hemorrhage :	-	-	-	-	1
- Pigment :	1	-	-	-	-
- Fibrosis :	1	-	-	-	1
ESOPHAGUS :	60	58	60	59	59
- Amyloid :	1	1	2	-	-
- Inflammation :	-	2	1	1	1
- Hemorrhage :	-	1	-	-	-

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
EYES :	59	59	59	56	58
- Disorganiz.; retina :	19	22	14	15	21
- Atrophy; retina :	8	6	7	8	10
- Mineralizat.; cornea:	1	-	2	3	1
- Mineralization; iris:	4	6	3	9	11
- Hemorrhage; retro-b.:	-	-	-	-	1
- Inflammation; retro.:	1	-	-	-	1
- Inflammat;vascular :	-	1	-	-	-
- Inflammation; cornea:	3	4	3	1	3
- Inflammation; uvea :	1	1	1	-	-
- Fibrosis; cornea :	1	-	-	-	-
- Amyloid; cornea :	1	-	-	-	-
- Degeneration; lens :	8	5	3	2	5
- Distended ocular gl.:	-	-	-	-	1
- Panophthalmitis :	1	-	-	-	1
- Phtisis bulbi :	-	1	-	-	-
- Synechia :	1	-	-	-	-
- Hemorrhage :	-	1	-	-	-
- Corneal epith.split.:	-	-	1	-	-
FEMUR :	60	60	60	60	60
- Hyperplasia;synovium:	1	-	-	-	-
- Arthropathy :	14	15	20	15	12
- Fibro-osseous lesion:	2	1	2	3	2
- Inflam;adj.tissue :	1	1	1	1	-
- Inflam;synovium :	1	-	-	-	2
FORESTOMACH :	60	60	60	60	60
- Inflammat.;adj.tiss.:	10	16	13	15	19
- Inflammat;vascular :	-	2	-	-	-
- Inflammat.;submucosa:	2	1	2	2	1
- Erosion/ulcer :	1	-	1	2	3
- Hyperkeratosis :	14	15	12	10	10
- Acanthosis :	11	12	10	9	7
- Cyst :	-	-	-	-	3
- Amyloid :	-	1	-	1	-
- Diverticulum :	-	-	-	1	-

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
GALL BLADDER :	52	49	47	48	54
- Hyperplasia;epithel.:	5	3	1	-	6
- Hyperplasia;angioma.:	1	-	-	-	-
- Dilation :	9	15	13	6	9
- Inflammation :	5	5	7	4	7
- Hemorrhage :	2	1	2	2	-
- Amyloid :	3	3	6	3	2
- Pigment :	1	-	-	-	-
- Cyst :	1	1	2	1	-
- Deg/necrosis:epith. :	-	-	1	-	-
HARDERIAN GLANDS :	60	60	60	57	60
- Hyperpl.;acin.c.foc.:	1	2	3	8	3
- Incr.;porph.deposit.:	20	26	29	24	14
- Atrophy; acinus :	-	-	-	1	-
- Inflammation :	9	12	12	11	11
- Amyloid :	1	1	-	1	1
- Bacteria :	1	-	-	-	-
- Cyst :	-	-	-	1	-
HEART :	60	60	60	60	60
- Amyloid :	16	16	16	17	17
- Fibrosis :	5	4	4	4	1
- Thrombus; atrium :	7	6	5	11	10
- Inflamm.; myocardium:	10	7	-	2	6
- Inflamm.; epicardium:	3	3	2	1	3
- Inflamm.; pericard. :	-	1	-	-	1
- Deg./necr.; myocard.:	1	2	-	1	-
- Inflammat;vascular :	4	3	1	1	3
- Infiltr.; granuloc. :	-	-	2	1	1
- Inflamm.;adjac.fat :	5	2	3	1	1
- Pigment :	1	1	-	-	-
- Hemorrhage :	1	2	-	2	1
- Angiectasis :	-	1	-	-	-
- Dilation;atrium :	3	4	2	2	-
- Dilation; ventricles:	-	-	1	-	1
- Bacteria :	1	-	-	-	-
- Mineralization :	1	-	-	-	-

NUMBER OF ANIMALS WITH NON-NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. DEATHS

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
ILEUM :	60	60	60	60	60
- Amyloid :	11	16	14	11	20
- Inflammat.;adj.tiss.:	1	-	-	-	1
- Dilation; lumen :	1	-	-	-	1
ILIAC LYMPH NODES :	6	-	3	2	3
- Inc. plasma cell nbr:	2	-	1	1	1
- Inc. follicle devlpt:	-	-	1	-	-
- Inc.granulocyte nbr :	-	-	-	-	1
- Hemopoiesis :	-	-	1	1	-
- Amyloid :	-	-	1	-	-
INGUINAL LYMPH NODES :	6	-	1	1	2
- Inc. follicle devlpt:	1	-	-	-	-
- Inc. plasma cell nbr:	3	-	-	1	1
JEJUNUM :	60	60	60	60	60
- Hyperplasia; crypt :	-	-	-	-	2
- Inflammation :	1	-	1	-	1
- Amyloid :	5	5	2	4	7

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
KIDNEYS :	60	60	60	58	60
- Nephropathy :	45	49	50	45	51
- Amyloid; interst. :	6	8	3	7	12
- Amyloid; glomerulus :	3	10	7	6	7
- Cyst :	19	15	16	11	20
- Dilation; tub. :	3	3	1	5	6
- Dilation; Bowm.sp. :	7	6	4	4	3
- Mineralization :	21	24	23	17	14
- Dilation; pelvis :	5	2	3	8	6
- Inflamm.; pelvis :	-	2	3	2	2
- Inflamm.; capsule :	1	-	1	1	-
- Infiltr.; mono.cells:	7	8	9	13	5
- Deg./necr.; papilla :	3	9	5	3	6
- Pigment :	5	3	3	7	4
- Abscess :	5	2	2	1	1
- Hemopoiesis :	-	-	2	-	1
- Inflammat;vascular :	3	2	3	1	-
- Hemorrhage :	-	1	-	-	-
- Congestion :	-	-	1	-	-
- Metaplasia; osseous :	-	-	1	-	1
- Bacteria :	3	-	-	-	-
- Scar; cortex :	3	-	-	-	1
LARYNX :	60	60	60	59	60
- Cyst :	2	3	2	3	6
- Deg/regen; myofibre :	-	1	-	-	1
- Inflammat;vascular :	-	2	1	-	-
- Infiltr.; granuloc. :	-	-	1	1	-
- Infiltr.; mono.cells:	2	1	2	1	2
- Hemorrhage :	-	1	-	1	1
- Mineralization :	-	-	1	-	-

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
LIVER :	60	59	60	58	60
- Alt.c.focus;baso. :	2	5	3	3	2
- Alt.c.focus;eosin. :	1	-	1	2	-
- Alt.c.focus;clear c.:	1	1	-	-	-
- Alt.c.focus;vac.c. :	-	-	1	2	2
- Hyperpl/hypertr;K.c.:	1	-	1	2	-
- Hyperpl.;angiomatous:	-	1	-	-	-
- Hypertrophy;hepatoc.:	10	4	11	19	29
- Hyperplasia;bile d. :	-	-	-	1	-
- Pigment :	16	5	4	12	12
- Inflammatory c.foci :	37	31	29	32	26
- Inflammation;portal :	-	-	-	1	2
- Infiltrate;lymph.c. :	1	-	-	1	-
- Fibrosis :	-	-	-	-	1
- Amyloid :	9	10	12	5	14
- Hemopoiesis :	14	12	8	11	11
- Glycogen content :	7	9	11	14	10
- Single c.nec/apopt. :	4	4	3	7	7
- Vacuolation;hepatoc.:	9	5	8	6	8
- Angiectasia :	-	3	-	1	-
- Focal necrosis :	2	1	1	-	2
- Deg/necr;hepatocyte :	-	-	-	-	1
- Lobar torsion/necr. :	-	-	-	2	-
- Atrophy;hepatocyte :	1	2	1	-	1
- Degen/necr;centril. :	-	1	-	-	1
- Tension lipidosis :	1	-	3	2	2
- Congestion :	2	-	-	-	1
- Hemorrhage :	-	-	-	-	1
- Hematoma :	-	-	-	2	-
- Cyst :	-	1	-	-	1
- Mineralization :	-	-	-	-	1
- Increased mitoses :	1	-	-	1	-

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
LUNGS :	60	60	60	60	60
- Hyperpl.;bronch-alv.:	5	2	1	5	1
- Infiltrate; lymphoid:	12	9	9	4	9
- Foamy macr.;alveolus:	20	20	17	20	23
- Inflam.;interst;chro:	9	4	3	5	7
- Inflam.;pleura;chron:	3	4	3	3	2
- Inflam.;br-alv;chron:	1	-	3	5	-
- Inflam.;perivascular:	1	-	-	-	-
- Inflam.;granulocytes:	2	1	1	1	2
- Infiltr.; eosino. :	-	-	1	-	-
- Edema :	1	-	-	-	2
- Congestion :	4	7	5	2	2
- Hemorrhage :	2	5	4	1	4
- Metaplasia; osseous :	1	-	1	-	-
- Mineralization :	1	1	1	-	3
- Mineral.; vasc.wall :	1	-	1	-	-
- Pigment :	1	-	-	1	2
- Amyloid :	-	2	1	-	-
- Cyst/Dilat.bronch.gl:	14	13	14	8	10
- Deg./necr.; bronchus:	1	1	-	1	1
MANDIBUL. LYMPH NODE :	60	60	59	59	60
- Inc. plasma cell nbr:	17	17	23	19	22
- Inc. follicle devlpt:	2	4	6	5	6
- Amyloid :	5	5	3	2	8
- Sin. red blood cells:	1	5	1	5	4
- Cystic degeneration :	-	1	2	2	-
- Incr.tingle bod.mac.:	1	1	-	1	-
- Pigment :	33	29	29	31	38
- Hemopoiesis :	4	1	3	4	3
- Lymphoid atrophy :	3	-	-	1	2
- Infiltr.granulocytes:	-	-	-	-	1
MEDIAST.LYMPH NODES :	2	1	1	2	3
- Inc. plasma cell nbr:	-	-	-	1	-
- Inc. follicle devlpt:	-	-	-	1	1
- Incr.paracortex :	-	1	-	-	1

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
MESENT. LYMPH NODE :	60	60	60	57	59
- Hyperplasia; angiom.:	-	1	-	1	-
- Hemopoiesis :	6	6	8	6	6
- Paracortex developm.:	-	1	-	-	-
- Amyloid :	9	11	15	7	18
- Cystic degeneration :	4	9	5	5	8
- Inc. plasma cell nbr:	-	-	1	-	-
- Sin. red blood cells:	17	18	16	21	22
- Inflammat;vascular :	-	1	1	-	-
- Incr.tingle bod.mac.:	-	3	-	3	-
- Pigment :	20	8	10	17	20
- Incr.granuloc.numb. :	2	-	1	1	-
- Inc.macroph.numbers :	1	-	2	-	-
- Inflammation :	-	-	-	-	2
- Lymphoid atrophy :	8	2	3	2	4
OPTIC NERVES :	59	59	59	55	55
- Vacuolation :	-	-	-	1	-
- Degeneration; axon :	-	1	-	-	-
PANCREAS :	60	60	59	58	59
- Hyperpl.; islet cell:	10	12	12	4	6
- Hypertrophy; acin.c.:	2	2	-	-	-
- Inflammation :	12	7	7	14	9
- Atrophy; lobule :	5	4	2	2	4
- Degranul.; acin.cell:	2	-	1	-	-
- Amyloid :	3	5	4	3	6
- Inflammat;vascular :	-	1	-	-	-
- Edema; interstitium :	1	-	-	-	-
- Abscess :	1	-	-	-	-
- Hemopoiesis :	-	-	-	1	-
- Lobular necrosis :	-	-	-	-	1
- Dilation; duct :	1	-	-	-	1
- Cyst :	-	-	1	-	-
PARATHYROID GLANDS :	58	57	56	57	55
- Hyperplasia; diffuse:	-	-	-	1	-
- Amyloid :	8	12	10	6	12
- Inflammation :	1	-	1	2	2
- Cyst :	-	2	-	-	-

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
PROSTATE :	60	60	60	58	59
- Hyperpl.; acin.cell :	4	5	2	5	3
- Inflammation :	33	31	35	29	34
- Edema :	-	-	1	-	-
- Inflammat;vascular :	-	2	1	-	-
- Atrophy; acinar :	-	1	-	-	-
- Amyloid :	-	6	6	4	2
- Abscess :	1	-	-	2	-
- Hemorrhage :	1	-	-	1	-
RENAL LYMPH NODES :	5	1	2	3	4
- Inc. plasma cell nbr:	2	-	-	1	1
- Inc.germ.cent.devlpt:	-	-	-	-	1
- Amyloid :	1	-	-	-	-
- Hemopoiesis :	-	-	1	-	-
- Pigment :	-	-	-	1	-
SALIVARY GLANDS :	60	60	60	59	60
- Inflammation :	30	25	27	32	27
- Fibrosis :	-	-	1	-	-
- Amyloid :	18	23	14	16	19
- Atrophy; acinar cell:	1	1	-	1	-
- Mineralization :	-	-	-	1	-
SCIATIC NERVE :	60	60	60	60	60
- Degeneration; nerve :	21	24	25	18	18
- Inflammation :	3	1	-	1	-
SEMINAL VESICLES :	60	60	60	58	59
- Hyperplasia :	2	-	2	-	1
- Inflammation :	33	28	27	28	24
- Hemorrhage :	-	-	1	2	-
- Dilation; lumen :	39	40	39	44	31
- Atrophy :	1	-	1	-	-
- Amyloid :	1	3	1	1	1
- Bacteria :	1	-	-	-	-
- Inflamm.; vascular :	-	1	-	-	-

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
SKELETAL MUSCLE :	60	60	60	60	60
- Inflammat;vascular :	-	1	-	-	-
- Inflammation;acute :	-	-	-	1	-
- Inflammation;subac. :	1	2	-	2	2
- Degen/necr;myofiber :	1	1	-	-	-
- Mineralization :	1	-	-	-	-
- Hemorrhage/congest. :	-	1	2	1	1
SKIN :	60	57	60	59	59
- Hyperplasia; angiom.:	-	1	-	-	-
- Hyperplasia; seb.gl.:	-	1	-	-	-
- Ulcer :	15	12	14	10	17
- Serocellular crust :	10	8	9	9	11
- Inflammation :	9	4	8	8	9
- Decr.nb.hair follic.:	1	1	-	1	-
- Cyst :	1	-	1	1	1
- Abscess :	-	-	-	1	-
- Acanthosis :	4	-	1	-	2
- Edema; subcutis :	-	1	1	-	-
SPINAL CORD :	60	60	60	60	59
- Developm.anomaly :	-	-	-	-	1
SPLEEN :	60	60	60	58	60
- Increased margin.zo.:	2	-	-	-	-
- Hemopoiesis :	44	43	47	43	41
- Amyloid :	9	13	12	7	12
- Lymphoid atrophy :	1	-	-	1	2
- Inc. follicle devlpt:	-	-	1	-	-
- Inc. PALS :	-	-	-	2	-
- Inc. tgb.bod. macro.:	-	2	1	1	-
- Pigment :	12	5	8	8	11
- Congestion :	-	1	1	2	1
- Inflammation :	3	1	6	-	3
- Inc. macrophage nbr :	-	-	1	-	-
- Hemorrhage/hematoma :	-	-	-	1	-
- Necrosis :	1	-	-	1	-
STERNUM :	1	-	-	-	-
- Fracture :	1	-	-	-	-

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
STOMACH :	60	60	60	60	60
- Hyperplasia;gland :	4	3	2	5	2
- Atrophy;gland :	-	-	-	-	1
- Amyloid :	10	15	8	6	14
- Inflammat.;adj.tiss.:	12	8	23	10	7
- Dilation; gland :	13	14	11	15	12
- Edema :	1	-	-	-	-
- Mineralization :	1	-	1	-	1
- Degen./necrosis :	1	1	1	1	1
- Infiltr.; mono.cells:	7	3	4	3	3
- Inflamm.;vascular :	-	1	1	1	-
- Bacteria :	1	-	-	-	-
- Hemorrhage :	-	-	-	1	-
- Pigment :	-	1	-	-	-
SUBCUTANEOUS TISSUE :	1	4	3	4	-
- Hyperplasia; br.fat :	-	-	-	1	-
- Abscess :	1	-	-	-	-
- Edema :	-	2	2	2	-
- Inflammation :	-	-	1	1	-
TEETH :	-	1	1	2	1
- Inflammation :	-	-	1	1	1
- Abnormal growth :	-	1	-	1	1

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
TESTES :	60	60	60	59	59
- Hyper.;Leyd.c.; foc.:	1	2	1	1	3
- Hyper.;Leyd.c.; dif.:	1	-	-	-	-
- Hyperpl.Sertoli cell:	1	-	-	-	-
- Hyperpl.; rete test.:	1	-	-	-	-
- Hypospermatogenesis :	40	47	32	39	42
- Deg./necr.; germ ce.:	1	-	1	1	1
- Vacuolation; tubule :	-	-	-	-	1
- Dilation; tub. lumen:	-	-	-	2	-
- Sloughing; germ cell:	-	-	-	1	1
- Infiltr.; granuloc. :	-	-	-	-	1
- Thrombus :	-	-	-	1	-
- Hemorrhage :	-	-	-	1	-
- Hypoplasia :	-	-	2	-	1
- Pigment :	-	-	-	-	2
- Amyloid :	-	1	-	-	1
- Sperm stasis :	8	4	5	3	2
- Cyst :	1	-	-	1	-
THYMUS :	56	55	54	53	56
- Hyperplasia;lymphoid:	9	1	3	4	3
- Lymphoid atrophy :	44	49	44	43	48
- Inflammation :	1	1	-	1	1
- Inc. tgb.bod. macro.:	1	-	1	-	1
- Amyloid :	2	3	1	1	3
- Hemorrhage; adj.tis.:	-	1	-	-	-
- Hemorrh./Hematoma :	1	1	2	-	1
- Hypertr;vasc.media :	1	-	-	-	-
THYROID GLANDS :	60	60	60	58	59
- Hyperpl.foc.follic. :	-	1	1	-	-
- Amyloid :	8	11	8	6	11
- Dilated/cystic foll.:	20	19	17	22	13
- Inflammation :	3	3	-	1	1
- Ectopic thymus :	-	-	1	-	-
TONGUE :	60	60	60	60	60
- Inflammat;vascular :	1	4	2	-	-
- Inflammation;acute :	1	-	-	3	-
- Inflammation;subac. :	2	4	1	-	1
- Amyloid :	4	2	2	1	2

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
TRACHEA :	60	60	60	59	58
- Inflammation :	1	-	1	-	-
- Cyst :	-	-	1	1	2
URETERS :	3	1	-	1	1
- Dilation; lumen :	3	1	-	1	1
- Inflammation; adj.t.:	1	-	-	-	-
URINARY BLADDER :	59	59	60	59	59
- Hyperpl.; uroth. :	5	1	-	2	1
- Inflamm.; mucosa :	19	21	27	19	24
- Inflamm.; muscle :	1	1	2	1	1
- Inflamm.; serosa :	2	3	-	4	1
- Dilation; lumen :	9	13	10	13	9
- Hemorrhage :	1	1	2	2	-
- Necrosis :	-	-	1	2	-
- Fibrosis; submuc. :	1	-	-	-	-
- Amyloid :	-	-	1	-	-
- Bacteria :	1	-	-	-	-
- Vacuol.; uroth. :	1	1	1	1	1

NUMBER OF ANIMALS WITH NON-NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. DEATHS

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
ABDOMINAL CAVITY :	-	-	-	-	1
- Fibrin :	-	-	-	-	1
ADIPOSE TISSUE :	-	2	2	-	4
- Fat tissue :	-	-	1	-	-
- Edema :	-	1	-	-	-
ADRENAL GLANDS :	60	60	60	60	60
- Hyperpl.; subcapsul.:	56	58	60	57	57
- Hyperpl.foc.medulla :	-	2	-	-	1
- Hypertr.cortic.cell :	6	6	4	1	2
- Hypertr.foc.medulla :	1	-	-	-	-
- Pigment; corticomed.:	14	21	10	12	9
- Angiectasis :	2	-	-	-	1
- Congestion :	1	1	1	1	1
- Amyloid :	3	4	5	3	2
- Hemopoiesis :	5	7	6	8	7
- Inflammation :	3	4	3	3	6
- Access.cortic.tissue:	5	5	9	2	8
- Vacuolation; cortex :	-	-	1	-	-
- Mineralization :	2	-	-	-	1
- Cyst :	-	2	-	-	-
AORTA :	60	60	60	59	60
- Inflamm.; adj. tis. :	11	13	9	15	6
- Inflammation; wall :	-	-	-	1	2
- Hemorrhage :	-	-	-	1	-
- Mineralization :	1	-	-	1	-
AXILLARY LYMPH NODES :	6	5	4	10	6
- Inc. plasma cell nbr:	4	2	1	6	3
- Inc. follicle devlpt:	3	1	-	-	2
- Sin. red blood cells:	-	-	-	-	1
- Hemangiectasis :	-	1	-	-	-
- Hemopoiesis :	-	-	-	1	1
- Pigment :	-	-	-	1	1
- Paracortex developm.:	1	-	-	-	-
- Incr.tingle bod.mac.:	-	-	-	1	-
- Amyloid :	-	1	-	-	-

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
BONE :	1	2	-	1	-
- METASTATIC SARCOMA :	1	-	-	-	-
- Inflammation :	-	1	-	1	-
- Fracture :	-	1	-	-	-
BONE MARROW :	59	60	59	60	59
- Inc. myeloid cells :	23	19	21	20	15
- Inc. erythroid cells:	7	2	2	3	2
- Pigment :	2	1	1	2	-
- Decreased cellulari.:	5	14	9	8	14
- Atrophy; serous :	1	-	-	-	1
- Necrosis :	-	-	-	-	1
BRAIN :	60	60	60	60	60
- Inflammat.;vascular :	-	1	-	-	-
- Inflammat.;meninge :	-	1	-	-	-
- Inflamm.;ventricle :	1	-	-	-	-
- Hemorrhage :	1	1	-	1	1
- Mineralization :	1	1	2	1	2
- Pigment :	1	-	-	-	-
- Compression :	1	-	-	-	-
CECUM :	60	60	60	60	60
- Edema :	-	3	-	1	-
- Amyloid :	-	-	1	1	-
- Inflammation :	-	-	3	1	-
- Pigment :	-	2	2	1	2
CERVICAL LYMPH NODES :	-	-	1	1	2
- Inc. plasma cell nbr:	-	-	-	1	-
CLITORAL GLANDS :	60	59	60	59	57
- Hyperp.; acinar cell:	-	-	-	1	-
- Inflammation :	8	9	11	10	12
- Dilation; gland :	6	8	6	7	5
- Cystic distension :	-	-	-	2	-
- Abscess :	1	-	-	-	1
COLON :	60	60	59	59	60
- Inflammation :	-	1	1	-	3
- Pigment :	-	1	-	-	-
- Dilation; lumen :	1	-	-	-	1

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
DUODENUM :	59	60	59	58	60
- Hyperplasia; avill. :	6	10	9	8	6
- Hyperplasia; Brun.g.:	1	-	1	1	2
- Degen./necrosis :	-	-	2	-	-
- Amyloid :	8	8	6	9	11
- Dilation; gland :	5	6	3	6	3
- Inflammat.;adj.tiss.:	2	2	3	2	2
- Infiltr.; mono.cells:	-	-	1	1	2
EARS :	5	1	1	2	-
- Ulcer :	5	1	-	1	-
- Sero-cellular crust :	5	1	-	1	-
- Inflammation :	5	1	1	1	-
- Acanthosis :	-	-	1	1	-
- Hyperkeratosis :	-	-	-	1	-
ESOPHAGUS :	60	60	60	60	60
- Hyperplasia;squam.c.:	-	-	-	-	1
- Hyperkeratosis :	-	-	-	-	1
- Inflammation :	1	1	2	3	2
EYES :	51	53	58	50	53
- Disorganiz.; retina :	16	21	20	16	13
- Atrophy; retina :	5	6	12	7	10
- Mineralizat.; cornea:	-	7	5	5	3
- Mineralization; iris:	1	5	2	5	2
- Inflammation; retro.:	-	1	1	-	2
- Inflammat;vascular :	-	-	-	1	-
- Inflammation; cornea:	-	4	4	3	1
- Inflammation; uvea :	-	2	2	2	1
- Degeneration; lens :	4	3	3	6	2
- Abscess :	-	-	-	1	-
- Synechia :	-	1	-	-	-
FEMUR :	60	60	59	60	60
- Hyperplasia;synovium:	1	-	-	-	-
- Arthropathy :	2	2	5	3	5
- Fibro-osseous lesion:	-	1	2	2	1
- Inflam;adj.tissue :	1	-	1	1	-
- Inflam;synovium :	1	-	-	-	-
- Inflammation;vascul.:	-	-	-	1	-

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
FORESTOMACH :	60	60	60	60	60
- Inflammat.;adj.tiss.:	14	17	16	12	13
- Inflammat;vascular :	1	-	-	-	-
- Inflammat.;submucosa:	-	-	3	-	3
- Inflammat.;epithel. :	-	1	-	-	-
- Erosion/ulcer :	1	1	3	-	2
- Hyperkeratosis :	2	3	6	2	2
- Acanthosis :	1	3	7	-	1
- Cyst :	-	1	-	-	-
GALL BLADDER :	45	48	48	43	40
- Hyperplasia;epithel.:	3	4	5	1	3
- Dilation :	18	15	23	20	14
- Inflammation :	7	7	3	8	8
- Hemorrhage :	1	-	-	-	1
- Amyloid :	1	1	1	-	-
- Pigment :	1	-	1	-	-
HARDERIAN GLANDS :	59	59	60	60	59
- Hyperpl.;acin.c.foc.:	3	2	1	2	-
- Incr.;porph.deposit.:	43	41	43	43	42
- Inflammation :	15	17	22	23	17
- Amyloid :	-	-	1	1	3
HEART :	60	60	60	60	60
- Amyloid :	8	7	9	9	12
- Fibrosis :	-	2	-	-	-
- Thrombus; atrium :	2	3	3	2	4
- Inflamm.; myocardium:	1	3	1	1	-
- Inflamm.; epicardium:	2	3	4	6	5
- Inflamm.; endocard. :	-	-	-	2	-
- Deg./necr.; myocard.:	-	-	-	1	-
- Inflammat;vascular :	-	2	1	2	1
- Infiltr.; granuloc. :	1	1	-	-	-
- Inflamm.;adjac.fat :	3	4	7	1	5
- Pigment :	-	1	-	1	1
- Dilation;atrium :	2	-	2	-	3
- Thrombus; valves :	1	-	-	-	-
- Mineralization :	-	-	1	-	-

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
ILEUM :	60	60	60	60	60
- Amyloid :	13	19	16	21	15
- Diverticulum :	1	-	-	-	-
- Hemorrhage :	-	1	-	-	-
- Inflammat.;adj.tiss.:	-	-	-	-	1
ILIAC LYMPH NODES :	5	3	6	6	4
- Inc. macrophage nbr :	-	-	1	-	-
- Hemorrhage :	1	-	-	-	-
- Mineralization :	1	-	-	-	-
- Hemopoiesis :	-	-	-	1	-
- Amyloid :	-	1	-	-	-
INGUINAL LYMPH NODES :	2	-	3	2	1
- Inc. plasma cell nbr:	-	-	-	-	1
JEJUNUM :	60	60	60	60	60
- Hyperplasia; crypt :	-	-	-	2	-
- Inflammation :	2	1	2	3	1
- Inflamm.;vascular :	-	-	-	-	1
- Amyloid :	1	5	6	7	6
- Degen./necrosis :	1	-	2	-	-

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
KIDNEYS :	60	60	60	59	57
- Nephropathy :	32	37	39	38	28
- Amyloid; interst. :	9	11	12	11	11
- Amyloid; glomerulus :	8	9	10	10	9
- Cyst :	5	7	5	5	6
- Dilation; tub. :	2	7	7	7	5
- Dilation; Bowm.sp. :	2	-	-	3	4
- Mineralization :	2	2	1	5	3
- Dilation; pelvis :	4	2	11	6	6
- Inflamm.; pelvis :	3	2	-	-	-
- Inflamm.; capsule :	1	-	-	-	-
- Infiltr.; mono.cells:	11	15	15	21	21
- Deg./necr.; papilla :	4	4	7	3	5
- Pigment :	6	6	6	4	9
- Abscess :	1	-	-	1	-
- Hemopoiesis :	1	2	-	1	1
- Inflammat;vascular :	1	-	1	-	-
- Hemorrhage :	-	1	-	-	1
- Metaplasia; osseous :	-	-	-	1	1
- Cast; granular :	-	1	-	-	-
- Scar; cortex :	-	-	1	-	-
LARYNX :	60	60	60	60	60
- Hyperpl.; respirato.:	-	-	-	1	-
- Cyst :	3	1	4	3	2
- Deg/regen; myofibre :	1	2	1	1	-
- Inflammat;vascular :	1	-	-	1	-
- Infiltr.; granuloc. :	2	1	2	3	2
- Infiltr.; mono.cells:	2	3	5	-	6
- Hemorrhage :	3	2	-	-	-
- Amyloid :	-	1	1	-	-

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
LIVER :	60	60	60	59	57
- Alt.c.focus;baso. :	2	-	1	-	-
- Alt.c.focus;eosin. :	1	1	-	1	1
- Alt.c.focus;clear c.:	-	-	2	2	-
- Hyperpl/hypertr;K.c.:	1	-	-	2	3
- Hypertrophy;hepatoc.:	3	5	11	17	25
- Pigment :	11	11	14	16	7
- Inflammatory c.foci :	24	30	19	35	28
- Inflammation;portal :	1	-	-	1	-
- Infiltrate;lymph.c. :	6	-	5	6	-
- Fibrosis :	-	-	-	1	-
- Amyloid :	10	11	7	6	9
- Hemopoiesis :	15	18	11	14	8
- Glycogen content :	11	15	12	11	13
- Single c.nec/apopt. :	2	3	4	4	3
- Vacuolation;hepatoc.:	1	2	2	3	4
- Focal necrosis :	3	4	3	1	1
- Deg/necr;hepatocyte :	2	-	-	2	-
- Atrophy;hepatocyte :	-	-	1	-	1
- Tension lipidosis :	-	2	-	-	-
- Congestion :	-	-	-	-	1
- Hemorrhage :	2	-	-	-	-
- Hematoma :	-	-	1	-	-
- Cyst :	-	-	-	1	1
- Mineralization :	-	-	-	-	1
- Increased mitoses :	-	1	-	-	-

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
LUNGS :	60	60	60	60	60
- Hyperpl.;bronch-alv.:	2	-	6	1	2
- Infiltrate; lymphoid:	7	15	18	17	12
- Foamy macr.;alveolus:	12	13	14	12	13
- Inflam.;interst;chro:	3	6	4	1	1
- Inflam.;pleura;chron:	4	1	-	5	5
- Inflam.;br-alv;chron:	-	-	-	1	2
- Inflam.;granulocytes:	1	1	-	1	1
- Edema :	-	1	1	-	1
- Congestion :	1	2	2	2	4
- Hemorrhage :	5	5	2	5	4
- Metaplasia; osseous :	-	-	-	-	1
- Mineralization :	1	3	3	1	-
- Mineral.; vasc.wall :	-	-	-	1	-
- Pigment :	-	2	4	1	2
- Amyloid :	-	-	-	-	1
- Cyst/Dilat.bronch.gl:	1	1	3	2	4
- Deg./necr.; bronchus:	-	3	1	-	-
- Hemopoiesis :	-	-	1	-	-
MAMMARY GLANDS AREA :	59	60	60	60	58
- Hyperpl.; acin.c. :	1	4	2	3	2
- Hyperpl.; atypical :	-	-	1	-	-
- Dilation; ac.lumen :	3	5	1	6	3
- Inflamm.;interst. :	1	2	4	2	5
MANDIBUL. LYMPH NODE :	60	59	60	60	60
- Inc. plasma cell nbr:	10	15	9	13	9
- Inc. follicle devlpt:	4	3	1	3	4
- Histiocytosis :	-	1	-	-	-
- Amyloid :	4	6	6	3	9
- Sin. red blood cells:	6	6	8	8	6
- Cystic degeneration :	-	2	-	1	-
- Incr.tingle bod.mac.:	-	1	-	1	-
- Pigment :	34	35	37	36	34
- Hemopoiesis :	6	2	4	5	5
- Lymphoid atrophy :	2	-	-	-	-
MANDIBULAR GLANDS :	-	-	-	1	-
- Hematoma :	-	-	-	1	-

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
MEDIAST.LYMPH NODES :	6	6	8	6	7
- Inc. plasma cell nbr:	-	1	-	-	-
- Inc. follicle devlpt:	-	1	-	-	-
- Sin. red blood cells:	2	-	-	-	-
- Hemangiectasis :	-	1	-	-	1
- Amyloid :	-	1	-	-	-
- Pigment :	1	-	-	-	-
- Hemopoiesis :	1	-	-	-	-
MESENT. LYMPH NODE :	59	59	57	58	55
- Hemopoiesis :	4	6	5	7	4
- Paracortex developm.:	1	2	2	-	1
- Amyloid :	11	13	10	12	12
- Cystic degeneration :	2	5	7	4	5
- Inc. plasma cell nbr:	-	2	1	-	2
- Sin. red blood cells:	15	12	17	17	12
- Incr.tingle bod.mac.:	1	2	1	-	-
- Pigment :	15	20	13	12	14
- Incr.granuloc.numb. :	2	-	-	-	-
- Inc.macroph.numbers :	-	1	1	-	-
- Inflammation :	1	-	-	-	-
- Lymphoid atrophy :	1	1	2	-	-
OPTIC NERVES :	58	58	60	58	57
- Inflammation :	1	1	-	1	-

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
OVARIES :	58	59	60	60	58
- Hyperp.; cyst./papi.:	1	2	2	-	-
- Hyper.; sex cord str:	6	7	3	4	4
- Atrophy :	9	9	13	7	9
- Cyst; ovarian :	43	49	47	47	46
- Cyst; paraovarian :	5	-	1	4	3
- Dilated/cystic bursa:	14	17	21	14	10
- Hemorrhagic follicle:	7	10	5	7	5
- Inflammation :	5	11	11	9	7
- Inflamm.; vascular :	-	-	1	-	-
- Amyloid :	5	7	5	5	9
- Pigment :	15	16	8	14	9
- Hemorrhage :	-	-	2	-	1
- Hematoma :	-	-	-	-	1
- Mineralization :	-	-	-	-	1
- Developm. anomaly :	1	-	-	-	-
- Necrosis; adj.fat :	-	-	-	-	1
- Abscess :	1	-	-	-	-
OVIDUCTS :	59	55	56	59	54
- Hyperplasia; glandu.:	1	2	2	1	-
- Inflammation :	1	2	2	2	2
- Amyloid :	-	-	-	-	1
- Cyst :	-	-	-	1	-
- Dilation; lumen :	-	1	-	-	-
PANCREAS :	58	60	59	58	56
- Hyperpl.; islet cell:	-	2	1	2	4
- Hypertrophy; acin.c.:	-	-	-	-	1
- Inflammation :	15	13	9	10	10
- Atrophy; lobule :	-	4	2	2	-
- Degranul.; acin.cell:	-	-	-	-	1
- Amyloid :	4	1	4	3	5
- Edema; interstitium :	2	1	3	3	-
- Single c.necr./apop.:	-	-	1	-	-
- Accesssory spleen :	1	-	-	-	-
- Dilation; duct :	-	1	-	-	-
- Vacuol.; acinar cell:	1	-	1	-	1
PARATHYROID GLANDS :	52	59	59	56	60
- Amyloid :	8	10	9	8	12
- Inflammation :	-	1	-	2	2

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
PEYER'S PATCHES :	38	41	46	39	36
- Hyperplasia;lymphoid:	-	-	2	1	3
- Hemorrhage :	-	1	-	-	-
PITUITARY GLAND :	60	59	60	60	59
- Hyperpl.foc.p.distal:	-	1	2	-	-
- Hyperpl.dif.p.interm:	2	-	2	-	-
- Hypertr. pars interm:	-	1	-	2	1
- Cyst :	3	1	1	2	3
- Hemorrhage :	1	1	-	-	-
- Infiltr.; mono.cells:	-	-	-	-	1
- Angiectasis :	-	-	-	1	2
- Pigment :	-	2	1	-	-
POPLITEAL LYMPH NODE :	2	1	1	2	1
- Incr.macroph.numbers:	-	1	-	-	-
- Paracortex developm.:	-	1	-	-	-
- Hemorrhage :	1	-	1	-	-
PORTAL LYMPH NODES :	4	5	2	5	6
- Necrosis :	1	-	-	1	-
- Inc. plasma cell nbr:	-	1	-	-	1
- Hemopoiesis :	-	-	-	-	1
- Hemorrhage :	1	-	-	1	-
- Amyloid :	-	1	-	-	-
RECTUM :	1	-	-	-	-
- Hemorrhage :	1	-	-	-	-
RENAL LYMPH NODES :	5	1	7	6	7
- Inc. plasma cell nbr:	-	-	-	-	1
- Inc.germ.cent.devlpt:	-	-	-	-	1
- Sin. red blood cells:	1	-	-	-	-
- Hemopoiesis :	-	-	1	-	-
SALIVARY GLANDS :	60	60	60	60	60
- Inflammation :	20	23	22	35	25
- Amyloid :	10	7	7	6	9
- Atrophy; acinar cell:	-	-	1	1	-
- Hemorrhage :	1	-	-	-	-

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
SCIATIC NERVE :	60	60	60	60	60
- Degeneration; nerve :	27	30	40	26	24
- Inflammation :	2	5	2	3	1
SKELETAL MUSCLE :	60	60	60	60	60
- Inflammation;acute :	1	1	-	-	-
- Inflammation;subac. :	-	4	4	1	5
- Degen/necr;myofiber :	-	-	1	-	-
SKIN :	60	60	60	60	58
- Ulcer :	10	9	9	12	6
- Serocellular crust :	10	5	7	9	7
- Inflammation :	1	2	3	2	2
- Decr.nb.hair follic.:	1	-	1	1	-
- Cyst :	-	-	-	-	1
- Abscess :	1	-	-	-	-
- Acanthosis :	-	1	-	2	-
- Edema; subcutis :	-	-	1	-	-
- Fibropl/fibr. dermis:	-	1	-	-	-
- Hemorrhage :	-	-	1	-	-
SPINAL CORD :	60	60	60	60	60
- Hemorrhage :	-	1	-	1	-
- Inflammation :	-	1	1	-	-
SPLEEN :	59	60	60	59	57
- Hyperp.; angiomatous:	-	-	1	-	-
- Increased margin.zo.:	1	3	5	6	3
- Hemopoiesis :	43	44	41	38	37
- Amyloid :	9	11	8	11	13
- Hemangiectasis :	-	-	-	-	1
- Inc. follicle devlpt:	1	-	-	-	-
- Inc. tgb.bod. macro.:	-	-	-	1	-
- Pigment :	12	23	14	10	12
- Congestion :	-	-	2	-	-
- Inflammation :	2	1	3	2	4
- Hemorrhage/hematoma :	-	-	1	-	-
- Abnormal shape :	-	-	-	1	-

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
STOMACH :	60	60	60	60	60
- Hyperplasia;gland :	-	1	-	2	1
- Amyloid :	6	10	9	9	8
- Inflammat.;adj.tiss.:	13	10	15	15	20
- Dilation; gland :	7	9	3	2	6
- Edema :	1	-	-	-	-
- Mineralization :	-	-	-	1	-
- Degen./necrosis :	4	-	-	1	4
- Infiltr.; mono.cells:	6	7	4	3	1
- Inflamm.;vascular :	1	-	1	1	-
- Pigment :	1	1	-	-	-
- Cyst :	1	1	1	1	-
SUBCUTANEOUS TISSUE :	6	6	3	1	6
- Edema :	6	6	3	1	5
- Inflammation :	1	2	1	-	4
TEETH :	-	1	-	-	-
- Decr.growth; teeth :	-	1	-	-	-
THYMUS :	56	60	58	56	55
- Hyperplasia;lymphoid:	11	10	13	11	6
- Lymphoid atrophy :	24	29	21	26	30
- Inflammation :	-	1	-	-	-
- Inc. tgb.bod. macro.:	-	2	-	1	-
- Pigment :	2	1	2	1	1
- Amyloid :	1	1	1	-	1
- Hemangiectasis :	1	-	1	-	2
- Hemorrh./Hematoma :	2	2	3	1	3
- Inflammation; vasc. :	-	-	1	-	-
- Hemopoiesis :	1	-	-	-	-
THYROID GLANDS :	60	60	59	60	60
- Hypertr.; follicular:	-	-	-	1	-
- Amyloid :	10	9	9	9	13
- Dilated/cystic foll.:	15	14	13	9	9
- Inflammation :	5	5	5	8	4
- Ectopic thymus :	1	1	-	1	-

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
TONGUE :	60	60	60	60	60
- Inflammat;vascular :	1	1	2	3	-
- Inflammation;acute :	2	3	1	2	-
- Inflammation;subac. :	3	4	2	3	1
- Amyloid :	2	1	-	-	3
- Granuloma :	-	-	-	1	-
- Hemorrhage :	-	-	1	-	-
TRACHEA :	60	60	60	60	60
- Inflammation :	1	2	2	1	-
- Hemorrhage :	-	-	1	-	-
URETERS :	-	-	2	2	1
- Dilation; lumen :	-	-	1	-	-
- Inflammation; adj.t.:	-	-	-	-	1
URINARY BLADDER :	58	58	58	60	57
- Hyperpl.; uroth. :	-	2	-	-	-
- Inflamm.; mucosa :	31	24	25	32	29
- Inflamm.; muscle :	1	2	-	-	1
- Inflamm.; serosa :	1	2	1	3	3
- Dilation; lumen :	2	-	2	2	1
- Amyloid :	-	-	-	1	-
- Vacuol.; uroth. :	-	-	1	-	-
UTERUS :	60	60	60	60	59
- Hyperpl.; gldcystic.:	56	54	56	57	48
- Hyperpl.; endometr. :	2	-	-	1	1
- Hyperpl.;stromal :	3	6	7	1	3
- Hyperpl.; angiomat. :	-	1	2	2	1
- Hyperpl.; fib./musc.:	2	1	2	-	-
- Metapl.; squam. cell:	4	2	5	2	1
- Adenomyosis :	1	1	1	-	-
- Inflammation :	5	6	4	5	5
- Amyloid :	7	3	1	5	2
- Hemorrhage :	4	4	2	3	2
- Hemangiectasis :	-	1	2	-	3
- Dilated lumen :	4	2	1	-	1
- Cyst :	3	1	1	1	1
- Inflamm.; vascular :	-	1	-	2	3
- Pigment :	-	1	-	1	1
- Granular cell focus :	-	-	-	-	1

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
VAGINA :	60	60	59	60	57
- Hyperp.;sq.c/hyperk.:	20	22	23	21	20
- Mucific.; epithelium:	4	11	8	4	6
- Hemorrhage :	1	1	-	-	-
- Amyloid :	1	1	-	1	2
- Cyst :	-	-	3	1	-
- Inflamm.; vascular :	1	-	-	1	-
- Infiltr.;granulocyte:	1	2	-	1	1

Appendix 3: Neoplastic lesions in male and female mice

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. DEATHS

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
ADRENAL GLANDS :	60	60	60	58	60
- METASTATIC CARCINOMA:	1	1	-	-	-
- METASTATIC SRACOMA :	1	-	-	-	-
- ADENOMA; CORTEX :	-	-	1	-	2
- ADENOMA; SUBCAPSULAR:	1	1	1	2	2
- PHAEOCHROMOCYTOMA :	-	-	1	2	-
AORTA :	60	60	60	60	59
- METASTATIC CARCINOMA:	-	2	-	1	1
- METASTATIC SARCOMA :	-	1	-	-	-
BONE :	-	1	-	1	1
- METASTATIC CARCINOMA:	-	1	-	-	-
BONE MARROW :	60	60	60	59	60
- METASTATIC CARCINOMA:	-	1	-	-	-
BRAIN :	60	60	59	58	60
- ASTROCYTOMA; MALIGN.:	-	-	-	-	1
CECUM :	59	60	60	60	60
- METASTATIC CARCINOMA:	-	-	-	-	1
COLON :	59	60	60	60	60
- ADENOCARCINOMA :	-	1	1	-	2
- ADENOMA :	-	-	-	1	-
DUODENUM :	60	58	60	58	60
- ADENOCARCINOMA :	1	-	-	-	-
- ADENOMA :	-	-	-	-	1
EPIDIDYMIDES :	60	60	60	59	59
- ADENOMA; LEYDIG CELL:	-	1	-	-	-
FEMUR :	60	60	60	60	60
- METASTATIC SARCOMA :	-	1	-	-	-
FORESTOMACH :	60	60	60	60	60
- METASTATIC CARCINOMA:	-	1	-	-	-

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
GALL BLADDER :	52	49	47	48	54
- METASTATIC CARCINOMA:	-	1	-	-	-
- METASTATIC SARCOMA :	1	-	-	-	-
- ADENOCARCINOMA :	-	-	-	-	1
- PAPILLOMA :	1	2	-	-	1
HARDERIAN GLANDS :	60	60	60	57	60
- METASTATIC CARCINOMA:	-	2	-	-	-
- ADENOCARCINOMA :	-	1	1	1	-
- ADENOMA :	6	2	2	3	2
HEART :	60	60	60	60	60
- METASTASTIC CARCINO.:	-	2	-	-	2
- METASTASTIC SARCOMA :	-	-	-	-	1
HEMOLYMPHORET. SYS. :	60	60	60	60	60
- LYMPHOMA; MALIGNANT :	6	6	7	3	7
- SARCOMA; HISTIOCYTIC:	-	1	1	2	-
- LEUKEMIA; GRANULOCYT:	-	-	-	1	-
- TUMOR; MAST C.; MAL.:	1	-	-	-	-
KIDNEYS :	60	60	60	58	60
- METASTATIC CARCINOMA:	-	2	-	1	1
- METASTATIC SARCOMA :	1	-	-	-	1
- ADENOMA; TUBULE :	2	-	-	1	2
LIVER :	60	59	60	58	60
- HEMANGIOSARCOMA :	-	1	1	1	3
- CARCINOMA;HEPATOCEL.:	7	4	5	8	7
- HEPATOBLASTOMA :	1	-	-	-	-
- METASTATIC SARCOMA :	2	1	-	-	-
- METASTATIC CARCINOMA:	-	1	-	-	-
- ADENOMA;HEPATOCELL. :	14	7	9	7	7
- HEMANGIOMA :	-	1	-	-	-
- TUMOR;ITO CELL;BEN. :	-	-	1	-	-
LUNGS :	60	60	60	60	60
- ADENOCARCINOMA :	6	8	9	13	11
- HEMANGIOSARCOMA :	-	1	-	-	-
- METASTASIS; SARCOMA :	1	-	-	-	1
- METASTASIS; CARCIN. :	1	2	-	-	-
- ADENOMA;BRONCHIO-ALV:	11	10	8	12	13

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
MANDIBULAR GLANDS :	1	-	-	-	-
- FIBROMA :	1	-	-	-	-
MESENT. LYMPH NODE :	60	60	60	57	59
- METASTATIC CARCINOMA:	-	1	-	-	-
- HEMANGIOSARCOMA :	-	1	-	-	-
MESENTERY :	1	-	-	-	-
- FIBROSARCOMA :	1	-	-	-	-
PANCREAS :	60	60	59	58	59
- METASTATIC SARCOMA :	1	-	-	-	1
- ADENOCARC.; ACIN.CEL:	-	1	-	-	-
PORTAL LYMPH NODES :	3	-	-	2	3
- METASTATIC SARCOMA :	1	-	-	-	-
PROSTATE :	60	60	60	58	59
- ADENOCARCINOMA :	1	-	-	-	-
- ADENOMA :	-	-	-	1	-
RENAL LYMPH NODES :	5	1	2	3	4
- METASTATIC SARCOMA :	1	-	-	-	-
SALIVARY GLANDS :	60	60	60	59	60
- METASTATIC CARCINOMA:	-	1	-	-	-
SEMINAL VESICLES :	60	60	60	58	59
- METASTASIS; CARCINO.:	-	-	-	-	1
- METASTATIC SARCOMA :	1	-	-	-	-
- TUMOR; GRANUL.C; MAL:	-	-	-	1	-
SKIN :	60	57	60	59	59
- METASTATIC CARCINOMA:	-	1	-	-	-
- METASTASTIC SARCOMA :	1	-	-	-	-
- HEMANGIOMA :	-	1	-	-	-
SPINAL CORD :	60	60	60	60	59
- METASTATIC SARCOMA :	-	-	-	-	1

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
SPLEEN :	60	60	60	58	60
- LEIOMYOSARCOMA :	1	-	-	-	-
- METASTATIC SARCOMA :	1	-	-	-	-
- METASTATIC CARCINOMA:	-	1	-	-	-
- HEMANGIOSARCOMA :	-	1	-	-	-
- HEMANGIOMA :	1	-	-	-	-
SUBCUTANEOUS TISSUE :	1	4	3	4	-
- OSTEOSARCOMA :	-	1	-	-	-
TEETH :	-	1	1	2	1
- FIBROMA; OSSIFYING :	-	-	-	1	-
TESTES :	60	60	60	59	59
- METASTATIC CARCINOMA:	-	1	-	-	-
- ADENOMA; LEYDIG CELL:	2	-	1	2	-
THYMUS :	56	55	54	53	56
- METASTATIC CARCINOMA:	-	1	-	1	1
THYROID GLANDS :	60	60	60	58	59
- CARCINOMA; FOLLIC. :	1	-	-	-	-
TONGUE :	60	60	60	60	60
- HEMANGIOSARCOMA :	-	-	-	-	1
URINARY BLADDER :	59	59	60	59	59
- LESION; MESENCH.PROL:	1	-	-	-	-

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
ADRENAL GLANDS :	60	60	60	60	60
- ADENOMA; CORTEX :	-	-	1	-	-
- PHAEOCHROMOCYTOMA :	-	-	-	-	2
AORTA :	60	60	60	59	60
- METASTATIC CARCINOMA:	-	-	-	-	1
- METASTATIC SARCOMA :	2	-	-	-	-
BONE :	1	2	-	1	-
- OSTEOSARCOMA :	1	-	-	-	-
BONE MARROW :	59	60	59	60	59
- HEMANGIOSARCOMA :	-	-	-	1	-
BRAIN :	60	60	60	60	60
- MENINGIOMA; MALIGN. :	-	1	-	-	-
BRONCHIAL LYMPH NODE :	-	-	1	3	-
- METASTATIC CARCINOMA:	-	-	-	1	-
CECUM :	60	60	60	60	60
- METASTATIC CARCINOMA:	-	-	1	-	-
COLON :	60	60	59	59	60
- ADENOCARCINOMA :	-	-	1	-	-
- METASTATIC CARCINOMA:	-	-	1	-	-
DIAPHRAGM :	2	1	-	-	-
- METASTATIC SARCOMA :	1	-	-	-	-
- METASTATIC CARCINOMA:	-	1	-	-	-
DUODENUM :	59	60	59	58	60
- METASTATIC CARCINOMA:	-	1	-	-	-
- ADENOMA :	-	-	1	-	-
FEMUR :	60	60	59	60	60
- METASTATIC SARCOMA :	1	-	-	-	-
FORESTOMACH :	60	60	60	60	60
- METASTATIC CARCINOMA:	-	1	-	-	-
- METASTATIC SARCOMA :	-	-	-	-	2
- MAST CELL TUMOR; BEN:	-	-	-	-	1

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
HARDERIAN GLANDS :	59	59	60	60	59
- ADENOCARCINOMA :	-	1	-	-	-
- ADENOMA :	4	3	1	1	4
HEART :	60	60	60	60	60
- METASTASTIC CARCINO.:	1	-	1	-	1
- METASTASTIC SARCOMA :	2	-	-	-	-
HEMOLYMPHORET. SYS. :	60	60	60	60	60
- LYMPHOMA; MALIGNANT :	21	16	23	16	19
- SARCOMA; HISTIOCYTIC:	3	6	3	5	4
- LEUKEMIA; GRANULOCYT:	-	1	-	-	-
ILEUM :	60	60	60	60	60
- ADENOCARCINOMA :	-	1	-	-	-
JEJUNUM :	60	60	60	60	60
- METASTATIC CARCINOMA:	1	-	-	-	-
KIDNEYS :	60	60	60	59	57
- METASTATIC CARCINOMA:	1	-	1	-	-
- METASTATIC SARCOMA :	-	-	-	-	1
LIVER :	60	60	60	59	57
- HEMANGIOSARCOMA :	2	-	-	-	-
- CARCINOMA;HEPATOCEL.:	1	-	-	1	-
- METASTATIC SARCOMA :	2	-	-	-	1
- METASTATIC CARCINOMA:	-	1	1	1	-
- ADENOMA;HEPATOCELL. :	-	3	2	2	1
LUNGS :	60	60	60	60	60
- ADENOCARCINOMA :	2	3	3	5	4
- METASTASIS; SARCOMA :	2	-	1	-	1
- METASTASIS; CARCIN. :	-	1	-	-	-
- ADENOMA;BRONCHIO-ALV:	6	3	2	4	6
MAMMARY GLANDS AREA :	59	60	60	60	58
- ADENOCARCINOMA :	1	1	-	1	2
- CARCINOMA; SQUAM.C. :	-	-	1	-	-
- ADENOMA :	1	-	-	-	-

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
MANDIBUL. LYMPH NODE :	60	59	60	60	60
- METASTATIC CARCINOMA:	-	-	1	-	-
MEDIAST.LYMPH NODES :	6	6	8	6	7
- METASTATIC SARCOMA :	-	-	-	-	1
MESENT. LYMPH NODE :	59	59	57	58	55
- METASTATIC CARCINOMA:	-	-	1	-	-
- HEMANGIOSARCOMA :	1	-	-	-	-
ORAL CAVITY :	-	1	-	-	-
- CARCINOMA; SQUAM.C. :	-	1	-	-	-
OVARIES :	58	59	60	60	58
- METASTATIC CARCINOMA:	1	-	1	-	-
- TUM.;SEX C;MIX;BEN. :	-	1	-	2	1
- ADENOMA; TUBULOSTRO.:	-	-	1	-	-
- LUTEOMA; BENIGN :	-	1	1	1	2
- TUM.; GRANUL.; BEN. :	-	1	-	-	-
- HEMANGIOMA :	-	1	1	-	1
- CYSTADENOMA :	1	2	2	1	-
PANCREAS :	58	60	59	58	56
- METASTATIC CARCINOMA:	-	-	1	-	-
PERITONEUM :	-	-	-	-	1
- SARCOMA :	-	-	-	-	1
PITUITARY GLAND :	60	59	60	60	59
- METASTATIS; CARCINO.:	-	-	1	-	-
- ADENOMA; P. DISTALIS:	2	-	-	2	-
PORTAL LYMPH NODES :	4	5	2	5	6
- METASTATIC SARCOMA :	-	-	-	-	1
RENAL LYMPH NODES :	5	1	7	6	7
- METASTATIC SARCOMA :	-	-	-	-	1
SALIVARY GLANDS :	60	60	60	60	60
- METASTATIC CARCINOMA:	-	-	1	-	-

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	60	60	60	60	60
SKELETAL MUSCLE :	60	60	60	60	60
- FIBROSARCOMA :	1	-	-	-	-
- METASTATIC CARCINOMA:	1	-	1	-	-
SKIN :	60	60	60	60	58
- METASTASTIC SARCOMA :	1	-	-	-	-
- CARCINOMA; SQUAMOUS :	-	-	-	-	1
- FIBROSARCOMA :	-	-	-	-	2
- OSTEOSARCOMA :	1	-	-	-	-
- HISTIOCYT.FIBR.MALIG:	-	-	-	1	-
- SARCOMA; NOS :	-	-	1	-	-
- HAIR FOLL. TUM. BEN.:	1	-	-	-	-
- BASAL CELL TUM. BEN.:	-	-	-	-	1
- PAPILLOMA; SQUAMOUS :	-	-	1	-	-
SPLEEN :	59	60	60	59	57
- METASTATIC SARCOMA :	1	-	-	-	2
- METASTATIC CARCINOMA:	-	-	1	-	-
- HEMANGIOSARCOMA :	-	1	-	-	1
STOMACH :	60	60	60	60	60
- METASTATIC SARCOMA :	-	-	-	-	2
- ADENOCARCINOMA :	-	1	-	-	-
- ADENOMA :	-	-	-	-	1
SUBCUTANEOUS TISSUE :	6	6	3	1	6
- HEMANGIOSARCOMA :	-	-	-	-	1
THYMUS :	56	60	58	56	55
- METASTATIC CARCINOMA:	-	-	1	1	1
- METASTATIC SARCOMA :	1	-	-	-	-
URINARY BLADDER :	58	58	58	60	57
- CARC.;TRANSIT.CELL :	-	-	-	1	-
- METASTATIC CARCINOMA:	-	-	1	-	-
UTERUS :	60	60	60	60	59
- SARCOMA; ENDOM.STRO.:	1	-	5	2	2
- LEIOMYOSARCOMA :	-	1	2	-	-
- HEMANGIOSARCOMA :	1	-	-	-	1
- ADENOCAR.; ENDOMETR.:	1	1	-	3	1
- METASTATIC CARCINOMA:	-	-	1	-	-
- LEIOMYOMA :	2	3	3	3	2
- FIBROMA :	-	-	-	1	-
- POLYP; ENDOM.STROMAL:	2	4	3	1	5
- HEMANGIOMA :	1	1	1	1	-
- SCHWANNOMA; BENIGN :	-	-	-	1	-
VAGINA :	60	60	59	60	57
- CARCINOMA;SQUAM.CELL:	-	-	1	-	-
- LEIOMYOMA :	-	1	1	-	-

Appendix 4: Non-neoplastic lesions in male and female SD-rats (Applicant's table from Study# GT-157-TX-38

NUMBER OF ANIMALS WITH NON-NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. DEATHS

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	50	50	50	50	50
ADIPOSE TISSUE :	1	1	1	2	1
- Degener./necrosis :	-	-	-	1	-
ADRENAL CORTICES :	50	50	50	50	50
- Hypertr.foc.cortic. :	10	10	15	11	13
- Hypertr.diff.cortic.:	-	-	-	2	2
- Hyperpl.; cortical :	8	5	6	2	8
- Cystic degeneration :	8	11	6	11	10
- Cyst :	-	-	1	-	-
- Vacuolation; focal :	10	10	13	12	8
- Vacuol.; diffuse :	-	-	-	-	1
- Congestion :	2	3	1	3	-
- Hemopoiesis :	3	-	-	-	-
ADRENAL MEDULLAS :	49	50	50	50	50
- Hyperplasia; diffuse:	-	-	1	-	-
- Hyperplasia; focal :	3	8	7	9	5
AORTA :	50	50	50	50	50
- Mineralization :	1	-	-	-	-
- Inflamm.; adj. tis. :	-	-	1	-	-
AXILLARY LYMPH NODES :	2	2	2	3	-
- Inc. plasma cell nbr:	1	2	1	-	-
- Lymphoid hyperplasia:	1	-	-	2	-
- Sin. red blood cells:	-	1	-	-	-
BONE MARROW :	50	50	50	50	50
- Increased cellulari.:	6	5	6	2	5
- Decreased cellulari.:	-	-	-	1	-
BRAIN :	50	50	50	50	50
- Dilatation;ventricle:	6	7	8	8	7
- Gliosis :	-	-	-	1	-
- Mineralization :	1	-	1	2	-
- Hemorrhage :	-	-	1	-	-
- Fibrosis; meninges :	1	-	-	-	-
CECUM :	50	50	50	50	50
- Inflammat.;adj.tiss.:	-	-	-	1	1

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	50	50	50	50	50
COLON :	50	50	50	50	50
- Erosion/ulcer :	-	-	-	1	-
- Polyarteritis Nodosa:	1	-	-	-	-
- Inflammat.;adj.tiss.:	-	-	-	1	-
DUODENUM :	50	50	50	50	50
- Degen./necrosis :	-	-	1	1	-
- Inflammation :	1	-	-	-	-
EARS :	11	6	5	5	3
- Chondropathy :	10	6	5	5	3
- Inflammation :	-	-	-	-	1
EPIDIDYMIDES :	50	50	50	50	50
- Reduced sperm cont. :	3	1	2	7	5
- Slou.cells;tub.lumen:	4	-	5	4	6
- Inflammation :	-	-	-	1	1
- Granuloma; spermatic:	1	-	-	-	-
- Dilation; tub. lumen:	-	1	-	-	-
ESOPHAGUS :	50	50	50	50	49
- Inflammat.;adj.tiss.:	1	1	1	2	1
EYES :	50	50	49	50	50
- Damaged :	1	1	1	1	1
- Enlarged eyeball :	1	-	-	-	-
- Panophthalmitis :	-	1	-	-	-
- Inflamm.; conjunct. :	1	1	1	-	1
- Hemorrhage :	6	6	1	7	5
- Degeneration; lens :	2	-	1	-	-
- Retinal detachment :	1	-	3	-	-
- Periph.atrophy;reti.:	1	4	-	3	7
- Diffuse atroph.;ret.:	1	-	-	-	-
- Focal atrophy;retin.:	1	-	1	2	-
FEMUR :	50	50	50	50	50
- Hyperplasia;cartil. :	1	-	-	-	-
- Retention;prim.spon.:	-	1	-	-	-
- Inflammation; joint :	-	-	1	2	1
- Degeneration; joint :	3	2	3	4	2
- Fibrosis :	-	-	-	-	1

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	50	50	50	50	50
FORELEG :	-	1	-	-	-
- Inflammation :	-	1	-	-	-
FORESTOMACH :	50	50	50	50	50
- Hyperplasia;squam.c.:	1	3	2	2	2
- Erosion/ulcer :	2	3	2	2	-
- Inflammation; mucos.:	1	1	3	2	1
- Cyst :	1	1	-	-	-
HARDERIAN GLANDS :	50	50	49	50	50
- Incr.;porph.deposit.:	4	7	3	4	5
- Infiltr.; mono.cells:	7	6	7	5	3
HEART :	50	50	50	50	50
- Hyperpl.; endocard. :	-	-	1	1	1
- Necr./fibr.; myofib.:	41	35	38	43	34
- Mineralization :	1	-	-	-	-
- Fibrosis :	-	-	-	1	-
- Thrombus; septic :	-	-	1	-	-
- Inflamm.; epicardium:	-	-	2	-	-
- Infiltr.; mono.cells:	1	1	2	-	1
- Hyperp.; mesothe.ce.:	-	-	-	1	-
HINDLEG :	4	7	8	7	5
- Inflammation :	4	7	8	7	5
ILEUM :	50	50	50	50	50
- Degen./necrosis :	-	2	-	-	-
- Inflammation; mucos.:	-	2	-	-	-
ILIAC LYMPH NODES :	-	1	3	3	-
- Lymphoid hyperplasia:	-	-	1	2	-
- Inc. plasma cell nbr:	-	1	-	1	-
- Sin. red blood cells:	-	1	-	-	-
- Lymphangiectasis :	-	-	-	1	-
JEJUNUM :	49	50	50	50	50
- Polyarteritis Nodosa:	1	-	-	-	-

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	50	50	50	50	50
KIDNEYS :	50	50	50	50	50
- Nephrop.;prog.; chr.:	32	35	34	39	33
- Hypertr.;tub.epith. :	1	-	-	-	-
- Hyal.drop.;tubul.ep.:	1	-	2	-	1
- Deg./necr.; papilla :	1	-	-	2	-
- Infil.; gran.;pye.c.:	3	2	4	5	1
- Infiltr.; mono.cells:	3	-	1	1	3
- Infiltr.; granuloc. :	1	-	-	1	1
- Inflamm.; papilla :	-	-	-	2	-
- Inflammation :	-	-	-	2	-
- Fat infiltration :	1	-	-	-	-
- Cyst :	4	-	-	-	3
- Hyperpl.; uroth. :	1	-	1	1	-
- Dilation; pelvis :	2	-	-	3	-
- Inflammation; adj.t.:	1	-	-	1	-
- Mineraliz.; cort. :	1	-	-	-	-
- Mineraliz.;pelvis :	1	4	-	2	-
- Hemorrhage :	-	-	1	-	-
LACRIMAL GLANDS :	-	1	-	-	-
- Harderian gl. acini :	-	1	-	-	-
LARYNX :	49	49	50	50	49
- Inflamm.; adj.tissue:	2	1	1	2	1
- Granuloma :	-	-	-	-	1
- Hemorrhage :	-	-	-	-	1
- Polyarteritis Nodosa:	1	-	-	-	-
- Degen./necr.;myofib.:	-	-	1	-	-
LIVER :	50	50	50	50	50
- Alt.c.focus;eosin. :	1	6	5	5	3
- Alt.c.focus;baso. :	13	13	10	6	8
- Alt.c.focus;clear c.:	29	17	22	19	25
- Fatty change :	-	1	3	2	-
- Alt.c.focus;vac.c. :	5	4	8	8	2
- Angiectasis :	7	3	6	6	7
- Focal necrosis :	5	2	6	7	2
- Single c.nec/apopt. :	1	-	1	1	-
- Deg/necr;hepatocyte :	1	2	-	1	1
- Inflammatory c.foci :	38	37	35	35	33
- Hyperplasia; bile d.:	16	21	23	16	11
- Vacuolation;peripor.:	5	9	12	11	4
- Vacuolation;centril.:	1	-	-	-	-
- Glycogen content :	2	-	-	-	1
- Hypertrophy;hepatoc.:	1	2	1	-	-
- Increased mitoses :	1	2	-	1	-
- Incr.cellul;sinusoid:	1	1	-	-	1
- Pigment :	-	3	-	-	-
- Fibrosis :	-	1	-	-	-
- Cystic degeneration :	8	4	10	4	7
- Cong/hem;agonal :	-	-	-	-	1
- Hemopoiesis :	3	-	1	4	2
- Tension lipidosis :	2	3	1	4	2
- Biliary cyst :	-	2	1	-	-
- Hemorrhage :	1	1	-	-	-
- Euth.pro/barb.lysis :	1	-	-	-	-
- Hepatodiaph.nodule :	-	-	-	1	-
- Medial thickening :	1	-	-	-	-

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	50	50	50	50	50
LUNGS :	50	50	50	50	49
- Hyperpl.;respiratory:	-	2	-	-	-
- Granuloma :	3	7	3	2	32
- Inflam.;br-alv;acute:	1	1	-	-	1
- Inflam.;br-alv;chron:	-	1	-	-	7
- Foamy macr.;alveolus:	12	18	8	10	13
- Inflam.;alveol;acute:	2	2	-	1	1
- Inflam.;alveol;chron:	4	-	1	-	4
- Hemorrhage :	1	-	1	1	1
- Edema :	1	4	2	4	3
- Agonal cong./hemorr.:	4	5	9	9	7
- Hyperpl.; II pneumoc:	1	-	1	1	-
- Bronchiectasis :	-	-	-	-	5
- Metaplasia; osseous :	4	-	2	2	1
- Atelectasis :	-	-	-	-	1
MAMMARY GLANDS AREA :	-	-	3	3	1
- Dilation; duct :	-	-	1	-	-
MANDIBUL. LYMPH NODE :	49	48	49	49	48
- Inc. plasma cell nbr:	49	46	47	48	46
- Dec.germ.cent.dvlpt :	2	-	1	3	1
- Sin. red blood cells:	5	2	2	1	2
- Agonal cong./hemorr.:	-	-	-	1	-
- Lymphangiectasis :	3	1	3	2	1
MESENT. LYMPH NODE :	49	50	50	50	50
- Sin. red blood cells:	2	2	3	2	1
- Lymphangiectasis :	-	1	-	-	-
- Fibrosis :	-	1	1	-	-
- Infiltr.; macroph. :	-	-	-	1	1
MESENTERY :	-	-	-	-	1
- Polyarteritis Nodosa:	-	-	-	-	1
OPTIC NERVES :	50	48	49	50	50
- Gliosis :	-	-	1	-	-

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	50	50	50	50	50
PANCREAS :	50	50	50	50	50
- Hyperpl.; acinar cel:	3	1	-	-	-
- Foc.;hypert.;basoph.:	2	-	2	1	-
- Atrophy; acinar cell:	9	8	6	9	7
- Degranul.; acin.cell:	1	-	-	-	-
- Hyperpl.; islet cell:	1	2	5	-	3
- Polyarteritis Nodosa:	1	-	1	1	-
- Infiltr.; mono.cells:	1	1	3	1	1
- Edema; interstitium :	-	-	-	1	-
- Inflamm.; adj.tissue:	-	-	1	-	-
- Necrosis; fat tissue:	-	-	-	1	-
PARATHYROID GLANDS :	47	43	45	46	44
- Hyperplasia; focal :	5	2	5	3	3
- Hyperplasia; diffuse:	-	-	1	-	-
PAROTID LYMPH NODES :	-	-	1	-	-
- Lymphoid hyperplasia:	-	-	1	-	-
PEYER'S PATCHES :	50	49	49	50	49
- Mineralization :	-	3	-	-	1
PITUITARY GLAND :	50	49	50	50	50
- Hyperpl.foc.p.distal:	8	9	7	4	8
- Hyperpl.foc.p.interm:	-	-	-	-	1
- Remnants; R. pouch :	-	-	-	-	1
- Hemorrhage :	-	-	2	1	-
- Cyst :	1	1	-	1	-
- Infiltr.; macroph. :	-	-	-	1	-
POPLITEAL LYMPH NODE :	3	5	12	8	1
- Inc. plasma cell nbr:	3	4	5	6	1
- Lymphoid hyperplasia:	1	5	8	2	-
- Sin. red blood cells:	-	1	-	-	-
PORTAL LYMPH NODES :	-	-	1	2	-
- Inc. plasma cell nbr:	-	-	-	1	-
- Sin. red blood cells:	-	-	-	1	-

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	50	50	50	50	50
PREPUTIAL GLANDS :	50	50	49	50	49
- Inflammation :	38	37	40	31	37
- Atrophy; lobular :	4	3	1	2	2
- Cystic distension :	1	1	1	3	1
PROSTATE :	50	49	50	50	50
- Hyperpl.; acin.cell :	-	-	1	-	-
- Inflammation :	39	37	38	45	38
- Atrophy; acinar :	2	-	1	3	2
- Infiltr.; mono.cells:	2	2	1	1	-
- Decr. secret.content:	1	2	-	1	-
- Dilation; gld lumen :	1	-	-	-	-
- Polyarteritis nodosa:	-	-	1	-	-
RENAL LYMPH NODES :	-	-	3	1	-
- Pigment :	-	-	1	-	-
- Sin. red blood cells:	-	-	-	1	-
- Inc. plasma cell nbr:	-	-	1	1	-
SALIVARY GLANDS :	49	49	49	50	49
- Atrophy; acinar cell:	-	1	-	-	-
- Inflam.; adj. tissue:	-	-	-	1	-
SCIATIC NERVE :	49	50	50	50	50
- Vacuolation; nerve :	-	1	-	-	-
SEMINAL VESICLES :	50	49	50	50	50
- Atrophy :	1	1	-	-	1
- Dilat.; coag.gld lu.:	-	1	-	-	1
- Dilation; gld lumen :	2	3	-	2	1
- Inflammation :	1	2	1	3	2
- Inflamm.; coag. gld :	-	-	1	1	4
- Infiltr.; mono.cells:	1	-	-	-	-
- Decr. secret.content:	3	3	4	3	4
SKELETAL MUSCLE :	49	50	50	50	50
- Inflammation;acute :	-	-	-	2	-
- Inflammation;chronic:	-	-	-	2	-
- Atrophy;myofiber :	2	2	2	3	3
- Fibrosis :	1	-	-	-	-

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	50	50	50	50	50
SKIN :	50	50	50	50	50
- Ulcer :	8	13	17	8	8
- Serocellular crust :	2	-	8	1	1
- Hyperkeratosis :	1	3	5	2	3
- Acanthosis :	1	1	3	-	3
- Inflammation :	1	3	5	2	4
- Fibrosis :	-	-	-	-	1
- Inflam. hair follic.:	1	1	1	2	1
- Atrophy; hair follic:	1	1	-	1	-
- Epidermal incl.cyst :	-	-	1	-	2
- Keratin cyst :	2	6	7	9	5
SPLEEN :	50	50	50	50	50
- Pigment; increased :	4	7	3	2	3
- Hemopoiesis; increa.:	9	6	9	11	5
- Lymphoid atrophy :	-	-	-	-	1
- Fibrosis :	-	-	-	-	1
- Necrosis :	1	-	-	-	-
- Infiltr.; macroph. :	-	-	-	2	-
- Congestion :	-	-	1	-	-
STOMACH :	50	50	50	50	50
- Erosion :	1	1	3	3	1
- Erosion/ulcer :	1	-	3	-	1
- Inflammation :	1	-	2	1	-
- Hemorrhage :	1	-	-	-	1
- Dilation; gland :	1	-	-	1	-
- Mineralization :	1	1	-	-	-
- Inflammat.;adj.tiss.:	1	-	-	-	-
- Polyarteritis Nodosa:	1	-	-	-	-
SUBCUTANEOUS TISSUE :	8	3	8	6	8
- Epidermal incl.cyst :	-	-	-	1	-
- Inflammation :	1	-	-	-	-
- Abscess :	-	-	1	1	1
TAIL :	1	1	1	-	-
- Inflammation :	1	1	1	-	-
TEETH :	1	2	-	1	1
- Developm.anomaly :	1	1	-	-	1

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	50	50	50	50	50
TESTES :	50	50	50	50	50
- Spermatids; swollen :	-	-	1	2	4
- Atrophy; Leydig cell:	-	-	-	-	1
- Deg./necr.; germ ce.:	-	-	1	-	-
- Necrosis; tubule :	-	-	1	-	-
- Hyper.;Leyd.c.; foc.:	-	2	-	2	2
- Atrophy/de.; tubular:	5	4	5	8	7
- Dilation; tub. lumen:	-	-	-	1	-
- Polyarteritis Nodosa:	-	-	1	-	-
- Inflamm.; vascular :	-	-	-	1	-
THYMUS :	50	49	50	50	50
- Lymphoid atrophy :	50	49	48	48	49
- Hemorrhage :	-	1	3	3	2
- Inflam.; adj. tissue:	1	-	1	-	-
- Cyst :	1	-	-	1	-
THYROID GLANDS :	49	49	50	49	49
- Hyperpl.foc.follic. :	1	2	2	1	1
- Hyperpl.diff. C cell:	12	14	14	11	14
- Hyperpl.foc. C cell :	2	5	1	4	4
- Dilated/cystic foll.:	-	1	1	-	1
- Ectopic thymus :	-	-	-	-	1
- Pigment :	2	-	-	1	-
- Fibrosis :	-	-	1	-	1
- Infiltr.; mono.cells:	1	-	-	-	-
TONGUE :	49	49	50	50	49
- Erosion/ulcer :	-	1	-	-	-
- Infiltrate;mon.inf.c:	-	-	-	-	1
- Granuloma :	1	-	-	-	-
- Polyarteritis nodosa:	-	-	1	-	-
TRACHEA :	50	50	50	50	49
- Inflamm.; adj.tissue:	-	1	-	-	-
URETERS :	1	-	-	3	-
- Dilation; lumen :	1	-	-	2	-
- Inflammation :	-	-	-	2	-
URINARY BLADDER :	50	49	50	49	50
- Hyperpl.; uroth. :	1	-	-	1	2
- Dilation; lumen :	3	-	-	4	-
- Inflamm.; muc. :	3	-	1	1	2
- Inflamm.; serosa :	-	-	1	1	-
- Hemorrh.; lumen :	-	-	1	-	-

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	50	50	50	50	50
ADIPOSE TISSUE :	-	-	-	2	-
- Degener./necrosis :	-	-	-	1	-
ADRENAL CORTICES :	50	50	50	50	50
- Hypertr.foc.cortic. :	6	6	9	5	8
- Hypertr.diff.cortic.:	2	2	1	3	2
- Hyperpl.; cortical :	7	3	7	5	4
- Cystic degeneration :	47	47	45	47	44
- Vacuolation; focal :	1	5	1	-	2
- Vacuol.; diffuse :	-	-	-	1	-
- Degener./necrosis :	1	-	1	1	-
- Atrophy; cortex :	-	1	1	-	-
- Congestion :	-	1	1	-	-
- Infiltr.; granuloc. :	-	-	-	-	1
- Infiltr.; mono.cells:	-	-	-	1	-
- Hemopoiesis :	2	2	-	-	1
ADRENAL MEDULLAS :	50	49	50	49	49
- Hyperplasia; focal :	3	3	6	7	3
AXILLARY LYMPH NODES :	2	1	1	-	2
- Inc. plasma cell nbr:	1	1	1	-	2
- Lymphoid hyperplasia:	2	1	-	-	-
BILE DUCT EXTRAHEP. :	-	-	-	-	1
- Dilated Lumen :	-	-	-	-	1
BONE :	-	1	-	1	-
- Callus :	-	1	-	-	-
- Degen./necrosis :	-	-	-	1	-
BONE MARROW :	50	50	50	50	50
- Increased cellulari.:	7	6	7	3	7
BRAIN :	50	50	50	50	50
- Dilatation;ventricle:	17	15	22	22	13
- Gliosis :	-	-	-	-	1
- Hemorrhage :	-	-	1	-	-
CECUM :	50	50	50	50	50
- Erosion/ulcer :	-	-	1	-	-
- Inflammation :	1	-	-	1	-

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	50	50	50	50	50
CLITORAL GLANDS :	49	48	48	48	49
- Keratin cyst :	-	-	1	-	-
- Inflammation :	38	33	26	31	29
- Atrophy; lobular :	-	1	-	-	-
- Infiltr.; macroph. :	-	-	-	1	-
- Mineralization :	1	-	-	1	-
- Cystic distension :	1	1	3	2	-
COLON :	50	50	50	50	50
- Erosion/ulcer :	-	-	1	-	-
- Inflammat.;adj.tiss.:	-	-	1	-	-
DUODENUM :	50	50	50	50	50
- Degen./necrosis :	-	-	-	-	1
- Polyarteritis Nodosa:	-	1	-	-	-
EARS :	-	1	1	2	-
- Chondropathy :	-	1	1	2	-
ESOPHAGUS :	50	50	50	50	50
- Inflammat.;adj.tiss.:	1	-	-	-	1
EYES :	50	50	50	50	50
- Inflamm.; conjunct. :	-	-	-	1	-
- Hemorrhage :	6	5	5	3	5
- Degeneration; lens :	1	-	1	1	-
- Retinal detachment :	-	-	-	1	-
- Periph.atrophy;reti.:	7	1	1	2	3
- Diffuse atroph.;ret.:	1	2	-	2	-
FEMUR :	50	50	50	50	50
- Granuloma :	-	1	-	-	-
- Inflammation; joint :	-	-	-	2	-
- Degeneration; joint :	-	2	1	2	-
- Fibrosis :	-	-	-	1	-
FORESTOMACH :	50	50	50	50	50
- Hyperplasia;squam.c.:	1	3	2	1	1
- Erosion/ulcer :	2	2	1	-	1
- Inflammation; mucos.:	1	2	2	-	1
- Cyst :	1	-	-	-	-

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	50	50	50	50	50
HARDERIAN GLANDS :	50	50	50	50	50
- Hyperpl.;acin.c.foc.:	-	-	-	-	1
- Incr.;porph.deposit.:	5	8	4	5	3
- Infiltr.; mono.cells:	2	4	3	6	5
HEART :	50	50	50	50	50
- Necr./fibr.; myofib.:	20	30	25	22	23
- Mineralization :	-	-	1	-	-
- Infiltr.; mono.cells:	1	1	-	-	1
HINDLEG :	-	-	-	1	2
- Inflammation :	-	-	-	1	2
ILEUM :	50	50	50	50	50
- Cyst :	-	-	-	-	1
ILIAC LYMPH NODES :	1	-	-	1	2
- Lymphoid hyperplasia:	1	-	-	-	1
- Inc. plasma cell nbr:	-	-	-	1	2
INGUINAL LYMPH NODES :	-	1	-	-	-
- Inc. plasma cell nbr:	-	1	-	-	-
KIDNEYS :	50	50	50	50	50
- Nephrop.;prog.; chr.:	21	24	29	25	27
- Hypertr.;tub.epith. :	-	1	-	-	1
- Hyal.drop.;tubul.ep.:	1	1	-	-	1
- Dilation; tub. :	-	-	-	-	1
- Infil.; gran.;pye.c.:	3	2	4	1	1
- Infiltr.; mono.cells:	2	2	1	2	-
- Inflamm.; papilla :	-	-	-	-	1
- Cyst :	3	-	1	1	1
- Hyperpl.; uroth. :	1	-	2	-	-
- Dilation; pelvis :	-	-	-	-	1
- Mineraliz.; cort. :	-	-	2	-	1
- Mineraliz.;papilla :	-	1	-	-	-
- Mineraliz.;pelvis :	9	12	10	10	3
- Mineraliz.;glom. :	-	-	-	-	1
- Leukocytosis :	-	-	1	-	-
- Polyarteritis Nodosa:	1	-	-	-	-
LARYNX :	50	50	50	50	50
- Inflamm.; adj.tissue:	-	-	1	-	2
- Hyperpl.; respirato.:	-	-	1	-	-

LIVER :	50	50	50	50	50
- Alt.c.focus;eosin. :	3	2	-	2	3
- Alt.c.focus;baso. :	31	28	33	31	27
- Alt.c.focus;clear c.:	16	17	20	20	19
- Fatty change :	-	1	1	1	1
- Alt.c.focus;vac.c. :	6	6	8	2	4
- Angiectasis :	3	6	9	4	7
- Focal necrosis :	1	1	1	2	1
- Single c.nec/apopt. :	-	1	-	-	-
- Deg/necr;hepatocyte :	2	2	4	1	4
- Inflammatory c.foci :	33	31	31	31	36
- Hyperplasia; bile d.:	22	21	19	14	22
- Vacuolation;peripor.:	13	11	10	11	11
- Vacuolation;centril.:	-	-	1	-	-
- Glycogen content :	-	1	3	1	2
- Hypertrophy;hepatoc.:	2	-	-	1	3
- Increased mitoses :	5	1	2	-	2
- Cytom/karyomegaly :	2	-	1	-	1
- Incr.cellul;sinusoid:	-	-	-	1	1
- Pigment :	2	-	1	2	-
- Fibrosis :	1	-	-	-	-
- Cystic degeneration :	-	1	-	1	2
- Hemopoiesis :	8	3	6	3	7
- Tension lipidosis :	5	2	7	4	3
- Deformation :	1	-	-	-	1
- Biliary cyst :	-	1	-	-	-
- Hemorrhage :	1	-	-	-	-
LUNGS :	50	50	50	50	50
- Granuloma :	2	2	3	1	5
- Inflam.;br-alv;acute:	-	-	-	-	1
- Inflam.;br-alv;chron:	1	-	1	1	-
- Foamy macr.;alveolus:	8	10	11	9	12
- Inflam.;alveol;acute:	1	1	-	-	1
- Inflam.;alveol;chron:	1	1	2	1	-
- Edema :	-	-	-	-	1
- Agonal cong./hemorr.:	4	2	3	3	2
- Hyperpl.; II pneumoc:	-	-	1	-	1
- Metaplasia; osseous :	-	-	1	-	2
- Metaplasia; chondro.:	-	-	-	1	-
- Cyst :	1	-	-	-	1
- Leukocytosis :	-	-	1	-	-
- Inflam.;perivascular:	-	-	-	2	-
- Thrombus :	-	-	1	-	1
- Polyarteritis Nodosa:	1	-	-	-	-
MAMMARY GLANDS AREA :	50	50	50	50	50
- Hyperpl.; atypical :	1	-	2	-	1
- Hyperpl.; lob.alv. :	3	3	4	3	2
- Dilation; ac.lumen :	7	6	6	3	6
- Dilation; duct :	22	16	21	21	17
- Inflamm.;interst. :	-	1	1	-	-
MANDIBUL. LYMPH NODE :	50	50	50	49	50
- Inc. plasma cell nbr:	48	49	50	49	50
- Dec.germ.cent.dvlpt :	1	6	6	6	2
- Sin. red blood cells:	2	1	5	2	2
- Lymphangiectasis :	-	2	1	-	-
MEDIAST.LYMPH NODES :	1	-	-	-	-
- Lymphoid hyperplasia:	1	-	-	-	-
- Sin. red blood cells:	1	-	-	-	-

MESENT. LYMPH NODE :	50	49	49	50	50
- Lymphoid atrophy :	-	1	-	-	1
- Sin. red blood cells:	3	1	-	1	2
- Lymphangiectasis :	-	-	-	-	1
- Fibrosis :	-	-	-	1	-
- Infiltr.; macroph. :	-	-	-	-	1

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	50	50	50	50	50
MESENTERY :	2	2	1	3	3
- Hyperplasia; mesoth.:	1	-	-	-	-
- Inflammation :	-	-	-	2	1
- Torsion :	-	-	-	1	-
- Polyarteritis Nodosa:	1	1	1	-	2
OPTIC NERVES :	50	50	50	49	49
- Gliosis :	-	-	-	1	-
- Degeneration; axon :	-	-	-	-	1
OVARIES :	50	50	50	50	50
- Hyper.; sex cord str:	9	7	4	7	5
- Cyst; follicular :	20	13	7	15	19
- Cyst :	6	15	11	9	10
- Dilated/cystic bursa:	4	3	2	3	-
- Atrophy :	-	-	-	-	1
PANCREAS :	50	50	50	50	50
- Hyperpl.; acinar cel:	-	1	-	1	-
- Foc.;hypert.;basoph.:	1	-	1	-	1
- Atrophy; acinar cell:	6	5	4	2	3
- Degranul.; acin.cell:	-	-	1	-	-
- Hyperpl.; islet cell:	1	1	1	1	1
- Polyarteritis Nodosa:	1	2	-	-	2
- Infiltr.; mono.cells:	2	1	2	2	2
- Inflamm.; adj.tissue:	1	-	-	-	1
PARATHYROID GLANDS :	46	46	47	46	47
- Hyperplasia; focal :	1	-	1	-	-
- Hyperplasia; diffuse:	-	-	-	-	1
- Agonal cong./hemorr.:	-	-	-	1	-
PEYER'S PATCHES :	46	48	50	48	48
- Mineralization :	2	3	2	3	1
PITUITARY GLAND :	50	49	50	50	49
- Hyperpl.diff.p.dist.:	3	1	1	-	-
- Hyperpl.foc.p.distal:	4	1	3	1	6
- Hemorrhage :	-	-	1	-	1
- Infiltr.; macroph. :	-	-	1	-	-
- Angiectasis :	2	1	2	-	1

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	50	50	50	50	50
POPLITEAL LYMPH NODE :	-	-	1	-	3
- Inc. plasma cell nbr:	-	-	-	-	3
- Lymphoid hyperplasia:	-	-	1	-	-
PORTAL LYMPH NODES :	1	-	-	-	-
- Lymphoid hyperplasia:	1	-	-	-	-
- Pigment :	1	-	-	-	-
SALIVARY GLANDS :	50	50	50	50	50
- Atrophy; acinar cell:	-	2	1	-	-
SKELETAL MUSCLE :	50	50	50	50	50
- Inflammation;chronic:	1	-	1	-	-
- Atrophy;myofiber :	2	-	-	-	-
SKIN :	50	50	50	50	50
- Ulcer :	3	2	4	3	5
- Serocellular crust :	3	3	1	1	-
- Hyperkeratosis :	3	5	4	3	2
- Acanthosis :	3	2	-	-	-
- Inflammation :	2	6	2	1	1
- Fibrosis :	-	1	-	-	-
- Inflam. hair follic.:	-	3	1	1	-
- Atrophy; hair follic:	1	2	1	3	2
- Keratin cyst :	5	8	1	7	7
SPINAL CORD :	49	50	50	50	49
- Pigment :	-	-	-	1	-
- Vacuolation; wh.mat.:	-	1	-	2	-
SPLEEN :	50	50	50	50	50
- Pigment; increased :	10	11	10	6	10
- Hemopoiesis; increa.:	12	11	13	15	13
- Lymphoid atrophy :	1	2	-	1	1
- Necrosis :	1	-	-	-	-
- Congestion :	-	2	-	-	-
- Hemosiderotic focus :	-	-	-	1	-
- Cyst :	-	1	-	-	-
- Adhesion :	-	-	-	1	-
- Inflamm.; adj.tissue:	1	-	-	-	-
- Abnormal shape :	1	-	-	-	-

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	50	50	50	50	50
STOMACH :	50	50	50	50	50
- Erosion :	1	2	2	2	-
- Erosion/ulcer :	-	2	-	1	-
- Hemorrhage :	-	-	-	-	1
- Inflammat.;adj.tiss.:	2	-	-	-	-
SUBCUTANEOUS TISSUE :	3	1	2	1	3
- Inflammation :	-	-	-	-	1
- Abscess :	1	-	-	-	-
TEETH :	1	-	3	2	2
- Developm.anomaly :	1	-	2	1	2
- Infiltr.;granuloc. :	1	-	1	1	-
THYMUS :	50	49	50	50	50
- Hyperplasia; epithe.:	1	1	1	-	-
- Lymphoid atrophy :	49	48	50	50	49
- Hemorrhage :	-	1	-	2	2
- Inflam.; adj. tissue:	-	-	-	1	-
- Cyst :	1	3	4	3	1
THYROID GLANDS :	50	50	50	50	50
- Hyperpl.foc.follic. :	-	-	-	1	-
- Hyperpl.diff. C cell:	19	18	15	21	18
- Hyperpl.foc. C cell :	7	5	5	1	4
- Dilated/cystic foll.:	-	1	2	1	-
- Ectopic thymus :	1	-	-	-	-
TONGUE :	50	50	50	50	50
- Polyarteritis nodosa:	1	-	-	-	-
TRACHEA :	50	50	50	50	50
- Degen.; epithelium :	-	-	-	-	1
- Inflammation :	-	-	-	-	1
- Inflamm.; adj.tissue:	-	-	-	-	1
URINARY BLADDER :	50	50	50	50	50
- Ulcer; urothelium :	1	-	-	-	1
- Dilation; lumen :	-	1	2	1	2
- Inflamm.; muc. :	-	-	-	-	1
- Vacuol.; uroth. :	1	-	-	-	-
UTERUS :	50	50	50	50	50
- Hyperp.; granular c.:	-	-	1	-	-
- Hypert.;strom.;cerv.:	1	1	-	2	2
- Hyperpl.; endometr. :	1	-	-	-	1
- Keratin cyst :	-	-	-	-	1
- Metapl.; squam. cell:	2	4	-	3	13
- Hyperpl.; fib./musc.:	-	-	1	-	-
- Atrophy :	-	-	-	-	1
- Hemorrhage :	-	-	-	1	-
- Dilated/cystic gland:	3	8	6	8	4
- Dilated lumen :	3	1	5	4	5
- Cyst :	1	1	-	-	1
- Hemangiectasis :	-	-	-	-	1
- Polyarteritis Nodosa:	1	-	-	-	-
VAGINA :	50	50	50	50	49
- Hyperp.; granular c.:	3	-	2	1	2
- Mucific.; epithelium:	17	25	21	26	16
- Exudates; lumen :	3	4	5	3	2

Appendix 5: Neoplastic lesions in male and female SD-rats (Applicant's table from Study# GT-157-TX-38

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. DEATHS

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	50	50	50	50	50
ADIPOSE TISSUE :	1	1	1	2	1
- LIPOSARCOMA :	-	-	-	1	-
- LIPOMA :	1	-	-	-	-
ADRENAL CORTICES :	50	50	50	50	50
- ADENOMA; CORTICAL :	4	3	5	2	-
ADRENAL MEDULLAS :	49	50	50	50	50
- PHEOCHROM.; MALIGN. :	2	2	-	-	1
- PHEOCHROM.; BENIGN :	5	5	2	3	4
BONE :	-	1	-	1	-
- OSTEOSARCOMA :	-	1	-	-	-
- METASTATIC SARCOMA :	-	-	-	1	-
BONE MARROW :	50	50	50	50	50
- METASTATIC SARCOMA :	-	-	-	1	-
BRAIN :	50	50	50	50	50
- ASTROCYTOMA; MALIGN.:	2	-	-	3	1
- ASTROCYTOMA; BENIGN :	-	-	-	-	1
- MENINGIOMA; BENIGN :	-	-	-	1	-
EARS :	11	6	5	5	3
- NEURAL CREST NEOPLA.:	1	-	-	-	-
FORESTOMACH :	50	50	50	50	50
- TUMOR;BASAL C.;BEN. :	1	-	-	-	-
- HEMANGIOMA :	1	-	-	-	-
HEART :	50	50	50	50	50
- SCHWANN. ENDOC. BEN.:	1	-	-	-	-
HEMOLYMPHORET. SYS. :	50	50	50	50	50
- LEUKEMIA; GRANULOCYT:	-	-	2	1	-
- LYMPHOMA; MALIGNANT :	-	-	-	1	-
- SARCOMA; HISTIOCYTIC:	1	1	1	1	1

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	50	50	50	50	50
KIDNEYS :	50	50	50	50	50
- CARCINOMA; TUBULE :	-	1	-	-	-
- ADENOMA; TUBULE :	-	-	1	-	-
- PAPIL.; TRANSIT.CELL:	-	-	-	1	-
- HEMANGIOMA :	-	1	-	-	-
LIVER :	50	50	50	50	50
- CARCINOMA;HEPATOCEL.:	2	1	3	1	1
- ADENOMA;HEPATOCELL. :	1	-	3	-	3
LUNGS :	50	50	50	50	49
- CARCIN.;BRONCHIO-ALV:	-	-	1	-	-
- ADENOMA;BRONCHIO-ALV:	-	-	-	1	-
- METASTASIS; SARCOMA :	-	1	-	2	-
MAMMARY GLANDS AREA :	-	-	3	3	1
- ADENOCARCINOMA :	-	-	-	1	1
- FIBROADENOMA :	-	-	1	1	-
- ADENOLIPOMA :	-	-	-	1	-
MANDIBUL. LYMPH NODE :	49	48	49	49	48
- METASTATIC SARCOMA :	-	1	-	-	-
MESENT. LYMPH NODE :	49	50	50	50	50
- LYMPHANGIOMA :	1	-	-	-	-
- HEMANGIOMA :	-	2	-	1	-
PANCREAS :	50	50	50	50	50
- ADENOCARC.; ACIN.CEL:	-	1	-	-	-
- ADENOMA; ACINAR CELL:	1	-	-	-	-
- ADENOMA; ISLET CELL :	1	4	-	4	2
PARATHYROID GLANDS :	47	43	45	46	44
- ADENOMA :	1	2	3	2	2
PERITONEUM :	1	-	1	-	1
- SARCOMA; NOS :	1	-	-	-	-
PITUITARY GLAND :	50	49	50	50	50
- ADENOMA; P. DISTALIS:	21	28	23	25	17
- ADENOMA; P. INTERM. :	-	-	-	-	1

SEX :					MALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	50	50	50	50	50
POPLITEAL LYMPH NODE :	3	5	12	8	1
- METASTATIC SARCOMA :	1	-	-	-	-
PROSTATE :	50	49	50	50	50
- ADENOCARCINOMA :	-	1	-	-	-
- FIBROSARCOMA :	-	1	-	-	-
- ADENOMA :	-	1	-	-	-
SALIVARY GLANDS :	49	49	49	50	49
- ADENOCARCINOMA :	-	-	1	-	-
SKIN :	50	50	50	50	50
- CARCINOMA; SQUAMOUS :	-	-	-	1	-
- BASAL CELL TUM. MAL.:	-	-	1	1	-
- BASAL CELL TUM. BEN.:	-	1	1	-	-
- PAPILLOMA; SQUAMOUS :	-	1	-	-	-
- KERATOACANTHOMA :	3	3	-	5	3
- ADENOMA; SEBACEOUS :	1	-	-	-	1
- FIBROMA :	-	2	1	2	4
- HAIR FOLL. TUM. BEN.:	1	1	1	1	2
- HEMANGIOMA :	1	-	-	-	-
SPLEEN :	50	50	50	50	50
- HEMANGIOSARCOMA :	-	-	1	-	-
- FIBROSARCOMA :	-	1	-	-	-
SUBCUTANEOUS TISSUE :	8	3	8	6	8
- FIBROSARCOMA :	1	-	-	-	2
- HISTIOC; FIBROUS;MAL:	1	-	-	1	-
- LIPOSARCOMA :	-	-	-	-	1
- SARCOMA NOS :	2	-	3	1	1
- FIBROMA :	1	1	3	1	3
- LIPOMA :	1	1	1	1	-
TEETH :	1	2	-	1	1
- ODONTOMA :	-	-	-	1	-
TESTES :	50	50	50	50	50
- ADENOMA; LEYDIG CELL:	3	-	-	2	3
THYMUS :	50	49	50	50	50
- MALIGNANT THYMOMA :	-	-	-	-	1
THYROID GLANDS :	49	49	50	49	49
- CARCINOMA; FOLLIC. :	-	2	2	-	1
- CARCINOMA; C CELL :	-	1	-	-	-
- ADENOMA; FOLLICULAR :	-	4	1	-	-
- ADENOMA; C CELL :	4	3	5	2	-

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	50	50	50	50	50
ADIPOSE TISSUE :	-	-	-	2	-
- HEMANGIOMA :	-	-	-	1	-
ADRENAL CORTICES :	50	50	50	50	50
- ADENOMA; CORTICAL :	2	2	4	3	1
ADRENAL MEDULLAS :	50	49	50	49	49
- PHEOCHROM.; BENIGN :	1	2	1	-	-
- PHEOCH.COMPLEX BEN. :	-	-	1	-	-
BRAIN :	50	50	50	50	50
- ASTROCYTOMA; MALIGN.:	1	-	-	-	-
- MENINGIOMA; MALIGN. :	-	-	-	1	-
- ASTROCYTOMA; BENIGN :	1	-	-	-	-
- METASTATIC CARCINOMA:	-	-	-	1	-
CLITORAL GLANDS :	49	48	48	48	49
- ADENOMA :	-	-	-	1	-
DUODENUM :	50	50	50	50	50
- LEIOMYOSARCOMA :	-	-	-	-	1
EYES :	50	50	50	50	50
- MELANOMA :	1	-	-	-	-
FORESTOMACH :	50	50	50	50	50
- TUMOR;BASAL C.;BEN. :	-	-	1	-	-
HEMOLYMPHORET. SYS. :	50	50	50	50	50
- SARCOMA; HISTIOCYTIC:	1	1	1	-	-
LARYNX :	50	50	50	50	50
- CARCINOMA; SQUAMOUS :	-	-	-	1	-
LIVER :	50	50	50	50	50
- CARCINOMA;HEPATOCEL.:	-	-	-	-	1
- ADENOMA;HEPATOCELL. :	2	6	3	5	1
LUNGS :	50	50	50	50	50
- METASTASIS; CARCIN. :	-	-	-	1	-

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	50	50	50	50	50
MAMMARY GLANDS AREA :	50	50	50	50	50
- ADENOCARCINOMA :	7	9	15	11	9
- ADENOCARC. IN FIBRO.:	2	3	4	1	1
- TUM.; MIX.; MAL. :	1	1	-	-	-
- ADENOMA :	-	-	1	2	2
- FIBROADENOMA :	18	20	20	20	20
- FIBROMA :	2	-	-	-	1
MANDIBUL. LYMPH NODE :	50	50	50	49	50
- METASTATIC SARCOMA :	-	1	-	-	-
MESENT. LYMPH NODE :	50	49	49	50	50
- LYMPHANGIOMA :	-	-	-	1	-
OVARIES :	50	50	50	50	50
- TUM.; GRANUL.; MAL. :	-	-	-	-	1
- TUM.; SERTOLI.; MAL.:	-	-	1	-	-
- TUM.; SERTOLI.; BEN.:	1	-	-	-	-
PANCREAS :	50	50	50	50	50
- ADENOMA; ACINAR CELL:	-	-	-	-	1
- ADENOMA; ISLET CELL :	3	-	-	-	-
PITUITARY GLAND :	50	49	50	50	49
- CARCINOMA; P. DISTAL:	-	1	-	1	-
- ADENOMA; P. DISTALIS:	33	39	40	42	33
SALIVARY GLANDS :	50	50	50	50	50
- ADENOCARCINOMA :	-	-	-	1	-
SKIN :	50	50	50	50	50
- BASAL CELL TUM. MAL.:	-	-	1	-	-
- PAPILLOMA; SQUAMOUS :	1	1	1	1	-
- KERATOACANTHOMA :	1	1	1	-	-
SPINAL CORD :	49	50	50	50	49
- GLIOMA; N.O.S.;MAL. :	-	1	-	-	-
SUBCUTANEOUS TISSUE :	3	1	2	1	3
- HISTIOC; FIBROUS;MAL:	1	-	-	-	1
- SARCOMA NOS :	-	-	1	-	-
- LIPOMA :	1	1	1	1	1

SEX :					FEMALE
DOSE GROUP:	1	2	3	4	5
NO.ANIMALS:	50	50	50	50	50
THYMUS :	50	49	50	50	50
- MALIGNANT THYMOMA :	1	-	-	-	-
- BENIGN THYMOMA :	-	1	-	-	1
THYROID GLANDS :	50	50	50	50	50
- CARCINOMA; FOLLIC. :	-	1	-	-	-
- CARCINOMA; C CELL :	1	-	-	-	1
- ADENOMA; FOLLICULAR :	-	-	-	1	-
- ADENOMA; C CELL :	4	5	3	2	3
URINARY BLADDER :	50	50	50	50	50
- LEIOMYOMA :	-	1	-	-	-
- METASTASIS; CARCIN. :	-	-	-	-	1
UTERUS :	50	50	50	50	50
- SARCOMA; ENDOM.STRO.:	-	1	1	-	2
- ADENOCAR.; ENDOMETR.:	-	1	-	-	-
- CARCINOMA; ADENOSQU.:	-	-	-	-	1
- POLYP; ENDOM. STROMA:	5	3	4	3	4
- TUM.; GRANULAR C. B.:	2	-	-	-	-
VAGINA :	50	50	50	50	49
- TUM.; GRANULAR C. B.:	5	5	7	1	2
- POLYP; STROMAL :	1	2	2	-	4
- PAPILLOMA; SQUA.CELL:	-	1	-	-	-

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/s/

SRUTHI T KING
04/15/2014

SUSHANTA K CHAKDER
04/15/2014

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 205494 **Applicant: Genzyme Corporation** **Stamp Date: 9/20/13**

Drug Name: Eliglustat (Cerdelga™/Genz-112638) **NDA Type: New NDA** **Submit Date: 9/19/13**

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	√		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	√		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	√		
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)?	√		
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).			N/A
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 submitted a rationale to justify the alternative route?	√		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	√		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	√		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	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?	√		The proposed labeling sections relevant to nonclinical studies may need to be revised during the labeling review.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	√		
11	Has the applicant addressed any abuse potential issues in the submission?			N/A
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A

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

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

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter. **None**

Tamal K. Chakrabortit, Ph.D. — October 15, 2013
Reviewing Pharmacologist — Date

Sushanta K. Chakder, Ph.D. — October 15, 2013
Supervisor — Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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/s/

TAMAL K CHAKRABORTI
10/15/2013

SUSHANTA K CHAKDER
10/15/2013