CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208854Orig1s000

PHARMACOLOGY REVIEW(S)

Comments on NDA 208854 naldemedine

From: A. Jacobs, AD
Date: 11/18/16

1. I concur that there are no pharm-tox related approval issues.
2. I have conveyed some other comments to the reviewer and they will be addressed as appropriate.

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
11/22/2016

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	NDA 208854
Supporting document/s:	SND 01
	SND 13
Applicant's letter date:	SND 01: March 23, 2016
	SND 13: July 15, 2016
CDER stamp date:	SDN 01: March 23, 2016
	SDN 13: July 15, 2016
Product:	Symproic™ (naldemedine tosylate) oral tablets
Indication:	Opioid-induced constipation (OIC) in adults with chronic non-cancer pain
Applicant:	Shionogi Inc.
Review Division:	Division of Gastroenterology and Inborn Errors Products (DGIEP)
Reviewer:	Tracy Behrsing, Ph.D.
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TABLE OF CONTENTS

1 Executive Summary

1.1 Introduction

Symproic™ (naldemedine tosylate; naldemedine) is an opioid antagonist that Shionogi Inc. seeks approval to market for the treatment of opioid-induced constipation (OIC) in adults with chronic non-cancer pain.

1.2 Brief Discussion of Nonclinical Findings

Naldemedine is an opioid antagonist, with Ki values for human mu, delta, and kappa opioid receptors of 0.34, 0.43, and 0.94 nM, respectively. Relative to naldemedine, each of the metabolites studied were less potent or had no binding affinities for mu, delta, and kappa receptors, although the Ki value of naldemedine 6-O-β-D-glucuronide for delta receptors (0.51 nM) approached that of the parent compound. While nor-naldemedine (a major metabolite in humans) showed weak agonistic activity for delta receptors, the EC_{50} value was approximately 340 times the human C_{max} for this metabolite at the recommended human dose of 0.2 mg once daily. In primary pharmacology studies in rats, naldemedine prevented morphine- and oxycodone-induced suppression of small intestinal transit (ED_{50} values of 0.02 – 0.23 mg/kg) and antagonized the inhibitory effect of morphine on castor oil-induced diarrhea (ED_{50} value of 0.01 mg/kg).

Repeat-dose toxicity studies with naldemedine were conducted in multiple species, with durations of up to 6 months in rats, and 9 months in dogs. In the 6-month toxicity study in rats, systemic exposures (AUC) at the NOAEL (100 mg/kg/day) was approximately 3600 times that in humans at the recommended human dose. In the 9-month toxicity study in dogs, systemic exposures (AUC) at the NOAEL (4 mg/kg/day) were approximately 345 times that in humans at the recommended human dose. In 1-month toxicity studies in rats, prolongation of diestrous occurred at doses as low as 0.3 mg/kg/day. While a NOEL for this effect (which may be related to treatment-related increases in prolactin) was not identified, recovery of the estrous cycle generally occurred even during the dosing period.

Naldemedine was negative in a bacterial reverse mutation test, a chromosomal aberration test using cultured Chinese hamster lung cells, and a rat micronucleus assay. In oral carcinogenicity studies of up to 2 years in duration, there were no drug-related neoplasms when naldemedine was orally administered to mice and rats at doses up to 100 mg/kg/day (approximately 17,500 times and 6,300 times the human AUC at the recommended human dose, respectively).

In male and female rats, there were no adverse effects on fertility or reproductive performance following oral administration of naldemedine at doses up to 1000 mg/kg/day (approximately 17,000 times the human AUC at the recommended dose). In females, irregular estrous cycles (prolongation of diestrous phase) and low number of estrous cases occurred at ≥10 mg/kg/day (approximately 179 times the human AUC at the recommended dose). However, the irregular estrous cycles recovered during the

pre-mating or mating period, and there was successful copulation with males except for a single female at 1000 mg/kg/day. In an oral embryofetal development study in rats with naldemedine, there were no effects on embryofetal development at doses up to 1000 mg/kg/day (the highest dose tested; approximately 23,000 times the human AUC at the recommended dose). In an oral embryofetal development study in rabbits with naldemedine, the NOAEL was <25 mg/kg/day for maternal toxicity and 100 mg/kg/day for embryofetal development (approximately 266 times the human AUC at the recommended dose). Effects in maternal animals at 400 mg/kg/day (approximately 844 times the human AUC at the recommended dose) included body weight loss / decreased body weight gain and food consumption, fetal loss, and premature delivery. Decreased fetal body weights also occurred at 400 mg/kg/day, and may be related to the observed maternal toxicity at this dose. In a pre- and postnatal development study in which pregnant rats were administered naldemedine at oral doses of 1, 30, and 1000 mg/kg/day, the NOAEL for maternal and developmental toxicity was 1 mg/kg/day (approximately 12 times the human AUC at the recommended dose). At doses greater than or equal to 30 mg/kg/day (approximately 626 times the human AUC at the recommended dose), there were effects on maternal body weight and food consumption, scattering of offspring in the cages (suggestive of poor nursing), and total litter loss. In F1 pups, there were decreases in the viability index on lactation day 4 at doses greater than or equal to 30 mg/kg/day and decreased body weights and delayed pinna unfolding at 1000 mg/kg/day.

1.3 Recommendations

1.3.1 Approvability

No nonclinical approvability issues have been identified.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

In response to high-level content and format labeling comments from FDA in the June 3, 2016 Filing Communication - No Filing Review Issues Identified, the Applicant submitted proposed revised text for the label (SDN 13). Nonclinical review of the Applicant's proposed Established Pharmacologic Class and text for sections 8.1, 8.2, 12.1, and 13.1 are provided below.

Applicant's Proposed Version (SDN 13):
8.1 Pregnancy
Risk Summary

(b) (4)

(b) (4)

Evaluation:
The proposed text should be modified as recommended below. Exposure comparisons for doses in the prenatal and postnatal development study in rats (relative to the human AUC value) were based on AUC values from a one-month repeat dose toxicity study in rats (Report No. R-297995-TB-048-L) and a supplemental one-month repeat dose toxicity study in rats (Report No. R-297995-TB-091-L).

Recommended Version:
8.1 Pregnancy

Risk Summary

There are no available data with naldemedine in pregnant women to inform a drug-associated risk of major birth defects and miscarriage. There is a potential for opioid withdrawal in a fetus when SYMPROIC is used in pregnant women *[see Clinical Considerations].* SYMPROIC should be used during pregnancy only if the potential benefit justifies the potential risk.

In a rat embryo-fetal development study, following oral administration of naldemedine during the period of organogenesis at doses resulting in systemic exposure approximately 23,000 times the human AUC at the recommended human dose of 0.2 mg/day, no developmental abnormalities were observed. In rabbits, there were no adverse effects on embryo-fetal development following oral administration of naldemedine during the period of organogenesis at doses resulting in systemic exposure approximately 226 times the human AUC at the recommended human dose of 0.2 mg/day *[see Data].* No effects on pre- and postnatal development were observed in rats at exposures 12 times human exposures at the recommended human dose.

The estimated background risk of major birth defects and miscarriage for the indicated population is unknown. All pregnancies have a background risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15- 20%, respectively.

Clinical Considerations

Fetal/Neonatal Adverse Reactions

Naldemedine crosses the placenta, and may precipitate opioid withdrawal in a fetus due to the immature fetal blood brain barrier. (b) (4)

(b) (4)

Data

Animal Data

In rats, there were no adverse effects on embryo-fetal development following oral administration of naldemedine during the period of organogenesis at doses up to 1000 mg/kg/day (approximately 23,000 times the human exposures (AUC) at the recommended human dose). In rabbits, there were no adverse effects on embryo-fetal development following oral administration of naldemedine during the period of organogenesis at doses up to 100 mg/kg/day (approximately 226 times the human exposures (AUC) at the recommended human dose). At 400 mg/kg/day (approximately 844 times the human exposures (AUC) at the recommended human dose), effects in maternal animals included body weight loss / decreased body weight gain and food

consumption, fetal loss, and premature delivery. Decreased fetal body weights at this dose may be related to the maternal toxicity observed.

In the pre- and postnatal development study, pregnant rats were administered naldemedine at oral doses up to 1000 mg/kg/day from gestation day 7 through lactation day 20. No effects on pre- and postnatal development were observed in rats at 1 mg/kg/day (approximately 12 times the human exposures (AUC) at the recommended human dose). A single dam died at parturition at 1000 mg/kg/day, and decreased body weights / body weight gain and food consumption, poor nursing, and total litter loss were noted at 30 and 1000 mg/kg/day (approximately 626 and 17,000 times the human exposures (AUC) at the recommended human dose, respectively). Decreases in the offspring viability index on Day 4 after birth were noted at 30 and 1000 mg/kg/day, and low body weights and delayed pinna unfolding in pups were noted at 1000 mg/kg/day.

Applicant's Proposed Version (SDN 13):
8.2 Lactation

(b) (4)

Evaluation:
The proposed text should be modified to include a section describing the nonclinical study that evaluated excretion into milk.

Recommended Version:
8.2 Lactation

Risk Summary
There is no information regarding the presence of naldemedine in human milk, the effects on the breastfed infant, or the effects on milk production. Naldemedine was present in the milk of rats *[see Data].* Because of the potential for serious adverse reactions, including opioid withdrawal in (b) (4) infants, a decision should be made to discontinue (b) (4) or discontinue the drug, taking into account the importance of the drug to the mother.

Data
Drug-related radioactivity was transferred into milk of lactating rats following a single oral dose of 1 mg/kg [carbonyl-^{14}C]-naldemedine (b) (4)

(b) (4)

Applicant's Proposed Version (SDN 13):
12.1 Mechanism of Action

Naldemedine is an antagonist of opioid binding at the mu-, delta-, and kappa-opioid receptors. Naldemedine functions as a peripherally-acting mu-opioid receptor antagonist in tissues such as the gastrointestinal tract, thereby decreasing the constipating effects of opioids without reversing the central nervous system (CNS)-mediated opioid effects.

Naldemedine is a derivative of naltrexone to which a side chain has been added that increases the molecular weight and the polar surface area, thereby reducing its ability to cross the blood-brain barrier (BBB); the CNS penetration of naldemedine is expected to be negligible at the recommended dose. Additionally, naldemedine is a substrate of the P-glycoprotein (P-gp) efflux transporter, which may also be involved in reducing naldemedine penetration into the CNS. Based on this, naldemedine is expected to exert its anti-constipating effects on opioids without reversing their CNS-mediated analgesic effects.

Evaluation:
The proposed text should be modified as recommended below.

Recommended Version:
12.1 Mechanism of Action
Naldemedine is an opioid antagonist with binding affinities for mu-, delta-, and kappa-opioid receptors. Naldemedine functions as a peripherally-acting mu-opioid receptor antagonist in the gastrointestinal tract, thereby decreasing the constipating effects of opioids. Naldemedine is a derivative of naltrexone to which a side chain has been added that increases the molecular weight and the polar surface area, thereby reducing its ability to cross the blood-brain barrier (BBB); (b) (4) (b) (4), naldemedine is a substrate of the P-glycoprotein (P-gp) efflux transporter, (b) (4)

Applicant's Proposed Version
13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility
Carcinogenesis
(b) (4)

Mutagenesis
Naldemedine was not genotoxic in the in vitro bacterial reverse mutation (Ames) assay, a chromosomal aberration assay with cultured Chinese hamster lung cells, and an in vivo micronucleus assay with rat bone marrow cells.

Impairment of Fertility

Naldemedine was found to have no effect on fertility or reproductive performance in male and female rats at oral doses up to 1000 mg/kg/day (b) (4) times the human AUC (b) (4) at the recommended human dose). (b) (4) prolongation of diestrous pha was noted at 10 mg/kg/day (b) (4) 179 times the human AUC (b) (4) at the recommended human dose.

Evaluation:
The proposed text should be modified as recommended below. Exposure comparisons for doses in the fertility and early embryonic development study (relative to the human AUC value) were based on AUC values from a one-month repeat dose toxicity study in rats (Report No. R-297995-TB-048-L) and a supplemental one-month repeat dose toxicity in rats (Report No. R-297995-TB-091-L).

Recommended Version:
13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility
Carcinogenesis
In 2-year carcinogenicity studies, there were no drug-related neoplastic findings following oral administration of naldemedine to mice and rats at doses up to 100 mg/kg/day (approximately 17,500 and 6,300 times the human exposures (AUC) at the recommended human dose, respectively).

Mutagenesis
Naldemedine was not genotoxic in the in vitro bacterial reverse mutation (Ames) assay, a chromosomal aberration assay with cultured Chinese hamster lung cells, and an in vivo (b) (4) micronucleus assay.

Impairment of Fertility
Naldemedine was found to have no effect on fertility or reproductive performance in male and female rats at oral doses up to 1000 mg/kg/day (approximately 17,000 times the human exposures (AUC) at the recommended human dose). In female rats, prolongation of diestrous phase was noted at 10 mg/kg/day (approximately 179 times the human exposures (AUC) at the recommended human dose).

2 Drug Information

2.1 Drug

Symproic™

CAS Registry Number: 1345728-04-2 (tosylate), 916072-89-4 (free base)

Generic Name: naldemedine tosylate

Code Name: S-297995B (tosylate), S-297995 (free base)

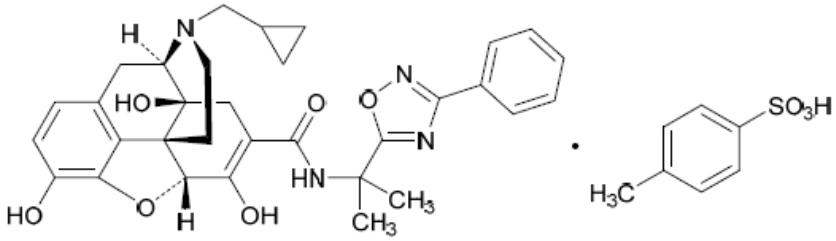
Chemical Name:

Tosylate: 17-(cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-3,6,14-trihydroxy-N-[2-(3-phenyl-1,2,4-oxadiazol-5-yl)propan-2-yl]morphinan-7-carboxamide 4-methylbenzenesulfonic acid

Free Base: 17-(cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy- 3,6,14-trihydroxy-*N*-[2-(3-phenyl-1,2,4-oxadiazol-5-yl)propan-2-yl]morphinan-7-carboxamide

Molecular Formula/Molecular Weight: $C_{32}H_{34}N_4O_6 \cdot C_7H_8O_3S$ / 742.84 (570.64 as free base)

Structure or Biochemical Description



Pharmacologic Class: opioid antagonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

- IND 107475 (Shionogi, Inc.: Treatment of opioid-induced constipation)

(b) (4)

2.3 Drug Formulation

Naldemedine tablets 0.2 mg are an immediate release oral formulation containing 0.26 mg of naldemedine tosylate (equivalent to 0.2 mg naldemedine). The components and composition of the tablets are shown in the Applicant's table below.

Table 1 Components and Composition of Naldemedine Tablets 0.2 mg

Component	Amount per Tablet (mg/tablet)	Function	Quality Standard
Naldemedine Tosylate [a]	0.26 (b) (4)	Active Ingredient	In-house standard
D-Mannitol	(b) (4)		USP/Ph.Eur./JP
Croscarmellose Sodium			NF/Ph.Eur./JP
Magnesium Stearate			NF/Ph.Eur./JP
			(b) (4)

a Equivalent to 0.2 mg of naldemedine.

(b) (4)

2.4 Comments on Novel Excipients

Mannitol, croscarmellose sodium, and magnesium stearate are compendial, listed in FDA's Inactive Ingredients Database, and used in approved oral drug products at levels that exceed the estimated maximum daily intake from naldemedine tablets at the proposed dose of 0.2 mg once daily.

(b) (4)

2.5 Comments on Impurities/Degradants of Concern

Drug Substance: Impurities classified as related substances included in the drug substance specification are as follows: (1) (b) (4) not more than (NMT) (b) (4) %, (2) (b) (4) NMT (b) (4) %, (3) Individual Others NMT (b) (4) %, and Total NMT (b) (4) %. The ICH Q3A identification and qualification thresholds for drug substances with a maximum daily dose ≤2 g/day are 0.10% or 1.0 mg/day (whichever is lower) and 0.15% or 1.0 mg/day

(whichever is lower), respectively. Thus, based on the recommended human dose of 0.26 mg naldemedine tosylate (equivalent to 0.2 mg naldemedine free base), the proposed specifications for specified impurities (b) (4) and unspecified impurities meet ICH Q3A limits.

(b) (4)

Actual and potential impurities formed during the manufacturing process were evaluated for mutagenic potential through screening with Derek Nexus (Derek Nexus 4.0.6; Nexus 1.7.6) and CASE Ultra ver. 1.5.0.1 software and experimental bacterial mutagenicity data. According to the Applicant, potential and actual mutagenic impurities arising from (b) (4) (b) (4) as shown in the Applicant's table below. Among these six impurities, the Applicant conducted a bacterial mutagenicity study for impurity (b) (4) Specifically, in Study No. (b) (4) was shown to test positive for bacterial mutagenicity, thereby confirming the positive prediction by the CASE Ultra software (mutagenicity study reviewed under Section 7.4 Other Genetic Toxicity Studies).

Table 2 List of Potential Mutagenic Impurities Arising from the Synthesis

Name	Structure [1]	Class [2]	Control Strategy [2]	Origin
(b) (4)				

The Applicant stated that the maximum exposure to mutagenic impurities was limited to the Threshold of Toxicological Concern of (b) (4) mcg/day for individual mutagenic impurities and (b) (4) mcg/day for total mutagenic impurities, which corresponds to limits of (b) (4) % for individual and total mutagenic impurities, respectively, based on a naldemedine tosylate dose of 0.26 mg/day. Three tosylate impurities (b) (4) can be determined by the related substances method. Therefore, while not included as specified impurities, the tosylate impurities are controlled by the drug substance specification of NMT (b) (4) % for unspecified impurities. (b) (4) are controlled as specified impurities (NMT (b) (4)
The Applicant states that the control strategy for (b) (4) was based on the establishment of a critical process parameter (b) (4) as well as historical batch data and spiking and purging data. Therefore, the Applicant did not propose a specification for (b) (4) in the final drug substance.

In addition to the six impurities identified in the Applicant's Table 2 above (List of Potential Mutagenic Impurities Arising from the Synthesis), the following impurities were predicted to be positive for mutagenicity by Case Ultra and/or plausible or equivocal for mutagenicity by Derek Nexus: (b) (4) The Applicant submitted in vitro bacterial mutagenicity studies for the following compounds: (b) (4) (mutagenicity studies reviewed under Section 7.4 Other Genetic Toxicity Studies). Based on negative experimental data in the submitted in vitro mutagenicity studies, these impurities were treated as non-mutagenic impurities. The remaining compounds identified as potential mutagens by in silico prediction were ultimately considered as non-mutagenic impurities since the alerting structures in those impurities were the same as in compounds which were tested and were non-mutagenic.

With respect to (b) (4) impurities, (b) (4) in the synthesis of the drug substance) were identified as potential impurities. The drug substance specification does not specify limits for any (b) (4) impurities, but instead includes a specification for residue on ignition of NMT (b) (4) % which the Applicant states will control (b) (4) impurities. Based on the recommended human dose (0.26 mg naldemedine tosylate; equivalent to 0.2 mg naldemedine free base), the proposed specification of (b) (4) % corresponds to a maximum daily intake of (b) (4) mcg/day. According to (b) (4), the permitted daily exposure (PDE) level for (b) (4) mcg/day. (b) (4) does not include a PDE level for (b) (4) and indicates that for (b) (4) for which PDEs were not established, if these (b) (4) impurities are present or included in the drug product they are addressed by other guidances and/or regional regulations and practices that may be applicable for (b) (4) The Agency for (b) (4) has derived intermediate and chronic duration oral minimal risk levels (MRLs)

for [redacted] that are both [redacted] mg/kg/day (or [redacted] mg/day for a 50 kg adult). The estimated maximum daily intake of [redacted] mcg/day) is substantially lower than the oral MRLs, and therefore exposure to [redacted] from the drug substance does not pose a safety concern.

Drug Product:
Four degradation products arising from the drug substance were identified by the Applicant: [redacted] The proposed specifications for [redacted] individual other impurities, and total impurities are NMT [redacted]%, respectively. [redacted] is controlled as part of individual other impurities. The ICH Q3B identification and qualification thresholds for drug substances with a maximum daily dose ≤1 mg/day are 1.0% or 5 mcg/day (whichever is lower) and 1.0% or 50 mcg/day (whichever is lower), respectively. Thus, based on the maximum recommended human dose (MRHD) of 0.26 mg naldemedine tosylate (equivalent to 0.2 mg naldemedine free base), the proposed specifications for specified impurities [redacted] and unspecified impurities meet ICH Q3B limits.

[redacted] impurities were evaluated as per [redacted], and total [redacted] impurity levels from all sources in the drug product were determined to be less than control thresholds identified by the Applicant for these impurities [redacted] Thus, these impurities were not included in the proposed drug product specification.

Potential [redacted] present in excipients are [redacted] in croscarmellose sodium [redacted] is controlled in croscarmellose sodium to NMT [redacted] ppm, which is less than the [redacted] ppm. The Applicant states that [redacted] is expected to be present in [redacted] at a maximum amount o [redacted] ppm, which is less than the [redacted] ppm.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed indication is for treatment of opioid-induced constipation (OIC) in adults with chronic non-cancer pain. The recommended dose is 0.26 mg naldemedine tosylate (equivalent to 0.2 mg naldemedine free base) once daily.

2.7 Regulatory Background

In Type C Meeting written responses dated March 15, 2013, the Division stated that the nonclinical studies listed in the End of Phase 2 Pre-Phase 3 briefing document appeared to be sufficient to support an NDA from DGIEP's standpoint; however, Controlled Substance Staff (CSS) may require additional nonclinical studies to address drug withdrawal and dependency potential. During a February 11, 2015 Type C Meeting, FDA advised Shionogi to perform three nonclinical studies (physical dependence study, self-administration study and drug discrimination study) and to assess abuse related signals or adverse events in all clinical studies. The Applicant agreed to provide characterization of the major metabolites regarding CNS penetration

as well as any information on any agonist activity. A Pre-NDA Meeting was held on October 5, 2015. The Division indicated that the nonclinical program appeared to be adequate to support the filing of an NDA. CSS provided comments related to the abuse potential evaluation.

3 Studies Submitted

3.1 Studies Reviewed

STUDY	**APPLICANT'S REPORT NUMBER** (CRO study number)
PHARMACOLOGY	
Primary Pharmacology	
Study of RSC-297995 monotosylate on specific binding and functional assay to opioid receptors in vitro	R-297995-EB-074-N
Preliminary study for functional assay on opioid receptors of RSC-297995 monotosylate in vitro	R-297995-EF-013-R (6005)
Exploratory study on type of antagonism of S-297995 monotosylate to opioids in functional assay using human mu opioid receptor	S-297995-EB-311-R
Study for specific binding and functional assay to opioid receptors of metabolites of S-297995 monotosylate in vitro	S-297995-EB-135-N
Study for binding kinetics of S-297995 monotosylate to rat mu opioid receptor	S-297995-EB-222-N
Study for binding kinetics of S-297995 monotosylate to human mu opioid receptor	S-297995-EB-224-N
Study for specific binding and functional assays on human kappa opioid receptor of S-297995 monotosylate and its metabolites in vitro	S-297995-EF-284-N
Binding and functional assays of S-297995 monotosylate to rat mu opioid receptor	S-297995-EB-225-N
Binding and functional assays of S-297995 monotosylate to rat delta and kappa opioid receptors	S-297995-EB-281-N
Antagonistic effect of RSC-297995 monotosylate on morphine-induced inhibition of small intestinal transit in rats	R-297995-EB-071-N
Effect of S-297995 monotosylate on orally administered morphine-induced constipation in rats	S-297995-EB-221-N
Antagonistic Effect of S-297995 monotosylate on oxycodone-induced inhibition of small intestinal transit in rats	S-297995-EF-260-N (7620)
Antagonistic effect on morphine-induced inhibition of castor-oil-induced diarrhea of RSC-297995 monotosylate in rats	R-297995-EB-092-N
Secondary Pharmacology	
Specificity study of RSC-297995 monotosylate via enzyme inhibition and radioligand receptor binding assays	R-297995-EF-081-N (AL-3795-G)
Effect of RSC-297995 monotosylate on morphine-induced analgesic effect in rat tail-flick test	R-297995-EB-072-N
Influence of S-297995 monotosylate on Morphine-induced Analgesic Effect in Rat Tail-Flick Test	S-297995-EB-181-N
Influence of S-297995 monotosylate on Morphine-induced Analgesic Effect in Rat Post-Operative Pain Model	S-297995-EB-274-N
Study on in vivo brain opioid receptor occupancy after oral administration of S-297995 monotosylate in rats	S-297995-EB-257-N
Effect of RSC-297995 monotosylate on precipitate withdrawal symptoms in	R-297995-EB-073-N

morphine-dependent mice	
Study on precipitated withdrawal symptoms of S-297995 monotosylate in morphine-dependent rats	S-297995-SB-270-N
Anti-emetic effect of RSC-297995 monotosylate on morphine-induced emesis in ferrets	R-297995-EF-082-N (6250)
Duration of anti-emetic effect of RSC-297995 monotosylate on morphine-induced emesis in ferrets	R-297995-EF-083-N
Effect of S-297995 monotosylate on emesis induced by orally-administered morphine in ferrets	Study No. S-297995-EF-253-N (7525)
Safety Pharmacology	
Effects of RSC-297995 monotosylate on action potential in guinea pig papillary muscles	R-297995-SF-078-L (B080434)
Effects of RSC-297995 monotosylate on ionic current in cells expressing hERG channels	R-297995-SF-079-L (B080435)
Effects of RSC-297995 monotosylate on central nervous system in rats	R-297995-SF-075-L (B080431)
Effects of RSC-297995 monotosylate on respiratory system in rats	R-297995-SF-077-L (B080433)
Effects of RSC-297995 monotosylate on cardiovascular system in conscious dogs	R-297995-SF-076-L (B080432)
PHARMACOKINETICS/ADME/TOXICOKINETICS	
Absorption	
Plasma concentration following single oral administration of [^{14}C]-RSC-297995 monotosylate in rats	R-297995-PB-059-N
Plasma concentration following single oral administration of [Carbonyl-^{14}C]-RSC-297995 monotosylate in rats	R-297995-PB-097-N
Dose-linearity of plasma concentration following single oral administration of RSC-297995 monotosylate in rats	R-297995-PB-070-N
Dose-linearity of plasma concentration following single oral administration of RSC-297995 monotosylate in rats with morphine-induced constipation	R-297995-PF-089-N (AL-3825-G)
Dose-linearity of plasma concentration following single oral administration of RSC-297995 monotosylate in rats with castor oil-induced diarrhea inhibited by morphine	R-297995-PF-090-N (AL-3826-G)
Plasma concentration of S-297995 and its metabolites after single oral administration of S-297995 monotosylate in Wistar rats	S-297995-PF-197-N (PBC055-191)
Plasma and brain concentration of S-297995 and its metabolites after single oral administration of S-297995 monotosylate in Wistar rats with morphine-induced analgesia	S-297995-PF-198-N (PBC055-190)
Plasma concentrations of naldemedine and morphine after a single oral administration of naldemedine tosylate	S-297995-PF-251-N (PBC055-270)
Pharmacokinetics and disposition following single administration of [^{14}C]-RSC-297995 monotosylate in dogs	R-297995-PB-068-N
Pharmacokinetics of radioactivity and S-297995 after single oral administration of [Carbonyl-^{14}C]-S-297995 monotosylate in dogs	S-297995-PB-177-N
Dose-linearity of plasma concentration of S-297995 after single oral administration of S-297995 monotosylate in dogs	S-297995-PB-161-N
Dose-linearity of plasma concentration following single oral administration of RSC-297995 monotosylate in ferrets with morphine-induced emesis	R-297995-PF-088-N (AL-3824-G)
Plasma concentrations of S-297995 and morphine after single oral administration of S-297995 monotosylate in ferrets with peroral morphine-induced emesis	S-297995-PF-250-N (PBC055-269)
Plasma concentration of radioactivity and S-297995 after repeated oral administration of [Carbonyl-^{14}C]-S-297995 monotosylate in rats	S-297995-PB-190-N
Distribution	

Quantitative whole-body autoradiography following single oral administration of [^{14}C]-RSC-297995 monotosylate in rats	R-297995-PB-057
Quantitative whole-body autoradiography following single oral administration of [Carbonyl-^{14}C]-RSC-297995 monotosylate in rats	R-297995-PB-096-N
Quantitative whole-body autoradiography after repeated oral administration of [Carbonyl-^{14}C]-S-297995 monotosylate in rats	S-297995-PB-192-N
Quantitative whole-body autoradiography after single oral administration of [Carbonyl-^{14}C]-S-297995 monotosylate in pregnant rats	S-297995-PF-238-N (YDL0025)
Quantitative whole body autoradiography after single oral administration of [Oxadiazole-^{14}C]-S-297995 monotosylate and [Carbonyl-^{14}C]-S-297995 monotosylate in pigmented rats	S-297995-PF-213-N (8223166)
In vitro plasma/blood cell partitioning of [^{14}C]-RSC-297995 monotosylate	R-297995-PB-023-N
In vitro protein binding of [^{14}C]-RSC-297995 monotosylate	R-297995-PB-024-N
Metabolism	
Study on the in vivo major metabolites of [^{14}C]-RSC0297995 monotosylate in rats	R-297995-PB-060-N
Preliminary toxicokinetics study of metabolite, S-297995-(7R)-7-hydroxide in rats following S-297995 oral administration and exploratory determination of S-297995-(7R)-7-hydroxide in dog plasma by LC/MS/MS	S-297995-TB-282-R
Study on the in vivo major metabolites of [Carbonyl-^{14}C]-RSC-297995 monotosylate in rats	R-297995-PB-099-N
Study on the in vivo major metabolites of [^{14}C]-RSC-297995 monotosylate in dogs	R-297995-PB-061-N
Study on the in vivo major metabolites of [^{14}C]-RSC-297995 monotosylate in ferrets	R-297995-PB-062-N
In vivo metabolite profiling of S-297995 after single oral administration of [Carbonyl-^{14}C]-S-297995 monotosylate in ferrets	S-297995-PB-156-N
In vivo metabolite profiling of S-297995 after single oral administration of [Carbonyl-^{14}C]-S-297995 monotosylate in dogs	S-297995-PB-136-N
In vivo Metabolite Profiling of S-297995 after Single Oral Administration of [Carbonyl-^{14}C]-S-297995 monotosylate in Rabbits	S-297995-PB-240-N
In vivo Metabolite Profiling of S-297995 after Single Oral Administration of [Carbonyl-^{14}C]-S-297995 monotosylate in Mice	S-297995-PB-241-N
Identification of Responsible Enzymes for [^{14}C]-S-297995 monotosylate Metabolism	S-297995-PF-200-N (8221179)
Study on the in vitro Major Metabolites of [^{14}]-RSC-297995 monotosylate in Human	R-297995-PB-063-N
Excretion	
Urinary, fecal, and biliary excretion following single oral administration of [^{14}C]-RSC-27995 monotosylate in rats	R-297995-PB-025-N
Urinary, fecal, and biliary excretion following single oral administration of [Carbonyl-^{14}C]-RSC-27995 monotosylate in rats	R-297995-PB-098-N
Urinary and fecal excretion of radioactivity after repeated oral administration of [Carbonyl-14C]-S-297995 monotosylate in rats	S-297995-PB-191-N
Enterohepatic circulation of radioactivity after single oral administration of [Carbonyl-^{14}C]-S-297995 monotosylate in rats	S-297995-PB-184-N
Excretion into milk of radioactivity after single oral administration of [carbonyl-^{14}C]-S-297995 monotosylate in nursing rats	S-297995-PF-239-N (YDL0026)

Pharmacokinetic Drug Interactions	
Study on P-glycoprotein mediated drug interaction of RSC-297995 monotosylate	R-297995-PF-067-N (8SHIOP1)
Substrate assessments for human transporters of S-297995 monotosylate	S-297995-PF-285-N (12SHIOP2)
Inhibitor assessments for human transports of S-297995 monotosylate	S-297995-PF-297-N (b) (4) -Shionogi-02-01-Oct2013)
Inhibitor assessment of Nor-S-297995 for human transporters	S-297995-PF-340-N (GE-1425-G)
Effects of RSC-297995 monotosylate on hepatic drug metabolizing enzymes in one-month oral toxicity study in rats	R-297995-PB-049-L
Effects of RSC-297995 monotosylate on hepatic drug metabolizing enzymes in one-month oral toxicity study in dogs	R-297995-PB-050-L
Other	
Determination of membrane permeability and solubility of S-297995 monotosylate	S-297995-PF-288-L
TOXICOLOGY	
Single-Dose Toxicity	
Rat	
Oral	R-297995-TB-047-L
Dog	
Oral	R-297995-TB-045-L
Repeat-Dose Toxicity	
Rabbit	
2-Week, Oral	S-297995-TF-119-L (250228A)
Mouse	
13-Week, Oral	S-297995-TF-226-L (YDL0024)
Rat	
2-Week, Oral	R-297995-TB-003-R
1-Month, Oral	R-297995-TB-048-L
1-Month, Oral	R-297995-TB-091-L
6-Month, Oral	R-297995-TF-108-L (SG08274)
Dog	
2-Week, Oral	R-297995-TB-002-R
1-Month, Oral	R-297995-TB-046-L
3-Month, Oral	S-297995-TF-109-L (SG08275)
9-Month, Oral	S-297995-TF-219-L (SG10119)
GENOTOXICITY	
Ames test	R-297995-TB-051-L
In vitro mammalian chromosome aberration test	R-297995-TF-052-L (G-07-070)
Mammalian erythrocyte micronucleus test	R-297995-TF-053-L (G-07-071)
Ames test, Impurity (b) (4)	(b) (4)
Ames test, Impurity	
Ames test, Impurity	
Ames test, Impurity	

Ames test, Impurity (b) (4)	(b) (4)
Ames test, synthetic intermediates (b) (4)	
Ames test, synthetic intermediate (b) (4)	
Ames test, synthetic intermediates (b) (4)	
Exploratory Ames test, Impurity (b) (4)	
CARCINOGENICITY	
Rat, oral	S-297995-TF-266-L (YDL0036)
Mouse, oral	S-297995-TF-265-L (YDL0037)
REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	
Rat	
Fertility and Early Embryonic Development, Oral	S-297995-TF-104-L (100428)
Embryofetal Development, Oral	S-297995-TF-146-L (2000129)
Prenatal and Postnatal Development, Oral	S-297995-TF-275-L (SG12011)
Rabbit	
Embryofetal Development, Oral, Dose Range-Finding	S-297995-TF-163-L (250228P)
Embryofetal Development, Oral	S-297995-TF-182-L (250228)
SPECIAL TOXICOLOGY	
Ocular irritation study of RSC-297995 monotosylate in rabbits	R-297995-TF-084-N (702128)
Dermal irritation study of RSC-297995 monotosylate in rabbits	R-297995-TF-085-N (702028)
Immunotoxicity study of S-297995 monotosylate in rats	S-297995-TB-234-L
Preliminary skin phototoxicity study of RSC-297995 monotosylate in hairless mice by oral dosing	R-297995-TB-006-R
Skin phototoxicity study of S-297995 monotosylate in hairless mice by oral dosing	S-297995-TB-249-L
Oral study for effects of RSC-297995 monotosylate on estrous cycle and plasma reproductive hormone concentration in female rats	R-297995-TF-105-R (P080796)
Single dose oral study for effects of S-297995 monotosylate on plasma prolactin concentration in rats	S-297995-TF-162-N (P090377)

3.2 Studies Not Reviewed

- Study reports for abuse-related nonclinical studies are not reviewed herein. Instead, the following nonclinical studies will be evaluated by Controlled Substances Staff:
  - Preliminary study on drug discrimination of naldemedine tosylate in rats (S-297995-TF-323-N; CRO Study No. SG14427)
  - Study on drug discrimination of S-297995 monotosylate in rats (S-297995-TF-334-L; CRO Study No. SG14428)

  - Preliminary study on psychological dependence liability of S-297995 monotosylate by intravenous self-administration in monkeys (S-297995-TF-322-N; CRO Study No. SG14425)
  - Study on psychological dependence liability of S-297995 monotosylate by intravenous self-administration in monkeys (S-297995-TF-335-L; CRO Study No. SG14426)
  - Preliminary study on physical dependence liability of S-297995 monotosylate in rats; CRO Study No. SG14429)
  - Study on physical dependence liability of S-297995 monotosylate in rats (S-297995-TF-333-L; CRO Study No. SG14430).
- The following analytical methods and method validation reports are not reviewed: R-297995-QB-020-N, R-297995-QB-095-N, R-297995-QB-069-N; R-297995-QF-086-N (CRO Study No. AL-3827-G), R-297995-QF-087-N (CRO Study No. AL-3828-G), R-297995-TB-039-N, R-297995-TB-040-N, R-297995-TF-101-N (CRO Study No. PBC055-095), R-297995-TF-102-N (CRO Study No. PBC055-096), R-297995-TF-112-N (CRO Study No. PBC055-106), S-297995-PF-185-N (CRO Study No. PBC055-188), S-297995-PF-186-N (CRO Study No. PBC055-189), S-297995-PF-248-N (CRO Study No. PBC055-259), S-297995-TB-246-N, S-297995-TF-230-L (CRO Study No. YDL0021), S-297995-TF-168-N (CRO Study No. PBC055-168), S-297995-TF-220-N (CRO Study No. PBC055-238), S-297995-QB-170-N, and S-297995-QB-205-N.
- In vitro studies pertinent to pharmacokinetics using human biomaterials submitted under module 5.3.2 will be reviewed by the clinical pharmacology reviewer. These studies include evaluation of plasma protein binding and hepatic metabolism and drug interaction studies (e.g., in vitro enzyme induction and inhibition). While the studies will be reviewed by the clinical pharmacology reviewer, the following study reports submitted under module 5.3.2 were also reviewed herein given that biomaterials from nonclinical species were included in the studies: Study No. R-297995-PB-024-N (In vitro protein binding) and Study No. R-297995-PB-023-N (In vitro plasma/blood cell partitioning). In addition, Study No. R-297995-PB-063-N (in vitro metabolism of [oxadiazole-^{14}C]-naldemedine tosylate in cryopreserved human hepatocytes) is reviewed herein.

3.3 Previous Reviews Referenced

- IND 107475 Pharmacology review dated July 19, 2011 (C. Wu, Ph.D., DGIEP)
- IND 107475 Pharmacology review dated November 23, 2011 (C. Wu, Ph.D., DGIEP)
- IND (b) (4) Pharmacology review dated November 6, 2013 (S. Chakder, Ph.D., DGIEP)

4 Pharmacology

4.1 Primary Pharmacology

Review of Study No. R-297995-EB-074-N from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Study of RSC-297995 monotosylate on Specific Binding and Functional Assay to Opioid Receptors *in vitro(Study # R-297995-EB-074-N)*

Methods: Binding affinities of RSC-297995 monotosylate, alvimopan and MNTX for mu, delta and kappa opioid receptors were investigated. [3H]-DAMGO for mu, [3H]-DADLE for delta and [3H]-U69,593 for kappa receptors were used as the radioligands. Recombinant human mu and delta receptors, and kappa receptor prepared from guinea pig cerebrum and cerebellum were used.

Results: RSC-297995 monotosylate showed potent binding affinities and antagonist activities for mu, delta, and kappa opioid receptors. Alvimopan showed selective binding affinity for mu receptor. Moreover, alvimopan showed antagonist activities for mu, delta, and kappa opioid receptors, while it had a partial inverse agonist activity for delta receptor. MNTX was demonstrated to be a mu selective antagonist. The Ki values for RSC-297995 monotosylate for human mu and delta, and guinea pig kappa receptors were 0.34 ± 0.03, 0.43 ± 0.08, and 2.64 ± 0.19 nM, respectively.. The functional K_b values (antagonist activity) of RSC-297995 monotosylate, alvimopan and MNTX for mu receptor were 0.50 ± 0.05, 0.26 ± 0.02 and 55.86 ± 6.07 nM, and those for kappa receptor were 3.13 ± 1.94, 21.37 ± 1.78 and 1681.42 ± 231.62 nM, and those for delta receptor were 0.27± 0.03, 3.18 ± 0.46 and 1082.71 ± 234.31 nM, respectively.

Preliminary Study for Functional Assay on Opioid Receptors of RSC-297995 monotosylate in vitro (Study No. R-297995-EF-013-R; CRO Study No. 6005).

Methods: Functional effects of RSC-297995 monotosylate on mu-, kappa-, and delta receptors were evaluated using isolated guinea pig ileum and mouse vas deferens preparations. For mu-, kappa-, and delta-receptors, respectively, antagonistic action of the test compound on DAMGO-induced, U-50488H-induced, and DPDPE-induced contraction inhibition elicited by electrical field stimulation was determined.

Results: RSC-297995 monotosylate showed concentration-dependent, non-competitive antagonistic action at mu-, kappa-, and delta- receptors. Ke values (defined as [test article] / [concentration ratio -1]) were 0.22 nmol/L, 0.24 nmol/L, and 0.06 nmol/L for mu-, kappa-, and delta-receptors, respectively, where the concentration ratio is the IC50 of an agonist in the presence of test article / IC50 of an agonist alone.

Exploratory Study on Type of Antagonism of S-297995 monotosylate to Opioids in Functional Assay using Human Mu Opioid Receptor (Study No. S-297995-EB-311-R).

Methods: Morphine, oxycodone, hydrocodone, and fentanyl-induced [^{35}S]-GTPγS binding was determined in the presence and absence of S-297995 monotosylate or naloxone using membrane preparations of CHO-K1 cells stably expressing human mu-opioid receptor.

Results: S-297995 monotosylate and naloxone showed concentration-dependent inhibition of [^{35}S]-GTPγS binding induced by each of the opioids evaluated. Based on

slope of Schild regression, naloxone was considered to exhibit competitive antagonism while S-297995 monotosylate exhibited non-competitive antagonism.

Review of Study No. S-297995-EB-135-N from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Study for Specific Binding and Functional Assay to Opioid Receptors of Metabolites of S-297995 monotosylate *in vitro (Study* # S-297995-EB-135-N*)*

Methods: Binding affinities of S-297995 6G, S-297995 3G, nor-S-297995, S-297995-carboxylic acid, benzamidine and naloxone for mu, delta and kappa opioid receptors were investigated.

Results: S-297995 6G, nor-S-297995, S-297995 carboxylic acid showed apparent affinities and antagonistic activities to mu, delta and kappa receptors. The binding affinity (Ki values) of S-297995 6G, S-297995 3G, nor-S-297995 and S-297995-carboxylic acid for mu receptor were 9.79, 191.78, 1.95 and 7.38 nM, respectively. The Ki values of S-297995 6G, S-297995 3G, nor-S-297995 and S-297995-carboxylic acid for delta receptor were 0.51, 158.59, 10.23 and 2.05 nM, respectively. The Ki values of S-297995 6G, S-297995 3G, nor-S-297995 and S-297995-carboxylic acid, for kappa receptors were 112.11, 1100.16, 71.66 and 18.59 nM, respectively. In the functional assays of the previous study, the K_b values for mu, delta and kappa receptors of S-297995 monotosylate were 0.5, 0.27 and 3.13 nM, respectively, showing potent antagonistic activities for all three opioid receptors. The K_b values for mu, delta and kappa receptors of S-297995 3G were >42.41, 301.13 and >472.22 nM, respectively. The K_b values for mu, delta and kappa receptors of S-297995 6G were 15.53, 0.70 and 51.52 nM, respectively, showing a comparable antagonistic activity for delta receptor to that of S-297995 monotosylate. The K_b values for mu, delta and kappa receptors of nor-S-297995 were 31.65, 112.36 and 80.14 nM, respectively. While, the K_b values for mu, delta and kappa receptors of S-297995-carboxylic acid were 14.11, 6.11 and 130.85 nM, respectively. The antagonistic activities of these metabolites were significantly weaker than those found for S-297995 monotosylate, while the antagonistic activity for delta receptor of S-297995 6G was almost comparable to that of S-297995 monotosylate. Nor-S-297995 also showed agonistic activity for delta receptor.

Study for Binding Kinetics of S-297995 monotosylate to Rat Mu Opioid Receptor (Study No. S-297995-EB-222-N; Study Report (Amendment)).

Methods: The binding kinetics of 0.5 nM [^{3}H]-S-297995 to the rat mu opioid receptor, relative to that of [^{3}H]-naloxone, were determined based on the time courses of association and disassociation.

Results: Association rate constants (K_{obs}) of [^{3}H]-S-297995 and [^{3}H]-naloxone for rat mu opioid receptor were 0.070 and 0.503 min^{-1}, respectively. The dissociation rate constants (K_{off}) of [^{3}H]-S-297995 and [^{3}H]-naloxone were 0.016 and 0.397 min^{-1}, respectively, and $t_{1/2}$ values (the time until binding of the radiolabelled ligand to the receptor decreases to 50% after addition of an excess amount of unlabeled ligand) were 43.8 and 1.745 min, respectively. Thus, the association and dissociation kinetics of [^{3}H]-S-297995 for rat mu opioid receptor were slower than that of [^{3}H]-naloxone.

Study for Binding Kinetics of S-297995 monotosylate to Human Mu Opioid Receptor (Study No. S-297995-EB-224-N; Study Report (Amendment)).

Methods: The binding kinetics of 0.5 nM [^{3}H]-S-297995 to the human mu opioid receptor were compared to those of [^{3}H]-naloxone, based on the time courses of association and dissociation.

Results: Association rate constants (K_{obs}) of [^{3}H]-S-297995 and [^{3}H]-naloxone for human mu opioid receptor were 0.045 and 0.233 min^{-1}, respectively. The dissociation rate constants (K_{off}) of [^{3}H]-S-297995 and [^{3}H]-naloxone were 0.023 and 0.266 min^{-1}, respectively, and $t_{1/2}$ values were 30.687 and 0.266 min, respectively. Therefore, the association and dissociation kinetics of [^{3}H]-S-297995 for human mu opioid receptor were slower than that of [^{3}H]-naloxone

Study for Specific Binding and Functional Assays on Human Kappa Opioid Receptor of S-297995 monotosylate and its Metabolites In Vitro (Study No. S-297995-EF-284-N; Amendment to the study report (II)).

Methods: Binding affinities and functional activities of S-297995 monotosylate, S-297995 6-O-β-D-glucuronide (S-297995 6G), 297995 3-O-β-D-glucuronide (S-297995 3G), Nor-S-297995, S-297995-carboxylic acid, benzamidine, and methylnaltrexone on human kappa opioid receptor were investigated.

Results: S-297995 monotosylate exhibited a potent binding affinity for kappa opioid receptor (Ki value = 0.94 nmol/L). The metabolites of S-297995 were less potent or had no significant binding affinity for kappa opioid receptor (Ki values of S-297995 6G, S-297995 3G, Nor-S-297995, S-297995-carboxylic acid, and benzamidine were 36.2, 915, 60.8, 151, and >6250 nmol/L, respectively). The Ki value of methylnaltrexone was 32.1 nmol/L. S-297995 monotosylate demonstrated potent antagonistic activity at kappa opioid receptor (Kb value = 0.44 nmol/L). The metabolites showed less potent antagonistic activity (Kb values of S-297995 6G, S-297995 3G, Nor-S-297995, S-297995-carboxylic acid, and benzamidine were 28.5, >270.68, >270.68, 201, and >270.68 nmol/L, respectively). The Kb value for methylnaltrexone was >270 nmol/L.

Binding and Functional Assays of S-297995 monotosylate to Rat Mu Opioid Receptor (Study No. S-297995-EB-225-N).

Methods: The binding affinity of S-297995 monotosylate for rat mu opioid receptor was determined using [^{3}H]-DAMGO as the radioligand. In functional assays, agonistic activities of S-297995 monotosylate and DAMGO (positive control) were determined using [^{53}S]-GTPɣS as the radioligand. In addition, antagonistic activity of S-297995 monotosylate was evaluated in the functional assay.

Results: In binding assays, the IC50 and Ki values of S-297995 monotosylate for rat mu opioid receptor were 3.21 and 1.40 nM, respectively. In assays conducted to assess agonist activity, the EC50 value of DAMGO for rat mu opioid receptor was 6.34 nM, compared to an EC50 value of >10000 nM for S-297995 monotosylate. Based on its EC50 value and Emax (percent of maximal stimulation in the [^{35}S]-GTPɣS binding) value of -5.98 nM, S-297995 did not exhibit agonistic activity for the rat mu opioid

receptor. S-297995 exhibited antagonistic activity with IC50 and Kb values for rat mu opioid receptor of 161.08 and 0.56 nM, respectively.

Binding and Functional Assays of S-297995 monotosylate to Rat Delta and Kappa Opioid Receptors (Study No. S-297995-EB-281-N; Study Report (Amendment)).

Methods: Binding affinities and functional activities of S-297995 monotosylate for rat delta and kappa opioid receptors were investigated.

Results: The IC50 and Ki values of S-297995 monotosylate for rat delta and kappa opioid receptors were 2.13 and 0.96 nM, and 5.43 and 2.16 nM, respectively. In the functional assay, S-297995 monotosylate did not exhibit agonistic activity. S-297995 monotosylate did however exhibit antagonistic activities with Kb values of 0.22 and 0.49 nM, respectively, for rat delta and kappa opioid receptors.

Review of Study No. R-297995-EB-071-N from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Antagonistic Effect of RSC-297995 monotosylate on Morphine-induced Inhibition of Small Intestinal Transit in Rats (Study # R-297995-EB-071-N)

Methods: 100 Rats were allocated into 10 study groups (10 animals/group) as following: Group 1: Vehicle (0.5% MC) + Saline; Group 2: Vehicle (0.5% MC) + Morphine 3 mg/kg; Group 3-9: RSC-297995 monotosylate 0.01 mg/kg-10 mg/kg (3-fold apart) + Morphine 3 mg/kg and Group 10: RSC-297995 monotosylate 10 mg/kg + Saline. Similar studies were designed for the reference substances Alvimopan and MNTX. Intestinal transit rate was measured with Evans blue.

Results: In all of experiments, the transit rates in the morphine control group (Group 2) significantly decreased compared with the vehicle control group (Group 1). Moreover, RSC-297995 monotosylate (10 mg/kg), alvimopan (30 mg/kg) and MNTX (30 mg/kg) (Group 10) did not affect the small intestinal transit, compared with vehicle control group (Group 1). RSC-297995 monotosylate (0.01-10 mg/kg), alvimopan (0.03-30 mg/kg) and MNTX (0.03-30 mg/kg) prevented the morphine-induced suppression of the small intestinal transit. RSC-297995 monotosylate showed a significant inhibition at the doses of 0.03 -10 mg/kg. Alvimopan showed a significant inhibition at the doses of 3-30 mg/kg. MNTX showed a significant inhibition at the doses of 10 and 30 mg/kg. The ED_{50} values (mg/kg) of these substances were as follows: RSC-297995 monotosylate; 0.03, alvimopan; 0.52, and MNTX; 4.47. The ED_{80} values (mg/kg) of these substances were as follows: RSC-297995 monotosylate; 0.14, alvimopan; 2.07, and MNTX; 17.86. These results suggest that RSC-297995 monotosylate is more effective than those of alvimopan or MNTX.

In conclusion, RSC-297995 monotosylate, alvimopan and MNTX prevented the morphine-induced transit suppression of the small intestine of rats. Especially RSC-297995 monotosylate showed the anti-constipation effect in lower doses than those of alvimopan and MNTX.

Effect of S-297995 monotosylate on Orally Administered Morphine-induced Constipation in Rats (Study No. S-297995-EB-221-N; Study Report (Amendment)).

Methods: In a study to optimize the dose of morphine, fasted male Crlj:WI rats (n=12/group) were orally administered vehicle (0.5% MC), followed by 3 to 30 mg/kg

morphine (or vehicle; distilled water) 15 minutes later. In a study conducted to evaluate S-297995 monotosylate, fasted male rats (n=12/group) were administered 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 mg/kg S-297995 monotosylate or vehicle (0.5% MC). Fifteen minutes after dosing with S-297995 monotosylate, the rats were orally administered 20 mg/kg morphine (or distilled water in the case of 1 of the 2 control groups).

Results: All doses of morphine significantly inhibited small intestinal transit, compared to rats administered 0.5% MC plus distilled water. A dose of 20 mg/kg morphine (which inhibited transit rates by ~54%, compared to controls) was therefore selected for the study using S-297995 monotosylate. S-297995 monotosylate prevented morphine-induced inhibition of small intestinal transit, with dose-dependent and statistically significant effects at ≥0.1 mg/kg S-297995 monotosylate. The ED50 and ED80 values for S-297995 monotosylate were 0.23 and 1.04 mg/kg, respectively.

Antagonistic Effect of S-297995 monotosylate on Oxycodone-induced Inhibition of Small Intestinal Transit in Rats (Study No. S-297995-EF-260-N; CRO Study No. 7620).

Methods: Oxycodone-induced inhibition of small intestinal transit in male Crlj: WI rats (n=10/group) were assessed following oral administration of 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, and 3 mg/kg S-297995 monotosylate.

Results: Oxycodone was shown to inhibit small intestinal transit. Administration of S-297995 monotosylate resulted in a dose-dependent prevention of oxycodone-induced suppression of small intestinal transit, with effects at all doses (statistically significant at ≥0.03 mg/kg S-297995 monotosylate). The ED50 and ED80 values for S-297995 monotosylate were 0.02 and 0.13 mg/kg, respectively.

Review of Study No. R-297995-EB-092-N from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Antagonistic Effect on Morphine-induced Inhibition of Castor Oil-induced Diarrhea of RSC-297995 monotosylate in Rats (Study # R-297995-EB-092-N)

Methods: Male rats were allocated into 9 study groups (n=11) as following: Group 1: Vehicle (0.5% MC) + Saline; Group 2: Vehicle (0.5% MC) + Morphine 1 mg/kg; Group 3-8: RSC-297995 monotosylate 0.003 mg/kg-1 mg/kg (3-fold apart) + Morphine 1 mg/kg; and Group 9: RSC-297995 monotosylate 1 mg/kg + Saline. Similar studies were designed for the reference substances Alvimopan and MNTX. RSC-297995 monotosylate and alvimopan were administered orally, and MNTX) was administered subcutaneously. Castor oil was intragastrically administered in a volume of 2 mL/rat at 45 min after the test and reference substances or vehicle (0.5% MC or 5% xylitol) administration. Morphine or saline was subcutaneously injected in a volume of 2 mL/kg at 15 min after the administration of castor oil. The castor oil-induced diarrhea was observed for 1 hr. The symptoms of diarrhea were scored as follows: 0, without diarrhea; 1, mild diarrhea containing loose bowels; 2, intense liquefied diarrhea.

Results: In all experiments, morphine (1 mg/kg) control group (Group 2) significantly decreased the castor oil induced diarrhea compared with the saline control group (Group 1) ($P<0.01$). RSC-297995 monotosylate at the doses of 0.003 and 0.01 mg/kg did not significantly antagonize the inhibitory effect of morphine on castor oil-induced diarrhea. RSC-297995 monotosylate at the doses of 0.03, 0.1, 0.3 and 1 mg/kg significantly antagonized the inhibitory effect of morphine on castor oil-induced diarrhea.

In conclusion, all of RSC-297995 monotosylate, alvimopan and MNTX antagonized the inhibitory effect of morphine on castor oil-induced diarrhea. Especially RSC-297995 monotosylate showed the anti-constipation effect in lower doses than those of alvimopan and MNTX.

4.2 Secondary Pharmacology

Review of Study No. R-297995-EB-081-N (CRO Study No. AL-3795-G) from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Specificity Study of RSC-297995 monotosylate via Enzyme Inhibition and Radioligand Receptor Binding Assays (Study # R-297995-EF-081-N)

Methods: The inhibitory effect of RSC-297995 monotosylate on various receptors, channels, transporters, and enzymes was investigated, and the inhibition ratios on the binding of specific ligand to each receptor or on the enzyme reaction of specific substrate were calculated.

Results: The inhibition ratio of RSC297995 monotosylate for opiate (Non-selective) receptor was 100% indicating more than 50% inhibition. In the other assay systems, the inhibition ratios of RSC297995 monotosylate were less than 50%. The results indicated that there was no inhibitory effect of RSC297995 on other receptors, channels, transporters or enzymes.

Review of Study No. R-297995-EB-072-N from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Effect of RSC-297995 monotosylate on Morphine-induced Analgesic Effect in Rat Tail-flick Test (Study # R-297995-EB-072-N)

Methods: Rats were allocated into seven study groups (n=10) as following: Group 1: Vehicle (0.5% MC) + Saline; Group 2: Vehicle (0.5% MC) + Morphine 6 mg/kg; Group 3-6: RSC-297995 monotosylate 1 mg/kg-30 mg/kg + Morphine 6 mg/kg; Group 7: RSC-297995 monotosylate 30 mg/kg + Saline. Similar studies were designed for the reference substances Alvimopan and MNTX. The test and reference substances were administered 1 hour before tail-flick test. Morphine or saline was subcutaneously administered 15 minutes after the administration of test or reference substances.

Results: All animals in morphine-treated groups reached the cut-off time of 20 seconds in the tail-flick test, showing an analgesic effect. RSC-297995 monotosylate (1 - 30 mg/kg, p.o.) and alvimopan (1-30 mg/kg, p.o.) did not show any reduction of the analgesic effect of morphine (6 mg/kg, s.c). MNTX (1-10 mg/kg, s.c.) also did not show any reduction on the analgesic effect of morphine. However, the highest dose of MNTX (30 mg/kg, s.c.) showed a significant reduction of the analgesic effect of morphine (6 mg/kg, s.c.). The highest dose of each substance itself (RSC-297995: 30 mg/kg and alvimopan: 30 mg/kg) did not show analgesic effect. The highest dose (30 mg/kg) of MNTX slightly, but significantly delayed the withdrawal latency by itself, however it was estimated not to affect the result, which inhibited the analgesic effect of morphine.

Influence of S-297995 monotosylate on Morphine-induced Analgesic Effect in Rat Tail-Flick Test (S-297995-EB-181-N).

Methods: Male Crlj: WI rats (n=10-11/group) were orally administered 3, 5, 7, 10, and 30 mg/kg S-297995 monotosylate or vehicle (0.5 w/v% MC). To evaluate the effect of S-297995 monotosylate, tail withdrawal latency was measured by tail-flick method at 1, 2, 4, 6, 7, and 24 h after administration of the test compound or vehicle. Morphine (6 mg/kg), or saline in the case of 1 of the 2 control groups, was administered forty-five minutes before the test. A cut-off time of 20 seconds was used to prevent tissue damage.

Results: All animals administered vehicle plus morphine reached the cut-off time, compared to 0/11 animals treated with vehicle and saline, demonstrating the analgesic effect of morphine. There was a statistically significant decrease in the number of animals reaching the cut-off time following treatment with 30 mg/kg S-297995 monotosylate, compared to animals treated with vehicle and morphine.). At 10 and 30 mg/kg, S-297995 monotosylate resulted in a statistically significant shortening of the normalized latency for tail withdrawal response (compared to animals dosed with vehicle and morphine) at 6 h and 4, 6, and 8 h, respectively. Thus, doses of ≥10 mg/kg S-297995 monotosylate inhibited the analgesic effect of morphine in a rat tail flick test.

Influence of S-297995 monotosylate on Morphine-induced Analgesic Effect in Rat Post-Operative Pain Model (Study No. S-297995-EB-274-N).

Methods: Male Crlj: WI rats (n=10-12/group) were orally administered 1, 3, 5, and 7 mg/kg S-297995 monotosylate or 0.1 mg/kg naloxone by subcutaneous injection. To evaluate the effect of S-297995 monotosylate on post-operative pain using an incisional

pain model, paw withdrawal thresholds in response to thermal stimuli were measured at 1, 2, 4, 6, 7, and 24 h after administration of S-297995 monotosylate and 1 h after administration of naloxone. Morphine (6 mg/kg), or saline in the case of 1 of the 2 control groups, was administered forty-five minutes prior the measurement at each time point. A cut-off time of 20 seconds was used to prevent tissue damage.

Results: At 5 and 7 mg/kg, treatment with S-297995 monotosylate significantly shortened the normalized latency (compared to animals dosed with vehicle and morphine) at 6 h and 4, 6, and 8 h, respectively. For comparison, treatment with 0.1 mg/kg naloxone significantly affected the analgesic effect of morphine at 1 hr. Thus, doses of ≥5 mg/kg S-297995 monotosylate inhibited the analgesic effect of morphine in a post-operative pain model.

Study on in vivo Brain Opioid Receptor Occupancy after Oral Administration of S-297995 monotosylate in Rats (Study No. S-297995-EB-257-N).

Methods: Fasted male Crlj: WI rats were administered 3, 10, and 30 mg/kg S-297995 monotosylate (expressed as S-297995) or vehicle (0.5 w/v% methylcellulose aqueous solution) by oral gavage. Opioid receptor occupancy, plasma, and brain concentrations of S-297995 were measured at 1, 4, 8, and 24 h after administration. [^{3}H]Diprenorphine (a non-selective opioid antagonist) was used as a tracer to evaluate the opioid receptor occupancy, and was intravenously injected approximately 30 min prior to each time point. Cerebral cortex, cerebellum, and plasma concentrations of S-297995 were determined using LC/MS/MS.

Results:
Opioid receptor occupancies in the cerebral cortex and thalamus were <6.1% following administration of 3 mg/kg S-297995. Following treatment with 10 and 30 mg/kg, the highest receptor occupancies in the cerebral cortex and thalamus were measured at 8 h. At 10 mg/kg, receptor occupancies in the cerebral cortex and thalamus were approximately 14% at 4 h and approximately 28% at 8 h. At 24 h after dosing with 10 mg/kg, receptor occupancies were 8.8 and 2.5%, respectively, in the cerebral cortex and thalamus. Following administration of 30 mg/kg, receptor occupancies in the cerebral cortex and thalamus were 38.5% and 23.9%, respectively, at 4 h, 57.1% and 34.6%, respectively, at 8 h, and 27.8% and 17.7%, respectively, at 24 h. Plasma concentrations of S-297995 were 229, 950, and 2590 ng/mL at 1 h after administration of 3, 10, and 30 mg/kg, respectively. Concentrations of S-297995 in the cerebral cortex were 3.4, 11.9, and 28.5 ng/g at 4 h after administration of 3, 10, and 30 mg/kg, respectively. Concentrations of S-297995 in the cerebellum at 4 h after administration were 5.05, 20.1, and 60.2 ng/g at 3, 10, and 30 mg/kg, respectively. Concentrations in the plasma decreased more rapidly as compared to the brain. Thus, the time at which maximum receptor occupancy in the brain was measured (8 h) was delayed relative to the time at which peak concentrations were measured in the brain.

Review of Study No. R-297995-EB-073-N from the IND 107475 nonclinical review dated July 19, 2011 is copied below (Dr. C. Wu, Ph.D.).

Effect of RSC-297995 monotosylate on Precipitate Withdrawal Symptoms in Morphine-dependent Mice (Study # R-297995-EB-073-N)

Methods: Morphine (25 mg/kg, s.c.) or saline (s.c.) was administered to the mice 5 times per day for 4 consecutive days. RSC-297995 monotosylate, alvimopan and MNTX (dosed at 0.01, 0.1, 1 and 10 mg/kg, n=11-13) or each vehicle and Naloxone (at 0.1 mg/kg) was administered 3 hr after the last injection of morphine or saline. After administration of the test substances, frequencies of diarrhea and jumping behavior were monitored as an index of peripheral and central mediated effects, respectively. Jumping was judged by whether hind paws were away from the chamber floor. Diarrhea was judged from collapsing shape of excrement on the floor in the chamber where filter paper was put.

Results: Administration of RSC-297995 monotosylate showed diarrhea but not jumping episode at the doses of 0.1 to 10 mg/kg, p.o. RSC-297995 monotosylate produced diarrhea in more than half of the animals in both 1 and 10 mg/kg treated groups. Statistically significant increase in withdrawal diarrhea was observed at the dose of 10 mg/kg, compared with saline-RSC-297995 monotosylate group ($P<0.01$). Also, statistically significant increase in withdrawal diarrhea was observed at the doses of 1 and 10 mg/kg, compared with vehicle-morphine group ($P<0.01$). Alvimopan also did not show jumping episode at doses of up to 10 mg/kg. However, alvimopan produced a significant diarrhea at the dose of 10 mg/kg ($P<0.01$) vs. vehicle-morphine group. MNTX produced a significant diarrhea at a dose of 10 mg/kg, compared with vehicle-morphine group ($P<0.01$) or saline-MNTX group ($P<0.01$). The jumping episode was also shown in 3 of 13 animals in the 10 mg/kg treated group but the incidence was not statistically significant compared with vehicle-morphine group or saline-MNTX group. Naloxone, as a positive control, produced a significant increase in both of jumping and diarrhea at the dose of 0.1 mg/kg in each experiment. Thus, the data showed that RSC-297995 monotosylate has a peripheral-preferred antagonistic activity to opioid receptor as well as those for alvimopan and MNTX.

Study on Precipitated Withdrawal Symptoms of S-297995 monotosylate in Morphine-Dependent Rats (Study No. S-297995-SB-270-N).

Methods: Morphine-dependent rats were orally administered 0.01, 0.03, 0.1, 0.3, 1, and 3 mg/kg S-297995 monotosylate (or vehicle, 0.5 w/v% methylcellulose aqueous solution) or subcutaneously administered 0.1 mg/kg naloxone or saline (n=8/group). The animals were observed for withdrawal symptoms immediately after dosing and at 1, 2, 4, 6, and 8 h after dosing. Parameters evaluated in this study included the numbers of jumping and wet dog shakes, diarrhea and teeth chattering, and weight loss.

Results: Administration of S-297995 monotosylate at up to 3 mg/kg did not result in jumping or wet dog shakes at any dose. However, administration of S-297995 monotosylate resulted in increased diarrhea (compared to vehicle controls), with statistically significant increases at the 1 and 3 mg/kg dose levels. There was also a statistically significant increase in weight loss following treatment with ≥0.3 mg/kg S-297995 monotosylate, compared to the vehicle control group. Finally, teeth chattering was increased after administration of 3 mg/kg S-297995 monotosylate. For comparison, 0.1 mg/kg naloxone resulted in increased jumping, wet dog shakes, teeth chattering, and diarrhea immediately after dosing and increased weight loss, compared

to vehicle controls. Therefore, treatment of morphine-dependent rats with S-297995 monotosylate resulted in withdrawal signs at ≥0.3 mg/kg.

Review of Study No. R-297995-EB-082-N (CRO Study No. 6250) from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Anti-emetic Effect of RSC-297995 monotosylate on Morphine-induced Emesis in Ferrets (Study # R-297995-EB-082-N)

Methods: The effects of oral administration of RSC-297995 monotosylate at 0.01, 0.03, 0.1 and 0.3 mg/kg, oral administration of Alvimopan at 0.03, 0.1 and 0.3 mg/kg and subcutaneous administration of Methylnaltrexone (MNTX) at 0.03, 0.1, 0.3 and 1 mg/kg on morphine-induced emesis were examined in ferrets.

Results: RSC-297995 monotosylate, at a dose of 0.01 mg/kg, showed no significant inhibitory actions on morphine-induced emesis, and at doses of 0.03, 0.1 and 0.3 mg/kg, showed a significant inhibitory action. Alvimopan, at doses of 0.03, 0.1, 0.3 and 1 mg/kg, showed a significant inhibitory action. MNTX, at doses of 0.03 and 0.1 mg/kg, showed no inhibitory actions on morphine-induced emesis, while doses of 0.3, 1 and 3 mg/kg, showed a significant inhibitory action.

Review of Study No. R-297995-EB-083-N (CRO Study No. 6251) from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Duration of Anti-emetic Effect of RSC-297995 monotosylate on Morphine-induced Emesis in Ferrets (Study # R-297995-EB-083-N)

Methods: The inhibitory effects of oral administration of RSC-297995 monotosylate (0.3 mg/kg, p.o.) and Alvimopan (1 mg/kg, p.o.) and subcutaneous administration of Methylnaltrexone (MNTX) (3 mg/kg, s.c.) on duration of morphine-induced emesis were examined in ferrets.

Results: RSC-297995 monotosylate decreased the number of incidence, increased latency and decreased duration in retching, vomiting and emesis, indicative of its sustained antiemetic effect on morphine-induced emesis until 8 hours after administration. In comparison, Alvimopan decreased the number of incidence and decreased duration of retching and emesis until 4 hours after administration, and increased the latency until 8 hours after administration. MNTX decreased the number of incidence of retching, vomiting and emesis, decreased duration of retching and emesis, and increased latency of vomiting until 0.5 hour after administration. At 4 hours after administration, increased latency of retching and emesis and decreased duration of vomiting were observed.

Effect of S-297995 monotosylate on Emesis Induced by Orally-administered Morphine in Ferrets (Study No. S-297995-EF-253-N; CRO Study No. 7525).

Methods: To investigate the anti-emetic effect of S-297995 monotosylate on morphine-induced emesis, male ferrets (n=10 total) were orally administered S-297995 monotosylate (0.01, 0.03, 0.1, and 0.3 mg/kg) or vehicle (0.5 w/v% methylcellulose solution). Morphine (1.2 mg/kg) was administered 30 minutes later and emetic symptoms were observed for 90 minutes.

Results: At 0.01 mg/kg S-297995 monotosylate, the average frequency of emesis, latency, and duration did not differ significantly from the vehicle control group. However, at ≥0.03 mg/kg S-207995 monotosylate, no emetic symptoms were observed in any of the animals after administration of morphine. The estimated ED50 of anti-retching, vomiting, and emesis were 0.016, 0.013, and 0.016 mg/kg, respectively.

4.3 Safety Pharmacology

Review of Study No. R-297995-SF-078-L (CRO Study No. B080434) from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Effects of RSC-297995 monotosylate on Action Potential in Guinea Pig Papillary Muscles (Study # R-297995-SF-078-L)

Methods: This was a GLP study. The effects of S-297995 monotosylate on action potential were investigated in isolated right ventricular papillary muscle from male Hartley guinea pigs (5 specimens/group). S-297995 monotosylate was dissolved in dimethylsulfoxide (DMSO) and applied onto the specimens at concentrations of 0.3, 3, and 30 μmol/L (0 [vehicle], 0.17, 1.71, and 17.1 μg/mL, respectively). Sotalol, a positive control, was given at 30 μmol/L. Action potential duration at 90% repolarization (APD_{90}), action potential duration at 30% repolarization (APD_{30}), difference in action potential duration between 90% and 30% repolarization (APD_{30-90}), resting membrane potential (RMP), action potential amplitude (APA) and maximum rate of depolization (MRD) were evaluated.

Results: At 0.3 μmol/L, S-297995 monotosylate had no effects on any parameters. At 3 μmol/L, S-297995 monotosylate significantly prolonged APD_{90} and APD_{30-90} by 2.1% and 5.6% of the initial values, respectively. At 30 μmol/L, S-297995 monotosylate prolonged APD_{30}, APD_{90}, and APD_{30-90} by 4.2%, 11.4%, and 24.8% of the initial values, respectively. In addition, S-297995 monotosylate increased RMP by 0.7% and decreased APA by 1.8% of the initial values at 30 μmol/L. These changes were statistically significant. The positive control, sotalol significantly prolonged APD_{90} and APD_{30-90} at 30 μmol/L. S-297995 monotosylate did not show clear effects

on MRD as shown in the table below. From the above results, S-297995 monotosylate prolonged the action potential duration in isolated guinea pig papillary muscles by more than 10% at 30 μmol/L.

Table 1 Effects of RSC-297995 monotosylate and sotalol on action potential parameters in isolated guinea pig papillary muscles

Test substance	Concentration (μmol/L)	Number of preparations	Percentage of value just before application					
			RMP	APA	APD_{30}	APD_{90}	APD_{30-90}[1]	MRD
Vehicle[2]	---	5	100.9 ± 0.9	100.0 ± 1.0	100.3 ± 1.4	99.8 ± 0.5	99.0 ± 1.4	99.3 ± 1.5
RSC-297995 monotosylate	0.3	5	100.7 ± 0.6	100.5 ± 0.4	98.8 ± 1.7	99.6 ± 0.6	101.0 ± 2.3	98.7 ± 3.1
	3	5	100.4 ± 1.0	99.4 ± 0.3	100.3 ± 2.4	102.1 ± 1.4*	105.6 ± 3.3*	100.6 ± 6.5
	30	5	99.3 ± 1.0*	98.2 ± 0.4*	104.2 ± 2.0*	111.4 ± 1.0*	124.8 ± 3.1*	95.4 ± 2.3
Sotalol	30	5	100.0 ± 1.1	99.8 ± 0.6	110.9 ± 5.8#	127.3 ± 5.4#	156.4 ± 6.5#	100.6 ± 1.7

Each value represents the mean ± S.D.
RMP : resting membrane potential, APA : action potential amplitude, APD_{30} and APD_{90} : action potential duration at 30% and 90% repolarization, respectively,
MRD : maximum rate of depolarization
[1] APD_{30-90} is the value subtracted APD_{30} from APD_{90}.
[2] 0.1 vol% dimethyl sulfoxide
Significantly different from the vehicle control value by Aspin-Welch's t test, $p<0.05$
* Significantly different from the vehicle control value by Dunnett's multiple comparison test, $p<0.05$

Review of Study No. R-297995-SF-079-L (CRO Study No. B080435) from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Effects of RSC-297995 monotosylate on Ionic Current in Cells Expressing hERG Channels (Study # B080435)

Methods: This was a GLP study. The electrophysiological effect of S-297995 monotosylate on rapidly activating component of delayed rectifier potassium currents (I_{Kr}) were investigated in cells expressing human ether-a-go-go related gene (hERG) channels. S-297995 monotosylate (Lot No. A81001) was dissolved in DMSO and applied to HEK293 cells (5 cells/group) expressing hERG channels at concentration of 0.3, 3, and 30 μmol/L (0.17, 1.71, and 17.1 μg/mL, respectively) to measure I_K, (hERG current) by the patch clamp method. E-4031 was used as the positive control.

Results: E-4031 at 0.1 μmol/L caused significant inhibition of hERG current with the relative current of 6.5%, and % inhibition was 93.3%. In comparison, S-297995 monotosylate inhibited peak tail current by 3.2%, 5.6%, and 33.1% of the baseline values at 0.3, 3, and 30 μmol/L, respectively, as shown in the table below, with statistical significance at 3 μmol/L or higher. The IC_{50} value was estimated to be higher than 30 μmol/L. These results indicated that S-297995 monotosylate had inhibitory effects on the hERG current at concentrations of 3 and 30 μmol/L.

Table 1 Effects of RSC-297995 monotosylate and E-4031 on tail peak current

Test substance	Concentration (μmol/L)	Number of preparations	% of pre-application	% inhibition
Vehicle[a]	---	5	96.4 ± 2.4	-
RSC-297995 monotosylate	0.3	5	93.3 ± 2.3	3.2
	3	5	91.0 ± 2.2*	5.6
	30	5	64.5 ± 3.4*	33.1
E-4031	0.1	5	6.5 ± 2.1#	93.3

% of pre-application : percentage of the value just before application

% inhibition : 100-([mean % of pre-application of the test substance or E-4031 group]/ [mean % of pre-application of the vehicle control group])×100

[a]0.1 vol% dimethyl sulfoxide

Each value represents the mean ± S.D.

* Significantly different from the vehicle control group by Williams' test, $p<0.025$

Significantly different from the control value by Student's t test, $p<0.05$

Review of Study No. R-297995-SF-075-L (CRO Study No. B080431) from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Effects of RSC-297995 monotosylate on Central Nervous System in Rats (Study # B080431)

Methods: This was a GLP study. The effects of S-297995 monotosylate on general behavior and neurobehavioral function were investigated in rats by means of functional observational battery (FOB). Male SD rats (age of 5-week old on the experimental day, 6 animals per group) were used for the study. S-297995 monotosylate was suspended in 0.5 w/v% MC and orally administered to rats at dose levels of 30, 100, and 300 mg/kg. General behavior and neurobehavioral function were observed using the functional observational battery before and 1, 2, 4, and 8 hours after single administration. Vehicle (0.5 w/v% MC) was administered to the control group.

Results: No treatment-related findings were observed in general behavior and neurobehavioral function at any time point up to 8 hours after oral administration of S-297995 monotosylate at 30, 100, and 300 mg/kg. It was concluded that S-297995 monotosylate had no effects on the central nervous system in rats at 30, 100, and 300 mg/kg.

Review of Study No. R-297995-SF-077-L (CRO Study No. B080433) from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.). Although the "Results" section states that there were no treatment-related effects at dose levels up to 1000 mg/kg, the maximum dose tested was 300 mg/kg.

Effects of RSC-297995 monotosylate on Respiratory System in Rats (Study # B080433)

Methods: This was a GLP study. The effects of S-297995 monotosylate on the respiratory system were investigated. Male SD rats (age of 5 weeks old on the experiment day) were used for the study. S-297995 monotosylate was suspended in 0.5 w/v% MC and orally administered to rats at dose levels of 30, 100, and 300 mg/kg. The effects of S-297995 monotosylate on

respiratory rate (RR), tidal volume (TV), and minute volume (MV) were measured in non-restraint conscious male rats with a whole body plethysmography system before and 1, 2, 4, and 8 hours after a single oral administration. Vehicle (0.5 w/v% MC) was administered to the control group.

Results: S-297995 monotosylate had no significant effects on RR, TV, or MV in rats at any doses tested. S-297995 showed no effects on any parameters at dose levels up to 1000 mg/kg.

Review of Study No. R-297995-SF-076-L (CRO Study No. B080432) from the IND 107475 nonclinical review dated July 19, 2011 is copied below (Dr. C. Wu, Ph.D.). The statistically significant changes in a subset of parameters evaluated in this study noted in Dr. Wu's review were concluded to be incidental and unrelated to treatment with the test compound. With respect to changes in blood pressure, the observed differences (compared to control) were transient, generally not dose-dependent, and did not correspond with Tmax. For QRS duration, the statistically significant changes were at the low and high dose only (there was not significant effect at the mid-dose), the increases in duration were small in magnitude, and the interval durations were considered to be within a normal range.

Effects of RSC-297995 monotosylate on Cardiovascular System in Conscious Dogs (Study # B080432)

Methods: This was a GLP study. The effects of S-297995 monotosylate on the cardiovascular system were investigated in conscious dogs. Male beagle dogs (age of 9 months at implantation of telemetry transmitter (n=4) were used for the study. S-297995 monotosylate was suspended in 0.5 w/v% MC at dose levels of 10, 30, and 100 mg/kg, and orally administered at 6- or 13-day intervals in a dose-escalation manner. Blood pressures (systolic, diastolic, and mean), heart rate, and electrocardiogram (ECG) parameters (PR interval, QRS duration, QT interval, and corrected QT interval [QTc]) were measured using telemetry systems before administration and 1, 2, 4, 8, and 24 hours after administration. Moreover, the concentrations of S-297995 monotosylate in dog plasma samples collected at 1, 2, 4, 8, and 24 hours after administration were determined by LC/MS/MS and toxicokinetic parameters were determined to investigate the systemic exposure level.

Results: Blood pressures in the 10 mg/kg RSC-297995 monotosylate group were comparable to those in the vehicle group. In the 30 mg/kg group, SBP, DBP, and MBP were significantly higher than those in the vehicle group by 22 to 25 mmHg at 2 hours after administration. In the 100 mg/kg group, SBP was significantly higher than that in the vehicle group; by 11 mmHg at 8 hours after administration. Heart rate showed no clear change from the vehicle group value in the RSC-297995 monotosylate 10, 30, and 100 mg/kg groups up to 24 hours after administration. Regarding QRS duration, the 30 mg/kg RSC-297995 monotosylate group showed no difference from the vehicle group. In the 10 and 100 mg/kg groups, QRS duration was significantly longer than that in the vehicle group; by 3 to 4 msec at 1 hour after administration. PR interval, QT interval, and QTc showed no clear change from the vehicle group value in the RSC-297995 monotosylate 10, 30, and 100 mg/kg groups up to 24 hours after administration. No abnormal arrhythmia was observed in the ECG up to 24 hours after administration of the vehicle or the test substance at 10, 30, or 100 mg/kg. The mean C_{max} values in the 10, 30 and 100 mg/kg groups were 6.86, 23.45 and 35.70 μg/mL, respectively. The corresponding mean $AUC_{0\text{-}24hr}$ values were 21.85, 91.04 and 182.70 μg·hr/mL, respectively, and T_{max} values in all groups were 1.0 hour. The mean plasma concentrations at 24 hours after administration (C_{24hr}) in the 10, 30 and 100 mg/kg groups were not estimated (too low concentration), 0.02, and 0.18 μg/mL, respectively.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption

Review of Study No. R-297995-PB-059-N from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Blood and Plasma Concentration after Single Oral Administration of [Oxadiazole-^{14}C]-S-297995 monotosylate in Rats (Study# R-297995-PB-059-N)

Methods: After single oral administration of [oxadiazole-14C]-S-297995 monotosylate (Lot No. 2007-0412-022-01) suspended with 0.5% methylcellulose (MC) aqueous solution at 1 mg/kg in male SD rats (the age of 9 weeks on the administration day; 4 animals were used) under nonfasted condition, blood and plasma concentrations of radioactivity and plasma concentration of S-297995 were measured at 15, 30 min, 1, 2, 4, 8, 12, 24 and 48 hr after dosing to determine pharmacokinetic parameters.

Results: The maximum plasma concentration (C_{max}) values of radioactivity (S-297995 equivalent) and unchanged S-297995 in plasma were 52.8 and 42.7 ng/mL, respectively. The T_{max} values of radioactivity (S-297995 equivalent) and unchanged S-297995 in plasma were both 1.5 hour. The area under the plasma concentration-time curve between 0 and infinity (AUC_{0-inf}) values of radioactivity and unchanged S-297995 in the plasma were 609 and 213 ng·hr/mL, respectively. The AUC_{0-inf} ratio of S-297995 to radioactivity was calculated to be 34.8%. The C_{max}, T_{max} and AUC_{0-inf} of radioactivity in blood were 41.8 ng/mL, 1.75 hour and 763 ng·hr/mL, respectively. The terminal elimination half-life ($t_{1/2}$) of radioactivity concentration in plasma was 4.18 hours, comparable with that in blood (4.32 hours) as shown in the Table below.

Table 2: Pharmacokinetic parameters of radioactivity in blood, plasma and blood cells and RSC-297995 in plasma following single oral administration of [^{14}C]-RSC-297995 monotosylate in rats at 1 mg/kg (as [^{14}C]-RSC-297995)

Pharmacokinetic Parameters	Radioactivity		RSC-297995	Radioactivity
	Blood	Plasma	Plasma	Blood cells
$Cmax_1$ (ng eq. of RSC-297995/mL or ng/mL)	39.5 ± 8.7	52.8 ± 11.7	42.7 ± 9.4	51.3 ± 3.9
$Cmax_2$ (ng eq. of RSC-297995/mL)	41.8 ± 1.6			
$Tmax_1$ (hr)	1.75 ± 0.50	1.50 ± 0.58	1.50 ± 0.58	8.00 ± 0.00
$Tmax_2$ (hr)	8.00 ± 0.00			
$t_{1/2,Z}$ (hr)[a)]	17.8 ± 9.7	4.18 ± 0.41	1.90 ± 0.42	4.41 ± 1.00
$t_{1/2,12-24}$ (hr)	4.32 ± 0.67			
AUC_{inf} (ng eq. of RSC-297995·hr/mL or ng·hr/mL)	763 ± 81	609 ± 70	213 ± 38	806 ± 95
MRT_{inf} (hr)	15.1 ± 3.6	8.41 ± 0.29	3.75 ± 0.31	11.3 ± 0.9
AUC_{inf} ratio (%)	-	-	34.8 ± 2.8	-

Data represent the mean ± S.D. of four rats.

a): Radioactivity in blood; $t_{1/2\ (24\text{-}48\ hr,\ 24\text{-}48\ hr,\ 12\text{-}24\ hr,\ 24\text{-}48\ hr)}$, Radioactivity in plasma; $t_{1/2\ (12\text{-}24\ hr)}$, RSC-297995 in plasma; $t_{1/2\ (4\text{-}8\ hr,\ 8\text{-}12\ hr,\ 4\text{-}8\ hr,\ 4\text{-}8\ hr)}$, Radioactivity in blood cells; $t_{1/2\ (12\text{-}24\ hr)}$

AUC_{inf} ratio (%) = (AUC_{inf} of RSC-297995 / AUC_{inf} of radioactivity in plasma) × 100

Review of Study No. R-297995-PB-097-N from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Plasma Concentration Following Single Oral Administration of [Carbonyl-^{14}C]-S-297995 monotosylate in Rats (Study# R-297995-PB-097-N)

Methods: After single oral administration of [Carbonyl-14C]-S-297995 monotosylate (Lot No. 2007-0412-022-01) suspended with 0.5% methylcellulose (MC) aqueous solution at 1 mg/kg in male SD rats (the age of 9 weeks on the administration day; 4 male animals were used) under nonfasted condition, plasma concentrations of radioactivity and plasma concentration of S-297995 at 15, 30 min, 1, 2, 4, 8, 12, 24 and 48 hr after dosing were measured to determine pharmacokinetic parameters.

Results: The maximum plasma concentration (C_{max}) values of radioactivity (S-297995 equivalent) and unchanged S-297995 in plasma were 79.8 ng/mL and 73.3 ng/mL, respectively at T_{max} 1.00 hr. The $t_{1/2,z}$ value of was 15.9 ± 6.5 hr. The AUC_{inf} values of radioactivity (S-297995 equivalent) and unchanged S-297995 in plasma were 849 and 293 ng·hr/mL, respectively. The AUC_{inf} ratio of RSC-297995 to radioactivity was 60.2 ± 6.7% as shown in the Table below.

Table 2: Pharmacokinetic parameters of radioactivity in blood, plasma and blood cells and RSC-297995 in plasma following single oral administration of [carbonyl-^{14}C]-RSC-297995 monotosylate in rats at 1 mg/kg (as free base)

Pharmacokinetic Parameters	Radioactivity		RSC-297995	Radioactivity
	Blood	Plasma	Plasma	Blood cells
Cmax (ng eq. of RSC-297995/mL or ng/mL)	55.6 ± 10.3	79.8 ± 13.9	73.3 ± 13.1	21.9 ± 5.5
Tmax (hr)	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.25 ± 0.50
$t_{1/2,Z}$ (hr)[a)]	13.1 ± 6.2	15.9 ± 6.5	1.77 ± 0.38	16.5 ± 9.4
AUC_{inf} (ng eq. of RSC-297995·hr/mL or ng·hr/mL)	360 ± 35	489 ± 49	293 ± 36	220 ± 42
MRT_{inf} (hr)	11.4 ± 5.0	12.1 ± 4.8	3.22 ± 0.55	17.4 ± 8.8
AUC_{inf} ratio (%)	-	-	60.2 ± 6.7	-

Data represent the mean ± S.D. of four rats.

a): Radioactivity in blood; $t_{1/2}$ (12-48 hr, 12-24 hr, 12-24 hr, 12-24 hr). Radioactivity in plasma; $t_{1/2}$ (12-24 hr, 12-24 hr, 12-48 hr, 12-48 hr). RSC-297995 in plasma; $t_{1/2}$ (4-8 hr, 4-8 hr, 4-12 hr, 4-12 hr). Radioactivity in blood cells; $t_{1/2}$ (12-24 hr)

AUC_{inf} ratio (%) = (AUC_{inf} of RSC-297995 / AUC_{inf} of radioactivity in plasma) × 100

Review of Study No. R-297995-PB-070-N from the IND 107475 nonclinical review dated July 19, 2011 is copied below (Dr. C. Wu, Ph.D.).

Dose-linearity of Plasma Concentration Following Single Oral Administration of RSC-297995 monotosylate in Rats (Study# R-297995-PB-070-N)

Methods: RSC-297995 monotosylate suspension was orally administered to rats at the doses of 0.3, 1, 3 and 10 mg/kg while the doses of 0.5 and 1 mg/kg were for intravenous administration. For oral administration study, the blood was collected at 15, 30 min, 1, 2, 4, 8, 12 and 24 hr after dosing. For intravenous administration study, the blood was collected at 2, 5, 15, 30 min, 1, 2, 4, 6, 8, 10 and 24 hr after dosing.

Results: The oral absorption of RSC-297995 monotosylate was rapid (Tmax ≤ 1.1 hr) and the Bioavailability was 25 - 32% following single oral administration at 0.3 - 3 mg/kg in non-fasted rats as shown in Table 4 below. The C_{max} values of S-297995 in plasma were 18.8, 37.9 and 168 ng/mL and AUC_{inf} values were 67.6, 172 and 674 ng·hr/mL, after single oral administration RSC-297995 of at 0.3, 1, 3 mg/kg, respectively. The pharmacokinetics of RSC-297995 was judged to be linear up to 1 mg/kg for intravenous administration and 3 mg/kg for oral administration. There was a negative food effect on the plasma concentration of RSC-297995 following single oral administration.

Table 4 Pharmacokinetic parameters of RSC-297995 following single oral administration of RSC-297995 monotosylate at 0.3, 1, 3 and 10 mg/kg (as RSC-297995) in non-fasted male rats

Pharmacokinetic parameters	Non-fasted			
	0.3 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg
Cmax (ng/mL)	18.8 ± 5.4	37.9 ± 3.4	168 ± 48	853 ± 175
Tmax (hr)	0.875 ± 0.250	1.00 ± 0.00	1.13 ± 0.63	0.625 ± 0.250
$t_{1/2,z}$ (hr)[a)]	1.87 ± 0.63	1.89 ± 0.54	1.66 ± 0.55	1.34 ± 0.34
MRT_{inf} (hr)	3.04 ± 0.95	3.32 ± 0.70	3.20 ± 0.59	2.65 ± 0.48
AUC_{inf} (ng·hr/mL)	67.6 ± 24.1	172 ± 33	674 ± 166	2650 ± 220
BA (%)	32.1 ± 11.4	24.5 ± 4.7	32.0 ± 7.9	37.7 ± 3.2

Data are expressed as the mean ± S.D. of four rats.

BA (%) = $Dose_{iv(0.5mg/kg)}/Dose_{po} \times AUC_{inf,po}/AUC_{inf,iv(0.5mg/kg)} \times 100$

The individual $AUC_{inf,po}$ at each dose and the mean $AUC_{inf,iv}$ at 0.5 mg/kg were used.

a): $t_{1/2,(1\text{-}4hr)}$ or $t_{1/2,(2\text{-}8hr)}$ or $t_{1/2,(4\text{-}12hr)}$

Review of Study No. R-297995-PF-089-N (CRO Study No. AL-3825-G) from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Dose-linearity of Plasma Concentration Following Single Oral Administration of RSC-297995 monotosylate in Rats with Morphine-induced Constipation (Study# R-297995-PF-089-N)

Methods: The plasma samples after a single administration of RSC-297995 monotosylate to rats with morphine-induced constipation at the doses of 0.03, 0.1, 0.3, or 1 mg/kg (as RSC-297995) were analyzed. The plasma concentrations of RSC-297995 were determined by liquid chromatography tandem mass spectrometry (LC/MS/MS).

Results: After a single oral administration of RSC-297995 monotosylate at the doses of 0.03, 0.1, 0.3, or 1 mg/kg (as RSC-297995) to rats with morphine-induced constipation, the C_{max} of RSC-297995 were 2.94, 10.2, 30.8, and 191 ng/mL, respectively. The $AUC_{0-\infty}$ of RSC-297995 were 11.5, 53.8, 114, and 590 ng·h/mL, respectively.

In conclusion, there was a tendency that both C_{max} and $AUC_{0-\infty}$ after a single oral administration of RSC-297995 to rats with morphine-induced constipation increased dose-proportionally in a dose range from 0.03 to 0.3 mg/kg.

Review of Study No. R-297995-PF-090-N (CRO Study No. AL-3826-G) from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Dose-linearity of Plasma Concentration Following Single Oral Administration of RSC-297995 monotosylate in Rats with Castor Oil-induced Diarrhea Inhibited by Morphine (Study# R-297995-PF-090-N)

Methods: The plasma samples after a single administration of RSC-297995 monotosylate to rats with castor oil-induced diarrhea inhibited by morphine at the doses of 0.01, 0.03, 0.1, or 0.3 mg/kg were analyzed. The plasma concentrations of RSC-297995 were determined by liquid chromatography tandem mass spectrometry (LC/MS/MS).

Results: After a single oral administration of RSC-297995 monotosylate at the doses of 0.01, 0.03, 0.1, or 0.3 mg/kg (as RSC-297995) to rats, the C_{max} values of RSC-297995 were 1.51, 5.56, 20.9, and 63.6 ng/mL, respectively. The $AUC_{0-\infty}$ values of RSC-297995 were 6.91, 16.5, 56.3, and 179 ng·h/mL, respectively.

In conclusion, there was a tendency that both C_{max} and $AUC_{0-\infty}$ after single oral administration of RSC-297995 to rats with caster oil-induced diarrhea inhibited by morphine increased dose-proportionally in a dose range from 0.01 to 0.3 mg/kg.

Plasma Concentration of S-297995 and its Metabolites after Single Oral Administration of S-297995 monotosylate in Wistar Rats [Study No. S-297995-PF-197-N; CRO Study No. PBC055-191; Amendment to the Final Report (No. 1)]

Methods: Fasted male rats were administered single oral doses of 1, 3, 5, 7, 10, and 30 mg/kg S-297995 monotosylate in 0.5 w/v% MC 400 solution (n=4/group; dosing volume 2 mL/kg). Blood samples were collected out to 24 h after dosing, and plasma concentrations of S-297995 and its metabolites were determined by LC/MS/MS.

Results: At the lowest dose tested (1 mg/kg), the Cmax value for S-297995 (77.6 ng/mL) was achieved at 0.75 h after dosing, and decreased to 0.0322 ng/mL at 24 h after dosing. Cmax values for metabolites S-297995 6-O-β-D-glucuronide (297995 6-G), S-297995 3-O-β-D-glucuronide (297995 3-G), Nor-S-297995, and S-297995-carboxylic acid following dosing with 1 mg/kg S-297995 were 0.275 ng/mL, 3.06 ng/mL, 3.88 ng/mL, and 0.145 ng/mL, respectively. Only Nor-S-297995 was detected at 24 h. With the exception of metabolite S-297995-carboxylic acid, exposure to S-297995 and its metabolites (based on concentration and AUC) increased in a dose-related manner from 1 to 30 mg/kg S-297995. Exposure to metabolite S-297995-carboxylic acid increased with increasing dose from 1 to 10 mg/kg, but the increase from 10 to 30 mg/kg was less than dose-proportional.

Review of Study No. R-297995-PF-198-N (CRO Study No. PBC055-190) from the IND 107475 nonclinical review dated July 19, 2011 is copied below (Dr. C. Wu, Ph.D.).

Plasma and Brain Concentration of S-297995 and its Metabolites after Single Oral Administration of S-297995 monotosylate in Wistar Rats with Morphine-induced Analgesia (Study# S-297995-PF-198-N)

Methods: Plasma and brain concentrations of S-297995 and its metabolites after single oral administration of S-297995 monotosylate at 3, 5, 7, 10, and 30 mg/kg in 4 male rats/group with morphine-induced analgesia were determined by LC/MS/MS method. PK parameters of S-297995 and its metabolites were calculated from the plasma and brain concentration at 1, 4, 8, and 24 hours after dosing.

Results: At 3 mg/kg, mean plasma concentration of S-297995 reached 167 ng/mL at 1.00 hour after administration, and decreased to 0.0827 ng/mL at 24 hours with a $t_{1/2,\ 8\text{-}24h}$ of 2.61 h. Both $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ were 819 ng·h/mL. Mean plasma Cmax concentrations of S-297995 6-G, S-297995 3-G, Nor-S-297995, and S-297995-carboxylic acid reached 0.202, 4.71, 10.7, and 0.115 ng/mL at 1.00, 1.00, 1.00, and 4.00 hours, respectively. Metabolites, except for Nor-S-297995 (0.275 ng/mL at 24 hours) were below LLOQ (0.04 ng/mL) at 24 hours. At 5 mg/kg, mean plasma concentration of S-297995 reached 300 ng/mL of Cmax at 1.00 hour after administration, and decreased to 0.145 ng/mL at 24 hours with a $t_{1/2,\ 8\text{-}24h}$ of 2.58 h. Both $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ were 1140 ng·h/mL. Mean plasma concentrations of S-297995 6-G, S-297995 3-G, Nor-S-297995, and S-297995-carboxylic acid reached 0.385, 7.07, 16.5, and 0.565 ng/mL of Cmax at 1.00, 1.00, 1.00, and 4.00 hours, respectively. Metabolites, except for Nor-S-297995 (0.311

ng/mL at 24 hours) were below LLOQ (0.04 ng/mL) at 24 hours. At 7 mg/kg, mean plasma concentration of S-297995 reached 433 ng/mL of Cmax at 1.00 hour after administration, and decreased to 0.241 ng/mL at 24 hours with a $t_{1/2, 8\text{-}24h}$ of 2.73 h. Both $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ were 2160 ng·h/mL. Mean plasma concentrations of S-297995 6-G, S-297995 3-G, Nor-S-297995, and S-297995-carboxylic acid reached 0.345, 8.16, 23.7, and 0.364 ng/mL of Cmax at 1.00 hour, respectively. Metabolites, except for Nor-S-297995 (0.601 ng/mL at 24 hours) were below LLOQ (0.04 ng/mL) at 24 hours. At 10 mg/kg, mean plasma concentration of S-297995 reached 636 ng/mL of Cmax at 1.00 hour after administration, and decreased to 0.364 ng/mL at 24 hours with a $t_{1/2, 8\text{-}24h}$ of 2.51 h. Both $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ were 3000 ng·h/mL. Mean plasma concentrations of S-297995 6-G, S-297995 3-G, Nor-S-297995, and S-297995-carboxylic acid reached 0.869, 12.7, 34.8, and 0.374 ng/mL of Cmax at 1.00, 1.00, 4.00, and 4.00 hours, respectively. Metabolites, except for Nor-S-297995 (0.732 ng/mL at 24 hours) were below LLOQ (0.04 ng/mL) at 24 hours. At 30 mg/kg, mean plasma concentration of S-297995 reached 1730 ng/mL of Cmax at 1.00 hour after administration, and decreased to 0.915 ng/mL at 24 hours with a $t_{1/2, 8\text{-}24h}$ of 2.32 h. Both $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ were 10500 ng·h/mL. Mean plasma concentrations of S-297995 6-G, S-297995 3-G, Nor-S-297995, and S-297995-carboxylic acid reached 1.90, 38.2, 89.1, and 2.86 ng/mL of Cmax at 1.00, 1.00, 4.00, and 4.00 hours, respectively. Plasma concentrations of S-297995 3-G, Nor-S-297995 and S-297995-carboxylic acid were 0.0373, 1.87 and 0.0938 ng/mL at 24 hours, respectively. S-297995 6-G was below LLOQ (0.04 ng/mL) at 24 hours. At 5 to 30 mg/kg, mean plasma concentration profiles of S-297995 were similar to that at 3 mg/kg. Cmax and AUC increased almost dose proportionally, and Tmax and $t_{1/2, 8\text{-}24h}$ were almost constant.

At 3 mg/kg, mean brain concentration of S-297995 reached 7.01 ng/g (B/P ratio; 0.0575) of Cmax at 4.00 hours after administration, and decreased to 1.86 ng/g (B/P ratio; 22.5) at 24 hours with a $t_{1/2, 8\text{-}24h}$ of 21.7 h. $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ were 80.4 and 139 ng·h/g, respectively. Mean brain concentrations of S-297995 3-G, Nor-S-297995, and S-297995-carboxylic acid reached 0.0355, 0.119, and 0.0451 ng/g of Cmax at 1.00, 4.00 and 8.00 hours, respectively. Metabolites, except for Nor-S-297995 (0.0565 ng/g at 24 hours), were below LLOQ (0.04 ng/g) at 24 hours. S-297995 6-G was not detected at any time point. At 5 mg/kg, mean brain concentration of S-297995 reached 8.44 ng/g (B/P ratio; 0.0281) of Cmax at 1.00 hour after administration, and decreased to 2.66 ng/g (B/P ratio: 18.3) as listed in the sponsor's table 2 and table 3) at 24 hours with a $t_{1/2, 8\text{-}24h}$ of 23.1 h. $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ were 108 and 197 ng·h/g, respectively. Mean brain concentrations of S-297995 3-G, Nor-S-297995, and S-297995-carboxylic acid reached 0.0523, 0.173, and 0.125 ng/g of Cmax at 1.00, 1.00, and 4.00 hours. Metabolites, except for Nor-S-297995 (0.0674 ng/g at 24 hours) were below LLOQ (0.04 ng/g) at 24 hours. S-297995 6-G was not detected at any time point. At 7 mg/kg, mean brain concentration of S-297995 reached 16.0 ng/g (B/P ratio; 0.0486) of Cmax at 4.00 hours after administration, and decreased to 4.13 ng/g (B/P ratio: 17.1) at 24 hours with a $t_{1/2, 8\text{-}24h}$ of 21.9 h. $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ were 184 and 315 ng·h/g, respectively. Mean brain concentrations of S-297995 3-G, Nor-S-297995, and S-297995-carboxylic acid reached 0.0719, 0.288, and 0.132 ng/g of Cmax at 1.00, 4.00, and 8.00 hours, respectively. Metabolites, except for Nor-S-297995 (0.130 ng/g at 24 hours) were below LLOQ (0.04 ng/g) at 24 hours. S-297995 6-G was not detected at any time point. At 10 mg/kg, mean brain concentration of S-297995 reached 27.9 ng/g (B/P ratio; 0.0687) of Cmax at 4.00 hours after administration, and decreased to 4.81 ng/g (B/P ratio: 13.2) at 24 hours with a $t_{1/2, 8\text{-}24h}$ of 13.6 h. $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ were 275 and 369

ng·h/g, respectively. Mean brain concentrations of S-297995 3-G, Nor-S-297995, and S-297995-carboxylic acid reached 0.108, 0.487, and 0.0776 ng/g of Cmax at 1.00, 4.00, and 4.00 hours, respectively. Metabolites, except for Nor-S-297995 (0.192 ng/g at 24 hours) were below LLOQ (0.04 ng/g) at 24 hours. S-297995 6-G was not detected at any time point. At 30 mg/kg, mean brain concentration of S-297995 reached 72.4 ng/g (B/P ratio; 0.0426) of Cmax at 4.00 hours after administration, and decreased to 7.85 ng/g (B/P ratio; 8.58) at 24 hours with a $t_{1/2, 8\text{-}24h}$ of 8.27 h. $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ were 683 and 777 ng·h/g, respectively. Mean brain concentrations of S-297995 3-G, Nor-S-297995, and S-297995-carboxylic acid reached 0.319, 1.15, and 0.327 ng/g of Cmax at 1.00, 4.00, and 4.00 hours, respectively. Nor-S-297995 and S-297995-carboxylic acid decreased to 0.462 and 0.0807 ng/g at 24 hours, respectively. S-297995 3-G was below LLOQ (0.04 ng/g) at 24 hours. S-297995 6-G was not detected at any time point. At 5 to 30 mg/kg, mean brain concentration profiles of S-297995 were similar to that at 3 mg/kg. Cmax and AUC increased almost dose proportionally and, Tmax and $t_{1/2, 8\text{-}24h}$ were almost constant. Thus, the data indicated that brain concentrations of S-297995 and its metabolites were lower than those in plasma. Although plasma and brain concentrations of S-297995 and its metabolites decreased to very low concentrations within 24 hours, the disappearance of S-297995 from the brain was slower than that from the plasma.

Plasma Concentrations of S-297995 and Morphine after Single Oral Administration of S-297995 monotosylate in Rats with Peroral Morphine-induced Emesis (Study No. S-297995-PF-251-N; CRO Study No. PBC055-270)

Methods: Fasted male Crlj:Wistar rats were administered single oral doses of 0.03, 0.1, 0.3, and 1 mg/kg S-297995 monotosylate (or vehicle, 0.5 w/v% methyl cellulose; dosing volume 2 mL/kg). Fifteen minutes after dosing, the animals were given single oral doses of 20 mg/kg morphine hydrochloride. Two groups of animals were included at each S-297995 dose level (n=4/group), and plasma concentrations of morphine (Groups 1-5) and S-297995 (Groups 6-9) were determined by LC/MS/MS.

Results: Exposure to S-297995 (based on AUC and Cmax) increased in a dose-proportional manner. Tmax ranged from 0.5 to 0.75 h and $t_{1/2,z}$ ranged from 2.02 to 3.06 h. Morphine Cmax values increased with increasing doses of S-297995 up to 0.3 mg/kg, and AUC values at all doses of S-297995 exceeded those of the vehicle control group. Summary data are provided in the Applicant's tables below.

Table 1-1 Plasma concentration and pharmacokinetics parameters (mean ± SD) of morphine after single oral administration of vehicle (0.5 w/v% MC) or S-297995 monotosylate (0.03, 0.1, 0.3, and 1 mg/kg as S-297995) and morphine hydrochloride (20 mg/kg)

Time after administration	Concentration (ng/mL)					
	vehicle (0.5 w/v% MC)*	0.03 mg/kg*	0.1 mg/kg*	0.3 mg/kg*	1 mg/kg*	
5 min	110 ± 78	333 ± 110	507 ± 268	645 ± 416	678 ± 584	
15 min	100 ± 48	394 ± 126	440 ± 53	729 ± 221	546 ± 147	
30 min	173 ± 13	299 ± 84	308 ± 124	521 ± 118	455 ± 102	
45 min	149 ± 42	249 ± 54	301 ± 81	425 ± 62	375 ± 47	
1 h	160 ± 79	191 ± 32	265 ± 70	342 ± 63	283 ± 60	
2 h	113 ± 27	158 ± 26	143 ± 32	122 ± 29	131 ± 48	
4 h	55.7 ± 35.5	77.9 ± 63.1	36.9 ± 24.4	25.5 ± 4.3	70.8 ± 82.4	
6 h	30.4 ± 22.4	48.9 ± 44.5	19.6 ± 14.4	45.0 ± 32.6	58.1 ± 62.0	
8 h	42.1 ± 11.5	26.3 ± 3.9	42.1 ± 19.6	38.8 ± 11.6	32.8 ± 16.0	
24 h	1.92 ± 3.83	1.58 ± 3.16	4.00 ± 2.67	2.01 ± 4.03	1.29 ± 2.57	
C_{max} (ng/mL)	206 ± 31	397 ± 120	585 ± 196	860 ± 311	766 ± 494	
T_{max} (h)	0.583 ± 0.391	0.208 ± 0.083	0.167 ± 0.096	0.167 ± 0.096	0.167 ± 0.096	
$t_{1/2,z}$ (h)	4.49 ± 2.14	3.35 ± 2.59	4.95 ± 2.45	3.17 ± 3.07	2.77 ± 1.96	
AUC_{0-t} (ng·h/mL)	674 ± 118	951 ± 191	1140 ± 350	1130 ± 80	1160 ± 420	
$AUC_{0-\infty}$ (ng·h/mL)	905 ± 190	1030 ± 180	1200 ± 340	1220 ± 110	1230 ± 410	

Data are the mean ± SD of the results from four animals.

*: Dose of vehicle or S-297995 monotosylate

Table 1-2 Plasma concentration and pharmacokinetics parameters (mean ± SD) of S-297995 after single oral administration of S-297995 monotosylate (0.03, 0.1, 0.3, and 1 mg/kg as S-297995) and morphine hydrochloride (20 mg/kg)

Time after administration	Concentration (ng/mL)			
	0.03 mg/kg*	0.1 mg/kg*#	0.3 mg/kg*	1 mg/kg*
5 min	0.885 ± 0.891	1.81 ± 1.22	7.79 ± 3.87	27.3 ± 20.1
15 min	2.46 ± 2.20	7.10 ± 3.09	23.7 ± 7.3	88.0 ± 36.6
30 min	3.46 ± 1.97	11.3 ± 2.9	32.6 ± 7.4	125 ± 21
1 h	3.10 ± 0.85	9.27 ± 2.56	25.8 ± 1.7	101 ± 16
2 h	1.83 ± 0.22	5.44 ± 2.22	17.4 ± 4.7	52.7 ± 16.0
4 h	1.13 ± 0.27	2.60 ± 0.90	9.11 ± 2.11	30.4 ± 4.2
6 h	0.638 ± 0.302	2.33 ± 0.54	7.29 ± 2.42	18.1 ± 6.0
8 h	0.553 ± 0.586	0.834 ± 0.308	2.14 ± 1.08	7.59 ± 2.36
24 h	N.C. ± N.C.	N.C. ± N.C.	0.0115 ± 0.0013	0.119 ± 0.160
C_{max} (ng/mL)	3.74 ± 1.71	11.3 ± 2.9	32.6 ± 7.4	125 ± 21
T_{max} (h)	0.750 ± 0.289	0.500 ± 0.000	0.500 ± 0.000	0.500 ± 0.000
$t_{1/2,z}$ (h)	3.06 ± 2.23	2.24 ± 0.61	2.02 ± 0.06	2.42 ± 0.66
AUC_{0-t} (ng·h/mL)	11.1 ± 2.5	31.7 ± 3.6	116 ± 14	390 ± 34
$AUC_{0-\infty}$ (ng·h/mL)	14.9 ± 6.8	34.6 ± 4.1	116 ± 14	390 ± 33

N.C.: Not calculated

Data are the mean ± SD of the results from four animals.

#: Data are the mean ± SD of the results from three animals, except for animal number 702.

*: Dose of S-297995 monotosylate

Pharmacokinetics and Disposition Following Single Administration of [^{14}C]-RSC-297995 monotosylate in Dogs (Study No. R-297995-PB-068-N).

Methods: Male dogs were administered [^{14}C]-RSC-297995 monotosylate by the oral, intravenous (i.v.), and intraduodenal routes of administration at single doses of 1 mg (as free base)/69.6 µCi/mL/kg, 0.5 mg (as free base)/34.8 µCi/0.2 mL/kg, and 1 mg/69.6 µCi/0.5 mL/kg, respectively. The dosing vehicle for oral and intraduodenal

administration was 0.5% methylcellulose aqueous solution, and the vehicle for i.v. administration was N,N-Dimethylacetamide/saline (1:9, v/v). Parameters evaluated were: blood, plasma, and blood cell concentration profiles of radioactivity; plasma concentration profile of RSC-297995; distribution radio of radioactivity in blood cells; and cumulative excretion of radioactivity in urine, feces, and bile. RSC-297995 concentrations were determined by LC/MS/MS.

Results: PK parameters of radioactivity and RSC-297995 following i.v. dosing under non-fasted conditions are summarized in the Applicant's table below. Plasma and blood radioactivity concentrations decreased in a bi-exponential manner, and as shown in the table, $t_{1/2,z}$ values in plasma, blood, and blood cells were similar (18 to 21.2 h). Distribution ratios of radioactivity in blood cells ranged from 9.3 to 35.6%. RSC-297995 concentrations in plasma were below the lower limit of quantification (BLQ) at 8 h after dosing, and the $t_{1/2,z}$ was substantially shorter (1h) than that of radioactivity concentration. The plasma AUC ratio of RSC-297995 to radioactivity was 40.4%.

Table 3 Pharmacokinetic parameters of radioactivity and RSC-297995 following single intravenous administration of [^{14}C]-RSC-297995 monotosylate at 0.5 mg/kg (as [^{14}C]-RSC-297995) in dogs

Pharmacokinetic parameters	Unit	Radioactivity			RSC-297995
		Blood	Plasma	Blood cells	Plasma
AUC_{inf}	ng eq. of RSC-297995·hr/mL or ng·hr/mL	1330 ± 110	1870 ± 130	680 ± 98	751 ± 80
CLtot	mL/hr/kg	379 ± 28	268 ± 18	746 ± 96	671 ± 72
$t_{1/2,z}$	hr	19.7 ± 0.9	21.2 ± 1.9	18.0 ± 3.6	1.00 ± 0.06
Vdss	mL/kg	6900 ± 370	4710 ± 510	17300 ± 4300	559 ± 83
MRT_{inf}	hr	18.3 ± 0.7	17.6 ± 2.2	23.0 ± 3.6	0.831 ± 0.055
AUC ratio to radioactivity	%	-	-	-	40.4 ± 5.8

Data are expressed as the mean ± S.D. of four dogs.
The $t_{1/2,z}$ of radioactivity and RSC-297995 concentration are calculated using logarithmic values of four points (10 – 48 hr) and three points (2 – 6 hr), respectively.
AUC ratio to radioactivity (%) = AUC_{inf} of RSC-297995 / AUC_{inf} of radioactivity × 100

PK parameters of radioactivity and RSC-297995 following oral dosing under non-fasted conditions are summarized in the Applicant's table below. The profiles of radioactivity concentrations in blood and plasma were similar, with $t_{1/2,z}$ values of 15 and 16.6 h, respectively. The Tmax for radioactivity concentration in blood cells was 4.75 h, and the $t_{1/2,z}$ value in blood cells was 12.6 h. Distribution ratios of radioactivity in blood cells increased with time up to 48 h after dosing, and ranged from 8.4 to 36.9% over the sampling periods evaluated. RSC-297995 concentrations in plasma were BLQ at 24 h after dosing, and the $t_{1/2,z}$ was substantially shorter (1.8 h) than that of radioactivity concentration. The plasma AUC ratio of RSC-297995 to radioactivity was 36.5%, and the oral bioavailability was estimated to be 59.5%.

Table 6 Pharmacokinetic parameters of radioactivity and RSC-297995 following single oral administration of [^{14}C]-RSC-297995 monotosylate at 1 mg/kg (as [^{14}C]-RSC-297995) in dogs

Pharmacokinetic parameters	Unit	Radioactivity			RSC-297995
		Blood	Plasma	Blood cells	Plasma
Cmax	ng eq. of RSC-297995/mL or ng/mL	146 ± 65	235 ± 100	48.6 ± 13.9	207 ± 113
Tmax	hr	2.75 ± 2.22	2.75 ± 2.22	4.75 ± 3.77	2.38 ± 2.50
$t_{1/2,z}$	hr	15.0 ± 0.9	16.6 ± 1.5	12.6 ± 0.9	1.82 ± 0.43
MRT_{inf}	hr	18.4 ± 1.8	18.3 ± 1.4	19.6 ± 2.4	3.84 ± 1.67
AUC_{inf}	ng eq. of RSC-297995·hr/mL or ng·hr/mL	1810 ± 100	2430 ± 100	1060 ± 110	885 ± 121
AUC ratio to radioactivity	%	-	-	-	36.5 ± 4.8
Bioavailability	%	-	-	-	59.5 ± 10.3

Data are expressed as the mean ± S.D. of four dogs.
The $t_{1/2,z}$ of radioactivity concentration are calculated using logarithmic values of three points (12 – 48 hr) or four points (10 – 48 hr).
The $t_{1/2,z}$ of RSC-297995 concentration are calculated using logarithmic values of three points (8 – 12 hr) or four points (6 – 12 hr), respectively.
AUC ratio to radioactivity (%) = AUC_{inf} of RSC-297995 / AUC_{inf} of radioactivity × 100
Bioavailability (%) = [AUC_{inf} (oral) of RSC-297995 / Dose (oral)] × [Dose (intravenous) / AUC_{inf} (intravenous) of RSC-297995] × 100

Following intraduodenal administration to dogs under fasted conditions, PK parameters of radioactivity in plasma showed that the Cmax and AUC_{inf} values (577 ng eq. of RSC-297995/mL and 4480 ng eq. of RSC-297995.h/mL, respectively) were greater than those following oral administration, and the Tmax value (0.5 h) was shorter than that following oral administration.

Cumulative excretion of radioactivity in urine and feces following i.v. and oral dosing was similar and indicated that most of the administered radioactivity was excreted into the feces. The cumulative urinary and fecal excretion of radioactivity up to 168 h after i.v. dosing was 24.3% and 68% of dose, respectively (total: 92.5%). The cumulative urinary and fecal excretion of radioactivity up to 168 h after oral dosing was 25.7% and 67% of dose, respectively (total: 92.8%). Following intraduodenal administration, urinary, biliary, and fecal excretion of radioactivity up to 48 h after dosing was 28.1%, 57.8%, and 9.3% of dose, respectively, indicating that the primary route of excretion was in the feces via bile. An absorption ratio of radioactivity was estimated to be 85.9%, based on the sum of urinary and biliary excretion ratio.

Pharmacokinetics of Radioactivity and S-297995 after Single Oral Administration of [Carbonyl-^{14}C]-S-297995 monotosylate in Dogs (Study No. S-297995-PB-177-N).

Methods: Male dogs were administered [Carbonyl-^{14}C]-S-297995 monotosylate by the oral and intraduodenal routes of administration at single doses of 1 mg (as free base)/72.2 µCi/mL/kg and 1 mg/72.2 µCi/0.5 mL/kg, respectively. The dosing vehicle was 0.5% methylcellulose aqueous solution, and parameters evaluated were: blood, plasma, and blood cell concentration profiles of radioactivity; plasma concentration

profile of RSC-297995; distribution radio of radioactivity in blood cells; and cumulative excretion of radioactivity in urine, feces, and bile. RSC-297995 concentrations were determined by LC/MS/MS.

Results: PK parameters of radioactivity and RSC-297995 following oral dosing are summarized in the Applicant's table below. The profiles of radioactivity concentrations in blood and plasma were similar, with $t_{1/2,z}$ values of 35.5 and 32.1 h, respectively. The Tmax for radioactivity concentration in blood cells was 2.5 h, and the $t_{1/2,z}$ value in blood cells was 47.6 h. Distribution ratios of radioactivity in blood cells increased with time up to 24 h after dosing, and ranged from 6.9 to 32.1% over the sampling periods evaluated. RSC-297995 concentrations in plasma were BLQ at 24 h after dosing, and the $t_{1/2,z}$ was shorter (1.74 h) than that of radioactivity concentration. The AUC ratio of RSC-297995 to radioactivity in plasma was 37.8%, and the oral bioavailability was estimated to be 50.6%.

Table 3 Pharmacokinetic parameters of radioactivity and S-297995 after single oral administration of [carbonyl-^{14}C]-S-297995 monotosylate at 1 mg/kg (as free base) in dogs

Pharmacokinetic parameters	Unit	Radioactivity			S-297995
		Blood	Plasma	Blood cells	Plasma
Cmax	ng eq. of S-297995/mL or ng/mL	211 ± 43	342 ± 63	48.4 ± 14.7	276 ± 61
Tmax	hr	1.75 ± 0.50	1.75 ± 0.50	2.50 ± 1.00	1.50 ± 0.58
$t_{1/2,z}$*	hr	35.5 ± 18.9	32.1 ± 9.7	47.6 ± 43.4	1.74 ± 0.72
MRT_{inf}	hr	36.8 ± 26.3	28.4 ± 11.6	63.8 ± 63.4	2.95 ± 0.78
AUC_{inf}	ng eq. of S-297995·hr/mL or ng·hr/mL	2210 ± 1130	2730 ± 580	1880 ± 2370	999 ± 69
AUC ratio to radioactivity	%	-	-	-	37.8 ± 7.3
Bioavailability	%	-	-	-	50.6 ± 2.5

Data are expressed as the mean ± S.D. of four dogs.
*: $t_{1/2,(6\text{-}10 \text{ or } 8\text{-}12 \text{ or } 12\text{-}48 \text{ hr})}$
AUC ratio to radioactivity (%) = AUC_{inf} of S-297995 / AUC_{inf} of radioactivity × 100
Bioavailability (%) = [AUC_{inf} (oral) of S-297995 / Dose (oral)] × [Dose (intravenous) / AUC_{inf} (intravenous) of S-297995] × 100

Following intraduodenal administration to dogs under fasted conditions, PK parameters of radioactivity in plasma indicated that Cmax and AUC_{inf} values (606 ng eq. of RSC-297995/mL and 6610 ng eq. of RSC-297995.h/mL, respectively) were greater than those following oral administration, and the Tmax value (0.5 h) was shorter than that following oral administration.

Cumulative excretion of radioactivity in urine and feces following oral dosing indicated that most of the administered radioactivity was excreted into the feces. The cumulative urinary and fecal excretion of radioactivity up to 168 h after oral dosing was 5.2% and 92.0% of dose, respectively (total: 97.2%). Following intraduodenal administration, urinary, biliary, and fecal excretion of radioactivity up to 48 h after dosing was 14.5%, 52.4%, and 26.0% of dose, respectively, indicating that the primary route of excretion was in the feces via bile. An absorption ratio of radioactivity after intraduodenal administration was estimated to be 66.9%, based on the sum of urinary and biliary excretion ratio.

Review of Study No. R-297995-PB-161-N from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Dose-linearity of Plasma Concentration of S-297995 after Single Oral Administration of S-297995 monotosylate in Dogs (Study# R-297995-PB-161-N)

Methods: A single intravenous administration of S-297995 monotosylate was given at a dose level of 0.5 and 1 mg/kg to 4 male beagle dogs of each group under non-fasted conditions, plasma concentrations of S-297995 at 15, 30 min, 1, 2, 4, 6, 8, 10, 12, 24 and 48 hr after dosing were measured to determine pharmacokinetic parameters. Whereas, after single oral administration of S-297995 monotosylate at 0.3, 1, 3 and 10 mg/kg to 4 male beagles/group under non-fasted conditions, plasma concentrations of S-297995 at 2, 5, 15, 30 min, 1, 2, 4, 6, 8, 10 and 24 hr after dosing were measured to investigate food effects on absorption of S-297995.

Results: Following single intravenous administration of S-297995 monotosylate at 0.5 and 1 mg/kg in non-fasted dogs, the pharmacokinetics of S-297995 was judged to be linear up to 1 mg/kg. After the dosing at 0.5 and 1 mg/kg, the plasma concentration of S-297995 rapidly decreased with $t_{1/2,z}$ values of 1.28 ± 0.03 and 1.24 ± 0.03 hr, respectively. The AUC_{inf} values at 0.5 and 1 mg/kg were 989 ± 72 and 1970 ± 190 ng·hr/mL, respectively. The CLtot values at 0.5 and 1 mg/kg were 508 ± 36 and 512 ± 47 mL/hr/kg, respectively. After single oral administration of S-297995 monotosylate at 0.3–10 mg/kg in non-fasted dogs, the pharmacokinetics of S-297995 was judged to be basically linear up to 3 mg/kg and non-linear at 10 mg/kg. The Tmax values were in the range of 1.13–1.75 hr. The Cmax values at 0.3, 1, 3 and 10 mg/kg were 57.9 ± 35.8, 213 ± 81, 963 ± 346 and 4110 ± 630 ng/mL, respectively. Thereafter, the plasma concentrations of S-297995 decreased with the $t_{1/2,z}$ values of 1.70–3.14 hr. The AUC_{inf} values at 0.3, 1, 3 and 10 mg/kg were 291 ± 45, 989 ± 127, 3700 ± 500, 19500 ± 2800 ng·hr/mL, respectively. The bioavailability values at 0.3, 1, 3 and 10 mg/kg were 48.9 ± 4.0%, 49.9 ± 3.5%, 62.4 ± 7.9% and 98.6 ± 11.2%, respectively.

Review of Study No. R-297995-PB-088-N (CRO Study No. AL-3824-G) from the IND 107475 nonclinical review dated July 19, 2011 is copied below (Dr. C. Wu, Ph.D.).

Dose-linearity of Plasma Concentration Following Single Oral Administration of RSC-297995 monotosylate in Ferrets with Morphine-induced Emesis (Study# R-297995-PB-088-N)

Methods: The plasma samples after a single oral administration of RSC-297995 monotosylate to ferrets with morphine-induced emesis at the doses of 0.01, 0.03, 0.1, or 0.3 mg/kg (as RSC-297995) were analyzed. The plasma concentrations of RSC-297995 were determined by liquid chromatography tandem mass spectrometry (LC/MS/MS).

Results: After a single oral administration of RSC-297995 monotosylate at the doses of 0.01, 0.03, 0.1, or 0.3 mg/kg (as RSC-297995) to ferrets with morphine-induced emesis, the C_{max} of RSC-297995 were 4.72, 5.01, 10.9, and 77.3 ng/mL, respectively. The $AUC_{0-\infty}$ of RSC-297995 were 9.65, 21.8, 52.7, and 291 ng•h/mL, respectively.

In conclusion, there was a tendency that both C_{max} and $AUC_{0-\infty}$ after a single oral administration of RSC-297995 to ferrets with morphine-induced emesis roughly increased as the dose increased from 0.01 to 0.3 mg/kg.

Plasma Concentrations of S-297995 and Morphine after Single Oral Administration of S-297995 monotosylate in Ferrets with Peroral Morphine-induced Emesis (Study No. S-297995-PF-250-N; CRO Study No. PBC055-269)

Methods: Male ferrets were administered single oral doses of 0.01, 0.03, 0.1, and 0.3 mg/kg S-297995 monotosylate (or vehicle, 0.5 w/v% methyl cellulose; dosing volume 2 mL/kg) five and a half hours after feeding. Thirty minutes after dosing, the animals were given single oral doses of 1.2 mg/kg morphine hydrochloride. Two groups of animals were included at each S-297995 dose level (n=4/group), and plasma concentrations of morphine (Groups 1-5) and S-297995 (Groups 6-9) were determined by LC/MS/MS.

Results: Exposure to S-297995 (based on AUC and Cmax) increased in a dose-proportional manner. Tmax was approximately 0.6 h and $t_{1/2,z}$ ranged from 1.14 - 2.7 h. Morphine Cmax and AUC values in groups administered S-297995 generally exceeded those of the vehicle control group, but were considered to be similar. Summary data are provided in the Applicant's tables below.

Table 1-1 Plasma concentration and pharmacokinetics parameters (mean ± SD) of morphine after single oral administration of vehicle (0.5 w/v% MC) or S-297995 monotosylate (0.01, 0.03, 0.1, and 0.3 mg/kg as S-297995) and morphine hydrochloride (1.2 mg/kg)

Time after administration	Concentration (ng/mL)				
	vehicle (0.5 w/v% MC)*	0.01 mg/kg*	0.03 mg/kg*	0.1 mg/kg*	0.3 mg/kg*
5 min	12.6 ± 7.1	25.7 ± 16.6	17.3 ± 17.8	38.6 ± 35.7	36.9 ± 32.6
15 min	48.9 ± 14.2	56.4 ± 31.7	65.8 ± 15.3	72.2 ± 19.3	75.2 ± 13.2
30 min	50.6 ± 22.5	64.9 ± 6.0	70.1 ± 9.3	59.5 ± 11.3	65.3 ± 15.0
1 h	35.5 ± 10.6	41.9 ± 5.6	42.0 ± 9.8	30.7 ± 2.5	42.9 ± 10.4
2 h	11.7 ± 3.1	9.92 ± 1.85	9.37 ± 2.43	7.08 ± 1.29	9.19 ± 2.14
4 h	1.45 ± 2.90	N.C. ± N.C.	1.51 ± 3.02	2.48 ± 4.96	2.95 ± 5.90
6 h	1.25 ± 2.51	N.C. ± N.C.	1.75 ± 3.50	3.06 ± 3.59	1.86 ± 3.72
8 h	N.C. ± N.C.	N.C. ± N.C.	1.27 ± 2.53	1.36 ± 2.72	2.67 ± 3.09
24 h	N.C. ± N.C.	N.C. ± N.C.	N.C. ± N.C.	N.C. ± N.C.	N.C. ± N.C.
C_{max} (ng/mL)	54.8 ± 16.6	72.4 ± 13.5	74.1 ± 8.0	72.7 ± 18.3	75.2 ± 13.2
T_{max} (h)	0.375 ± 0.144	0.375 ± 0.144	0.438 ± 0.125	0.313 ± 0.125	0.250 ± 0.000
$t_{1/2,z}$ (h)	0.964 ± 0.428	0.545 ± 0.068	0.878 ± 0.756	1.33 ± 1.14	1.68 ± 0.83
AUC_{0-t} (ng·h/mL)	71.4 ± 32.1	75.7 ± 6.6	89.1 ± 33.5	83.8 ± 16.4	99.1 ± 21.0
$AUC_{0-\infty}$ (ng·h/mL)	82.9 ± 29.8	83.6 ± 7.9	97.5 ± 37.9	94.4 ± 21.6	114 ± 25

N.C.: Not calculated

Data are the mean ± SD of the results from four animals.

*: Dose of vehicle or S-297995 monotosylate

Table 1-2 Plasma concentration and pharmacokinetics parameters (mean ± SD) of S-297995 after single oral administration of S-297995 monotosylate (0.01, 0.03, 0.1, and 0.3 mg/kg as S-297995) and morphine hydrochloride (1.2 mg/kg)

Time after administration	Concentration (ng/mL)			
	0.01 mg/kg*#	0.03 mg/kg*	0.1 mg/kg*	0.3 mg/kg*
5 min	0.303 ± 0.294	1.06 ± 0.79	2.82 ± 1.24	4.79 ± 4.59
15 min	1.59 ± 0.98	6.99 ± 3.49	17.4 ± 3.4	48.0 ± 18.8
30 min	2.43 ± 1.21	8.36 ± 2.76	24.9 ± 3.2	81.9 ± 17.0
1 h	2.69 ± 0.64	6.61 ± 0.37	23.7 ± 8.3	79.2 ± 23.9
2 h	1.23 ± 0.31	3.28 ± 0.93	13.1 ± 4.9	40.3 ± 14.7
4 h	0.233 ± 0.102	0.643 ± 0.225	3.05 ± 2.08	9.82 ± 5.21
6 h	0.0663 ± 0.0306	0.206 ± 0.077	1.07 ± 0.91	3.52 ± 2.24
8 h	0.0259 ± 0.0098	0.0892 ± 0.0286	0.449 ± 0.416	1.65 ± 1.20
24 h	N.C. ± N.C.	N.C. ± N.C.	0.00295 ± 0.00590	0.0254 ± 0.0081
C_{max} (ng/mL)	2.75 ± 0.69	8.93 ± 2.17	26.6 ± 6.3	86.3 ± 16.3
T_{max} (h)	0.667 ± 0.289	0.563 ± 0.315	0.625 ± 0.250	0.625 ± 0.250
$t_{1/2,z}$ (h)	1.14 ± 0.27	1.30 ± 0.26	1.51 ± 0.59	2.70 ± 0.28
AUC_{0-t} (ng·h/mL)	5.77 ± 1.70	16.4 ± 2.8	61.5 ± 25.2	203 ± 65
$AUC_{0-\infty}$ (ng·h/mL)	5.81 ± 1.72	16.5 ± 2.8	61.8 ± 25.1	203 ± 65

N.C.: Not calculated

Data are the mean ± SD of the results from four animals.

#: Data are the mean ± SD of the results from three animals, except for animal number 601.

*: Dose of S-297995 monotosylate

Plasma Concentration of Radioactivity and S-297995 after Repeated Oral Administration of [Carbonyl-^{14}C]-S-297995 monotosylate in Rat (Study No. S-297995-PB-190-N).

Methods: Male rats were administered oral doses of 1 mg (as free base)/69.6 µCi/2mL/kg/day [Carbonyl-^{14}C]-S-297995 monotosylate once daily for 7, 13, and 14 days under non-fasted condition. Blood samples were collected at 0.25, 0.5, 1, 2, 4, 8, 12, 24, and 48 h after single administration; 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, and 48 h after the 7th and 14th administration; 24 h after the 1st, 3rd, 5th, 7th, 9th, 11th, and 13th administration. Parameters evaluated were: plasma concentrations of radioactivity and S-297995, blood concentration of radioactivity, and distribution ratio and concentration of radioactivity in blood cells. S-297995 concentrations were measured using LC/MS/MS.

Results: Plasma Cmax values of radioactivity after the 7th and 14th administrations were comparable (62.4 and 61.2 ng eq. of S-297995/mL, respectively), and higher than the Cmax after single administration (37.7 ng eq. of S-297995/mL). Similarly, the plasma Cmax value S-297995 after the 7th and 14th administrations were comparable (48.8 and 47.8 ng/mL, respectively), and higher than Cmax after single administration (33.7 ng/mL). The AUC_{0-24h} of radioactivity in plasma after the 7th and 14th administrations were 449 and 505 ng eq. of S-297995.h/mL, respectively, compared to the AUC_{0-24h} of 302 ng eq. of S-297995.h/mL after single administration. The AUC_{0-24h} of S-297995 in plasma after single, 7th, and 14th administrations were 217, 228, and 268 ng.h/mL, respectively. The AUC radios of S-297995 to radioactivity were 69.2, 51.3, and 53.3% after single, 7th, and 14th administration of the test compound, respectively. Furthermore, the $t_{1/2,z}$ of radioactivity in plasma was longer than that of S-297995, suggesting the presence of metabolite(s) which are eliminated slower than S-297995.

Blood concentrations of radioactivity showed similar findings to those in plasma, and trough concentrations of radioactivity in plasma, blood, and blood cells after repeated oral administration up to the 13th dose. Finally, distribution ratios of radioactivity in blood cells were 18.9-45.6, 21.6-48.4, and 29.4-63.2% after single, 7th, and 14th administration, respectively. Based on the findings of this study, it was concluded that extensive accumulation of radioactivity and S-297995 does not occur after repeated oral dosing in rats of up to 14 days in duration.

Distribution

Review of Study No. R-297995-PB-057-N from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Quantitative Whole-Body Autoradiography Following Single Oral Administration of [^{14}C]-RSC-297995 Monotosylate in Rats (Study# R-297995-PB-057-N)

Methods: Whole-body autoradiograms at 0.25, 1, 4, 8, 24 and 72 hr after single oral administration of [^{14}C]-RSC-297995 monotosylate at 1 mg (as free base) /kg were conducted in male rats. The concentrations of radioactivity for the dosing formulation, blood and plasma samples were measured with a liquid scintillation counter.

Results: After single oral administration of [^{14}C]-RSC-297995 monotosylate to rats, the radioactivity was widely distributed in the whole-body and the concentrations of radioactivity in the most tissues reached the maximum at 8 hr. The plasma and whole blood concentrations as well as the partitioning ratios into blood cells are summarized the Table 1 below.

Table 1 Radioactivity concentrations (plasma, whole blood) and the partitioning ratios into blood cells following single oral administration of [^{14}C]-RSC-297995 monotosylate at 1 mg/kg (as free base) in rats

Time (hr)	Concentration (ng eq. of RSC-297995/g)		Partitioning ratio into blood cells (%)
	Plasma	Whole Blood	
0.25	8.74	6.42	20.4
1	57.1	41.5	18.2
4	24.0	20.7	31.2
8	32.0	38.4	51.2
24	2.49	3.11	54.8
72	N.D.	1.23	N.C.

n = 1 at each time point, N.D.: not detected, N.C.: not calculated

The tissues observed high levels of radioactivity were the renal cortex, submaxillary gland, liver and parotid gland. At 72 hr post-dosing, the radioactivity in the all tissues was undetectable except for the liver and blood where radioactivity surely decreased with time and $t_{1/2,terminal}$ values were within 36 hr. It was concluded that there would be no residues of radioactivity in the specified tissues. The radioactivity was not detected in the brain at any time point, suggesting that no drug-derived radioactivity penetrated the blood/brain barrier. The levels of radioactivity in different organs/tissues are listed in the Table 2 below.

Table 2 Tissue concentrations of radioactivity following single oral administration of [^{14}C]-RSC-297995 monotosylate at 1 mg/kg (as free base) in rats

Tissues	Concentration (ng eq. /g)					
	0.25 hr	1 hr	4 hr	8 hr	24hr	72 hr
Adrenal gland.	N.D.	143	65.7	106	N.D.	N.D.
Blood in the heart	N.D.	34.6	14.7	40.1	N.D.	N.D.
Blood in the hepatic vein	N.D.	52.1	15.2	45.4	N.D.	N.D.
Blood in the portal vein	N.D.	97.2	30.8	74.3	N.D.	N.D.
Blood in the renal vein	N.D.	N.C.	N.C.	N.C.	N.D.	N.D.
Bone marrow	N.D.	60.3	35.3	73.3	N.D.	N.D.
Brown fat	N.D.	56.0	26.5	105	N.D.	N.D.
Cervical lymph node	N.D.	50.2	39.2	87.5	N.D.	N.D.
Cerebellum	N.D.	BLQ	BLQ	BLQ	N.D.	N.D.
Cerebrum	N.D.	BLQ	BLQ	BLQ	N.D.	N.D.
Exorbital lacrimal gland.	N.D.	80.8	83.4	236	N.D.	N.D.
Harderian gland.	N.D.	102	180	315	N.D.	N.D.
Heart	N.D.	62.1	40.8	195	N.D.	N.D.
Hypophysis	N.D.	N.C.	176	269	N.D.	N.D.
Intestinal wall	N.D.[1)]	N.C.	N.C.	N.C.	N.D.	N.D.
Liver	130	649	611	525	104	26.0
Lung	N.D.	95.6	59.6	79.8	N.D.	N.D.
Pancreas	N.D.	80.8	57.0	127	N.D.	N.D.
Parotid gland.	N.D.	64.0	66.1	457	N.D.	N.D.
Pineal body	N.D.	N.C.	N.C.	N.C.	N.D.	N.D.
Preputial gland	N.D.	84.0	51.7	120	N.D.	N.D.
Prostate	N.D.	49.6	28.2	69.9	N.D.	N.D.
Rectal mucosa	N.D.	88.1	52.0	972	70.8	N.D.
Renal cortex	N.D.	149	101	164	23.6	N.D.
Renal cortico medullary	N.D.	N.C.	N.C.	N.C.	N.C.	N.D.
Renal medulla	N.D.	122	80.4	183	BLQ[2)]	N.D.
Seminal vesicle	N.D.	10.3	9.28	31.8	N.D.	N.D.
Skeletal muscle	N.D.	29.8	21.3	69.6	N.D.	N.D.
Skin	N.D.	26.3	2.41	34.1	N.D.	N.D.
Spinal cord	N.D.	BLQ	BLQ	BLQ[2)]	N.D.	N.D.
Spleen	N.D.	90.3	77.7	84.2	N.D.	N.D.
Submaxillary gland	N.D.	139	118	628	23.8	N.D.
Testis	N.D.	BLQ[2)]	3.72	54.9	29.4	N.D.
Thymus	N.D.	28.9	34.9	144	N.D.	N.D.
Thyroid	N.D.	N.C.	N.C.	N.C.	N.D.	N.D.
White fat	N.D.	BLQ	BLQ[2)]	6.52	N.D.	N.D.

n = 1 at each time point
N.D.: not detected (undiscernible from background), BLQ: below limit of quantification
N.C.: not calculated (not distinguished from the around tissue)
1) N.D. or N.C., 2) BLQ or N.C.

Review of Study No. R-297995-PB-096-N from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Quantitative Whole-body Autoradiography Following Single Oral Administration of [Carbonyl-^{14}C]-RSC-297995 monotosylate in Rats (Study# R-297995-PB-096-N)

Methods: Whole-body autoradiograms at 0.25, 1, 4, 8, 24 and 72 hr after single oral administration of [carbonyl-^{14}C]-RSC-297995 monotosylate at 1 mg (as free base) /kg were conducted in male rats. The concentrations of radioactivity for the dosing formulation, blood and plasma samples were measured with a liquid scintillation counter.

Results: After single oral administration of [carbonyl-^{14}C]-RSC-297995 monotosylate to rats, the radioactivity was widely distributed in the whole-body and the concentrations of radioactivity in the most tissues were reached the maximum at 1 to 4 hr. The tissues observed high levels of radioactivity were the rectal mucosa, liver, Harderian gland, hypophysis and exorbital lacrimal gland. At 72 hr post-dosing, the radioactivity in the all tissues was not detected, except for the liver and renal cortico-medullary junction. The liver and renal cortico-medullary junction showed relatively long $t_{1/2,terminal}$, however, the concentration of radioactivity in these tissues were very low at 72 hr post-dosing as shown in the Table 2 below. Radioactivity in plasma was higher than that in blood in the heart at 1 to 4 hours after dosing with ratio of 1.32 and 1.41, respectively). The concentrations of radioactivity in most tissues were higher than those in plasma and blood as shown in the Table below. The radioactivity was not detected in the brain at any time point, suggesting that no drug-derived radioactivity penetrated the blood/brain barrier.

Table 2 Tissue concentrations of radioactivity following single oral administration of [carbonyl-^{14}C]-RSC-297995 monotosylate at 1 mg/kg (as free base) in rats

Tissues	Concentration (ng eq. of RSC-297995/g)					
	0.25hr	1hr	4hr	8hr	24hr	72hr
Adrenal gland	N.D.	108	88.6	9.27	N.D.	N.D.
Blood in the heart	N.D.	29.9	19.9	BLQ	N.D.	N.D.
Blood in the hepatic vein	N.D.	53.8	31.1	BLQ	N.D.	N.D.
Blood in the portal vein	26.7	94.5	38.8	BLQ	N.D.	N.D.
Blood in the renal vein	N.D.	32.9	26.3	BLQ	N.D.	N.D.
Bone marrow	N.D.	49.9	53.7	N.D.	N.D.	N.D.
Brown fat	N.D.	48.0	39.5	N.D.	N.D.	N.D.
Cerebellum	N.D.	BLQ	BLQ	N.D.	N.D.	N.D.
Cerebrum	N.D.	BLQ	BLQ	N.D.	N.D.	N.D.
Cervical lymph node	N.D.	44.0	47.7	N.D.	N.D.	N.D.
Exorbital lacrimal gland	N.D.	60.3	124	22.5	N.D.	N.D.
Harderian gland	N.D.	76.0	304	196	35.6	N.D.
Heart	N.D.	51.9	42.4	N.D.	N.D.	N.D.
Hypophysis	N.D.	137	184	102	57.8	N.D.
Intestinal wall	N.D.	110	N.C.	17.7	N.D.	N.D.
Liver	178	708	665	773	43.2	23.7
Lung	N.D.	85.6	107	32.1	N.D.	N.D.
Pancreas	N.D.	82.5	64.3	26.5	BLQ	N.D.
Parotid gland	N.D.	53.4	50.3	N.D.	N.D.	N.D.
Pineal body	N.D.	93.3	N.C.	N.D.	N.D.	N.D.
Preputial gland	N.D.	77.0	96.4	49.0	N.D.	N.D.
Prostate	N.D.	33.2	56.5	N.D.	N.D.	N.D.
Rectal mucosa	N.D.	51.0	104	4244	56.2	N.D.
Renal cortex	23.7	112	96.6	29.6	BLQ	N.D.
Renal cortico-medullary junction	N.C.	N.C.	N.C.	60.1	23.1	21.0
Renal medulla	9.43	86.3	75.9	11.0	BLQ	N.D.
Renal papilla	BLQ[1)]	115	75.8	31.6	BLQ	N.D.
Seminal vesicle	N.D.	BLQ	17.8	N.D.	N.D.	N.D.
Skeletal muscle	N.D.	27.3	25.6	N.D.	N.D.	N.D.
Skin	N.D.	24.0	22.8	N.D.	N.D.	N.D.
Spinal cord	N.D.	BLQ	BLQ	N.D.	N.D.	N.D.
Spleen	N.D.	88.6	79.4	20.3	N.D.	N.D.
Submaxillary gland	N.D.	110	99.5	17.4	N.D.	N.D.
Testis	N.D.	BLQ	15.3	26.5	BLQ	N.D.
Thymus	N.D.	28.3	33.9	N.D.	N.D.	N.D.
Thyroid	N.D.	N.C.	N.C.	N.D.	N.D.	N.D.
White fat	N.D.	4.05	BLQ	BLQ	N.D.	N.D.

n = 1 at each time point
N.D.: not detected (undiscernible from background), BLQ: below limit of quantification
N.C.: not calculated (not distinguished from the around tissue)
BLQ[1)]: BLQ or N.D.

Quantitative Whole-body Autoradiography after Repeated Oral Administration of [Carbonyl-^{14}C]-S-297995 monotosylate in Rats (Study No. S-297995-PB-192-N).

Methods: Male Crl:SD(SD) rats were orally administered [Carbonyl-^{14}C]-S-297995 monotosylate at 1 mg/kg (as free base) once daily for up to 14 days. Quantitative whole-body autoradiography was conducted at 24 h after the 1st, 7th, and 13th administrations and at 1, 4, 8, 24, 72, 168, 672, and 1008 h after the 14th administration. Additional groups were administered single nasal or intravenous doses of [Carbonyl-^{14}C]-S-297995 monotosylate and autoradiography was conducted at 72 and 168 h after dosing. Radioactivity concentrations in tissues, blood, and plasma and qualitative evaluation of distribution of radioactivity in nasal bone were determined.

Results: Concentrations of radioactivity in plasma, blood, and tissues after 1, 7, 13, and 14 oral doses of [Carbonyl-^{14}C]-S-297995 monotosylate are summarized in the

Applicant's table 1 below. Drug-derived radioactivity was either not detected or below the lower limit of quantification in the cerebellum and cerebrum at all time points. As shown in the Applicant's table 2 below, concentration ratios of tissue to plasma radioactivity after the 14th administration were highest in the liver, nasal bone, rectal mucosa, and renal cortico-medullary junction. The elimination half-lives of radioactivity ($t_{1/2,\ terminal}$) after 14th administration in plasma and blood were 95.7 and 426.3 h, respectively, and $t_{1/2,terminal}$ values were also long (23.1-186 h) in the epididymis, exorbital lacrimal gland, liver, preputial gland, rectal mucosa, renal cortex, renal cortico-medullary junction, skin, spleen, and thyroid. However, the concentration of radioactivity in these tissues decreased and was not detected at 672 h after the 14th administration (with the exception of the nasal bone). Additional experiments conducted to investigate high concentrations of radioactivity detected in nasal bone suggested that distribution of high radioactivity in the nasal bone may be due to back efflux of the dosing formulation.

Table 1 The concentrations of radioactivity in plasma, blood and tissues after single and 7th, 13th and 14th oral administration of [carbonyl-^{14}C]-S-297995 monotosylate at 1 mg/kg in rats

Tissue	Concentration of radioactivity (ng eq. of S-297995/g)										
	1st-24 hr	7th-24 hr	13th-24 hr	14th							
				1 hr	4 hr	8 hr	24 hr	72 hr	168 hr	336 hr	672 hr	1008 hr
Blood	2.72	6.36	6.58	21.1	44.3	25.1	8.06	4.68	8.32	3.97	3.28	1.30
Plasma	1.12	4.18	3.69	21.3	49.8	29.5	3.94	1.46	1.22	N.D.*	N.D.*	N.D.*
Adrenal gland	N.D.	N.D.	N.D.	63.8	156	74.2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Blood in the heart	N.D.	N.D.	N.D.	19.8	41.5	25.2	BLQ or N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Blood in the hepatic vein	N.D.	N.D.	N.D.	23.5	63.3	28.6	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Blood in the portal vein	N.D.	N.D.	N.D.	34.4	79.1	42.3	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Blood in the renal vein	N.D.	N.D.	N.D.	25.9	35.2	22.5	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Bone marrow	N.D.	N.D.	N.D.	38.7	79.5	41.4	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Brown fat	N.D.	BLQ or N.D.	3.37	38.8	75.7	27.7	9.40	N.D.	N.D.	N.D.	N.D.	N.D.
Cervical lymph node	N.D.	N.D.	N.D.	28.4	70.7	52.8	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Cerebellum	N.D.	N.D.	N.D.	N.D.	BLQ	BLQ	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Cerebrum	N.D.	N.D.	N.D.	N.D.	BLQ	BLQ	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Connective tissue	N.D.	N.D.	N.D.	N.C.	37.2	24.8	N.C.	N.D.	N.D.	N.D.	N.D.	N.D.
Epididymis	N.D.	BLQ or N.D.	N.D.	N.C.	58.6	56.2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Exorbital lacrimal gland	N.D.	BLQ	22.0	40.3	124	110	BLQ	N.D.	N.D.	N.D.	N.D.	N.D.
Eye ball	N.D.	N.D.	N.D.	BLQ	11.5	15.1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

Blood and plasma concentrations of radioactivity were determined by LSC. Tissues concentration of radioactivity was determined by QWBA.
n=1 at each time point
N.D.: not detected (indiscernible from background on the images), *N.D.: (<approximately 0.647 ng eq. of S-297995/g)
BLQ: below the lower limit of quantification (<approximately 16.0 ng eq. of S-297995/g), N.C.: not calculated

Table 1 (continued) The concentrations of radioactivity in plasma, blood and tissues after single and 7th, 13th and 14th oral administration of [carbonyl-^{14}C]-S-297995 monotosylate at 1 mg/kg in rats

Tissue	Concentration of radioactivity (ng eq. of S-297995/g)											
	1st-24 hr	7th-24 hr	13th-24 hr	14th								
				1 hr	4 hr	8 hr	24 hr	72 hr	168 hr	336 hr	672 hr	1008 hr
Harderian gland	24.5	75.3	30.3	79.5	382	450	10.4	N.D.	N.D.	N.D.	N.D.	N.D.
Heart	N.D.	N.D.	N.D.	31.0	70.2	42.1	BLQ or N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Hypophysis	N.D.	N.D.	N.D.	76.7	243	168	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Intestinal wall	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.D.	N.D.	N.D.	N.D.	N.D.
Liver	69.9	241	365	685	1064	826	473	318	104	24.7	N.D.	N.D.
Lung	N.D.	BLQ or N.D.	BLQ or N.D.	46.7	158	122	4.69	N.D.	N.D.	N.D.	N.D.	N.D.
Lymph fluid	N.D.	N.D.	N.D.	N.C.	N.C.	N.C.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Nasal bone	N.D.	291	4502	4978	7414	3935	2810	67.9	2242	4281	N.D.	2264
Pancreas	N.D.	BLQ or N.D.	BLQ or N.D.	42.2	104	58.3	BLQ	N.D.	N.D.	N.D.	N.D.	N.D.
Parotid gland	N.D.	BLQ or N.D.	N.D.	32.8	72.5	45.1	BLQ	N.D.	N.D.	N.D.	N.D.	N.D.
Pineal body	N.D.	N.D.	163	N.C.	109	68.1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Preputial gland	N.D.	N.D.	42.9	67.9	129	86.7	64.8	N.D.	N.D.	N.D.	N.D.	N.D.
Prostate	N.D.	N.D.	N.D.	N.C.	N.C.	N.C.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Rectal mucosa	122	N.C.	156	509	405	264	728	-	42.9	N.D.	N.D.	N.D.
Renal cortex	BLQ or N.D.	36.3	68.3	141	204	127	58.6	58.3	30.8	8.43	N.D.	N.D.
Renal cortico-medullary junction	34.0	153	246	239	346	226	178	138	107	53.7	N.D.	N.D.

Blood and plasma concentrations of radioactivity were determined by LSC. Tissues concentration of radioactivity was determined by QWBA.
n=1 at each time point
N.D.: not detected (indiscernible from background on the images), BLQ: below the lower limit of quantification (<approximately 16.0 ng eq. of S-297995/g)
N.C.: not calculated, -: not estimated because the tissue concentration in each section was N.R (not represented).

Table 1 (continued) The concentrations of radioactivity in plasma, blood and tissues after single and 7th, 13th and 14th oral administration of [carbonyl-^{14}C]-S-297995 monotosylate at 1 mg/kg in rats

Tissue	Concentration of radioactivity (ng eq. of S-297995/g)											
	1st-24 hr	7th-24 hr	13th-24 hr	14th								
				1 hr	4 hr	8 hr	24 hr	72 hr	168 hr	336 hr	672 hr	1008 hr
Renal medulla	BLQ	BLQ	BLQ	47.9	131	58.8	BLQ	BLQ	BLQ	BLQ	N.D.	N.D.
Renal papilla	6.43	BLQ	6.99	93.6	112	82.1	BLQ	11.1	BLQ	BLQ	N.D.	N.D.
Seminal vesicle	N.D.	N.D.	N.D.	N.C.	22.6	29.1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Skeletal muscle	N.D.	BLQ or N.D.	BLQ	6.36	41.8	22.6	BLQ or N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Skin	N.D.	7.48	6.39	17.0	40.6	26.5	22.9	N.D.	N.D.	N.D.	N.D.	N.D.
Spinal cord	N.D.	N.D.	N.D.	N.D.	BLQ	BLQ	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Spleen	N.D.	BLQ or N.D.	5.18	69.8	139	94.5	20.7	13.1	BLQ or N.D.	BLQ or N.D.	N.D.	N.D.
Sublingual gland	N.D.	N.D.	N.C. or N.D.	N.C. or N.D.	N.C.	N.C.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Submaxillary gland	N.D.	N.D.	BLQ or N.D.	54.8	150	67.6	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Testis	BLQ	9.05	18.4	21.6	39.3	50.4	16.1	N.D.	N.D.	N.D.	N.D.	N.D.
Thymus	N.D.	N.D.	N.D.	N.C.	46.9	34.3	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Thyroid	N.D.	40.3	35.6	62.5	150	86.2	18.2	54.3	21.4	N.D.	N.D.	N.D.
Trachea	N.D.	-	N.D.	N.C.	N.C.	N.C.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
White fat	N.D.	N.D.	N.D.	N.C.	N.C.	N.C.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

Blood and plasma concentrations of radioactivity were determined by LSC. Tissues concentration of radioactivity was determined by QWBA.
n=1 at each time point
N.D.: not detected (indiscernible from background on the images), BLQ: below the lower limit of quantification (<approximately 16.0 ng eq. of S-297995/g)
N.C.: not calculated, -: not estimated because the tissue concentration in each section was N.R. (not represented).

Table 2 Concentration ratios of tissue to plasma of radioactivity (Kp) after single and 7th, 13th and 14th oral administration of [carbonyl-^{14}C]-S-297995 monotosylate at 1 mg/kg in rats

Tissue	Concentration ratio of tissue to plasma of radioactivity (Kp)											
	1st-24 hr	7th-24 hr	13th-24 hr	14th								
				1 hr	4 hr	8 hr	24 hr	72 hr	168 hr	336 hr	672 hr	1008 hr
Adrenal gland	N.C.	N.C.	N.C.	2.99	3.14	2.51	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Blood in the heart	N.C.	N.C.	N.C.	0.93	0.83	0.85	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Blood in the hepatic vein	N.C.	N.C.	N.C.	1.10	1.27	0.97	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Blood in the portal vein	N.C.	N.C.	N.C.	1.61	1.59	1.43	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Blood in the renal vein	N.C.	N.C.	N.C.	1.22	0.71	0.76	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Bone marrow	N.C.	N.C.	N.C.	1.82	1.60	1.40	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Brown fat	N.C.	N.C.	0.91	1.82	1.52	0.94	2.38	N.C.	N.C.	N.C.	N.C.	N.C.
Cervical lymph node	N.C.	N.C.	N.C.	1.33	1.42	1.79	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Cerebellum	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Cerebrum	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Connective tissue	N.C.	N.C.	N.C.	N.C.	0.75	0.84	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Epididymis	N.C.	N.C.	N.C.	N.C.	1.18	1.90	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Exorbital lacrimal gland	N.C.	N.C.	5.96	1.89	2.49	3.73	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Eye ball	N.C.	N.C.	N.C.	N.C.	0.23	0.51	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.

n=1 at each time point
N.C.: not calculated

Table 2 (continued) Concentration ratios of tissue to plasma of radioactivity (Kp) after single and 7th, 13th and 14th oral administration of [carbonyl-^{14}C]-S-297995 monotosylate at 1 mg/kg in rats

Tissue	Concentration ratio of tissue to plasma of radioactivity (Kp)											
	1st-24 hr	7th-24 hr	13th-24 hr	14th								
				1 hr	4 hr	8 hr	24 hr	72 hr	168 hr	336 hr	672 hr	1008 hr
Harderian gland	21.83	18.01	8.21	3.73	7.68	15.22	2.64	N.C.	N.C.	N.C.	N.C.	N.C.
Heart	N.C.	N.C.	N.C.	1.46	1.41	1.43	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Hypophysis	N.C.	N.C.	N.C.	3.60	4.89	5.67	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Intestinal wall	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Liver	62.24	57.70	98.76	32.16	21.38	27.99	119.93	217.36	85.48	N.C.	N.C.	N.C.
Lung	N.C.	N.C.	N.C.	2.19	3.18	4.13	1.19	N.C.	N.C.	N.C.	N.C.	N.C.
Lymph fluid	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Nasal bone	N.C.	69.70	1218.65	233.61	149.00	133.25	712.97	46.37	1843.48	N.C.	N.C.	N.C.
Pancreas	N.C.	N.C.	N.C.	1.98	2.10	1.97	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Parotid gland	N.C.	N.C.	N.C.	1.54	1.46	1.53	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Pineal body	N.C.	N.C.	44.01	N.C.	2.20	2.31	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Preputial gland	N.C.	N.C.	11.62	3.18	2.58	2.94	16.43	N.C.	N.C.	N.C.	N.C.	N.C.
Prostate	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Rectal mucosa	108.44	N.C.	42.26	23.87	8.15	8.94	184.75	N.C.*	35.25	N.C.	N.C.	N.C.
Renal cortex	N.C.	8.68	18.48	6.63	4.11	4.31	14.88	39.85	25.35	N.C.	N.C.	N.C.
Renal cortico-medullary junction	30.23	36.73	66.51	11.21	6.96	7.65	45.13	94.29	88.27	N.C.	N.C.	N.C.

n=1 at each time point
N.C.: not calculated
*: not calculated because the tissue concentration in each section was N.R. (not represented).

Table 2 (continued) Concentration ratios of tissue to plasma of radioactivity (Kp) after single and 7th, 13th and 14th oral administration of [carbonyl-^{14}C]-S-297995 monotosylate at 1 mg/kg in rats

Tissue	Concentration ratio of tissue to plasma of radioactivity (Kp)											
	1st-24 hr	7th-24 hr	13th-24 hr	14th								
				1 hr	4 hr	8 hr	24 hr	72 hr	168 hr	336 hr	672 hr	1008 hr
Renal medulla	N.C.	N.C.	N.C.	2.25	2.63	1.99	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Renal papilla	5.73	N.C.	1.89	4.39	2.25	2.78	N.C.	7.60	N.C.	N.C.	N.C.	N.C.
Seminal vesicle	N.C.	N.C.	N.C.	N.C.	0.45	0.98	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Skeletal muscle	N.C.	N.C.	N.C.	0.30	0.84	0.76	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Skin	N.C.	1.79	1.73	0.80	0.82	0.90	5.81	N.C.	N.C.	N.C.	N.C.	N.C.
Spinal cord	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Spleen	N.C.	N.C.	1.40	3.28	2.80	3.20	5.24	8.94	N.C.	N.C.	N.C.	N.C.
Sublingual gland	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Submaxillary gland	N.C.	N.C.	N.C.	2.57	3.01	2.29	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Testis	N.C.	2.17	4.97	1.01	0.79	1.71	4.08	N.C.	N.C.	N.C.	N.C.	N.C.
Thymus	N.C.	N.C.	N.C.	N.C.	0.94	1.16	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Thyroid	N.C.	9.64	9.63	2.93	3.02	2.92	4.62	37.11	17.58	N.C.	N.C.	N.C.
Trachea	N.C.	N.C.*	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
White fat	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.

n=1 at each time point
N.C.: not calculated
*: not calculated because the tissue concentration in each section was.N.R. (not represented).

Quantitative Whole-body Autoradiography after Single Oral Administration of [Carbonyl-^{14}C]-S-297995 monotosylate in Pregnant Rats (Study No. S-297995-PF-238-N; CRO Study No. YDL0026).

Methods: Pregnant female Crl:SD(SD) rats were administered single oral doses of [Carbonyl-^{14}C]-S-297995 monotosylate at 1 mg/kg (as free base; 49.6 µCi/mg as free base) on day 18 of gestation. Quantitative whole-body autoradiography was conducted at 1, 4, 8, 24, and 48 h administration and concentrations of radioactivity in tissues, blood, and plasma were determined.

Results: Concentrations of drug-related radioactivity in plasma, blood, and tissues after a single dose of [Carbonyl-^{14}C]-S-297995 monotosylate are summarized in the Applicant's figure below. As shown, maximum concentrations of radioactivity were measured in most tissues at 1 and 4 h after dosing and radioactivity concentrations in most maternal tissues were equal to or greater than those in cardiac whole-blood at up to 8 h after dosing. Concentrations of radioactivity in fetal tissues were lower than those in maternal cardiac whole blood, the amnion membrane, and placenta, and below the limit of quantification in fetal tissues by 24 h after dosing. In maternal animals, radioactivity was detected in the cerebrum at 4 h after dosing only (tissue: plasma radioactivity concentration ratio = 0.09). Radioactivity was below the limit of quantification in the fetal brain at all time points. At 48 h, radioactivity was measured only in the renal cortex and medulla, liver, large intestine wall, and amnion membrane. Based on the findings of this study, it was concluded that placental transfer was low.

Table 1 Concentrations of radioactivity in tissues determined by QWBA following a single oral administration of [carbonyl-^{14}C]-S-297995 monotosylate (1 mg free base/kg) to pregnant female albino rats

Tissue type	Tissue/organ	Animal number, sex and sacrifice time				
		1F 1 hour	2F 4 hours	3F 8 hours	4F 24 hours	5F 48 hours
Circulatory	Aorta	**0.073**	0.061	0.034	BLQ	BLQ
	Plasma[a]	**0.082**	0.076	0.020	BLQ	BLQ
	Whole-blood[b]	**0.056**	0.055	0.015	BLQ	BLQ
	Whole-blood (cardiac)	**0.070**	0.052	0.018	0.009	BLQ
	Vena cava	**0.062**	0.045	BLQ	BLQ	BLQ
Nervous	Cerebellum	BLQ	BLQ	BLQ	BLQ	BLQ
	Cerebrum	BLQ	0.007	BLQ	BLQ	BLQ
	Choroid plexus	0.190	**0.230**	0.059	BLQ	BLQ
	Meninges	**0.131**	0.120	0.044	BLQ	BLQ
	Pineal body	**0.208**	0.114	0.041	BLQ	BLQ
	Spinal cord	BLQ	BLQ	BLQ	BLQ	BLQ
Ocular	Eye (lens)	BLQ	BLQ	BLQ	BLQ	BLQ
	Uveal tract/retina	0.061	**0.085**	0.056	0.008	BLQ
Visceral organs	Kidney cortex	**0.278**	0.239	0.080	0.018	0.029
	Kidney medulla	**0.167**	0.167	0.038	0.028	0.012
	Liver	1.15	**1.37**	0.523	0.095	0.048
	Lung	0.146	**0.190**	0.082	0.008	BLQ
	Myocardium	**0.114**	0.098	0.026	BLQ	BLQ
	Spleen	0.192	**0.215**	0.082	0.011	BLQ
Glandular/secretory	Adrenal gland	**0.226**	0.179	0.042	0.018	BLQ
	Exorbital lacrimal gland	0.191	**0.214**	0.130	0.012	BLQ
	Harderian gland	0.230	0.573	**0.671**	BLQ	BLQ
	Intra-orbital lacrimal gland	0.224	**0.225**	0.141	BLQ	BLQ
	Mandibular lymph nodes	**0.110**	0.087	0.040	BLQ	BLQ
	Mesenteric lymph nodes	**0.136**	0.115	0.062	BLQ	BLQ
	Mucous gland	0.113	**0.124**	0.045	0.010	BLQ
	Nasal mucosa	0.011	0.020	**0.029**	0.012	BLQ
	Pancreas	0.148	**0.152**	0.045	BLQ	BLQ
	Pituitary	0.234	**0.337**	0.101	BLQ	BLQ
	Parotid salivary gland	0.094	**0.134**	0.037	BLQ	BLQ
	Salivary gland	0.217	**0.297**	0.109	BLQ	BLQ
	Thymus	0.079	**0.099**	0.053	BLQ	BLQ
	Thyroid	0.118	**0.141**	0.050	BLQ	BLQ
Reproductive	Clitoral gland	0.040	0.168	**0.195**	BLQ	BLQ
	Mammary gland	**0.083**	0.080	0.045	BLQ	BLQ
	Ovary	0.048	**0.057**	0.023	0.007	BLQ
	Uterus wall	**0.107**	0.053	0.040	BLQ	BLQ

Results are expressed as µg equivalents/g of tissue
For all QWBA measurements:
Upper and lower limits of quantification = 38.1 and 0.007 µg equivalents S-297995 free base/g of tissue, respectively
BLQ Tissue radioactivity concentration below the lower limit of quantification
QWBA Quantitative whole-body autoradiography
[a] Determined by liquid scintillation analysis
[b] Determined by sample oxidation and liquid scintillation analysis
[c] Tissue corrected for quenching
Values in bold represent maximum concentrations achieved (T_{max})

Table 1 continued

Tissue type	Tissue/organ	Animal number, sex and sacrifice time				
		1F 1 hour	2F 4 hours	3F 8 hours	4F 24 hours	5F 48 hours
Musculo-skeletal	Bone marrow	**0.109**	0.101	0.034	BLQ	BLQ
	Bone surface[c]	**0.018**	BLQ	BLQ	BLQ	BLQ
	Fat (abdominal)[c]	0.025	**0.058**	0.022	BLQ	BLQ
	Fat (brown)	**0.074**	0.066	0.022	BLQ	BLQ
	Muscle (skeletal)	0.038	**0.053**	0.016	BLQ	BLQ
	Skin	0.018	**0.030**	0.025	BLQ	BLQ
	Tongue	0.077	**0.082**	0.024	BLQ	BLQ
	Trachea	0.032	**0.049**	0.024	BLQ	BLQ
Excretory	Stomach wall	0.098	**0.256**	0.057	BLQ	BLQ
	Small intestine wall	**1.16**	0.241	0.099	0.041	BLQ
	Caecum wall	0.074	**0.342**	0.251	0.056	BLQ
	Large intestine wall	0.167	**0.553**	0.403	0.259	0.137
	Urinary bladder wall	0.045	0.051	**0.342**	BLQ	BLQ
In utero	Amnion membrane	0.105	**0.697**	0.291	0.060	0.026
	Foetus (whole)	0.007	**0.014**	0.011	BLQ	BLQ
	Foetal blood	**0.014**	0.011	BLQ	BLQ	BLQ
	Foetal brain	BLQ	BLQ	BLQ	BLQ	BLQ
	Foetal kidney	**0.016**	0.011	BLQ	BLQ	BLQ
	Foetal liver	0.014	**0.017**	0.013	BLQ	BLQ
	Foetal lung	0.007	**0.017**	0.015	BLQ	BLQ
	Placenta	**0.073**	0.067	0.028	BLQ	BLQ

Results are expressed as µg equivalents/g of tissue
For all QWBA measurements:
Upper and lower limits of quantification = 38.1 and 0.007 µg equivalents S-297995 free base/g of tissue, respectively
BLQ Tissue radioactivity concentration below the lower limit of quantification
QWBA Quantitative whole-body autoradiography
[a] Determined by liquid scintillation analysis
[b] Determined by sample oxidation and liquid scintillation analysis
[c] Tissue corrected for quenching
Values in bold represent maximum concentrations achieved (T_{max})

Quantitative Whole-body Autoradiography after Single Oral Administration of [Oxadiazole-^{14}C]-S-297995 monotosylate and [Carbonyl-^{14}C]-S-297995 monotosylate in Pigmented Rats (Study No. S-297995-PF-213-N; CRO Study No. 8223166).

Methods: Male Long Evans (HsdBlu:LE) rats were administered single oral doses of [Oxadiazole-^{14}C]-S-297995 monotosylate and [Carbonyl-^{14}C]-S-297995 monotosylate at 1 mg/kg (as free base; the radioactive dose was 69.6 µCi (as free base)/kg). Quantitative whole-body autoradiography was conducted at 1, 4, 8, 24, 72, 168, 336, and 840 h administration and concentrations of radioactivity in tissues, blood, and plasma were determined.

Results: Maximum concentrations of radioactivity were detected in plasma at 1 h after dosing with both test articles, as shown in the Applicant's tables 3 and 4 below. Although radioactivity concentrations decreased to a large extent by 8 h after dosing with [Carbonyl-^{14}C]-S-297995 monotosylate, concentrations remained relatively high at 8 h after dosing with [Oxadiazole-^{14}C]-S-297995 monotosylate. Concentrations of radioactivity in tissues as determined by autoradiography are summarized in the Applicant's tables 5 and 6 below. Maximum concentrations of radioactivity were detected in most tissues by 8 and 1 h after dosing with [Oxadiazole-^{14}C]-S-297995 monotosylate and [Carbonyl-^{14}C]-S-297995 monotosylate, respectively. Radioactivity

was not detected or below the limit of quantitation in the cerebellum, cerebrum, medulla, and olfactory lobe, but was detected in the choroid plexus. By 72 h, radioactivity was not detected or below the limit of quantitation with the exception of the eye, eye uveal tract, liver, and pigmented skin. The detection of radioactivity in pigmented ocular tissue (i.e., eye uveal tract) at 840 h after dosing suggested an affinity for melanin, and the estimated half-life values in the uveal tract ranged from 309 and 447 h. The highest tissue:plasma ratios following administration of [Oxadiazole-^{14}C]-S-297995 monotosylate were in the uveal tract, bile, eye, cecum, and liver. The highest tissue:plasma ratios following administration of [Carbonyl-^{14}C]-S-297995 monotosylate were in the liver, uveal tract, Harderian gland, pituitary gland, and bile.

Table 3
Concentrations of radioactivity in plasma at specified times after a single oral administration of [Oxadiazole-^{14}C]-S-297995 monotosylate to male rats (Group 1, 1 mg/kg)

	ng Equivalents [Oxadiazole-^{14}C]-S-297995/g							
	Animal Number (Sacrifice Time)							
Sample	B20358 (1 h)	B20359 (4 h)	B20360 (8 h)	B20361 (24 h)	B20362 (72 h)	B20363 (168 h)	B20364 (336 h)	B20365 (840 h)
Plasma	42.1	36.7	32.1	2.39	BLQ	BLQ	BLQ	BLQ

BLQ Below the limit of quantitation.
h Hour(s).
Note: The lower limit of quantitation for plasma was 1.23 ng equivalants [Oxadiazole-^{14}C]-S-297995/g.

Table 4
Concentrations of radioactivity in plasma at specified times after a single oral administration of [Carbonyl-^{14}C]-S-297995 monotosylate to male rats (Group 2, 1 mg/kg)

	ng Equivalents [Carbonyl-^{14}C]-S-297995/g							
	Animal Number (Sacrifice Time)							
Sample	B20366 (1 h)	B20367 (4 h)	B20368 (8 h)	B20369 (24 h)	B20370 (72 h)	B20371 (168 h)	B20372 (336 h)	B20373 (840 h)
Plasma	51.8	32.3	1.73	1.40	BLQ	BLQ	BLQ	BLQ

BLQ Below the limit of quantitation.
h Hour(s).
Note: The lower limit of quantitation for plasma was 1.23 ng equivalants [Carbonyl-^{14}C]-S-297995/g.

Table 5
Concentrations of radioactivity in tissues determined by whole-body autoradiography at specified times after a single oral administration of [Oxadiazole-^{14}C]-S-297995 monotosylate to male rats (Group 1, 1 mg/kg)

	ng Equivalents [Oxadiazole-^{14}C]-S-297995/g							
	Animal Number (Sacrifice Time)							
Matrix	B20358 (1 Hour)	B20359 (4 Hours)	B20360 (8 Hours)	B20361 (24 Hours)	B20362 (72 Hours)	B20363 (168 Hours)	B20364 (336 Hours)	B20365 (840 Hours)
Adrenal gland	113	144	131	BLQ	ND	ND	ND	ND
Arterial walls	60.8	63.1	78.5	ND	ND	ND	ND	ND
Bile	2850	2200	260	ND	ND	ND	ND	ND
Blood	29.0	27.2	34.7	ND	ND	ND	ND	ND
Bone	BLQ	BLQ	BLQ	ND	ND	ND	ND	ND
Bone marrow	54.6	70.3	84.5	ND	ND	ND	ND	ND
Brain cerebellum	BLQ	BLQ	BLQ	ND	ND	ND	ND	ND
Brain cerebrum	BLQ	BLQ	BLQ	ND	ND	ND	ND	ND
Brain choroid plexus	63.8	42.1	29.3	ND	ND	ND	ND	ND
Brain medulla	BLQ	BLQ	BLQ	ND	ND	ND	ND	ND
Brain olfactory lobe	BLQ	BLQ	BLQ	ND	ND	ND	ND	ND
Bulbo-urethral gland	67.1	95.7	201	ND	ND	ND	ND	ND
Cecum	59.1	484	423	84.7	ND	ND	ND	ND
Contents, cecum	43.0	6010	16200	218	BLQ	ND	ND	ND
Contents, esophageal	360	518	83.4	BLQ	ND	ND	ND	ND
Contents, large intestine	BLQ	9630	25900	592	BLQ	ND	ND	ND
Contents, small intestine	60400	26200	2860	59.1	BLQ	ND	ND	ND
Contents, stomach	12700	12700	269	BLQ	ND	ND	ND	ND

BLQ Below the limit of quantitation (<13.1 ng equivalents [Oxadiazole-^{14}C]-S-297995/g).
ND Not detectable (sample shape not discernible from background or surrounding tissue).

Table 5 (continued)
Concentrations of radioactivity in tissues determined by whole-body autoradiography at specified times after a single oral administration of [Oxadiazole-^{14}C]-S-297995 monotosylate to male rats (Group 1, 1 mg/kg)

	ng Equivalents [Oxadiazole-^{14}C]-S-297995/g							
	Animal Number (Sacrifice Time)							
Matrix	B20358 (1 Hour)	B20359 (4 Hours)	B20360 (8 Hours)	B20361 (24 Hours)	B20362 (72 Hours)	B20363 (168 Hours)	B20364 (336 Hours)	B20365 (840 Hours)
Diaphragm	50.8	103	107	BLQ	ND	ND	ND	ND
Epididymis	18.2	35.5	80.6	16.7	ND	ND	ND	ND
Esophagus	497	90.8	322	BLQ	ND	ND	ND	ND
Exorbital lacrimal gland	77.8	110	188	16.7	ND	ND	ND	ND
Eye	20.3	73.1	93.7	99.1	115	50.2	20.4	BLQ
Eye lens	BLQ	BLQ	BLQ	16.2	BLQ	ND	ND	ND
Eye uveal tract	102	386	461	346	541	246	115	48.9
Fat (abdominal)	BLQ	30.2	14.0	ND	ND	ND	ND	ND
Fat (brown)	54.9	50.7	86.1	ND	ND	ND	ND	ND
Harderian gland	104	181	177	25.9	ND	ND	ND	ND
Intra-orbital lacrimal gland	97.8	127	172	13.3	ND	ND	ND	ND
Kidney	123	147	193	25.1	BLQ	BLQ	ND	ND
Kidney cortex	129	149	157	24.9	ND	ND	ND	ND
Kidney medulla	91.0	156	280	24.7	ND	ND	ND	ND
Large intestine	48.5	87.2	161	49.6	ND	ND	ND	ND
Liver	859	819	504	77.4	48.4	17.1	BLQ	ND
Lung	96.6	143	78.3	ND	ND	ND	ND	ND
Lymph nodes	49.8	71.8	83.3	ND	ND	ND	ND	ND
Muscle	28.0	33.6	71.5	ND	ND	ND	ND	ND
Myocardium	51.1	76.5	167	BLQ	ND	ND	ND	ND

BLQ Below the limit of quantitation (<13.1 ng equivalents [Oxadiazole-^{14}C]-S-297995/g).
ND Not detectable (sample shape not discernible from background or surrounding tissue).

Table 5 (continued)
Concentrations of radioactivity in tissues determined by whole-body autoradiography at specified times after a single oral administration of [Oxadiazole-^{14}C]-S-297995 monotosylate to male rats (Group 1, 1 mg/kg)

	ng Equivalents [Oxadiazole-^{14}C]-S-297995/g							
	Animal Number (Sacrifice Time)							
Matrix	B20358 (1 Hour)	B20359 (4 Hours)	B20360 (8 Hours)	B20361 (24 Hours)	B20362 (72 Hours)	B20363 (168 Hours)	B20364 (336 Hours)	B20365 (840 Hours)
Nasal turbinates	24.2	32.3	73.7	14.1	ND	ND	ND	ND
Pancreas	69.6	102	158	ND	ND	ND	ND	ND
Pituitary gland	193	578	188	25.7	ND	ND	ND	ND
Preputial gland	50.1	56.3	94.8	NR	BLQ	ND	ND	ND
Prostate gland	27.7	59.7	51.7	16.4	ND	ND	ND	ND
Salivary gland	80.4	206	731	36.6	ND	ND	ND	ND
Seminal vesicle	19.3	24.9	35.9	ND	ND	ND	ND	ND
Skin (nonpigmented)	21.7	23.6	36.4	BLQ	BLQ	ND	ND	ND
Skin (pigmented)	22.4	27.1	65.6	22.6	16.8	19.5	BLQ	BLQ
Small intestine	475	209	270	14.4	ND	ND	ND	ND
Spinal cord	BLQ	BLQ	BLQ	ND	ND	ND	ND	ND
Spleen	72.8	88.2	81.7	BLQ	ND	ND	ND	ND
Stomach	72.4	75.4	122	BLQ	ND	ND	ND	ND
Testis	BLQ	23.0	53.8	27.5	ND	ND	ND	ND
Thymus	29.0	56.5	116	ND	ND	ND	ND	ND
Thyroid	66.1	50.2	79.3	ND	ND	ND	ND	ND
Urinary bladder	54.4	94.1	176	16.5	ND	ND	ND	ND
Urine	163	1240	1490	209	ND	ND	ND	ND

BLQ Below the limit of quantitation (<13.1 ng equivalents [Oxadiazole-^{14}C]-S-297995/g).
ND Not detectable (sample shape not discernible from background or surrounding tissue).
NR Not represented (tissue not present in section).

Table 6
Concentrations of radioactivity in tissues determined by whole-body autoradiography at specified times after a single oral administration of [Carbonyl-^{14}C]-S-297995 monotosylate to male rats (Group 2, 1 mg/kg)

	ng Equivalents [Carbonyl-^{14}C]-S-297995/g							
	Animal Number (Sacrifice Time)							
Matrix	B20366 (1 Hour)	B20367 (4 Hours)	B20368 (8 Hours)	B20369 (24 Hours)	B20370 (72 Hours)	B20371 (168 Hours)	B20372 (336 Hours)	B20373 (840 Hours)
Adrenal gland	142	82.7	BLQ	BLQ	ND	ND	ND	ND
Arterial walls	62.0	43.1	ND	ND	ND	ND	ND	ND
Bile	3810	1690	ND	ND	ND	ND	ND	ND
Blood	37.6	22.2	ND	ND	ND	ND	ND	ND
Bone	BLQ	BLQ	ND	ND	ND	ND	ND	ND
Bone marrow	58.2	49.4	ND	ND	ND	ND	ND	ND
Brain cerebellum	BLQ	BLQ	ND	ND	ND	ND	ND	ND
Brain cerebrum	BLQ	BLQ	ND	ND	ND	ND	ND	ND
Brain choroid plexus	32.2	91.5	32.5	ND	ND	ND	ND	ND
Brain medulla	BLQ	BLQ	ND	ND	ND	ND	ND	ND
Brain olfactory lobe	BLQ	BLQ	ND	ND	ND	ND	ND	ND
Bulbo-urethral gland	74.7	46.7	ND	ND	ND	ND	ND	ND
Cecum	52.9	280	38.8	NR	ND	ND	ND	ND
Contents, cecum	64.2	8200	24000	NR	BLQ	ND	ND	ND
Contents, esophageal	839	642	BLQ	ND	ND	ND	ND	ND
Contents, large intestine	26.4	2580	37600	2160	BLQ	ND	ND	ND
Contents, small intestine	68400	31300	1110	25.4	BLQ	ND	ND	ND
Contents, stomach	9700	24400	48.1	ND	ND	ND	ND	ND

BLQ Below the limit of quantitation (<14.0 ng equivalents [Carbonyl-^{14}C]-S-297995/g).
ND Not detectable (sample shape not discernible from background or surrounding tissue).
NR Not represented (tissue not present in section).

Table 6 (continued)
Concentrations of radioactivity in tissues determined by whole-body autoradiography at specified times after a single oral administration of [Carbonyl-^{14}C]-S-297995 monotosylate to male rats (Group 2, 1 mg/kg)

	ng Equivalents [Carbonyl-^{14}C]-S-297995/g							
	Animal Number (Sacrifice Time)							
Matrix	B20366 (1 Hour)	B20367 (4 Hours)	B20368 (8 Hours)	B20369 (24 Hours)	B20370 (72 Hours)	B20371 (168 Hours)	B20372 (336 Hours)	B20373 (840 Hours)
Diaphragm	58.8	48.9	ND	ND	ND	ND	ND	ND
Epididymis	27.3	23.9	28.0	ND	ND	ND	ND	ND
Esophagus	193	35.7	59.8	BLQ	ND	ND	ND	ND
Exorbital lacrimal gland	102	55.4	BLQ	ND	ND	ND	ND	ND
Eye	22.8	49.0	106	80.9	47.7	31.2	16.6	BLQ
Eye lens	BLQ	BLQ	BLQ	BLQ	ND	ND	ND	ND
Eye uveal tract	136	244	571	407	222	148	72.0	59.6
Fat (abdominal)	BLQ	BLQ	ND	ND	ND	ND	ND	ND
Fat (brown)	54.2	36.8	ND	ND	ND	ND	ND	ND
Harderian gland	136	212	284	42.9	ND	ND	ND	ND
Intra-orbital lacrimal gland	95.8	64.0	BLQ	ND	ND	ND	ND	ND
Kidney	129	102	35.7	19.8	BLQ	14.1	ND	ND
Kidney cortex	136	105	36.3	22.3	BLQ	15.9	ND	ND
Kidney medulla	116	92.5	34.9	BLQ	BLQ	BLQ	ND	ND
Large intestine	56.6	101	66.0	ND	ND	ND	ND	ND
Liver	976	806	672	116	55.0	BLQ	BLQ	ND
Lung	123	106	20.4	ND	ND	ND	ND	ND
Lymph nodes	51.9	40.4	ND	ND	ND	ND	ND	ND
Muscle	37.3	26.1	ND	ND	ND	ND	ND	ND
Myocardium	62.1	37.0	ND	ND	ND	ND	ND	ND

BLQ Below the limit of quantitation (<14.0 ng equivalents [Carbonyl-^{14}C]-S-297995/g).
ND Not detectable (sample shape not discernible from background or surrounding tissue).

Table 6 (continued)
Concentrations of radioactivity in tissues determined by whole-body autoradiography at specified times after a single oral administration of [Carbonyl-^{14}C]-S-297995 monotosylate to male rats (Group 2, 1 mg/kg)

	ng Equivalents [Carbonyl-^{14}C]-S-297995/g							
	Animal Number (Sacrifice Time)							
Matrix	B20366 (1 Hour)	B20367 (4 Hours)	B20368 (8 Hours)	B20369 (24 Hours)	B20370 (72 Hours)	B20371 (168 Hours)	B20372 (336 Hours)	B20373 (840 Hours)
Nasal turbinates	BLQ	35.7	BLQ	ND	ND	ND	ND	ND
Pancreas	79.9	61.6	19.0	ND	ND	ND	ND	ND
Pituitary gland	137	128	139	ND	ND	ND	ND	ND
Preputial gland	54.5	60.5	22.5	BLQ	ND	ND	ND	ND
Prostate gland	38.4	26.3	ND	ND	ND	ND	ND	ND
Salivary gland	116	80.1	59.1	ND	ND	ND	ND	ND
Seminal vesicle	14.6	22.6	ND	ND	ND	ND	ND	ND
Skin (nonpigmented)	21.1	17.5	BLQ	BLQ	BLQ	ND	ND	ND
Skin (pigmented)	27.2	26.8	29.1	20.1	15.0	BLQ	ND	ND
Small intestine	45.1	200	69.4	ND	ND	ND	ND	ND
Spinal cord	BLQ	BLQ	ND	ND	ND	ND	ND	ND
Spleen	83.4	66.6	BLQ	ND	ND	ND	ND	ND
Stomach	77.5	64.3	BLQ	ND	ND	ND	ND	ND
Testis	BLQ	14.1	20.4	BLQ	ND	ND	ND	ND
Thymus	36.9	32.2	ND	ND	ND	ND	ND	ND
Thyroid	63.9	60.5	BLQ	16.1	ND	ND	ND	ND
Urinary bladder	30.9	172	ND	ND	ND	ND	ND	ND
Urine	61.8	508	ND	ND	ND	ND	ND	ND

BLQ Below the limit of quantitation (<14.0 ng equivalents [Carbonyl-^{14}C]-S-297995/g).
ND Not detectable (sample shape not discernible from background or surrounding tissue).

Review of Study No. R-297995-PB-023-N from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

***In vitro* Plasma/Blood Cell Partitioning of [^{14}C]-RSC-297995 monotosylate (Study# R-297995-PB-023-N)**

Methods: [^{14}C]-RSC-297995 monotosylate was added to blood obtained from male Crl:CD(SD) rat, Beagle dog and healthy human volunteers at concentrations of 0.02, 0.2 and 2 µg/mL (as [^{14}C]-RSC-297995). The concentrations of radioactivity in blood and plasma samples were measured with Radio-HPLC analysis following the incubation with [^{14}C]-RSC-297995 for 15 min at 37°C.

Results: The *in vitro* distribution to blood cells of [^{14}C]-RSC-297995 was in the range of 13.5-20.3% in rat, dog and human blood, showing no marked differences among the species. Additionally, in all species tested, there were no concentration-dependent changes at 0.02-2 µg/mL. The concentration ratios of whole blood to plasma in the rat, dog and human were 0.689-0.698, 0.624-0.658 and 0.632-0.651, respectively. The percent distributions of radioactivity in blood cells were 13.9-15.5%, 16.2-20.3% and 13.5-16.3% in the rat, dog and human, respectively. There was no species difference and concentration-dependent change of the percent distributions in all species.

Review of Study No. R-297995-PB-024-N from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

***In vitro* Protein Binding of [^{14}C]-RSC-297995 monotosylate (Study# R-297995-PB-024-N)**

Methods: [^{14}C]-RSC-297995 monotosylate was added to sera from the CD(SD) rat, Wistar rat, dog, ferret and refined human protein solutions at concentrations of 0.02, 0.2 and 2 µg/mL. Protein binding assay was performed by ultrafiltration method. It was confirmed that the protein binding of [^{14}C]-RSC-297995 monotosylate reached equilibrium instantaneously, therefore, the protein samples containing [^{14}C]-RSC-297995 monotosylate were put into the filtration kit, and centrifuged at 2,500 rpm for fixed time at 37°C without incubation. The concentrations of radioactivity in the protein sample and filtrate were determined with a liquid scintillation counter, and then the ratio of unbound drug (f_u) and the ratio of protein binding were calculated by the following equation (2) and (3), respectively..

$$f_u = \frac{C_{filtrate}}{C_{protein\ sample}} \quad \cdots Eq.(2)$$

$$Binding\ ratio(\%) \quad 1 \quad f_u) \ 100 \quad \cdots Eq.(3)$$

Results: In the CD(SD) rat, Wistar rat, dog, ferret and human, the protein binding ratios of [^{14}C]-RSC-297995 at concentrations of 0.02-2 µg/mL in sera obtained under fasted condition were 89.2-90.5%, 88.9-91.0%, 92.7%, 89.3-89.9% and 93.2-94.2%, respectively. The binding ratios of [^{14}C]-RSC-297995 in 4% albumin solution were 95.3-96.0%, which were the highest values in all proteins tested, at concentrations of 0.02-2 µg/mL. The binding ratios of [^{14}C]-RSC-297995 in 0.08% α$_1$-acid glycoprotein solution were 22.7-25.9% at these concentrations. The binding ratios of [^{14}C]-RSC-297995 in 1% γ-globulin solution were 17.2-19.5% at these concentrations. [^{14}C]-RSC-297995 was stable in the serum of all species during the incubation.

Metabolism

Review of Study No. R-297995-PB-060-N from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Study on the *in vivo* Major Metabolites of [^{14}C]-RSC-297995 monotosylate in Rats (Study# R-297995-PB-060-N)

Methods: [14C]-RSC-297995 monotosylate was administered and the samples were collected in 4 rat groups as shown in the Table below. The concentrations of radioactivity for the testing samples were measured with a liquid scintillation counter. The dose at 5 mg/kg of [14C]-RSC-297995 was selected for Group A study. The dose at 1 mg/kg of [14C]-RSC-297995 was selected for Group B and C study. The dose at 100 mg/kg of [14C]-RSC-297995 was selected for Group D study. In group A, the blood was collected at 1 and 4 hr after administration. In group B and C, the urine was collected at 0-6 hr, 6-24 hr and 24-48 hr after administration. In group C, the bile was collected at 0-6 hr, 6-24 hr and 24-48 hr after administration. In group B and C, the feces were collected at 0-24 hr and 24-48 hr after administration. In group D, the feces were collected at 0-24 hr after administration. The metabolites were detected and analyzed by radio-HPLC and LC/MS/MS.

Group	Purpose	Treatment	Number of animals (Rat No.)
A	Plasma Collection	Intact	4 (060-001~060-004, 2 at each sampling point)
B	Urine and feces collection	Intact	2 (060-005, 060-006)
C	Urine, bile and feces collection	Bile duct-cannulation	2 (060-007, 060-008)
D	Urine and feces collection at high dose	Intact	3 (060-009~060-011)

Results: Following single oral administration of [^{14}C]-RSC-297995 monotosylate to rats, metabolites in the plasma, urine, bile and feces were analyzed. The major component of 1-hr plasma radioactivity was unchanged RSC-297995. In addition, RSC-297995 3-*O*-β-D-glucuronide and Nor-RSC-297995 existed as metabolites. On the other hand, the major component of 4-hr plasma was benzamidine. In the bile, RSC-297995 3-*O*-β-D-glucuronide existed as the major metabolites. Also, Nor-RSC-297995 and RSC-297995 6-*O*-β-D-glucuronide were detected. In the urine, most of the radioactivity was attributed to benzamidine. In the feces, most of the radioactivity was also attributed to benzamidine. RSC-297995 and its metabolites, RSC-297995 3-*O*-β-D-glucuronide, Nor-RSC-297995 and RSC-297995 6-*O*-β-D-glucuronide, were not stable in the feces pellet, although RSC-297995 was almost stable in urine. In addition, benzamidine was barely detected in the bile. Therefore, these results suggest that benzamidine was produced by enterobacteria from unabsorbed RSC-297995 and metabolites excreted *via* bile.

Preliminary Toxicokinetics Study of Metabolite, S-297995-(7R)-7-hydroxide in Rats following S-297995 Oral Administration and Exploratory Determination of S-297995-(7R)-7-hydroxide in Dog Plasma by LC/MS/MS (Study No. S-297995-TB-282-R)

Methods: Male and female Crl:CD(SD) rats were administered 30 and 100 mg/kg/day S-297995 monotosylate (as S-297995) in 0.5 w/v% methylcellulose by oral gavage once daily for 2 weeks (n=4/sex/group). Blood samples were collected prior to dosing (Day 14 only), and at 0.5 ,1 ,2, 4, 8, and 24 h after dosing on Days 1 and 14, and plasma was analyzed for S-297995-(7R)-7-hydroxide using LC/MS/MS. In addition, an exploratory determination of S-297995-(7R)-7-hydroxide in dog plasma samples was conducted. For this evaluation, plasma samples from dogs dosed with 20 mg/kg/day S-297995 monotosylate (as S-297995) in a 9-month oral toxicity study (Study No. S-297995-TF-219-L) analyzed using LC/MS/MS.

Results: TK parameters for S-297995-(7R)-7-hydroxide in rats are summarized in the Applicant's tables below. Tmax ranged from 0.5-1.3 h, and $AUC_{0\text{-}24h}$ increased with increasing dose level.

<Day 1>

Sex	Dose (mg/kg/day)	$AUC_{0\text{-}24hr}$ (μg·hr/mL)	C_{max} (μg/mL)	T_{max} (hr)	C_{24hr} (μg/mL)
Male	30	29.4	15.4	0.5	NE
	100	71.3	28.5	0.6	NE
Female	30	18.0	9.54	0.5	NE
	100	75.0	18.3	0.8	NE

<Day 14>

Sex	Dose (mg/kg/day)	$AUC_{0\text{-}24hr}$ (μg·hr/mL)	C_{max} (μg/mL)	T_{max} (hr)	C_{24hr} (μg/mL)
Male	30	25.1	6.21	0.8	NE
	100	103	17.1	1.0	0.0237
Female	30	13.1	5.40	0.5	NE
	100	84.4	13.9	1.3	0.0118

NE: Not estimated, the mean and standard deviation values were not calculated when more than half of the individual values were below the lower limit of quantification for plasma concentration.

TK parameters from S-297995-(7R)-7-hydroxide in dogs are summarized in the Applicant's table below.

<Day 273>

Sex	Dose (mg/kg/day)	$AUC_{0\text{-}24hr}$ (μg·hr/mL)	C_{max} (μg/mL)	T_{max} (hr)	C_{24hr} (μg/mL)
Male	20	368	65.7	0.9	0.441
Female	20	320	62.7	0.7	0.312

Review of Study No. R-297995-PB-099-N from the IND 107475 nonclinical review dated November 23, 2011 is copied below (C. Wu, Ph.D.).

Study on the *in vivo* Major Metabolites of [Carbonyl-^{14}C]-RSC-297995 monotosylate in Rats (Study# R-297995-PB-099-N)

Methods: Metabolite profiling in the rat plasma, urine, bile and feces following single oral administration of [carbonyl-^{14}C]-RSC-297995 monotosylate was analyzed. The plasma samples were collected at 1-, 4-, 6- and 8-hr after dosing.

Results: Following single oral administration of [carbonyl-^{14}C]-RSC-297995 monotosylate to rats, metabolites in the plasma, urine, bile and feces were analyzed. The major component of radioactivity of 1-, 4-, 6- and 8-hr plasma was unchanged RSC-297995. RSC-297995

3-*O*-β-D-glucuronide, nor-RSC-297995 and benzamidine existed in plasma. In the urine, most of the radioactivity was attributed to unchanged RSC-297995. Benzamidine existed in rat urine. In the bile, RSC-297995 3-*O*-β-D-glucuronide existed as the major metabolites. Also, nor-RSC-297995 and RSC-297995 6-*O*-β-D-glucuronide were detected. In the feces, most of the radioactivity was attributed to RFM6, a metabolite with molecular ion at *m/z* 471, speculated to be the carboxylic form of after leaving benzamidine from RSC-297995. The metabolite was barely detected in the plasma. Benzamidine was detected in the feces.

Review of Study No. R-297995-PB-061-N from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Study on the *in vivo* Major Metabolites of [^{14}C]-RSC-297995 monotosylate in Dogs (Study# R-297995-PB-061-N)

Methods: Metabolite profiling in the dog plasma, urine, bile and feces following single oral administration of [Oxadiazole-^{14}C]-RSC-297995 monotosylate at 1 mg/69.6 µCi/1 mL/kg was analyzed. The plasma and blood cell samples were collected at 1-, 4-, and 8-hr after administration and urine, feces and bile samples were collected 0-24 hr after administration.

Results: The major components of 1- and 4-hr plasma radioactivity were unchanged RSC-297995. On the other hand, the major component of 8-hr plasma radioactivity was benzamidine. The major component of 1-hr blood cells radioactivity was unchanged RSC-297995. Also, the major component of 4- and 8-hr blood cells radioactivity was benzamidine. In the urine, most of the radioactivity was attributed to benzamidine. In the bile, RSC-297995 3-*O*-β-D-glucuronide, Nor-RSC-297995 and RSC-297995 6-*O*-β-D-glucuronide existed as major metabolites. In addition, metabolites which might have morphinan structure and oxadiazole cleavage structures were detected. In the feces, most of the radioactivity was also attributed to benzamidine.

Study on the *in vivo* Major Metabolites of [^{14}C]-RSC-297995 monotosylate in Ferrets (Study No. R-297995-PB-062-N).

Methods: Male ferrets were administered a single oral dose of 1 mg (as free base /69.6 µCi/mL/kg [Oxadiazole-^{14}C]-RSC 297995 monotosylate under non-fasted condition. Blood samples were collected at 1 and 4 h after administration for determination of metabolites in plasma. Metabolite profiling was conducted by radio-HPLC, and the parent compound and metabolites were identified by LC/MS/MS.

Results: At 1 and 4 h, unchanged RSC-297995 accounted for 72% and 34%, respectively, of the radioactivity. An additional peak (RSC-297995-FPM1) accounted for 34% of the radioactivity in plasma collected at 4 h. Based on LC/MS/MS analysis, RSC-297995-FPM1 was identified as benzamidine. Other metabolites (RSC-297995 3-O-β-D-glucuronide, RSC-297995 6-O-β-D-glucuronide, and Nor-RSC-297995) were not detected. Thus, at 1 h, unchanged RSC-297995 accounted for the majority of radioactivity in plasma and at 4 h, unchanged RSC-297995 and benzamidine were the primary components of radioactivity in plasma.

In vivo Metabolite Profiling of S-297995 after Single Oral Administration of [Carbonyl-^{14}C]-S-297995 monotosylate in Ferrets (Study No. S-297995-PB-156-N).

Methods: Male ferrets were administered a single oral dose of 5 mg (as free base /361 µCi/2 mL/kg [Carbonyl-^{14}C]-S-297995 monotosylate under non-fasted condition. Blood samples were collected at 1 and 4 h after administration for determination of metabolites in plasma. Metabolite profiling was conducted by radio-HPLC, and the parent compound and metabolites were identified by LC/MS/MS.

Results: Unchanged S-297995 accounted for 81% and 86% of the radioactivity in the 1 and 4 h plasma samples, respectively. S-297995-FPM2 accounted for 5% and 2% of the radioactivity in the 1 and 4 h plasma samples, respectively, and S-297995-FPM3 accounted for 2% of the radioactivity in each of the 1 and 4 h plasma samples. Based on LC/MS/MS analysis, S-297995-FPM2 was identified as S-297995 3-O-β-D-glucuronide. S-297995-FPM3 was estimated to be a metabolite containing a hydroxyl group in the morphinane moiety, with a molecular weight 16 Da larger than S-297995. Finally, while not detected as radioactive peaks, benzamidine, S-297995 6-O-β-D-glucuronide, Nor-S-297995, and two other metabolites (estimated to be hydroxylated form in the morphinane moiety) were also detected.

Review of Study No. R-297995-PB-136-N from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

***In vivo* Metabolite Profiling of S-297995 after Single Oral Administration of [Carbonyl-^{14}C]-S-297995 monotosylate in Dogs (Study# R-297995-PB-136-N)**

Methods: Metabolite profiling in the dog plasma, urine, bile and feces following single oral administration of [Carbonyl-^{14}C]-RSC-297995 monotosylate at 5 mg/361 µCi/2 mL/kg was analyzed. The plasma and blood cell samples were collected at 1-, 4-, and 8-hr after administration and urine, feces and bile samples were collected 0-24 hr after administration.

Results: The major components of 1-, 4- and 8-hr plasma radioactivity were unchanged S-297995. Nor-S-297995 and three metabolites were detected, however, the amount of these metabolites were less than 4% of total radioactivity in plasma. The major component of 1-, 4- and 8-hr blood cells radioactivity was also unchanged S-297995. In the bile, S-297995 3-*O*-β-D-glucuronide, Nor-S-297995 and S-297995 6-*O*-β-D-glucuronide existed as major metabolites. Also, an oxidative metabolite with the molecular ion of *m/z* 587 was barely detected, and metabolites subsequently oxidized and/or conjugated with glucuronic acid with the molecular ion of *m/z* 779 were detected as major metabolites. In addition, a metabolite with the molecular ion of *m/z* 589 which has a morphinane structure and was speculated to be *N-O* bond cleavaged structure was barely detected, and metabolites subsequently oxidized and/or conjugated with glucuronic acid with the molecular ions of *m/z* 605, 621 and 765 were also detected in the bile as major metabolites. In the feces, the peak speculated to be S-297995 carboxylic acid was detected as one of the most prominent peaks, and metabolites which might be related to benzamidine leaving structures from metabolites were detected in bile.

***In vivo* Metabolite Profiling of S-297995 after Single Oral Administration of [Carbonyl-^{14}C]-S-297995 monotosylate in Rabbits (Study No. S-297995-PB-240-N).**

Methods: Female rabbits were administered a single oral dose of 5 mg (as free base) /248 µCi/5 mL/kg [Carbonyl-^{14}C]-S-297995 monotosylate under non-fasted condition. Blood samples were collected at 0.5 and 2 h after administration for determination of

metabolites in plasma. Metabolite profiling was conducted by radio-HPLC, and the parent compound and metabolites were identified by LC/MS/MS.

Results: Two major radioactive peaks were detected in plasma. At 0.5 and 2 h, S-297995-BPM2 accounted for 62% and 70% of the radioactivity in plasma, respectively. At 0.5 and 2 h, S-297995-BPM1 accounted for 4 and 5% of the radioactivity, respectively. Based on LC/MS/MS analysis, S-297995-BPM2 was identified as S-297995 3-O-β-D-glucuronide, and S-297995-BPM1 appeared to be a glucuronide of Nor-S-297995 (although the exact structure was not determined). While not detected as radioactive peaks, unchanged S-297995, S-297995 6-O-β-D-glucuronide, Nor-S-297995, and benzamidine were also detected. Thus, 297995 3-O-β-D-glucuronide was the major metabolite detected in rabbit plasma at 0.5 and 2 h after dosing.

***In vivo* Metabolite Profiling of S-297995 after Single Oral Administration of [Carbonyl-^{14}C]-S-297995 monotosylate in Mice (Study No. S-297995-PB-241-N).**

Methods: Male mice were administered a single oral dose of 5 mg (as free base) /248 µCi/10 mL/kg [Carbonyl-^{14}C]-S-297995 monotosylate under non-fasted condition. Blood samples were collected at 0.5 and 2 h after administration for determination of metabolites in plasma. Metabolite profiling was conducted by radio-HPLC, and the parent compound and metabolites were identified by LC/MS/MS.

Results: At 0.5 and 2 h after administration, unchanged S-297995 accounted for 70% and 45% of the radioactivity in plasma. Six radioactive peaks (S-297995-UPM1 through UPM6) were identified, with S-297995-UPM1 accounting for 6 and 17% of the radioactivity in 0.5 and 2 h samples, S-297995-UPM4 accounting for 6 and 9% of the radioactivity in 0.5 and 2 h samples, and the other metabolites accounting for less than 4% of the radioactivity. S-297995-UPM4 was identified as Nor-S-297995, and S-297995-UPM1 appeared to be a glucuronide of Nor-S-297995 (although the exact structure was not determined). While not detected as radioactive peaks, the following metabolites were identified by LC/MS: S-297995 3-G, S-297995 6-G, S-297995-carboxylic acid, S-297995-(7S)-7-hydroxide, and benzamidine. S-297995-UPM2 appeared to be a glucuronide containing a hydroxyl group in the morphinane moiety of S-297995 (exact structure unknown), S-297995-UPM3 appeared to be a glucuronide containing 30 Da larger group in the morphinane moiety of S-297995 (exact structure unknown), S-297995-UPM-5 was estimated to be hydroxylated form in the morphinane moiety, and S-297995-UPM6 appeared to be a metabolite containing 34 Da larger group in the morphinane moiety of S-297995 (exact structure unknown).

Identification of the Responsible Enzymes for [^{14}C]-S-297995 monotosylate Metabolism (Study Report No. S-297995-PF-200-N; CRO Study No. 8221179).

Methods: In this study, effects of CYP isozyme-selective inhibitors on the metabolism of 50 µM [^{14}C]-S-297995 were evaluated using pooled human liver microsomes. In addition, 50 µM [^{14}C]-S-297995 was incubated with recombinant human cDNA expressed cytochrome P450 (CYP) isozymes or UGT isozymes (Supersomes^{TM}) to

determine the contribution of individual enzymes to the metabolism of the test article. The reactions were initiated with NADPH and/or UDPGA, and after incubation the reactions were terminated by addition of acetonitrile. Samples were analyzed by radio-HPLC.

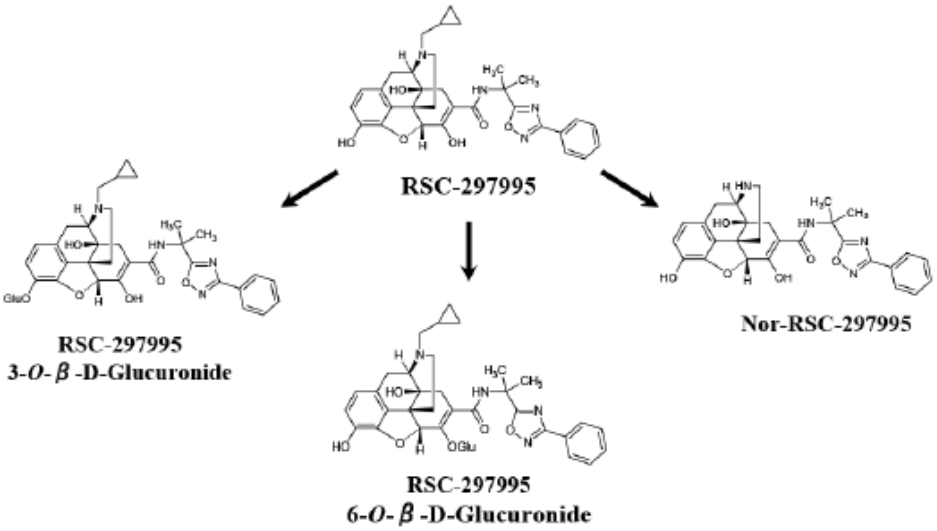
Results: When incubated with human liver microsomes, [^{14}C]-S-297995 was metabolized to Nor-S-297995, S-297995 3-O-β-D-glucuronide, and S-297995 6-O-β-D-glucuronide. Formation of Nor-S-297995 was inhibited (with 84.3% inhibition from respective control) in the presence of ketoconazole (a CYP3A4 inhibitor). When incubated with recombinant cDNA expressed human CYP isozymes, Nor-S-297995 accounted for 4.04% of radioactivity and formation of this metabolite was primarily mediated by CYP3A4 Supersomes™. This metabolite was also identified after incubation with CYP2C19 Supersomes™ (0.44% of the radioactivity). Thus, CYP3A4 was considered to be a predominant enzyme responsible for the metabolism of S-297995 to Nor-S-297995. In studies with recombinant cDNA expressed human UGT isozymes, the formation of S-297995-3-O-β-D-glucuronide and S-297995 6-O-β-D-glucuronide were primarily mediated by UGT1A3 isozyme. These metabolites accounted for 18.1 and 2.23% of the radioactivity, respectively.

Review of Study No. R-297995-PB-063-N from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Study on the *in vitro* Major Metabolites of [^{14}C]-RSC-297995 monotosylate in Human (Study# R-297995-PB-063-N)

Methods: Metabolite profiling of reaction mixture after incubation of [^{14}C]-RSC-297995 monotosylate at 5 and 50 µmol/L with cryopreserved human hepatocytes was analyzed with Radio-HPLC, LC/MS/MS.

Results: RSC-297995 3-*O*-β-D-glucuronide, RSC-297995 6-*O*-β-D-glucuronide and Nor-RSC-297995 were identified as the major metabolites. The *in vitro* prominent metabolic pathways of RSC-297995 in cryopreserved human hepatocytes were postulated as shown below. The major metabolic pathways of RSC-297995 are conjugate reactions with glucuronic acid at 3- or 6-hydroxyl group in morphinan structure and *N*-dealkylation at methylcyclopropane group.



The in vitro prominent metabolic pathways of RSC-297995 in cryopreserved human hepatocytes

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Excretion

Review of Study No. R-297995-PB-025-N from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Urinary, Fecal and Biliary Excretion Following Single Oral Administration of [^{14}C]-RSC-297995 monotosylate in Rats (Study# R-297995-PB-025-N)

Methods: Radioactivity in urine, feces and bile following single oral administration of [^{14}C]-RSC-297995 monotosylate 1 mg (as free base)/2.58 MBq (69.6 µCi)/2 mL/kg in both intact rats and bile duct-cannulated rats was determined by a liquid scintillation counter.

Results: In the intact rats, more than 97% of the radioactivity administered was recovered until 48 hr after dosing. The cumulative excretions in urine and feces within 168 hr after dosing were 49.2 % and 49.1 %, respectively. In the bile duct-cannulated rats, more than 97% of the radioactivity administered was recovered until 48 hr after dosing. The cumulative excretions in urine, bile and feces until 48 hr after dosing were 44.8%, 28.2% and 24.4 %, respectively. Thus, the test drug is predominantly excreted through urine.

Review of Study No. R-297995-PB-098-N from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Urinary, Fecal and Biliary Excretion Following Single Oral Administration of [Carbonyl-^{14}C]-RSC-297995 monotosylate in Rats (Study# R-297995-PB-098-N)

Methods: Radioactivity in urine, feces and bile following single oral administration of [Carbonyl-^{14}C]-RSC-297995 monotosylate 1 mg (as free base)/2.67 MBq (72.2 µCi)/2 mL/kg in both intact rats (n=4) and bile duct-cannulated rats (n=5) was determined by a liquid scintillation counter.

Results: In the intact rats, more than 98% of the radioactivity administered was recovered within 48 hr after dosing. The cumulative excretions in urine and feces within 168 hr after dosing were 1.5 % and 97.4 %, respectively. In the bile duct-cannulated rats, the cumulative excretion ratios in urine, bile and feces up to 48 hr after dosing were 2.5%, 31.3% and 57.6 %, respectively. Therefore, the absorption rate of RSC-297995 monotosylate was calculated to be about 34%. RSC-297995 was mainly excreted to feces *via* bile in rats.

Urinary and Fecal Excretion of Radioactivity after Repeated Oral Administration of [Carbonyl-14C]-S-297995 monotosylate in Rats (Study No. S-297995-PB-191-N).

Methods: Male Sprague-Dawley [Crl:CD(SD)] rats were administered 1mg/kg (as free base) [Carbonyl-^{14}C]-S-297995 monotosylate once daily for 14 days by oral gavage. Urine and feces were collected at 24 after each dose and at 24 h intervals up to 168 h after the final administration for measurement of radioactivity.

Results: Cumulative total excretion of radioactivity within 24 h after the 1st and 14th dose ranged from 93.7 – 97.5% (urine: 1.1-2.0%; feces: 92.6-96.6%). Cumulative total excretion of radioactivity within 168 h after administration on the 14th day was 99.3% (urine: 2.0%, feces: 97.3%). Thus, excretion of radioactivity after repeated oral administration in rats was primarily through the feces, with the total recovery of administered radioactivity being 99.3%.

Review of Study No. R-297995-PB-184-N from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Enterohepatic Circulation of Radioactivity after Single Oral Administration of [Carbonyl-14C]-S-297995 monotosylate in Rats (Study# S-297995-PB-184-N)

Methods: [Carbonyl-^{14}C]-RSC-297995 monotosylate 1 mg (as free base)/2.58 MBq (69.6 µCi)/2 mL/kg was administered to male donor SD rats (n=3). Recipient rats receiving the bile from the donor rats were connected to the donors via cannulas as a troop. Radioactivity in samples of urine (0–6, 6–24 and 24–48 hr) after dosing, feces (0–24 and 24–48 hr) after dosing and gastrointestinal contents and carcass (48 hr) after dosing from both donor and recipient rats was determined by a liquid scintillation counter.

Results: In the recipient rats, cumulative excretions of radioactivity in the urine, bile and feces collected within 48 hr after single oral administration were 0.2 ± 0.1%, 0.3 ± 0.1% and 25.6 ± 6.1% of dose, respectively. The remaining radioactivity in the gastrointestinal contents and carcass at 48 hr after dosing was 1.0 ± 0.2% and 0.5 ± 0.2% of dose, respectively. The total recovery in the recipient rats within 48 hr after oral administration was 27.7 ± 6.3% of dose. The sum of total recovery in the donor and recipient rats was 97.7 ± 1.2% of dose. The rate of re-absorption was calculated to be 1.1 ± 0.1% of dose (4.1 ± 0.9% of radioactivity recovered in recipient rat). Furthermore, the rate of enterohepatic circulation to amount of radioactivity recovered in recipient rat was calculated to be 1.3 ± 0.9% (0.3 ± 0.1% of dose).

Excretion into Milk of Radioactivity after Single Oral Administration of [carbonyl-^{14}C]-S-297995 monotosylate in Nursing Rats (Study No. S-297995-PF-239-N; CRO Study No. YDL0026).

Methods: Female nursing Sprague-Dawley rats were administered single oral doses of 1 mg/kg (as free base) [carbonyl-^{14}C]-S-297995 monotosylate approximately 12 days after parturition. Milk samples were collected at 1, 2, 4, 8, and 24 h after dosing for measurement of radioactivity. At sacrifice, terminal whole blood samples were also collected for measurement of radioactivity in plasma.

Results: Maximum concentrations of radioactivity were detected in plasma and milk at 1 h after dosing (115 and 74.6 ng equivalents/g, respectively). At 2 h after dosing, concentrations of radioactivity in milk and plasma were similar (68 and 68.9 ng equivalents/g, respectively). Radioactivity decreased over time and was below the limit of quantification in both plasma and milk at 24 h. The terminal half-lives of radioactivity in plasma and milk were calculated to be 1.9 and 2 h, respectively, and the milk:plasma AUC_{24} ratio for total radioactivity was 0.94.

Pharmacokinetic Drug Interactions

Substrate Assessments for Human Transporters of S-297995 Monotosylate (Study No. S-297995-PF-285-N; CRO Study No. 12SHIOP2)

Methods: The purpose of this study was to evaluate whether S-297995 monotosylate is a substrate of hepatic (OATP1B1, OATP1B3, OCT1) or renal (OAT1, OAT3, OCT2) uptake transporters or apical efflux transporter BCRP. Human uptake transport assays were conducted using transporter-transfected human embryonic kidney epithelial cells (HEK293) treated with 0.5 and 2 µM S-297995 for 2, 5, and 10 minutes. Apical efflux transport was assessed using bi-directional assays using Caco-2 and CPT-B1 cells (a

sub-clone of Caco-2 cells with downregulated BCRP function) and S-297995 concentrations of 0.5, 2, and 10 µM.

Results: In uptake transporter substrate assessments, ratios of the influx rates of S-297995 in transporter-transfected cells relative to vector control-transfected cells ranged from 0.8 to 1.3 with the exception of OCT2, for which ratios ranged from 1.4 to 1.8. For comparison, ratios of influx rates for probe substrates in transporter-transfected cells relative to vector control-transfected cells ranged from 7.8 to 76.6. Based on influx rate ratios of S-297995 less than 2.0, the test compound was concluded to not be a substrate of these uptake transporters. In the BCRP substrate assessments, efflux ratios of 0.5, 2, and 10 µM S-297995 were 14.9, 19.5, and 15.4, respectively, in Caco-2 cells and 135, 69.7, and 140, respectively, in CPT-B1 cells. For comparison, the efflux ratios of BCRP probe substrate E3S were 64.5 and 28.3 in Caco-2 and CPT-B1 cells, respectively. The efflux ratios of S-297995 in Caco-2 cells indicate that the test compound is subject to efflux transport. Because efflux transport was increased in CPT-B1 cells, compared to Caco-2 cells, this suggests that S-297995 is not a substrate of BCRP. Because digoxin (a P-gp substrate) also demonstrated an increased efflux ratio in CPT-B1 cells, compared to Caco-2 cells, this study suggests that the compound is a P-gp (but not BCRP) substrate.

Study on P-glycoprotein Mediated Drug Interaction of RSC-297995 monotosylate (Study No. R-297995-PF-067-N; CRO Study No. 8SHIOP1).

Methods: The objective of this study was to evaluate whether RSC-297995 is a substrate or inhibitor of P-glycoprotein (P-gp) using a bi-directional permeability assay. In this study, transport of 0.2, 1, and 5 µM RSC-297995 in the apical-to-basolateral (AP-to-BL) and basolateral-to-apical (BL-to-AP) directions was measured in Caco-2 cells. For comparison, bi-directional permeability of digoxin (a P-gp substrate) was also measured, and bi-directional permeability of 0.2 µM RSC-297995 and digoxin were determined the presence and absence of P-gp inhibitors (10 µM cyclosporine A and ketoconazole). To determine whether RSC-297995 is an inhibitor of P-gp, the bi-directional transport of digoxin in Caco-2 cells was measured in the presence and absence of 5 µM RSC-297995, 10 µM cyclosporine A, or 10 µM ketoconazole. The apparent permeability (Papp), recovery, and efflux ratio values were calculated.

Results: Papp values for 0.2, 1, and 5 µM RSC-297995 in the A-to-B direction were $0.97x10^6$, $0.96x10^6$, and $1.38x10^6$ cm/s, respectively, compared to $0.46x10^6$ cm/s for 10 µM digoxin. In the B-to-A direction, Papp values for 0.2, 1, and 5 µM RSC-297995 were $31.7x10^6$, $37.8x10^6$, and $37.8x10^6$ cm/s, respectively, compared to $16.9x10^6$ cm/s for 10 µM digoxin. Efflux ratios for 0.2, 1, and 5 µM RSC-297995 were 32.7, 39.4, and 24.9, respectively, compared to 36.8 for 10 µM digoxin. In the presence of P-gp inhibitors, efflux ratios for 0.2 µM RSC-297995 were substantially lower (1.6 and 1.7) compared to the efflux ratio in the absence of P-gp inhibitors (26.3), similar to findings for digoxin. In the presence of 5 µM RSC-297995, the efflux ratio of digoxin was decreased slightly (29.5 versus 36.8), while the presence of cyclosporine A and ketoconazole caused substantial decreases in the digoxin efflux ratio values. The remaining efflux of digoxin

in the presence of RSC-297995 was 79.8%, compared to 0.1% and 1.9% in the presence of cyclosporine A and ketoconazole, respectively. Overall, this study demonstrated that RSC-297995 is a P-gp substrate.

Inhibitor Assessments for Human Transporters of S-297995 monotosylate (Study No. S-297995-PF-297-N; CRO Study No. (b) (4) Shionogi-02-01Oct2013).

Methods: The purpose of this study was to evaluate whether S-297995 monotosylate inhibits transport activities of BCRP, OATP1B1, OATP1B3, OCT1, OCT2, OAT1, and OAT3. Inhibitory effects of S-297995 monotosylate were evaluated using MDCKII-BCRP cells, MDCKII-OAT3 cells, and recombinant OATP1B1, OATP1B3, OCT1, OCT2, OAT1, and OAT3 expressing Chinese hamster ovary (CHO) cells. In studies to evaluate inhibitory effects on BCRP, S-297995 monotosylate was tested at a concentration of 5 µmol/L and for all other transporters, S-297995 monotosylate was tested at 1 and 5 µmol/L. In the uptake transporter inhibition experiments, values were calculated on a relative scale, with 100% defined as transport of a probe substrate in the absence of S-297995 monotosylate and 0% defined as the absence of transporter activity.

Results: In MCKII-BCRP cells, the net efflux ratios of prazosin in the presence and absence of S-297995 monotosylate were 13.1 and 13.6, respectively, compared to 0.8 for the reference inhibitor, suggesting that the test compound was not an inhibitor of BCRP. In OATP1B1, OATP1B3, OCT1, and OAT1 expressing CHO cells, relative activities for uptake of the respective probe substrates in the presence of 5 µM S-297995 monotosylate were 89.5%, 86.6%, 97.5%, and 88.9%. In OCT2 and OAT3 expressing CHO cells, uptake of the respective probe substrates was inhibited in a dose-dependent manner with relative activities in the presence of 5 µM S-297995 monotosylate of 70.5% and 58.9%. Overall, it was concluded 5 µM S-297995 monotosylate showed inhibitory effects for OCT2 and OAT3, whereas inhibition at the other transporters was characterized as negligible.

Inhibitor Assessments of Nor-S-297995 for Human Transporters (Study No. S-297995-PF-340-N; CRO Study No. GE-1425-G).

Methods: The purpose of this study was to evaluate whether Nor-S-297995 is an inhibitor of P-gp, BCRP, OATP1B1, OATP1B3, OCT1, OCT2, OAT1, and OAT3. Inhibitory effects of Nor-S-297995 on transport of typical substrates were evaluated using Caco-2 cells expressing P-gp and BCRP. Inhibitory effects of Nor-S-297995 on uptake of typical substrates were evaluated using recombinant OATP1B1, OATP1B3, OCT1, OCT2, OAT1, and OAT3 expressing human embryonic kidney 293 (HEK293) cells. In these experiments, Nor-S-297995 was tested at concentrations of 1 and 20 nmol/L.

Results: In Caco-2 cells, permeability coefficients for P-gp mediated and BCRP transport of substrates were similar in the presence and absence of Nor-S-297995 indicating that Nor-S-297995 exhibited no inhibitory effect on P-gp or BCRP. In

OATP1B1, OATP1B3, OCT1, OCT2, OAT1, and OAT3 expressing cells, the cleared volumes of typical substrates in the presence of 20 nmol/L Nor-S-297995 were 93.5%, 94.2%, 62.4%, 101.6%, 93.5%, and 108.2% of controls, respectively. Overall, it was concluded that 20 nM Nor-S-297995 showed inhibitory effects at OCT1.

Effects of RSC-297995 monotosylate on Hepatic Drug Metabolizing Enzymes in One-Month Oral Toxicity Study in Rats (Study No. R-297995-PB-049-L).

Methods: Hepatic microsomes were prepared from rats administered oral doses of 30, 100, and 1000 mg/kg/day RSC-297995 monotosylate for one month in Study No. R-297995-TB-048-L. Microsomal protein concentration and CYP content were determined. Furthermore, ethoxyresorufin O-deethylase (EROD), testosterone 6β-hydroxylase (T6β-OHase), 16α-hydroxylase (T16α-OHase), 16β-hydroxylase (T16β-OHase), and 2α-hydroxylase (T2α-OHase) activities were assessed as markers of activity for CYP1A, CYP3A, CYP2C11/CYP2B, CYP2B, and CYP2C11, respectively.

Results: Following one-month of oral dosing with 1000 mg/kg/day, there were statistically significant decreases in protein (-14%, compared to control) and T6β-OHase, T16α-OHase, and T2α-OHase activities (-61%, -46%, and -48%, respectively, compared to controls). In females, there were statistically significant increases in T6β-OHase at all doses (up to 160% of control) and T16β-OHase activities at 100 and 1000 mg/kg/day (up to 161% of control). Small, but statistically significant, decreases in protein occurred at 30 and 100 mg/kg/day (-13%, compared to control). Following a one-month recovery period after dosing with the test compound, there were no apparent treatment-related changes compared to controls. Overall, this study suggested that repeated dosing with naldemedine monotosylate affected several CYP activities. In males, RSC-297995 monotosylate decreased the expression of CYP3A and CYP2C11/CYP2B and/or inhibited their enzymatic activities whereas in females, the test compound appeared to induce CYP3A and CYP2B.

Effects of RSC-297995 monotosylate on Hepatic Drug Metabolizing Enzymes in One-Month Oral Toxicity Study in Dogs (Study No. R-297995-PB-050-L).

Methods: Hepatic microsomes were prepared from dogs administered oral doses of 1, 3, 10, and 50 mg/kg/day RSC-297995 monotosylate for one month in Study No. R-297995-TB-046-L. Microsomal protein concentration and CYP content were determined. In addition, ethoxyresorufin O-deethylase (EROD), testosterone 6β-hydroxylase (T6-β-OHase), and testosterone 16-α-hydroxylase (T16α-OHase) activities were assessed as markers of activity for CYP1A, CYP3A12, and CYP2B11/2C21, respectively.

Results: Following one-month of oral dosing, there was a statistically significant increase in protein at all dose levels in males (+21 to +27%, compared to controls). There were no statistically significant changes in enzyme activity at any dose. In females, there were statistically significant decreases in EROD activity at all dose levels (-40 to -77%, compared to control) and T16α-OHase activity at 50 mg/kg/day (-41%,

compared to control). While statistically insignificant, P450 content was decreased at 50 mg/kg/day (-54%, compared to control). The findings were reversible however, based on the lack of significant differences (compared to controls) in hepatic microsomes prepared from RSC-297995 monotosylate treated dogs followed by a one-month recovery period.

Other

Determination of Membrane Permeability and Solubility S-297995 Monotosylate (Study No. S-297995-PF-288-L).

Methods: The purpose of this study was to determine the solubility of S-297995 monotosylate and the permeability of S-297995 across Caco-2 cell monolayers. The aqueous solubility, S-297995 monotosylate was evaluated at pH 1.0, 3.5, 4.5, 5.5, and 7.5 using the shake-flask method. For the permeability study, Caco-2 monolayers were treated with 14,140, and 1400 nM S-297995 on the apical side for apical to basolateral (A-to-B) assessment or basolateral side for B-to-A assessment. The cell monolayers were incubated at 37ºC and samples were collected at 15, 30, and 45 min for the receivers and 0 and 45 min for the donors. Minoxidil and atenolol were also tested in the unidirectional permeability study (A-to-B) as reference compounds (these compounds were used as a high permeability internal standard and monolayer integrity marker compound, respectively). The apparent permeability (Papp) and efflux ratio were calculated.

Results: In the solubility study, mean dissolved concentrations of S-297995 (free base) at pH 1.0, 3.5, 4.5, 5.5, and 7.5 were 0.397, 0.101, 0.0826, 0.135, and 0.420 mg/mL. These concentrations exceed the concentration equivalent to the highest proposed dose (0.2 mg S-297995) dissolved in 250 mL (0.0008 mg/mL). Thus it was concluded that the highest dose strength (0.2 mg of S-297995) can be dissolved in less than 250 mL of aqueous medium across the pH range of 1.0 to 7.5. In the unidirectional permeability study, the Papp values for S-297995 in the A-to-B direction were $1.99x10^{-6}$ and $1.63x10^{-6}$ cm/s at 140 and 1400 nM, respectively. The permeability of S-297995 was lower than that of co-dosed minoxidil. In the bidirectional permeability experiment, the Papp values for the A-to-B direction were $1.91x10^{-6}$ and $1.95x10^{6}$ at 140 and 1400 nM, respectively, and the Papp values for the B-to-A direction were $37.4x10^{-6}$, $32.1x10^{-6}$, and $37.9x10^{-6}$ cm/s at 14, 140, and 1400 nM, respectively. The efflux ratios were 16.8 and 19.4 at 140 and 1400 nM, respectively, suggesting that S-297995 is a substrate of one or more efflux transporters.

5.2 Toxicokinetics

TK data are reviewed with the associated general toxicology, carcinogenicity, and reproductive and developmental toxicology studies under Sections 6, 8, and 9 below.

6 General Toxicology

6.1 Single-Dose Toxicity

Review of Study No. R-297995-TB-047-L from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Title: **Single Dose Oral Toxicity Study of RSC-297995 monotosylate in Rats (Study Number: R-297995-TB-047-L)**

Methods: This study was to determine the potential toxicity and toxicokinetics (TK) of S-297995 monotosylate following a single dose in rats. Groups of Sprague-Dawley (SD) rats (5 animals/sex/group, age of 6 weeks at the time of administration) were given 0 (control), 500, and 2000 mg/kg S-297995 monotosylate (Lot No. A81001) in 0.5 w/v% methylcellulose (MC) aqueous solution once by oral gavage under fasted condition. For the assessment of TK, additional rats (4 animals/sex/group) were treated in the same way as the toxicity animals. The observation period for the toxicity animals was 14 days after dosing, and the following items were examined: clinical observations, body weight, and gross pathology. Blood samples were collected at 4 hours after administration for the control group and at 0.5, 1, 2, 4, 8, and 24 hours after administration for the S-297995 monotosylate dosing groups. This study was conducted in compliance with GLP.

Results: There were no deaths throughout the study period. In clinical observations, compound-colored feces were observed in all animals of the 2000 mg/kg group on the administration day and the next day. As compared to the control animals, a suppression of body weight increase was evident on the next day of administration in males of the 500 and 2000 mg/kg groups and in females of the 2000 mg/kg group. However, the body weights in these animals became comparable thereafter to those in the control group. At necropsy after the 14-day observation period, no gross lesion was observed in any animals. On the basis of these results, it was concluded that the lethal dose of S-297995 monotosylate in both male and female rats was estimated to be more than 2000 mg/kg.

Review of Study No. R-297995-TB-045-L from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Title: **Single Oral Toxicity Study of RSC-297995 monotosylate in Dogs (Study Number: R-297995-TB-045-L)**

Methods: This study was to determine the potential toxicity and TK of S-297995 monotosylate in a single dose in dogs. S-297995 monotosylate (dose volume: 10 mL/kg, suspension in 0.5% MC) was administered orally to male and female beagle dogs (1 animal/sex/group, age of 9 months at the time of administration) once at dose levels of 0 (control), 200, and 1000 mg/kg. After administration, the animals were observed for 14 days. The following parameters were examined in this study: clinical observations, body weight, food consumption, urinalysis, hematology, blood chemistry and TK. Blood samples were collected at the following intervals:

0.25, 0.5, 1, 2, 4, 7, and 24 hours after dosing. This study was conducted in compliance with GLP.

Results: There were no deaths during the observation period. In clinical observations, vomiting or vomitus, soon followed by salivation, was observed shortly after dosing in all animals of the 200 and 1000 mg/kg groups, with higher frequency in a male of the 1000 mg/kg group. The vomiting/vomitus was no longer observed 2-hour post-dose, except for the male of the 1000 mg/kg group that showed vomitus until 7 hours after dosing. This male showed slight decrease in body weight on the following day. Stool containing pale yellowish test article-like material was observed on the day after dosing in the male and female of the 1000 mg/kg group, but there were no test article-related findings afterward. In blood chemistry, dose-related increase of alkaline phosphatase (ALP) activity, accompanied with high values of total bilirubin (T.Bil) in some animals, was observed in all animals of the 200 and 1000 mg/kg groups on the day after dosing, suggesting effects on hepatobiliary system. These changes ameliorated/disappeared during the 14-day observation period. There were no test-article related changes in food consumption and hematology. The lethal dose of S-297995 monotosylate in both male and female dogs was estimated to be more than 1000 mg/kg.

6.2 Repeat-Dose Toxicity

Rabbit

Study title: Two-Week Oral Toxicity Study of S-297995 monotosylate in Non-Pregnant Rabbits, Amendment to Final Report (No. 1)

Study no:	S-297995-TF-119L (CRO Study No. 250228A)
Study report location:	
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 8, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	S-297995 monotosylate, lot# A81001, 97.5%

Key Study Findings

Treatment-related findings at 100 mg/kg/day were limited to a transient decease in food consumption at the beginning of the study only. At 300 mg/kg/day, findings included decreased fecal volume (single observation only), body weight gain (statistically significant for Days 1 – 2 only), and food consumption (statistically significant on Day 2 only). At 1000 mg/kg/day, treatment-related findings included decreased fecal volume, body weight, body weight gain, and food consumption (associated with body weight loss). Based on these findings, the maximum dose level for the "Dose Range-Finding Oral Study for Effects of S-297995 monotosylate on Embryo-Fetal Development in Rabbits" was set at 600 mg/kg/day.

Methods

Doses:	100, 300, and 1000 mg/kg/day (as S-297995)
Frequency of dosing:	Once daily
Route of administration:	Oral (gavage)
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5 w/v% methylcellulose aqueous solution
Species/Strain:	Kbl:JW rabbits
Number/Sex/Group:	3 females/group
Age:	16 weeks
Weight:	3.00 – 3.40 kg
Satellite groups:	No
Unique study design:	No
Deviation from study protocol:	Protocol deviations did not affect the quality or integrity of the study.

Observations and Results

Mortality

Mortality checks were conducted twice daily during the study, and once on the day of necropsy. There were no mortalities.

Clinical Signs

Animals were observed for clinical signs twice daily, and once of the day of necropsy.

Clinical signs were limited to decreased fecal volume in 1/3 and 3/3 animals at 300 and 1000 mg/kg/day, respectively. This finding was observed on only a single occasion (prior to dosing on Day 3) at 300 mg/kg/day whereas the frequency of this finding at 1000 mg/kg/day was greater.

Body Weights

Body weights were recorded once daily.

At 1000 mg/kg/day, there was a statistically significant decrease in body weight (compared to controls). On Day 15, mean body weights at 100, 300, and 1000 mg/kg/day were 95.6%, 95.0%, and 83.4% of controls, respectively.

At 300 and 1000 mg/kg/day, there were statistically significant decreases in mean body weight gain. At 300 mg/kg/day, a statistically significant decrease in mean body weight occurred from Days 1 to 2 only (body weight gain was -0.11 kg compared to -0.03 in controls). At 1000 mg/kg/day, statistically significant decreases occurred through the majority of the study. Mean body weight gain from Day 1 to 14 was 0.19, 0.10, 0.10, and -0.31 kg in the control, 100, 300, and 1000 mg/kg/day dose groups, respectively.

Feed Consumption

Food consumption was measured daily.

Statistically significant decreases in mean food consumption (compared to controls) occurred at all dose levels. At 100 mg/kg/day, mean food consumption was significantly decreased on Days 2, 4, 7, and 9. At 300 mg/kg/day, mean food consumption was significantly decreased on Day 2 only. At 1000 mg/kg/day, mean food consumption was significantly decreased on Days 2 – 14. On Day 2, mean food consumption was 182, 105, 96, and 92 g in the control, 100, 300, and 1000 mg/kg/day groups, respectively. On Day 14, mean food consumption was 182, 161, 179, and 54 g/day in the control, 100, 300, and 1000 mg/kg/day groups, respectively.

Gross Pathology

Animals were necropsied on Day 15 (one day after the last administration of the test compound).

No treatment-related findings were identified.

Toxicokinetics

Blood was collected at 0.5, 1, 2,4, 8, and 24 h after administration on Day 1, and before dosing and at 0.5, 1, 2, 4, 8, and 24 h after administration on Day 14. Frozen plasma samples were shipped to the TK analysis test site (b) (4) Samples were analyzed by LC/MS/MS for S-297995 concentration.

Cmax was observed 0.5 to 3 h after dosing, and exposures (based on Cmax and AUC) increased with increasing dose. The Applicant's table below summarized TK parameters for S-297995 on Days 1 and 14.

Table 1-1 Concentrations of S-297995 in plasma and its TK parameters on Day 1 of repeated oral administration of S-297995 to rabbit for two weeks

Group	Dose (mg/kg/day)	Sex	Animal No.	Concentration (μg/mL) 0.5h	1h	2h	4h	8h	24h	Cmax (μg/mL)	Tmax (h)	AUC_{0-24h} (μg·h/mL)
1	0	Female	F01151	ND	ND	ND	ND	ND	ND	NC	NC	NC
			F01152	ND	ND	ND	ND	ND	ND	NC	NC	NC
			F01153	ND	ND	ND	ND	ND	ND	NC	NC	NC
		Mean		0.00	0.00	0.00	0.00	0.00	0.00	NC	NC	NC
		SD		0.00	0.00	0.00	0.00	0.00	0.00	NC	NC	NC
2	100	Female	F02251	1.21	0.589	0.237	0.0319	ND	ND	1.21	0.5	1.50
			F02252	1.50	1.09	0.348	0.0765	ND	ND	1.50	0.5	2.32
			F02253	2.08	1.66	0.512	0.116	ND	ND	2.08	0.5	3.40
		Mean		1.60	1.11	0.366	0.0748	0.00	0.00	1.60	0.5	2.41
		SD		0.44	0.54	0.138	0.0421	0.00	0.00	0.44	0.0	0.95
3	300	Female	F03351	8.32	5.14	1.39	0.321	0.0244	ND	8.32	0.5	11.3
			F03352	5.41	1.88	0.639	0.120	0.0456	ND	5.41	0.5	5.89
			F03353	5.69	2.83	1.49	0.192	ND	ND	5.69	0.5	7.78
		Mean		6.47	3.28	1.17	0.211	0.0233	0.00	6.47	0.5	8.32
		SD		1.61	1.68	0.47	0.102	0.0228	0.00	1.61	0.0	2.75
4	1000	Female	F04451	9.05	6.40	4.15	1.60	0.0895	0.0341	9.05	0.5	21.5
			F04452	22.3 *#	22.6 *#	4.99	0.473	0.102	0.0247	22.6	1.0	38.2
			F04453	14.5 *#	9.90	5.87	1.04	0.0566	0.166	14.5	0.5	28.5
		Mean		15.3	13.0	5.00	1.04	0.0827	0.0749	15.4	0.7	29.4
		SD		6.7	8.5	0.86	0.56	0.0235	0.0790	6.8	0.3	8.4

*: Analytical samples were diluted 10-fold with blank rabbit plasma.
#: Reanalysis values
ND: Not Detected
NC: Not Calculated

Table 1-2 Concentrations of S-297995 in plasma and its TK parameters on Day 14 of repeated oral administration of S-297995 to rabbit for two weeks

Group	Dose (mg/kg/day)	Sex	Animal No.	Concentration (μg/mL) Pre	0.5h	1h	2h	4h	8h	24h	Cmax (μg/mL)	Tmax (h)	AUC_{0-24h} (μg·h/mL)
1	0	Female	F01151	ND	ND	ND	ND	ND	ND	ND	NC	NC	NC
			F01152	ND	ND	ND	ND	ND	ND	ND	NC	NC	NC
			F01153	ND	ND	ND	ND	ND	ND	ND	NC	NC	NC
		Mean		0.00	0.00	0.00	0.00	0.00	0.00	0.00	NC	NC	NC
		SD		0.00	0.00	0.00	0.00	0.00	0.00	0.00	NC	NC	NC
2	100	Female	F02251	ND	1.26	0.463	0.207	0.0603	ND	ND	1.26	0.5	1.47
			F02252	ND	2.10	0.618	0.306	0.0552	ND	ND	2.10	0.5	2.14
			F02253	ND	2.40	0.811	0.358	0.0665	ND	ND	2.40	0.5	2.54
		Mean		0.00	1.92	0.631	0.290	0.0607	0.00	0.00	1.92	0.5	2.05
		SD		0.00	0.59	0.174	0.077	0.0057	0.00	0.00	0.59	0.0	0.54
3	300	Female	F03351	ND	3.68	5.08	2.51	0.401	0.0487	ND	5.08	1.0	11.1
			F03352	ND	8.78	3.50	1.36	0.183	0.0404	ND	8.78	0.5	10.0
			F03353	ND	3.97	2.14	2.10	0.367	0.0346	ND	3.97	0.5	8.19
		Mean		0.00	5.48	3.57	1.99	0.317	0.0412	0.00	5.94	0.7	9.76
		SD		0.00	2.86	1.47	0.58	0.117	0.0071	0.00	2.52	0.3	1.47
4	1000	Female	F04451	0.0652	3.72	4.67	6.70	8.02	2.22	0.0507	8.02	4.0	62.1
			F04452	0.582	3.90	5.95	8.04	10.7 *#	2.91	0.473	10.7	4.0	83.6
			F04453	0.0661	4.93	14.9 *#	14.5 *#	7.25	1.45	0.0560	14.9	1.0	72.1
		Mean		0.238	4.18	8.51	9.75	8.66	2.19	0.193	11.2	3.0	72.6
		SD		0.298	0.65	5.57	4.17	1.81	0.73	0.242	3.5	1.7	10.8

*: Analytical samples were diluted 10-fold with blank rabbit plasma.
#: Reanalysis values
ND: Not Detected
NC: Not Calculated

Dosing Solution Analysis

Samples of the dosing solutions used on the first and last dosing days were analyzed for S-297995 concentration. Concentrations ranged from 98.0 to 99.2% of nominal concentrations and the values of RSD were between 0.0 and 2.0%. Thus, the concentrations and homogeneity of the dosing solutions were deemed to be acceptable.

Mouse

Study title: Preliminary Carcinogenicity Study (Gavage) of S-297995 monotosylate in Mice for 2 Weeks*

Study no.:	S-297995-TB-218-R
Study report location:	EDR
Conducting laboratory and location:	Developmental Research Laboratories, Shionogi & Co., Ltd. Osaka, Japan
Date of study initiation:	April 26, 2010
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	S-297995 monotosylate, A81001, 97.5% (on anhydrous basis)

*Reviewer Comment: Although the title of the study is “carcinogenicity study”, this is a 2-week dose range finding study.

Methods

Doses:	Experiment 1: 30, 100, and 300 mg/kg/day, expressed as S-297995 Experiment 2: 1000 mg/kg/day, expressed as S-297995
Frequency of dosing:	Once daily
Route of administration:	Oral (gavage)
Dose volume:	10 ml/kg
Formulation/Vehicle:	0.5% methylcellulose aqueous solution
Species/Strain:	Mouse / Crlj:CD1(ICR)
Number/Sex/Group:	Experiment 1: 10/sex/group (main study), 33/sex/S-297995 dose level (TK) Experiment 2: 18/sex
Age:	6 weeks
Weight:	Males: 28.4 – 35.1 g Females: 20.3 – 27.8 g
Satellite groups:	Yes (TK)
Unique study design:	No
Deviation from study protocol:	Not specified

Brief Overview of Study Findings

This was a preliminary, non-GLP toxicity study, for which limited toxicology parameters were evaluated (e.g., histopathological examination of high dose animals was not conducted). In experiment 1, at 300 mg/kg/day, there were statistically significant increases (+8.6%, compared to controls) in body weights of females and food consumption (up to +28.6%) in males. Statistically significant changes in clinical chemistry parameters in males included decreased amylase and increased total cholesterol at ≥100 mg/kg/day (up to -27.9% and +34.8%, respectively, compared to controls), and increased albumin and A/G ratio at 300 mg/kg/day (+11% and +50%, respectively, compared to controls). In females, total cholesterol was increased (+39%, compared to controls) and urea nitrogen was decreased (-27.2%, compared to controls) at 300 mg/kg/day. Statistically significant increases in absolute and relative liver weights in females at 300 mg/kg/day (+37.7% and +27%, respectively, compared to controls), correlated with findings of enlargement of the liver observed at necropsy (2/10 females) and mild hepatocellular hypertrophy observed microscopically (4/10 females). Mild hepatocellular hypertrophy was also observed in 4/10 males at 300 mg/kg/day, compared to an incidence of 0/10 in controls. In experiment 2, dosing with 1000 mg/kg/day for 2 weeks was considered to be well-tolerated. Overall, the primary target organ of toxicity in this study appeared to be the liver. Based on the absence of mortality and clinical findings, 1000 mg/kg/day was chosen as the high dose for the 13-week dose range finding study in mice.

Review of Study No. S-297995-TF-226-L (CRO Study No. YDL0024) from the IND 107475 nonclinical review dated November 23, 2011 is copied below (C. Wu, Ph.D.). Although Dr. Wu's review states that the study title is "Thirteen-Week Repeated-Dose Oral Toxicity Study of S-297995 in Mice", the study report shows that the study title is

"Preliminary Carcinogenicity Study (Gavage) of S-297995 monotosylate in Mice for 13 Weeks." It is noted that although the title of the study is "carcinogenicity study", this is a 13-week oral toxicity study.

Study title: Thirteen-Week Repeated-Dose Oral Toxicity Study of S-297995 in Mice

Study no.:	S-297995-TF-226-L
Study report location:	Electronic Submission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 13, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RSC-297995 monotosylate, Lot No. A81001, 97.5% purity

Key Study Findings

Mice received daily oral dosing with S-297995 at 100, 300 or 1000 mg/kg/day. The treatment related bodyweight gain was observed all treatment groups. Main histopathological changes found in the liver were centrilobular hypertrophy in all treated groups males and 300 and 1000 mg/kg/day females and periportal vacuolization mainly in animals given 1000 mg/kg/day in both sexes. In addition, reduced / absent corpora lutea was observed in the ovaries in the majority of females given 1000 mg/kg/day and in 6 females given 300 mg/kg/day, and oestrus and metoestrus morphological characteristics was observed in the vagina mainly in the treated groups. The only histopathological finding observed at 100 mg/kg/day was centrilobular hypertrophy in the liver, but was not dose-dependent. In addition, an elevated alkaline phosphatase level (2-fold) was also noted in females at 1000mg/kg/day. The no-observed-adverse-effect level (NOAEL) in this study was considered to be 100 mg/kg/day with $AUC_{0\text{-}24h}$ of 194 μg·hr/mL for males and 165 μg·hr/mL for females. The primary target organ of toxicity for S-297995 in mice was the liver.

Methods

Doses:	S-297995 was given at 0 (vehicle control), 100, 300 and 1000 mg/kg/day.
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% methylcellulose aqueous solution (0.5 w/v% MC)
Species/Strain:	Crl:CD-1 mice
Number/Sex/Group:	12 animals/sex/group (main study)
Age:	37-43 days
Weight:	21 to 37 g for males and 21 to 28 g for females
Satellite groups:	60 animals/sex/group for TK study
Unique study design:	none
Deviation from study protocol:	There were 4 minor deviations which did not affect the integrity or validity of the study.

Observations and Results

Mortality

There was no death in any group.

Clinical Signs

In Weeks 8 to 13, head shaking (comprising of head movements to the left and right) was seen in animals in all treated groups immediately after dosing, however there was no dose relationship and there was a single incident in the female control group. This sign was observed in a total of 1 female in the control group, 6 males and 6 females treated at 100 mg/kg/day, 6 males and 4 females treated at 300 mg/kg/day and 5 males and 9 females treated at 1000 mg/kg/day.

Body Weights

Overall, higher mean body weights were observed in all treated male groups and in females 6 weeks after receiving 300 or 1000 mg/kg/day as shown in the Tables below. The mean bodyweight gains reached up to 1.34X in males and 1.31X in females compared with control with statistical significance attained for both sexes treated at 300 or 1000 mg/kg/day at week 13.

Table 1 Male mouse body weight changes during 3 month study

Dose group (mg/kg/day)	Week 0 (g)	Week 6 (g)	Week 13 (g)	Change Week 0-13
0	31.9±2.67	40.4±3.46	44.5±4.23	12.6±2.51
100	33.1±2.22	43.0±2.87	47.2±3.85	14.2±2.36
300	30.2±3.86	42.2±2.95	47.1±3.01	16.9±3.78**

1000	31.6±1.59	43.2±1.79	48.0±2.44	16.4±2.43**

Table 2 Female mouse body weight changes during 3 month study

Dose group (mg/kg/day)	Week 0 (g)	Week 6 (g)	Week 13 (g)	Change 0-13 week
0	23.5±1.81	29.5±2.79	33.9±3.22	10.4±2.23
100	25.4±1.54	33.5±2.88	36.1±2.33	10.7±1.45
300	24.3±1.59	34.4±2.94	36.6±2.64	12.3±2.42*
1000	24.3±1.25	33.2±2.53	37.8±1.91	13.5±1.65**

Food Consumption

There were no S-297995-related effects on food consumption in all treated groups as compared with control throughout the study.

Ophthalmoscopy

Not performed.

Hematology

The hematological examination at termination revealed statistically significantly lower hemoglobin values for males receiving 300 mg/kg/day (0.95X) and both sexes given 1000 mg/kg/day (0.93X) with an associated decrease in MCHC in those groups (down to 0.95X) and lower mean MCH in males given 300 mg/kg/day (0.95X) and females given 1000 mg/kg/day (0.96X). In addition, a lower mean red blood cell count (0.95X) and a higher reticulocyte count (1.21X) were observed in males receiving 1000 mg/kg/day. In all treated female groups and males given 1000 mg/kg/day higher mean white blood cell counts (up to 3X) were seen.

Clinical Chemistry

The biochemical examination at termination revealed higher mean ALP values for males and females (up to 1.54X) treated at 1000 mg/kg/day with statistical significance attained for the females only. In addition, statistically significantly lower mean urea values were seen for all treated male groups and females given 1000 mg/kg/day (0.74X). Lower mean triglyceride values were also observed for all treated groups (0.41X). Statistically significantly higher mean potassium values were seen for all treated female groups and males receiving 1000 mg/kg/day (up to 1.12X) and statistically significantly lower mean phosphorus values were observed for males given 300 or 1000 mg/kg/day (0.79X).

Urinalysis

Not reported.

Gross Pathology

An S-297995-related gross finding was enlarged liver found in 7 out of 12 female mice given 1000 mg/kg/day, but not in males.

Organ Weights

At termination, statistically significant lower mean heart weights were seen for males receiving 300 mg/kg/day and males and females receiving 1000 mg/kg/day (0.85X). In addition, statistically significant higher mean liver weights were observed for females at 300 mg/kg/day and males and females at 1000 mg/kg/day (up to 1.41X) and statistically significant higher mean spleen weights were seen for males given 1000 mg/kg/day (1.30X). Statistically significant lower ovary weights were also observed for females given 300 or 1000 mg/kg/day (0.42X). A lower mean uterus and cervix weight was also seen for females receiving 1000 mg/kg/day (0.79X).

Histopathology

Adequate Battery

Yes

Peer Review

Yes

Histological Findings

All animals at the final sacrifice (Groups 1 through 4) were evaluated. The following tissues or organs were examined histologically: adrenals, aorta, bone with bone marrow smears, brain (cerebellum, cerebrum, midbrain, medulla), cecum, colon, duodenum, epididymides, esophagus, eyes, femur, gall bladder, heart, ileum, jejunum, kidneys, larynx, liver (section from 2 main lobes), lungs (section from two major lobes including bronchi), lymph nodes -Axillary-mandibular-mesenteric, optic nerves, ovaries (with oviduct), pancreas, Peyer's patches, pituitary, prostate, rectum, salivary glands – mandibular, sciatic nerves, seminal vesicles, skeletal muscle, skin with mammary glands (caudal area), small intestine, spinal cord (transverse and longitudinal sections at the cervical, thoracic and lumbar levels), spleen, sternum, stomach, testes, thymus, thyroid with parathyroids, tongue, trachea, ureters, urinary bladder, uterus with cervix, and vagina and gross lesions.

An increased incidence of centrilobular hypertrophy in the liver was observed in all treated groups in males and 300 and 1000 mg/kg/day group females. This change was occasionally accompanied by an increased incidence of hepatocyte periportal vacuolation mainly in animals given 1000 mg/kg/day as shown in Table 1 below.

Summary of treatment related findings in the liver for animals killed after 13 weeks of treatment

Group/sex Dose (mg/kg/day)	1M 0	2M 100	3M 300	4M 1000	1F 0	2F 100	3F 300	4F 1000
Hepatocyte Hypertrophy, Centrilobular								
Minimal	2	7	8	5	0	0	3	5
Slight	0	1	3	6	0	0	0	0
Moderate	0	0	1	1	0	0	0	0
Total	2	8	12	12	0	0	3	5
Hepatocyte Vacuolation, Periportal								
Minimal	1	0	3	6	1	2	5	2
Slight	0	0	0	3	2	2	0	8
Moderate	0	0	0	2	0	0	0	1
Total	1	0	3	11	3	4	5	11
Number of animals examined	12	12	12	12	12	12	12	12

Reduced / absent corpora lutea in the ovaries was observed in the majority of females given 1000 mg/kg/day and in 6 females given 300 mg/kg/day. This change was accompanied by a shift towards the proportion of females that showed oestrus or metoestrus morphological characteristics in the vaginal epithelium as shown in the Table below.

Summary of treatment related findings in the ovaries and vagina for females killed after 13 weeks of treatment

Group/sex Dose (mg/kg/day)	1F 0	2F 100	3F 300	4F 1000
Ovaries				
Reduced /Absent Corpora Lutea				
Presence	0	0	6	10
Total	0	0	6	10
Number of animals examined	12	12	12	12
Vagina (Cycle Staging)				
Proestrus	7	5	2	1
Oestrus	2	1	7	5
Metoestrus	2	6	3	6
Dioestrus	1	0	0	0
Number of animals examined	12	12	12	12

Special Evaluation

None

Toxicokinetics

As shown in Table below, maximum mean plasma concentrations (Cmax) of S-297995 on Day 91 were 21.2 µg/mL for males and 20.6 µg/mL for females at 100 mg/kg/day. S-297995 exposure $AUC_{0\text{-}24}$ was 194 µg•hr/mL for males and 165 µg•hr/mL for females at 100 mg/kg/day.

Dose level (mg/kg/day)	C_{max} (µg/mL) Day 1 Males	Day 1 Females	Day 28 Males	Day 28 Females	Day 91 Males	Day 91 Females
100	26.4	23.7	26.6	31.9	21.2	20.6
300	41.5	50.8	38.0	40.3	43.8	46.6
1000	67.7	72.3	87.6	97.7	78.6	70.8

Dose level (mg/kg/day)	AUC_{0-24h} (µg.h/mL) Day 1 Males	Day 1 Females	Day 28 Males	Day 28 Females	Day 91 Males	Day 91 Females
100	111	132	154	170	194	165
300	270	291	388	332	475	362
1000	590	611	1220	1410	787	865

The AUC_{0-24} values of three metabolites Benzamidine, S-297995 3-O-beta-D-glucuronide and S-297995 6-O-beta-D-glucuronide on Day 91 were less than 2% of parent S-297995 for both males and females. AUC_{0-24} for Nor-S-297995, a major metabolite of S-297995, was 8.7% and 16.2% of the parent compound for females and males, respectively. Thus, the mouse metabolic profiles were similar to those observed in human study where Nor-S-297995 was the primary metabolite found in humans and was detected at more than 10% of unchanged S-297995 in humans.

Rat

Review of Study No. R-297995-TB-003-R from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Study title: Preliminary Two-Week Oral Toxicity Study of RSC-297995 monotosylate in Rats

Study no.:	R-297995-TB-003-R
Study report location:	Electronic submission
Conducting laboratory and location:	Developmental Research Laboratories, Shionogi & Co., Ltd. Osaka, Japan
Date of study initiation:	July 4, 2007
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	RSC-297995 monotosylate, Lot No. A81001, 97.5% purity

Key Study Findings

This was a non-GLP study. There were no deaths during the study. Significant decrease in body weight was noted in a dose-dependent manner in 200 and 1000 mg/kg groups of both sexes on Day 2. Slight to severe decrease in food consumption was observed on Day 3 in males and females of all treated groups. Slightly lower mean values in food consumption continued from Days 3 to 13 in males of the 1000 mg/kg group, but sporadically in other groups. No test substance-related changes in hematology, clinical chemistry, organ weight, gross pathology or histopathology were noted except for decreased colloid in the thyroid and hypertrophy of pale granular cell in anterior pituitary in males of the 200 and 1000 mg/kg groups. The no observed

adverse effect level (NOAEL) was considered to be 40 mg/kg/day. No target organ of toxicity was identified.

Methods

Doses:	S-297995 monotosylate was given at 0 (vehicle control), 8, 40, 200, and 1000 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	By oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	Methylcellulose aqueous solution (0.5 w/v% MC) was used as the vehicle
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	4 animals/sex/group
Age:	6 weeks old
Weight:	191.5 to 217.8 g for males and 140.3 to 162.9 g for females
Satellite groups:	3 animals/sex/group (control and 1000 mg/kg/day) (TK study)
Unique study design:	None
Deviation from study protocol:	Not specified

Review of Study No. R-297995-TB-048-L from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Study title:	One-Month Oral Toxicity Study of RSC-297995 monotosylate in Rats
Study no.:	R-297995-TB-048-L
Study report location:	Electronic submission
Conducting laboratory and location:	Developmental Research Laboratories, Shionogi & Co., Ltd. Japan
Date of study initiation:	March 11, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RSC-297995 monotosylate, Lot No. A81001, 97.5% purity

Key Study Findings

There were no deaths throughout the study. Slight to severe decrease in food consumption were noted in a dose-dependent manner in all treatment groups of both sexes on Day 2. Prolongation of diestrus, lasting maximally 18 days, was observed in females of all dosing groups. However, the abnormal cycle recovered in almost all cases even in the administration period, and there were no histopathological lesions in functionally associated organs such as ovary, uterus, and mammary gland. No test article-related changes were observed

in ophthalmoscopy, hematology and histopathology. The no observed adverse effect level (NOAEL) was considered to be 1000 mg/kg/day. No target organ of toxicity was identified.

Methods

Doses:	S-297995 monotosylate was given at 0 (vehicle control), 30, 100, and 1000 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	By gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	Methylcellulose aqueous solution (0.5 w/v% MC) was prepared as vehicle
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	10 animals/sex/group (main study), 4 animals/sex/group in TK study
Age:	6 weeks
Weight:	195.2 to 218.9 g for males and 137.6 to 174.4 g for females
Satellite groups:	5 animals/sex/group (control and 1000 mg/kg/day) (recovery)
Unique study design:	None
Deviation from study protocol:	8 minor deviations were reported which did not affect the study outcome of the study.

Observations and Results

Mortality

There were no deaths during the study.

Clinical Signs

Three males and two females of the 1000 mg/kg/day group showed salivation just before or after dosing sporadically during the administration period, although it disappeared soon. Other clinical signs were loss of fur and/or crust in back of neck in two males and one female of the 1000 mg/kg/day group. Prolongation of diestrus, lasting maximally 18 days, was observed in females of all dosing groups. However, the abnormal cycle recovered in almost all cases even in the administration period, and there were no histopathological lesions in functionally associated organs such as ovary, uterus, and mammary gland. There was no clinical sign in any animals through the recovery study.

Body Weights

In agreement with the food consumption changes, body weight slightly decreased in all treatment groups of both sexes on Day 2 (-4%, -7%, and -11% in males and -3, -5, and -7% in females at 30, 100, and 1000 mg/kg/day groups, respectively). The body weight turned to

increase from Day 4 onward, but the mean body weight values in males were still lower than control group throughout the administration period in all groups. No clear effects were seen in female body weight from Day 7 onward as shown in the Figure below. From these results, the body weight changes were understood secondary to decreased food consumption. However, the differences in mean final body weight to the control group in males on Day 31 remained slight (-5%, -10%, and -9% at 30, 100, and 1000 mg/kg/day groups, respectively).

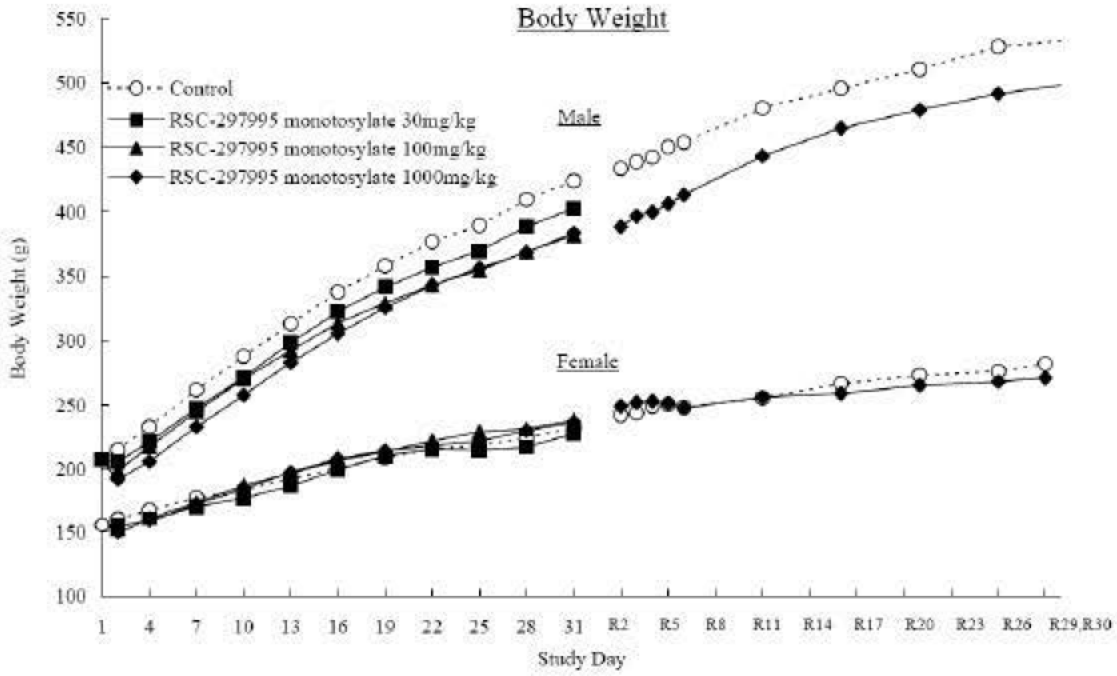

Figure 1-1 One-Month Oral Toxicity Study of RSC-297995 monotosylate in Rats (R-297995-TB-048-L)

Feed Consumption

Slight to severe decrease in food consumption were noted in a dose-dependent manner in all treatment groups of both sexes on Day 2. In males, slightly lower food consumption continued throughout the administration period in all treatment groups. No clear effects in food consumption were seen in females from Day 7 onward.

Ophthalmoscopy

No test article-related changes were observed in ophthalmoscopy.

ECG

N/A

Hematology

Blood samples were collected at scheduled necropsy one day after the final administration (Day 31) and the final day of recovery period. No test article-related changes were observed in hematology.

Clinical Chemistry

Only noticeable clinical chemistry change was the dose-dependent decrease in Triglyceride (TG) observed in males of the 10, 100 and 1000 mg/kg/day group (-23%, -46% and -60%, respectively) as compared with control group. But the statistically significant decrease in TG was observed in males of the 100 and 1000 mg/kg/day group ($P<0.05$ and $P<0.01$, respectively).

Urinalysis

In the 1000 mg/kg/day group, there were statistically significant decreases in urine volume (2.0±0.6 ml vs. 3.6±1.3 ml for controls) accompanied with increased specific gravity (1.053±0.013 vs. 1.027±0.008) and increase in protein positive case in males. In female in the 1000 mg/kg/day, increase of protein positive case was noted but there were not statistically significant. No remarkable changes were observed in the recovery period.

Gross Pathology

There were no test substance-related findings in any dosing animals. Dark red color in the liver and spleen, dark red and enlarged kidney were noted in one male (Animal No. 4102) of the 1000 mg/kg/day group because of a death under anesthesia.

Organ Weights

In the 1000 mg/kg/day group, there were statistically significant increases in absolute (18.288±2.143 g vs. 16.552±1.542 g of controls for the males, 9.226±1.037 g vs. 7.9±1.3 g of controls for the females) and relative weight (4.72±0.34% vs. 3.94±0.27% of controls for the males, 3.93±0.27% vs. 3.48±0.23% of controls for the females) of liver in both sexes and pituitary (6.14±1.09% vs. 5.15±0.58% of controls) in females. These may not considered to be toxicologically significant, because no abnormal changes were observed in blood chemistry and/or histopathology in the 1000 mg/kg/day group.

Histopathology

Adequate Battery

Yes

Peer Review

Yes

Histological Findings

No test article-related changes were observed in histology including the liver and female reproductive organs at the administration and the recovery periods.

Special Evaluation

Prolongation of estrus cycles (duration of estrus and following estrus were over 5 days) were observed in 5/10, 6/10 and 8/15 females of the 30, 100 and 1000 mg/kg/day groups, respectively in the administration period. But these changes did not continue and disappeared within 18 days even in the administration period. During the recovery period, the prolongation of estrus cycles of 2/5 females in 1000 mg/kg/day group were continued from administration period, but these was recovered at early stage (Day R6 or R7). Another prolongation of estrus cycles were observed in one female of Control and 1000 mg/kg/day groups.

Toxicokinetics

On day 1, the mean Cmax values of male animals in the 30, 100, and 1000 mg/kg/day groups were 3.17, 10.78, and 32.03 µg/mL, respectively. The corresponding $AUC_{0\text{-}24hr}$ values were 7.84, 36.27, and 281.18 µg·hr/mL, and the Tmax values were 0.5, 0.6, and 2.0 hours, respectively. On day 30, the mean Cmax values in the 30, 100, and 1000 mg/kg/day groups were 3.71, 12.53, and 48.68 µg/mL, respectively. The corresponding $AUC_{0\text{-}24hr}$ values were 11.32, 61.79, and 524.43 µg·hr/mL, and the Tmax values were 0.5, 1.8, and 3.0 hours, respectively, as shown in the Table below.

Male:

Day	Dose level mg/kg/day		$AUC_{0\text{-}24hr}$ µg·hr/mL	Cmax µg/mL	Tmax hr	C_{24hr} µg/mL
1	30	Mean	7.84	3.17	0.5	NE
		SD	0.63	0.69	0.0	NE
		N	4	4	4	4
	100	Mean	36.27	10.78	0.6	NE
		SD	3.46	1.84	0.3	NE
		N	4	4	4	4
	1000	Mean	281.18	32.03	2.0	2.76
		SD	69.52	10.04	0.0	2.11
		N	4	4	4	4
30	30	Mean	11.32	3.71	0.5	NE
		SD	1.25	0.46	0.0	NE
		N	4	4	4	4
	100	Mean	61.79	12.53	1.8	NE
		SD	17.70	2.60	0.5	NE
		N	4	4	4	4
	1000	Mean	524.43	48.68	3.0	1.82
		SD	169.92	14.06	1.2	3.28
		N	4	4	4	4

On Day 1, the mean Cmax values of female animals in the 30, 100, and 1000 mg/kg/day groups were 3.73, 12.85, and 28.20 µg/mL, respectively. The corresponding $AUC_{0\text{-}24hr}$ values were 9.22, 41.97, and 204.14 µg·hr/mL, and the Tmax values were 0.5, 0.9, and 2.0 hours, respectively as shown in the Table below. On Day 30, the mean Cmax values in the 30, 100, and 1000 mg/kg/day groups were 4.26, 10.58, and 42.38 µg/mL, respectively. The corresponding $AUC_{0\text{-}24hr}$ values were 10.60, 36.44, and 286.63 µg·hr/mL, and the Tmax values were 0.5, 0.9, and 2.0 hours, respectively.

Female:

Day	Dose level mg/kg/day		$AUC_{0\text{-}24hr}$ µg·hr/mL	Cmax µg/mL	Tmax hr	C_{24hr} µg/mL
1	30	Mean	9.22	3.73	0.5	NE
		SD	0.42	0.94	0.0	NE
		N	4	4	4	4
	100	Mean	41.97	12.85	0.9	NE
		SD	3.18	2.55	0.3	NE
		N	4	4	4	4
	1000	Mean	204.14	28.20	2.0	3.67
		SD	34.64	3.46	0.0	4.14
		N	4	4	4	4
30	30	Mean	10.60	4.26	0.5	NE
		SD	1.33	0.64	0.0	NE
		N	4	4	4	4
	100	Mean	36.44	10.58	0.9	NE
		SD	6.68	1.19	0.3	NE
		N	4	4	4	4
	1000	Mean	286.63	42.38	2.0	0.14
		SD	80.63	5.66	0.0	0.08
		N	4	4	4	4

Review of Study No. R-297995-TB-091-L from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Study title: One-Month Oral Toxicity Study of RSC-297995 monotosylate in Rats (Supplement)

Study no.:	R-297995-TB-091-L
Study report location:	Electronic submission
Conducting laboratory and location:	Developmental Research Laboratories, Shionogi & Co., Ltd. Japan
Date of study initiation:	June 26, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RSC-297995 monotosylate, Lot No. A81001, 97.5% purity

Key Study Findings

The objective of the present study was to determine doses at which there was no prolongation of estrus cycles seen in the previous one-month study; this study focused on estrus cycle observation in female animals given lower doses of the test substance for one month.

Increased incidences of prolongation of diestrus (including proestrus) was observed for maximally 11 days during the administration period, and the incidences were 3/10, 1/10, 4/10, and 3/10 animals in the 0.3, 1, 3, and 10 mg/kg/day groups, respectively. Although the observed changes were not clearly dose-related and the toxicokinetics analyses showed minimum but dose-dependent exposure except 0.3 mg/kg/day group, it was considered that such higher incidences were not the case for control animals, and thus they were considered test substance-related. It was also considered that the test substance had weak effects on the estrus cycle regulation at all doses tested, since it did not continue throughout the dosing period. The no-observed-effect level (NOEL) was not able to be determined.

Methods

Doses:	S-297995 monotosylate was given at 0 (vehicle control), 0.3, 1, 3 and 10 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	By gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	Methylcellulose aqueous solution (0.5 w/v% MC) was prepared as vehicle
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	10 females/group (main study)
Age:	6 weeks
Weight:	137.6 to 174.4 g
Satellite groups:	5 animals/female/group (TK study)
Unique study design:	None
Deviation from study protocol:	8 minor deviations were reported.

Observations and Results

Mortality

There were no deaths during the study.

Clinical Signs

There was no clinical sign in any animals through the study.

Body Weights

There was no body weight change in any animals through the study.

Special Evaluation

Prolongation of estrus cycles (duration of estrus cycle was over 5 days) was observed in 1/10, 3/10, 1/10, 4/10, and 3/10 females of the control, 0.3, 1, 3, and 10 mg/kg/day groups, respectively. Almost all these changes did not continue for a long time and the diestrus (including proestrus) lasted only maximally 11 days even in the administration period. Although there was no clear dose relationship, the sponsor considered that the observed incidences in the RSC-297995 dosing groups were not the case in control animals, and thus they were test substance-related.

Toxicokinetics

The mean Cmax and $AUC_{0\text{-}24hr}$ values of RSC-297995 increased approximately dose-proportionally between the 1 and 10 mg/kg/day groups, but those in the 0.3 mg/kg/day group were not able to be determined since most of the collected samples concentrations were below the lower limit of quantification (0.02 µg/mL). Three metabolites, Nor-RSC-297995, RSC-297995 3-*O*-β-D-glucuronide, and RSC-297995 6-*O*-β-D-glucuronide were also detected, but the mean Cmax and AUC values at 0.3, 1, and 3 mg/kg/day were not able to be calculated because of the shortage of plasma concentrations satisfying the lower limit of quantification. In the 10 mg/kg/day group, percentages of Nor-RSC-297995, RSC-297995 3-*O*-β-D-glucuronide, and RSC-297995 6-*O*-β-D-glucuronide to those of RSC-297995 were 1%, 2.6%, and 0.1%, respectively on Day 30.

Review of Study No. R-297995-TF-108-L (CRO Study No. SG08274) from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Study title:	Six-Month Oral Toxicity Study of RSC-297995 monotosylate in Rats
Study no.:	R-297995-TF-108-L
Study report location:	Electronic submission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Oct. 20, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RSC-297995 monotosylate, Lot No. A81001, 97.5% purity

Key Study Findings

The 6-month administration of RSC-297995 monotosylate in rats caused consistent suppression of body weight gain throughout the dosing period in males and female of the 1000 mg/kg group. This body weight change was not directly associated with the food consumption changes, and no other adverse effects were observed in any parameters examined. The animals of the high-dose group (1000 mg/kg) also showed abnormal eating behavior, but no drug-withdrawal signs were seen during the recovery period. There were test article-related findings in hematology, blood chemistry and organ weight, but they were minimal or secondary to the body weight change or lack of histopathological correlate. The suppressed body weight tended to recover during the recovery period. Under the conditions of this study, the no observed adverse effect level (NOAEL) of RSC-297995 was considered to be 100 mg/kg/day for both males and females.

Methods

Doses:	S-297995 monotosylate was given at 0 (vehicle control), 10, 100, and 1000 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	By gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	Methylcellulose aqueous solution (0.5 w/v% MC) was prepared as vehicle
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	12 animals/sex/group (main study), 6 animals/sex/group (control and 1000 mg/kg/day; recovery)
Age:	6 weeks
Weight:	195.2 to 218.9 g for males and 137.6 to 174.4 g for females
Satellite groups:	6 animals/sex/group (TK study)
Unique study design:	None
Deviation from study protocol:	5 minor deviations were reported.

Observations and Results

Mortality

There were no deaths during the study.

Clinical Signs

Salivation was observed for 1 of the 12 males in the 100 mg/kg group and for 14 of the 18 males and 4 of the 18 females in the 1000 mg/kg group at 1 hour post-dosing during Days 9-176 of dosing. Of these animals, 4 males in the 1000 mg/kg group exhibited increased amounts of spilt feed during Days 24-160 of dosing. Soiled fur (yellowish brown) was observed in 2 males and 5 females in the 1000 mg/kg group from the periurethral orifice or gluteal region to the base of the tail or in the caudal abdominal region between Day 3 of dosing and the day of terminal necropsy. Soiled fur (yellowish brown) from the gluteal region to the base of the tail was still observed in 1 of these females on Days 1-28 of recovery.

Body Weights

During the dosing period, the body weight increased with time in all treatment groups. However, slight but statistically significant lower body weight values (−11% and −13% in males and females as shown in the Fig 1 and Fig 2 below, respectively, on Day 182, against the control means) were noted in males in the 1000 mg/kg group from Day 7 of dosing and in females in this group from Day 35 of dosing as compared to the control group. During the recovery period,

statistically significant lower values were still noted in males in the 1000 mg/kg group from Day 1 of recovery. However, body weight gains in males and females in this group were greater than the control group and their body weights.

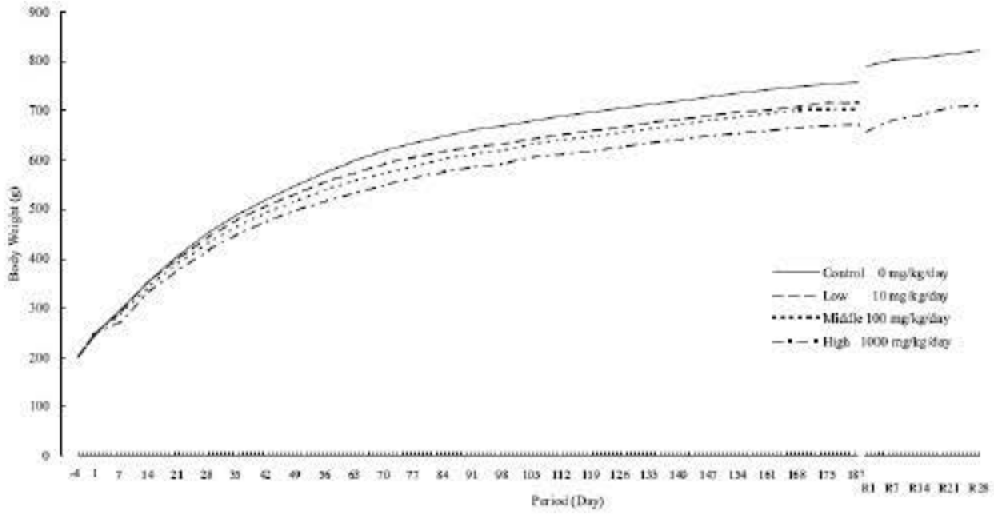

Fig. 1 Six-Month Oral Toxicity Study of RSC-297995 monotosylate in Rats
Body Weight (Male)

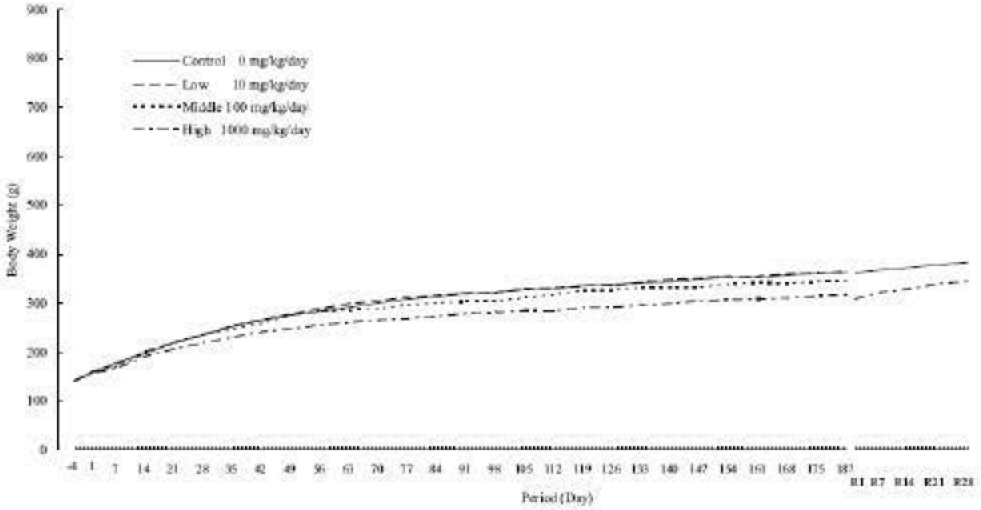

Fig. 2 Six-Month Oral Toxicity Study of RSC-297995 monotosylate in Rats
Body Weight (Female)

Feed Consumption

During the dosing period, statistically significant decreases in food consumption were noted in males and females in the 10, 100 and 1000 mg/kg groups on Days 7 (-7.3%, -13.6% and

-25.6%, respectively for males, -8.8%, -16% and -22.7%, respectively for females) and/or Days 14 (-8.2% for male 100 mg group and -8% for male 1000 mg group) of dosing as compared to the control group. During the recovery period, a statistically significant decrease in food consumption in males on Day 28 of recovery and statistically significant increases in females on Days 7-21 of recovery were noted in the 1000 mg/kg group as compared to the control group. However, these changes were slight and were therefore considered not to be treatment-related.

Ophthalmoscopy

No test article-related changes were observed in ophthalmoscopy.

ECG

Not conducted

Hematology

At termination of the dosing period, the following statistically significant changes were noted in males as compared to the control group: dose-dependent prolongation of PT in the 100 mg/kg group (14.7±4.1 sec.) and 1000 mg/kg groups (18.6±6.5 sec.) vs. controls (10.9±2.2 sec.) and prolongation of APTT in the 1000 mg/kg group (22.0±1.2 sec. vs. 20.2±1.3 sec. of controls). These changes may not be toxicologically significant since these were slight changes within or slightly exceeding the ranges of historical control data and hemorrhage or thrombus formation was not observed at necropsy or histopathology. At termination of the recovery period, the following statistically significant changes were noted in females in the 1000 mg/kg group as compared to the control group: decreases in the red blood cell count ($7.44\pm0.45x10^6/\mu l$ vs. $8.02\pm0.32x10^6/\mu l$ of controls), hemoglobin concentration (13.7±0.5 g/dL vs. 15.1±0.3 g/dL of controls) and hematocrit (40.4±2.1% vs. 44.4±1.1% of controls) and shortening of PT (7.0±0.2 sec. vs. 7.4±0.3 sec. of controls). However, these changes were considered not to be treatment-related since they were slight or were within the ranges of historical control data.

Clinical Chemistry

At termination of the dosing period, statistically significant increases were noted in total cholesterol in males (89±14 mg/dL vs. 72±18 mg/dL of controls) and females and in amylase and phospholipids in females in the 1000 mg/kg group as compared to the control group. At termination of the recovery period, the following statistically significant changes were noted in the 1000 mg/kg group as compared to the control group: increases in total protein and albumin in males and a decrease in lactate dehydrogenase (LD) and an increase in glucose in females.

Urinalysis

No treatment-related urinary findings were noted in any animal at termination of the dosing or recovery period.

Gross Pathology

No treatment-related gross pathological lesions were observed in any animal at termination of the dosing or recovery period except for the sporadic incidence, such as, callosity

on the footpads of the hindlimbs (an effect of long-term housing) was observed in 1 male and 1 female in the control group and in 2 males in the 1000 mg/kg group.

Organ Weights

At termination of the dosing period: Statistically significant decreases or a tendency to decrease in the absolute and relative weights of the thymus was recorded in females in the 100 mg/kg group (158±40 mg vs. 202±24 mg for controls) and in males and females in the 1000 mg/kg group (143±36 mg vs. 192±48 mg for controls in females and 147±37 mg vs. 202±24 mg for controls in females) At termination of the recovery period: A tendency of decreases in the absolute (117±33 mg vs. 176±81 mg of controls) and relative (36.8±7.8 mg% vs. 47.5±15.5 mg% of controls) weights of the thymus in females was noted in the 1000 mg/kg group. These changes were not accompanied by the histopathological lesions.

Histopathology

Adequate Battery

Yes

Peer Review

Yes

Histological Findings

No treatment-related histopathological lesions were observed in any animal at termination of the dosing or recovery period. At termination of the dosing period: some of gross pathological lesions were histopathologically identified as follows: dark reddish spots on the lungs (Animal No. 1M01) were focal hemorrhage and inflammatory cell infiltration, callosity on the footpads of the hindlimbs (Animal Nos. 1M06, 4M01, 4M05 and 1F02) was moderate pododermatitis. At termination of the recovery period: some gross pathological lesions were histopathologically identified as follows: callosity on the footpads of the hindlimbs (Animal Nos. 1M14 and 4M14) was moderate pododermatitis, large part of the pituitary (Animal No. 4F15) was hyperplasia in the pars distalis of the pituitary.

Special Evaluation

None

Toxicokinetics

Maximum plasma concentrations (Cmax) in 10 to 1000 mg/kg/day dose groups on Days 1, 91 and 182 were increased with dose as shown in the Table below, but the rate of increase was less than the dose ratio. No large difference of Cmax was observed in sex. Areas under the plasma concentration-time curve from 0 to 24 hours ($AUC_{0\text{-}24h}$) in 10 to 1000 mg/kg/day dose groups on Days 1, 91 and 182 were increased with dose, but the rate of increase was less than the

dose ratio. AUC0-24h value of the female tended to be less than that of the male on Day 1 (AUC_{0-24h}: 0.5- to 0.7-fold). On Day 91 of dosing, AUC_{0-24h} value of the female tended to be less than that of the male at 100 mg/kg/day (AUC_{0-24h}: 0.6-fold). On Day 182 of dosing, no large difference of AUC0-24h was observed in sex. Cmax and AUC0-24h values on Day 182 tended to be relatively higher than those on Day 1 (Cmax: 1.2- to 2.7-fold and AUC_{0-24h}: 1.5- to 3.3-fold). Tmax of Day 182 was similar to these of Day 1. Cmax, AUC_{0-24h} and Tmax of Day 182 were similar to those of Day 91, respectively.

	Male			Female		
Dose (mg/kg)	**10**	**100**	**1000**	**10**	**100**	**1000**
Number of animals	**4**	**4**	**4**	**4**	**4**	**4**
Mean C_{max} (μg/mL)						
Day 1	0.76	8.33	25.3	0.99	10.1	22.7
Day 91	2.03	12.5	43.7	3.53	20.1	48.8
Day 182	1.93	10.2	31.0	2.71	18.7	47.8
Mean AUC_{0-24hr} (μg·hr/mL)						
Day 1	3.43	35.9	288	2.26	23.8	145
Day 91	6.56	95.9	561	6.91	58.4	513
Day 182	6.51	67.9	420	7.36	61.5	453
Mean t_{max} (hr)						
Day 1	0.5	0.8	4.5	0.6	0.9	1.3
Day 91	0.6	1.5	4.0	0.5	0.8	1.8
Day 182	0.6	2.5	5.5	0.6	1.0	2.0

Dog

Review of Study No. R-297995-TB-002-R from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Study title: Preliminary Two-week Oral Toxicity Study of RSC-297995 monotosylate in Dogs

Study no.:	R-297995-TB-002-R
Study report location:	Electronic submission
Conducting laboratory and location:	Developmental Research Laboratories, Shionogi & Co., Ltd. Japan
Date of study initiation:	July 3, 2007
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	RSC-297995 monotosylate, Lot No. A81001, 97.5% purity

Key Study Findings

This is a non-GLP preliminary 2-week toxicity study in dogs (1 animal/sex/group). Main clinical findings in the study were increased incidences of vomitus observed in a male and a female of the highest dose (100 mg/kg) group. No test substance-related changes were observed in body weight, food consumption, electrocardiography, urinalysis, hematology, bone marrow analysis (at necropsy). Increases in ALP and ALT activities were observed in a male and a female of the 100 mg/kg group. The absolute and relative weights of the liver were greater in females of the 3 and 10 mg/kg group, and the relative liver weight was greater in a female of the 100 mg/kg

group than the control. There were some histopathologic changes in the liver in the high dose group.

Methods

Doses:	S-297995 monotosylate was given at 0 (vehicle control), 3, 10 and 100 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	By gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	Methylcellulose aqueous solution (0.5 w/v% MC) was used as the vehicle
Species/Strain:	Beagle dog
Number/Sex/Group:	1 animals/sex/group
Age:	8 months
Weight:	9.6 to 10.5 kg for males and 8.85 to 10.30 kg for females
Satellite groups:	none
Unique study design:	None
Deviation from study protocol:	Not specified

This was a preliminary toxicity study with limited number of animals.and all toxicology parameters were not evaluated.

Review of Study No. R-297995-TB-046-L from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Study title: One-Month Oral Toxicity Study of RSC-297995 monotosylate in Dogs

Study no.:	R-297995-TB-046-L
Study report location:	Electronic submission
Conducting laboratory and location:	Developmental Research Laboratories, Shionogi & Co., Ltd. Japan
Date of study initiation:	March 7, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RSC-297995 monotosylate, Lot No. A81001, 97.5% purity

Key Study Findings

The toxicologically significant changes of RSC-297995 monotosylate were vomiting and hepatotoxicity observed in the 50 mg/kg group. No observed adverse effect level (NOAEL) is estimated as 10 mg/kg/day under the conditions of the present study. The liver is a target organ of toxicity.

Methods

Doses:	S-297995 monotosylate was given at 0 (vehicle control), 1, 3, 10 and 50 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	By gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	Methylcellulose aqueous solution (0.5 w/v% MC) was prepared as vehicle
Species/Strain:	Beagle dog
Number/Sex/Group:	5 animals/sex/group (main study)
Age:	9 months
Weight:	9.0 to 10.8 kg for males and 8.25 to 10.35 kg for females
Satellite groups:	2 animals/sex/group (control and 50 mg/kg/day) (recovery)
Unique study design:	None
Deviation from study protocol:	15 minor deviations were reported.

Observations and Results

Mortality

There were neither deaths nor moribund states throughout the study.

Clinical Signs

Vomiting/vomitus and salivation, were observed in both male and female animals of the 50 mg/kg group throughout the administration period. The frequency was increased maximally 7 days in males and 8 days in females for 30 dosing days, meanwhile 1 or 2 days in the control and 10 mg/kg or lower dose groups in most cases. These changes were not observed in the recovery period.

Body Weights

Initial mean body weights for control males and females were 10.0 kg and 9.19 kg, respectively. At week 4, mean body weight for males and females were 10.13 kg and 9.28 kg, respectively. There were no test substance-related changes in the body weight throughout the study.

Feed Consumption

There were no test substance-related changes in food consumption throughout the study.

Ophthalmoscopy

There were no test substance-related changes in ophthalmoscopic findings throughout the study.

ECG

There were no test substance-related changes in electrocardiographic findings throughout the study. On Day 16, first-degree atrioventicular heart block was observed in one male (animal No. 2102) of the 1 mg/kg group; however, this event was not dose-related. On Day 28, bradycardia was observed in one female (animal No. 5202) of the 50 mg/kg group; however, the heart rate of this animal during the acclimation period was rather low comparing with the one usually observed in normal animals.

Hematology

Females of the 50 mg/kg group showed low values of RBC (6.04±0.59 $x10^6$/μL vs. 7.05±0.46 $x10^6$/μL of controls), HGB (14±1.3 vs. 16.5±0.9 g/dL of controls), and HCT (39.9±3.6% vs. 47.4±2.2% of controls) on Day 28 in comparison with the acclimation period. These values were approximately 20% lower than the pre-dosing values in each animal. In males, similar changes were observed in HGB (14±0.9 vs. 15.4±0.6 g/dL of controls), and HCT (40.2±2.7% vs. 44.1±1.6% of controls on Day 28).

Clinical Chemistry

Increased ALT (114±29 vs. 35±4 U/L of controls on day 10 and 161±161 vs. 37±8 U/L of controls on day 28 for males; 80±31 vs. 32±5 U/L of controls on day 10 and 77±27 vs. 34±7 U/L of controls on day 28 for females) and ALP (555±87 vs. 298±59 U/L of controls on day 10 and 1061±622 vs. 285±58 U/L of controls on day 28 for males; 341±74 vs. 287±61 U/L of controls on day 10 and 692±205 vs. 286±63 U/L of controls on day 28 for females) were observed in both males and females of the 50 mg/kg group on Days 10 and 28. The mean values of ALT and ALP were approximately 2 to 4 times higher than those in the control group. These changes were statistically significant, except for ALT in males on Day 28 and ALP in females on Day 10. Some of these animals also showed increased AST but not biologically meaningful, and the increased total cholesterol (T.Cho) in both males (179±33 vs. 119±20 mg/dL of controls) and females (203±30 vs. 138±8 mg/dL of controls) on Day 28 and were statistically significant. These statistically significant changes were dose-dependent as indicated by the linear trend test suggesting the liver toxicity. Increased ALT, ALP, or T.Cho were also observed in a few animals of the 3 and 10 mg/kg groups. The extent of these changes was slighter than that in the 50 mg/kg group.
These changes ameliorated or disappeared during the recovery period.

Urinalysis

There were no test substance-related changes in urinalysis data throughout the study.

Gross Pathology

Abnormal findings in animals necropsied at the end of the administration period included: Enlargement of the liver in 1/3 male of the 10 mg/kg group; small in size of the thymus in 1/3 male and 1/3 female of the 1 mg/kg group and in 2/3 females of the 50 mg/kg group.

Organ Weights

There were no test substance-related changes in absolute and relative weights of any organs.

Histopathology

Adequate Battery

Yes

Peer Review

Yes

Histological Findings

The test substance-related changes were observed in the liver and spleen in males or females of the 50 mg/kg group. Single cell necrosis of hepatocyte was observed in all males and females of the 50 mg/kg group at the end of the administration period, and the lesion was associated with inflammatory cell infiltration in 2/3 males. The severity of lesions was mild in all cases. Mild increase in hemosiderin deposition was observed in Kupffer cells of the liver and in the spleen of 1/3 female of the 50 mg/kg group at the end of the administration period. The increase in hemosiderin deposition was also observed at the end of the recovery period in the spleen of 1/2 female in the 50 mg/kg group. Mild or moderate decrease of lymphocyte in the thymus cortex was observed in several animals of the RSC-297995 monotosylate dosing groups at the end of the administration or recovery periods, in addition with the reduction in weight and small in size (See section 10.12) of thymus. However, the lesion was also seen in the control group, and the pathological changes in thymus were not considered test substance-related.

Table. Incidence of Histopathologic Observation

Tissues with Diagnoses	MALES			FEMALES		
	Control	10 mg/kg	50 mg/kg	Control	10 mg/kg	50 mg/kg
Liver						
Cellular infiltration	0/3	0/3	2/3	1/3	0/3	1/3
Single cell necrosis	0/3	0/3	3/3	0/3	0/3	3/3
hemosiderin deposition (Kupffer cells)	0/3		0/3	0/3		1/3
Spleen						
hemosiderin deposition	0/3		0/3	0/3		1/3
Thymus						
Cortex, decreased lymphocyte	0/3	1/3	1/3	1/3	0/3	3/3

Special Evaluation

There were no test substance-related changes in myelogram.

Toxicokinetics

The $AUC_{0\text{-}24hr}$ and C_{max} of S-297995 increased with nearly dose proportional manner in general as shown in the table below. The mean t_{max} and C_{24hr} values suggested relatively rapid absorption and excretion of this compound in dogs. There were no obvious repeated dosing effects and sexual differences. Three metabolites, Nor-S-297995, S-297995 3-G, and S-297995 6-G were also detected, accounting for below 5%, below 3%, and below 1% at $AUC_{0\text{-}24hr}$ basis compared with S-297995, respectively on Day 30, suggesting minor contribution to the toxicity.

[S-297995]

	Male				Female			
Dose (mg/kg)	**1**	**3**	**10**	**50**	**1**	**3**	**10**	**50**
Number of animals	**3**	**3**	**3**	**5**	**3**	**3**	**3**	**5**
Mean C_{max} (μg/mL)								
Day 1	0.65	1.92	5.78	31.04	0.64	1.80	6.78	21.16
Day 9	0.64	2.44	7.18	19.80	0.71	1.90	5.57	20.72
Day 30	0.52	2.29	6.48	20.12	0.57	2.04	7.47	22.24
Mean C_{24hr} (μg/mL)								
Day 1	–	–	–	0.03	–	–	–	0.04
Day 9	–	–	–	0.07	–	–	–	0.05
Day 30	–	–	–	0.15	–	–	–	0.11
Mean $AUC_{0\text{-}24hr}$ (μg·hr/mL)								
Day 1	1.01	3.63	18.40	93.86	1.02	2.81	15.37	98.29
Day 9	1.04	4.58	24.51	121.57	1.07	3.28	14.41	103.86
Day 30	1.26	5.03	23.95	142.80	1.27	4.24	19.60	130.09
Mean t_{max} (hr)								
Day 1	0.3	0.4	0.5	1.3	0.4	0.4	0.4	0.9
Day 9	0.3	0.4	0.7	1.4	0.4	0.4	0.4	1.0
Day 30	0.6	0.5	0.6	1.1	0.5	0.4	0.7	1.1

Review of Study No. R-297995-TF-109-L (CRO Study No. SG08275) from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Study title:	Three-Month Oral Toxicity Study of RSC-297995 monotosylate in Dogs
Study no.:	S-297995-TF-109-L
Study report location:	Electronic submission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 8, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RSC-297995 monotosylate, Lot No. A81001, 97.5% purity

Key Study Findings

Slight single cell necrosis in the liver and adipose tissues atrophy were observed at 30 mg/kg/day as target organ toxicities in addition to some in-life observations. Favorable recovery was confirmed after the 1-month drug withdrawal. Therefore, the NOAEL was estimated 5 mg/kg/day under the condition of the present study.

Methods

Doses:	S-297995 monotosylate was given at 0 (vehicle control), 1, 5 and 30 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	By gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	Methylcellulose aqueous solution (0.5 w/v% MC)
Species/Strain:	Beagle dog
Number/Sex/Group:	4 animals/sex/group (main study)
Age:	10-12 months
Weight:	9.88 to 12.88 kg for males and 9.40 to 12.18 kg for females
Satellite groups:	3 animals/sex/group (control and 30 mg/kg/day) (recovery)
Unique study design:	None
Deviation from study protocol:	15 minor deviations were reported.

Observations and Results

Mortality

All animals survived during the study.

Clinical Signs

Vomitus was observed for 1 of the 7 females in the control group, 1 of the 4 animals/sex in the 1 mg/kg group, 3 of the 4 males and 2 of the 4 females in the 5 mg/kg group and all 7

animals/sex in the 30 mg/kg group during the dosing period as shown in the Table below. The incidence of vomitus was comparable to that commonly noted in beagle dogs in many of these animals, but was slightly higher in 1 male in the 5 mg/kg group and 4 animals/sex in the 30 mg/kg group, suggesting effects of the test article. However, the total incidences of vomitus (1-12) were still of small values as compared to total incidences of observations (273), and in most cases there were no effects on body weights or food consumption in these animals, excluding 1 female (Animal No. 74) in the 30 mg/kg group. Therefore, this finding was considered to be of no toxicological significance at least at 5 mg/kg. Salivation was observed for 4 males and 3 females in the 30 mg/kg group immediately prior to or after dosing or at 1 hour post-dosing from Day 37 of dosing. In addition, scant feces were observed in 1 female in this group on Days 48 and 49 of dosing. No marked clinical signs were observed in any animal during the recovery period.

Table. Individual total incidences of vomitus / total incidences of observations during 3-month dosing period

Dose (mg/kg/day)	Male	Female
Control	0, 0, 0, 0, 0, 0, 0 / 273	0, 0, 0, 0, 0, 1, 0 / 273
1	0, 2, 0, 0 / 273	1, 0, 0, 0 / 273
5	1, 0, 1, 7 / 273	2, 2, 0, 0 / 273
30	7, 12, 3, 3, 5, 1, 5 / 273	3, 1, 3, 6, 8, 8, 7 / 273

Data are shown in order of Animal Nos.

Vaginal hemorrhage was observed for 14 females during the dosing and recovery periods, regardless of the dose levels. This sign was due to estrus commonly observed in female dogs and the incidence or onset times were not dose-related.

Body Weights

Initial mean body weights for control males and females were 11.54 kg and 11.29 kg, respectively. On day 91 after dosing, mean body weight for males and females were 10.44 kg and 10.36 kg, respectively. No statistically significant changes were noted in any treated group as compared to the control group during the dosing period or during the recovery period as shown in the figure below.

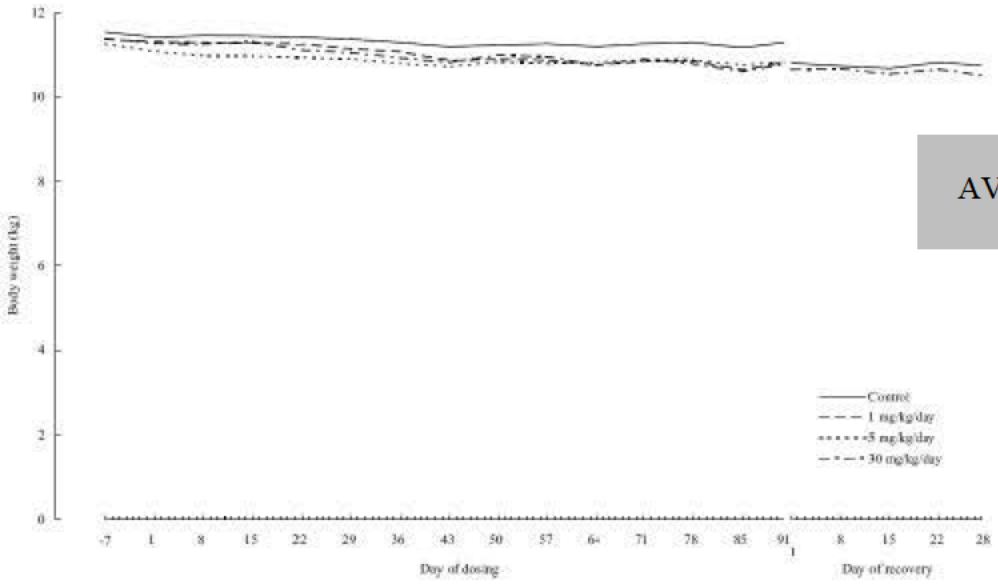

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Fig. 1 Three-Month Oral Toxicity Study of S-297995 monotosylate in Dogs
Mean body weight (males)

Feed Consumption

Food consumption was decreased in 1 female (Animal No. 74) in the 30 mg/kg group and was below 80 g vs. ± 300g food consumption of controls on Days 41-43, 45-47, 52 and 83 of dosing. The sponsor did not explain the observation. No statistically significant differences were noted in any treated group as compared to the control group during the dosing period. No marked food consumption changes were evident in any animal in the 30 mg/kg group during the recovery period.

Ophthalmoscopy

No treatment-related ophthalmological abnormalities were evident in any animal during Week 13 of dosing or Week 4 of recovery.

ECG

No treatment-related abnormalities were evident in any ECG parameter in any animal during the dosing or recovery period.

Hematology

Hematological examinations during Week 4 of dosing revealed the following statistically significant differences (Approx. 10%-15%) from the control means in the 30 mg/kg group: decreases in the red blood cell count (6.6.2±0.37 $x10^6/\mu L$ vs. 7.33±0.40 $x10^6/\mu L$ of controls in

males; 6.42±0.5 x10^6/µL vs. 7.34±0.51 x10^6/µL of controls in females) hemoglobin concentration (15.4±0.8 vs. 16.6±0.7 g/dL of controls in males; 15.3±1.0 vs. 17.4±1.1 g/dL of controls in females) and hematocrit value (44.9±2.3% vs. 49.3±2.4% of controls in males; 43.4±3.5% vs. 50.8±3.7% of controls in females) in males and females and decreases in the reticulocyte count (21.2±8.9 x10^9/L vs. 62.3±18.8 10^9/L of control)/ reticulocyte ratio (0.3±0.2% vs. 0.9±0.3% of controls) in males. During Week 13 of dosing, the following statistically significant findings were noted in the 30 mg/kg group: decreases in the reticulocyte count/ratio in males (18.5±6.4x 10^9/L vs. 50.0±23.5 x10^9/L of control); ratio (0.3±0.1% vs. 0.7±0.3% of controls) and females [count (17.8±9.4 x10^9/mL vs. 45.9±30.4 x10^9/L of control)] and decreases in the red blood cell count (6.41±0.36 x10^6/µL vs. 7.46±0.55 x10^6/µL of controls), hemoglobin concentration (14.9±0.7 vs. 16.8±1.3 g/dL of controls and hematocrit value (41.7±1.9% vs. 47.7±3.7% of controls) in males. The decreased reticulocyte count/ratio may not appear to have significantly toxicological importance since the hematopoietic cell concentration did not decrease in the active hematopoietic tissue, i.e., marrow of the sternum, and since the myeloid/erythroid cell ratio did not decrease in the marrow of the rib. No marked changes were noted in any animal during Week 4 of recovery.

Clinical Chemistry

Statistically significant increases (2-3 fold) in ALT, GGT, ALP and total cholesterol (30% increase) and statistically significant decreases in amylase (40%) were noted in males and females in the 30 mg/kg group during Week 4 of dosing as compared to the control group, but no changes in albumin (ALB) or A/G ratio were observed. During Week 13 of dosing, changes similar to but slightly more advanced than those during Week 4 of dosing were noted in the 30 mg/kg group and the differences from the control values tended to be increased as shown in Table 2 and Table 3 below. In addition, an increase in total bilirubin (33%) was noted in 1 female (Animal No. 74) in this group. The aforementioned changes during the dosing period were no longer evident during Week 4 of recovery.

Table2 Mean Clinical Chemistry Data in Male Dogs at Week 13

	CHO mg/dL	ALT IU/L	AST IU/L	ALP IU/L	Amy IU/L	T Bili mg/dL	GGT IU/L	ALB g/dL	A/G %
Group1 (0 mg)									
Mean	120	46	31	237	962	0.06	4.5	2.5	0.64
SD	20	7.0	5.0	131	287	0.01	3.6	0.17	0.08
Group2 (1 mg)									
Mean	124	44	33	205	725	0.06	4.3	2.4	0.67
SD	25	10	4.0	60	127	0.01	0.7	0.16	0.11
Group3 (5 mg)									

Mean	114	57	36	439	702	0.06	4.6	2.5	0.70
SD	7	31	5.0	180	88	0.01	0.5	0.24	0.03
Group4 (30 mg)									
Mean	155*	99*	29	984**	618*	0.07	8.2*	2.3	0.59
SD	29	63	5	406	135	0.07	2.7	0.21	0.07

Table3 Mean Clinical Chemistry Data in female Dogs at Week 13

	CHO mg/dL	ALT IU/L	AST IU/L	ALP IU/L	Amy IU/L	T Bili mg/dL	GGT IU/L	ALB g/dL	A/G %
Group1 (0 mg)									
Mean	126	42	32	237	1011	0.06	3.8	2.5	0.64
SD	11	5.0	5.0	64	223	0.01	0.6	0.17	0.08
Group2 (1 mg)									
Mean	131	44	30	254	920	0.06	4.6	2.4	0.67
SD	27	9	4.0	94	199	0.01	0.8	0.16	0.11
Group3 (5 mg)									
Mean	140	40	33	337	821	0.06	4.3	2.5	0.70
SD	20	14	3.0	78	144	0.01	0.4	0.24	0.03
Group4 (30 mg)									
Mean	168*	165*	42	1090**	611**	0.08	7.6**	2.3	0.59
SD	23	165	12	743	115	0.05	3.8	0.21	0.07

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

Urinalysis

There were no test substance-related changes in urinalysis data throughout the study.

Gross Pathology

No treatment-related gross pathological lesions were observed in any animal at termination of the dosing or recovery period.

Organ Weights

There were no test substance-related changes in absolute and relative weights of any organs.

Bone Marrow Examinations

No treatment-related changes were noted in any animal at termination of the dosing or recovery period.

Histopathology

Adequate Battery

Yes

Peer Review

Yes

Histological Findings

Treatment-related histopathological lesions were observed in the femoral bone marrow, adipose tissue and liver at termination of the dosing period as follows: Atrophy of the adipose tissue and deposition of the gelatinous material in the femoral bone marrow were observed in 1 animal/sex in the 30 mg/kg group to a slight or moderate degree. Slight atrophy of the pericardial and perirenal adipose tissues was observed in these animals, indicating effects of the test article on the adipose tissues. Slight single cell necrosis in the liver was observed in 1 animal of both sexes in the 30 mg/kg group. In addition, slight extramedullary hematopoiesis was observed in the liver in 3 males and 1 female in the 30 mg/kg group. The lesions in the femoral bone marrow, adipose tissue and liver observed at termination of the dosing period were no longer evident at termination of the recovery period.

Special Evaluation

None.

Toxicokinetics

The Tmax values on Days 1, 28 and 91 of dosing at 1 to 30 mg/kg/day were 0.4 to 1.4 hours and seemed to be independent of dose levels and the dosing period as shown in the table below. The Cmax values on Days 1, 28 and 91 of dosing increased almost dose-proportionally. The $AUC_{0\text{-}24h}$ values on Days 1, 28 and 91 of dosing increased more than dose-proportionally. Plasma S-297995 concentrations were not affected by repeated dosing except for the 30 mg/kg group, at which the $AUC_{0\text{-}24h}$ tended to increase by repeated dosing. No large difference in the Cmax, $AUC_{0\text{-}24h}$ or Tmax was observed between the sexes. S-297995 was not detected in any plasma sample from the control group.

	Male			Female		
Dose (mg/kg)	**1**	**5**	**30**	**1**	**5**	**30**
Number of animals	**4**	**4**	**7**	**4**	**4**	**7**
Mean C_{max} (µg/mL)						
Day 1	0.383	2.99	12.0	0.457	2.93	11.9
Day 28	0.523	3.16	13.7	0.346	2.79	12.6
Day 91	0.552	3.79	13.9	0.455	2.67	13.6
Mean AUC_{0-24hr} (µg·hr/mL)						
Day 1	0.705	6.41	47.2	0.726	6.88	50.0
Day 28	0.767	6.71	70.1	0.551	5.69	44.8
Day 91	0.880	8.28	90.9	0.724	5.90	78.7
Mean t_{max} (hr)						
Day 1	0.6	0.4	0.8	0.5	0.5	1.1
Day 28	0.4	0.5	0.9	0.4	0.5	0.6
Day 91	0.4	0.4	1.4	0.4	0.5	1.3

Study title: Nine-Month Oral Toxicity Study of S-297995 monotosylate in Dogs

Study no.:	S-297995-TF-219-L (CRO Study No. SG10119)
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 28, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	S-297995 monotosylate, lot # A81001, 97.5%

Key Study Findings

Treatment with S-297995 monotosylate produced changes in clinical chemistry parameters at all doses, with the correlating histopathological findings in the liver at 20 mg/kg/day. Clinical chemistry changes at the high dose included increases in ALT, ALP, and GGT in both males and females (compared to controls). In high dose females, there were increases in other parameters such as AST, cholesterol, and phospholipids. Histopathological findings in high dose animals included slight single cell necrosis and increases in Kupffer cells with brownish pigment deposition in high dose animals. While a single male each in the low- and mid-dose groups exhibited decreases in body and organ weights and histopathological findings (e.g., atrophy of adipose tissue, deposition of material in the bone marrow), such findings were not observed at the high dose and there was no apparent dose-relationship as changes at the low dose were noted in more organs than at the mid dose. Following the 1-month recovery period, no treatment-related findings were observed. The NOAEL was considered to be 4 mg/kg/day and the target organ was the liver.

Methods

Doses:	1, 4, and 20 mg/kg (as S-297995)
Frequency of dosing:	Once daily
Route of administration:	Oral (gavage)
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5 w/v% methylcellulose aqueous solution
Species/Strain:	Beagle dog
Number/Sex/Group:	Main study: 4/sex/group, Recovery: 3/sex (control and high dose groups only)
Age:	9-10 months
Weight:	Males: 9.10-11.80 kg, Females: 8.24-10.88 kg
Satellite groups:	Yes (Recovery)
Unique study design:	No
Deviation from study protocol:	Protocol deviations did not affect the quality or integrity of the study.

Observations and Results

Mortality

Animals were observed three times per day during the dosing period, and twice daily during other periods of the study.

There were no mortalities.

Clinical Signs

Cage-side observations were conducted three times per day during the dosing period, and twice daily during other periods of the study.

Clinical observations (e.g., vomitus, vaginal hemorrhage considered a likely sign of estrus) were considered to be incidental and unrelated to the treatment compound based on the low incidence and/or occurrence across groups (including controls). In a single high dose female, protrusion of the nictitating membrane was observed from Day 217 (and throughout the recovery period). From Day 267, this animal also exhibited hyperemia of the bulbar conjunctiva. These findings were limited to one eye, and persisted through the recovery period.

Body Weights

Animals were weighed once weekly, and 1 day prior to necropsy.

There were no statistically significant effects on mean body weights in males or females, compared to controls. Body weight decreases in a single male each from the low- and mid-dose groups (with minimum values of 0.84- and 0.86-fold Day 1 values) were considered incidental based on a lack of dose-relationship and tendency towards decreased body weights in these animals prior to initiation of dosing.

Feed Consumption

Food consumption was recorded daily.

No treatment-related effects on food consumption were observed.

Ophthalmoscopy

Ophthalmological examinations were conducted once pretest, during Weeks 13, 26, and 39 of dosing, and during Week 4 of the recovery period.

No treatment-related findings were identified. As discussed above, in a single high dose female, protrusion of the nictitating membrane and hyperemia of the bulbar conjunctiva was observed. In addition, in a single high dose male, detachment of the retinal vessels in a single eye was observed during Weeks 26 and 39. The finding was described as not progression and considered to be the result of an accident, rather than treatment with the test compound.

ECG

ECGs were recorded twice pretest, during Weeks 13, 26, and 39 of dosing, and during Week 4 of recovery. The following parameters were determined: PR intervals, QRS duration, QT intervals, and RR duration. QTc intervals were calculated using Fridericia's correction formula and heart rate was determined.

There were no treatment-related findings. Although there were statistically significant changes in mean values for a subset of parameters (increased heart rate; shortening of PR and QT intervals and QRS and RR duration, compared to controls) at a subset of the timepoints, these were considered to be incidental based on a lack of dose relationship and/or comparison to pretest values.

Hematology

Samples were collected twice pretest, during Weeks 4, 13, 26, and 39 of dosing, and Weeks 2 and 4 of the recovery period for analysis of standard hematological parameters. Prothrombin time, activated partial thromboplastin time, and fibrinogen concentration were also determined.

While statistically significant changes in a subset of parameters (compared to controls) were observed during the dosing and recovery phases, the findings were considered incidental based on a lack of dose-relationship, the transient nature of the changes, and/or comparison to pretest values. In the single low- and mid-dose males which exhibited decreased body weights, there were decreases in red blood cell (RBC) count, hemoglobin, and hematocrit during Week 39. In high dose males, eosinophils were significantly increased during Weeks 13 and 26 (up to +76% of controls), but eosinophils were also significantly higher (compared to controls) pretest. Also in high

dose males, there was a statistically significant increase in prothrombin time during Week 26 (+9%, compared to controls). In high dose females, there were statistically significant increases in platelets during weeks 13 and 26 (up to +30% compared to controls) but values were similar to those measured pretest. High dose females also had significantly increased WBC count (up to +24% compared to controls) during Weeks 4 and 26, but values were similar to pretest values.

During the recovery period, there were statistically significant decreases in mean RBC count (up to -13%), HGB (up to -11%), and HCT (up to -11%) in high dose males (compared to controls). In high dose males, fibrinogen was significantly decreased (-24%, compared to controls) during recovery week 2. However, fibrinogen levels were decreased in both controls and high dose males relative to pretest values and there were no significant changes (compared to controls) during the dosing period. In high dose females, prothrombin time was increased (+13% compared to controls) during recovery week 4 but there were no significant effects on this parameter during the dosing period and higher values were measured in high dose females during the pretest period.

Clinical Chemistry

Samples collected twice pretest, during Weeks 4, 13, 26, and 39 of dosing, and Weeks 2 and 4 of the recovery period were for standard clinical chemistry parameters.

In high dose males, there were increases in mean ALT (up to +62%; statistically significant during Weeks 4 and 13) and ALP values (up to +151%; statistically significant during Weeks 26 and 39), compared to controls. While the group means were not significantly different from controls, the following changes were noted: increased bilirubin in a single male each in the mid- and high dose groups, and increased GGT in 2 high dose males during the dosing period.

In high dose females, there were increases in mean values for ALT (up to +151%; significant during Weeks 13 and 39), ALP (up to +318%; significant during Weeks 4, 13, 26, and 39), GGT (up to +54%; significant during Weeks 4, 13, 26, and 39), and AST (+46%; significant during Week 39), compared to controls. Also in high dose females, there were statistically significant increases in creatinine (+21%, compared to controls, during Week 39), cholesterol (up to +47%, compared to controls; significant during Weeks 4, 13, 26, and 39), phospholipids (up to +27%, compared to controls; significant during Weeks 4 and 13 only), and total protein (up to +7%, compared to controls; significant during Weeks 13 and 26). While the group means were not significantly different from controls, the following changes were noted: increased cholesterol and phospholipids in a single low-dose female, increased GGT values in 2 mid-dose females, increased ALP values in a single mid-dose female, and increased bilirubin in a single high dose female during the dosing period.

Statistically significant changes in other parameters during a subset of timepoints appeared to be incidental based on comparison to pretest values and/or variability

among groups and over time. There were no statistically significant changes in high dose males or females (compared to controls) during the recovery period.

Urinalysis

Urinalysis was conducted once pretest, during Weeks 19 and 39 of dosing, and Week 4 of the recovery period.

In high dose females, there was as statistically significant increase in mean urine volume (+61%, compared to controls) and decrease in specific gravity (1.033 compared to 1.047 for controls) during Week 39. Values for these parameters were similar to controls during the recovery phase.

Gross Pathology

At termination, complete macroscopic examinations were conducted.

At the end of the dosing period, gross findings included a pituitary cyst, dark reddish contents in the stomach, and small spleen each in a single low dose male. A light brown focus was observed on the lung of a single mid-dose male, and there was dark brown discoloration of the kidneys in a single high dose male. Gross findings observed only in females treated with the test compound included a nodule-like thickening of the left uterine horn (single low-dose female), a cyst on the ovary (single mid-dose animal), and light brown focus on the anterior lobe of the lung (single high dose female). In recovery animals, gross findings were limited to protrusion of the nictitating membrane in a single high dose female.

Organ Weights

At termination, weights of organs identified in the Applicant's table pasted below under "Histopathology" were recorded. Organ weights relative to terminal body weight ("relative weights") were calculated.

There were no statistically significant changes in organ weights in males at the end of the dosing period. In the single low- and mid-dose males which exhibited body weight decreases, absolute and relative thymus, spleen, and prostate weights were decreased relative to controls. The absolute and relative thyroid weights were significantly increased in high dose females (+75% and +78%, respectively, compared to controls). There were however, no histopathological correlates for this finding in the thyroid. In addition, absolute and relative thymus weights were significantly decreased in the low dose group (up to -60%, compared to controls). In recovery males, mean relative spleen weights were significantly decreased (-18%, compared to controls). In recovery females, relative thyroid and pancreas weights were significantly increased (+54% and +15%, respectively, compared to controls).

Histopathology

Histopathological examination of tissues/organs identified in the Applicant's table below was conducted. Bone marrow smears were prepared but not evaluated.

Organ/tissue	Fixation	Organ weight	Specimen preparation	
			HE-stained	Note
Heart	O	O	O	Left ventricular papillary muscle, right ventricular wall and an area including the coronary artery and aortic valve
Aorta (thoracic)	O	–	O	
Sternum	O	–	O	
Sternal bone marrow		–		
Femurs	O (R&L)	–	O (L)	Including distal end of the articular cartilage
Femoral bone marrow	O (R)	–	O (R)	
Thymus	O	O	O	
Spleen	O	O	O	
Cervical lymph nodes	O (R&L)	–	O (L)	
Submandibular lymph nodes	O (R&L)	–	O (L)	
Mesenteric lymph nodes	O	–	O	
Trachea	O	–	O	
Bronchi	O (R&L)	O (R&L separated)	O (R&L)	Left anterior and right posterior lobes
Lungs				
Tongue	O	–	O	
Submandibular glands	O (R&L)	O (R&L combined)	O (L)	
Sublingual glands	O (R&L)	–	O (L)	
Parotid glands	O (R&L)	–	O (L)	
Esophagus	O	–	O	
Stomach	O	–	O	Cardia, body and pylorus
Duodenum	O	–	O	
Jejunum	O	–	O	
Ileum	O	–	O	
Peyer's patches	O	–	O	
Cecum	O	–	O	
Colon	O	–	O	
Rectum	O	–	O	
Liver	O	O (with bile-drained gallbladder)	O	Left lateral lobe and right medial lobe including the gallbladder
Gallbladder			O	
Pancreas	O	O	O	
Kidneys	O (R&L)	O (R&L separated)	O (R&L)	PAS-stained specimens (L) were also prepared.
Urinary bladder	O	–	O	
Ureters	O (R&L)	–	O (L)	
Pituitary	O	O	O	
Thyroids	O (R&L)	O (R&L separated)	O (R&L)	
Parathyroids			O (L)	
Adrenals	O (R&L)	O (R&L separated)	O (R&L)	
Testes	O (R&L)	O (R&L separated)	O (R&L)	
Epididymides	O (R&L)	O (R&L separated)	O (R&L)	

Organ/tissue	Fixation	Organ weight	Specimen preparation	
			HE-stained	Note
Prostate	O	O	O	
Ovaries	O (R&L)	O (R&L separated)	O (R&L)	
Uterus	O	O	O	
Vagina	O	–	O	
Brain	O	O	O	Cerebrum [frontal, parietal (including basal ganglia and hippocampus) and occipital lobes]; cerebellum; pons; and medulla oblongata
Spinal cord (thoracic)	O	–	O	
Sciatic nerve	O (L)	–	O (L)	
Eyes	O (R&L)	–	O (R&L)	
Optic nerves	O (R&L)	–	O (R&L)	
Lacrimal glands	O (R&L)	–	O (L)	
Skeletal muscle (thigh)	O (L)	–	O (L)	
Skin (abdominal)	O	–	O	
Mammary glands (females only)	O	–	O	
Organs/tissues with gross lesions	O	–	O	

O: Conducted –: Not conducted
R&L: Both the right and left organs/tissues were conducted.
L: Either the right or left organ/tissue (usually the left) was conducted.
R: Either the right or left organ/tissue (usually the right) was conducted.

Adequate Battery: Yes

Peer Review: No

Histological Findings
Treatment-related findings were observed in the liver of high dose males and females. In high dose males, findings in the liver at the end of the dosing period which occurred at an increased incidence included single cell necrosis (slight severity; 2/4 animals), increases in Kupffer cells with pigment deposition (slight severity; 1/4 animals), and focal mononuclear cell infiltration (slight severity; 1/4 animals). In females, findings in the liver at the end of the dosing period included single cell necrosis (slight severity; 2/4 animals), increases in Kupffer cells with pigment deposition (slight severity; 2/4 animals), and focal mononuclear cell infiltration (slight severity; 2/4 animals).

Other findings in males and females (including those correlating to gross findings) were considered to be incidental. In the single low- and mid-dose males that exhibited decreased body weights and thymus, spleen, and prostate weights, histopathological findings were noted to include slight atrophy of the adipose tissues (pericardial and perirenal) and mucosal surface of the stomach epithelium, deposition of a gelatinous material in the bone marrow (slight), and moderate, diffuse atrophy of the thymus. In the low dose animal there was also slight atrophy of the adipose tissue in the bone marrow, slight diffuse atrophy of the spleen, slight atrophy of hepatocytes, and moderate atrophy of the prostate epithelium.

No treatment-related effects were observed at the end of recovery. In a single high dose female, there was slight hyperplasia of the nictitating gland and severe mononuclear cell infiltration of the mucosa/submucosa of the nictitating membrane.

Special Evaluation

None.

Toxicokinetics

Blood samples were collected for TK analysis out to24 h post-dosing on Days 1, 136, and 273 of dosing. Plasma samples were analyzed for S-297995 and benzamidine concentrations using LC/MS/MS.

TK parameters for S-297995 and benzamidine are summarized in the Applicant's tables below. As shown, mean Tmax values for S-297995 and benzamidine were ~0.5 to 1 h and ~7 h, respectively. Mean $AUC_{0\text{-}24h}$ values for S-297995 increased in a more than dose proportional manner as the dose increased from 4 to 20 mg/kg/day. Mean Cmax and $AUC_{0\text{-}24h}$ values for benzamidine were lower than those of S-297995.

S-297995

Summary table 1 (for S-297995)

		Mean Cmax (μg/mL)			Mean Tmax (h)			Mean $AUC_{0\text{-}24h}$ (μg·h/mL)			Mean C_{24h} (μg/mL)		
		Dose level (mg/kg/day)											
		1	4	20	1	4	20	1	4	20	1	4	20
Male	Day 1 of dosing	0.423	2.25	8.04	0.38	0.38	0.79	0.735	4.08	36.7	NC	NC	NC
	Day 136 of dosing	0.400	2.92	9.89	0.63	0.38	1.14	0.856	6.56	56.4	NC	NC	NC
	Day 273 of dosing	0.295	2.32	10.0	0.63	0.50	0.86	0.707	5.85	55.9	NC	NC	0.0158
Female	Day 1 of dosing	0.434	2.16	8.74	0.50	0.44	0.75	1.04	5.87	33.6	NC	NC	NC
	Day 136 of dosing	0.609	2.40	8.98	0.63	0.44	0.93	1.22	7.03	48.9	NC	NC	NC
	Day 273 of dosing	0.401	1.90	8.35	0.56	0.50	0.89	0.991	5.97	40.5	NC	NC	NC

NC: Not calculated

Benzamidine

Summary table 2 (for Benzamidine)

		Mean Cmax (ng/mL)			Mean Tmax (h)			Mean $AUC_{0\text{-}24h}$ (ng·h/mL)			Mean C_{24h} (ng/mL)		
		Dose level (mg/kg/day)											
		1	4	20	1	4	20	1	4	20	1	4	20
Male	Day 1 of dosing	3.18	11.0	78.3	7.00	5.75	7.00	40.1	147	1070	0.349	1.82	22.1
	Day 136 of dosing	3.71	16.7	78.8	5.75	7.00	7.00	55.1	285	1340	1.08	6.16	37.0
	Day 273 of dosing	4.16	19.2	83.5	7.00	7.00	7.00	66.3	303	1390	1.57	6.62	37.6
Female	Day 1 of dosing	3.15	10.3	58.4	7.00	7.00	7.00	39.0	125	775	0.457	0.911	13.3
	Day 136 of dosing	3.32	10.1	97.0	7.00	7.00	7.00	47.4	146	1600	0.696	2.05	40.7
	Day 273 of dosing	4.53	11.0	84.8	7.00	7.00	6.00	66.1	158	1350	1.10	2.43	32.3

Dosing Solution Analysis

Samples from the first and last dosing solution preparations were analyzed for S-297995 concentration and evaluation of homogeneity.

The concentrations of S-297995 in all dosing formulations ranged from 94.0 to 99.8% of nominal concentrations, and thus were acceptable.

7 Genetic Toxicology

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

Review of Study No. R-297995-TB-051-L from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Study title:	Reverse Mutation Test of RSC-297995 monotosylate with Bacteria
Study no.:	R-297995-TB-051-L
Study report location:	Electronic submission
Conducting laboratory and location:	Developmental Research Laboratories, Shionogi & Co., Ltd. Japan
Date of study initiation:	March 3, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RSC-297995, Lot No. A81001, 97.5%

Key Study Findings

The number of revertant colonies in the test substance treated groups was almost the same as that in the negative control (DMSO) group and the test substance did not produce two-fold or more increase in the revertant colonies over the negative control group at any dose of any tester strain with or without S9 mix in either tests.

Methods

Strains:	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 *Escherichia coli* WP2*uvrA*
Concentrations in definitive study:	156, 313, 625, 1250, 2500 and 5000 µg/plate For TA98 and TA1537 without S9 mix, a dose of 78.1 ug/plate was used
Basis of concentration selection:	The dose selection was based on preliminary cytotoxicity in which RSC-297995 concentrations of 5, 15, 50, 150, 500, 1500 and 5000 µg/plate were used. Cytotoxicity was observed at 1500 µg/plate and above.
Negative control:	Solvent dimethyl sulfoxide (DMSO)
Positive control:	The positive control used for all strains in the presence of metabolic activation was 2-aminoanthracene (2AA). In the absence of metabolic activation, the following substances were used as positive controls: TA100- **2**-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF2), TA1535- sodium azide (NaN_3), WP2uvrA and TA98- **2**-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF2), and TA1537- 9-aminoacridine (9AA).
Formulation/Vehicle:	The solvent dimethyl sulfoxide (DMSO) was used as a vehicle for the test drug and also used for dissolving and diluting the test substance
Incubation & sampling time:	The tester strains were incubated with the test substance and positive or negative controls by the plate incorporation method. The plates were incubated for at 37^0C for 48 hours before counting the revertant colonies.

Study Validity

The study was considered valid if the number of revertant colonies for the negative controls for each strain were within the historical control range from the conducting laboratory, and the mean value for the positive controls for each tester strain exhibited at least 2-fold increase over the vehicle control for that strain in the absence or presence of metabolic activation. Based on these criteria, the study was valid.

Results

Incubation of the bacterial stains with RSC-297995 did not cause two-fold or greater increases in the number of revertants for any strain, either in the presence or absence of metabolic activation. The positive controls, on the other hand, showed two-fold or higher increases in the number of revertants when compared with the negative control. The number of revertant colonies for different strains in the absence or presence of metabolic activation is

shown in the Table below. Thus, RSC-297995 was not genotoxic in the bacterial reverse mutation assay (Ames test).

Metabolic activaton	Test substance Dose (μg/plate)	Number of revertants (number of colonies/plate, mean)				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
Without S9 mix (-S9)	Negative control	116 112 (114)	12 12 (12)	34 33 (34)	19 17 (18)	13 11 (12)
	78.1	NT	NT	NT	21 15 (18)	12 13 (13)
	156	96 104 (100)	8 6 (7)	46 33 (40)	18 15 (17)	14 14 (14)
	313	114 92 (103)	11 8 (10)	34 22 (28)	10 13 (12)	5 7 (6)
	625	103 107 (105)	7 10 (9)	33 34 (34)	22 18 (20)	7 10 (9)
	1250	100 101 (101)	10 13 (12)	27 42 (35)	15 17 (16)	7 * 10 * (9)
	2500 +	85 * 56 * (71)	5 * 3 * (4)	24 * 28 * (26)	17 * 17 * (17)	2 * 2 * (2)
	5000 +	31 * 7 * (19)	0 * 1 * (1)	21 * 23 * (22)	NT	NT
With S9 mix (+S9)	Negative control	120 110 (115)	13 15 (14)	44 39 (42)	19 24 (22)	23 21 (22)
	156	88 118 (103)	11 11 (11)	28 35 (32)	28 20 (24)	22 18 (20)
	313	121 97 (109)	8 7 (8)	34 49 (42)	18 30 (24)	17 18 (18)
	625	108 108 (108)	9 6 (8)	35 43 (39)	11 28 (20)	18 10 (14)
	1250	111 105 (108)	15 12 (14)	32 37 (35)	26 27 (27)	9 9 (9)
	2500	77 * 100 * (89)	6 * 6 * (6)	34 30 (32)	36 36 (36)	11 * 3 * (7)
	5000 +	27 * 28 * (28)	2 * 2 * (2)	30 * 25 * (28)	21 * 28 * (25)	4 * 3 * (4)
Positive controls (-S9)	Substance	AF-2	NaN_3	AF-2	AF-2	9AA
	Dose (μg/plate)	0.01	0.5	0.01	0.1	80
	Number of colonies/plate	587 614 (601)	315 343 (329)	220 254 (237)	881 741 (811)	762 529 (646)
Positive controls (+S9)	Substance	2AA	2AA	2AA	2AA	2AA
	Dose (μg/plate)	1	2	10	0.5	2
	Number of colonies/plate	973 958 (966)	259 260 (260)	776 753 (765)	292 286 (289)	266 317 (292)

NT: Not tested,
*: Rreduction of the bacterial background lawn, +: Precipitation derived from the test substance,
Negative control: Dimethyl sulfoxide (DMSO, 100 μL/plate),
AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide, NaN_3: Sodium azide, 9AA: 9-Aminoacridine hydrochloride, 2AA: 2-Aminoanthracene

7.2 *In Vitro* Assays in Mammalian Cells

Review of Study No. R-297995-TB-052-L (CRO Study No. G-07-070) from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Study title: Chromosomal aberration test of RSC-297995 monotosylate using cultured Chinese Hamster Lung (CHL/IU) Cells

Study no.:	R-297995-TF-052-L
Study report location:	Electronic submission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 3, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RSC-297995, lot no. A81001, 97.5% purity

Key Study Findings

RSC-297995 monotosylate did not induce chromosomal aberrations in CHL/IU cells in the presence or absence of metabolic activation.

Methods

Cell line:	Chinese hamster lung fibroblast cells (CHL/IU)
Concentrations in definitive study:	105, 158, 237, 356. 533 and 800 µg/mL for short treatment (6 hrs), and 46.8, 70.2, 105, 158, 237 µg/mL for long treatment (24 hr.)
Basis of concentration selection:	The sponsor stated that the doses were selected on the basis of the preliminary cytoxicity study, and the high doses caused an inhibition of cell growth by >50%.
Negative control:	Solvent dimethyl sulfoxide (DMSO)
Positive control:	Mitomycin C (MMC) was used as the positive control substance in the short-term treatment without metabolic activation and the continuous treatment. In the short-term treatment with metabolic activation, cyclophosphamide (CP) was used as the positive control substance.
Formulation/Vehicle:	DMSO was used as a vehicle for the drug.
Incubation & sampling time:	Cells were treated with S-297995 monotosylate for 6 hours (followed by reculture for 18 hours) in the presence or absence of S9 mix, or were continuously treated for 24 hours in the absence of S9 mix.

Study Validity

The assay was valid as the number of cells with structural abnormalities in the negative control groups was <3%, and the positive controls caused significant increases in the number of cells with aberrations which appeared to be same levels of the historical control data, in the presence or absence of metabolic activation.

Results

In all treatment groups with RSC-297995, cells with structural chromosomal aberrations or polyploid cells were not statistically significantly increased at any dose as shown in the Table 1, Table 2, and Table 3 below. Positive controls, MMC in the short-term and the continuous treatment groups without S9 mix (Table 1 and Table 3) and CP in the short-term treatment group with S9 mix (Table 2), significantly induced structural chromosomal aberrations. Incidences in the negative (solvent) and positive controls were the same level as the historical control data. Therefore, this study showed that RSC-297995 monotosylate did not induce chromosomal aberrations in CHL/IU cells under the present test conditions.

Table 1 Chromosome analysis of Chinese hamster lung (CHL/IU) cells treated with RSC-297995 monotosylate (RSC-297995B) for 6 hours without S9 mix

Group	Concentration (μg/mL)	S9 mix	Time of exposure (hrs)	Concurrent [2] cell growth (%)	Mitotic [3] index (%)	Number of cells analyzed	Number of structural aberrations: gap	cth	cte	csh	cse	mul [4]	total	Others [5]	Number of cells with aberrations: +gap (%)	-gap (%)	Number [6] of polyploid cells (%)
Negative [1] (DMSO)	0	−	6 - (18)	100	NA	100	2	1	0	0	0	0	3	1	3 (3.0)	1 (1.0)	0 (0.0)
						100	1	0	0	1	1	0	3	0	3 (3.0)	2 (2.0)	0 (0.0)
						200	3	1	0	1	1	0	6	1	6 (3.0)	3 (1.5)	0 (0.0)
RSC-297995B	105	−	6 - (18)	99	NA	not observed											
RSC-297995B	158	−	6 - (18)	93	NA	not observed											
RSC-297995B	237	−	6 - (18)	89	NA	100	0	1	0	0	0	0	1	0	1 (1.0)	1 (1.0)	1 (1.0)
						100	1	1	1	1	0	10	14	0	5 (5.0)	4 (4.0)	0 (0.0)
						200	1	2	1	1	0	10	15	0	6 (3.0)	5 (2.5)	1 (0.5)
RSC-297995B	356	−	6 - (18)	78	NA	100	0	1	1	0	0	0	2	1	2 (2.0)	2 (2.0)	0 (0.0)
						100	0	1	0	0	0	0	0	0	0 (0.0)	0 (0.0)	0 (0.0)
						200	0	1	1	0	0	0	2	1	2 (1.0)	2 (1.0)	0 (0.0)
RSC-297995B	533	−	6 - (18)	56	2.8, 4.2	100	1	4	3	0	0	0	8	0	6 (6.0)	5 (5.0)	0 (0.0)
						100	1	1	1	0	0	0	3	1	3 (3.0)	2 (2.0)	0 (0.0)
						200	2	5	4	0	0	0	11	1	9 (4.5)	7 (3.5)	0 (0.0)
RSC-297995B	800	−	6 - (18)	14	NA	not made chromosome specimen											
MMC	0.1	−	6 - (18)	NA	NA	100	5	22	44	0	1	0	72	0	48 (48.0)	46 (46.0)	0 (0.0)
						100	3	24	59	1	0	0	87	0	49 (49.0)	46 (46.0)	0 (0.0)
						200	8	46	103	1	1	0	159	0	97 (48.5)	92 *(46.0)	0 (0.0)

Abbreviations: gap, chromatid gap and chromosome gap; cth, chromatid break; cte, chromatid exchange; csh, chromosome break; cse, chromosome exchange (dicentric and ring); mul, multiple aberrations; +gap, total number of cells with aberrations including gaps; -gap, total number of cells with aberrations excluding gaps; DMSO, Dimethyl sulfoxide; MMC, mitomycin C; NA, not analyzed.

1) DMSO was used as a solvent and added at the level of 1 vol% per dish. 2) Cell confluency, representing cytotoxicity, was measured with a Monocellater.[TM] 3) Metaphase frequency was calculated by counting 500 cells in each dish. 4) When the number of aberrations in a cell was more than 9, the cell was scored as having 10 aberrations. 5) Others, such as attenuation and premature chromosome condensation, were excluded from the number of structural aberrations. 6) Two hundred cells were analyzed in each group.

*, Significantly different from the negative control at p=0.01 (one-side) by Fisher's exact probability test.

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Table 2 Chromosome analysis of Chinese hamster lung (CHL/IU) cells treated with RSC-297995 monotosylate (RSC-297995B) for 6 hours with S9 mix

Group	Concentration (μg/mL)	S9 mix	Time of exposure (hrs)	Concurrent cell growth [2] (%)	Mitotic index [3] (%)	Number of cells analyzed	Number of structural aberrations: gap	ctb	cte	csb	cse	mul [4]	total	Others [5]	Number of cells with aberrations: +gap (%)	-gap (%)	Number [6] of polyploid cells (%)
Negative [1] (DMSO)	0	+	6 - (18)	100	NA	100	0	0	1	0	3	0	4	0	2 (2.0)	2 (2.0)	0 (0.0)
						100	0	1	0	1	0	0	2	0	2 (2.0)	2 (2.0)	0 (0.0)
						200	0	1	1	1	3	0	6	0	4 (2.0)	4 (2.0)	0 (0.0)
RSC-297995B	105	+	6 - (18)	105	NA	not observed											
RSC-297995B	158	+	6 - (18)	104	NA	not observed											
RSC-297995B	237	+	6 - (18)	103	NA	100	1	0	9	0	0	0	10	1	3 (3.0)	2 (2.0)	1 (1.0)
						100	0	2	0	1	0	0	3	0	3 (3.0)	3 (3.0)	0 (0.0)
						200	1	2	9	1	0	0	13	1	6 (3.0)	5 (2.5)	1 (0.5)
RSC-297995B	356	+	6 - (18)	97	NA	100	0	1	0	0	0	0	1	0	1 (1.0)	1 (1.0)	1 (1.0)
						100	2	0	1	0	0	0	3	0	3 (3.0)	1 (1.0)	1 (1.0)
						200	2	1	1	0	0	0	4	0	4 (2.0)	2 (1.0)	2 (1.0)
RSC-297995B	533	+	6 - (18)	64	4.8, 5.4	100	0	1	0	0	0	0	1	0	1 (1.0)	1 (1.0)	0 (0.0)
						100	2	4	4	1	0	0	11	0	5 (5.0)	5 (5.0)	0 (0.0)
						200	2	5	4	1	0	0	12	0	6 (3.0)	6 (3.0)	0 (0.0)
RSC-297995B	800	+	6 - (18)	35	Tox, Tox	not observed because of severe cytotoxicity											
CP	10	+	6 - (18)	NA	NA	100	3	8	11	0	0	0	22	1	19 (19.0)	18 (18.0)	0 (0.0)
						100	3	12	28	0	0	0	43	1	31 (31.0)	30 (30.0)	0 (0.0)
						200	6	20	39	0	0	0	65	2	50 (25.0)	48 *(24.0)	0 (0.0)

Abbreviations: gap, chromatid gap and chromosome gap; ctb, chromatid break; cte, chromatid exchange; csb, chromosome break; cse, chromosome exchange (dicentric and ring); mul, multiple aberrations; +gap, total number of cells with aberrations including gaps; -gap, total number of cells with aberrations excluding gaps; DMSO, Dimethyl sulfoxide; CP, cyclophosphamide; Tox, cytotoxic; NA, not analyzed.

1) DMSO was used as a solvent and added at the level of 1 vol% per dish. 2) Cell confluency, representing cytotoxicity, was measured with a Monocellator.™ 3) Metaphase frequency was calculated by counting 500 cells in each dish. 4) When the number of aberrations in a cell was more than 9, the cell was scored as having 10 aberrations. 5) Others, such as attenuation and premature chromosome condensation, were excluded from the number of structural aberrations. 6) Two hundred cells were analyzed in each group.

*, Significantly different from the negative control at p<0.01 (one-side) by Fisher's exact probability test.

Table 3 Chromosome analysis of Chinese hamster lung (CHL/IU) cells continuously treated with RSC-297995 monotosylate (RSC-297995B) for 24 hours without S9 mix

Group	Concentration (μg/mL)	Time of exposure (hrs)	Concurrent [2] cell growth (%)	Mitotic [3] index (%)	Number of cells analyzed	Number of structural aberrations: gap	ctb	cte	csb	cse	mul [4]	total	Others [5]	Number of cells with aberrations: +gap (%)	-gap (%)	Number [6] of polyploid cells (%)
Negative [1] (DMSO)	0	24	100	NA	100	0	1	0	4	0	0	5	1	3 (3.0)	3 (3.0)	0 (0.0)
					100	1	1	0	0	0	0	2	0	2 (2.0)	1 (1.0)	0 (0.0)
					200	1	2	0	4	0	0	7	1	5 (2.5)	4 (2.0)	0 (0.0)
RSC-297995B	46.8	24	98	NA	not observed											
RSC-297995B	70.2	24	86	NA	100	2	4	4	6	0	0	16	0	6 (6.0)	4 (4.0)	1 (1.0)
					100	1	2	0	0	0	0	3	0	3 (3.0)	2 (2.0)	0 (0.0)
					200	3	6	4	6	0	0	19	0	9 (4.5)	6 (3.0)	1 (0.5)
RSC-297995B	105	24	71	NA	100	0	1	0	0	0	0	1	0	1 (1.0)	1 (1.0)	0 (0.0)
					100	0	0	0	0	1	0	1	0	1 (1.0)	1 (1.0)	0 (0.0)
					200	0	1	0	0	1	0	2	0	2 (1.0)	2 (1.0)	0 (0.0)
RSC-297995B	158	24	55	1.2, 1.0	100	2	3	0	0	0	0	5	0	5 (5.0)	3 (3.0)	0 (0.0)
					100	0	4	0	0	0	0	4	1	3 (3.0)	3 (3.0)	1 (1.0)
					200	2	7	0	0	0	0	9	1	8 (4.0)	6 (3.0)	1 (0.5)
RSC-297995B	237	24	38	Tox, Tox	not observed because of severe cytotoxicity											
MMC	0.05	24	NA	NA	100	2	26	58	2	1	0	89	0	53 (53.0)	52 (52.0)	0 (0.0)
					100	3	24	48	0	0	10	85	2	52 (52.0)	49 (49.0)	0 (0.0)
					200	5	50	106	2	1	10	174	2	105 (52.5)	101 *(50.5)	0 (0.0)

Abbreviations: gap, chromatid gap and chromosome gap; ctb, chromatid break; cte, chromatid exchange; csb, chromosome break; cse, chromosome exchange (dicentric and ring); mul, multiple aberrations; +gap, total number of cells with aberrations including gaps; -gap, total number of cells with aberrations excluding gaps; DMSO, Dimethyl sulfoxide; MMC, mitomycin C; Tox, cytotoxic; NA, not analyzed.

1) DMSO was used as a solvent and added at the level of 1 vol% per dish. 2) Cell confluency, representing cytotoxicity, was measured with a Monocellator.™ 3) Metaphase frequency was calculated by counting 500 cells in each dish. 4) When the number of aberrations in a cell was more than 9, the cell was scored as having 10 aberrations. 5) Others, such as attenuation and premature chromosome condensation, were excluded from the number of structural aberrations. 6) Two hundred cells were analyzed in each group.

*, Significantly different from the negative control at p<0.01 (one-side) by Fisher's exact probability test.

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

Review of Study No. R-297995-TF-053-L (CRO Study No. G-07-071) from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Study title: Micronucleus Test of RSC-297995 Monotosylate With Rat Bone Marrow

Cells

Study no:	R-297995-TF-053-L
Study report location:	Electronic submission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 4, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RSC-297995, lot no. A81001, 97.5% purity

Key Study Findings

RSC-297995 monotosylate neither induced micronuclei in rat bone marrow cells nor suppressed their proliferation.

Methods

Doses in definitive study:	250, 500, 1000 and 2000 mg/kg/day were administered in 24-hour
Frequency of dosing:	Twice a day
Route of administration:	Oral
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5 w/v% methylcellulose 400 cP solution (0.5 w/v% MC)
Species/Strain:	Male Sprague-Dawley rats [Crl:CD(SD), SPF]
Number/Sex/Group:	5 rats
Satellite groups:	None
Basis of dose selection:	The sponsor stated that the doses were selected on the basis of the earlier 2-week oral toxicity study, and the high doses caused significant decreases of body weight and food consumption in males of the 200 and 1000 mg/kg groups. Therefore, the highest dose was fixed at 2000 mg/kg/day.
Negative control:	Vehicle control (0.5 w/v% MC)
Positive control:	Cyclophosphamide monohydrate (CP, 20 mg/kg)

Study Validity

The study was valid as the vehicle control group had less than 0.2% micronucleated PCEs and the positive control group had a statistically significant higher mean percent MNPCEs than the vehicle control group at 1% level, which are within the fluctuation range of their historical data (Mean ± 3 S.D.) in conducting facility: (b) (4). One thousand erythrocytes (ERYs) per rat (500 ERYs per person) were observed by two persons and the number of PCEs was recorded.

Results

There were no rats showing abnormal signs after dosing. However, all of the rats in the RSC-297995 monotosylate treated group showed a decrease in body weight especially at

beginning of dosing period. Decreases of mean body weight in 250, 500, 1000 and 2000 mg/kg/day groups were -6.1%, -5.9%, -6.3% and -7.2%, respectively vs. 3% increase of control group.

The frequency of MNPCEs in any RSC-297995 monotosylate treated group up to 2000 mg/kg/day was not significantly higher than that in the negative control group and a dose dependency was not observed. However, a significant increase in the frequency of MNPCEs was observed in the positive control group. It suggested that this test system was appropriate for an evaluation of the clastogenic effect and/or spindle toxicity *in vivo*. The proportion of PCEs in the total ERYs in any RSC-297995 monotosylate treated group and the positive control group was not significantly different from that of the negative control group. Thus, dosing of the test article does not suppress the proliferation of bone marrow cells. These results demonstrated that RSC-297995 monotosylate neither induces micronuclei in rat bone marrow cells nor suppresses their proliferation.

Table 1 Results of the micronucleus test in male CD(SD) rats after double oral administrations of RSC-297995 monotosylate

Group	Animal No.	% of MNPCEs[a]	% of PCEs in ERYs[b]
Negative control 0.5 w/v% MC 20 mL/kg/day	1	0.05	61.8
	2	0.20	41.8
	3	0.15	51.9
	4	0.15	62.3
	5	0.00	56.0
	Total	11 10000	2738 5000
	Mean ± S.D.	0.11 ± 0.08	54.8 ± 8.4
	Min. - Max.	0.00 - 0.20	41.8 - 62.3
RSC-297995 monotosylate 500 mg/kg/day	11	0.20	61.0
	12	0.10	58.5
	13	0.30	46.3
	14	0.15	60.8
	15	0.00	59.0
	Total	15 10000	2856 5000
	Mean ± S.D.	0.15 ± 0.11	57.1 ± 6.1
	Min. - Max.	0.00 - 0.30	46.3 - 61.0
RSC-297995 monotosylate 1000 mg/kg/day	16	0.30	58.4
	17	0.05	63.5
	18	0.15	45.7
	19	0.05	59.7
	20	0.15	58.0
	Total	14 10000	2853 5000
	Mean ± S.D.	0.14 ± 0.10	57.1 ± 6.7
	Min. - Max.	0.05 - 0.30	45.7 - 63.5
RSC-297995 monotosylate 2000 mg/kg/day	21	0.00	46.4
	22	0.10	45.2
	23	0.05	57.0
	24	0.05	61.9
	25	0.10	63.2
	Total	6 10000	2737 5000
	Mean ± S.D.	0.06 ± 0.04	54.7 ± 8.5
	Min. - Max.	0.00 - 0.10	45.2 - 63.2
Positive control CP 20 mg/kg	26	2.20	53.4
	27	2.95	47.8
	28	2.95	53.3
	29	2.50	57.7
	30	2.15	65.4
	Total	255 10000 *	2776 5000
	Mean ± S.D.	2.55 ± 0.39	55.5 ± 6.5
	Min. - Max.	2.15 - 2.95	47.8 - 65.4

a, % of micronucleated polychromatic erythrocytes in polychromatic erythrocytes observed
, % of polychromatic erythrocytes in erythrocytes observed
0.5 w/v% MC, 0.5 w/v% Methylcellulose 400 cP solution
CP, Cyclophosphamide monohydrate (single oral administration)
*, Significantly higher than the negative control at 1% level.

7.4 Other Genetic Toxicity Studies

Study title: Bacterial Reverse Mutation Test of (b) (4) an Impurity of S-297995

Study no.: (b) (4)
Study report location: EDR
Conducting laboratory and location: Shionogi & Co., Ltd., Osaka, Japan
Date of study initiation: July 7, 2014
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: (b) (4) Lot # 2014-6159-001-01, 96.3% (on anhydrous

basis)

Key Study Findings

Under the conditions tested, (b) (4) was negative in the *in vitro* bacterial reverse mutation assay using the plate incorporation method both with and without metabolic activation.

Methods

Strains:	S. typhimurium strains TA98, TA100, TA1535, and TA1537 E. coli strain WP2uvrA
Concentrations in definitive study:	20.6, 61.7, 185, 556, 1670, and 5000 µg/plate
Basis of concentration selection:	Concentrations were selected based on results of a non-GLP bacterial reverse mutation test of (b) (4)
Negative control:	Dimethylsulfoxide (DMSO)
Positive control:	The positive control in the presence of S9 mix was 2-Aminoanthracene (2AA). The positive controls in the absence of S9 mix were 9-Aminoacridine hydrochloride (9AA) for tester strain TA1537, 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) for tester strains TA98, TA100, and WP2uvrA, and sodium azide (NaN_3) for tester strain TA1535.
Formulation/Vehicle:	DMSO
Incubation & sampling time:	The tester strains were incubated with the test substance and positive and negative controls using the plate incorporation method, with and without metabolic activation. S9 mix was prepared using S9 fraction from a rat liver homogenate (male SD rats treated with phenobarbital and 5,6-benzoflavone). Plates were incubated for 48 h at 37ºC before counting the number of revertant colonies.

Study Validity

The study was considered valid if the mean negative and positive control values were within acceptable historical ranges based on historical control data, the numbers of revertant colonies for the positive controls were greater than twice the number for the respective negative controls, and contamination was not detected. Based on these criteria, the study was valid. The test substance was considered to be positive if there was a ≥2-fold increase in the number of revertant colonies over the negative control in

at least 1 of the 5 tester strains, and the number of revertant colonies increases with concentration in that tester strain.

Results

In the absence of S9 mix, cytotoxicity was observed at 556 µg/plate in TA98, at 556 and 1670 µg/plate in TA1537, and at 1670 and 5000 µg/plate in WP2uvrA. In the presence of S9 mix, cytotoxicity was observed at 1670 µg/plate in TA1537 and at 5000 µg/plate for WP2uvrA. Precipitation was observed at ≥556 µg/plate in the presence and absence of S9 mix.

With one exception, two-fold or greater increases in the number of revertants (compared to the number for negative controls) did not occur when (b) (4) was incubated with any of the tester strains regardless of metabolic activation. In the presence of S9 mix, there was a 2-fold increase in the number of revertants in tester strain TA1535 at 20.6 µg/plate (b) (4) (mean number of revertants = 14), compared to the negative control (mean number of revertants = 7). However, the number of revertant colonies at the next higher concentration (61.7 µg/plate (b) (4)) was equivalent to the number in the negative control. Therefore, there was no dose-dependent increase. Furthermore, the mean colonies per plate in TA1535 at 20.6 µg/plate (b) (4) (20) was within the range of historical control values for this testing facility (range: 5-19, mean =11). Therefore, (b) (4) was concluded to be negative under the conditions of the study. Summary results are presented in the Applicant's table below

Table 1 Results of the Mutagenicity Test of (b) (4)

Metabolic activation	Test substance / Dose (μg/plate)	Number of revertants (number of colonies/plate, mean)									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		WP2*uvrA*		TA98		TA1537	
Without Activation (-S9)	Negative control	106 / 103 / 106	105	10 / 4 / 12	9	40 / 30 / 27	32	18 / 20 / 16	18	11 / 8 / 13	11
	20.6	124 / 110 / 143	126	13 / 10 / 13	12	26 / 31 / 40	32	23 / 29 / 18	23	12 / 12 / 10	11
	61.7	121 / 112 / 138	124	16 / 9 / 7	11	26 / 29 / 28	28	25 / 19 / 21	22	12 / 14 / 18	15
	185	120 / 114 / 130	121	14 / 9 / 16	13	35 / 27 / 30	31	18 / 10 / 26	18	14 / 8 / 13	12
	556 ‡	92 / 100 / 97	96	5 / 14 / 3	7	25 / 39 / 30	31	0 *† / 0 *† / 0 *†	0	0 *† / 0 *† / 0 *†	0
	1670 ‡	77 / 86 / 95	86	8 / 1 / 2	4	15 * / 14 * / 21 *	17	23 / 13 / 14	17	0 † / 0 † / 0 †	0
	5000 ‡	ND / ND / ND		ND / ND / ND		ND * / ND * / ND *		ND / ND / ND		ND / ND / ND	
With Activation (+S9)	Negative control	103 / 106 / 92	100	4 / 9 / 7	7	43 / 45 / 40	43	14 / 16 / 19	16	19 / 25 / 21	22
	20.6	88 / 123 / 112	108	9 / 20 / 12	14	37 / 34 / 36	36	33 / 20 / 32	28	16 / 22 / 30	23
	61.7	128 / 97 / 128	118	4 / 11 / 5	7	33 / 42 / 36	37	14 / 18 / 22	18	25 / 24 / 26	25
	185	109 / 130 / 135	125	6 / 11 / 10	9	50 / 37 / 44	44	26 / 32 / 25	28	19 / 15 / 21	18
	556 ‡	135 / 131 / 113	126	8 / 10 / 6	8	27 / 34 / 29	30	28 / 16 / 20	21	22 / 16 / 6	15
	1670 ‡	97 / 103 / 111	104	6 / 5 / 6	6	21 / 22 / 23	22	15 / 15 / 10	13	0 † / 0 † / 0 †	0
	5000 ‡	ND / ND / ND		ND / ND / ND		ND * / ND * / ND *		ND / ND / ND		ND / ND / ND	
Positive controls (-S9)	Substance	AF-2		NaN_3		AF-2		AF-2		9AA	
	Dose (μg/plate)	0.01		0.5		0.01		0.1		80	
	Number of colonies/plate	477 / 412 / 509	466	326 / 265 / 284	292	112 / 101 / 143	119	491 / 483 / 468	481	848 / 837 / 815	833
Positive controls (+S9)	Substance	2AA		2AA		2AA		2AA		2AA	
	Dose (μg/plate)	1		2		10		0.5		2	
	Number of colonies/plate	985 / 1110 / 812	1002	371 / 265 / 333	323	871 / 799 / 794	821	330 / 278 / 311	306	245 / 168 / 177	197

(b) (4)

*: Cytotoxicity (reduction of the bacterial background lawn)
†: Cytotoxicity (reduction in the numbers of revertant colonies)
ND: No data obtained because heavy precipitates obscured counting colonies
‡: Precipitation derived from the test substance
Negative control: Dimethyl sulfoxide (DMSO, 100 μL/plate), AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide, NaN_3: Sodium azide, 9AA: 9-Aminoacridine hydrochloride, 2AA: 2-Aminoanthracene

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Study title: Bacterial Reverse Mutation Test of (b) (4) an Impurity of S-297995

Study no.:	(b) (4)
Study report location:	EDR
Conducting laboratory and location:	Shionogi & Co., Ltd, Osaka, Japan
Date of study initiation:	February 23, 2015
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) Lot # 2013-6319-019-01, 100.0% (on anhydrous basis)

Key Study Findings

Under the conditions tested, (b) (4) was negative in the *in vitro* bacterial reverse mutation assay using the plate incorporation method both with and without metabolic activation.

Methods

Strains:	S. typhimurium strains TA98, TA100, TA1535, and TA1537 E. coli strain WP2uvrA
Concentrations in definitive study:	20.6, 61.7, 185, 556, 1670, and 5000 µg/plate
Basis of concentration selection:	Concentrations were selected to include ≥4 concentrations without cytotoxicity. Concentrations were selected as 3-fold serial dilutions from a maximum concentration of 5000 µg/plate.
Negative control:	Dimethylsulfoxide (DMSO)
Positive control:	The positive control in the presence of S9 mix was 2-Aminoanthracene (2AA). The positive controls in the absence of S9 mix were 9-Aminoacridine hydrochloride (9AA) for tester strain TA1537, 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) for tester strains TA98, TA100, and WP2uvrA, and sodium azide (NaN_3) for tester strain TA1535.
Formulation/Vehicle:	DMSO
Incubation & sampling time:	The tester strains were incubated with the test substance and positive and negative controls using the plate incorporation method, with and without S9 mix. S9 mix was prepared using S9 fraction from a rat liver homogenate (male SD rats treated with phenobarbital and 5,6-benzoflavone). Plates were incubated for 48 h at 37ºC before counting the number of revertant colonies.

Study Validity

Criteria for a valid study were as follows: 1) the mean negative and positive control values were within acceptable historical ranges based on historical control data, 2) the numbers of revertant colonies for the positive controls were ≥2-fold greater than the number for the respective negative controls, and 3) contamination was not detected. Based on these criteria, the study was valid. The test substance was considered to be positive if there was a ≥2-fold increase in the number of revertant colonies over the negative control in at least 1 of the 5 tester strains, and when the number of revertant colonies increases with concentration in that tester strain.

Results

In this study, cytotoxicity was not observed in any of the tester strains in the presence or absence of S9 mix at up to 5000 µg/plate [redacted] (b) (4) Precipitation was observed at ≥61.7 µg/plate in the absence of S9 mix and at ≥556 µg/plate in the presence of S9 mix. A two-fold or greater increase in the number of revertant colonies did not occur in any of the tester strains at up to 5000 µg/plate regardless of metabolic activation. Therefore, the test compound was concluded to be negative under the conditions of the study. Summary results of the mutagenicity test are presented in the Applicant's table below.

Table 1 Result of the Mutagenicity Test of [redacted] (b) (4)

Metabolic activaton	Test substance Dose (µg/plate)	Number of revertants (number of colonies/plate, mean)				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	$WP2_{uvrA}$	TA98	TA1537
Without Activation (-S9)	Negative control	135 127 (120) 99	12 14 (13) 12	26 37 (32) 33	17 11 (14) 13	14 13 (13) 12
	20.6	131 116 (125) 128	16 18 (16) 15	33 37 (35) 36	22 15 (19) 21	6 10 (9) 10
	61.7 †	111 116 (118) 127	11 7 (10) 13	42 43 (38) 29	21 9 (19) 26	8 9 (8) 8
	185 ‡	85 119 (115) 140	7 9 (7) 6	33 37 (36) 37	13 16 (14) 13	3 10 (6) 5
	556 ‡	109 101 (106) 108	6 6 (8) 11	43 39 (39) 34	15 7 (12) 14	8 14 (10) 9
	1670 ‡	108 110 (105) 98	8 15 (11) 9	22 22 (20) 17	11 16 (13) 11	5 6 (6) 6
	5000 ‡	80 92 (86) 86	11 10 (12) 15	35 24 (27) 22	8 7 (8) 8	10 8 (9) 9
With Activation (+S9)	Negative control	121 125 (121) 117	20 22 (18) 13	27 29 (31) 37	12 32 (23) 25	10 18 (12) 9
	20.6	134 138 (134) 130	20 19 (19) 18	29 36 (36) 42	32 26 (27) 24	10 12 (13) 16
	61.7	122 122 (121) 119	10 22 (15) 12	33 46 (40) 42	21 27 (24) 23	9 16 (12) 11
	185	113 120 (119) 123	14 28 (20) 19	41 54 (46) 42	30 29 (26) 20	19 10 (15) 15
	556 ‡	130 154 (145) 151	13 15 (14) 13	39 40 (39) 37	20 14 (17) 17	15 10 (13) 13
	1670 ‡	125 107 (120) 128	18 21 (16) 8	37 27 (30) 27	25 16 (23) 28	14 12 (12) 10
	5000 ‡	95 111 (105) 110	13 20 (18) 20	26 25 (27) 31	13 25 (19) 20	18 11 (15) 15
Positive controls (-S9)	Substance	AF-2	NaN_3	AF-2	AF-2	9AA
	Dose (µg/plate)	0.01	0.5	0.01	0.1	80
	Number of colonies/plate	455 434 (445)	467 441 (454)	198 193 (196)	402 434 (418)	1227 1221 (1224)
Positive controls (+S9)	Substance	2AA	2AA	2AA	2AA	2AA
	Dose (µg/plate)	1	2	10	0.5	2
	Number of colonies/plate	1380 1310 (1345)	521 497 (509)	867 909 (888)	433 450 (442)	319 303 (311)

†: Precipitation derived from the test substance after the overlaid agars solidify
‡: Precipitation derived from the test substance after the overlaid agars solidify and incubation
Negative control: Dimethyl sulfoxide (DMSO, 100 µL/plate), AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide, NaN_3: Sodium azide, 9AA: 9-Aminoacridine hydrochloride, 2AA: 2-Aminoanthracene

Study title: Bacterial Reserve Mutation Test of (b) (4) an Impurity of S-297995

Study no.:	(b) (4)
Study report location:	EDR
Conducting laboratory and location:	Shionogi & Co., Ltd, Osaka, Japan
Date of study initiation:	February 23, 2015
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) Lot # 2013-6319-025-01, 90.9% (on anhydrous basis)

Key Study Findings

Under the conditions tested, (b) (4) tested negative in the *in vitro* bacterial reverse mutation assay using the plate incorporation method both with and without metabolic activation.

Methods

Strains:	S. typhimurium strains TA98, TA100, TA1535, and TA1537 E. coli strain WP2uvrA
Concentrations in definitive study:	20.6, 61.7, 185, 556, 1670, and 5000 µg/plate
Basis of concentration selection:	Concentrations were selected to include ≥4 concentrations without cytotoxicity. Concentrations were selected as 3-fold serial dilutions from a maximum concentration of 5000 µg/plate.
Negative control:	Dimethylsulfoxide (DMSO)
Positive control:	The positive control in the presence of S9 mix was 2-Aminoanthracene (2AA). The positive controls in the absence of S9 mix were 9-Aminoacridine hydrochloride (9AA) for tester strain TA1537, 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) for tester strains TA98, TA100, and WP2uvrA, and sodium azide (NaN_3) for tester strain TA1535.
Formulation/Vehicle:	DMSO
Incubation & sampling time:	The tester strains were incubated with the test substance and positive and negative controls using the plate incorporation method, in the presence and absence of metabolic activation. S9 mix was prepared using S9 fraction from a rat liver homogenate (male SD rats treated with phenobarbital and 5,6-benzoflavone). Plates were incubated for 48 h at 37ºC before counting the number of revertant colonies.

Study Validity

Criteria for a valid study were the following: 1) the mean negative and positive control values were within acceptable ranges based on historical control data, 2) the numbers of revertant colonies for the positive controls were two-fold or greater than numbers for the respective negative controls, and 3) contamination was not detected. Based on these criteria, the current study was considered to be valid. The test substance was judged to be positive if there was a ≥2-fold increase in the number of revertant colonies over the negative control in at least 1 of the 5 tester strains, and when the number of revertant colonies increases with concentration in that tester strain.

Results

Cytotoxicity was not observed at any concentration in any strain regardless of metabolic activation, while precipitation was observed at ≥185 µg/plate without metabolic activation and at ≥1670 µg/plate in the presence of metabolic activation. The highest concentration (5000 µg/plate) was not evaluated since precipitation interfered with the colony counting. In regard to the number of revertants, no two-fold or greater increases were observed following incubation with any concentration of the test compound (relative to the negative control) either in the presence or absence of metabolic activation. Thus, the test compound tested negative under the conditions of the study. Summary results are presented in the Applicant's table below.

Table 1 Result of the Mutagenicity Test of (b) (4)

Metabolic activaton	Test substance Dose (µg/plate)	Number of revertants (number of colonies/plate, mean)				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
Without Activation (-S9)	Negative control	131 118 (127) 133	10 8 (9) 9	40 39 (39) 37	21 32 (26) 25	13 6 (11) 15
	20.6	130 138 (137) 144	6 7 (8) 10	38 41 (38) 36	24 30 (25) 20	11 12 (13) 15
	61.7	132 161 (137) 118	10 11 (11) 12	36 42 (42) 47	26 13 (22) 28	18 7 (12) 11
	185 †	128 123 (126) 128	6 9 (9) 12	35 34 (36) 38	20 15 (19) 22	8 13 (10) 8
	556 ‡	124 114 (120) 121	11 6 (9) 9	38 40 (34) 23	21 18 (18) 14	12 12 (14) 18
	1670 ‡	131 118 (122) 116	7 10 (8) 8	18 36 (25) 22	15 26 (18) 12	13 18 (14) 11
	5000 ‡	ND	ND	ND	ND	ND
With Activation (+S9)	Negative control	125 140 (132) 132	9 13 (11) 11	42 37 (39) 38	25 16 (23) 29	12 14 (14) 16
	20.6	162 140 (151) TE	10 14 (11) 8	39 50 (45) 46	24 33 (26) 22	8 12 (11) 13
	61.7	160 174 (160) 147	16 14 (15) 14	44 52 (42) 30	26 29 (29) 33	10 14 (12) 12
	185	154 188 (165) 154	14 8 (10) 8	43 49 (46) 47	29 35 (33) 35	9 8 (9) 11
	556	151 168 (157) 152	15 18 (17) 18	48 34 (42) 44	32 28 (29) 28	10 9 (10) 11
	1670 ‡	162 151 (152) 143	7 12 (10) 12	32 49 (41) 41	26 23 (25) 27	6 12 (9) 10
	5000 ‡	ND	ND	ND	ND	ND
Positive controls (-S9)	Substance	AF-2	NaN_3	AF-2	AF-2	9AA
	Dose (µg/plate)	0.01	0.5	0.01	0.1	80
	Number of colonies/plate	471 477 (474)	324 369 (347)	159 168 (164)	481 395 (438)	918 992 (955)
Positive controls (+S9)	Substance	2AA	2AA	2AA	2AA	2AA
	Dose (µg/plate)	1	2	10	0.5	2
	Number of colonies/plate	1480 1390 (1435)	499 504 (502)	904 912 (908)	509 407 (458)	268 278 (273)

†: Precipitation derived from the test substance after the overlaid agars solidify (TA1535, TA1537 and WP2*uvrA*)
‡: Precipitation derived from the test substance after the overlaid agars solidify and incubation (all tester strains)
ND: No data obtained because of heavy precipitates
TE: No data obtained because of technical error
Negative control: Dimethyl sulfoxide (DMSO, 100 µL/plate), AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide, NaN_3: Sodium azide, 9AA: 9-Aminoacridine hydrochloride, 2AA: 2-Aminoanthracene

Study title: Bacterial Reverse Mutation Test of [redacted]

Study no.:	[redacted] (Report translated from Japanese)
Study report location:	EDR
Conducting laboratory and location:	[redacted]
Date of study initiation:	October 12, 2010
GLP compliance:	According to the Applicant, the study was conducted according to GLP regulations for [redacted] The study report states that the study was conducted in compliance with [redacted]
QA statement:	Yes
Drug, lot #, and % purity:	[redacted] Lot # 00901K, >98%

Key Study Findings

Under the conditions tested, [redacted] tested negative in the *in vitro* bacterial reverse mutation assay using the pre-incubation method both with and without metabolic activation.

Methods

Strains:	S. typhimurium TA98, TA100, TA1535, and TA1537 E. coli WP2uvrA
Concentrations in definitive study:	313, 625, 1250, 2500, 5000 µg/plate
Basis of concentration selection:	In a dose-finding test, concentrations of 1.2, 4.9, 20, 78, 313, 1250, and 5000 µg/plate were tested. Since no cytotoxicity was observed at up to 5000 µg/plate, this was selected as the maximum concentration for the definitive study.
Negative control:	Dimethyl sulfoxide (DMSO)
Positive control:	In the presence of metabolic activation, 2-Aminoanthracene (2AA) was used as the positive control. In the absence of metabolic activation, the positive controls were 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) for tester strains TA98, TA100, and WP2uvrA, sodium azide (NaN_3) for tester strain TA1535, and 6-Chloro-9-[3-(2-chloroethylamino)-propylamino] -2-methoxyacridine Dihydrochloride (ICR-191) for tester strain TA1537.
Formulation/Vehicle:	DMSO
Incubation & sampling time:	The tester strains were incubated with the test substance and positive and negative controls using the pre-incubation method, in the presence and absence of metabolic activation. Briefly, tester strains were incubated with the test substance for 20 min at 37°C prior to addition of agar. Plates were then incubated for 48 h at 37ºC before counting the number of revertant colonies. S9 mix was prepared with S9 fraction from a rat liver homogenate (male SD rats treated with phenobarbital and 5,6-benzoflavone).

Study Validity

The study was considered to be valid since the numbers of revertant colonies for the negative controls were within the acceptable range based on historical control data, the positive controls induced over 2-fold the number of revertant colonies (compared to the negative control), and there was no contamination observed. The test substance was considered to be positive if the number of revertant colonies was increased by 2-fold or

more following treatment with the test substance, compared to the negative control, and concentration dependency and reproducibility were observed.

Results

In the dose-finding and definitive test, no bacterial growth inhibition or precipitation were observed in any of the tester strains regardless of metabolic activation. Furthermore, the numbers of revertant colonies in all strains incubated with up to 5000 µg/plate (b) (4) were less than twice those in negative controls. As such, the test compound was concluded to be negative under the conditions of the study. Summary data from the definitive test are presented in the Applicant's tables below.

Table 2 Study No. : (b) (4)

Results of Test (Main test)

Name of the test substance: (b) (4)

Test period		From October 28, 2010 to November 1, 2010				
With (+) or without (−) S9 Mix	Dose (µg/plate)	Number of revertant colonies/plate				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
S9 Mix (−)	Negative control	93 108 102 (101)	11 12 13 (12)	36 43 43 (41)	18 19 16 (18)	7 6 7 (7)
	313	100 101 (101)	14 12 (13)	43 42 (43)	18 18 (18)	7 6 (7)
	625	96 86 (91)	11 8 (10)	42 41 (42)	17 18 (18)	7 7 (7)
	1250	83 101 (92)	11 11 (11)	43 40 (42)	19 16 (18)	8 8 (8)
	2500	103 104 (104)	11 12 (12)	45 39 (42)	15 15 (15)	7 7 (7)
	5000	105 105 (105)	9 10 (10)	38 42 (40)	17 15 (16)	6 5 (6)
S9 Mix (+)	Negative control	99 98 104 (100)	12 12 9 (11)	49 41 47 (46)	33 30 30 (31)	18 19 17 (18)
	313	93 111 (102)	10 11 (11)	48 44 (46)	27 24 (26)	19 17 (18)
	625	98 84 (91)	10 10 (10)	45 41 (43)	29 24 (27)	15 17 (16)
	1250	101 101 (101)	11 8 (10)	44 44 (44)	24 26 (25)	15 13 (14)
	2500	105 104 (105)	12 9 (11)	49 49 (49)	25 28 (27)	19 18 (19)
	5000	116 116 (116)	12 9 (11)	40 48 (44)	36 31 (34)	18 17 (18)
Positive control without S9 Mix	Name	AF-2	NaN_3	AF-2	AF-2	ICR-191
	Dose (µg/plate)	0.01	0.5	0.01	0.1	1.0
	Number of colonies/plate	502 536 (519)	442 443 (443)	125 122 (124)	499 484 (492)	2344 1752 (2048)
Positive control with S9 Mix	Name	2AA	2AA	2AA	2AA	2AA
	Dose (µg/plate)	1.0	2.0	10.0	0.5	2.0
	Number of colonies/plate	843 878 (861)	272 281 (277)	1122 1113 (1118)	378 393 (386)	133 106 (120)

[Notes]
Positive controls:
AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
NaN_3: Sodium azide
ICR-191: 6-Chloro-9-[3-(2-chloroethylamino)-propylamino]-2-methoxyacridine dihydrochloride
2AA: 2-Aminoanthracene
( ): Mean number of colonies

Study title: Reverse Mutation Test with Bacteria on [redacted] a Synthetic Intermediate of S-297995

Study no.:	[redacted] (Report translated from [redacted]
Study report location:	EDR
Conducting laboratory and location:	[redacted]
Date of study initiation:	October 22, 2010
GLP compliance:	According to the Applicant, the study was conducted according to GLP regulations [redacted] The study report states that the study was conducted in compliance with the following GLP principle [redacted]
QA statement:	
Drug, lot #, and % purity:	[redacted] Lot # 2010-6090-190-01, 98.77%

Key Study Findings

Under the conditions tested, [redacted] tested negative in the *in vitro* bacterial reverse mutation assay using the pre-incubation method in the presence and absence of metabolic activation.

Methods

Strains:	S. typhimurium strains TA98, TA100, TA1535, and TA1537 E. Coli strain WP2uvrA
Concentrations in definitive study:	156, 313, 625, 1250, 2500, and 5000 µg/plate
Basis of concentration selection:	In a dose-finding test, concentrations of 4.88, 19.5, 78.1, 313, 1250, and 5000 µg/plate were tested. Bacterial growth inhibition was observed at 5000 µg/plate under all test conditions except for tester strain WP2uvrA without S9 mix. Precipitation was observed at 5000 µg/plate, with and without S9 mix.
Negative control:	Dimethyl sulfoxide (DMSO)
Positive control:	In the presence of S9 mix, 2-aminoanthracene (2AA) was used as the positive control. In the absence of S9 mx, positive controls were 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) for tester strains TA98, TA100, and WP2uvrA, sodium azide (NaN_3) for tester strain TA1535, and ICR-191 for tester strain TA1537.
Formulation/Vehicle:	DMSO
Incubation & sampling time:	The tester strains were incubated with the test substance and positive and negative controls using the pre-incubation method in the presence and absence of metabolic activation. Briefly, tester strains were incubated with the test substance for 20 min at 37°C prior to addition of agar. Plates were then incubated for 48 h at 37ºC before counting the number of revertant colonies. S9 mix was prepared using S9 fraction from the liver of male Sprague Dawley rats administered phenobarbital and 5,6-benzoflavone.

Study Validity

The current study was considered to be valid since the numbers of revertant colonies in the positive controls were greater than twice that of negative controls, the numbers of revertant colonies in the negative and positive controls were within historical control ranges for the testing facility, and there was no evidence of contamination. Criteria for the test substance to be judged as positive were as follows: the number of revertant colonies increased to twice or more than that of the negative control and when there were concentration-related and/or reproducible responses.

Results

In the definitive test, bacterial growth inhibition was observed at ≥2500 µg/plate for all tester strains regardless of metabolic activation, with the exception of tester strain WP2uvrA without S9 mix. Precipitation was observed at the highest concentration (5000 µg/plate) both with and without S9 mix. In the dose-range finding test and definitive test, the number of revertant colonies in all strains incubated with up to 5000 µg/plate (b) (4) were less than twice that in negative controls in the presence and absence of S9 mix. Thus, the test compound was concluded to be negative under the conditions of the study. Summary results of the definitive test are presented in the Applicant's table below.

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Table 2 Results of the main test

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Test substance: (b) (4)

Test Period	From November 16, 2010 to November 18, 2010									
With(+)or without(-) S9 mix	Test substance dose (μg/plate)	Number of revertant colonies per plate								
		Base-pair substitution type						Frameshift type		
		TA100		TA1535		WP2*uvrA*		TA98		TA1537
-S9 mix	Negative control	114 105 110	(110)	11 14 13	(13)	31 26 26	(28)	18 21 18	(19)	7 8 7	(7)
	156	98 108	(103)	13 13	(13)	20 29	(25)	13 19	(16)	11 10	(11)
	313	96 121	(109)	12 10	(11)	22 21	(22)	20 15	(18)	13 6	(10)
	625	94 121	(108)	12 12	(12)	22 26	(24)	20 21	(21)	12 8	(10)
	1250	78 88	(83)	11 11	(11)	19 21	(20)	19 14	(17)	5 7	(6)
	2500	80* 66*	(73)	4* 8*	(6)	11 24	(18)	13* 21*	(17)	6* 5*	(6)
	5000 †	79* 61*	(70)	6* 4*	(5)	24 20	(22)	18* 11*	(15)	2* 3*	(3)
+S9 mix	Negative control	109 91 109	(103)	10 12 11	(11)	28 30 25	(28)	27 36 30	(31)	16 18 20	(18)
	156	102 96	(99)	10 10	(10)	35 21	(28)	19 35	(27)	23 26	(25)
	313	117 111	(114)	10 10	(10)	29 28	(29)	27 22	(25)	19 18	(19)
	625	122 111	(117)	7 12	(10)	34 20	(27)	33 34	(34)	18 14	(16)
	1250	104 96	(100)	8 10	(9)	29 27	(28)	25 23	(24)	18 19	(19)
	2500	98* 94*	(96)	8* 2*	(5)	13* 20*	(17)	17* 17*	(17)	10* 10*	(10)
	5000 †	92* 89*	(91)	5* 12*	(9)	23* 28*	(26)	26* 23*	(25)	6* 6*	(6)
Positive control -S9 mix	Chemical	AF-2		NaN_3		AF-2		AF-2		ICR-191
	Dose(μg/plate)	0.01		0.5		0.01		0.1		0.5
	Number of revertant colonies/plate	673 610	(642)	301 314	(308)	362 405	(384)	394 349	(372)	1563 1569	(1566)
Positive control +S9 mix	Chemical	2AA		2AA		2AA		2AA		2AA
	Dose(μg/plate)	1		2		10		0.5		2
	Number of revertant colonies/plate	1460 1436	(1448)	295 299	(297)	557 478	(518)	295 324	(310)	571 556	(564)

[Notes]
( ): The mean number of colonies per plate.
†: Precipitation of the test substance was observed in all plates for each tester strain.
*: Bacterial growth inhibition was observed.
AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
NaN_3: Sodium azide
2AA: 2-Aminoanthracene
(b) (4)
Negative control: Dimethyl sulfoxide

Study title: Reverse Mutation Test with Bacteria on [redacted] Synthetic Intermediates of S-297995

Study no.:	[redacted] (Report translated from [redacted])
Study report location:	EDR
Conducting laboratory and location:	[redacted]
Date of study initiation:	August 25, 2011
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	(1) [redacted], Lot # 2007-6016-071-01, Purity not specified (2) [redacted], Lot # 2011-6004-071-01, Purity not specified (3) [redacted], Lot # 2010-6008-060-01, Purity not specified (4) [redacted], Lot # 2010-6127-099-02, Purity not specified (5) [redacted], Lot # 2011-6036-054-01, Purity not specified

Key Study Findings

Under the conditions of the study, [redacted] tested negative in the *in vitro* bacterial reverse mutation assay using the pre-incubation method both with and without metabolic activation. However, [redacted] tested positive in all tester strains both with and without metabolic activation.

Methods

Strains:	S. typhimurium strains TA98, TA100, TA1535, and TA1537 E. coli strain WP2uvrA
Concentrations in definitive study:	(b) (4) 313, 625, 1250, 2500, and 5000 µg/plate for all tester strains, with and without S9 mix (b) (4): -S9 mix: 19.5, 39.1, 78.1, 156, 313, 625, 1250, 2500, and 5000 µg/plate for tester strain TA100; 78.1, 156, 313, 625, 1250, 2500, and 5000 µg/plate for tester strains TA98, TA1535, and WP2uvrA; 4.88, 9.77, 19.5, 39.1, 78.1, 156, 313, 625, 1250, 2500, 5000 µg/plate for tester strain TA1537 +S9 mix: 78.1, 156, 313, 625, 1250, 2500, and 5000 µg/plate for tester strains TA98, TA100, TA1535, and WP2uvrA; 19.5, 39.1, 78.1, 156, 313, 625, 1250, 2500, and 5000 µg/plate for TA1537 (b) (4): -S9 mix: 313 625, 1250, 2500, 5000 µg/plate +S9 mix: 39.1, 78.1, 156, 313, 625, 1250 µg/plate
Basis of concentration selection:	In a dose-range finding test, each of the compounds were tested at concentrations of 4.88, 19.5, 78.1, 313, 1250, and 5000 µg/plate. Concentrations for the definitive test were selected based on cytotoxicity, precipitation, or an increase in the number of revertant colonies >2-times that in the negative control. Findings from the dose-range finding test are discussed under Results.
Negative control:	Dimethyl sulfoxide (DMSO)
Positive control:	In the presence of S9 mix, 2-Aminoanthracene (2AA) was used as a positive control for all tester strains. In the absence of S9 mix, positive controls were 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) for tester strains TA98, TA100, and WP2uvrA, sodium azide (NaN_3) for tester strain TA1535, and ICR-191 for tester strain

	TA1537.
Formulation/Vehicle:	DMSO
Incubation & sampling time:	The study was conducted using the pre-incubation method, in the presence and absence of S9 mix. Briefly, tester strains were incubated with the test substance and positive and negative controls for 20 min at 37°C prior to addition of agar. Plates were then incubated for 48 h at 37ºC before counting the number of revertant colonies. S9 mix was prepared using S9 fraction from the livers of male SD rats administered phenobarbital and 5,6-benzoflavone.

Study Validity

The study was considered to be valid based upon the following: (1) the numbers of revertant colonies in the negative and positive controls were within historical control ranges at the testing facility, (2) the numbers of revertant colonies in the positive controls were >2-times those in respective negative controls, and (3) there was no evidence of contamination. Based on these criteria, the current study was considered to be valid. Criteria for a test substance to be considered positive were as follows: the number of revertant colonies increased to ≥2-time that in the negative control and when the responses were concentration-dependent and/or reproducible.

Results

Results from the dose-range finding test and definitive test for each test compound are presented below.

(b) (4) In the dose-range finding and definitive tests, neither bacterial growth inhibition nor precipitation were observed in any of the tester strains at up to 5000 µg/plate, regardless of metabolic activation. In both the dose-range finding and definitive tests, the number of revertant colonies at all (b) (4) concentrations were less than twice the number in negative controls under all test conditions. Results from the definitive test are presented in the Applicant's table below.

Table 6 Results of the main test (b) (4)

Test substance: (b) (4)

Test Period		From September 16, 2011 to September 20, 2011				
With(+)or without(-) S9 mix	Test substance dose (μg/plate)	Number of revertant colonies per plate				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
-S9 mix	Negative control	113 107 (110) 110	8 14 (12) 14	22 22 (22) 22	20 13 (17) 17	8 10 (10) 11
	313	116 111 (114)	14 11 (13)	25 13 (19)	16 22 (19)	10 8 (9)
	625	103 119 (111)	12 10 (11)	13 24 (19)	17 19 (18)	10 5 (8)
	1250	107 105 (106)	11 18 (15)	26 25 (26)	18 28 (23)	7 7 (7)
	2500	92 111 (102)	12 13 (13)	28 25 (27)	21 26 (24)	5 4 (5)
	5000	90 73 (82)	7 18 (13)	18 22 (20)	15 15 (15)	6 5 (6)
+S9 mix	Negative control	111 116 (114) 115	20 11 (13) 7	35 39 (36) 33	34 21 (30) 34	21 19 (19) 18
	313	119 113 (116)	11 8 (10)	18 29 (24)	33 35 (34)	15 8 (12)
	625	140 104 (122)	13 15 (14)	26 21 (24)	31 31 (31)	14 19 (17)
	1250	110 103 (107)	18 18 (18)	24 24 (24)	25 33 (29)	10 15 (13)
	2500	150 127 (139)	15 19 (17)	13 21 (17)	27 32 (30)	17 17 (17)
	5000	90 53 (72)	7 4 (6)	12 11 (12)	26 22 (24)	24 7 (16)
Positive control -S9 mix	Chemical	AF-2	NaN_3	AF-2	AF-2	ICR-191
	Dose(μg/plate)	0.01	0.5	0.01	0.1	0.5
	Number of revertant colonies/plate	600 678 (639)	402 362 (382)	334 321 (328)	573 573 (573)	1131 1190 (1161)
Positive control +S9 mix	Chemical	2AA	2AA	2AA	2AA	2AA
	Dose(μg/plate)	1	2	10	0.5	2
	Number of revertant colonies/plate	989 921 (955)	199 201 (200)	724 605 (665)	318 345 (332)	184 193 (189)

[Notes]
( ): The mean number of colonies per plate.
AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
NaN_3: Sodium azide
2AA: 2-Aminoanthracene
(b) (4) synthetic intermediates of S-297995
Negative control: Dimethyl sulfoxide

(b) (4) In the dose-range finding test, precipitation was observed at 5000 μg/plate, in the presence and absence of metabolic activation. In the definitive test, precipitation was observed at ≥2500 both with and without S9 mix. In both the dose-range finding and definitive tests, the numbers of revertant colonies at al (b) (4) concentrations were less than twice the number in negative controls under all test conditions. Results from the definitive test are presented in the Applicant's table below.

Table 7 Results of the main test (b) (4)

Test substance (b) (4)

Test Period		From September 16, 2011 to September 20, 2011				
With(+)or without(-) S9 mix	Test substance dose (μg/plate)	Number of revertant colonies per plate				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
-S9 mix	Negative control	123 94 (101) 87	12 10 (11) 10	38 24 (34) 39	21 15 (18) 18	12 2 (7) 8
	313	91 106 (99)	6 4 (5)	18 31 (25)	13 17 (15)	8 8 (8)
	625	80 99 (90)	11 5 (8)	20 24 (22)	15 21 (18)	10 5 (8)
	1250	87 103 (95)	12 11 (12)	14 26 (20)	18 13 (16)	8 6 (7)
	2500 †	57 73 (65)	9 7 (8)	31 24 (28)	17 12 (15)	7 4 (6)
	5000 †	80 71 (76)	8 7 (8)	15 13 (14)	10 18 (14)	4 8 (6)
+S9 mix	Negative control	110 93 (108) 122	6 13 (9) 7	33 46 (41) 43	35 31 (35) 38	18 17 (16) 12
	313	135 110 (123)	11 6 (9)	43 24 (34)	31 40 (36)	25 19 (22)
	625	132 105 (119)	6 7 (7)	36 45 (41)	31 20 (26)	15 17 (16)
	1250	91 98 (95)	6 12 (9)	24 38 (31)	22 24 (23)	15 14 (15)
	2500 †	86 94 (90)	9 10 (10)	30 25 (28)	26 22 (24)	9 16 (13)
	5000 †	81 70 (76)	7 9 (8)	23 17 (20)	28 29 (29)	10 12 (11)
Positive control -S9 mix	Chemical	AF-2	NaN_3	AF-2	AF-2	ICR-191
	Dose(μg/plate)	0.01	0.5	0.01	0.1	0.5
	Number of revertant colonies/plate	793 726 (760)	320 329 (325)	329 333 (331)	526 539 (533)	1225 1153 (1189)
Positive control +S9 mix	Chemical	2AA	2AA	2AA	2AA	2AA
	Dose(μg/plate)	1	2	10	0.5	2
	Number of revertant colonies/plate	1180 1267 (1224)	308 272 (290)	686 818 (752)	358 358 (358)	175 190 (183)

[Notes]
( ): The mean number of colonies per plate.
†: Precipitation of the test substance was observed in all plates for each tester strain.
AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
NaN_3: Sodium azide
2AA: 2-Aminoanthracene
(b) (4) synthetic intermediates of S-297995
Negative control: Dimethyl sulfoxide

(b) (4) In the dose-finding and definitive tests, bacterial growth inhibition and precipitation were not noted under any of the testing conditions. However, in both the dose-finding and definitive tests, the numbers of revertant colonies in the (b) (4) treatment groups were increased to 2-fold or more than the numbers in respective negative control groups, in all tester strains both with and without S9 mix.

In the dose-finding test, in the absence of S9 mix, the numbers of revertants were twice or more than that in respective negative controls at ≥313 µg/plate in strains TA100, TA1535, WP2uvrA, and TA98, and ≥19.5 µg/plate in strain TA1537. In the dose-finding test with S9 mix, the numbers of revertants were twice or more than that in respective negative controls at ≥313 µg/plate in strains TA100 and WP2uvrA, at ≥1250 in strains TA1535 and TA98, and at ≥78.1 µg/plate in strain TA1537.

In the definitive test without S9 mix, the numbers of revertants were twice or more than that in respective negative controls at ≥78.1 µg/plate in strain TA100, ≥313 µg/plate in strain TA1535, ≥625 µg/plate in strain WP2uvrA, ≥156 µg/plate in strain TA98, and ≥9.77 µg/plate in strain TA1537. In the presence of S9 mix, the numbers of revertants were twice or more than that in respective negative controls at ≥156 µg/plate in strain TA100, ≥313 µg/plate in strain TA1535, ≥625 µg/plate in strains WP2uvrA and TA98, and ≥78.1 µg/plate in strain TA1537.

Based on the observed increases in the number of revertants in the (b) (4) treatment groups (compared to those in negative controls), the dose-relationship of the observed increases, and the reproducibility of this finding, (b) (4) was judged to be positive under the conditions of the study. The highest relative mutagenic activity (calculated as the difference in the mean number of revertant colonies in the (b) (4) treatment group and the mean number of revertants in the negative control divided by the dose) was reported to be 1.47 x 10^3 revertants/mg. The Applicant's tables below summarize the findings of the dose-finding and definitive tests for (b) (4) and the relative mutagenic activity calculated for (b) (4)

Table 3 Results of the dose-range finding test (b) (4)

Test substance (b) (4)

Test Period	From August 30, 2011 to September 2, 2011					
With(+)or without(-) S9 mix	Test substance dose (μg/plate)	Number of revertant colonies per plate				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
-S9 mix	Negative control	113 112 (108) 99	12 11 (10) 8	17 29 (21) 17	19 24 (19) 15	6 7 (8) 10
	4.88	109 139 (124) 124	13 10 (12) 13	25 19 (22) 25	13 22 (18) 18	14 11 (13) 12
	19.5	127 124 (126) 128	6 13 (10) 11	26 32 (29) 35	18 20 (19) 19	12 19 (16) 17
	78.1	216 214 (215) 214	12 8 (10) 10	28 28 (28) 28	31 18 (25) 26	29 29 (29) 29
	313	549 503 (526) 526	35 27 (31) 31	48 47 (48) 49	42 33 (38) 39	94 81 (88) 89
	1250	979 1001 (990) 1001	75 58 (67) 67	82 84 (83) 84	100 98 (99) 97	212 189 (201) 203
	5000	1199 1138 (1169) 1171	94 124 (109) 109	206 196 (201) 201	202 220 (211) 211	457 469 (463) 463
+S9 mix	Negative control	123 121 (114) 99	6 10 (9) 10	20 27 (25) 28	33 29 (32) 34	14 12 (12) 11
	4.88	82 113 (98) 98	14 8 (11) 11	42 18 (30) 30	32 27 (30) 31	13 13 (13) 13
	19.5	113 112 (113) 114	14 10 (12) 12	41 28 (35) 35	40 29 (35) 36	13 25 (19) 19
	78.1	167 156 (162) 162	3 6 (5) 6	43 35 (39) 39	35 33 (34) 34	31 33 (32) 32
	313	553 485 (519) 519	17 17 (17) 17	41 59 (50) 58	73 36 (55) 37	80 66 (73) 73
	1250	1172 644 (908) 1172	36 32 (34) 34	102 110 (106) 110	145 140 (143) 144	230 220 (225) 225
	5000	1917 1326 (1622) 1622	88 79 (84) 84	262 265 (264) 265	211 202 (207) 207	577 518 (548) 548
Positive control -S9 mix	Chemical	AF-2	NaN_3	AF-2	AF-2	ICR-191
	Dose(μg/plate)	0.01	0.5	0.01	0.1	0.5
	Number of revertant colonies/plate	786 779 (783) 779	259 243 (251) 251	334 342 (338) 338	462 542 (502) 502	1313 1269 (1291) 1291
Positive control +S9 mix	Chemical	2AA	2AA	2AA	2AA	2AA
	Dose(μg/plate)	1	2	10	0.5	2
	Number of revertant colonies/plate	943 988 (966) 966	248 250 (249) 249	655 628 (642) 642	390 343 (367) 367	243 214 (229) 229

[Notes]
( ): The mean number of colonies per plate.
AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
NaN_3: Sodium azide
2AA: 2-Aminoanthracene
(b) (4) synthetic intermediates of S-297995
Negative control: Dimethyl sulfoxide

Table 8 Results of the main test (b) (4)

Test substance (b) (4)

Test Period	From September 16, 2011 to September 20, 2011									
With(+)or without(-) S9 mix	Test substance dose (μg/plate)	Number of revertant colonies per plate								
		Base-pair substitution type						Frameshift type		
		TA100		TA1535		WP2*uvrA*		TA98		TA1537
-S9 mix	Negative control	123 94 (101) 87		12 10 (11) 10		38 24 (34) 39		21 15 (18) 18		12 2 (7) 8
	4.88	—		—		—		—		11 13 (12)
	9.77	—		—		—		—		17 17 (17)
	19.5	139 134 (137)		—		—		—		15 11 (13)
	39.1	159 164 (162)		—		—		—		14 22 (18)
	78.1	179 227 (203)		21 17 (19)		36 38 (37)		27 21 (24)		29 34 (32)
	156	299 362 (331)		26 13 (20)		46 40 (43)		35 41 (38)		46 58 (52)
	313	480 545 (513)		36 29 (33)		28 63 (46)		53 43 (48)		107 69 (88)
	625	770 807 (789)		48 49 (49)		72 67 (70)		66 72 (69)		164 175 (170)
	1250	928 1072 (1000)		82 94 (88)		91 104 (98)		122 132 (127)		235 199 (217)
	2500	1418 1277 (1348)		97 124 (111)		170 156 (163)		188 183 (186)		326 294 (310)
	5000	1605 1394 (1500)		119 116 (118)		252 235 (244)		221 223 (222)		436 394 (415)
+S9 mix	Negative control	110 93 (108) 122		6 13 (9) 7		33 46 (41) 43		35 31 (35) 38		18 17 (16) 12
	19.5	—		—		—		—		24 22 (23)
	39.1	—		—		—		—		31 13 (22)
	78.1	179 164 (172)		11 12 (12)		40 46 (43)		27 22 (25)		29 34 (32)
	156	270 267 (269)		12 8 (10)		48 29 (39)		46 46 (46)		46 49 (48)
	313	432 503 (468)		22 19 (21)		62 41 (52)		71 55 (63)		82 82 (82)
	625	829 861 (845)		22 28 (25)		92 92 (92)		92 99 (96)		123 142 (133)
	1250	1199 1149 (1174)		33 22 (28)		128 118 (123)		127 109 (118)		214 261 (238)
	2500	1470 1468 (1469)		49 40 (45)		216 173 (195)		175 175 (175)		383 364 (374)
	5000	1648 1467 (1558)		80 87 (84)		232 266 (249)		233 215 (224)		571 580 (576)
Positive control -S9 mix	Chemical	AF-2		NaN_3		AF-2		AF-2		ICR-191
	Dose(μg/plate)	0.01		0.5		0.01		0.1		0.5
	Number of revertant colonies/plate	793 726 (760)		320 329 (325)		329 333 (331)		526 539 (533)		1225 1153 (1189)
Positive control +S9 mix	Chemical	2AA		2AA		2AA		2AA		2AA
	Dose(μg/plate)	1		2		10		0.5		2
	Number of revertant colonies/plate	1180 1267 (1224)		308 272 (290)		686 818 (752)		358 358 (358)		175 190 (183)

[Notes]
( ): The mean number of colonies per plate.
AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
NaN_3: Sodium azide
2AA: 2-Aminoanthracene
(b) (4) synthetic intermediates of S-297995
Negative control: Dimethyl sulfoxide

Table 11 Relative mutagenic activity (b) (4)

Test substance (b) (4)

	Tester strain	-S9 mix		+S9 mix	
		Specific activity[a] (Revertants/mg)	Dose (μg/plate)	Specific activity[a] (Revertants/mg)	Dose (μg/plate)
Dose-range finding test	TA100	1.34×10^{3}	313	1.29×10^{3}	313
	TA1535	6.71×10^{1}	313	2.00×10^{1}	1250
	WP2*uvrA*	8.63×10^{1}	313	7.99×10^{1}	313
	TA98	6.40×10^{1}	1250	8.88×10^{1}	1250
	TA1537	4.10×10^{2}	19.5	2.56×10^{2}	78.1
Main test	TA100	1.47×10^{3}	156	1.18×10^{3}	625
	TA1535	7.03×10^{1}	313	3.83×10^{1}	313
	WP2*uvrA*	5.76×10^{1}	625	8.16×10^{1}	625
	TA98	1.28×10^{2}	156	9.76×10^{1}	625
	TA1537	1.02×10^{3}	9.77	2.11×10^{2}	313

a) Maximum value of each tester strain

(b) (4) synthetic intermediates of S-297995

(b) (4). In the dose-range finding test, precipitation was observed at ≥1250 μg/plate with and without metabolic activation, and bacterial growth inhibition was observed at ≥1250 μg/plate in the presence of S9 mix. In the definitive test, precipitation was observed at ≥1250 μg/plate in the absence of S9 mix and at 1250 μg/plate with S9 mix. Bacterial growth inhibition was observed in the definitive test with metabolic activation at ≥625 μg/plate in strains TA100, TA1535, TA98, and TA1537, and at 1250 μg/plate in strain WP2uvrA. In the dose-range finding and definitive tests, the numbers of revertant colonies in the (b) (4) treatment groups were less than twice those in respective negative controls, regardless of metabolic activation. Thus, the test compound was concluded to be negative under the conditions of the study. Summary results for the definitive test are presented in the Applicant's table below.

Table 9 Results of the main test (b) (4)

Test substance: (b) (4)

Test Period	From September 16, 2011 to September 20, 2011									
With(+)or without(-) S9 mix	Test substance dose (μg/plate)	Number of revertant colonies per plate								
		Base-pair substitution type						Frameshift type		
		TA100		TA1535		WP2*uvrA*		TA98		TA1537	
-S9 mix	Negative control	113 107	(110)	8 14	(12)	22 22	(22)	20 13	(17)	8 10	(10)
		110		14		22		17		11	
	313	87 74	(81)	14 10	(12)	28 26	(27)	17 17	(17)	6 3	(5)
	625	104 85	(95)	7 13	(10)	24 13	(19)	13 26	(20)	6 7	(7)
	1250 †	85 100	(93)	10 14	(12)	14 19	(17)	15 13	(14)	7 1	(4)
	2500 †	84 112	(98)	11 3	(7)	21 15	(18)	17 12	(15)	6 7	(7)
	5000 †	93 87	(90)	9 8	(9)	16 14	(15)	13 11	(12)	10 5	(8)
+S9 mix	Negative control	111 116	(114)	20 11	(13)	35 39	(36)	34 21	(30)	21 19	(19)
		115		7		33		34		18	
	39.1	126 148	(137)	12 12	(12)	27 24	(26)	43 34	(39)	13 18	(16)
	78.1	132 134	(133)	13 18	(16)	35 28	(32)	41 39	(40)	18 20	(19)
	156	112 122	(117)	12 7	(10)	19 27	(23)	31 35	(33)	18 12	(15)
	313	103 113	(108)	7 7	(7)	40 25	(33)	26 35	(31)	11 13	(12)
	625	41* 50*	(46)	6* 2*	(4)	21 33	(27)	24* 14*	(19)	1* 2*	(2)
	1250 †	0* 0*	(0)	0* 0*	(0)	8* 8*	(8)	0* 0*	(0)	0* 0*	(0)
Positive control -S9 mix	Chemical	AF-2		NaN_3		AF-2		AF-2		ICR-191	
	Dose(μg/plate)	0.01		0.5		0.01		0.1		0.5	
	Number of revertant colonies/plate	600 678	(639)	402 362	(382)	334 321	(328)	573 573	(573)	1131 1190	(1161)
Positive control +S9 mix	Chemical	2AA		2AA		2AA		2AA		2AA	
	Dose(μg/plate)	1		2		10		0.5		2	
	Number of revertant colonies/plate	989 921	(955)	199 201	(200)	724 605	(665)	318 345	(332)	184 193	(189)

[Notes]
( ): The mean number of colonies per plate.
†: Precipitation of the test substance was observed in all plates for each tester strain.
*: Bacterial growth inhibition was observed.
AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
NaN_3: Sodium azide
2AA: 2-Aminoanthracene
(b) (4) synthetic intermediates of S-297995
Negative control: Dimethyl sulfoxide

(b) (4). In the dose-range finding and definitive tests, precipitation was observed at 5000 μg/plate in the presence and absence of S9 mix. At all doses tested, the numbers of revertant colonies in (b) (4) treatment groups were less than twice those in respective negative controls under all test conditions. Thus, the test compound

was concluded to be negative. Results of the definitive test are summarized in the Applicant's table below.

Table 10 Results of the main test (b) (4)

Test substance: (b) (4)

Test Period		From September 16, 2011 to September 20, 2011				
With(+)or without(-) S9 mix	Test substance dose (μg/plate)	Number of revertant colonies per plate				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
-S9 mix	Negative control	123 94 (101) 87	12 10 (11) 10	38 24 (34) 39	21 15 (18) 18	12 2 (7) 8
	313	112 122 (117)	8 15 (12)	32 38 (35)	21 19 (20)	7 10 (9)
	625	126 124 (125)	7 15 (11)	31 40 (36)	18 18 (18)	8 4 (6)
	1250	126 127 (127)	11 6 (9)	33 35 (34)	25 21 (23)	6 11 (9)
	2500	144 136 (140)	8 8 (8)	26 26 (26)	14 21 (18)	7 4 (6)
	5000 †	113 94 (104)	7 8 (8)	24 26 (25)	14 21 (18)	3 2 (3)
+S9 mix	Negative control	110 93 (108) 122	6 13 (9) 7	33 46 (41) 43	35 31 (35) 38	18 17 (16) 12
	313	128 156 (142)	8 12 (10)	34 40 (37)	36 28 (32)	22 18 (20)
	625	153 116 (135)	11 8 (10)	47 34 (41)	35 28 (32)	7 17 (12)
	1250	113 124 (119)	8 11 (10)	28 24 (26)	34 27 (31)	19 17 (18)
	2500	130 152 (141)	14 11 (13)	43 33 (38)	29 26 (28)	18 12 (15)
	5000 †	137 109 (123)	9 4 (7)	30 31 (31)	26 20 (23)	13 7 (10)
Positive control -S9 mix	Chemical	AF-2	NaN_3	AF-2	AF-2	ICR-191
	Dose(μg/plate)	0.01	0.5	0.01	0.1	0.5
	Number of revertant colonies/plate	793 726 (760)	320 329 (325)	329 333 (331)	526 539 (533)	1225 1153 (1189)
Positive control +S9 mix	Chemical	2AA	2AA	2AA	2AA	2AA
	Dose(μg/plate)	1	2	10	0.5	2
	Number of revertant colonies/plate	1180 1267 (1224)	308 272 (290)	686 818 (752)	358 358 (358)	175 190 (183)

[Notes]
( ): The mean number of colonies per plate.
†: Precipitation of the test substance was observed in all plates for each tester strain.
AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
NaN_3: Sodium azide
2AA: 2-Aminoanthracene
(b) (4) synthetic intermediates of S-297995
Negative control: Dimethylsulfoxide

Study title: Bacterial Reverse Mutation Test of (b) (4) a Synthetic Intermediate of S-297995

Study no.:	(b) (4)
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 5, 2012
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	(b) (4) Lot # B14001, Purity not specified

Key Study Findings

Under the conditions tested, (b) (4) tested negative in the *in vitro* bacterial reverse mutation assay using the pre-incubation method both with and without metabolic activation.

Methods

Strains:	S. typhimurium strains TA98, TA100, TA1535, and TA1537 E. coli strain WP2uvrA
Concentrations in definitive study:	39.1, 78.1, 156, 313, 625, 1250 µg/plate
Basis of concentration selection:	In a dose-finding test, concentrations of 4.88, 19.5, 78.1, 313, 1250, and 5000 µg/plate were tested. Bacterial growth inhibition was observed at ≥1250 µg/plate and precipitation was observed at 5000 µg/plate.
Negative control:	Dimethyl sulfoxide (DMSO)
Positive control:	In the presence of S9 mix, 2-Aminoanthracene (2AA) was used as a positive control for all tester strains. In the absence of S9 mix, positive controls were 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) for tester strains TA98, TA100, and WP2uvrA, sodium azide (NaN_3) for tester strain TA1535, and ICR-191 for tester strain TA1537.
Formulation/Vehicle:	DMSO
Incubation & sampling time:	The study was conducted using the pre-incubation method, with and without S9 mix. Briefly, tester strains were incubated with the test substance and positive and negative controls for 20 min at 37°C prior to addition of agar. Plates were then incubated for 48 h at 37ºC before counting the number of revertant colonies. S9 mix was prepared using S9 fraction from the livers of male SD rats administered phenobarbital and 5,6-benzoflavone.

Study Validity

The study was considered to be valid based on the following: (1) the numbers of revertant colonies in the positive controls were more than twice that of negative controls, (2) the numbers of revertant colonies in the negative and positive controls were within historical control ranges for the testing facility, and (3) there was no evidence of contamination. A test substance was judged to be positive if the numbers of revertant colonies was increased to twice or more than that of respective negative controls and the responses were concentration-related and/or reproducible.

Results

In the definitive test, bacterial growth inhibition was observed at 1250 µg/plate in all tester strains without S9 mix and at ≥625 µg/plate with S9 mix. No precipitation was observed at any concentration. In both the dose-finding and definitive tests, (b) (4) did not produce an increase in the number of revertant colonies of two-fold or greater, compared to the respective negative controls, regardless of metabolic activation. Thus, (b) (4) was concluded to be negative under the conditions of the study. The Applicant's table below summarizes finding from the definitive test.

Table 2 Results of the main test

Test substance: (b) (4)

Test Period		From November 2, 2012 to November 5, 2012				
With(+)or without(-) S9 mix	Test substance dose (µg/plate)	Number of revertant colonies per plate				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
-S9 mix	Negative control	116 107 (107) 99	12 10 (9) 6	31 21 (27) 29	19 22 (22) 25	20 13 (17) 19
	39.1	97 94 (96)	5 14 (10)	31 21 (26)	19 28 (24)	20 18 (19)
	78.1	113 136 (125)	15 11 (13)	26 21 (24)	15 20 (18)	18 17 (18)
	156	96 84 (90)	10 12 (11)	22 20 (21)	13 15 (14)	15 22 (19)
	313	103 80 (92)	10 6 (8)	24 27 (26)	8 15 (12)	21 14 (18)
	625	93 86 (90)	4 4 (4)	31 27 (29)	15 10 (13)	8 13 (11)
	1250	116* 107* (112)	2* 9* (6)	24* 26* (25)	20* 23* (22)	10* 0* (5)
+S9 mix	Negative control	96 102 (103) 112	6 11 (7) 4	33 29 (30) 27	35 25 (28) 25	25 17 (19) 15
	39.1	115 98 (107)	6 5 (6)	27 25 (26)	32 25 (29)	21 13 (17)
	78.1	115 92 (104)	6 6 (6)	31 20 (26)	26 22 (24)	19 21 (20)
	156	144 109 (127)	8 8 (8)	25 34 (30)	24 22 (23)	20 8 (14)
	313	97 96 (97)	8 8 (8)	35 20 (28)	21 22 (22)	10 4 (7)
	625	27* 28* (28)	4* 3* (4)	11* 19* (15)	22* 14* (18)	2* 1* (2)
	1250	0* 0* (0)	0* 0* (0)	12* 9* (11)	5* 2* (4)	0* 0* (0)
Positive control -S9 mix	Chemical	AF-2	NaN_3	AF-2	AF-2	ICR-191
	Dose(µg/plate)	0.01	0.5	0.01	0.1	0.5
	Number of revertant colonies/plate	931 906 (919)	162 181 (172)	345 355 (350)	513 419 (466)	1479 1385 (1432)
Positive control +S9 mix	Chemical	2AA	2AA	2AA	2AA	2AA
	Dose(µg/plate)	1	2	10	0.5	2
	Number of revertant colonies/plate	1038 1004 (1021)	255 225 (240)	567 595 (581)	221 189 (205)	189 164 (177)

[Notes]
- ( ): The mean number of colonies per plate.
- *: Bacterial growth inhibition was observed.
- AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
- NaN_3: Sodium azide
- 2AA: 2-Aminoanthracene

Study title: Reverse Mutation Test with Bacteria on [redacted] Synthetic Intermediates of S-297995

Study no.:	[redacted]
Study report location:	EDR
Conducting laboratory and location:	[redacted]
Date of study initiation:	January 23, 2012
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	(1) [redacted] Lot # 2010-6089-109-01, Purity not specified (2) [redacted] Lot # 2010-6089-115-01, Purity not specified

Key Study Findings

Under the conditions of the study, [redacted] tested negative in the *in vitro* bacterial reverse mutation assay using the pre-incubation method both with and without metabolic activation.

Methods

Strains:	S. typhimurium TA98, TA100, TA1535, and TA1537 E. coli WP2uvrA
Concentrations in definitive study:	(b) (4) 313, 625, 1250, 2500, and 5000 µg/plate (b) (4): 9.77, 19.5, 39.1, 78.1, 156, and 313 µg/plate
Basis of concentration selection:	In a dose-range finding test, concentrations of 4.88, 19.5, 78.1, 313, 1250, and 5000 µg/plate for each test substance were used. In addition, a second dose-finding test was conducted with (b) (4) concentrations of 9.77, 19.5, 39.1, 78.1, 156, and 313 µg/plate, based on bacterial growth inhibition observed at ≥313 µg/plate and precipitation observed at 5000 µg/plate in the first dose-finding test. In the second dose-finding test, inhibition of bacterial growth was observed at ≥156 µg/plate in the absence of S9 mix and at 313 µg/plate in the presence of S9 mix.
Negative control:	Dimethyl sulfoxide (DMSO)
Positive control:	In the presence of S9 mix, 2-Aminoanthracene (2AA) was used as a positive control for all tester strains. In the absence of S9 mix, positive controls were 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) for tester strains TA98, TA100, and WP2uvrA, sodium azide (NaN_3) for tester strain TA1535, and ICR-191 for tester strain TA1537.
Formulation/Vehicle:	DMSO
Incubation & sampling time:	The study was performed using the pre-incubation method, with pre-incubation for 20 min. at 37°C, followed by the addition of agar and incubation for 48 h at 37ºC. S9 mix was prepared using S9 fraction from the livers of male SD rats administered phenobarbital and 5,6-benzoflavone.

Study Validity

The current study was considered to be valid based on the following criteria: (1) the numbers of revertant colonies in the positive controls were more than twice those of negative controls, (2) the numbers of revertant colonies in the negative and positive controls were within acceptable ranges based on historical control data for the testing

facility, and (3) there was no evidence of contamination. A test substance was judged to be positive when the numbers of revertant colonies were increased to twice or more than that of respective negative controls and the responses were concentration-related and/or reproducible.

Results

(b) (4) In the definitive test, bacterial growth inhibition or precipitation were not observed at any concentration. In both the dose-range finding and definitive tests, the number of revertant colonies in the (b) (4) treatment groups were less than twice those in respective negative controls, regardless of metabolic activation. Thus, (b) (4) was concluded to test negative under the conditions of the study. The Applicant's table below summarizes the findings of the definitive test.

Table 4 Results of the main test (b) (4)

Test substance (b) (4)

Test Period		From February 8, 2012 to February 10, 2012				
With(+)or without(-) S9 mix	Test substance dose (μg/plate)	Number of revertant colonies per plate				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
-S9 mix	Negative control	117 105 (111) 110	10 10 (10) 10	29 12 (20) 19	20 14 (19) 24	7 3 (5) 4
	313	87 107 (97)	8 12 (10)	32 19 (26)	10 14 (12)	8 6 (7)
	625	121 109 (115)	6 11 (9)	28 20 (24)	12 18 (15)	3 7 (5)
	1250	119 93 (106)	8 15 (12)	25 15 (20)	8 17 (13)	6 5 (6)
	2500	113 110 (112)	7 12 (10)	11 18 (15)	14 12 (13)	4 7 (6)
	5000	79 99 (89)	15 14 (15)	18 11 (15)	14 11 (13)	8 5 (7)
+S9 mix	Negative control	93 113 (107) 116	6 8 (8) 10	20 21 (21) 21	18 20 (19) 18	6 8 (7) 7
	313	121 121 (121)	11 7 (9)	20 20 (20)	24 25 (25)	11 10 (11)
	625	126 116 (121)	12 3 (8)	35 19 (27)	18 21 (20)	3 6 (5)
	1250	124 122 (123)	5 4 (5)	26 19 (23)	14 17 (16)	11 8 (10)
	2500	126 126 (126)	12 14 (13)	25 22 (24)	15 28 (22)	10 11 (11)
	5000	130 106 (118)	11 7 (9)	20 24 (22)	24 22 (23)	7 12 (10)
Positive control -S9 mix	Chemical	AF-2	NaN_3	AF-2	AF-2	ICR-191
	Dose(μg/plate)	0.01	0.5	0.01	0.1	0.5
	Number of revertant colonies/plate	799 678 (739)	321 327 (324)	389 335 (362)	575 595 (585)	1375 1287 (1331)
Positive control +S9 mix	Chemical	2AA	2AA	2AA	2AA	2AA
	Dose(μg/plate)	1	2	10	0.5	2
	Number of revertant colonies/plate	1022 966 (994)	285 265 (275)	701 770 (736)	399 330 (365)	164 181 (173)

[Notes]
- ( ): The mean number of colonies per plate.
- AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
- NaN_3: Sodium azide
- 2AA: 2-Aminoanthracene

(b) (4) In the definitive test, inhibition of bacterial growth inhibition was observed at ≥156 and 313 µg/plate in all tester strains in the absence and presence of S9 mix, respectively. In the two dose-range finding tests and the definitive test, the number of revertant colonies in the (b) (4) treatment groups were less than twice those in respective negative controls, regardless of metabolic activation. Thus, (b) (4) was concluded to test negative under the conditions of the study. Results of the definitive test are presented in the Applicant's table below.

Table 5 Results of the main test (b) (4)

Test substance: (b) (4)

Test Period		From February 22, 2012 to February 24, 2012				
With(+)or without(-) S9 mix	Test substance dose (µg/plate)	Number of revertant colonies per plate				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
-S9 mix	Negative control	96 91 (96) 100	4 14 (9) 10	29 27 (26) 21	18 14 (15) 13	5 5 (6) 9
	9.77	104 102 (103) -	6 10 (8) -	21 20 (21) -	27 19 (23) -	11 7 (9) -
	19.5	85 104 (95) -	4 4 (4) -	19 14 (17) -	17 17 (17) -	4 7 (6) -
	39.1	78 81 (80) -	3 10 (7) -	26 24 (25) -	13 18 (16) -	5 5 (5) -
	78.1	79 86 (83) -	6 5 (6) -	13 14 (14) -	12 11 (12) -	3 11 (7) -
	156	53* 49* (51) -	6* 6* (6) -	10* 13* (12) -	14* 14* (14) -	1* 1* (1) -
	313	11* 7* (9) -	0* 0* (0) -	16* 11* (14) -	8* 7* (8) -	0* 0* (0) -
+S9 mix	Negative control	92 113 (103) 104	14 5 (13) 19	24 24 (24) 24	29 19 (23) 22	14 12 (12) 10
	9.77	111 111 (111) -	7 18 (13) -	26 21 (24) -	29 31 (30) -	19 10 (15) -
	19.5	135 144 (140) -	6 12 (9) -	26 19 (23) -	21 15 (18) -	10 6 (8) -
	39.1	109 123 (116) -	11 10 (11) -	26 19 (23) -	18 20 (19) -	12 8 (10) -
	78.1	106 130 (118) -	14 8 (11) -	24 13 (19) -	22 24 (23) -	7 5 (6) -
	156	98 119 (109) -	7 12 (10) -	20 15 (18) -	18 22 (20) -	11 11 (11) -
	313	34* 41* (38) -	0* 1* (1) -	14* 16* (15) -	14* 15* (15) -	1* 1* (1) -
Positive control -S9 mix	Chemical	AF-2	NaN_3	AF-2	AF-2	ICR-191
	Dose(µg/plate)	0.01	0.5	0.01	0.1	0.5
	Number of revertant colonies/plate	909 843 (876) -	338 334 (336) -	356 349 (353) -	476 386 (431) -	1763 1643 (1703) -
Positive control +S9 mix	Chemical	2AA	2AA	2AA	2AA	2AA
	Dose(µg/plate)	1	2	10	0.5	2
	Number of revertant colonies/plate	1168 1040 (1104) -	245 252 (249) -	919 909 (914) -	404 342 (373) -	222 240 (231) -

[Notes]
- ( ): The mean number of colonies per plate.
- *: Bacterial growth inhibition was observed.
- AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
- NaN_3: Sodium azide
- 2AA: 2-Aminoanthracene

Study title: Exploratory Bacterial Reverse Mutation Test of (b) (4)

Study no.:	(b) (4)
Study report location:	EDR
Conducting laboratory and location:	Shionogi & Co., Ltd, Osaka, Japan
Date of study initiation:	July 19, 2007
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	(b) (4) Lot # 01, Purity not specified

Key Study Findings

Under the conditions of the study, (b) (4) tested negative in the *in vitro* bacterial reverse mutation assay using the pre-incubation method both with and without metabolic activation.

Methods

Strains:	S. typhimurium TA98, TA100, TA1535, and TA1537 E. coli WP2uvrA
Concentrations in definitive study:	TA98 and TA100: 6.68, 20.6, 61.7, 185, 556, 1667, and 5000 µg/plate TA1535, WP2uvrA, and TA1537: 61.7, 185, 556, 1667, and 5000 µg/plate
Basis of concentration selection:	Not stated. However, the maximum concentration was 5000 µg/plate and lower concentrations were based on 3-fold differences.
Negative control:	Dimethyl sulfoxide (DMSO)
Positive control:	In the presence of S9 mix, 2-Aminoanthracene (2AA) was used as a positive control for all tester strains. In the absence of S9 mix, positive controls were 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) for tester strains TA98, TA100, and WP2uvrA, sodium azide (NaN_3) for tester strain TA1535, and 9-aminoacridine hydrochloride (9AA) for tester strain TA1537.
Formulation/Vehicle:	DMSO
Incubation & sampling time:	In this study, the pre-incubation method was used, with pre-incubation for 20 min. at 37°C, followed by addition of agar and incubation for 48 h at 37ºC. The test was conducted with and without a metabolic activation system (S9 mix).

Study Validity

Criteria for study validity were not stated. However, the numbers of revertant colonies in the positive controls were substantially greater than 2-times those in respective negative controls. The test substance was considered to be positive if the compound produces ≥2-fold increases in the number of revertant colonies over the negative control group, in at least 1 of 5 tester strains.

Results

In this study, growth inhibition of all tester strains was observed at 5000 µg/plate in all tester strains. At up to 5000 µg/plate (b) (4) the numbers of revertant colonies were less than twice the numbers in respective negative controls regardless of metabolic activation. Thus, (b) (4) was concluded to be negative under the conditions of the study. Results of the study are presented in the Applicant's table below.

Exploratory Bacterial Reverse Mutation Test of (b) (4)

Result

Compound	Dose (μg/plate)	S9	Revertant colonies per plate									
			TA100		TA1535		WP2*uvrA*		TA98		TA1537	
Negative control	0	-	114		11		34		25		10	
			96	(105)	14	(13)	29	(32)	22	(24)	15	(13)
(b) (4)	6.86	-	90						21			
			95	(93)		NT		NT	19	(20)		NT
	20.6	-	93						29			
			88	(91)		NT		NT	28	(29)		NT
	61.7	-	103		7		35		20		13	
			90	(97)	13	(10)	24	(30)	29	(25)	9	(11)
	185	-	89		10		27		17		12	
			97	(93)	6	(8)	30	(29)	28	(23)	13	(13)
	556	-	115		9		26		17		12	
			108	(112)	16	(13)	35	(31)	16	(17)	15	(14)
	1667	-	82		11		27		24		13	
			90	(86)	10	(11)	23	(25)	21	(23)	10	(12)
	5000	-	0		8		16		11		5	
			46	(23)*	7	(8)*	7	(12)*	10	(11)*	2	(4)*
	PC	-	431		485		184		365		315	
			418	(425)	434	(460)	168	(176)	347	(356)	371	(343)
Negative control	0	+	101		10		34		28		26	
			106	(104)	15	(13)	45	(40)	38	(33)	21	(24)
(b) (4)	6.86	+	101						27			
			99	(100)		NT		NT	36	(32)		NT
	20.6	+	92						28			
			107	(100)		NT		NT	24	(26)		NT
	61.7	+	121		15		41		34		23	
			106	(114)	8	(12)	38	(40)	29	(32)	21	(22)
	185	+	100		9		45		27		25	
			116	(108)	11	(10)	42	(44)	33	(30)	22	(24)
	556	+	99		16		43		28		25	
			108	(104)	11	(14)	48	(46)	41	(35)	30	(28)
	1667	+	96		7		36		39		19	
			98	(97)	13	(10)	29	(33)	32	(36)	28	(24)
	5000	+	62		3		13		13		3	
			67	(65)*	9	(6)*	8	(11)*	17	(15)*	9	(6)*
	PC	+	1010		256		964		221		208	
			952	(981)	248	(252)	910	(937)	230	(226)	236	(222)

Negative control: DMSO (100 μL/plate)

NT: Not tested * : Growth inhibition of the tester strain was observed.

· Parentheses: mean of two plates

· PC: Positive control (μg/plate)

without S9 mix; TA100: AF-2 (0.01), TA1535: NaN_3 (0.5), WP2*uvrA*: AF-2 (0.01), TA98: AF-2 (0.1), TA1537: 9AA (80)

with S9 mix; TA100: 2AA (1), TA1535: 2AA (2), WP2*uvrA*: 2AA (10), TA98: 2AA (0.5), TA1537: 2AA (2)

[AF-2: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, NaN_3: sodium azide, 9AA: 9-aminoacridine hydrochloride, 2AA: 2-aminoanthracene.]

8 Carcinogenicity

Study title: 104-Week Carcinogenicity Study of S-297995 Monotosylate in Rats by Gavage

Study no.:	YDL0036 (Sponsor Reference Number: S-297995-TF-266-L; Study No. YDL0036)
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 5, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	S-297995 monotosylate; Batch # B15001; Content 99.7% (on anhydrous basis)
CAC concurrence:	Yes (ECAC meeting minutes re: dose selection dated November 17, 2011, Attachment 1; Recommendations re: early termination procedures sent to the Applicant on November 7, 2013)

Key Study Findings

- There was no statistically significant effect on mortality in either sex.
- All surviving females from all dose groups were terminated early (from Week 103) when the number of surviving females in the control group reached 15 animals.
- In males, there were treatment-related decreases in body weight gain (compared to controls) at all doses. In females, body weight gain from Week 0 to 103 in the low-, mid-, and high dose groups was reported to be 82%, 86%, and 79%, respectively, of the control group (statistically significant at high dose only).
- In males, there were statistically significant treatment-related decreases in food consumption (compared to controls) at all dose levels.
- There were no drug-related neoplastic findings in male or female rats (ECAC meeting minutes dated July 21, 2016, Attachment 2).

Adequacy of Carcinogenicity Study

The carcinogenicity study conduct was acceptable. The dose selection was based on recommendations from the Executive CAC (November 7, 2011). Based on the high dose having an AUC value > 25 times that in humans, the Division and CDER Executive Carcinogenicity Assessment Committee (ECAC) concurred with the Applicant's proposed high dose of 100 mg/kg/day. During the conduct of the study, the Division reviewed the Applicant's proposed termination plan and provided recommendations regarding early termination procedures (IND 107475, S. Chakder, Ph.D., November 6, 2013). It is noted that all surviving females from all dose groups were terminated early

(from Week 103) when the number of surviving females in the control group reached 15 animals.

Appropriateness of Test Models

The Crl:CD(SD) rat is an acceptable test model for the carcinogenicity study.

Evaluation of Tumor Findings

FDA Statistical Analysis of Tumor Data: The tumor data were analyzed for dose response relationships across the control, low-, mid-, and high dose groups, and pairwise comparisons of each of the three treated groups (low-, mid-, and high dose) against the vehicle control group, using the Poly-k method. For the adjustment of multiple testing, common and rare tumors were tested for dose response relationship at significance levels of 0.005 and 0.025, respectively. For multiple pairwise comparisons of treated groups with controls, test levels of 0.01 and 0.05 were used for common and rare tumors, respectively.

In males and females, there were no drug-related neoplastic findings at up to 100 mg/kg/day S-297995 monotosylate.

Methods

Doses:	0, 10, 30, 100 mg/kg/day (as free base; see Applicant's table below)
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral (Gavage)
Formulation/Vehicle:	0.5% w/v methylcellulose (MC) aqueous solution
Basis of dose selection:	AUC ratio (based on the high dose having an AUC value > 25 times that in humans; Rat AUC values based on 6-month oral toxicity and TK study (Sponsor's Study No. R-297995-TF-108-L; CRO Study No. SG08274)
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	Toxicology: 65/sex/group Satellite: 3/sex/group
Age:	33 to 40 days of age at start of treatment
Animal housing:	5 animals of the same sex per cage (main study); 3 animals of the same sex per cage (satellite)
Paradigm for dietary restriction:	No diet restriction
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	Yes (for TK analysis)
Deviation from study protocol:	Protocol deviations did not affect the quality or integrity of the study.

The following table copied from the Applicant's study report shows the study group assignments.

Group	Treatment	Dose# (mg/kg/day)	Number of animals			
			Main study		Satellite study†	
			Male	Female	Male	Female
1	Control	0	65	65	3	3
2	S-297995	10	65	65	3	3
3	S-297995	30	65	65	3	3
4	S-297995	100	65	65	3	3

Expressed in terms of the free base. A weighing factor of 1.31 was used.
† Satellite animals used for toxicokinetic sampling only.

Observations and Results

Mortality

Animals were checked twice daily for signs of morbidity and mortality.

Results: The Applicant's table below summarizes mortality in males and females. Since the number of surviving females in the control group reached 15 animals/sex/group, all surviving females from all dose groups were terminated early from Week 103. Surviving males were terminated at Week 105.

	Group and sex							
	1M	2M	3M	4M	1F	2F	3F	4F
Dose (mg/kg/day)	0	10	30	100	0	10	30	100
Group size	65	65	65	65	65	65	65	65
No. of deaths Weeks 1 to 104	36	33	39	37	50	46	46	48
No. of survivors to terminal necropsy	29	32	26	28	15	19	19	17
% Survival	45	49	40	43	23	29	29	26

As summarized in the Applicant's table below, the most common factors contributing to death were identified as pituitary adenomas (anterior pituitary of both sexes) and fibroadenomas or adenocarcinomas (female mammary glands), and there was no dose-relationship for these findings.

Summary of factors contributory to death for decedents

Group/sex	1M	2M	3M	4M	1F	2F	3F	4F
Dose (mg/kg/day)	0	10	30	100	0	10	30	100
Group size	65	65	65	65	65	65	65	65
Pituitary adenoma	10	8	11	11	27	27	25	24
Mammary fibroadenoma	0	0	0	0	6	9	9	5
Mammary adenocarcinoma	0	0	0	0	8	4	5	5

Independent analysis of survival data by the FDA statistical reviewer (H. Chen) showed that the numbers of rats surviving to terminal necropsy were 29 (44.62%), 32 (49.23%), 26 (40.00%), and 28 (43.08%) for control, low-, mid-, and high dose males, respectively,

and 15 (23.08%), 19 (29.23%), 19 (29.23%), and 17 (26.15%) for control, low-, mid-, and high dose females, respectively. The FDA statistical reviewer's survival analysis determined that there were no statistically significant differences in mortality in either sex. Results of the intercurrent mortality comparison for male and female rats are shown below in Tables 1A and 1B, respectively (copied from the statistical review from H. Chen).

Table 1A: Intercurrent Mortality Rate in Male Rats

	0 mg/kg/day Vehicle Control		10 mg/kg/day Low		30 mg/kg/day Mid		100 mg/kg/day High	
Week / Type of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	Cum %
0 - 52	3	4.62	1	1.54	2	3.08	5	7.69
53 - 78	12	23.08	10	16.92	10	18.46	7	18.46
79 - 92	8	35.38	8	29.23	11	35.38	10	33.85
93 - 104	13	20.00	13	20.00	16	24.62	13	20.00
Accidental Death			1	1.54			2	3.08
Terminal sacrifice	29	44.62	32	49.23	26	40.00	28	43.08
Total	65		65		65		65	

Test	All Dose Groups	Vehicle Control vs. Low	Vehicle Control vs. Mid	Vehicle Control vs. High
Dose-Response (Likelihood Ratio)	0.8320	0.4625	0.7225	0.9396
Homogeneity (Log-Rank)	0.7271	0.4587	0.7201	0.9392

#All Cum. % Cumulative Percentage except for Terminal sacrifice;

Table 1B: Intercurrent Mortality Rate in Female Rats

	0 mg/kg/day Vehicle Control		10 mg/kg/day Low		30 mg/kg/day Mid		100 mg/kg/day High	
Week / Type of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	Cum %
0 - 52	2	3.08	1	1.54	2	3.08	3	4.62
53 - 78	15	26.15	15	24.62	18	30.77	19	33.85
79 - 92	20	56.92	17	50.77	10	46.15	18	61.54
93 - 103	13	20.00	13	20.00	16	24.62	8	12.31
Terminal sacrifice	15	23.08	19	29.23	19	29.23	17	26.15
Total	65		65		65		65	

Test	All Dose Groups	Vehicle Control vs. Low	Vehicle Control vs. Mid	Vehicle Control vs. High
Dose-Response (Likelihood Ratio)	0.5649	0.5028	0.3997	0.8534
Homogeneity (Log-Rank)	0.6921	0.4960	0.3917	0.8510

#All Cum. % Cumulative Percentage except for Terminal sacrifice;

The Kaplan-Meier curves for survival rates of all treatment groups (from the statistical review from H. Chen) are shown below in Figures 1A and 1B for male and female rats, respectively.

Figure 1A: Kaplan-Meier Survival Functions for Male Rats

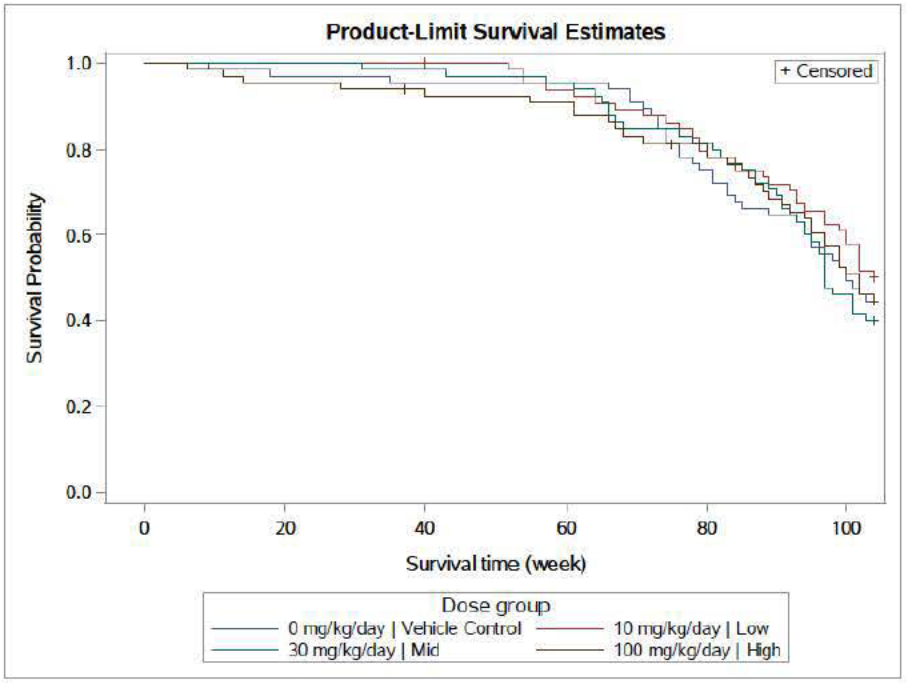
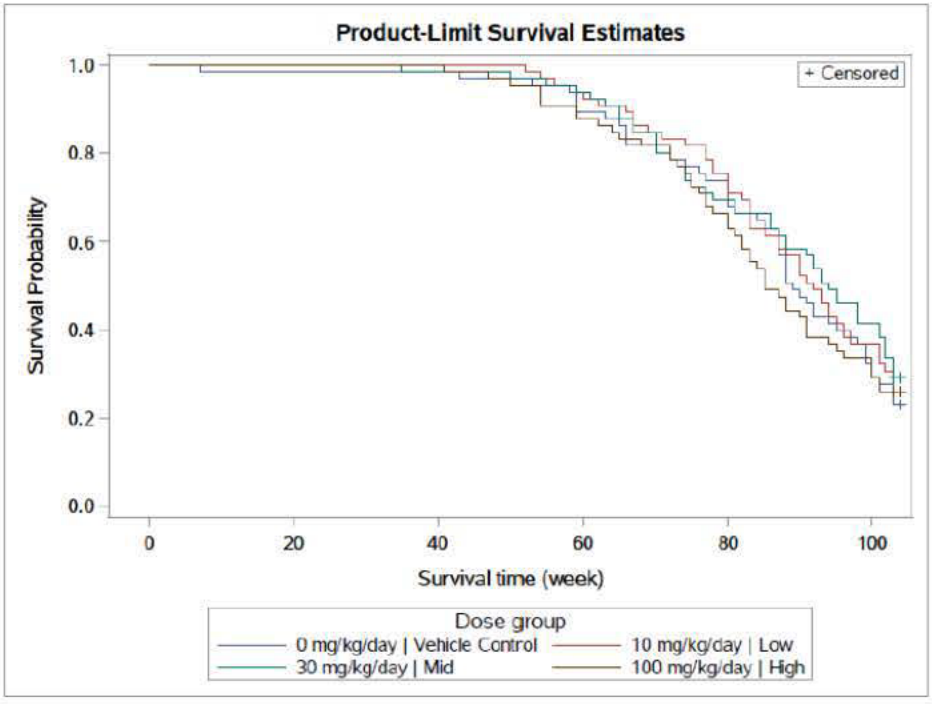


Figure 1B: Kaplan-Meier Survival Functions for Female Rats



Clinical Signs

Animals were observed for clinical signs daily during the first week of treatment, twice weekly during Weeks 2 to 4, once weekly during Weeks 5 to 13, every other week during Weeks 14 to 52, and once every 4 weeks thereafter. Detailed physical

examinations and palpation for masses were conducted weekly. The mass number and location, date, size, and description of new masses were recorded.

Results: Treatment-related clinical signs associated with dosing included chin rubbing, a finding that was observed in all S-297995 treatment-groups throughout the study. The incidence of this finding increased in a dose-related manner and was considered to be related to the taste of the formulation. There also was an increased incidence of salivation associated with dosing, generally in high dose males and females.

The numbers of animals with palpable masses, total number of masses, and mean time of onset were similar in males across all treatment-groups. In females, the total numbers of masses in the control, low-, mid-, and high dose groups were 92, 107, 114, and 98, respectively. Although the incidences of masses in the low- and mid- dose groups were increased relative to the control group, the incidence in the high dose group was similar to the control group and less than the low- and mid-dose groups. Thus, this finding was considered to be incidental. The number of females in the control, low-, mid-, and high dose groups with masses were 45, 50, 52, and 48, respectively, and the mean time of onset was 71, 69, 74, and 71 weeks, respectively.

Body Weights

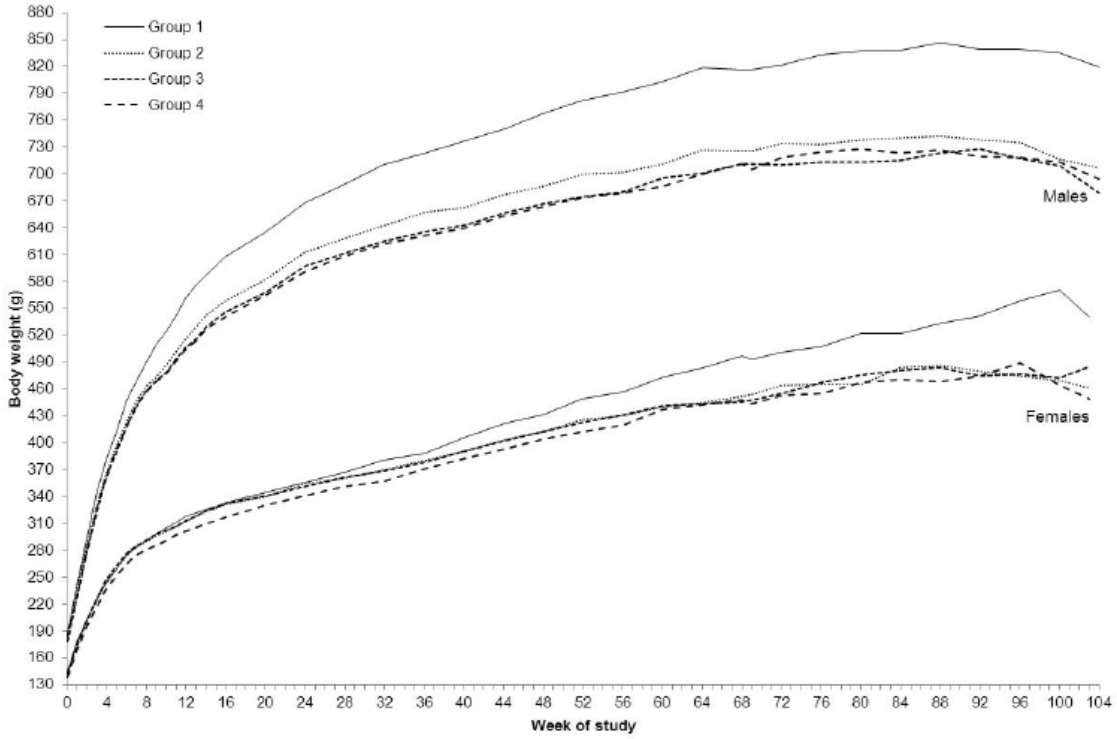
Body weights were recorded at Week -1, the first day of treatment, weekly intervals for the first 16 weeks of treatment, once every 4 weeks thereafter, and prior to necropsy.

Results: In males, treatment-related effects on body weights occurred at all doses. In males, body weight gain from Week 0 to 104 in the low-, mid-, and high dose groups was reported to be 83%, 80%, and 81%, respectively, of the control group (statistically significant at all doses). In females, body weight gain from Week 0 to 103 in the low-, mid-, and high dose groups was reported to be 82%, 86%, and 79%, respectively, of the control group (statistically significant at high dose only). There were statistically significant decreases in body weight gain (compared to controls) in females during other intervals however (Week 28 – 52 and Week 52-103).

The Applicant's figure below shows mean body weights in males and females over the course of the study period.

Body weight - group mean values (g)

Group	:	1	2	3	4
Compound	:	Control	S-297995	S-297995	S-297995
Dose (mg/kg/day)	:	0	10	30	100



Feed Consumption

Food consumption was recorded for Week -1, weekly for the first 16 weeks of treatment, and once every 4 weeks thereafter.

Results: In males, there were statistically significant treatment-related decreases in food consumption (related to controls) at all dose levels. Mean food consumption values (Week 1 – 104) in low-, mid-, and high dose males were 93%, 92%, and 93% of controls, respectively. In females, there were no treatment-related effects on food consumption.

Ophthalmoscopy

Ophthalmological examinations were conducted prior to initiation of treatment (all animals), and at Weeks 52 and 100 (20/sex of control and high dose groups).

Results: There were no treatment-related findings.

Clinical Pathology

Blood samples were collected from 20 fasted animals/sex (if possible) during Week 103/104 (females) and Week 104 (males) prior to dosing. Samples were analyzed for the following hematological and coagulation parameters: hematocrit, hemoglobin concentration, erythrocyte count, absolute reticulocyte count, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume, total white cell count,

differential white blood cell count, platelet count, morphology, prothrombin time, and activated partial thromboplastin time. In addition, samples were analyzed for the following clinical chemistry parameters: alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, total bilirubin, urea, creatinine, glucose, total cholesterol, triglycerides, sodium, potassium, chloride, calcium, inorganic phosphorous, total protein, and albumin. When possible, blood samples were obtained from animals terminated during the treatment period, and these samples were analyzed for clinical chemistry parameters and a limited list of hematological parameters. Blood films were prepared for all samples for examination of abnormalities by light microscopy. Urine samples were collected from 20 animals/sex/group (if possible) during Week 103 for urinalysis.

Results:

Hematology: In males, there were statistically significant increases in basophil count at all doses at Week 104 (+60%, +40%, and +60% of control values in the low-, mid-, and high dose groups, respectively). Other statistically significant changes in the high dose male group only included increased hematocrit (+7.6%), hemoglobin (+6.7%), red blood cells (+8.7%), and percent basophils (+33%) and decreased percent reticulocytes (-52%), compared to controls. All remaining statistically significant changes in hematological parameters for males did not occur in a dose-related manner. In females, there were no statistically significant changes in hematological parameters at Week 103/104.

Clinical Chemistry: During Week 104, there were statistically significant changes in the following parameters in males (compared to controls): (1) increased creatinine at 30 and 100 mg/kg/day (+24% and +12%, respectively), (2) decreased triglycerides at 100 mg/kg/day (-41%), and (3) increased potassium at 100 mg/kg/day (+23%). In females, during Week 104 there were statistically significant decreases in triglycerides (-40%, compared to controls) and calcium (-4.5%, compared to controls) at 100 mg/kg/day.

Urinalysis: In high dose males, there was a small but statistically significant increase in urine pH (8.0 versus 7.3 in the control group). There was also decreased total potassium at all doses (up to -41% of controls), but the changes were not dose-related. In females, there were statistically significant changes in several parameters at all dose levels (compared to controls), but the changes were not dose-dependent (decreased urine volume by up to -43%, decreased chloride, sodium, and potassium by up to -44%, -42%, and -42%, respectively).

Changes in hematological and clinical chemistry parameters in animals sacrificed prematurely were considered to be related to the general poor health status of the animals rather than treatment with the test compound.

Gross Pathology

Main study male and female animals were sacrificed following completion of 103 and 104 weeks of treatment, respectively. Necropsies (including examination of the external surface and orifices were conducted on all main study animals.

Results: No treatment-related gross pathological changes were identified in any group.

Histopathology

At necropsy, organs and tissues listed in the Applicant's table below were collected, weighed, and preserved for histopathological examination (main study animals only). Masses and lymph nodes draining the regions adjacent to masses were also preserved. Histopathological evaluation was conducted for all main study animals sacrificed at the end of the treatment period and those that died prematurely. In addition, satellite animal number 202 (30 mg/kg/day) was evaluated to determine potential reasons for the deterioration of this animal, necessitating premature sacrifice.

Tissue and regions examined	Necropsy Fix	Histology	Pathology Light microscopy
Abnormalities	*	*	*
Adrenals	*	*	*
Aorta - thoracic	*	*	*
Brain (cerebellum, cerebrum, midbrain)	*	*	*
Caecum	*	*	*
Colon	*	*	*
Duodenum	*	*	*
Epididymides	*	*	*
Eyes	*	*	*
Femur (femorotibial joint)	b)	*	*
Harderian glands	*	*	*
Head	a)	#	#
Heart (including auricular and ventricular regions)	*	*	*
Ileum	*	*	*
Jejunum	*	*	*
Kidneys	*	*	*
Lachrymal glands	*	*	*
Larynx	*	*	*
Liver (section from two lobes)	*	*	*
Lungs (section from two major lobes including bronchi)	*	*	*
Lymph nodes - mandibular	*	*	*
- mesenteric	*	*	*
- left axillary	*	*	*
Oesophagus	*	*	*
Optic nerves	*	*	*
Ovaries (with oviduct)	*	*	*
Pancreas	*	*	*
Peyer's patches	*	*	*
Pituitary	*	*	*
Preputial/clitoral glands	*	*	*
Prostate	*	*	*
Rectum	*	*	*
Salivary glands - submandibular	*	†	†
- parotid	*	#	#
- sublingual	*	#	#
Sciatic nerves	*	†	†
Seminal vesicles	*	*	*
Skeletal muscle	*	†	†
Skin with mammary glands (inguinal area)	*	*	*
Spinal cord (transverse and longitudinal sections at the cervical, thoracic and lumbar levels)	*	*	*
Spleen	*	*	*
Sternum	*	*	*
Stomach	*	*	*
Testes	*	*	*
Thymus	*	*	*
Thyroid with parathyroids	*	*	*
Tongue	*	*	*
Trachea	*	*	*
Ureters	*	*	*
Urinary bladder	*	*	*
Uterus with cervix	*	*	*
Vagina	*	*	*
Zymbals gland with external ear	*	#	#

a) Including nasal cavity, paranasal sinuses and nasopharynx.
b) Both hindlimbs retained, one sectioned where appropriate.
* Samples fixed or sections examined microscopically.
# Not examined.
† Only one examined.

Peer Review
A histopathology peer review was conducted.

Neoplastic

Males: The following table (copied from the FDA statistical review; H. Chen) summarizes tumor types with p-values ≤0.05 for dose response relationship and/or pairwise comparisons of treated groups and vehicle control group.

Table 2. Summary Table of Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship and/or Pairwise Comparisons of Treated Groups and Vehicle Control Group in Rats

Organ name	Tumor name	0 mg Vehicle (C) P - Trend	10 mg Low (L) P - C vs. L	30 mg Mid (M) P - C vs. M	100 mg High (H) P - C vs. H
Male: H-Poietic Tumor	Malignant Lymphoma	0/48 (65)	0/50 (65)	2/49 (65)	3/48 (65)
		0.0227 $	NC	0.2526	0.1211
Male: Liver	Hepatocellular Adenoma/ Hepatocellular Carcinoma	0/48 (65)	0/50 (65)	1/49 (65)	3/47 (65)
		0.0170 @	NC	0.5052	0.1171

& X/YY (ZZ): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
$ = Statistically significant at 0.025 level in rare tumor for test of dose response relationship;
@ = Not statistically significant at 0.005 level in common tumor for test of dose response relationship;
NC = Not calculable.

The incidence of malignant lymphoma was numerically higher at ≥30 mg/kg/day, as compared to controls, and the incidence exceeded the historical control range for the testing facility of 0.0 – 3.1% (mean 0.75%). However, based on the lack of statistical significance in pairwise comparisons (p=0.1211; FDA statistics review), this tumor type does not meet CDER criteria for statistical significance.

Although a p-value of 0.0170 was noted for the dose response relationship of combined hepatocellular adenoma/carcinoma, this trend was not statistically significant as per CDER criteria since this tumor combination was considered to be common based on historical control data. In addition, pairwise comparison of this tumor combination at the high dose versus control did not meet statistical significance as per CDER criteria (p = 0.1171; FDA statistics review).

Females: In females, there were no statistically significant findings for any tumor types at any dose.

Non Neoplastic
In males, there was an increased incidence of alveolitis in the lung at all dose levels, compared to controls. The incidence of this finding was 9/65, 12/65, 17/65, and 18/65 animals in the control, low-, mid-, and high dose groups, respectively. Also in the lung of males, the incidence of increased alveolar macrophages was greater at 30 and 100 mg/kg/day (22/65 and 27/65 animals, respectively) than the incidence in controls (16/65). In the stomach of males, the incidences of epithelial hyperplasia and submucosal inflammation of the nonglandular region were increased. The incidence of epithelial hyperplasia of the nonglandular region was 4/65, 5/65, 7/65, and 10/65 animals in the control, low-, mid-, and high dose groups, respectively. The incidence of

submucosal inflammation in the nonglandular region was 9/65 high dose animals, compared to 5/65 control animals.

As summarized in the Applicant's table below, there was an increased incidence of reduced or absent corpora lutea in the ovaries at all dose levels, compared to controls. The incidence of this finding in all treatment groups (including controls) exceeded the historical control range (4.5 – 7.7%). Also in the ovaries, the incidence of cysts was increased, compared to controls (11/65, 17/65, 17/65, and 16/65 control, low-, mid-, and high dose animals, respectively).

Summary of incidental findings in the ovaries after 104 weeks of treatment

Group/sex Dose (mg/kg/day)	1F 0	2F 10	3F 30	4F 100
Reduced or absent corpora lutea				
Present	35	51	50	47
Percentage	53.8	78.5	76.9	72.3
Number of tissues examined	65	65	65	65

In the thymus of females, the incidences of cysts and epithelial hyperplasia were increased, compared to controls (although not dose-related in all cases). The incidence of cysts was 10/63, 12/63, 12/65, and 17/65 in the control, low-, mid-, and high dose groups respectively. The incidence of epithelial hyperplasia was 11/63, 21/63, 18/65, and 21/65 animals in the control, low-, mid-, and high dose groups, respectively. In addition, in the stomach, the incidence of submucosal inflammation in the nonglandular region was increased at the high dose (6/65 animals, compared to 1/65, 3/65, and 1/65 control, low-, and mid-dose animals). In the mammary gland, the incidence of galatocele(s) was 8/65 and 10/65 mid- and high dose animals, compared to 4/65 control and low-dose animals.

Toxicokinetics

Blood samples were collected from satellite animals at 1, 2, and 24 h after dosing during Weeks 13 and 26. Plasma samples were analyzed for S-297995 and metabolites using a validated LC-MS/MS method. Metabolites evaluated in the study were: benzamidine, Nor-S-297995, S-297995 3-O-β-D-glucuronide, and S-297995 6-O-β-D-glucuronide.

Results: According to the TK report, if plasma concentrations were not quantifiable at all 3 sampling times at all 3 dose levels, Cmax and AUC values were calculated for information purposes but not used for assessment of exposure. Instead, quantifiable mean plasma concentration data were used as indices of exposure for S-297995, Nor-S-297995, S-297995 3-O-beta-D-glucuronide, and S-297995 6-O-beta-D-glucuronide. For benzamidine, Cmax and AUC values for benzamidine were calculated and used to assess exposure in the report. Summary data are presented in the following tables copied directly from the study report.

S-297995

Dose level (mg/kg/day)	C_{1h} (ng/mL) Week 13 Males	Week 13 Females	Week 26 Males	Week 26 Females	C_{2h} (ng/mL) Week 13 Males	Week 13 Females	Week 26 Males	Week 26 Females
10	1300	1470	763	1300	714	739	493	634
30	3130	5580	3060	6950	2070	2650	2320	3580
100	6680	9380	8800	13200	6790	4450	8700	8150

Benzamidine

Dose level (mg/kg/day)	C_{max} (ng/mL) Week 13 Males	Week 13 Females	Week 26 Males	Week 26 Females	AUC_{0-24h} (ng.h/mL) Week 13 Males	Week 13 Females	Week 26 Males	Week 26 Females
10	23.6	6.14	18.7	8.24	351	117	259	127
30	48.3	43.4	55.7	25.4	905	695	791	391
100	57.1	75.2	160	155	1010	1320	2280	2400

Nor-S-297995

Dose level (mg/kg/day)	C_{1h} (ng/mL) Week 13 Males	Week 13 Females	Week 26 Males	Week 26 Females	C_{2h} (ng/mL) Week 13 Males	Week 13 Females	Week 26 Males	Week 26 Females
10	70.4	66.5	50.9	71.3	43.0	26.0	-	34.3
30	167	92.7	183	154	122	51.7	177	77.0
100	611	139	1240	301	659	85.3	1750	230

S-297995 3-O-beta-D-glucuronide

Dose level (mg/kg/day)	C_{1h} (ng/mL) Week 13 Males	Week 13 Females	Week 26 Males	Week 26 Females	C_{2h} (ng/mL) Week 13 Males	Week 13 Females	Week 26 Males	Week 26 Females
10	72.4	164	88.7	245	32.9	52.8	38.2	54.3
30	228	340	362	769	99.3	151	211	239
100	2730	744	9200	2320	1930	382	10100	1320

S-297995 6-O-beta-D-glucuronide

Dose level (mg/kg/day)	C_{1h} (ng/mL) Week 13 Males	Week 13 Females	Week 26 Males	Week 26 Females	C_{2h} (ng/mL) Week 13 Males	Week 13 Females	Week 26 Males	Week 26 Females
30	16.4	10.2	13.6	15.1	-	-	-	-
100	54.3	19.6	104	42.3	31.7	6.53	111	20.0

TK parameters for naldemedine (S-297995) and its metabolites are summarized in the Applicant's table below copied directly from the Applicant's Toxicology Written Summary (2.6.6.5.2 Carcinogenicity Study in Rats).

Table 2.6.6-11 Toxicokinetic Parameters in Carcinogenicity Study in Rats

			Dose (mg/kg/day)					
			Male			Female		
Analyte	Parameter	Week	10	30	100	10	30	100
Naldemedine	C_{max} (μg/mL)	13	1.30	3.13	7.05	1.47	5.58	9.38
		26	0.763	3.17	9.42	1.30	6.95	13.2
	AUC_{0-24hr} (μg·hr/mL)	13	9.52	27.0	84.8	9.98	36.3	61.0
		26	6.44	29.7	109	8.59	48.2	107
Nor-Naldemedine	C_{max} (μg/mL)	13	0.0704	0.167 [(a)]	0.702	0.0665	0.0927 [(a)]	0.139
		26	0.0509	0.201 [(a)]	1.75	0.0713	0.154	0.306
	AUC_{0-24hr} (μg·hr/mL)	13	0.565	1.57	8.18	0.365	0.686	1.12
		26	0.297	2.21	21.5	0.465	1.04	2.95
Naldemedine 3-G	C_{max} (μg/mL)	13	0.0724	0.228 [(a)]	2.79	0.164	0.340	0.744
		26	0.0887	0.362 [(a)]	10.1	0.245	0.769	2.32
	AUC_{0-24hr} (μg·hr/mL)	13	0.450	1.37 [(a)]	25.1	0.769	2.13	5.19
		26	0.528	2.79 [(a)]	125	0.869	3.51	17.5
Naldemedine 6-G	C_{max} (μg/mL)	13	N.C.	0.0164 [(a)]	0.0543	N.C.	0.0102	0.0196
		26	N.C.	0.0136 [(a)]	0.123	N.C.	0.0151	0.0423
	AUC_{0-24hr} (μg·hr/mL)	13	N.C.	0.0164 [(a)]	0.419	N.C.	0.0102	0.0947
		26	N.C.	0.0508 [(a)]	1.38	N.C.	0.0384	0.272
Benzamidine	C_{max} (μg/mL)	13	0.0236	0.0483 [(a)]	0.0571	0.00614	0.0434	0.0752
		26	0.0187	0.0557 [(a)]	0.160	0.00824	0.0254	0.155
	AUC_{0-24hr} (μg·hr/mL)	13	0.351	0.905 [(a)]	1.01	0.117	0.695	1.32
		26	0.259	0.791 [(a)]	2.28	0.127	0.391	2.40

N.C., Not calculated because of BLQ (< 0.005 μg/mL for naldemedine 6-G).

(a) This mean value was calculated from 2 animals.

Dosing Solution Analysis

Samples were collected from the dosing solutions used in Weeks 1, 13, 26, 27 (Group 3 only for TK sampling), 39, 52, 65, 81, 91, 103, and 105 for determination of the test article concentration using a validated analytical method. The homogeneity and stability of the high dose formulation in refrigerated storage were evaluated out to 12 days. According to the report, as part of a previous study, the homogeneity and stability of formulations of 1 and 134 mg/mL were shown to be stable for 8 days when stored at 4ºC.

Results: Mean concentrations of S-297995 ranged from -9.0% to +3.0% of nominal concentrations, and thus were considered acceptable. After 12 days of refrigerated storage, mean concentrations of S-297995 in high dose formulation samples were within 1% of Day 0 values and the coefficient of variation was less than 1%.

Study title: 104-Week Carcinogenicity Study of S-297995 Monotosylate in Mice by Gavage

Study no.:	YDL0037 (Sponsor Reference Number: S-297995-TF-265-L; Study No. YDL0037)
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 5, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	S-297995 monotosylate; Batch # B15001; Content 99.7% (on anhydrous basis)
CAC concurrence:	Yes (ECAC meeting minutes re: dose selection dated November 17, 2011, Attachment 1)

Key Study Findings

- There was no statistically significant effect on mortality in either sex.
- There were no statistically significant effects on body weight gain in either sex.
- In males only, there was a small (+6%, compared to control) increase in food consumption at the high dose.
- There were no drug-related neoplastic findings in male or female mice (ECAC meeting minutes dated July 21, 2016, Attachment 2).

Adequacy of Carcinogenicity Study

The carcinogenicity study conduct was acceptable. The dose selection was based on recommendations from the Executive CAC (November 7, 2011). Based on the high dose having an AUC value > 25 times that in humans, the Division and CDER Executive Carcinogenicity Assessment Committee (ECAC) concurred with the Applicant's proposed high dose of 100 mg/kg/day.

Appropriateness of Test Models

The Crl:CD1(ICR) mouse is an acceptable test model for the carcinogenicity study.

Evaluation of Tumor Findings

FDA Statistical Analysis of Tumor Data: The tumor data were analyzed for dose response relationships across the control, low-, mid-, and high dose groups, and pairwise comparisons of each of the three treated groups (low-, mid-, and high dose) against the vehicle control group, using the Poly-k method. For the adjustment of multiple testing, common and rare tumors were tested for dose response relationship at significance levels of 0.005 and 0.025, respectively. For multiple pairwise

comparisons of treated groups with controls, test levels of 0.01 and 0.05 were used for common and rare tumors, respectively.

In males and females, there were no drug-related neoplastic findings at up to 100 mg/kg/day S-297995 monotosylate.

Methods

Doses:	0, 10, 30, 100 mg/kg/day (as free base; see Applicant's table below)
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral (Gavage)
Formulation/Vehicle:	0.5% w/v methylcellulose (MC) aqueous solution
Basis of dose selection:	AUC ratio (based on the high dose having an AUC value > 25 times that in humans; Mouse AUC values based on 13-week oral toxicity and TK study (Sponsor's Study No. S-297995-TF-108-L; CRO Study No. YDL0024)
Species/Strain:	Crl:CD1(ICR) mice
Number/Sex/Group:	Toxicology: 60/sex/group Satellite: 18/sex/group
Age:	36 to 42 days of age at start of treatment
Animal housing:	3 animals of the same sex per cage
Paradigm for dietary restriction:	No diet restriction
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	Yes (for TK analysis)
Deviation from study protocol:	Protocol deviations did not affect the quality or integrity of the study.

The following table copied from the Applicant's study report shows the study group assignments.

Group	Treatment	Dose# (mg/kg/day)	Number of animals			
			Main study		Satellite study†	
			Male	Female	Male	Female
1	Control	0	60	60	18	18
2	S-297995	10	60	60	18	18
3	S-297995	30	60	60	18	18
4	S-297995	100	60	60	18	18

Expressed in terms of the free base. A weighing factor of 1.31 was used.
† Satellite animals used for toxicokinetic sampling only during Weeks 13 and 26 of the study.

Observations and Results

Mortality

Animals were checked twice daily for signs of morbidity and mortality.

Results: The Applicant's table below summarizes mortality in males and females.

Group distribution of deaths during the study

Group/sex	1M	2M	3M	4M	1F	2F	3F	4F
Dose (mg/kg/day)	0	10	30	100	0	10	30	100
Group size	60	60	60	60	60	60	60	60
Decedents	32	34	29	37	36	39	37	33
Accidental death	0	0	1	0	1	1	0	1
Terminal sacrifice	28	26	30	23	23	20	23	26

As summarized in the Applicant's table below, the most common factors contributing to death were malignant lymphoma, skin tumors/lesions (fibrosarcomas, ulcerative dermatitis), brochiolo-alveolar carcinomas, genital/lower urinary tract lesions (obstructive uropathy; males only), and ovarian lesions (hemorrhage/hemorrhagic cysts). In males, the incidence of malignant lymphoma was increased to a similar extent at all dose levels (compared to controls). In females, although no dose-relationship was apparent, the incidence of ulcerative dermatitis was increased at ≥30 mg/kg/day and the incidence of ovarian lesions was increased at all dose levels (compared to controls).

Summary of most common factors contributory to death in decedent animals during the study

Group/sex	1M	2M	3M	4M	1F	2F	3F	4F
Dose (mg/kg/day)	0	10	30	100	0	10	30	100
Malignant lymphoma	3	8	7	8	11	12	8	9
Fibrosarcoma, skin	7	3	3	2	1	4	1	1
Bronchiolo-alveolar carcinoma	3	4	1	1	2	1	2	0
Ulcerative dermatitis	1	1	2	0	2	2	8	4
Obstructive uropathy	5	7	6	8	0	0	0	0
Haemorrhage/Haemorrhagic cyst, Ovary	0	0	0	0	1	3	5	3
Number of animals examined	32	34	29	37	36	39	37	33

Independent analysis of survival data by the FDA statistical reviewer (H. Chen) showed that the numbers of mice surviving to terminal necropsy were 28 (46.67%), 26, (43.33%), 30 (50.00%), and 23 (38.33%) for control, low-, mid-, and high dose males, respectively, and 23 (38.33%), 20 (33.33%), 23 (38.33%), and 26, (43.33%) for control, low-, mid-, and high dose females, respectively. The FDA statistical reviewer's survival analysis determined that there were no statistically significant mortality findings in either sex. Results of the intercurrent mortality comparison for male and female mice are shown below in Tables 3A and 3B, respectively (copied from the statistical review from H. Chen).

Table 3A: Intercurrent Mortality Rate in Male Mice

	0 mg/kg/day Vehicle Control		10 mg/kg/day Low		30 mg/kg/day Mid		100 mg/kg/day High	
Week / Type of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	Cum %
0 - 52	6	10.00	3	5.00	4	6.67	6	10.00
53 - 78	10	26.67	11	23.33	5	15.00	14	33.33
79 - 92	8	40.00	11	41.67	13	36.67	7	45.00
93 - 104	8	13.33	9	15.00	7	11.67	10	16.67
Accidental Death					1	1.67		
Terminal sacrifice	28	46.67	26	43.33	30	50.00	23	38.33
Total	60		60		60		60	

Test	All Dose Groups	Vehicle Control vs. Low	Vehicle Control vs. Mid	Vehicle Control vs. High
Dose-Response (Likelihood Ratio)	0.3248	0.8017	0.5011	0.3702
Homogeneity (Log-Rank)	0.4793	0.8007	0.4987	0.3672

#All Cum. % Cumulative Percentage except for Terminal sacrifice;

Table 3B: Intercurrent Mortality Rate in Female Mice

	0 mg/kg/day Vehicle Control		10 mg/kg/day Low		30 mg/kg/day Mid		100 mg/kg/day High	
Week / Type of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	Cum %
0 - 52	9	15.00	4	6.67	6	10.00	9	15.00
53 - 78	5	23.33	9	21.67	12	30.00	8	28.33
79 - 92	11	41.67	10	38.33	6	40.00	8	41.67
93 - 104	11	18.33	16	26.67	13	21.67	8	13.33
Accidental Death	1	1.67	1	1.67			1	1.67
Terminal sacrifice	23	38.33	20	33.33	23	38.33	26	43.33
Total	60		60		60		60	

Test	All Dose Groups	Vehicle Control vs. Low	Vehicle Control vs. Mid	Vehicle Control vs. High
Dose-Response (Likelihood Ratio)	0.5322	0.9090	0.9838	0.6764
Homogeneity (Log-Rank)	0.9325	0.9081	0.9837	0.6740

#All Cum. % Cumulative Percentage except for Terminal sacrifice;

The Kaplan-Meier curves for survival rates of all treatment groups (from the FDA statistical review of H. Chen are shown below in Figures 2A and 2B for male and female mice, respectively.

Figure 2A: Kaplan-Meier Survival Functions for Male Mice

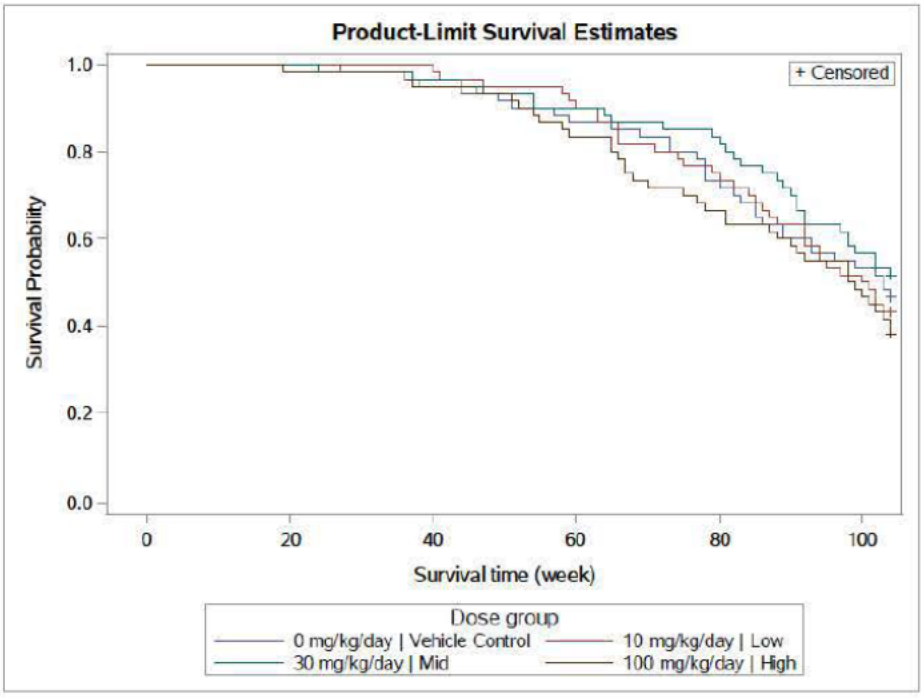
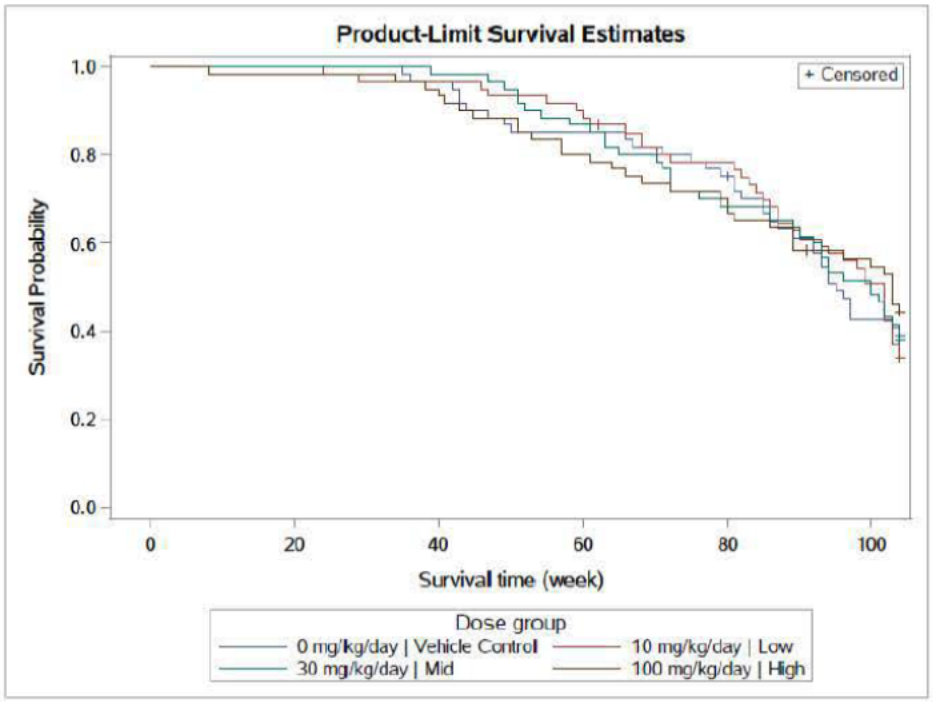


Figure 2B: Kaplan-Meier Survival Functions for Female Mice



Clinical Signs

Animals were observed for clinical signs associated with dosing daily during the first week of treatment, twice weekly during Weeks 2 to 4, once weekly during Weeks 5 to

13, every other week during Weeks 14 to 52, and once every 4 weeks thereafter. Detailed physical examinations and palpation for masses were conducted weekly. The mass number and location, date, size, and description of new masses were recorded.

Results: During a subset of weeks, following dosing there was an increased incidence of chin rubbing (high dose males and females; primarily during Week 7) and head shaking (all doses; males and females; primarily from Weeks 7 - 12), compared to controls. During Week 7, the incidence of chin rubbing after dosing was 8 and 7 males and females each, compared to 0 controls. During Week 7, the incidence of head shaking after dosing was 9, 9, and 6 males and 1, 7, and 18 females in the low-, mid-, and high dose groups, respectively, compared to 0 controls.

There was no apparent treatment-related effect on the number of animals with palpable masses, total number of masses, or the mean time of onset in either sex.

Body Weights

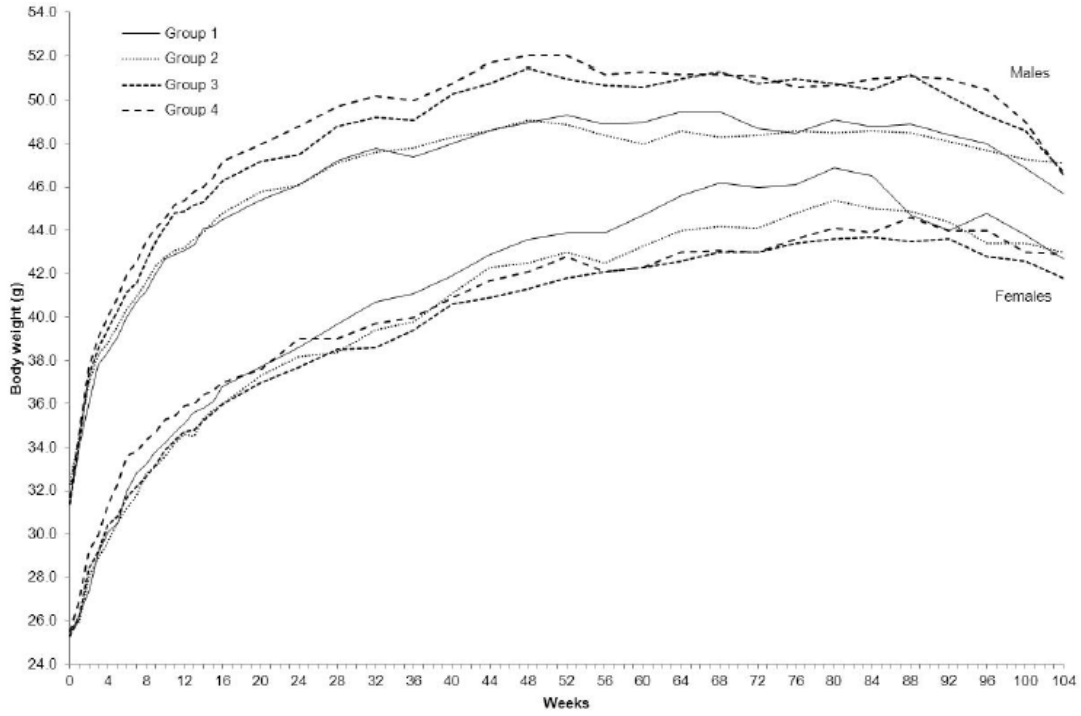
Body weights were recorded at Week -1, the first day of treatment, weekly intervals for the first 16 weeks of treatment, once every 4 weeks thereafter, and prior to necropsy.

Results: In males, there were no treatment-related effects on body weight. Although body weight gain was generally greater in males at ≥30 mg/kg/day (compared to controls), body weight gain in males from Week 0 to 104 in the low-, mid-, and high dose groups was reported to be 101%, 106%, and 104%, respectively, of the control group (not statistically significant at any dose). In females, there were no treatment-related effects on body weight. Although body weight gains were generally lower than controls, body weight gain in females from Week 0 to 104 in the low-, mid-, and high dose groups was reported to be 98%, 97%, and 98%, respectively, of the control group (not statistically significant at any dose).

The Applicant's figure below shows mean body weights in males and females over the course of the study period.

Body weight - group mean values (g)

Group	:	1	2	3	4
Compound	:	Control	S-297995	S-297995	S-297995
Dose (mg/kg/day)	:	0	10	30	100



Feed Consumption

Food consumption was recorded for Week -1, weekly for the first 16 weeks of treatment, and once every 4 weeks thereafter.

Results: In males, there was a small (but statistically significant) increase in food consumption at the high dose. Mean food consumption from Week 1 – 104 was 103%, 104%, and 106%, of controls, respectively. In females, there were no statistically significant differences in food consumption compared to controls.

Clinical Pathology

Blood samples were collected at terminal sacrifice and from decedents (if possible). Samples were analyzed for the following hematological parameters: erythrocyte count, total white cell count, and differential white blood cell count (neutrophils, lymphocytes, eosinophils, basophils, monocytes, and large unstained cells). Blood films were prepared for examination of abnormalities by light microscopy.

Results:
In males, there was a statistically significant decrease in neutrophils and percent neutrophils at 100 mg/kg/day (-34% and 29%, respectively, compared to controls). There also was a small but statistically significant increase in percent lymphocytes at 100 mg/kg/day (+14%, compared to controls). There were no apparent treatment-related changes in hematological parameters in females.

Gross Pathology

Detailed necropsies (including examination of the external surface and orifices) were conducted on all animals.

Results: In the liver, an increased incidence of masses was observed, compared to the incidence in controls. In males, the increased incidence occurred at all doses with no apparent dose relationship. In females, the incidence of this finding was greatest in the low dose group, and there was no dose relationship. The tables below summarize the incidence of masses in the liver observed in animals prematurely sacrificed or found dead, animals terminated after 104 weeks of treatment, and the overall incidence in the study.

Males: Incidence of masses in the liver/number of animals examined (%)

	Control	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Animals prematurely sacrificed or found dead	3/32 (9.4%)	8/34 (23.5%)	9/30 (30%)	8/37 (21.6%)
Terminal sacrifice	2/28 (7.1%)	5/26 (19.2%)	21/30 (70%)	6/23 (26.1%)
Overall	5/60 (8.3%)	13/60 (21.7%)	30/60 (50%)	14/60 (23.3%)

Females: Incidence of masses in the liver/number of animals examined (%)

	Control	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Animals prematurely sacrificed or found dead	0/37 (0%)	3/40 (7.5%)	0/37 (0%)	1/34 (2.9%)
Terminal sacrifice	1/23 (4.3%)	1/20 (5%)	0/23 (0%)	0/26 (0%)
Overall	1/60 (1.7%)	4/60 (6.7%)	0/60 (0%)	1/60 (1.7%)

In males, an increased incidence of distended and discolored seminal vesicles was observed at all doses, relative to the incidence in controls, as summarized in the tables below.

Incidence of findings in the seminal vesicles/number of animals examined (%)

	Control	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Distended				
Animals prematurely sacrificed or found dead	13/32 (40.6%)	15/34 (44.1%)	21/30 (70%)	23/37 (62.2%)

Terminal sacrifice	16/28 (57.1%)	22/26 (84.6%)	24/30 (80%)	22/23 (95.7%)
Overall	29/60 (48.3%)	37/60 (61.7%)	45/60 (75%)	45/60 (75%)
Discolored				
Animals prematurely sacrificed or found dead	0/22 (0%)	2/34 (5.9%)	5/30 (16.7%)	3/37 (8.1%)
Terminal sacrifice	2/28 (7.1%)	9/26 (34.6%)	4/30 (13.3%)	7/23 (30.4%)
Overall	2/60 (3.3%)	11/60 (18.3%)	9/60 (15%)	10/60 (16.7%)

Histopathology

At necropsy, organs and tissues listed in the Applicant's table below were preserved for histopathological examination. Masses and lymph nodes draining the regions adjacent to masses were also preserved.

Tissue and regions examined	Necropsy	Histology	Pathology
	Fix		Light microscopy
Abnormalities	*	*	*
Adrenals	*	*	*
Aorta - thoracic	*	*	*
Brain (cerebellum, cerebrum, midbrain)	*	*	*
Caecum	*	*	*
Colon	*	*	*
Duodenum	*	*	*
Epididymides	*	*	*
Eyes	*	*	*
Femur (femorotibial joint)	b)	*	*
Gall bladder	*	*	*
Harderian glands	*	*	*
Head	a)	#	#
Heart (including auricular and ventricular regions)	*	*	*
Ileum	*	*	*
Jejunum	*	*	*
Kidneys	*	*	*
Lachrymal glands	*	*	*
Larynx	*	*	*
Liver (section from two lobes)	*	*	*
Lungs (section from two major lobes including bronchi)	*	*	*
Lymph nodes - mandibular	*	*	*
- mesenteric	*	*	*
- left axillary	*	*	*
Oesophagus	*	*	*
Optic nerves	*	*	*
Ovaries (with oviduct)	*	*	*
Pancreas	*	*	*
Peyer's patches	*	*	*
Pituitary	*	*	*
Preputial/clitoral glands	*	*	*
Prostate	*	*	*
Rectum	*	*	*

Tissue and regions examined	Necropsy	Histology	Pathology
	Fix		Light microscopy
Salivary glands - submandibular	*	†	†
- parotid	*	#	#
- sublingual	*	#	#
Sciatic nerves	*	†	†
Seminal vesicles	*	*	*
Skeletal muscle (thigh)	*	†	†
Skin with mammary glands (inguinal area)	*	*	*
Spinal cord (transverse and longitudinal sections at the cervical, thoracic and lumbar levels)	*	*	*
Spleen	*	*	*
Sternum	*	*	*
Stomach	*	*	*
Testes	*	*	*
Thymus	*	*	*
Thyroid with parathyroids	*	*	*
Tongue	*	*	*
Trachea	*	*	*
Ureters	*	*	*
Urinary bladder	*	*	*
Uterus with cervix	*	*	*
Vagina	*	*	*
Zymbals gland with external ear	*	#	#

In addition, carcass was retained.
a) Including nasal cavity, paranasal sinuses and nasopharynx.
b) Both hindlimbs retained, one sectioned where appropriate.
* Samples fixed or sections examined microscopically.
# Not examined.
† Only one examined.

Peer Review

A histopathology peer review was conducted.

Neoplastic

Analysis of tumor data by the FDA statistical reviewer showed no statistically significant dose response relationships or pairwise comparisons in either sex.

While not statistically significant, an increased incidence of hepatocellular neoplasms was noted in males. In addition, there was an increased incidence of subcapsular cell adenoma in the adrenal gland of males at ≥30 mg/kg/day. However, no dose relationship was apparent. For reference, the Applicant's tables below summarize the incidence of these findings in males.

Summary of hepatocellular neoplasms for all animals

Group/sex	1M	2M	3M	4M	1F	2F	3F	4F
Dose (mg/kg/day)	0	10	30	100	0	10	30	100
Hepatocellular carcinoma	0	0	3	0	0	0	0	1
Hepatocellular adenoma	4	8	10	10	0	0	0	0
Number of tissues examined	60	60	60	60	60	60	60	60

Summary of adrenal neoplasms for all animals

Group/sex Dose (mg/kg/day)	1M 0	2M 10	3M 30	4M 100	1F 0	2F 10	3F 30	4F 100
Subcapsular Cell Adenoma	1	1	8	4	0	1	3	0
Number of tissues examined	59	59	58	60	60	60	59	60

Non Neoplastic

In males, there was an increased incidence of increased colloid of the seminal vesicles at all doses, compared to controls. The incidence of this finding is summarized in the Applicant's table below.

Summary of selected findings in the seminal vesicles for all animals

Group/sex Dose (mg/kg/day)	1M 0	2M 10	3M 30	4M 100
Increased colloid				
Slight	15	22	26	22
Moderate	7	10	15	22
Total	22	32	41	44
Number of tissues examined	60	60	59	60

While not dose-related, there also was an increased incidence of thickening/fibrosis of the seminal vesicles wall (0/60, 8/60, 5/60, and 2/60 control, low-, mid-, and high dose males, respectively). In the epididymis, reduced numbers of spermatozoa were observed in 4/60, 8/60, 9/60, and 8/60 control, low-, mid-, and high dose males, respectively. Absent spermatozoa were observed in 1/60, 8/60, 8/60, and 5/60 control, low-, mid-, and high dose males. Mineralization in the epididymis was observed in 3/60 mid- and high dose males each, compared to 0/60 control and low- dose males.

In females, there was an increased incidence in tubular casts in the kidneys in animals treated with S-297995 (2/60, 3/60, 10/60, and 11/60 animals in the control, low-, mid-, and high dose groups, respectively). The incidence of this finding in males from all groups (including controls) ranged from 8/60 to 12/60 animals, however.

Toxicokinetics

Blood samples were collected from satellite animals at 1, 2, and 24 h after dosing during Weeks 13 and 26. Plasma samples were analyzed for S-297995 and metabolites using a validated LC-MS/MS method. Metabolites evaluated in the study were: benzamidine, Nor-S-297995, S-297995 3-O-β-D-glucuronide, and S-297995 6-O-β-D-glucuronide.

Results: According to the TK report, since mean plasma concentrations were not quantifiable at all 3 sampling times at all 3 dose levels, Cmax and AUC values were calculated for information purposes but were not used for assessment of exposure.

Instead, quantifiable mean plasma concentration data at 1 and 2 h after dosing were used as indices of systemic exposure. Summary data are presented in the following tables copied directly from the study report.

S-297995

Dose level	C_{1h} (ng/mL)				C_{2h} (ng/mL)			
	Week 13		Week 26		Week 13		Week 26	
(mg/kg/day)	Males	Females	Males	Females	Males	Females	Males	Females
10	2190	3410	2540	5080	2900	3070	2580	3660
30	8030	10500	8110	10000	5690	6940	8370	10800
100	18700	21200	27400	23300	14200	14600	27100	23800

Benzamidine

Dose level	C_{1h} (ng/mL)				C_{2h} (ng/mL)			
	Week 13		Week 26		Week 13		Week 26	
(mg/kg/day)	Males	Females	Males	Females	Males	Females	Males	Females
10	4.12	3.54	4.73	4.19	13.5	15.0	17.2	24.5
30	19.7	18.6	11.1	16.9	26.1	40.6	37.7	32.9
100	23.7	34.2	31.4	45.8	97.4	109	78.2	93.5

Nor-S-297995

Dose level	C_{1h} (ng/mL)				C_{2h} (ng/mL)			
	Week 13		Week 26		Week 13		Week 26	
(mg/kg/day)	Males	Females	Males	Females	Males	Females	Males	Females
10	143	204	220	266	295	200	242	296
30	517	689	658	560	692	681	1280	699
100	1180	1180	2080	1250	1980	1190	2300	2130

S-297995 3-O-beta-D-glucuronide

Dose level	C_{1h} (ng/mL)				C_{2h} (ng/mL)			
	Week 13		Week 26		Week 13		Week 26	
(mg/kg/day)	Males	Females	Males	Females	Males	Females	Males	Females
10	-	-	-	-	6.84	-	-	-
30	36.6	23.4	37.9	58.0	12.2	14.5	31.0	13.0
100	80.6	61.1	150	103	107	42.2	241	156

S-297995 6-O-beta-D-glucuronide

Dose level	C_{1h} (ng/mL)				C_{2h} (ng/mL)			
	Week 13		Week 26		Week 13		Week 26	
(mg/kg/day)	Males	Females	Males	Females	Males	Females	Males	Females
30	8.55	10.6	6.56	12.4	-	8.43	11.4	10.5
100	17.3	25.5	28.5	25.8	29.9	21.8	52.1	61.9

TK parameters for naldemedine (S-297995) and its metabolites are summarized in the Applicant's table below copied directly from the Applicant's Toxicology Written Summary (2.6.6.5.1 Carcinogenicity Study in Mice).

Table 2.6.6-10 Toxicokinetic Parameters in Carcinogenicity Study in Mice

			Dose (mg/kg/day)					
			Male			Female		
Analyte	Parameter	Week	10	30	100	10	30	100
Naldemedine	C_{max} (µg/mL)	13	2.90	8.03	18.7	3.41	10.5	21.2
		26	2.58	8.37	27.4	5.08	10.8	23.8
	AUC_{0-24hr} (µg·hr/mL)	13	35.8	73.5	182	38.7	90.3	189
		26	32.2	104	339	47.2	134	297
Nor-Naldemedine	C_{max} (µg/mL)	13	0.295	0.692	1.98	0.204	0.689	1.19
		26	0.242	1.28	2.30	0.296	0.699	2.13
	AUC_{0-24hr} (µg·hr/mL)	13	3.54	8.48	24.0	2.50	8.52	14.9
		26	3.00	15.4	28.5	3.67	8.60	25.7
Naldemedine 3-G	C_{max} (µg/mL)	13	0.00684	0.0366	0.107	N.C.	0.0234	0.0611
		26	N.C.	0.0379	0.241	0.00425 [a]	0.0580	0.156
	AUC_{0-24hr} (µg·hr/mL)	13	0.0806	0.177	1.31	N.C.	0.190	0.546
		26	N.C.	0.394	2.92	0.00425 [b]	0.208	1.90
Naldemedine 6-G	C_{max} (µg/mL)	13	N.C.	0.00855	0.0299	N.C.	0.0106	0.0255
		26	N.C.	0.0114	0.0521	N.C.	0.0124	0.0619
	AUC_{0-24hr} (µg·hr/mL)	13	N.C.	0.533	0.361	N.C.	0.108	0.276
		26	N.C.	0.138	0.628	N.C.	0.133	0.738
Benzamidine	C_{max} (µg/mL)	13	0.0135	0.0261	0.0974	0.0150	0.0406	0.109
		26	0.0172	0.0377	0.0782	0.0245	0.0329	0.0935
	AUC_{0-24hr} (µg·hr/mL)	13	0.159	0.329	1.20	0.176	0.511	1.41
		26	0.206	0.464	1.00	0.298	0.415	1.24

N.C., not calculated because of BLQ (< 0.005 µg/mL for naldemedine 3-G and naldemedine 6-G)..

(a) This mean value was calculated from 2 animals.

(b) This mean value was calculated from only 1 plasma concentration.

Dosing Solution Analysis

Samples were collected from the dosing solutions used in Weeks 1, 13, 26, 39, 52, 65, 78, 91, and 103 for determination of the test article concentration using a validated analytical method. According to the report, as part of a previous study, the homogeneity and stability of formulations of 1 and 134 mg/mL were shown to be stable for 8 days when stored at 4ºC.

Results: Mean concentrations of S-297995 ranged from -7.0% to +2.0% of nominal concentrations, and thus were considered acceptable.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Review of Study No. R-297995-TF-104-L (CRO Study No. 100428) from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.). In addition to the findings discussed in Dr. Wu's review, it is noted that in the 10, 100, and 1000 mg/kg groups, there was a dose-dependent increase in the incidence of irregular estrous cycles (prolongation of estrous cycles with continued diestrous at the beginning of the dosing period) and the number of estrous cases was lower than in the control group. However, with the exception of a single high dose female, all females with irregular cycles had estrous cases during the pre-mating or mating period and/or successfully mated during the mating period.

Study title: Oral Study for Effects of S-297995 monotosylate on Fertility and Early Embryonic Development to Implantation in Rats

Study no.:	S-297995-TF-104-L
Study report location:	Electronic submission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 19, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RSC-297995, lot no. A81001, 97.5% purity

Key Study Findings

In the Segment I fertility and early embryonic development study with S-297995 in rats, the drug was administered to male and female animals at 1, 10, 100 and 1000 mg/kg doses. S-297995 had no effects on the fertility and general reproductive performance of male and female rats at oral doses up to 1000 mg/kg.

Methods

Doses:	0, 1, 10, 100, and 1000 mg/kg/day
Frequency of dosing:	Once a day
Dose volume:	10 mL/kg
Route of administration:	Oral by gavage
Formulation/Vehicle:	0.5 w/v% methylcellulose aqueous solution
Species/Strain:	Rats [Crl: CD (SD), SPF]
Number/Sex/Group:	20
Satellite groups:	None
Study design:	S-297995 monotosylate at 0, 1, 10, 100, and 1000 mg/kg/day was administered orally by gavage to male rats daily for a total of 70 - 73 days beginning 28 days before mating and continuing until the day before necropsy and to female rats daily for 14 days before mating and continuing until Day 7 of pregnancy, and the effects of S-297995 monotosylate on reproductive functions of parental animals and early embryonic development were assessed.
Deviation from study protocol:	The sponsor stated 4 minor deviations from the SOP.

Observations and Results

Mortality

No dead or moribund males and females were noted in any group.

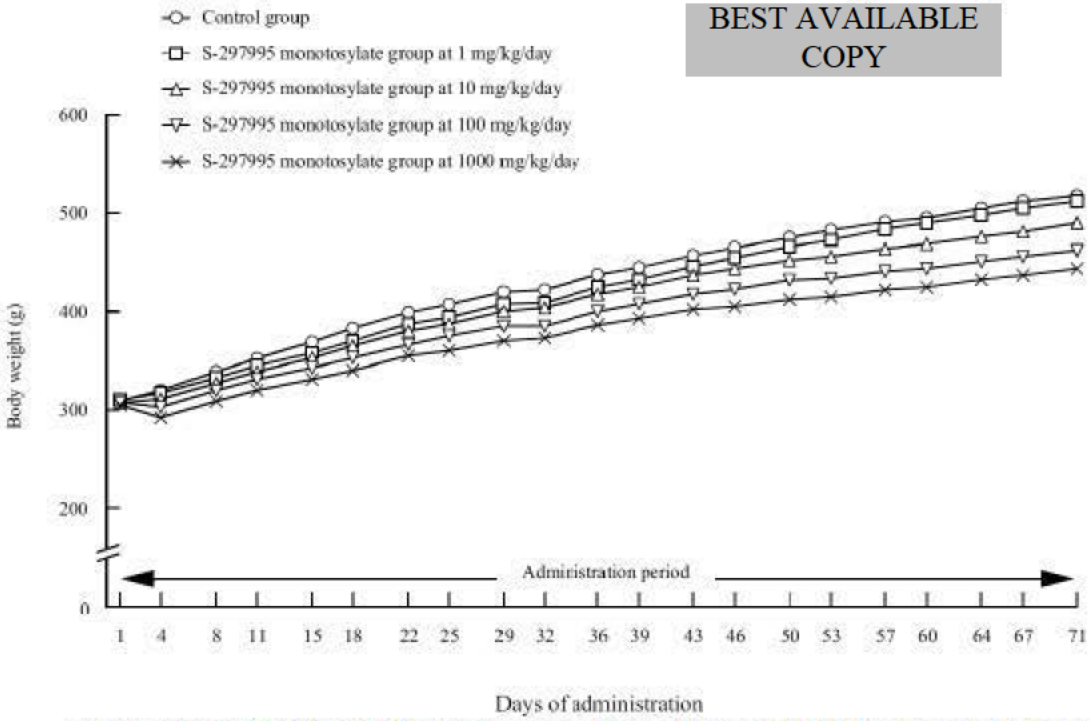
Clinical Signs

In the 1000 mg/kg group, salivation was noted sporadically throughout the dosing period in all males. No abnormal signs were noted in the 1 mg/kg, 10 mg/kg, 100 mg/kg, or control group.

Body Weight

The initial mean body weights in male rats were 309 g and 518g on day 71. No significant differences from the control group were seen in the 1 mg/kg group on any day of measurement. In the 10 mg/kg group, body weight was significantly lower than in the control group on Days 8, 11, 15, 18, 22, 25, 53, 64, 67, and 71 of administration. Although no significant differences from the control group were seen, body weight in this group tended to be lower on

Days 4, 29, 32, 36, 39, 43, 46, 50, 57, and 60 of administration. In the 100 and 1000 mg/kg groups, body weight was significantly lower than in the control group on Days 4 - 71 of administration as shown in the figure below.



The initial mean body weights of female rats were 235 g and 280g on day 18. During the pre-mating and mating periods, no significant differences from the control group were seen in the body weight in the 1, 10, or 100 mg/kg group. In the 1000 mg/kg group, body weight was significantly lower than in the control group on Day 4 of administration. During the pregnancy period, no significant differences from the control group were seen in the 1, 10, 100, or 1000 mg/kg group on any day of measurement.

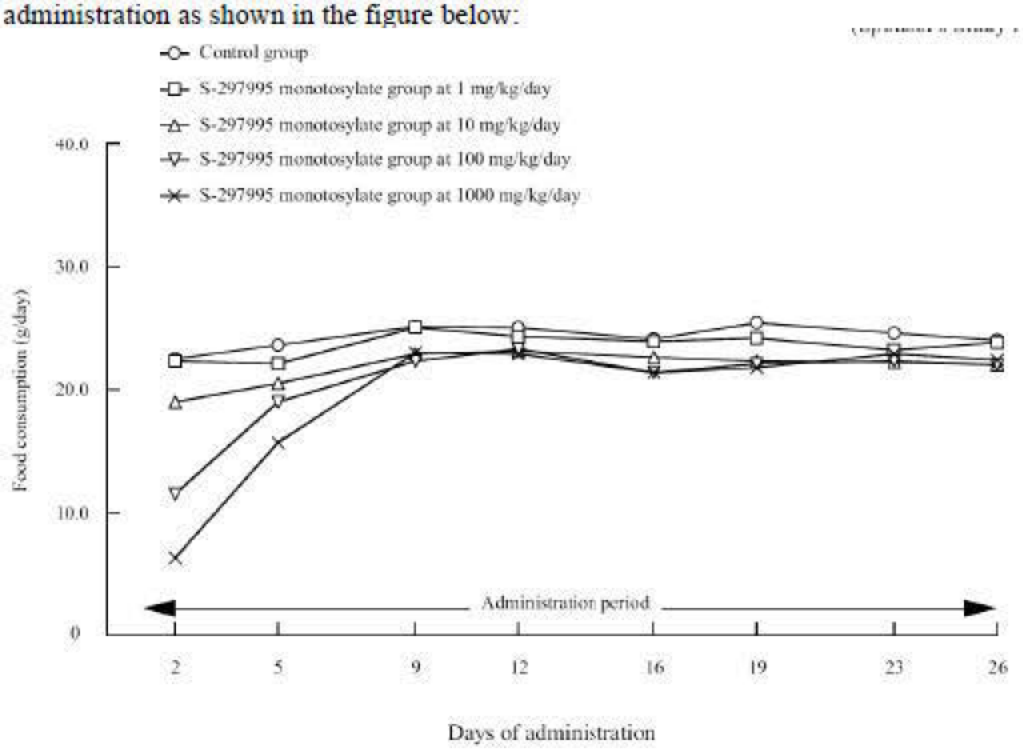
Feed Consumption

In, males, no significant differences from the control group were seen in the 1 mg/kg group on any day of measurement. In the 10 and 100 mg/kg groups, food consumption was significantly lower than in the control group on Days 2, 5, 9, 19, and 23 of administration. Although no significant differences from the control group were seen, food consumption in these groups tended to be lower on Days 12 and 16 of administration. In the 1000 mg/kg group, food consumption was significantly lower than in the control group on Days 2, 5, 9, 16, and 19 of administration. Although no significant difference from the control group was seen, food consumption in this group tended to be lower on Days 12 and 23 of administration as shown in the table below.

Group	Control	S-297995 monotosylate			
mg/kg/day	0	1	10	100	1000
Number of males	20	20	20	20	20
Days of administration					
2	22.5 ± 3.8	22.3 ± 4.9	19.0 ± 2.8 #	11.5 ± 2.7 ##	6.3 ± 1.7 ##
5	23.6 ± 3.1	22.1 ± 2.3	20.5 ± 2.2 **	19.0 ± 3.0 **	15.7 ± 3.0 **
9	25.1 ± 2.3	25.0 ± 2.6	22.9 ± 3.0 *	22.3 ± 2.5 **	23.0 ± 2.4 *
12	25.0 ± 3.2	24.3 ± 3.2	23.1 ± 2.6	23.3 ± 3.2	22.9 ± 1.8
16	24.1 ± 2.1	23.9 ± 3.3	22.6 ± 2.0	21.4 ± 4.0	21.4 ± 2.9 ##
19	25.4 ± 2.7	24.2 ± 3.0	22.3 ± 2.5 **	22.1 ± 2.1 **	21.8 ± 2.3 **
23	24.6 ± 2.7	23.2 ± 3.6	22.2 ± 2.7 *	22.3 ± 2.5 *	22.9 ± 2.5
26	24.0 ± 2.3	23.8 ± 2.6	22.0 ± 2.5	22.0 ± 2.3	22.4 ± 3.1

Each value shows mean (g/day) ± S.D.
Significantly different from the control group (#: p<0.05, ##: p<0.01 by Steel's test).
Significantly different from the control group (*: p<0.05, **: p<0.01 by Dunnett's test).

In females, no significant differences in food consumption from the control group were seen in the 1 mg/kg group throughout the test. In the 10 and 100 mg/kg groups, food consumption was significantly lower than in the control group on Days 2, 5, 9, 19, and 23 of administration. Although, no significant differences from the control group were seen, food consumption in these groups tended to be lower on Days 12 and 16 of administration. In the 1000 mg/kg group, food consumption was significantly lower than in the control group on Days 2, 5, 9, 16, and 19 of administration. Although no significant difference from the control group was seen, food consumption in this group tended to be lower on Days 12 and 23 of administration as shown in the figure below:



Toxicokinetics

The sponsor did not conduct the TK study.

Sperm Analysis

No significant differences from the control group were seen in any of the parameters (sperm mobility and sperm morphology) of the sperm analysis in the 1 mg/kg group. In the 10, 100, and 1000 mg/kg groups, the abnormal sperm tail rate was significantly higher than in the control group. However, the values in the 10 and 100 mg/kg groups were within the range of the background data obtained at the testing facility. In the 1000 mg/kg group, markedly high abnormal tail rate was noted in only 1 male (Male No. M05510) with softened epididymides, but the group mean value was 0.1% (within the range of the background data) when the value of this male was excluded from calculation. If this animal is included, the mean abnormal tail rate at 1000 mg/kg would be 1.0±3.9%, which was significantly higher than controls ($p<0.05$). In the 10, 100, and 1000 mg/kg groups, no significant differences from the control group were seen in any of the other parameters. Regarding the males which failed to copulate or whose female partners failed to conceive even in the additional mating (Male No. M03311 in the 10 mg/kg group and Male No. M05510 in the 1000 mg/kg group), a low value of sperm motility, a high number of morphologically abnormal sperms, and a low number of sperms were noted.

Group	Control	S-297995 monotosylate			
mg/kg/day	0	1	10	100	1000
Number of males	20	20	20	20	20
Sperm motility					
Motile sperm rate (%)	85.2 ± 3.6	82.1 ± 19.6	82.2 ± 19.4	86.2 ± 5.1	81.4 ± 19.7
Progressive sperm rate (%)[a]	34.2 ± 6.4	33.5 ± 10.8	33.0 ± 9.5	35.9 ± 10.1	35.2 ± 11.5
Path velocity (VAP) (μm/s)	160.0 ± 9.2	157.9 ± 16.7	153.2 ± 24.0	159.7 ± 15.2	152.8 ± 38.1
Straight line velocity (VSL) (μm/s)	101.2 ± 6.7	99.7 ± 11.3	97.8 ± 13.7	100.7 ± 12.4	97.1 ± 23.8
Curvilinear velocity (VCL) (μm/s)	351.0 ± 14.1	345.3 ± 39.9	337.7 ± 54.9	346.1 ± 33.9	331.2 ± 81.2
Amplitude of lateral head displacement (ALH) (μm)	23.3 ± 1.1	22.0 ± 5.2	21.7 ± 5.2	23.0 ± 1.2	21.6 ± 5.3
Beat cross frequency (BCF) (Hz)	32.4 ± 1.4	32.2 ± 2.6	33.6 ± 4.4	32.6 ± 1.9	30.7 ± 7.3
Sperm morphology					
Abnormal sperm rate (%)[b]	1.8 ± 0.9	6.6 ± 20.7	5.7 ± 15.5	2.8 ± 2.3	7.5 ± 20.5
Abnormal head rate (%)[b]	1.7 ± 0.9	6.5 ± 20.7	5.5 ± 15.2	2.6 ± 2.3	6.5 ± 16.7
Abnormal tail rate (%)[b]	0.0 ± 0.2	0.1 ± 0.2	0.2 ± 0.3 #	0.2 ± 0.3 #	1.0 ± 3.9 #
Weight of left cauda epididymis (mg)	303 ± 27	301 ± 49	299 ± 34	318 ± 36	299 ± 56
Number of sperms in left cauda epididymis ($\times 10^6$)	259.7 ± 47.0	241.3 ± 79.1	245.7 ± 62.0	279.5 ± 74.1	241.0 ± 89.3
Number of sperms/g weight of left cauda epididymis ($\times 10^6$)	859 ± 141	790 ± 261	821 ± 216	872 ± 156	770 ± 273

Each value shows mean ± S.D.
a): [Number of progressive sperms (VAP>150 μm/s and VSL/VAP×100>55%) /total number of sperms]×100.
b): (Number of abnormal sperms/number of sperms examined)×100.
Significantly different from the control group (#: $p<0.05$ by Steel's test).

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Necropsy

Softening of the left testis and left epididymis was noted in 1 male in the 1 mg/kg group (Male No. M02219). In the 1000 mg/kg group, softening of the right and left testes and right and left epididymides was noted in 1 male (Male No. M05510), and softening of the left testis and left epididymis was noted in 1 male (Male No. M05513). These findings may not be considered to be test-drug related, since the findings were noted only in a few males. No abnormal necropsy findings were noted in the 10 mg/kg, 100 mg/kg, or control group.

Effects on Mating and fertility

No significant differences from the control group were seen in the copulation index in the 1, 10, 100, or 1000 mg/kg group. In the 1000 mg/kg group, 1 pair (Male No. M05510 and Female No. F05560) failed to copulate. All pairs copulated in the control, 1 mg/kg, 10 mg/kg, and 100 mg/kg groups.

Group	Control	S-297995 monotosylate			
mg/kg/day	0	1	10	100	1000
Number of females	20	20	20	20	20
Number of estrous cases during administration (14 days) (Mean ± S.D.)	3.6 ± 0.5	3.3 ± 0.7	2.6 ± 0.8 ##	2.2 ± 1.5 ##	1.8 ± 1.4 ##
Number of females	20	20	20	20	20
Number of females which copulated	20	20	20	20	19
Copulation index (%) a)	100.0	100.0	100.0	100.0	95.0
Number of days till copulation after start of pairing (Mean ± S.D.)	2.4 ± 1.6	3.8 ± 2.2 #	3.9 ± 4.2	3.1 ± 1.5	2.5 ± 1.0
Number of pregnant females	20	20	19	19	18
Fertility index (%) b)	100.0	100.0	95.0	95.0	94.7

a): (Number of females which copulated/number of females which paired)×100.
b): (Number of pregnant females/number of females which copulated)×100.
Significantly different from the control group (#: p<0.05, ##: p<0.01 by Steel's test).

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No significant differences from the control group were seen in the number of corpora lutea, number of implantation sites, number of pre-implantation losses, pre-implantation loss rate, or implantation rate in the 1, 10, 100, or 1000 mg/kg group as shown in the table below.

Group	Control	S-297995 monotosylate			
mg/kg/day	0	1	10	100	1000
Number of dams	20	20	19	19	18
Number of corpora lutea					
Total	312	320	300	310	286
Mean ± S.D. per dam	15.6 ± 1.9	16.0 ± 2.3	15.8 ± 2.0	16.3 ± 2.2	15.9 ± 2.3
Number of implantation sites					
Total	290	279	277	298	278
Mean ± S.D. per dam	14.5 ± 1.8	14.0 ± 4.2	14.6 ± 2.0	15.7 ± 1.7	15.4 ± 2.5
Implantation rate a)					
Mean % ± S.D. per dam	93.2 ± 8.0	85.5 ± 21.4	92.4 ± 8.5	96.5 ± 5.1	96.8 ± 5.9
Number of pre-implantation losses b)					
Total	22	41	23	12	8
Mean ± S.D. per dam	1.1 ± 1.3	2.1 ± 2.5	1.2 ± 1.3	0.6 ± 1.0	0.4 ± 0.7
Pre-implantation loss rate c)					
Mean % ± S.D. per dam	6.8 ± 8.0	14.5 ± 21.4	7.6 ± 8.5	3.5 ± 5.1	3.2 ± 5.9
Number of dead embryos					
Total	13	24	16	17	11
Mean ± S.D. per dam	0.7 ± 0.8	1.2 ± 1.8	0.8 ± 1.1	0.9 ± 0.9	0.6 ± 0.6
Post-implantation loss rate d)					
Mean % ± S.D. per dam	4.4 ± 5.5	7.5 ± 10.7	5.6 ± 7.1	5.8 ± 5.7	4.4 ± 4.6
Number of live embryos					
Total	277	255	261	281	267
Mean ± S.D. per dam	13.9 ± 1.8	12.8 ± 3.8	13.7 ± 2.1	14.8 ± 2.0	14.8 ± 2.8

a): (Number of implantation sites/number of corpora lutea) × 100.
b): Number of corpora lutea - number of implantation sites.
c): (Number of pre-implantation losses/number of corpora lutea) × 100.
d): (Number of dead embryos/number of implantation sites) × 100.

9.2 Embryonic Fetal Development

Review of Study No. R-297995-TF-146-L (CRO Study No. 2000129) from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.). In addition to the findings noted in Dr. Wu's review, body weight loss and decreased body weight gain (compared to controls) were observed in maternal animals at all doses.

Study title: Oral Study for Effects of S-297995 monotosylate on Embryo-Fetal Development in Rats

Study no.:	S-297995-TF-146-L
Study report location:	Electronic submission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 21, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	S-297995B, Lot No. A81001, purity: 97.5%

Key Study Findings

No observed adverse effect levels for F0 dams were considered to be less than 10 mg/kg/day for general toxicity with reduced body weight, and food consumption, while 1000 mg/kg/day for reproductive function and for F1 litter and 1000 mg/kg/day for embryo-fetal development. There were no effects of S-297995 on embryo-fetal development in the test system, thus, S-297995 was not teratogenic.

Methods

Doses:	S-297995 at 0, 10, 100, and 1000 mg/kg/day was administered orally by gavage to female rats
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral
Formulation/Vehicle:	0.5 w/v% methylcellulose aqueous solution
Species/Strain:	Crl: CD (SD) rats
Number/Sex/Group:	20 female rats/group
Satellite groups:	3 pregnant females/TK group, total 4 groups
Study design:	S-297995 monotosylate at 0, 10, 100, and 1000 mg/kg/day (as the parent entity) was administered orally by gavage to female rats daily during the period of organogenesis (Days 7 – 17 of pregnancy), and the effects of S-297995 monotosylate on dams and embryo-fetal development were assessed. Each test group consisted of 19 or 20 pregnant females. Plasma concentrations of S-297995 were also determined using TK satellite animals (3 pregnant females per group).
Deviation from study protocol:	The sponsor had planned to receive female animals ranging in weight from 200 to 260 g the day after receipt, but actually the female animals ranged in weight from 220 to 262 g; body weights of 2 females

exceeded the intended range. The deviation was minor, and the females whose body weights deviated had no abnormalities in general signs or body weight changes during the quarantine period.

Observations and Results

Mortality

No dead dams, moribund dams, or dams which aborted or delivered prematurely were noted in any group. In the control group, 1 female did not conceive.

Clinical Signs

No abnormal signs were noted in the 10 mg/kg, 100 mg/kg, or the control group. In the 1000 mg/kg group, salivation was noted sporadically in 9 females on Days 15 - 17 of pregnancy. This finding is not considered to be a toxicologically significant effect, since it was noted only transiently after administration.

Body Weight

As shown in the Table below, the mean body weight of dams in the 10 mg/kg group was significantly lower than in the control group on Day 18 of pregnancy. In the 100 and 1000 mg/kg groups, body weight was significantly lower than in the control group on Days 8 - 19 of pregnancy.

Table 3. Body weights of dams

Group	Control	S-297995 monotosylate		
mg/kg/day	0	10	100	1000
Number of dams	19	20	20	20
Days of pregnancy				
0	273 ± 14	273 ± 14	273 ± 12	273 ± 12
3	292 ± 15	292 ± 16	290 ± 12	292 ± 16
6	304 ± 13	305 ± 17	303 ± 13	305 ± 16
7	309 ± 12	309 ± 18	306 ± 14	307 ± 19
8	311 ± 14	304 ± 17	290 ± 13 **	289 ± 18 **
9	315 ± 15	308 ± 18	296 ± 13 **	291 ± 18 **
10	320 ± 16	311 ± 19	301 ± 14 **	298 ± 20 **
11	327 ± 14	317 ± 19	311 ± 14 *	304 ± 18 **
12	332 ± 16	322 ± 19	316 ± 14 **	309 ± 17 **
13	336 ± 17	327 ± 20	319 ± 13 **	313 ± 16 **
14	341 ± 16	332 ± 20	324 ± 14 **	318 ± 18 **
15	347 ± 17	338 ± 20	331 ± 14 *	325 ± 18 **
16	359 ± 17	347 ± 21	339 ± 13 **	333 ± 19 **
17	371 ± 19	359 ± 23	348 ± 14 **	346 ± 20 **
18	386 ± 21	370 ± 24 *	363 ± 14 **	358 ± 21 **
19	401 ± 22	392 ± 27	385 ± 13 #	380 ± 24 #
20	418 ± 23	408 ± 27	404 ± 15	400 ± 25

Each value shows mean (g) ± S.D.
Significantly different from the control group (*: p<0.05, **: p<0.01 by Dunnett's test).
Significantly different from the control group (#: p<0.05 by Steel's test).

Feed Consumption

In the 10, 100 and 1000 mg/kg groups, food consumption was significantly lower than in the control group on Days 8 - 18 of pregnancy in a dose-dependent manner. In the 1000 mg/kg group, food consumption (31.4 ± 4.7 g/day) was significantly higher than in the control group (27.1 ± 3.4 g/day) on Day 20 of pregnancy as shown in the Table 4 below. This finding is considered to be recovered after withdrawal and not toxicologically significant.

Table 4. Food consumption in rats

Group	Control	S-297995 monotosylate		
mg/kg/day	0	10	100	1000
Number of dams	19	20	20	20
Days of pregnancy				
1	19.6 ± 4.0	19.2 ± 2.7	19.4 ± 3.4	18.8 ± 2.0
4	23.9 ± 2.5	22.5 ± 3.7	22.8 ± 3.4	23.2 ± 3.9
7	25.2 ± 3.1	25.2 ± 3.6	23.9 ± 3.2	23.9 ± 4.0
8	24.7 ± 3.6	19.1 ± 2.5 **	12.4 ± 2.6 **	8.4 ± 2.1 **
9	25.3 ± 4.1	20.6 ± 2.5 **	15.8 ± 2.8 **	11.5 ± 3.4 **
10	26.8 ± 2.7	21.1 ± 4.0 **	18.6 ± 2.7 **	14.9 ± 2.6 **
11	25.9 ± 2.6	20.6 ± 3.0 **	21.4 ± 2.8 **	16.7 ± 3.6 **
12	26.6 ± 3.6	22.9 ± 2.7 ##	22.3 ± 2.1 ##	19.2 ± 4.3 ##
13	26.8 ± 3.2	24.4 ± 2.7 *	23.2 ± 2.7 **	21.1 ± 3.4 **
14	27.0 ± 2.6	23.9 ± 4.1 *	23.2 ± 2.8 **	21.9 ± 3.8 **
15	26.1 ± 2.4	23.6 ± 3.3 *	23.0 ± 3.3 **	21.8 ± 3.3 **
16	28.4 ± 3.1	24.4 ± 2.9 **	23.8 ± 2.9 **	23.4 ± 2.9 **
17	29.0 ± 3.5	25.3 ± 2.9 **	23.3 ± 3.9 **	24.6 ± 2.5 **
18	29.5 ± 3.0	24.2 ± 4.2 **	25.3 ± 4.2 **	26.0 ± 4.4 *
19	28.3 ± 3.5	30.1 ± 3.9	30.2 ± 2.9	30.9 ± 4.5
20	27.1 ± 3.4	26.8 ± 3.4	29.1 ± 4.3	31.4 ± 4.7 **

Each value shows mean (g/day) ± S.D.
Significantly different from the control group (*: p<0.05, **: p<0.01 by Dunnett's test).
Significantly different from the control group (##: p<0.01 by Steel's test).

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Toxicokinetics

As shown in the Table below, the mean Tmax on Day 7 of pregnancy at 10, 100, and 1000 mg/kg was 0.8, 1.0, and 2.7 hours, respectively. The mean Cmax at 10, 100, and 1000 mg/kg was 1.23, 9.58, and 15.6 µg/mL, respectively. The mean $AUC_{0\text{-}24hr}$ at 10, 100, and 1000 mg/kg was 5.66, 58.4, and 163 µg·hr/mL, respectively. The mean Tmax on Day 17 of pregnancy at 10, 100, and 1000 mg/kg was 1.0, 4.7, and 6.7 hours, respectively. The mean Cmax at 10, 100, and 1000 mg/kg was 1.39, 9.84, and 29.3 µg/mL, respectively. The mean $AUC_{0\text{-}24hr}$ at 10, 100, and 1000 mg/kg was 8.78, 126, and 391 µg·hr/mL, respectively. Thus, Tmax seemed to be slightly affected by dose level and dosing period. Increases in Cmax and $AUC_{0\text{-}24hr}$ between 10 and 100 mg/kg on both days were almost dose-proportional. However, Cmax and $AUC_{0\text{-}24hr}$ between 100 and 1000 mg/kg increased less than dose- proportionally. Cmax on Day 17 of pregnancy was slightly higher than on Day 7 of pregnancy at 1000 mg/kg. $AUC_{0\text{-}24hr}$ on Day 17 of pregnancy was slightly higher than on Day 7 of pregnancy at 100 and 1000 mg/kg.

Test group	Dose (mg/kg/day)	Sex	Cmax (μg/mL)		Tmax (hr)		$AUC_{0\text{-}24hr}$ (μg·hr/mL)	
			Day 7	Day 17	Day 7	Day 17	Day 7	Day 17
1	0	Mean (n=3)	NC	NC	NC	NC	NC	NC
		SD	NC	NC	NC	NC	NC	NC
2	10	Mean (n=3)	1.23	1.39	0.8	1.0	5.66	8.78
		SD	0.41	0.41	0.3	0.0	1.36	1.21
3	100	Mean (n=3)	9.58	9.84	1.0	4.7	58.4	126
		SD	1.41	0.30	0.0	3.1	2.4	12
4	1000	Mean (n=3)	15.6	29.3	2.7	6.7	163	391
		SD	1.9	6.4	1.2	2.3	30	91

NC: Not Calculated

Necropsy

No abnormal necropsy findings were noted in any group. Compared with the control group, no significant differences were seen in the number of corpora lutea, number of implantation sites, number of pre-implantation losses, pre-implantation loss rate, or implantation rate in the 10, 100, or 1000 mg/kg group as shown in the Table below.

Table 7. Observation of fetuses from dams

Group	Control	S-297995 monotosylate		
mg/kg/day	0	10	100	1000
Number of dams	19	20	20	20
Number of corpora lutea				
Total	286	293	303	306
Mean ± S.D. per dam	15.1 ± 1.6	14.7 ± 2.5	15.2 ± 1.7	15.3 ± 1.5
Number of implantation sites				
Total	266	272	291	296
Mean ± S.D. per dam	14.0 ± 1.9	13.6 ± 3.2	14.6 ± 1.4	14.8 ± 1.4
Implantation rate[a]				
Mean % ± S.D. per dam	92.8 ± 7.4	91.9 ± 14.9	96.3 ± 5.0	96.9 ± 4.5
Number of pre-implantation losses[b]				
Total	20	21	12	10
Mean ± S.D. per dam	1.1 ± 0.9	1.1 ± 1.6	0.6 ± 0.8	0.5 ± 0.7
Pre-implantation loss rate[c]				
Mean % ± S.D. per dam	7.3 ± 7.4	8.1 ± 14.9	3.7 ± 5.1	3.2 ± 4.5
Number of post-implantation losses				
Total	16	9	12	14
Mean ± S.D. per dam	0.8 ± 0.8	0.5 ± 0.6	0.6 ± 0.7	0.7 ± 1.4
Implantation scars	0	0	0	0
Early resorptions	16	9	12	12
Late resorptions	0	0	0	0
Macerated fetuses	0	0	0	2
Dead fetuses	0	0	0	0
Post-implantation loss rate[d]				
Mean % ± S.D. per dam	6.0 ± 6.1	3.5 ± 5.0	4.0 ± 4.4	5.0 ± 11.0
Number of live fetuses				
Total	250	263	279	282
Mean ± S.D. per dam	13.2 ± 2.0	13.2 ± 3.3	14.0 ± 1.3	14.1 ± 2.1
Sex ratio[e] (mean ± S.D. per dam)	0.52 ± 0.12	0.53 ± 0.12	0.54 ± 0.13	0.52 ± 0.14

a): (Number of implantation sites/number of corpora lutea) × 100.
b): Number of corpora lutea - number of implantation sites.
c): (Number of pre-implantation losses/number of corpora lutea) × 100.
d): (Number of post-implantation losses/number of implantation sites) × 100.
e): Number of males/(number of males + number of females).

(Continued)

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No external abnormalities were noted in the live fetuses in any group as shown in the Table below. Compared with the control group, no significant differences were seen in placental

weight of either sex in the 10, 100, or 1000 mg/kg group. No abnormal placental morphology was noted in the live fetuses in any group.

Group	Control	S-297995 monotosylate		
mg/kg/day	0	10	100	1000
Number of dams	19	20	20	20
Mean fetal body weight (g)				
Male (mean ± S.D. per dam)	3.85 ± 0.27	3.88 ± 0.20	3.82 ± 0.30	3.95 ± 0.27
Female (mean ± S.D. per dam)	3.66 ± 0.23	3.63 ± 0.19	3.61 ± 0.23	3.74 ± 0.25
Mean placental weight (g)				
Male (mean ± S.D. per dam)	0.49 ± 0.04	0.49 ± 0.04	0.49 ± 0.05	0.49 ± 0.06
Female (mean ± S.D. per dam)	0.49 ± 0.04	0.47 ± 0.04	0.47 ± 0.05	0.47 ± 0.06
Number of fetuses with external abnormalities	0	0	0	0
Mean % ± S.D. per dam[f]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Number of dams noted [%][f]	0 [0.0]	0 [0.0]	0 [0.0]	0 [0.0]
Number of abnormal placentae	0	0	0	0
Mean % ± S.D. per dam[h]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Number of dams noted [%][f]	0 [0.0]	0 [0.0]	0 [0.0]	0 [0.0]

f): (Number of fetuses with external abnormalities/number of live fetuses) × 100.
g): Not statistically analyzed.
h): (Number of abnormal placentae/number of placentae examined) × 100.

Compared with the control group, no significant differences were seen in the incidence of fetuses with visceral abnormalities in the 10, 100, or 1000 mg/kg group. Visceral abnormalities were noted as follows: membranous ventricular septum detect and short brachiocephalic artery in 1 fetus each in the control group; malpositioned right subclavian branch in 1 fetus in the 10 mg/kg group; short brachiocephalic artery in 1 fetus and malpositioned right subclavian branch in 2 fetuses in the 100 mg/kg group; and malpositioned right subclavian branch in 1 fetus and a combination of absent kidney and absent ureter in 2 fetuses in the 1000 mg/kg group as shown in the Table below. Compared with the control group, no significant differences were seen in the incidence of fetuses with visceral variations in the 10, 100, or 1000 mg/kg group. Visceral variations were noted as follows: supernumerary liver lobe, dilated renal pelvis, and dilated ureter in 1 fetus each in the control group; and dilated ureter in 2 fetuses in the 10 mg/kg group. These findings are not considered to be adverse effects of the test article, since the visceral variations were not dose-dependent changes. Compared with the control group, no significant differences were seen in the incidence of any type of visceral variation in the 10, 100, or 1000 mg/kg group.

Table 8. Visceral examination of fetuses

Group	Control	S-297995 monotosylate		
mg/kg/day	0	10	100	1000
Number of dams	19	20	20	20
Number of fetuses examined	120	128	134	136
Abnormalities				
Number of fetuses with abnormalities	2	1	3	3
Mean % ± S.D. per dam[a]	1.8 ± 5.3	0.7 ± 3.2	2.7 ± 8.5	2.4 ± 7.9
Number of dams noted [%][b]	2 [10.5]	1 [5.0]	2 [10.0]	2 [10.0]
Membranous ventricular septum defect	1	0	0	0
Mean % ± S.D. per dam	0.9 ± 3.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Number of dams noted [%][b]	1 [5.3]	0 [0.0]	0 [0.0]	0 [0.0]
Short brachiocephalic artery	1	0	1	0
Mean % ± S.D. per dam	0.9 ± 3.8	0.0 ± 0.0	0.8 ± 3.7	0.0 ± 0.0
Number of dams noted [%][b]	1 [5.3]	0 [0.0]	1 [5.0]	0 [0.0]
Malpositioned right subclavian branch	0	1	2	1
Mean % ± S.D. per dam	0.0 ± 0.0	0.7 ± 3.2	1.8 ± 5.7	0.7 ± 3.2
Number of dams noted [%][b]	0 [0.0]	1 [5.0]	2 [10.0]	1 [5.0]
Absent kidney	0	0	0	2
Mean % ± S.D. per dam	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.7 ± 7.4
Number of dams noted [%][b]	0 [0.0]	0 [0.0]	0 [0.0]	1 [5.0]
Absent ureter	0	0	0	2
Mean % ± S.D. per dam	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.7 ± 7.4
Number of dams noted [%][b]	0 [0.0]	0 [0.0]	0 [0.0]	1 [5.0]

a): (Number of fetuses with visceral abnormalities/number of fetuses examined)×100. (Continued)
b): Not statistically analyzed.

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No skeletal abnormalities were noted in any group. Compared with the control group, no significant differences were seen in the incidence of fetuses with skeletal variations in the 10, 100, or 1000 mg/kg group. Skeletal variations were noted as follows: bipartite ossification of thoracic centrum and cervical rib in 1 fetus each and short supernumerary rib in 20 fetuses in the control group; bipartite ossification of thoracic centrum in 4 fetuses, short supernumerary rib in 11 fetuses, and cervical rib in 2 fetuses in the 10 mg/kg group; bipartite ossification of thoracic centrum and cervical rib in 2 fetuses each, full supernumerary rib in 1 fetus, and short supernumerary rib in 13 fetuses in the 100 mg/kg group; and short supernumerary rib in 11 fetuses in the 1000 mg/kg group. These findings are not considered to be adverse effects of the test article, since the skeletal variations were not dose-dependent changes. Compared with the control group, no significant differences were seen in the incidence of any type of skeletal variation in the 10, 100, or 1000 mg/kg group. Compared with the control group, no significant differences were seen in the degree of ossification of any bone or incidence of unossified bones in the 10, 100, or 1000 mg/kg group.

Group	Control	S-297995 monotosylate		
mg/kg/day	0	10	100	1000
Number of dams	19	20	20	20
Number of fetuses examined	120	128	134	136
Variations				
Number of fetuses with variations	2	2	0	0
Mean % ± S.D. per dam[c]	1.6 ± 4.9	1.4 ± 6.4	0.0 ± 0.0	0.0 ± 0.0
Number of dams noted [%][b]	2 [10.5]	1 [5.0]	0 [0.0]	0 [0.0]
Supernumerary liver lobe	1	0	0	0
Mean % ± S.D. per dam	0.8 ± 3.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Number of dams noted [%][b]	1 [5.3]	0 [0.0]	0 [0.0]	0 [0.0]
Dilated renal pelvis	1	0	0	0
Mean % ± S.D. per dam	0.9 ± 3.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Number of dams noted [%][b]	1 [5.3]	0 [0.0]	0 [0.0]	0 [0.0]
Dilated ureter	1	2	0	0
Mean % ± S.D. per dam	0.9 ± 3.8	1.4 ± 6.4	0.0 ± 0.0	0.0 ± 0.0
Number of dams noted [%][b]	1 [5.3]	1 [5.0]	0 [0.0]	0 [0.0]

b): Not statistically analyzed.
c): (Number of fetuses with visceral variations/number of fetuses examined)×100.

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Review of Study No. R-297995-TF-163-L (CRO Study No. 250228P) from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Study title: Dose Range-Finding Oral Study for Effects of S-297995 monotosylate on Embryo-Fetal Development in Rabbits

Study no.:	S-297995-TF-163-L
Study report location:	Electronic submission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 5, 2009
GLP compliance:	Not indicated
QA statement:	Not indicated
Drug, lot #, and % purity:	S-297995B, Lot No. A81001; purity: 97.5%

Key Study Findings

In the 60 and 200 mg/kg groups, body weight gain and food consumption were slightly low at the beginning of the dosing period, and slight body weight loss was also noted. In the 600 mg/kg group, decreased fecal volume and low body weight gain were noted throughout most of the dosing period, and one dam aborted. In this group, food consumption was markedly decreased throughout the dosing period, and body weight loss was also noted.

Methods S-297995 monotosylate at 0, 60, 200, and 600 mg/kg/day was administered orally by gavage to female rabbits daily during the period of organogenesis (Days 6 - 18 of pregnancy), and the effects of S-297995 monotosylate on dams and embryo-fetal development were assessed. Each test group consisted of 6 pregnant females.

Doses:	0, 60, 200, and 600 mg/kg/day S-297995 was administered.
Frequency of dosing:	Once daily
Dose volume:	5 mL/kg
Route of administration:	Oral
Formulation/Vehicle:	0.5 w/v% methylcellulose aqueous solution
Species/Strain:	female Kbl: JW rabbits
Number/Sex/Group:	6 pregnant females/group
Satellite groups:	None
Study design:	The test drug administered orally by gavage to 3 groups at 60, 200, and 600 mg/kg/day as compared to the vehicle control.
Deviation from study protocol:	The relative higher humidity of the animal room deviated from the acceptable range.

Observations and Results

Mortality

No dead dams, moribund dams, or dams which aborted or delivered prematurely were noted in the 60 mg/kg, 200 mg/kg, or control group. In the 600 mg/kg group, 1 dam (No. F04454) aborted on Day 20 of pregnancy. Five dead pups, 7 resorbed embryos, and 5 placentas were noted in the dam which aborted.

Clinical Signs

No abnormal signs were noted in the 60mg/kg, 200 mg/kg, or control group. In the 600 mg/kg group, decreased fecal volume was noted in 3 dams (Dam Nos. F04451, F04454, and F04455), and 1 of the 3 dams (Dam No. F04454) aborted.

Body Weight

No significant differences from the control group were seen in the body weight in the 60 or 200 mg/kg group on any day of measurement. Although no significant differences from the control group were seen, body weight in the 600 mg/kg group tended to be lower on Days 7 - 20 of pregnancy. Compared with the control group, body weight gain on Days 7, 8, and 10 of pregnancy, which was calculated on the basis of body weight on Day 6 of pregnancy, was significantly lower in the 60mg/kg group. Compared with the control group, body weight gain on Days 8, 9, 10, and 12 of pregnancy was significantly lower in the 200mg/kg group. In these groups, compared with the first dosing day, body weight loss was noted at the beginning of the dosing period. In the 600 mg/kg group, body weight gain on Days 7 - 20 of pregnancy was significantly lower than in the control group. In this group, compared with the first dosing day, body weight loss was noted throughout the dosing period as shown in the Table below.

Table 4. Body weight gain in dams

Group	Control	S-297995 monotosylate			
mg/kg/day	0	60	200	600	
Number of dams	6	6	6	6	
Days of pregnancy					
6-7	0.01 ± 0.02	-0.06 ± 0.03 #	-0.04 ± 0.06	-0.07 ± 0.02 #	
6-8	0.02 ± 0.03	-0.08 ± 0.05 **	-0.10 ± 0.05 **	-0.15 ± 0.05 **	
6-9	0.04 ± 0.03	-0.05 ± 0.06	-0.06 ± 0.07 *	-0.13 ± 0.09 **	
6-10	0.06 ± 0.02	-0.05 ± 0.06 #	-0.02 ± 0.05 #	-0.14 ± 0.11 #	
6-11	0.07 ± 0.03	-0.01 ± 0.07	0.00 ± 0.06	-0.17 ± 0.14 #	
6-12	0.10 ± 0.03	0.02 ± 0.08	0.03 ± 0.05 #	-0.14 ± 0.16 #	
6-13	0.12 ± 0.03	0.05 ± 0.08	0.06 ± 0.06	-0.13 ± 0.18 #	
6-14	0.15 ± 0.06	0.10 ± 0.08	0.09 ± 0.05	-0.08 ± 0.16 **	
6-15	0.17 ± 0.07	0.14 ± 0.08	0.11 ± 0.08	-0.08 ± 0.19 **	
6-16	0.21 ± 0.06	0.14 ± 0.07	0.12 ± 0.07	-0.06 ± 0.20 #	
6-17	0.22 ± 0.07	0.15 ± 0.07	0.13 ± 0.08	-0.08 ± 0.21 #	
6-18	0.23 ± 0.07	0.14 ± 0.08	0.16 ± 0.07	-0.11 ± 0.23 #	
6-19	0.25 ± 0.08	0.18 ± 0.07	0.18 ± 0.06	-0.13 ± 0.24 #	
6-20	0.27 ± 0.09	0.25 ± 0.05	0.24 ± 0.05	-0.03 ± 0.33 #	(5)
6-22	0.30 ± 0.10	0.31 ± 0.06	0.31 ± 0.04	0.03 ± 0.41	(5)
6-24	0.34 ± 0.12	0.37 ± 0.07	0.36 ± 0.03	0.08 ± 0.46	(5)
6-26	0.37 ± 0.11	0.40 ± 0.08	0.38 ± 0.06	0.09 ± 0.50	(5)
6-28	0.40 ± 0.11	0.44 ± 0.12	0.41 ± 0.07	0.10 ± 0.54	(5)

Each value shows mean (kg) ± S.D.
Significantly different from the control group (#: $p<0.05$ by Steel's test).
Significantly different from the control group (*: $p<0.05$, **: $p<0.01$ by Dunnett's test).
Figures in parentheses indicate number of dams.

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Feed Consumption

Compared with the control group, food consumption was significantly lower in the 60 mg/kg group on Days 7, 8, 9, 10, 11, and 12 of pregnancy. In the 200 mg/kg group, compared with the control group, food consumption was significantly lower on Days 1, 7, 8, 9, 10, and 12 of pregnancy. In the 600 mg/kg group, compared with the control group, food consumption was significantly lower on Days 7, 8, 9, 10, 11, 12, 13, 14, and 17 of pregnancy. Although there were no significant differences from the control group, food consumption tended to be lower in this group on Days 15, 16, 18, and 19 of pregnancy as shown in the Figure below. In the 600 mg/kg group, markedly decreased food consumption was noted in 2 dams (Dam Nos. F04454 [aborted] and F04455) from the day after the start of the dosing period to the day of necropsy.

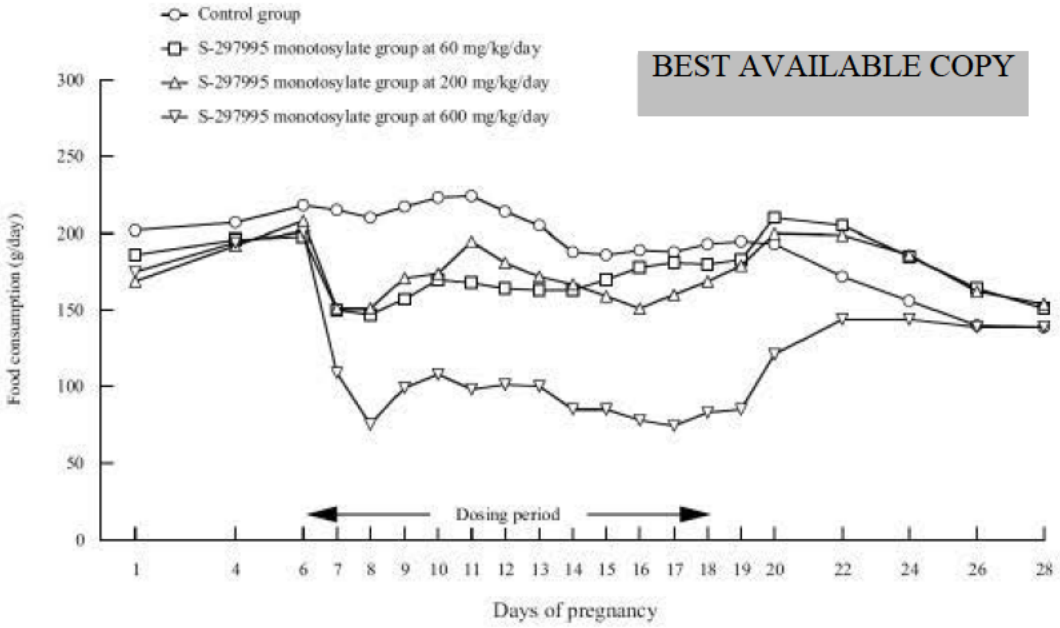

Fig. 2. Food consumption in dams.

Toxicokinetics

Not conducted

Necropsy

No abnormal necropsy findings were noted in any group. Compared with the control group, no significant differences were seen in the number of corpora lutea, number of implantation sites, implantation rate, number of pre-implantation losses, or pre-implantation loss rate in any S-297995 monotosylate group as shown in the Table below.

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Group	Control	S-297995 monotosylate		
mg/kg/day	0	60	200	600
Number of dams	6	6	6	5
Number of corpora lutea				
Total	56	64	54	41
Mean ± S.D. per dam	9.3 ± 1.2	10.7 ± 1.2	9.0 ± 1.7	8.2 ± 1.1
Number of implantation sites				
Total	52	62	46	34
Mean ± S.D. per dam	8.7 ± 1.4	10.3 ± 0.8	7.7 ± 2.7	6.8 ± 2.9
Implantation rate[a]				
Mean % ± S.D. per dam	92.9 ± 8.4	97.2 ± 4.3	82.8 ± 18.6	81.3 ± 31.0
Number of pre-implantation losses[b]				
Total	4	2	8	7
Mean ± S.D. per dam	0.7 ± 0.8	0.3 ± 0.5	1.3 ± 1.2	1.4 ± 2.2
Pre-implantation loss rate[c]				
Mean % ± S.D. per dam	7.1 ± 8.4	2.8 ± 4.3	17.2 ± 18.6	18.7 ± 31.0
Number of post-implantation losses				
Total	1	2	1	3
Mean ± S.D. per dam	0.2 ± 0.4	0.3 ± 0.5	0.2 ± 0.4	0.6 ± 0.5
Implantation scars	0	0	0	0
Early resorption	1	1	0	1
Late resorption	0	1	0	2
Macerated fetuses	0	0	0	0
Dead fetuses	0	0	1	0
Post-implantation loss rate[d]				
Mean % ± S.D. per dam	2.1 ± 5.1	3.2 ± 4.9	1.5 ± 3.7	7.3 ± 6.8
Number of live fetuses				
Total	51	60	45	31
Mean ± S.D. per dam	8.5 ± 1.5	10.0 ± 0.9	7.5 ± 2.4	6.2 ± 2.5
Sex ratio[e] (mean ± S.D. per dam)	0.47 ± 0.16	0.50 ± 0.23	0.45 ± 0.22	0.43 ± 0.29

(Continued)

a): (Number of implantation sites/number of corpora lutea) × 100.
b): Number of corpora lutea - number of implantation sites.
c): (Number of pre-implantation losses/number of corpora lutea) × 100.
d): (Number of post-implantation losses/number of implantation sites) × 100.
e): Number of males/(number of males + number of females).

Regarding fetal growth, no significant differences from the control group were seen in fetal body weight of either sex in any S-297995 monotosylate group. In the 600 mg/kg group, however, fetal body weights in 1 dam in which food consumption was markedly decreased were low. Regarding fetal morphology, no external abnormalities were noted in live fetuses in any group. No adverse effects of the test article were noted in placental morphology. No significant differences from the control group were seen in placental weight in any S-297995 monotosylate group as shown in the Table below. In the 600 mg/kg group, however, placental weight in 1 dam in which food consumption was markedly decreased was low.

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Group	Control	S-297995 monotosylate		
mg/kg/day	0	60	200	600
Number of dams	6	6	6	5
Mean fetal body weight (g)				
Male (mean ± S.D. per dam)	36.1 ± 5.2	37.6 ± 3.3	39.7 ± 3.9	33.6 ± 13.7 (4)
Female (mean ± S.D. per dam)	34.8 ± 6.1	36. ± 3.9	37.7 ± 3.7	35.8 ± 10.5
Mean placental weight (g)				
Male (mean ± S.D. per dam)	5.61 ± 0.48	4.9 ± 0.33	5.80 ± 1.03	5.04 ± 1.58 (4)
Female (mean ± S.D. per dam)	5.23 ± 0.38	4.8 ± 0.32	5.69 ± 1.01	5.48 ± 1.33
Number of fetuses with external abnormalities	0	0	0	0
Mean % ± S.D. per dam[f]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Number of dams noted [%][g]	0 [0.0]	0 [0.0]	0 [0.0]	0 [0.0]
Number of abnormal placentae	0	0	0	0
Mean % ± S.D. per dam[h]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Number of dams noted [%][g]	0 [0.0]	0 [0.0]	0 [0.0]	0 [0.0]

f): (Number of fetuses with external abnormalities/number of live fetuses examined) × 100.
g): Not statistically analyzed.
h): (Number of abnormal placentae/number of placentae examined) × 100.
Figures in parentheses indicate number of dams.

Study title: Oral Study for Effects of S-297995 monotosylate on Embryo-Fetal Development in Rabbits

Study no.:	S-297995-TF-182-L (CRO Study No. 250228)
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 24, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	S-297995 monotosylate, Lot No. A81001, 97.5% (on an anhydrous basis)

Key Study Findings

In maternal animals, treatment with ≥25 mg/kg/day resulted in decreased body weight, body weight gain, and food consumption (compared to controls), with body weight losses particularly at the beginning of the dosing period at 25 and 100 mg/kg/day. At 400 mg/kg/day only, additional treatment-related findings in maternal animals included decreased fecal volume (compared to controls), abortion (two dams on GDs 21 and 22), and premature delivery (single dam on GD 27). In the offspring, there were decreased fetal body weights at 400 mg/kg/day (compared to controls), a finding that may be related to maternal toxicity at this dose. No external, visceral, or skeletal abnormalities or variations were noted at any dose level. Based on findings in this study, the NOAEL is <25 mg/kg/day for maternal toxicity and 100 mg/kg/day for embryofetal development.

Methods

Doses:	25, 100, and 400 mg/kg/day (as S-297995)
Frequency of dosing:	Once daily
Dose volume:	5 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	0.5% w/v% methylcellulose aqueous solution
Species/Strain:	Rabbits/Kbl:JW,SPF)
Number/Sex/Group:	20 females/group
Satellite groups:	No
Study design:	Pregnant females were dosed once daily during the period of organogenesis (gestation day (GD) 6 to 18), and the effects of S-97995 monotosylate on dams and embryofetal development was assessed.
Deviation from study protocol:	Deviations did not affect the quality or integrity of the study.

Observations and Results

Mortality

Viability checks of dams were conducted twice daily during the dosing period and once a daily during the non-dosing period.

No mortalities occurred in dams of any dose group.

Clinical Signs

Dams were observed for clinical signs twice daily during the dosing period and once a daily during the non-dosing period.

Two high dose dams aborted on GDs 21 and 22. Among these animals, one dam delivered 5 dead pups and 4 placentae and 1 placenta was observed in the uterus. The other dam delivered 8 dead pups and placentae, and 2 dead fetuses and 5 placentae were observed in the uterus. In addition, a single high dose female prematurely delivered on GD 27, with 2 dead pups, 2 resorbed embryos, and 6 placentae.

Decreased fecal volume (compared to controls) was observed in 6 high dose dams, including the two dams which aborted and single dam who delivered prematurely.

Body Weight

Body weights of dams were recorded on Days 0, 3, and 6, daily during the dosing period (GD 7 – 18), and on Days 19, 20, 22, 24, 26, and 28.

There were statistically significant decreases in body weight at all doses (compared to controls). At 25 mg/kg/day, there was a statistically significant decrease in body weight on GD 11 only (-3%, compared to controls). At 100 and 400 mg/kg/day, body weights were significantly decreased on GDs 7-15 (up to -4%, compared to controls) and on GDs 7-20 (up to -9%), respectively.

There were body weight losses and decreased body weight gains (compared to controls) at all dose levels. At 25, 100, and 400 mg/kg/day, body weight losses occurred from GD 6 through 12, GD 6 through 12, and GD 6 through 19, respectively. At 25 and 100 mg/kg/day, body weight gain was significantly lower than controls from GD 6 through 19 and at 400 mg/kg/day, body weight gain was significantly lower from GD 6 through 20. From GD 6-19, body weight gain at 25 and 100 mg/kg/day was decreased by 43% and 53% compared to controls. From GD 6-19, controls gained 0.21 kg, while the high dose group lost 0.07 kg.

Feed Consumption

Food consumption was determined for Day 0, 3, and 6, daily during the dosing period (GD 7 – 18), and on Days 19, 21, 23, 25, and 27.

There were statistically significant decreases in food consumption at all doses (compared to controls). At 25 mg/kg/day, there was a statistically significant decrease in food consumption on GD 7 – 12. At 40 mg/kg/day, food consumption was

significantly decreased from GD 7-11, GD 13-15, and GD 17. At 400 mg/kg/day, food consumption was significantly decreased from GD 7 – 19. On GD 7, food consumption in the low-, mid-, and high dose groups was decreased by 16%, 41%, and 61%, respectively, compared to controls.

Toxicokinetics

Blood was collected from 5 dams/group at 0.5, 1, 2,4, 8, and 24 h after dosing on GD 6 (prior to initiation of dosing) and before dosing and at 0.5, 1, 2, 4, 8, and 24 h after dosing on GD 18. Plasma samples from 3 pregnant females/group were frozen, shipped to the test site (b) (4) and analyzed for S-297995, Nor-S-297995, S-297995 3-G, and S-297995 6-G.

Exposure to S-297995 and its metabolites was demonstrated at all dose levels on GD 6 and 18. TK parameters are summarized in the Applicant's table below.

Summary table 1

		Cmax (μg/mL)			Tmax (h)			AUC_{0-24h} (μg·h/mL)		
		Dose level (mg/kg/day)								
Analyte	Day of pregnancy	25	100	400	25	100	400	25	100	400
S-297995	6	0.173	2.37	6.45	0.5	0.5	0.5	0.205	2.80	11.4
	18	0.351	3.46	8.65	0.5	0.5	0.5	0.366	3.82	14.3
Nor-S-297995	6	0.0398	0.179	0.543	0.5	0.5	0.8	0.0331	0.323	1.20
	18	0.0609	0.212	0.557	0.5	0.5	1.0	0.0633	0.358	1.22
S-297995 3-G	6	5.34	26.8	64.7	0.5	0.5	1.0	8.64	53.5	155
	18	7.52	32.4	51.1	0.5	0.5	1.0	12.3	57.2	144
S-297995 6-G	6	0.0122	0.105	0.519	0.5	0.5	1.0	0.0173	0.203	1.02
	18	0.0191	0.136	0.278	0.5	0.5	1.2	0.0270	0.218	0.588

Dosing Solution Analysis

Samples of the dosing formulations used for the first and last days of dosing were analyzed for the test compound concentration. Concentrations were reported to be between 96.5-98.5% of nominal values, and the relative standard deviation ranged from 0.5-2.2%. Thus, it was concluded that the concentrations and homogeneity of the dosing formulations were acceptable.

Necropsy

Dams were euthanized on GD 28, and the number of corpora lutea and implantation sites were counted. For females with no evidence of implantation, the uterus was stained to reconfirm the females were not pregnant.

No treatment-related findings were noted. Two dams were non-pregnant (a single control and high dose dam, each).

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

At scheduled necropsy, the number of corpora lutea, implantation sites, post-implantation losses, and live fetuses were counted. The placentae of live fetuses were also macroscopically examined and weighed. The implantation rate, pre-implantation loss rate, and post-implantation loss rates were calculated.

There were no statistically significant effects on the numbers of corpora lutea, implantation sites, pre-implantation losses, post-implantation losses, or live fetuses, or the rates of implantation, pre-implantation loss, or post-implantation loss. Placental weights were also not significantly different at any dose, compared to controls. Summary data are presented in the Applicant's table below.

(Sponsor's Study No.: S-297995-TF-182-L)

Table 7. Observation of fetuses from dams

Group	Control	S-297995 monotosylate		
mg/kg/day	0	25	100	400
Number of dams	19	20	20	16
Number of corpora lutea				
Total	169	189	184	145
Mean ± S.D. per dam	8.9 ± 2.5	9.5 ± 1.8	9.2 ± 1.8	9.1 ± 1.8
Number of implantation sites				
Total	152	175	170	133
Mean ± S.D. per dam	8.0 ± 3.0	8.8 ± 2.3	8.5 ± 2.5	8.3 ± 2.3
Implantation rate[a)]				
Mean % ± S.D. per dam	87.4 ± 13.4	91.1 ± 11.2	90.4 ± 16.1	90.1 ± 15.0
Number of pre-implantation losses[b)]				
Total	17	14	14	12
Mean ± S.D. per dam	0.9 ± 0.9	0.7 ± 0.8	0.7 ± 1.0	0.8 ± 0.9
Pre-implantation loss rate[c)]				
Mean % ± S.D. per dam	12.6 ± 13.4	8.9 ± 11.2	9.6 ± 16.1	9.9 ± 15.0
Number of post-implantation losses				
Total	9	16	17	5
Mean ± S.D. per dam	0.5 ± 1.0	0.8 ± 0.8	0.9 ± 1.0	0.3 ± 0.6
Implantation scars	0	0	0	0
Early resorption	9	8	9	2
Late resorption	0	5	6	3
Macerated fetuses	0	1	2	0
Dead fetuses	0	2	0	0
Post-implantation loss rate[d)]				
Mean % ± S.D. per dam	9.6 ± 23.4	9.4 ± 9.8	8.9 ± 10.3	3.2 ± 6.3
Number of live fetuses				
Total	143	159	153	128
Mean ± S.D. per dam	7.5 ± 3.5	8.0 ± 2.4	7.7 ± 2.3	8.0 ± 2.1
Sex ratio[e)] (mean ± S.D. per dam)	0.47 ± 0.26	0.52 ± 0.16	0.51 ± 0.18	0.52 ± 0.21

a): (Number of implantation sites/number of corpora lutea) × 100.
b): Number of corpora lutea - number of implantation sites.
c): (Number of pre-implantation losses/number of corpora lutea) × 100.
d): (Number of post-implantation losses/number of implantation sites) × 100.
e): Number of males/(number of males + number of females).

(Continued)

(Sponsor's Study No.: S-297995-TF-182-L)

Table 7. (Continued) Observation of fetuses from dams

Group	Control	S-297995 monotosylate		
mg/kg/day	0	25	100	400
Number of dams	19	20	20	16
Mean fetal body weight (g)				
Male (mean ± S.D. per dam)	40.0 ± 4.8 (17)	39.4 ± 4.9	39.7 ± 4.3	36.9 ± 5.9 (15)
Female (mean ± S.D. per dam)	40.2 ± 4.1	39.5 ± 5.0	39.1 ± 3.6	36.1 ± 5.1 *
Mean placental weight (g)				
Male (mean ± S.D. per dam)	5.92 ± 0.68 (17)	5.87 ± 0.88	5.92 ± 0.83	5.44 ± 0.72 (15)
Female (mean ± S.D. per dam)	6.19 ± 1.05	5.72 ± 0.89	5.58 ± 0.77	5.38 ± 0.44
Number of fetuses with external abnormalities	0	1	0	0
Mean % ± S.D. per dam[f)]	0.0 ± 0.0	1.0 ± 4.5	0.0 ± 0.0	0.0 ± 0.0
Number of dams noted [%][g)]	0 [0.0]	1 [5.0]	0 [0.0]	0 [0.0]
Exencephaly	0	1	0	0
Mean % ± S.D. per dam	0.0 ± 0.0	1.0 ± 4.5	0.0 ± 0.0	0.0 ± 0.0
Number of dams noted [%][g)]	0 [0.0]	1 [5.0]	0 [0.0]	0 [0.0]
Number of abnormal placentae	0	0	0	0
Mean % ± S.D. per dam[h)]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Number of dams noted [%][g)]	0 [0.0]	0 [0.0]	0 [0.0]	0 [0.0]

f): (Number of fetuses with external abnormalities/number of live fetuses examined) × 100.
g): Not statistically analyzed.
h): (Number of abnormal placentae/number of placentae examined) × 100.
Significantly different from the control group (*: $p<0.05$ by Dunnett's test).
Figures in parentheses indicate number of dams.

Offspring (Malformations, Variations, etc.)

Fetuses were weighed, sexed, examined for external, visceral, and skeletal findings.

There were statistically significant differences in the sex ratio. However, at 400 mg/kg/day, there was a statistically significant decrease in female fetal body weights (-10.2%, compared to controls) and a trend toward lower body weights in males.

External abnormalities were limited to exencephaly in a single fetus at 25 mg/kg/day. Based on the observation of this finding in only a single fetus and lack of similar findings at the mid- and high dose, this was not considered to be a treatment-related effect.

As summarized in the Applicant's table below, there were no statistically significant increases in the incidence of visceral abnormalities, and no visceral variations were observed at any dose. Visceral abnormalities considered to be incidental based on the low incidence and historical data for the testing facility included included small lung (single fetus; control group) and retroesophageal subclavian artery (single fetus; 400 mg/kg/day group).

Table 8. Visceral examination of fetuses

Group	Control	S-297995 monotosylate		
mg/kg/day	0	25	100	400
Number of dams	19	20	20	16
Number of fetuses examined	143	159	153	128
Abnormalities				
Number of fetuses with abnormalities	1	0	0	1
Mean % ± S.D. per dam[a)]	0.7 ± 2.9	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 2.5
Number of dams noted [%][b)]	1 [5.3]	0 [0.0]	0 [0.0]	1 [6.3]
Small lung	1	0	0	0
Mean % ± S.D. per dam	0.7 ± 2.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Number of dams noted [%][b)]	1 [5.3]	0 [0.0]	0 [0.0]	0 [0.0]
Retroesophageal subclavian artery	0	0	0	1
Mean % ± S.D. per dam	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 2.5
Number of dams noted [%][b)]	0 [0.0]	0 [0.0]	0 [0.0]	1 [6.3]
Variations				
Number of fetuses with variations	0	0	0	0
Mean % ± S.D. per dam[c)]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Number of dams noted [%][b)]	0 [0.0]	0 [0.0]	0 [0.0]	0 [0.0]

a): (Number of fetuses with visceral abnormalities/number of fetuses examined)×100.
b): Not statistically analyzed.
c): (Number of fetuses with visceral variations/number of fetuses examined)×100.

There were no statistically significant increases in the incidence of skeletal abnormalities or variations at up to 400 mg/kg/day, as summarized in the Applicant's table below. Skeletal abnormalities considered to be incidental based on low incidence, lack of dose relationship, and historical data for the testing facility included fused thoracic centrum and lumbar centrum (single fetus; control group), absent skull (single fetus; 25 mg/kg/day group), and absent lumbar vertebra (single fetus; 100 mg/kg/day group). Skeletal variations observed in the study included full supernumerary rib, short supernumerary rib, cervical rib, and supernumerary lumbar vertebra in all groups (including controls) and peduncular fused sternebrae in the low- and mid- dose groups only.

Table 9. Skeletal examination of fetuses

Group	Control	S-297995 monotosylate		
mg/kg/day	0	25	100	400
Number of dams	19	20	20	16
Number of fetuses examined	143	159	153	128
Abnormalities				
Number of fetuses with abnormalities	1	1	1	0
Mean % ± S.D. per dam[a)]	0.9 ± 3.8	1.0 ± 4.5	0.6 ± 2.5	0.0 ± 0.0
Number of dams noted [%][b)]	1 [5.3]	1 [5.0]	1 [5.0]	0 [0.0]
Absent skull	0	1	0	0
Mean % ± S.D. per dam	0.0 ± 0.0	1.0 ± 4.5	0.0 ± 0.0	0.0 ± 0.0
Number of dams noted [%][b)]	0 [0.0]	1 [5.0]	0 [0.0]	0 [0.0]
Absent lumbar vertebra	0	0	1	0
Mean % ± S.D. per dam	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 2.5	0.0 ± 0.0
Number of dams noted [%][b)]	0 [0.0]	0 [0.0]	1 [5.0]	0 [0.0]
Fused thoracic centrum and lumbar centrum	1	0	0	0
Mean % ± S.D. per dam	0.9 ± 3.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Number of dams noted [%][b)]	1 [5.3]	0 [0.0]	0 [0.0]	0 [0.0]

a): (Number of fetuses with skeletal abnormalities/number of fetuses examined)×100.
b): Not statistically analyzed.

(Continued)

Table 9. (Continued) Skeletal examination of fetuses

Group	Control	S-297995 monotosylate		
mg/kg/day	0	25	100	400
Number of dams	19	20	20	16
Number of fetuses examined	143	159	153	128
Variations				
Number of fetuses with variations	44	60	48	49
Mean % ± S.D. per dam[c)]	29.2 ± 30.0	39.2 ± 28.6	32.4 ± 25.4	40.6 ± 26.7
Number of dams noted [%][b)]	13 [68.4]	20 [100.0]	18 [90.0]	16 [100.0]
Peduncular fused sternebra	0	2	1	0
Mean % ± S.D. per dam	0.0 ± 0.0	1.4 ± 6.4	0.7 ± 3.2	0.0 ± 0.0
Number of dams noted [%][b)]	0 [0.0]	1 [5.0]	1 [5.0]	0 [0.0]
Full supernumerary rib	12	19	17	24
Mean % ± S.D. per dam	8.9 ± 16.7	13.8 ± 24.7	10.7 ± 17.5	17.9 ± 24.8
Number of dams noted [%][b)]	7 [36.8]	10 [50.0]	7 [35.0]	9 [56.3]
Short supernumerary rib	35	39	30	28
Mean % ± S.D. per dam	22.4 ± 21.9	23.5 ± 22.2	20.1 ± 19.3	23.8 ± 22.8
Number of dams noted [%][b)]	13 [68.4]	18 [90.0]	13 [65.0]	13 [81.3]
Cervical rib	5	7	3	4
Mean % ± S.D. per dam	3.7 ± 11.6	5.1 ± 11.9	2.7 ± 6.9	3.9 ± 10.6
Number of dams noted [%][b)]	3 [15.8]	5 [25.0]	3 [15.0]	2 [12.5]
Supernumerary lumbar vertebra	2	6	7	8
Mean % ± S.D. per dam	2.5 ± 8.1	4.9 ± 11.0	4.7 ± 9.8	6.9 ± 18.5
Number of dams noted [%][b)]	2 [10.5]	4 [20.0]	5 [25.0]	3 [18.8]

b): Not statistically analyzed.
c): (Number of fetuses with skeletal variations/number of fetuses examined)×100.

(Continued)

Table 9. (Continued) Skeletal examination of fetuses

Group	Control	S-297995 monotosylate		
mg/kg/day	0	25	100	400
Number of dams	19	20	20	16
Number of fetuses examined	143	159	153	128
Number of ossified bones				
Sternebrae				
Mean ± S.D. per dam	5.9 ± 0.1	5.9 ± 0.2	5.8 ± 0.2	5.9 ± 0.2
Metacarpal bone of forepaw				
Mean ± S.D. per dam	10.0 ± 0.1	10.0 ± 0.1	10.0 ± 0.1	9.9 ± 0.2
Proximal phalanx of forepaw				
Mean ± S.D. per dam	10.0 ± 0.0	10.0 ± 0.2	10.0 ± 0.0	10.0 ± 0.0
Middle phalanx of forepaw				
Mean ± S.D. per dam	7.9 ± 0.2	7.9 ± 0.3	7.9 ± 0.4	7.9 ± 0.2
Metatarsal bone of hind paw				
Mean ± S.D. per dam	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0
Proximal phalanx of hind paw				
Mean ± S.D. per dam	8.0 ± 0.0	8.0 ± 0.1	8.0 ± 0.0	8.0 ± 0.0
Middle phalanx of hind paw				
Mean ± S.D. per dam	8.0 ± 0.0	8.0 ± 0.1	8.0 ± 0.1	8.0 ± 0.1
Sacrococcygeal centrum				
Mean ± S.D. per dam	19.0 ± 0.4	18.9 ± 0.4	19.0 ± 0.3	19.0 ± 0.4
Unossified bones				
Number of fetuses with unossified bones	0	1	1	1
Mean % ± S.D. per dam[d)]	0.0 ± 0.0	1.0 ± 4.5	0.6 ± 2.5	0.6 ± 2.5
Number of dams noted [%][b)]	0 [0.0]	1 [5.0]	1 [5.0]	1 [6.3]
Unossified hyoid bone	0	0	1	1
Mean % ± S.D. per dam	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 2.5	0.6 ± 2.5
Number of dams noted [%][b)]	0 [0.0]	0 [0.0]	1 [5.0]	1 [6.3]
Unossified pubis	0	0	0	1
Mean % ± S.D. per dam	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 2.5
Number of dams noted [%][b)]	0 [0.0]	0 [0.0]	0 [0.0]	1 [6.3]
Unossified talus	0	1	1	0
Mean % ± S.D. per dam	0.0 ± 0.0	1.0 ± 4.5	0.6 ± 2.5	0.0 ± 0.0
Number of dams noted [%][b)]	0 [0.0]	1 [5.0]	1 [5.0]	0 [0.0]

b): Not statistically analyzed.
d): (Number of fetuses with unossified bones/number of fetuses examined)×100.

9.3 Prenatal and Postnatal Development

Study title: Oral Study for Effects of S-297995 monotosylate on Pre- and Postnatal Development, Including Maternal Function, in Rats

Study no.:	S-297995-TF-275-L (CRO Study No. SG12011)
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 22, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	S-297995 monotosylate, lot# A81001, 97.5%

Key Study Findings

In maternal animals, there were treatment-related decreases (compared to controls) in body weights / body weight gain and food consumption during gestation at ≥30 mg/kg/day and a single high dose animal died on PND 22. Additional findings at ≥30 mg/kg/day included scattering of offspring in the cages (suggestive of poor nursing) and total litter loss in 5 and 3 dams at 30 and 1000 mg/kg/day, respectively. In F_1 pups, there were decreases in the viability index on lactation day 4 at ≥30 mg/kg/day, and decreased body weights and delayed pinna unfolding at 1000 mg/kg/day. Based on these findings, the NOAEL for maternal and developmental toxicity was 1 mg/kg/day.

Methods

Doses:	1, 30, and 1000 mg/kg/day (as S-297995)
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	0.5 w/v% methylcellulose aqueous solution
Species/Strain:	Rat (SPF)/Crl:CD(SD)
Number/Sex/Group:	22 females/group
Satellite groups:	No
Study design:	Pregnant female rats were administered the test compound from gestation day (GD) 7 to lactation day (LD) 20.
Deviation from study protocol:	Protocol deviations did not affect the quality or integrity of the study.

F_0 Dams:

Survival:

Dams were observed three times per day during the dosing period, and once daily during other periods.

On GD 22, a single high dose female was found dead. A total of 10 newborns (6 live and 4 dead) were observed with the dam, and gross pathology revealed 8 dead fetuses in the uterus, dark reddish discoloration of the lungs, foamy fluid in the trachea and bronchi, and a small thymus.

Clinical signs:
Cage-side observations of dams were conducted three times per day during the dosing period, and once daily during other periods of the study.

During LDs 0-4, scattering of offspring in the cages (considered to be indicative of poor nursing) were observed for 4 dams each in the 30 and 1000 mg/kg/day dose groups. In addition, total litter loss occurred in 5 and 3 dams, respectively, in the mid- and high dose groups.

Body weight:
Body weights were recorded once daily on days 0, 4, and 7-20 of gestation and days 0, 4, 7, 11, 14, 18, and 21 of lactation.

During gestation, there were statistically significant decreases in body weights and body weight gain in the 30 and 1000 mg/kg/day treatment groups, compared to controls. On GD 20, mean body weights of dams in the 30 and 1000 mg/kg/day groups were 94% and 93%, respectively, of the mean control weight. Body weight gain at 30 and 1000 mg/kg/day on GD was 81% and 74%, respectively, of controls. During the beginning of the lactation periods, body weights continued to be decreased in the mid- and high dose groups relative to controls. Decreased body weights were significantly different than controls at 30 mg/kg/day on LD 0 only, and LD 0, 4, and 7 at 1000 mg/kg/day. Body weight gain in the mid- and high dose groups increased from LD 11 and was significantly increased on LD 21 (1.9-fold the body weight gain in controls).

Feed consumption:
Consumption of food was recorded on days 0, 4, 7-19, and 20 of gestation and days 0, 4, 7, 11, 14, 18, and 21 of lactation.

There was a statistically significant decrease in food consumption at 30 and 1000 mg/kg/day, compared to controls during the gestation period (e.g., -28% and -61% at 30 and 1000 mg/kg/day, respectively, on GD 8). During the lactation period, food consumption was not significantly different than that of controls.

Uterine content:
There were no statistically significant effects on the duration of gestation, gestation index, number of implantation sites, or delivery index (compared to controls). However, the number of dead pups in the 30 and 1000 mg/kg/day groups was elevated (compared to controls) and there was a trend towards a decreased birth index at these dose levels. Summary data for reproductive observations of dams at delivery are presented in the Applicant's tables below.

Table 6 Reproductive observations of dams (F0) at delivery

		Control	S-297995 monotosylate (mg/kg/day) 1	30	1000
Number of dams		22	22	22	22
Number of dams with no delivery		1	0	0	0
Number of dams which died before delivery		0	0	0	1
Number of dams which delivered		21	22	22	21
Number of dams with a gestation duration of					
21 days		4	2	1	6
22 days		16	19	19	14
23 days		1	1	2	1
Duration of gestation (days, Mean ± S.D.)	[R]	21.9 ± 0.5	22.0 ± 0.4	22.0 ± 0.4	21.8 ± 0.5
Number of dams with live newborns		21	22	21	20
Gestation index [a]	[C]	95.5	100.0	95.5	90.9
Number of implantation sites (Mean ± S.D.)	[D]	15.7 ± 2.2	15.5 ± 2.5	15.5 ± 2.4	15.6 ± 1.5
Number of newborns (Mean ± S.D.)	[D]	15.0 ± 1.9	14.9 ± 2.5	14.4 ± 2.1	14.6 ± 1.6
Delivery index (Mean ± S.D.) [b]	[R]	95.7 ± 4.6	96.5 ± 4.2	93.3 ± 6.3	94.1 ± 7.2
Number of live newborns (Mean ± S.D.)	[R]	14.5 ± 2.1	14.7 ± 2.4	12.9 ± 3.6	13.4 ± 3.6
Birth index (Mean ± S.D.) [c]	[R]	92.7 ± 6.7	95.5 ± 4.8	84.7 ± 20.9	86.9 ± 22.7
Body weight in live male newborns (g, Mean ± S.D.)	[D]	6.35 ± 0.30	6.49 ± 0.43	6.24 ± 0.51 [f]	6.03 ± 0.37 [g] *
Body weight in live female newborns (g, Mean ± S.D.)	[D]	6.05 ± 0.29	6.14 ± 0.42	5.93 ± 0.52 [f]	5.70 ± 0.40 [g] *
Male proportion (%, Mean ± S.D.)	[R]	46.2 ± 12.6	51.7 ± 12.9	52.5 ± 13.6 [f]	49.9 ± 14.1 [g]
Number of dead newborns		10	4	32	25
Stillbirths		7	4	27	21
Death [e]		3	0	5	4
Cannibalism		0	0	0	0
Number of live newborns with external malformations: (%, Mean ± S.D.) [d]	[R]	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)	0 (0.0 ± 0.0) [f]	0 (0.0 ± 0.0) [g]

Percentages and indices were calculated as follows:
[a] (Number of dams with live newborns / Number of dams) × 100
[b] (Number of newborns / Number of implantation sites) × 100, litter basis
[c] (Number of live newborns / Number of implantation sites) × 100, litter basis
[d] (Number of live newborns with external malformations / Number of live newborns) × 100, litter basis
[e] Newborns which died immediately after birth. [f] Calculated from 21 dams
[g] Calculated from 20 dams
*: P<0.05, significantly different from control
Method of statistical analysis: [D]: Dunnett's test [R]: mean rank test of the Dunnett type [C]: Chi-square (χ^2) test

Necropsy observation:
Gross necropsies of the dams (F_0) were performed at termination on LD 21. Parameters evaluated included gross pathology and the number of implantation sites. A single control group animal that failed to deliver was terminated on Day 25 of gestation. Dams with total litter loss were terminated on the day in which the total litter loss (deaths of all offspring) occurred.

Gross findings in the single high dose female that died on GD 22 are summarized under "Survival." At scheduled termination of dams necropsied at weaning, there were no treatment-related findings. In dams for which there was total litter loss (4 and 2 females in the mid- and high dose groups, respectively), small thymus and spleen were noted.

Toxicokinetics:
Not conducted

Dosing solution analysis:
Dosing solution samples were collected at the first and last preparations for verification of concentrations and homogeneity through HPLC analysis. Actual concentrations were 97.7-104.0% of nominal concentrations and the relative standard deviation was 0.0-0.6%. Therefore, the concentrations and homogeneity were deemed acceptable.

F1 Generation:

Survival:

The total number of offspring and offspring viability was recorded at birth. Dead newborns were classified as stillbirths, postnatal deaths, or cannibalized newborn animals, and examined for external morphology. Litters with greater than 8 offspring were culled to 8 pups (4/sex) on PND 4.

There were no statistically significant differences in the number of newborns or sex ratio of live newborns at up to 1000 mg/kg/day, compared to controls. As discussed under "Uterine Content" above and depicted in the Applicant's figure, the number of dead newborns in the 30 and 1000 mg/kg/day dose groups was numerically higher than that in controls and there was a trend towards a decreased birth index at these dose levels. In addition, on LD 4, there was a decrease in the viability index at 30 and 1000 mg/kg/day (compared to controls; statistically significant at 30 mg/kg/day only). Viability of F_1 offspring is summarized in the Applicant's table below. There were no treatment-related effects on the weaning index or survival after weaning.

Table 9 Viability of offspring (F1)

		Control	S-297995 monotosylate (mg/kg/day) 1	30	1000
Number of live offspring					
Day of lactation					
0		305 (21)	324 (22)	284 (21)	282 (20)
4		302 (21)	309 (22)	225 (18)	241 (18)
4 a)		168 (21)	173 (22)	136 (17)	141 (18)
7		168 (21)	173 (22)	136 (17)	141 (18)
11		168 (21)	173 (22)	136 (17)	141 (18)
14		168 (21)	173 (22)	136 (17)	141 (18)
18		168 (21)	173 (22)	136 (17)	141 (18)
21		168 (21)	173 (22)	136 (17)	141 (18)
Viability index on Day 4 b)	[R]	99.0 ± 2.5	95.7 ± 13.3	77.8 ± 39.1 *	84.4 ± 32.6
Weaning index c)	[R]	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

a) After culling
Indices were calculated as follows:
b) (Number of live offspring on Day 4 of lactation / Number of live newborns) × 100, litter basis
c) (Number of live offspring on Day 21 of lactation / Number of live offspring after culling on Day 4 of lactation) × 100, litter basis
( ): Number of dams with live offspring
*: P<0.05, significantly different from control
Method of statistical analysis: [R]: mean rank test of the Dunnett type

Clinical signs:

F_1 offspring were observed for clinical signs once daily from the day of birth until weaning. Clinical observations were recorded once daily until the day of necropsy for animals selected for reproductive and behavioral evaluations.

There was an increased incidence of subnormal body temperature, no milk in the stomach, and total litter loss at 30 and 1000 mg/kg/day, compared to controls. Clinical signs of F_1 offspring pre-weaning are summarized in the Applicant's table below. There were no treatment-related clinical signs in males or females after weaning, or in females during gestation.

Table 7 Clinical observations of offspring (F1) prior to weaning

	Control	S-297995 monotosylate (mg/kg/day) 1	30	1000
Number of dams	21	22	22	21
Number of dams whose offspring had abnormal signs	3	6	11	8
Number of dams whose offspring had the following signs				
Subnormal body surface temperature of all/some offspring	0	0	4	2
No milk in the stomach of all/some offspring	0	0	4	3
A small amount of milk in the stomach of some offspring	0	0	1	1
Death of some offspring	3	6	6	5
Death of all offspring	0	0	5	3

Body weight:

Body weights of F_1 offspring were recorded at birth and at 4, 7, 11, 14, 18, and 21 days of age. Body weights of F1 animals after weaning were recorded at 28, 35, 42, 49, 56, 63, and 70 days of age. Mated females were weighted on Days 0, 7, and 13 of gestation.

As summarized in Applicant's table below, at birth and prior to weaning, there were statistically significant decreases in F_1 offspring body weights in the 1000 mg/kg/day dose group, compared to controls. There were no treatment-related changes in body weight (compared to controls) after weaning.

Table 8 Body weight of offspring (F1) prior to weaning

			Control		S-297995 monotosylate (mg/kg/day) 1		30		1000	
Male										
Day after birth	0	[D]	6.35 ± 0.30	(21)	6.49 ± 0.43	(22)	6.24 ± 0.51	(21)	6.03 ± 0.37 *	(20)
	4 a)	[D]	10.45 ± 0.91	(21)	10.29 ± 1.31	(22)	10.16 ± 1.44	(17)	9.47 ± 1.32 *	(18)
	4 b)	[D]	10.54 ± 0.87	(21)	10.45 ± 1.30	(22)	10.26 ± 1.46	(17)	9.68 ± 1.40	(18)
	7	[D]	17.5 ± 1.3	(21)	17.5 ± 1.9	(22)	17.3 ± 1.8	(17)	16.4 ± 2.1	(18)
	11	[D]	28.8 ± 1.9	(21)	29.1 ± 2.1	(22)	28.4 ± 2.4	(17)	26.7 ± 2.3 *	(18)
	14	[D]	37.5 ± 2.5	(21)	38.2 ± 2.7	(22)	36.9 ± 2.8	(17)	35.1 ± 2.6 *	(18)
	18	[D]	48.7 ± 3.3	(21)	49.3 ± 3.1	(22)	48.2 ± 3.9	(17)	45.1 ± 3.1 **	(18)
	21	[D]	61.7 ± 3.5	(21)	62.5 ± 4.2	(22)	61.3 ± 5.7	(17)	58.0 ± 5.3 *	(18)
Female										
Day after birth	0	[D]	6.05 ± 0.29	(21)	6.14 ± 0.42	(22)	5.93 ± 0.52	(21)	5.70 ± 0.40 *	(20)
	4 a)	[D]	10.04 ± 0.90	(21)	9.88 ± 1.22	(22)	9.72 ± 1.33	(17)	9.02 ± 1.26 *	(18)
	4 b)	[D]	10.29 ± 0.99	(21)	9.99 ± 1.15	(22)	9.80 ± 1.27	(17)	9.33 ± 1.49 *	(18)
	7	[D]	17.0 ± 1.4	(21)	16.7 ± 1.6	(22)	16.5 ± 1.6	(17)	15.8 ± 2.1	(18)
	11	[D]	28.2 ± 2.0	(21)	28.1 ± 1.9	(22)	27.3 ± 1.9	(17)	26.0 ± 2.5 **	(18)
	14	[D]	36.6 ± 2.4	(21)	36.9 ± 2.3	(22)	35.7 ± 2.2	(17)	34.2 ± 2.8 **	(18)
	18	[D]	47.5 ± 3.1	(21)	47.7 ± 2.6	(22)	46.4 ± 3.1	(17)	44.1 ± 3.3 **	(18)
	21	[D]	60.1 ± 3.6	(21)	59.9 ± 3.3	(22)	59.0 ± 4.1	(17)	56.3 ± 5.1 *	(18)

Each value represents Mean ± S.D. (g)
a) Before culling b) After culling
( ): Number of dams
*: P<0.05, **: P<0.01, significantly different from control
Method of statistical analysis: [D]: Dunnett's test

Feed consumption:

Not evaluated

Physical development:

Animals were observed daily from 2-4 days of age for pinna unfolding. Observations for growth of hair and eruption of incisors were conducted at 11 and 14 days of age, and observations for eyelid opening were conducted at 14 and 18 days of age. Males were observed for preputial separation at 42 and 49 days of age, and females were observed for vaginal opening at 35 and 42 days of age.

At 3 days of age, there was a decrease in the incidence of pinna unfolding at 1000 mg/kg/day (compared to controls; statistically significant for females only). There were no treatment-related effects on the incidence of growth of hair, eruption of incisors, or eyelid opening. Physical development data for F_1 offspring are summarized in the Applicant's table below.

Table 10 Physical development of offspring (F1)

		Control	S-297995 monotosylate (mg/kg/day) 1	30	1000
Male					
Number of dams		21	22	19	19
Number of offspring [a]: (%, Mean ± S.D.)					
Pinna unfolding 2 days after birth	[R]	52/141 (37.2 ± 39.0)	94/158 (59.1 ± 41.0)	51/123 (42.0 ± 34.1)	42/120 (30.1 ± 36.9)
Pinna unfolding 3 days after birth	[R]	127/140 (90.0 ± 23.1)	141/157 (90.4 ± 29.4)	112/115 (93.1 ± 24.0) [b]	92/118 (78.4 ± 33.8) [b]
Pinna unfolding 4 days after birth	[R]	140/140 (100.0 ± 0.0)	157/157 (100.0 ± 0.0)	113/113 (100.0 ± 0.0) [c]	118/118 (100.0 ± 0.0) [b]
Growth of abdominal hair 11 days after birth	[R]	84/84 (100.0 ± 0.0)	91/91 (100.0 ± 0.0)	68/68 (100.0 ± 0.0) [c]	72/72 (100.0 ± 0.0) [b]
Growth of abdominal hair 14 days after birth	[R]	84/84 (100.0 ± 0.0)	91/91 (100.0 ± 0.0)	68/68 (100.0 ± 0.0) [c]	72/72 (100.0 ± 0.0) [b]
Eruption of upper incisor 11 days after birth	[R]	78/84 (92.9 ± 19.6)	87/91 (95.5 ± 14.7)	66/68 (97.1 ± 8.3) [c]	65/72 (90.8 ± 20.2) [b]
Eruption of upper incisor 14 days after birth	[R]	84/84 (100.0 ± 0.0)	91/91 (100.0 ± 0.0)	68/68 (100.0 ± 0.0) [c]	72/72 (100.0 ± 0.0) [b]
Eyelid opening 14 days after birth	[R]	5/84 (6.0 ± 19.2)	14/91 (15.9 ± 29.4)	6/68 (8.8 ± 24.9) [c]	9/72 (11.9 ± 20.5) [b]
Eyelid opening 18 days after birth	[R]	84/84 (100.0 ± 0.0)	91/91 (100.0 ± 0.0)	68/68 (100.0 ± 0.0) [c]	72/72 (100.0 ± 0.0) [b]
Female					
Number of dams		21	22	19	19
Number of offspring [a]: (%, Mean ± S.D.)					
Pinna unfolding 2 days after birth	[R]	79/162 (46.2 ± 38.6)	90/153 (58.5 ± 41.8)	47/124 (38.6 ± 37.9)	39/124 (31.9 ± 37.2)
Pinna unfolding 3 days after birth	[R]	150/162 (93.4 ± 17.9)	140/153 (92.6 ± 22.0)	110/117 (88.3 ± 31.3)	93/123 (73.9 ± 33.1) [b] *
Pinna unfolding 4 days after birth	[R]	162/162 (100.0 ± 0.0)	152/152 (100.0 ± 0.0)	112/112 (100.0 ± 0.0) [c]	123/123 (100.0 ± 0.0) [b]
Growth of abdominal hair 11 days after birth	[R]	84/84 (100.0 ± 0.0)	82/82 (100.0 ± 0.0)	68/68 (100.0 ± 0.0) [c]	69/69 (100.0 ± 0.0) [b]
Growth of abdominal hair 14 days after birth	[R]	84/84 (100.0 ± 0.0)	82/82 (100.0 ± 0.0)	68/68 (100.0 ± 0.0) [c]	69/69 (100.0 ± 0.0) [b]
Eruption of upper incisor 11 days after birth	[R]	81/84 (96.4 ± 9.0)	80/82 (97.7 ± 7.4)	59/68 (86.3 ± 29.7) [c]	61/69 (87.5 ± 26.1) [b]
Eruption of upper incisor 14 days after birth	[R]	84/84 (100.0 ± 0.0)	82/82 (100.0 ± 0.0)	68/68 (100.0 ± 0.0) [c]	69/69 (100.0 ± 0.0) [b]
Eyelid opening 14 days after birth	[R]	9/84 (10.7 ± 21.8)	8/82 (9.1 ± 23.8)	6/68 (8.5 ± 14.9) [c]	11/69 (16.2 ± 24.7) [b]
Eyelid opening 18 days after birth	[R]	84/84 (100.0 ± 0.0)	82/82 (100.0 ± 0.0)	68/68 (100.0 ± 0.0) [c]	69/69 (100.0 ± 0.0) [b]

[a] Number of offspring in which development occurred / Number of offspring observed
[b] Calculated from 18 offspring [c] Calculated from 17 offspring
*: P<0.05, significantly different from control
Method of statistical analysis: [R]: mean rank test of the Dunnett type

There were no statistically significant differences in the incidence of preputial separation or vaginal opening at either timepoint evaluated in the study (compared to controls).

Neurological assessment:

Early behavior of offspring was evaluated at 11 and 14 days of age. Parameters evaluated were back righting and geotaxis. One male and female from each litter was selected at 21 days of age for behavioral evaluations after weaning. At 4 weeks of age, the following sensory function parameters were evaluated: visual function (visual placing response and pupillary reflex), auditory function (Preyer's reflex), and pain sensitive function (pain response). An open field test was conducted at 5 weeks of age, and conditioned avoidance response was evaluated at 6-7 weeks of age.

There were no treatment-related effects on back righting or geotaxis, sensory function parameters, or conditional avoidance response. In the open field test, there was a statistically significant increase in the mean frequency of defecation in male F_1 offspring at 1000 mg/kg/day (0.7 versus 0.1 in controls). However, because the increase was limited to the second trial only and there were no treatment-related changes in other

parameters, the increase was considered to be incidental. For comparison, during the first trial, the mean incidence of defecation in males was 0.4, 0.5, 0.4, and 0.3 in the control, 1, 30, and 1000 mg/kg/day groups, respectively.

Reproduction:

One male and female from each litter was selected at 21 days of age for evaluation of reproductive function after weaning. Vaginal smears were collected from 14 days prior to initiation of mating until the day of copulations. The mean length of the estrous cycle and number of cycles were calculated based on estrous phases over 15 days. From 10-13 weeks of age, males and non-sibling females in the same group were mated on a one-to-one basis for a maximum of 14 days. For pairs that failed to mate, a second mating period was continued for up to 4 days after the first mating period.

There were no treatment-related effects on the mean estrous cycle length or number of cycles (compared to controls). There also were no statistically significant effects on the mating index, fertility index, or pre-coital period. Summary data for the 1st and 2nd mating periods are provided in the Applicant's table below. At 30 and 1000 mg/kg/day, a single female each did not become pregnant, compared to 0 animals in the control and 1 mg/kg/day groups.

Table 24 Mating and fertility of offspring (F1)

		Control	S-297995 monotosylate (mg/kg/day) 1	30	1000
Number of offspring paired		21	22	17	18
Number of offspring which successfully mated					
1st mating period: (%) [a]	[C]	21 (100.0)	21 (95.5)	17 (100.0)	16 (88.9)
1st and 2nd mating periods: (%) [a]	[C]	21 (100.0)	22 (100.0)	17 (100.0)	18 (100.0)
Number of pregnancies					
1st mating period: (%) [b]	[C]	21 (100.0)	21 (100.0)	16 (94.1)	15 (93.8)
1st and 2nd mating periods: (%) [b]	[C]	21 (100.0)	22 (100.0)	16 (94.1)	17 (94.4)
Pre-coital period (days)					
Number of offspring		21	22	17	18
Mean ± S.D.	[D]	3.3 ± 2.4	2.9 ± 2.8	3.9 ± 2.9	3.3 ± 2.2

Percentages were calculated as follows:
[a] Mating index: (Number of offspring which successfully mated / Number of offspring paired) × 100
[b] Fertility index: (Number of pregnancies / Number of offspring which successfully mated) × 100
Not significantly different from control
Method of statistical analysis: [C]: Chi-square (χ^2) test
[D]: Dunnett's test

Necropsy Evaluation:

At 21 days of age, offspring (other than those selected for reproduction and behavioral assessments) were terminated and gross pathology was evaluated. At 10 weeks of age, F_1 animals used for behavioral observations were terminated and gross pathology was evaluated. After completion of cesarean section of mated F_1 females, F_1 males were terminated and gross pathology was evaluated. On Day 13 of gestation, cesarean section was performed on mated F_1 females. Gross pathology, evidence of implantations, and the number of corpora lutea were determined. The number of implantations and number of live and dead embryos were determined. Dead embryos were classified as early (early death after implantation), resorb (unformed embryos), or dead (formed embryos with placentae).

There were no treatment-related gross findings in F_1 offspring at weaning or 10 weeks of age (behavioral evaluation animals). There also were no treatment-related findings in males after evaluation of mating ability. At cesarean section, there were no gross abnormalities in the any animals (including the single female each at 30 and 1000 mg/kg/day which did not become pregnant), or treatment-related effects on the number of corpora lutea, number of implantations, pre- or postimplantation loss, or number of live embryos (compared to controls). Summary data are shown in the Applicant's table below.

Table 29 Reproductive observations of dams (F1)

			S-297995 monotosylate (mg/kg/day)		
		Control	1	30	1000
Number of dams		21	22	16	17
Number of corpora lutea (Mean ± S.D.)	[D]	16.2 ± 2.1	16.0 ± 2.0	16.4 ± 2.2	15.6 ± 1.9
Number of implantations (Mean ± S.D.)	[R]	14.6 ± 1.8	15.5 ± 1.7	14.9 ± 2.1	13.9 ± 3.6
Pre-implantation loss: (%, Mean ± S.D.) [a)]	[R]	9.1 ± 10.7	2.9 ± 5.4	8.3 ± 13.3	11.1 ± 23.0
Number of live embryos (Mean ± S.D.)	[R]	13.7 ± 2.3	14.6 ± 1.8	14.6 ± 2.3	13.4 ± 3.6
Post-implantation loss: (%, Mean ± S.D.) [b)]	[R]	19 (6.5 ± 8.5)	18 (5.3 ± 6.8)	6 (2.8 ± 4.8)	9 (3.5 ± 6.8)
Early	[R]	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)
Resorb	[R]	19 (6.5 ± 8.5)	18 (5.3 ± 6.8)	6 (2.8 ± 4.8)	9 (3.5 ± 6.8)
Dead	[R]	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)

Percentages were calculated as follows:
[a)] [(Number of corpora lutea - Number of implantations) / Number of corpora lutea] × 100, litter basis
[b)] (Post-implantation loss / Number of implantations) × 100, litter basis
Not significantly different from control
Method of statistical analysis: [D]: Dunnett's test
[R]: mean rank test of the Dunnett type

Toxicokinetics:
Not conducted

10 Special Toxicology Studies

Review of Study No. R-297995-TF-084-N (CRO Study No. 702128) from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Title: **Ocular Irritation Study of RSC-297995 monotosylate in Rabbits**

Methods: RSC-297995 monotosylate was given to the eyes of male rabbits (Kbl: JW) once, and the ocular irritation potential of RSC-297995 monotosylate was assessed. For the present study, the dosage was set at 0.1 g per eye, and it was given to the left eyes of 6 rabbits. The eyes of the animals (n=3) in the eye-washed group were washed 20 - 30 seconds after application, and the eyes of the animals (n=3) in the non-eye-washed group were left as they were. The right eyes of all animals were untreated and served as the untreated controls. Ocular observation was performed 1, 24, 48, and 72 hours and 4 and 7 days after application.

Results: In the non-eye-washed group, redness (irritation score: 2), chemosis (irritation score: 2), and discharge (irritation score: 2 or 3) were noted in the conjunctivae 1 hour after application. These findings diminished with the lapse of time and disappeared by 48 hours after application. The index of acute ocular irritation (I.A.O.I.) 1 hour after application, which was calculated for each day of evaluation, was 13.33. In the eye-washed group, redness (irritation score: 1) and chemosis (irritation score: 1) were noted in the conjunctivae 1 hour after application. These findings disappeared by 24 hours after application. The index of acute ocular irritation (I.A.O.I.) 1 hour after application, which was calculated for each day of evaluation, was 4.00. From the above results, RSC-297995 monotosylate was classified as a substance which was "slightly irritating," the second lowest potential when the severity was classified into 6 (the AFNOR's evaluation criteria). In the eye-washed group, alleviating effects of eye washing were noted, since the mean score 1 hour after application was lower than in the non-eye-washed group, and ocular irritation disappeared earlier than in the non-eye-washed group.

Review of Study No. R-297995-TF-085-N (CRO Study No. 702028) from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Title: **Dermal Irritation Study of RSC-297995 monotosylate in Rabbits**

Methods: RSC-297995 monotosylate was administered percutaneously to male rabbits (Kbl: JW), and the primary skin irritation potential of RSC-297995 monotosylate was assessed. For the present study, 0.5 g of RSC-297995 monotosylate was applied to the intact and abraded skin (applied area: 2.5 × 2.5 cm) of 3 rabbits for 24 hours under occluded conditions, and the application sites were observed macroscopically for dermal reactions 24 hours after the start of application (about 30 minutes after removal of the applied materials) and 48 and 72 hours after the start of application.

Results: No dermal reactions such as erythema, eschar formation or edema formation were noted in the intact or abraded skin at any time of observation, and the primary irritation index was 0.00. Moreover, no abnormalities in general signs or body weight changes were observed in any animal during the observation period. Thus, RSC-297995 was not a dermal irritant.

Immunotoxicity Study of S-297995 monotosylate in Rats: Determination of Specific Antibody Formation against T-cell Dependent Antigen (Study No. S-297995-TB-234-L)

Methods: Crl:CD(SD) rats (n=10/sex/group) were administered 30, 100, and 1000 mg/kg/day S-297995 monotosylate (or vehicle, 0.5 w/v% methylcellulose aqueous

solution; dosing volume 10 mL/kg) by oral gavage for 1 month. Following administration of the test compound on Day 24, animals were immunized once by intravenous injection of 0.2 mg/0.5 mL Hemocyanin, Keyhole Limpet (KLH) in saline solution. On the day of necropsy (Day 31), serum samples were collected for determination of IgM class anti-KLH antibody titers by ELISA.

Results: S-297995 produced salivation in 3 and 5 high dose males and females, respectively. There were transient, statistically significant decreases in body weight at all dose levels (Day 4 only in females; beginning on Day 4 in males). Although anti-KLH antibody titers in the test compound groups were higher than those in the control groups, there were no statistically significant differences in the antibody titer at up to 1000 mg/kg/day S-297995 (compared to controls) and no dose-relationship was apparent (the highest mean titers were measured at 100 mg/kg/day). Thus, it was concluded that under the conditions of the study, the test compound did not affect T-cell dependent antibody formation in rats. Summary data are shown in the Applicant's tables below.

Antibody titers in male rats

Group	n	Mean	Std. Dev.	Minimum	Median	Maximum
Control	10	885.27	583.33	311.34	793.66	2310.13
S-297995 monotosylate (30 mg/kg/day)	10	1664.71	997.99	559.90	1458.73	3847.89
S-297995 monotosylate (100 mg/kg/day)	10	1790.56	1653.06	332.15	947.83	5493.51
S-297995 monotosylate (1000 mg/kg/day)	10	1377.79	1108.60	352.12	872.47	3627.55

n: number of animals in group
Data were obtained from enzyme-linked immunosorbent assay (Experimental date: November 9-10, 2010).
No significantly difference was observed between control-group and each dosing group.
Method of statistical analysis : Dunnett's multiple comparison test

Antibody titers in female rats

Group	n	Mean	Std. Dev.	Minimum	Median	Maximum
Control	10	737.09	518.24	283.43	557.28	1947.51
S-297995 monotosylate (30 mg/kg/day)	10	1234.49	1011.51	437.51	947.86	3467.37
S-297995 monotosylate (100 mg/kg/day)	10	2240.99	3296.21	139.97	903.85	10999.17
S-297995 monotosylate (1000 mg/kg/day)	10	1232.59	1217.18	177.43	813.01	3886.72

n: number of animals in group
Data were obtained from enzyme-linked immunosorbent assay (Experimental date: November 11-12, 2010).
No significantly difference was observed between control-group and each dosing group.
Method of statistical analysis : Dunnett's multiple comparison test

Preliminary Skin Phototoxicity Study of S-297995 monotosylate in Hairless Mice by Oral Dosing (Study No. S-297995-TB-006-R)

Methods: Female hairless Hos:HR-1 mice were administered single oral (gavage) doses of 100, 200, and 300 mg/kg S-297995 monotosylate (or vehicle, 0.5 w/v% methyl cellulose; dosing volume 5 mL/kg). 8-methoxypsoralen (8-MOP; 30 mg/kg) was administered to separate groups of mice as a positive control. Each S-297995 dose level included 2 groups of animals (n=3/group), one of which was irradiated with UV light (10 J/cm^2, 290 – 400 nm) immediately after dosing for 2 h and 20 min. The vehicle and positive control groups included 3 animals each, and these groups received UV irradiation. Gross observation of the skin for erythema, eschar and thickening of skin was conducted at 6 h and 20 mins, 1 day, and 2 days after administration. Satellite groups were administered 30 and 300 mg/kg S-297995 and included for toxicokinetic analysis (n=15/group). Blood samples were collected out to 24 h after dosing, and concentrations of S-297995 in plasma were analyzed by LC/MS/MS.

Results: While marked erythema and thickening of the skin was observed in the positive control group, there were no macroscopic changes in animals treated with S-297995 or the vehicle control. There also were no apparent effects on body weight or clinical observations in animals administered the test compound. In TK animals, AUC_{0-24h} values at 30 and 300 mg/kg were 10.47 and 118.80 mcg.h/mL, respectively. Cmax values at 30 and 300 mg/kg were 3.42 and 26.87 mcg/mL, respectively. Tmax values were 1.0 h.

Skin Phototoxicity Study of S-297995 monotosylate in Hairless Mice by Oral Dosing (Study No. S-297995-TB-249-L)

Methods: Female hairless Hos:HR-1 mice were administered single oral (gavage) doses of 30 and 300 mg/kg S-297995 monotosylate (or vehicle, 0.5 w/v% methylcellulose aqueous solution; dosing volume 10 mL/kg). 8-methoxypsoralen (8-MOP; 8 mg/kg) was administered to separate groups of mice as a positive control. Each S-297995 dose level included 2 groups of animals, one which was not exposed to UV irradiation (n=5) and one which was irradiated with UV light (10 J/cm^2 in a range of wavelength 290 – 400 nm) for 1 h beginning 0.5 h after dosing (n=10). The vehicle and positive control groups included 2 groups of 5 animals each (with and without UV-irradiation). The skin was assessed and graded using the specified criteria with gross examination and palpation out to 48 h after completion of the UV-irradiation. Satellite groups (exposed to UV-irradiation) were included for toxicokinetic analysis (control n=4; 30 mg/kg n=20; 300 n=20). Blood samples were collected out to 24 h after dosing, and concentrations of S-297995 in plasma were analyzed by LC/MS/MS.

Results: No dermal reactions were observed in mice administered the test compound or vehicle control; whereas, treatment-related skin reactions were observed in animals administered 8-MOP and treated with UV-irradiation. In addition, S-297995 produced no apparent effects on body weight or clinical observations. Thus, under the conditions of the study, S-297995 was considered to not have skin phototoxic potential. In TK animals, AUC_{0-24h} values at 30 and 300 mg/kg were 9.73 and 163 mcg.h/mL, respectively. Cmax values at 30 and 300 mg/kg were 4.40 and 30.4 mcg/mL, respectively. Tmax values were 1.0 h.

Review of Study No. R-297995-TF-105-R (CRO Study No. P080796) from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Title: **Oral Study for Effects of RSC-297995 monotosylate on Estrous Cycle and Plasma Reproductive Hormone Concentration in Female Rats (Study# P080796)**

Methods: RSC-297995 monotosylate was orally administered at dose levels of 0 and 1000 mg/kg daily for 16 days to Sprague-Dawley female rats [Crl:CD(SD), n=20], and the estrous cycle and the plasma LH, FSH, prolactin, estradiol and progesterone levels at 1, 2 and 4 hours after dosing on day 1 and day 9 of dosing and in the morning on day 2 of dosing were assessed.

Results: No mortalities or any clinical signs were noted among the females in any group. However, body weights were decreased in the 1000 mg/kg from day 4 of dosing and were significantly lower in this group than in the control group on day 4 of dosing and later (250.9± 12.8 g vs. 264.0± 13.9 g of control at day 16). The count of estrus was significantly lower in the 1000 mg/kg group (2.95±1.23) than in the control group (3.80±0.52). The estrous cycle was slightly prolonged in the 1000 mg/kg group (4.12 vs. 4.08 days of controls), although this change was statistically insignificant.

Effects of RSC-297995 on female hormone levels were reported on:

Day 1 dosing (expected metestrus phase): the prolactin level at 4 hours after dosing was significantly higher in the 1000 mg/kg group (94.29 ng/mL) than in the control group (3.17 ng/mL). The progesterone levels at 1 and 2 hours after dosing were significantly higher (30%-34%, $p<0.01$) in the 1000 mg/kg group than in the control group. The estradiol levels in the 1000 mg/kg group were below the lower limit of quantification (BLQ) at all time points, and significant differences were observed at 1 and 4 hours after dosing as compared with the control levels (1 hour: 11.7 pg/mL, 4 hour: BLQ).

Day 2 of dosing (expected diestrus phase): progesterone level was significantly higher in the 1000 mg/kg group (17.93 ng/mL) than in the control group (8.41 ng/mL). The proractin and estradiol levels in the 1000 mg/kg group were BLQ, whereas those in the control group were 4.26 ng/mL and 13.3 pg/mL, respectively, although these were statistically insignificant.

Day 3 of dosing (expected proestrus phase): the LH, FSH, prolactin, estradiol and progesterone levels were measured on day 3 of dosing (proestrus phase), but no significant differences between the control and 1000 mg/kg groups were noted at any time point.

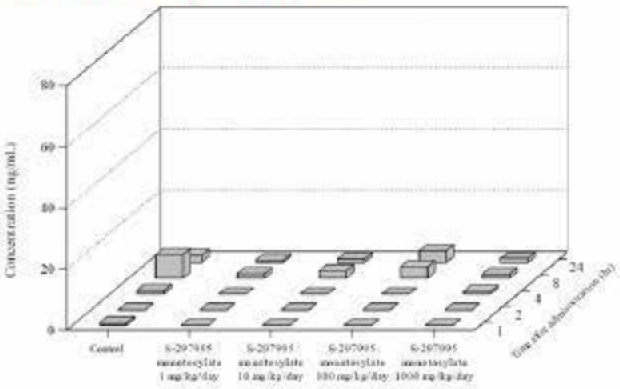
Day 9 of dosing (expected metestrus phase): the FSH levels at 2 (162%) and 4 (230%) hours after dosing were significantly higher in the 1000 mg/kg group than in the control group. The estradiol level in the 1000 mg/kg group was BLQ at all time points, although no significant differences were observed as compared with the control value (BLQ to 11.2 ng/mL). There was no significant difference of the LH, prolactin and progesterone levels between the control and the 1000 mg/kg groups. Taken together, treatment with 1000 mg/kg/day induced an irregular estrous cycle, increased markedly the prolactin, FSH and the progesterone level. No remarkable changes were observed in the LH and estradiol levels.

Review of Study No. S-297995-TF-162-N (CRO Study No. P090377) from the IND 107475 nonclinical review dated July 19, 2011 is copied below (C. Wu, Ph.D.).

Title: **Single Dose Oral Study for Effects of S-297995 monotosylate on Plasma Prolactin Concentration in Rats (Study# P090377)**

Methods: S-297995 monotosylate was orally administered at dose of 0, 1, 10, 100 and 1000 mg/kg to male and female rats [Crl:CD(SD), 10 week-old, 10/sex/group], and the plasma prolactin level was assessed at 1, 2, 4, 8 and 24 hours after dosing.

Results: In the males, no obvious prolactin fluctuation was observed in any group at any time points as shown in Figure 1 below.



BEST AVAILABLE COPY

Figure 1 Plasma prolactin levels in male rats

In contrast, the prolactin levels in the females at 8 hours after dosing were significantly higher in the 10, 100 and 1000 mg/kg groups (67.85, 58.98 and 66.13 ng/mL, respectively) than in the control group (2.92 ng/mL) as shown in the figure below. The plasma prolactin level at 4 hours after dosing was significantly lower in the 100 mg/kg group than in the control group; however, this may not considered to be test article-related based on the lack of dose-dependency.

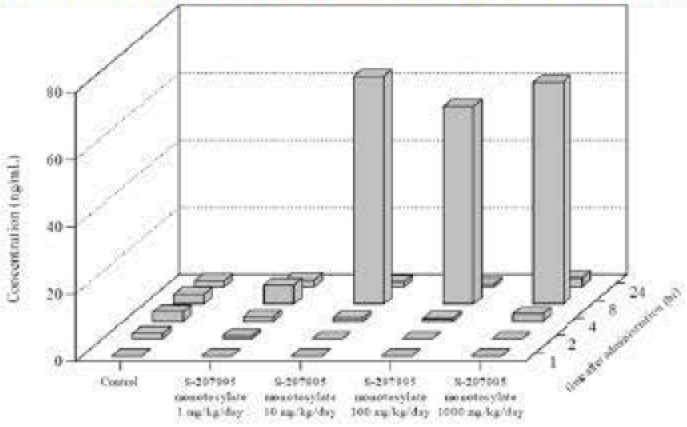


Figure 2 Plasma prolactin levels in female rats - Expected metestrus phase -

11 Integrated Summary and Safety Evaluation

Symproic™ (naldemedine tosylate; naldemedine) is an opioid antagonist. Under NDA 208854, the Applicant is seeking approval of Symproic™ for the treatment of opioid-induced constipation (OIC) in adults with chronic non-cancer pain at a recommended

human dose of 0.2 mg naldemedine (as free base; equivalent to 0.26 mg naldemedine tosylate) once daily. Naldemedine is a derivative of naltrexone (another opioid receptor antagonist) to which a side chain has been added. The Applicant states that addition of the side chain results in a molecule with a greater polarity and lower lipid solubility than naltrexone, thus reducing its ability to cross the blood-brain barrier. Furthermore, naldemedine is a substrate of the P-glycoprotein (P-gp) efflux transporter, which may also reduce its ability to penetrate the central nervous system (CNS). Therefore, the Applicant asserts that naldemedine is expected to function as an opioid receptor antagonist in peripheral tissues (particularly the enteric nervous system in the gastrointestinal tract), without reversing opioid CNS-mediated analgesic effects. In support of the NDA, the Applicant has conducted pharmacology, pharmacokinetics/ ADME/toxicokinetics, single- and repeat dose general toxicology, genotoxicity, carcinogenicity, reproductive and developmental toxicology, and special toxicology studies.[1]

Naldemedine is an opioid antagonist, with Ki values for human mu, delta, and kappa opioid receptors of 0.34, 0.43, and 0.94 nM, respectively. Functional Kb values (antagonist activity) of naldemedine for human mu, delta, and kappa receptors were 0.5, 0.27, and 0.44 nM, respectively. Compared to naloxone, naldemedine exhibited slower association and dissociation kinetics to mu receptor, and the compound exhibited non-competitive antagonism of mu receptor activation by opioids. Among those studied, each of the metabolites had less potent binding affinities for mu, delta, and kappa receptors relative to the parent compound, while one metabolite (benzamidine) had no significant binding affinities for these receptors. Although less potent, the Ki value of S-297995 6-O-β-D-glucuronide (S-297995 6-G) for delta receptors (0.51 nM) approached that of the parent compound. While antagonistic activities of the metabolites at mu, delta, and kappa receptors were less potent than naldemedine, the antagonistic activity of S-297995 6-G for delta receptors approached that of the parent compound (Kb value = 0.7 nM). Metabolite nor-S-297995 (nor-naldemedine) showed weak agonistic activity for delta receptors with an EC_{50} of 96 nM (equivalent to 49.61 ng/mL which is 340 times the human C_{max} for this metabolite (0.146 ng/mL) at the recommended human dose).

When tested in *in vivo* pharmacology studies in rats, naldemedine prevented morphine- and oxycodone-induced suppression of small intestinal transit (ED_{50} values ranging from 0.02 to 0.23 mg/kg) and antagonized the inhibitory effect of morphine on castor oil-induced diarrhea in rats (ED_{50} value of 0.01 mg/kg). An anti-emetic effect on morphine-induced emesis in ferrets was demonstrated with significant findings at ≥0.03 mg/kg naldemedine. In the rat tail-flick test and post-operative pain model, inhibition of the analgesic effects of morphine occurred at doses ≥10 mg/kg and ≥5 mg/kg, respectively. In morphine-dependent mice and rats, naldemedine caused withdrawal symptoms considered to be peripherally-mediated (i.e., diarrhea and/or weight loss) at ≥1 mg/kg and ≥0.3 mg/kg, respectively. Centrally-mediated withdrawal signs associated with naldemedine treatment were limited to teeth chattering at ≥3 mg/kg in morphine-

[1] The test article used in nonclinical studies was generally naldemedine monotosylate. The NOAELs identified for general toxicology, carcinogenicity, and reproductive and developmental toxicity studies are expressed as naldemedine.

dependent rats (other effects such as jumping behavior or wet-dog shakes were not observed). There were no significant inhibitory effects when naldemedine was screened against various receptors, channels, transporters, and enzymes with the exception of opiate (non-selective) receptor.

In safety pharmacology studies, naldemedine did not have any effects on the central nervous and respiratory systems at oral doses of up to 300 mg/kg. In the cardiovascular system study in dogs, there were no clear treatment-related effects following oral administration of up to 100 mg/kg. In a hERG channel patch clamp study, naldemedine inhibited the peak tail current by 5.6% and 33.1% at 3 and 30 µmol/L, respectively (IC50 value was estimated to be higher than 30 µmol/L). In addition, naldemedine prolonged the action potential duration in isolated guinea pig papillary muscles by more than 10% at 30 µmol/L. Based on the human Cmax value of naldemedine (2 ng/mL) at the recommended clinical dose, safety margins for the concentrations at which effects were observed in vitro (3 and 30 µmol/L; equivalent to 1.71 and 17.1 µg/mL, respectively) are 855 and 8550, respectively.

Pharmacokinetics of naldemedine were studied in rodents and nonrodents. Following single oral administration of naldemedine at 0.3 to 3 mg/kg to rats, oral bioavailability ranged from 25 to 32% and the elimination half-life ranged from 1.7 to 1.9 h. In dogs, bioavailability ranged from 49 to 62% and the elimination half-life ranged from 1.7 to 3.1 h. There were no substantial differences in serum protein binding across species, with values ranging from 88.9 to 94.2%. In tissue distribution studies, drug-derived radioactivity was widely distributed in rats, although no radioactivity was detected in the brain. Following single oral administration of naldemedine to rats with morphine-induced analgesia at 5 to 30 mg/kg, concentrations of the parent compound and metabolites in the brain were lower than in plasma; and, while concentrations decreased substantially within 24 h after dosing, disappearance of naldemedine from the brain was slower than that from plasma. Administration of radiolabelled naldemedine to pregnant and nursing rats demonstrated a low level of placental transfer and excretion into the milk, respectively. In male pigmented rats, radioactivity was distributed to pigmented tissue suggesting an affinity for melanin. In an in vitro study using human hepatocytes, naldemedine 3-O-β-D- glucuronide (naldemedine 3-G), naldemedine 6-O-β-D-glucuronide (naldemedine 6-G), and nor-naldemedine were identified as the main metabolites, and each of these metabolites were detected in rat and dog plasma. Metabolism to naldemedine 3-G and naldemedine 6-G appear to be mediated by UGT1A3, and nor-naldemedine appears to be mediated by CYP3A4. Naldemedine-(7R)-7-hydroxide is formed by oxidation of the parent compound, and naldemedine-carboxylic acid and benzamidine appear to be produced by enterobacteria. After single oral administration of [oxadiazole-^{14}C]-S-297995 to rats and dogs, excretion of radioactivity in urine until 168 hours after dosing was approximately 49% and 26%, respectively. The sum of urinary and biliary excretion until 48 hours was approximately 73% and 86% in bile duct-cannulated rats and dogs, respectively. After single oral administration of [carbonyl-^{14}C]-S-297995 in rats and dogs, excretion of the radioactivity in urine until 168 h after dosing was 1.5% and 5.2%, respectively. The sum of urinary and biliary excretion until 48 hours after dosing was approximately 34% and

67% in bile duct-cannulated rats and dogs, respectively, with excretion occurring mainly in the feces via bile. Because benzamidine was a major metabolite in urine after oral dosing with [oxadiazole-^{14}C]-S-297995 in rats and dogs, higher levels of radioactivity in urine observed with [oxadiazole-^{14}C]-S-297995 were attributed to urinary excretion of benzamidine (a metabolite which is not traced by the radiolabel of carbonyl-^{14}C-S-297995). As mentioned above, naldemedine was demonstrated to be a P-gp substrate.

Repeat-dose toxicity studies with naldemedine were conducted in multiple species, with durations of up to 13-weeks in mice, 6-months in rats, and 9-months in dogs. Liver was the primary target organ in mice and dogs, and there were effects on the estrous cycle and body weight suppression in rats. Briefly, in a 13-week study in mice, the NOAEL was 100 mg/kg/day. The primary target organ was the liver (histopathological findings included centrilobular hepatocyte hypertrophy and periportal vacuolation). Reduced/absence corpora lutea in the ovaries was observed at ≥300 mg/kg/day, and oestrus and metoestrus morphological characteristics were observed in the vagina. In a 6-month toxicity study in rats, the NOAEL was 100 mg/kg/day based on suppression of body weight gain at 1000 mg/kg/day. In 3- and 9-month repeat-dose toxicity studies in dogs, the liver was the target organ, with the NOAEL in the 9-month study identified as 4 mg/kg/day based on histopathological changes (single cell necrosis and increases in Kupffer cells with pigment deposition) and corresponding clinical chemistry changes (e.g., increased ALT, ALP, GGT, cholesterol, and phospholipids). In the 3-month toxicity study in dogs, adipose tissue atrophy was observed with a NOAEL of 5 mg/kg/day. Overall, the NOAEL from the 6-month toxicity study in rats (100 mg/kg/day) produced systemic exposures approximately 3630 times that in humans at the recommended human dose (based on AUC)[2]. The NOAEL from the 9-month toxicity study in dogs (4 mg/kg/day) produced systemic exposures approximately 345 times that in humans at the recommended dose (based on AUC).

In addition to findings noted above in the 6-month toxicity study in rats, abnormal estrous cycles (prolongation of diestrous) were observed in 1-month rat toxicity studies. While a NOEL for this finding was not identified in a 1-month toxicity study with doses ranging from 30 to 1000 mg/kg/day, the increased incidence of abnormal estrous cycle was considered to be a non-adverse effect based on the lack of histopathological changes in the ovaries, uterus, and mammary gland, and since there was recovery of the estrous cycle generally even during the dosing period. In a supplemental 1-month toxicity study with 0.3 to 10 mg/kg/day naldemedine, a NOEL was also not identified for this finding. However, the overall incidences were low and did not show a dose-response, and the changes generally recovered even during the dosing period. In studies conducted to investigate reproductive hormone levels, treatment-related increases in prolactin, progesterone, and FSH levels were observed, and it was considered that irregular estrous cycle findings may be attributable to treatment-related increases in prolactin levels.

[2] The human AUC value of naldemedine at the intended human dose used for exposure ratio comparison was 0.01694 µg.h/mL.

Naldemedine was negative in a bacterial reverse mutation test, a chromosomal aberration test using cultured Chinese hamster lung cells, and a rat micronucleus assay.

The carcinogenic potential of naldemedine was assessed in oral carcinogenicity studies of up to 2 years in duration in rats and mice. In these studies, there were no drug-related neoplasms when the test compound was administered to mice and rats at doses up to 100 mg/kg/day (a dose estimated to produce systemic exposures approximately 17,500 times and 6,300 times the human AUC at the recommended dose, respectively).

In an oral fertility and early embryonic development study, male and female rats were administered naldemedine at 1, 10, 100, and 1000 mg/kg/day. The NOAEL for fertility and reproductive performance was 1000 mg/kg/day (a dose resulting in systemic exposures approximately 16,900 times the human AUC at the recommended dose).[3] In females, irregular estrous cycles (prolongation of diestrous phase) and low number of estrous cases occurred at ≥10 mg/kg/day (approximately 179 times the human AUC at the recommended dose). However, the irregular estrous cycles recovered during the pre-mating or mating periods, and there was successful copulation with males except for a single female at 1000 mg/kg/day. The NOAELs for male and female systemic toxicity in this study were 1 and 100 mg/kg/day, respectively.

In an embryofetal development study in rats with naldemedine, there were no effects on embryofetal development at doses up to 1000 mg/kg/day (the highest dose tested; a dose resulting in systemic exposure approximately 23,000 times the human AUC at the recommended human dose). The NOAEL for maternal toxicity in this study was <10 mg/kg/day based on reduced body weight and food consumption. In an embryofetal development study in rabbits with naldemedine, the NOAEL for maternal toxicity was <25 mg/kg/day and the NOAEL for embryofetal development was 100 mg/kg/day (a dose resulting in systemic exposure approximately 226 times the human AUC at the recommended human dose). At 400 mg/kg/day (approximately 844 times the human AUC at the recommended dose), effects in maternal animals included body weight loss / decreased body weight gain and food consumption, fetal loss, and premature delivery. Decreased fetal body weights at this dose may be related to the maternal toxicity observed.

In a pre- and postnatal development study, pregnant rats were administered naldemedine at oral doses of 1, 30, and 1000 mg/kg/day. The NOAEL for maternal and developmental toxicity was 1 mg/kg/day (a dose resulting in systemic exposure approximately 12 times the human AUC at the recommended human dose).[4] At 1000

[3] Exposure comparisons for doses in the fertility and early embryonic development study (relative to the human AUC value) were based on AUC values from a one-month repeat dose toxicity study in rats (Report No. R-297995-TB-048-L) and a supplemental one-month repeat dose toxicity in rats (Report No. R-297995-TB-091-L).

[4] Exposure comparisons for doses in the prenatal and postnatal development study in rats (relative to the human AUC value) were based on AUC values from a one-month repeat dose toxicity study in rats. (Report No. R-297995-TB-048-L) and a supplemental one-month repeat dose toxicity in rats (Report No.

mg/kg/day (approximately 16,900 times the human AUC at the recommended human dose), mortality occurred in a single maternal animal. At dose of 30 mg/kg/day (approximately 626 times the human AUC at the recommended human dose) and greater, there were effects on maternal body weight and food consumption, scattering of offspring in the cages (suggestive of poor nursing), and total litter loss. In F1 pups, there were decreases in the viability index on lactation day 4 at ≥30 mg/kg/day, and decreased body weights and delayed pinna unfolding at 1000 mg/kg/day.

Nor-naldemedine was identified by the Applicant as a major metabolite in humans based on exposure to this metabolite in plasma being greater than 10% of naldemedine exposure. In a human mass balance study, nor-naldemedine accounted for 9 to 13% of total systemic exposure of naldemedine in plasma after administration of a single 2 mg dose of [carbonyl-^{14}C]-naldemedine or [oxadiazole-^{14}C]-naldemedine. The Applicant also reported that this metabolite accounted for 6.66% to 9.28% of total radioactivity exposure using both labelled compounds. Furthermore, the Applicant stated that the AUC ratio of nor-naldemedine to naldemedine was 18.1 to 26.1% in a phase 1 study with healthy subjects and doses ranging from 3 to 30 mg. In nonclinical species, exposure to nor-naldemedine was demonstrated through toxicokinetic evaluation in a subset of the repeat-dose toxicity studies (1 month toxicity studies in rats and dogs), the embryonic fetal development study in rabbits, and the rat and mouse carcinogenicity studies. Because systemic exposures to nor-naldemedine were not evaluated in the chronic 6- and 9-month rat and dog studies, exposures to this metabolite at the NOAEL (or a dose similar to the NOAEL) were evaluate using TK data from the 1 month toxicity studies in these species. In the 1 month toxicity study in rats, systemic exposures to nor-naldemedine at the NOAEL from the 6-month toxicity study (100 mg/kg/day) were approximately ≥232 times the human AUC for this metabolite[5] at the recommended human dose. In the 1 month toxicity study in dogs, systemic exposures to nor-naldemedine at 3 mg/kg/day (a dose slightly lower than the NOAEL in the 9 month study (4 mg/kg/day)) were approximately ≥33 times the human AUC for this metabolite. In the rat and mouse carcinogenicity studies, systemic exposures to nor-naldemedine at the highest dose tested (100 mg/kg/day) were estimated to be approximately 1200 times and 10500 times, respectively, the human AUC for this metabolite at the recommended dose. Finally, in the embryonic fetal development study in rabbits, systemic exposure to nor-naldemedine at the NOAEL for embryonic development (100 mg/kg/day) was approximately 146 times the human AUC for this metabolite at the recommended dose. Thus, based on exposure to this metabolite demonstrated in nonclinical species, no significant safety concerns were identified for nor-naldemedine at the proposed human dose of the drug.

With respect to impurity safety evaluation, in vitro bacterial reverse mutation assays were conducted for numerous potential drug substance impurities. In these tests, a single impurity (b) (4) was positive and will be controlled as a specified impurity (b) (4)

R-297995-TB-091-L).

[5] The human AUC value of nor-naldemedine at the intended human dose used for exposure ratio comparison was 2.457 ng.h/mL.

(b) (4). Another impurity, (b) (4) was predicted to be positive for mutagenicity by in silico screening and will be controlled through a specification for (b) (4) of no more than (b) (4) Three tosylate impurities (b) (4) will be controlled by the drug substance specification of no more than (b) (4) % for unspecified impurities, and (b) (4) will be controlled based on establishment of a critical process parameter as well as historical batch data and spiking and purging data. The proposed limit of no more than (b) (4) % corresponds to an intake (b) (4) µg/day based on the recommended naldemedine monotosylate dose of 0.26 mg/day (equivalent to 0.2 mg/day naldemedine free base), and therefore is acceptable according to ICH M7.

Overall, the Applicant has submitted adequate nonclinical studies of naldemedine monotosylate in support of the NDA. Thus, no nonclinical approvability issues have been identified. Recommended edits to the Applicant's proposed labeling are presented under section 1.3.3 (Labeling).

12 Appendix/Attachments

Attachment 1: ECAC meeting minutes dated November 17, 2011

Executive CAC
Date of Meeting: November 15, 2011

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Dan Mellon, Ph.D., DAAAP, Alternate Member
Sushanta Chakder, Ph.D., DGIEP, Supervisor
Charles Wu, Ph.D., DGIEP Presenting Reviewer

Author of Minutes: Charles Wu, 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 #: 107,475
Submit date: October 11, 2011
Drug Name: S-297995
Sponsor: Shionogi Inc.

Background
S-297995, an opioid receptor antagonist, is being developed for the treatment of gastrointestinal disorders in opioid-treated patients. The anticipated therapeutic dose of S-297995 is ≤ 10 mg/day. The sponsor submitted the rationale for the dose selection for proposed 2-year oral carcinogenicity studies in SD rats and CD-1 mice.

Rat Carcinogenicity Study Protocol and Dose Selection
The sponsor proposed to conduct a 2-year oral (gavage, 10 mL/kg) carcinogenicity study of S-297995 at 0 (vehicle), 10, 30, and 100 mg/kg/day in Sprague-Dawley rats (n = 65/sex/dose). S-297995 will be administered by oral gavage once daily, in 0.5% (w/v) methylcellulose aqueous solution. A complete necropsy will be conducted on all animals and a standard battery of tissues/organs will be processed for histopathological examinations with a peer review.

In a 6-month oral repeat dose toxicity study with S-297995, SD rats received daily oral dosing at 10, 100 and 1000 mg/kg/day. The plasma exposure levels [AUC0-24h] at 100 mg/kg/day were 67.9 μg·hr/mL for males and 61.5 μg·hr/mL for females. The plasma exposure level in humans following administration of the 10 mg dose (anticipated clinical dose) was 1.24 μg·hr/mL. Thus, the estimated AUC multiples for rats to humans are 55-fold for males and 50-fold for females.

Based on the AUC ratio, the 100 mg/kg/day dose was proposed as the high dose for the 2-year carcinogenicity study in rats. The proposed low and mid doses are 10 and 30 mg/kg/day, respectively.

Mouse Carcinogenicity Study Protocol and Dose Selection
The sponsor proposed to conduct a 2-year oral (gavage, 10 mL/kg) carcinogenicity study of S-297995 at 0 (vehicle), 10, 30, and 100 mg/kg/day in CD-1 mice (n = 65/sex/dose). S-297995 will be administered by oral gavage once daily, in 0.5% (w/v) methylcellulose aqueous solution. A complete necropsy will be conducted on all animals and a standard battery of tissues/organs will be processed for histopathological examinations with a peer review.

In a 3-month oral repeat dose toxicity study with S-297995, CD-1 mice received daily oral dosing at 100, 300 and 1000 mg/kg/day. The plasma exposures [AUC0-24h] at 100 mg/kg/day were 194 µg·hr/mL for males and 165 µg·hr/mL for females. The plasma exposure in humans following administration of the 10 mg dose (anticipated clinical dose) was 1.24 µg·hr/mL. Thus, the estimated AUC multiples for mice to humans are 156-fold for males and 133-fold for females. Based on the AUC ratio, the 100 mg/kg/day dose was proposed as the high dose for the 2-year carcinogenicity study in mice. The proposed low and mid doses are 10 and 30 mg/kg/day, respectively.

Executive CAC Recommendations and Conclusions:

Rat:

The Committee concurred with the sponsor's proposed doses of 0, 10, 30, and 100 mg/kg/day, by oral gavage, based on the high dose having an AUC value > 25 times that in humans.

Mouse:

The Committee concurred with the sponsor's proposed doses of 0, 10, 30, and 100 mg/kg/day, by oral gavage, based the high dose having an AUC value > 25 times that in humans.

For both studies, the Committee notes that if the clinical dose changes such that the AUC ratio is no longer at least 25-fold, then the study may not be acceptable.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\ /Division File, DGIEP
/S. Chakder, Ph.D., Supervisor, DGIEP
/C. Wu, Ph.D., Primary Reviewer, DGIEP
/ M. Scherer, Project Manager, DGIEP
/A. Seifried, OND IO

Reference ID: 3046179

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

/s/

DAVID JACOBSON KRAM
11/17/2011

Attachment 2: ECAC meeting minutes dated July 21, 2016

Executive CAC
Date of Meeting: July 19, 2016

Committee: Karen Davis Bruno, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Tim McGovern, Ph.D., OND IO, Member
Mukesh Summan, Ph.D., DBRUP, Alternate Member
Sushanta Chakder, Ph.D., DGIEP, Pharm Tox Supervisor
Tracy Behrsing, Ph.D., DGIEP, Presenting Reviewer

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA #: 208854
Drug Name: Naldemedine tosylate; S-297995 monotosylate
Sponsor: Shionogi, Inc.

Background:
S-297995 monotosylate is an opioid antagonist under development for the treatment of opioid-induced constipation (OIC) in adults with chronic non-cancer pain.

Rat Carcinogenicity Study
In a 104-week carcinogenicity study in Crl:CD(SD) rats, animals were administered S-297995 monotosylate by oral gavage at doses of 10, 30, or 100 mg/kg/day in 0.5% w/v methylcellulose aqueous solution. The control animals received 0.5% w/v methylcellulose aqueous solution by oral gavage. Dose selection was based on the high dose having an AUC value > 25 times that in humans, and these doses received prior concurrence from the ECAC. In this study, all surviving females from all dose groups were terminated early (from Week 103) when the number of surviving females in the control group reached 15 animals. This differs from the usual execCAC recommendation of terminating a sex when control reaches 20. There were no drug-related neoplastic findings in male or female rats.

Mouse Carcinogenicity Study
In a 104-week carcinogenicity study in Crl:CD1(ICR) mice, S-297995 monotosylate was administered by oral gavage. Animals were treated with doses of 10, 30, or 100 mg/kg/day in 0.5 % w/v methylcellulose aqueous solution. The control animals received 0.5% w/v methylcellulose aqueous solution by oral gavage. Dose selection was based on the high dose having an AUC value >25 times that in humans, and the doses received prior concurrence from the ECAC. There were no drug-related neoplastic findings in male or female mice.

Executive CAC Recommendations and Conclusions:

Rat:

- The Committee concurred that the study was adequate, noting prior agreement with the protocol.
- The Committee concluded that there were no drug related neoplasms for both males and females.

Mouse:

- The Committee concurred that the study was adequate, noting prior agreement with the protocol.
- The Committee concluded that there were no drug related neoplasms for both males and females.

Karen Davis Bruno, Ph.D.
Chair, Executive CAC

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