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APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	205435 and 205436
Supporting document/s:	SD #1
Applicant's letter date:	10/18/2013
CDER stamp date:	10/21/2013
Product:	Tedizolid (TR-701 FA)
Indication:	Acute Bacterial Skin and Skin Structure Infections (ABSSSI)
Applicant:	Trius Therapeutics
Review Division:	Division of Anti-infective Products
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TABLE OF CONTENTS

Table of Tables

1 Executive Summary

1.1 Introduction

TR-701 is a prodrug in the oxazolidinone antibiotic class of drugs which also includes the marketed antibiotic linezolid. The active metabolite of TR-701, TR-700 inhibits protein synthesis in bacteria and is active against clinically relevant gram-positive bacteria and some fastidious gram-negative bacteria. TR-701 was shown to be efficacious in several *in vivo* infection models. Under the conditions of the studies, TR-701 was 3-5 fold more potent than linezolid.

1.2 Brief Discussion of Nonclinical Findings

- Taken as a whole, the nonclinical toxicology data for TR-701 and TR-700 suggests relative safety for clinical administration of the clinical therapeutic dose of 200 mg/day TR-701 for up to 14 days. TR-701 was immunotoxic in animals studies at high doses suggesting immune cells should be monitored in patients. TR-701 has also shown a potential to produce toxicities associated with mitochondrial protein synthesis inhibition and MAO inhibition, as well as effects consistent with transient neural impairment, but at exposures much higher than that expected to occur at the clinical therapeutic dose. The weight of evidence suggests TR-701 and TR-700 are not genotoxic. While TR-701 should not impair male or female fertility at therapeutic doses, it caused maternal and fetal toxicity in rodent embryo-fetal studies suggesting it should be restricted for administration to pregnant women.
- In a fertility study, oral TR-701 had no adverse effects on the fertility or reproductive performance, including spermatogenesis, in male rats at doses associated with a TR-700 plasma AUC approximately 5-fold greater than the plasma AUC value in humans. TR-701 also had no adverse effects on the fertility or reproductive performance of adult female rats at a dose associated with a TR-700 plasma AUC exposure approximately 4-fold higher than that in humans at the oral therapeutic dose.
- In embryo-fetal studies, TR-701 was shown to produce fetal developmental toxicities in mice, rats, and rabbits. Fetal developmental effects occurring in mice in the absence of maternal toxicity included reduced fetal weights and an increased incidence of costal cartilage anomalies. In rats, decreased fetal weights and increased skeletal variations including reduced ossification of the sternabrae, vertebrae, and skull were observed at doses associated with maternal toxicity (reduced maternal body weights and mortality). In rabbits, reduced fetal weights but no malformations or variations were observed at doses associated with reduced maternal body weights and abortions. The no observed adverse effect levels (NOAELs) for fetal toxicity in mice (5 mg/kg/day), and maternal and fetal toxicity in rats (2.5 mg/kg/day), and rabbits (1 mg/kg/day) were associated with TR-700 plasma AUC values approximately equivalent to (mice and rats) or 0.04 fold (rabbit) the AUC value associated with the oral human therapeutic dose.

- In a pre-postnatal study in rats, there were no adverse maternal or offspring effects when female rats were treated during pregnancy and lactation with the highest tested dose of 3.75 mg/kg/day TR-701 which was associated with plasma TR-700 exposures approximately equivalent to the human plasma AUC exposure.
- TR-701 was negative for genotoxicity in all *in vitro* assays (bacterial reverse mutation (Ames), Chinese hamster lung (CHL) cell chromosomal aberration) and in all *in vivo* tests (mouse bone marrow micronucleus, rat liver unscheduled DNA synthesis). TR-700 was positive in an *in vitro* CHL cell chromosomal aberration assay, but negative for genotoxicity in other *in vitro* assays (Ames, mouse lymphoma mutagenicity) and *in vivo* in a mouse bone-marrow micronucleus assay. The weight of evidence suggests TR-701 and TR-700 have limited potential to be genotoxic in humans.
- In safety pharmacology studies (neural, cardiovascular, respiratory, renal and GI) limited TR-701-related effects occurred only at high doses. Hexobarbital-induced sleep time was significantly increased with an oral dose of 100 mg/kg TR-701. Spontaneous locomotor activity in mice was significantly reduced with oral administration of 30 and 100 mg/kg TR-701. A high-oral dose of 100 mg/kg TR-701 significantly increased urinary sodium and chloride concentrations, and mean gastric volume was significantly reduced by 39% and mean total gastric acidity was reduced 48% (not statistically significant) while gastric pH remained unchanged.
- TR-701 produced no significant effects in cardiovascular (hERG, isolated rat heart, and ECG in dog) and respiratory safety pharmacology studies.
- Serotonin syndrome and monoamine oxidase (MAO) inhibition have been reported for linezolid. *In vitro* studies with TR-701 and TR-700 indicated that TR-700 was a weak inhibitor of MAO-A and MAO-B with IC_{50} values comparable to linezolid. However, in a mouse head-twitch experiment and a tyramine-challenge experiment in rats, linezolid doses comparable to the human therapeutic dose produced positive results (increased head twitch or increased mean arterial pressure), but TR-701 doses associated with plasma TR-700 C_{max} and AUC values greatly exceeding the clinical equivalents did not.
- Oral and IV TR-701 was rapidly and extensively metabolized to the active metabolite, TR-700. The bioavailability of TR-700 after oral administration of TR-701 was > 60% in mice, rats, and dogs. The plasma $t_{1/2}$ of TR-700 ranged from 2-8 hours in rodents and was generally < 1 hour in dogs. TR-700 plasma exposure following oral dosing was similar between genders for mice and dogs, but female exposure was roughly twice as high as male exposure in rats necessitating different dose ranges in toxicology studies. In rats, mice and dogs, TR-700 accumulated in plasma only moderately (generally < 3 fold) with repeated-dosing for as long as 3 months.
- Distribution studies with [^{14}C]-TR-701 with both IV and oral administration in rats and dogs indicated that radioactivity distributed to many tissues with the lowest concentrations in eye vitreous and brain. Protein binding of TR-701 and TR-700 were high ranging from 75-98% in plasma from mice, rats, dogs, and humans.

- Metabolite profiles for rats, dogs, and humans were similar. The primary metabolite for all three species was TR-700 (tedizolid). All of the plasma metabolites in humans were represented in both rats and dogs. TR-701 was not metabolized by CYP-450 enzymes and did not strongly inhibit or induce CYP-450 isozyme activity.
- The primary route of excretion was in feces (80% in rats, 90% in dogs) with less excretion in urine (20% in rats, 10% in dogs).
- The nonclinical toxicity of TR-701 was investigated in rats and dogs in 2-week, 1-month and/or 3-month studies by both the intravenous and oral routes. The major toxicities were hematopoietic (more pronounced in the rat and including decreased RBC, WBC, platelets and bone marrow hypocellularity), gastrointestinal, and injection site reactions (dog only). The systemic toxicities were dose and duration dependent, reversible, and occurred at TR-700 plasma exposures between 4- and 10-times higher than those seen in humans.
- In a 1-month rat immunotoxicity study, oral TR-701 was shown to significantly reduce cell counts, splenic T and B cells, IgG titer and IgG-mediated plaque formation at TR-700 plasma exposures 4-8 times the human exposure.
- At longer durations and higher doses in the rat, toxicities to the liver (increased liver enzymes and hepatocellular centrilobular degeneration and atrophy), renal tubular degeneration, and reproductive organ degeneration and atrophy in both males and females were observed.
- Inhibition of mitochondrial protein synthesis could account for the observed hematopoietic effects. In *in vitro* experiments using mitochondria isolated from rat heart, TR-700 was 20-25 fold more potent than linezolid in inhibiting mitochondrial protein synthesis. However, in another experiment, TR-700 did not distribute into mitochondrial subcellular compartments in isolated macrophages concentrating instead more in phagolysosomes and cytosolic fractions.
- The potential for peripheral and optic neuropathy previously associated with nonclinical administration and prolonged clinical administration of linezolid, was evaluated in a 9-month neurotoxicity study for oral TR-701 administered daily to pigmented rats. The results of this study indicated that TR-701 doses corresponding to plasma TR-700 exposures approximately 7-8 times the clinical plasma exposure did not change functional observational battery reactions or locomotor activity or produce peripheral nerve or ocular histopathology in rats. Clinical signs consistent with transitory neurotoxicity in the 1-month IV rat toxicology study occurred only at C_{max} and AUC exposures in excess of 17 times that expected with clinical administration.

1.3 Recommendations

1.3.1 Approvability

Yes

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Applicant's Suggested Labelling (Section 8)

8.1 Pregnancy
Pregnancy Category C

[redacted] (b) (4)

There are no adequate and well-controlled studies of TRADENAME in pregnant women. TRADENAME should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Reviewer's Suggested Labeling (Section 8)

8.1 Pregnancy
Pregnancy Category C

There are no adequate and well-controlled studies of SIVEXTRO in pregnant women. SIVEXTRO should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

In embryo-fetal studies, tedizolid phosphate was shown to produce fetal developmental toxicities in mice, rats, and rabbits. Fetal developmental effects occurring in mice in the absence of maternal toxicity included reduced fetal weights and an increased incidence of costal cartilage anomalies. In rats, decreased fetal weights and increased skeletal variations including reduced ossification of the sternabrae, vertebrae, and skull were observed [redacted] (b) (4) In rabbits, reduced fetal weights but no malformations or variations were observed at doses associated with [redacted] (b) (4) The no observed adverse effect levels (NOAELs) for fetal toxicity in mice (5 mg/kg/day), and maternal and fetal toxicity in rats (2.5 mg/kg/day), and rabbits (1 mg/kg/day) were associated with tedizolid plasma under the curve (AUC) values approximately equivalent to (mice and rats) or 0.04 fold (rabbit) the tedizolid AUC value associated with the oral human therapeutic dose.

In a pre-postnatal study, there were no adverse maternal or offspring effects when female rats were treated during pregnancy and lactation with tedizolid phosphate at the

highest tested dose of 3.75 mg/kg/day, with plasma tedizolid exposures approximately equivalent to the human plasma AUC exposure at a clinical dose of 200 mg/day.

Sponsor's Suggested Labelling (Section 13)

13 Nonclinical Toxicology

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term carcinogenicity studies have not been conducted with tedizolid phosphate.

Tedizolid phosphate was negative for genotoxicity in all *in vitro* assays (bacterial reverse mutation (Ames), Chinese hamster lung (CHL) cell chromosomal aberration) and in all *in vivo* tests (mouse bone marrow micronucleus, rat liver unscheduled DNA synthesis). Tedizolid, (b) (4) generated from tedizolid phosphate after metabolic activation (*in vitro* and *in vivo*), was also tested for genotoxicity (b) (4)

In a fertility study, oral tedizolid phosphate had no adverse effects on the fertility or reproductive performance, including spermatogenesis, (b) (4) male rats at (b) (4) the maximum tested (50 mg/kg/day) with a plasma tedizolid AUC value approximately 5-fold greater than the plasma AUC value in humans at the oral therapeutic dose. Tedizolid

phosphate also had no adverse effects on the fertility or reproductive performance of adult female rats at dosages up to the maximum tested (15 mg/kg/day). Plasma tedizolid exposure (AUCs) at this NOAEL in female rats was approximately 4-fold higher than that in humans at the oral therapeutic dose.

Reviewer's Suggested Labelling (Section 8)

13 Nonclinical Toxicology

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

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In a fertility study, oral tedizolid phosphate had no adverse effects on the fertility or reproductive performance, including spermatogenesis, (b) (4) male rats at (b) (4) the maximum tested (50 mg/kg/day) (b) (4) with a tedizolid plasma AUC approximately 5-fold greater than the plasma AUC value in humans at the oral therapeutic dose. Tedizolid phosphate also had no adverse effects on the fertility or reproductive performance of adult female rats at dosages up to the maximum tested (15 mg/kg/day). Plasma tedizolid exposure (AUCs) at this NOAEL in female rats was approximately 4-fold higher than that in humans at the oral therapeutic dose.

13.2 Animal Toxicity and/or Pharmacology

Repeated-oral dosing of tedizolid phosphate in rats produced dose- and time-dependent (b) (4) bone marrow hypocellularity (myeloid, erythroid, and megakaryocytes), (b) (4) These effects showed evidence of reversibility and occurred at plasma tedizolid exposure levels (AUC) ≥ 6-fold greater than the plasma exposure associated with the human therapeutic dose. In a 1-month immunotoxicology study in rats, repeated-oral dosing of tedizolid phosphate was shown to significantly reduce splenic B cells and T cells and reduce plasma IgG titers. These effects occurred at plasma tedizolid exposure levels (AUC) ≥ 3 fold greater than the expected human plasma exposure associated with the therapeutic dose.

(b) (4)

(b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number: 856867-55-5

Generic Name: Tedizolid phosphate

Code Name: TR-701 free acid (FA)

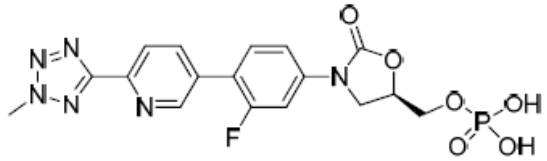
Chemical Name

IUPAC: [(5*R*)-(3-{3-Fluoro-4-[6-(2-methyl-2*H*-tetrazol- 5-yl)pyridin-3-yl]phenyl}-2-oxooxazolidin- 5-yl]methyl hydrogen phosphate.

CAS: 2-Oxazolidinone, 3-[3-fluoro-4-[6-(2-methyl- 2*H*-tetrazol-5-yl)-3-pyridinyl]phenyl]- 5-[(phosphonooxy)methyl]-, (5*R*)-

Molecular Formula/Molecular Weight: $C_{17}H_{16}FN_6O_6P$/450.32 g/mole

Structure or Biochemical Description



Pharmacologic Class

Antibacterial

2.2 Relevant INDs, NDAs, BLAs and DMFs

INDs 77872 and 106307

2.3 Drug Formulation

The drug substance formulations for the oral tablet and the intravenous product are shown in Table 3 and Table 4 respectively.

Table 3: Formulation for TR-701 FA Tablet (NDA 205435). (Sponsor's Table)

Ingredient	Quality Standard	Function	200 mg Tablet	
			Weight (mg/unit)	% (w/w)
Tedizolid Phosphate[a]	In-house	Active Ingredient	200	(b) (4)
Microcrystalline Cellulose	NF			(b) (4)
Mannitol[b]	USP			
Povidone	USP			
Crospovidone	NF			
(b) (4)				
Magnesium Stearate[d]	NF			
(b) (4)				
Total Coated Tablet Weight				(b) (4)

Table 4: Formulation for TR-701 Phosphate for Injection (NDA 205436). (Sponsor's Table)

Component	Quality Standard	Function	Unit Formula
Tedizolid Phosphate	In house	Active Ingredient	(b) (4)
Mannitol	USP	(b) (4)	105 mg
Sodium Hydroxide	USP		(b) (4)
Hydrochloric Acid	NF		
(b) (4)	NF		
Water for Injection[b]	USP		

Abbreviations: NF=National Formulary; qs=quantity sufficient; USP=United States Pharmacopeia

(b) (4)

[b]Water for Injection is essentially removed during lyophilization.

2.4 Comments on Novel Excipients

In the oral tablet, the only excipient that has not been previously used in an approved oral product (b) (4) shown in Table 3 (b) (4) However, all of the listed components of (b) (4) polyvinyl alcohol, titanium dioxide, polyethylene glycols, and talc have been used (b) (4) in previously approved products. The final component, yellow iron oxide, has been used in many approved products.

In the intravenous product, 105 mg of mannitol is included (b) (4) This amount is qualified by the use of mannitol in many approved intravenous products (b) (4) according to the FDA Inactive Ingredients Search for Approved Products database.

2.5 Comments on Impurities/Degradants of Concern

The drug substance specifications for TR-701 FA are shown in Table 5.

Table 5: Tedizolid Phosphate Drug Substance Specification. (Sponsor's Table)

Test	Acceptance Criteria	Analytical Procedure
Appearance	White to yellow solid	Visual
(b) (4)	(b) (4)	IR (USP <197>)
		HPLC (AP-010)
Assay		HPLC (AP-010)
(b) (4)	(b) (4)	HPLC (AP-010)
	NMT	
	NMT	
	NMT	
	NMT	
	NMT	
	NMT	
	NMT	
	NMT	
	NMT	
	NMT	UPLC-MS (AP-028)
Residual Solvents		
(b) (4)	NMT	GC (AP-011)
	NMT	
	NMT	
	NMT	GC (AP-016)
	NMT	GC (AP-011 and AP-016)
	NMT	GC (AP-029)
	NMT	GC (AP-029)

Table 5 cont.

Test	Acceptance Criteria	Analytical Procedure
Heavy Metals		
(b) (4)	NMT (b) (4)	ICP-OES (AP-012)
	NMT	
	NMT	
	NMT	
	NMT	GF-AAS (AP-013)
Water Content	NMT	Karl Fischer (USP <921> Method Ic)
(b) (4)		
Particle Size (b) (4)	(b) (4) NMT	Laser Diffraction (AP-015 and USP <429>)
Residue on Ignition	NMT	USP <281>
Bacterial Endotoxins	NMT	USP <85>
Microbial Limits Total aerobic microbial count Total yeast and molds count	 NMT NMT	USP <61>

Abbreviations: CFU=colony forming units; EU=Endotoxin Units; GC=Gas Chromatography; GF-AAS=Graphite Furnace-Atomic Absorption Spectroscopy; HPLC=high performance liquid chromatography; ICP-OES=Inductively Coupled Plasma–Optical Emission Spectroscopy; NMT= not more than; UPLC-MS=Ultra Performance Liquid Chromatography-Mass Spectrometry; (b) (4) (b) (4)

Organic Impurities

The qualification data supporting the acceptance criteria for the different TR-701 FA impurities are summarized below.

(b) (4) These two impurities were isolated and administered to rats at an intravenous dose of 0.25 mg/kg/day in a 2-week toxicology study (TOX-13-0701-075; reviewed in Section 10). (b) (4) Thus both (b) (4) impurities were qualified (b) (4) This impurity appears as a (b) (4) impurity in the tedizolid disodium phosphate batch CMLW-304/07-TR3 which was administered intravenously in doses of 10, 30, and 90 mg/kg/day to male rats and 5, 15, and 45 mg/kg/day to female rats for 28 days in Study No.: TOX-08-0701-009 (reviewed in Section 6.2). The tedizolid disodium phosphate NOAEL was 30 mg/kg/day in males and 15 mg/kg/day in females. (b) (4)

(b) (4) For females the qualified percentage is (b) (4) the (b) (4) acceptance level proposed by the Sponsor. Consequently, the Sponsor has been requested (b) (4) the acceptance level (b) (4)

(b) (4) This impurity was included as a (b) (4) impurity in the tedizolid disodium phosphate batch OXA-005 which was administered in oral doses of 100, 200, and 400 mg/kg/day to male and female Beagle dogs for 4 weeks in Study No.: TOX-07-0701-013A (reviewed in Section 6.2). The tedizolid disodium phosphate NOAEL was 400 mg/kg/day in males and females. (b) (4)

(b) (4) This impurity was included as a (b) (4) impurity in tedizolid disodium phosphate batch OXA-005 in the same 4-week dog study (Study No.: TOX-07-0701-013A) discussed above (b) (4)

(b) (4) This impurity was purified (90.5% pure by weight) and administered to rats intravenously in doses of 0.5, and 2 mg/kg/day for 14 days in Study No.: TOX-10-0701-001 (reviewed in Section 10). (b) (4)

Genotoxic Impurities

An extensive analysis to determine potential genotoxic impurities was performed. (b) (4) The Sponsor has indicated that all three impurities will be tested in each batch of the drug substance with the acceptance criteria shown in Table 6. The acceptance criteria (b) (4) are based on criteria described in the draft FDA Guidance for Industry: "Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended

Approaches." According to this guidance, the acceptable qualification threshold for supporting a marketing application is 1.5 µg per person per day for each genotoxic impurity. (b) (4)

The (b) (4) acceptance criteria (b) (4) is based on the recommended levels in the ICH Q3C Guidance; "Impurities: Residual Solvents – Tables and List."

Table 6: Acceptance Criteria (b) (4) in the Tedizolid Phosphate Drug Substance Specification. (Sponsor's Table)

Impurity	Proposed Tedizolid Phosphate Drug Substance Acceptance Criteria	Justification
(b) (4)	NMT (b) (4)	Based on the dose (200 mg) and a Threshold of Toxicological Concern (TTC) value of 1.5 µg/day intake
	NMT	ICH Q3C (R5) Impurities: Guideline for Residual Solvents
	NMT	Based on the dose (200 mg) and a Threshold of Toxicological Concern (TTC) value of 1.5 µg/day intake

Abbreviations: NMT=not more than

Residual Solvents

Three of the residual solvents, (b) (4) are (b) (4) in the ICH Q3C Tables and Lists for which the limits are not more than (NMT) (b) (4) The Sponsor's proposed acceptance criteria for the three solvents, NMT (b) (4) are all within the (b) (4) limit in the ICH Q3C Guidance.

One residual solvent, (b) (4) which is not listed in the ICH Q3C tables, has been detected at levels (b) (4) in 10 batches of tedizolid phosphate. The proposed acceptance criteria by the Sponsor (b) (4) is NMT (b) (4) This threshold (b) (4) would seem to be acceptable based on the results of a published rat toxicology studies described on the National Toxicology Program (US Department of Health and Human Services) website in which no toxicity was observed (b) (4)

Drug Product Degradation Impurities

For both the TR-701 FA Tablet and IV products, the degradation impurities shown below in Table 7 are the only drug product impurities other than those controlled in the drug substance. The acceptance criteria and qualification thresholds are summarized. The drug substance impurities are not monitored beyond in the drug product specification in accordance with the ICH Harmonized Tripartate Guideline Q6A Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances."

Table 7: Product Degradants Acceptance Criteria and Qualification

Product Degradant	Acceptance Criteria	Release Range	Qualified level
[redacted] (b) (4)			

[b] Guidance for Industry Q3B(R2): "Impurities in New Drug Products."

2.6 Proposed Clinical Population and Dosing Regimen

Proposed Clinical Population: Patients ≥ 18 years of age suffering from acute bacterial skin and skin structure infections (ABSSSI).
Proposed Dosing Regimen: The proposed clinical IV or oral dose of tedizolid phosphate (TR-701) is 200 mg administered once daily for 6 days. The IV infusion will take place over 1 hour. The pharmacokinetic parameters for the active metabolite, tedizolid (TR-700) associated with the 200 mg/day clinical dose of tedizolid phosphate are shown below in Table 8.

Table 8: Pharmacokinetic Parameters of Tedizolid following 200 mg QD Oral/IV Tedizolid Phosphate

Pharmacokinetic Parameters of Tedizolid	Oral		IV	
	Single Dose	Steady State	Single Dose	Steady State
C_{max} (µg/mL)	2.0 (0.7)	2.2 (0.6)	2.3 (0.6)	3.0 (0.7)
T_{max} (h)	2.5 (1.0 – 8.0)	3.5 (1.0 – 6.0)	1.1 (0.9 – 1.5)	1.2 (0.9 - 1.5)
AUC (µg·h/mL)	23.8 (6.8)	25.6 (8.4)	26.6 (5.2)	29.2 (6.2)
CL or CL/F (L/hr)	6.9 (1.7)	8.4 (2.1)	6.4 (1.2)	5.9 (1.4)

2.7 Regulatory Background

The oral TR-701 tablet was initially reviewed in IND 77872 and the IV formulation in IND 106307

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology
Safety Pharmacology

1. Effect of TR-701 on Blood Pressure, Heart Rate, and ECG in Dogs Using a Radiotelemetry System (Study No.: SPH-07-0701-017).
2. Effect of TR-701 on General Behavior in Rats (Study No.: SPH-07-0701-018).
3. Effects of TR-701 on Spontaneous Locomotor Activity in Mice (Study No.: SPH-07-0701-019).
4. Effect of TR-701 on Motor Coordination in Mice (Study No.: SPH-07-0701-020).
5. The Effect of TR-700 on hERG Tail Current (Study No.: SPH-07-0701-067).
6. Effect of TR-701 on Hexobarbital-induced Sleep Time in Mice (Study No.: SPH-07-0701-068).
7. Effect of TR-701 on Analgesia in Mice Exposed to Acetic Acid (Study No.: SPH-07-0701-069).
8. Effect of TR-701 on Analgesia in Mice Evaluated by the Hot Plate Test (Study No.: SPH-07-0701-070).
9. Effect of TR-701 on Pentylenetetrazole-Induced Convulsions in Mice (Study No.: SPH-07-0701-071).
10. Effect of TR-701 on Electric-Shock Induced Convulsions in Mice (Study No.: SPH-07-071-073).
11. Effect of TR-701 on Body Temperature in Mice (Study No.: SPH-07-0701-074).
12. Effect of TR-701 on Respiration Rate and Volume in Rats (Study No.: SPH-07-0701-075).
13. Effect of TR-701 on Gastrointestinal Transport in Mice (Study No.: SPH-07-0701-076).
14. Effect of TR-701 on Gastric Secretion in Rats (Study No.: SPH-07-0701-077).
15. Effect of TR-701 on Renal Function in Rats (Study No.: SPH-07-0701-078).
16. Assessing the Effects of TR-701 on the Autonomic Nervous System Using Isolated Guinea Pig Ileum (Study No.: SPH-07-0701-079).
17. Effect of TR-701 on Cardiac Function of Isolated Rat Heart (Study No.: SPH-07-0701-080).
18. Effect of TR-700 on Cardiac Function of Isolated Rat Heart (Study No.: SPH-07-0701-081).
19. Effect of TR-701 on hERG Channel Tail Currents (Study No.: SPH-07-0701-083).

Pharmacokinetics

Absorption

1. Pharmacokinetics of TR-701 and TR-700 Following Intravenous or Oral Administration to Mice (Study No.: PDM-07-0701-023).
2. Pharmacokinetics of TR-701 and TR-700 After Single Intravenous and Oral Administration of TR-701 to Beagle Dogs (Study No.: PDM-07-0701-025).
3. Pharmacokinetics of TR-701 and TR-700 Following Intravenous or Oral Administration in Rats (Study No.: PDM-07-0701-027).
4. A Single-dose Oral (Gavage) Pharmacokinetic Study of TR-701 FA in Long Evans Rats (Study No.: PDM-11-0701-021).

Distribution

1. TR-700 Levels in Plasma, Bone Marrow, and Liver Tissues of Mice Following Once-Daily Oral Administration of TR-701 for Three Days (Study No.: PDM-07-0701-026).

2. Tissue Distribution of TR-701 in Rats Upon Single Intravenous Dose Administration (Study No.: PDM-07-0701-029).
3. Tissue Distribution and Excretion Balance in Male and Female Beagle Dogs Following Intravenous and Oral Administration of [^{14}C]-TR-701 (Study No.: PDM-08-0701-020A).
4. Tissue Distribution Using Quantitative Whole Body Autoradiography and Excretion Balance in Male and Female Rats Following Intravenous and Oral Administration of [^{14}C]-TR-701 (Study No.: PDM-08-0701-021A).
5. Protein Binding of TR-700 in Heparinized Mouse, Rat, Dog, and Human Plasma (Study No.: PDM-13-070-080).
6. Protein Binding of TR-701 and TR-700 in EDTA-Treated Mouse, Rat, Dog, and Human Plasma (Study No.: PDM-13-070-081).
7. Study of the Cellular Uptake and Subcellular Distribution of the Oxazolidinone Tedizolid in Murine J774 Macrophages: Lack of Association with Mitochondria (Study No.: PDM-13-0701-082).

Metabolism

1. In Vitro Metabolism Stability of TR-701 and TR-700 (Study No.: PDM-0701-07-028).
2. Effect of Twice-Daily Oral Dosing of TR-701 for 14 Days on Rat Liver Microsomal Enzyme Activity (Study No.: PDM-07-0701-031).
3. Metabolic Stability of TR-701 and Formation of TR-700 Using a Rat Liver S9 Fraction (Study No.: PDM-09-0701-010).
4. Assessment of Systemic Exposure to TR-700 Enantiomers After Single-Dose Oral Administration of TR-701 FA to Sprague Dawley Rats (Study No.: PDM-13-0701-083).
5. Tissue Distribution and Excretion Balance in Male and Female Beagle Dogs Following Intravenous and Oral Administration of [^{14}C]-TR-701 (Study No.: PDM-08-0701-020A).
6. Tissue Distribution Using Quantitative Whole Body Autoradiography and Excretion Balance in Male and Female Rats Following Intravenous and Oral Administration of [^{14}C]-TR-701 (Study No.: PDM-08-0701-021A).

Excretion

1. Measuring Biliary Excretion of TR-700 After a Single Intravenous Administration of TR-701 to Rats (Study No.: PDM-07-0701-030).
2. Tissue Distribution and Excretion Balance in Male and Female Beagle Dogs Following Intravenous and Oral Administration of [^{14}C]-TR-701 (Study No.: PDM-08-0701-020A).
3. Tissue Distribution Using Quantitative Whole Body Autoradiography and Excretion Balance in Male and Female Rats Following Intravenous and Oral Administration of [^{14}C]-TR-701 (Study No.: PDM-08-0701-021A).

Toxicology

General Toxicity

Single-dose

1. Single Oral Dose Toxicity Study of TR-701 in Mice (Study No.: TOX-07-0701-007).
2. Single Oral Dose Toxicity Study of TR-701 in Rats (TOX-07-0701-008).

3. Single Intravenous Dose Toxicity Study of TR-701 in Mice (TOX-07-0701-009).
4. Single Intravenous Dose Toxicity Study of TR-701 in Rats (TOX-07-0701-010).

Oral Repeated-dose

1. 4-week Repeated Oral Dose Toxicity Study of DA-7218 in Rats (TOX-07-0701-014A).
2. A 3-month Oral (Gavage) Toxicity and Toxicokinetic Study of TR-701 FA in Sprague Dawley Rats with a 28-day Recovery Period (Study No.: TOX-11-0701-027).
3. A 1-, 3-, 6-, and 9-month Oral (Gavage) Neurotoxicity Study of TR-701 FA in Long Evans Rats with a 3-month Recovery Period (Study No.: TOX-11-0701-028).
4. Repeated Dose 4-week Oral Toxicity Study of DA-7218 in Beagle Dogs (Study No.: TOX-07-0701-013A).
5. A 3-month Oral (Gavage) Toxicity and Toxicokinetic Study of TR-701 FA in Beagle Dogs with a 28-day Recovery Period (Study No.: TOX-11-0701-026).

Intravenous Repeated-dose

1. A 28-day Intravenous (Injection) Toxicity Study of TR-701 with a 28-day Recovery Period in Sprague Dawley Rats (Study No.: TOX-08-0701-009).
2. A 14-day (Twice-daily) Intravenous 30-Minute Infusion Toxicity Study of TR-701 in Beagle Dogs (Study No.: TOX-08-0701-019).

Genotoxicity

In Vitro

1. TR-700 Bacterial Reverse Mutation Assay (Study No.: TOX-07-0701-005A).
2. TR-701 Bacterial Reverse Mutation Assay (Study No.: TOX-07-0701-001).
3. TR-700 In Vitro Chromosome Aberration Test (Study No.: TOX-07-0701-086).
4. TR-701 In Vitro Mammalian Chromosome Aberration Test (TOX-07-0701-021).
5. TR-700 In Vitro Mouse Lymphoma Cell Mutation Assay (Study No.: TOX-07-0701-089).

In Vivo

1. TR-701 Mammalian Erythrocyte Micronucleus Test in Male Mice (Study No.: TOX-07-0701-084).
2. TR-701 Mammalian Erythrocyte Micronucleus Test in Female Mice (Study No.: TOX-07-0701-085).
3. TR-700 Mammalian Erythrocyte Micronucleus Test in Male Mice (Study No.: TOX-07-0701-022).
4. TR-700 Mammalian Erythrocyte Micronucleus Test in Female Mice (Study No.: TOX-07-0701-087).
5. TR-701 In Vivo Unscheduled DNA Synthesis Test in Rats (Study No.: TOX-07-0701-090).

Reproductive and Developmental Toxicology

1. A Study of the Effects of TR-701 Free Acid on Fertility and Early Embryonic Development in Rats. (Study No.: TOX-08-0701-026).
2. A Study of the Effects of TR-701 on Embryo/Fetal Development in Rats (Study No.: TOX-08-0701-004).
3. A Dose Range-Finding Study of the Effects of TR-701 on Embryo/Fetal Development in Rabbits (Study No.: TOX-08-0701-012).

4. An Oral (Gavage) Study of the Effects of TR-701 FA on Embryo-Fetal Development in Mice (Study No.: TOX-09-0701-019).
5. An Oral (Gavage) Study of the Effects of TR-701 on Pre- and Postnatal Development Including Maternal Function in Rats (Study No.: TOX-11-0701-025).

Special Toxicology Studies

Immunotoxicity

1. Subacute Oral Immunotoxicity Study in Sprague Dawley Rats (4 Weeks Administration by Gavage) (Study No.: TOX-12-0701-060).

Local Tolerance

1. Acute Perivascular, Intramuscular, and Subcutaneous Irritation Study of TR-701 FA in Rabbits (Study No.: TOX-12-0701-058).

Mechanistic Studies

1. Effect of TR-700 and TR-701 on Rat Heart Mitochondrial Protein Synthesis (Study No.: SPH-08-0701-028).

Impurities

1. A 14-Day Intravenous (Bolus) Injection Impurity Safety Qualification Study in Sprague Dawley Rats (Study No.: TOX-13-0701-075).
2. A 14-Day Intravenous (Bolus) Toxicity Study (b) (4) in Female Sprague Dawley Rats (Study No.: TOX-10-0701-001).
3. Intermediate 6 Reference Standard (b) (4) *Salmonella-E. coli*/Mammalian Microsome Reverse Mutation Assay (Study No: TOX-11-0701-015).
4. (b) (4) Mammalian Microsome Reverse Mutation Assay (Study No.: TOX-12-0701-035).
5. (b) (4) Salmonella-E.Coli Mammalian Microsome Reverse Mutation Assay (Study No.: TOX-12-0701-036).
6. Bacterial Reverse Mutation Assay (Study No: TOX-13-0701-078).

3.2 Studies Not Reviewed

1. An Escalating-Dose Oral (Gavage) Tolerability and Toxicokinetic Study of TR-701 FA in Beagle Dogs (Study No.: TOX-11-0701-024).
2. Single Dosage Phototoxicity Study to Determine the Effects of Oral (Gavage) Administration of TR-701 FA on Eyes and Skin in Pigmented Rats (Study No.: TOX-10-0701-011).
3. MultiCASE Mutagenicity Assessment for 5-Bromo-2-Cyanopyridine (Study No.: TOX-12-0701-047).
4. In Silico Mutagenicity Evaluation of Tedizolid and Related Impurities (Study No.: TOX-13-0701-077).
5. A 7-Day Intravenous (60- or 120-Minute) Infusion Irritation Study of TR-701 in Beagle Dogs (Study No.: TOX-09-0701-001).
6. A 7-Day Intravenous (120-Minute) Infusion Vascular Irritation Study of TR-701 in Sprague-Dawley Rats (Study No.: TOX-09-0701-006).
7. A 7-Day Intravenous (10-Minute) Infusion Vascular Irritation Study of TR-701 FA in Male Sprague Dawley Rats (Study No.: TOX-10-0701-002).
8. A Dose Range-Finding Study of the Effects of TR-701 on Embryo/Fetal Development in Mice (Study No.: TOX-08-0701-027)

9. A Seven-Day Tolerability Study of Orally Administered TR-701 in Non-Pregnant New Zealand White Rabbits (Study No.: TOX-08-0701-007).
10. Selectivity and Specificity of TR-700 in Radioligand Binding and Biochemical Assays (Study No.: SPH-07-0701-040).
11. In Vitro Determination of IC50 values for TR-700 Versus Monamine Oxidase A and Monamine Oxidase B (Study No: SPH-07-0701-015A).
12. Evaluation of TR-701 and TR-700 In Vitro Receptor Binding and Inhibitory Activity Versus Monamine Oxidase A and Monamine Oxidase B (Study No.: SPH-08-0701-011A).
13. MAO-A/MAO-B: Determination of Reversible/Irreversible Inhibitory Effects of TR-700 (Study No.: PHA-12-0701-030).
14. MAO-B: Determination of Reversible/Irreversible Inhibitory Effects of TR-700 (Study No.: SPH-12-0701-061).
15. Evaluation of Trius Compound TR-701 FA Against Known MAO Inhibitors and an SSRI on Head Twitch Behavior and Monoamine Content in Mouse Brain (Study No.: PHA-12-0701-031A).
16. Effect of TR-701 in Combination with Tyramine on In Vivo Cardiovascular Function in Rats (Study No.: SPH-07-0701-016).
17. TR-701: Tyramine Challenge Assay in Conscious, Radiotelemetry-Instrumented Male Sprague Dawley Rats (SPH-08-0701-008).
18. An Escalating-dose Intravenous (Slow Push) Injection Tolerability and Toxicokinetic Study of TR-701 in Beagle Dogs (TOX-08-0701-018).
19. 28-day Repeat Oral Dose Toxicity Study of TR-701 in Beagle Dogs (Dose Range Finding) (Study No.: TOX-07-0701-012).
20. 14-day Repeat Oral Dose Study of TR-701 in Rats (TOX-07-0701-011).
21. A 14-day Intravenous (Injection) Toxicity Study of TR-701 in Sprague Dawley Rats (Study No.: TOX-08-0701-005).
22. A 14-day Comparative Toxicity Study Between an Intravenous (Slow Push) Injection and a 60-Minute Intravenous Infusion of TR-701 in Sprague Dawley Rats (Study No.: TOX-08-0701-024).
23. A Comparative Oral and 60-minute Intravenous Infusion Toxicity Study in Female Sprague Dawley Rats for TR-701FA and Linezolid (Study No.: TOX-09-0701-002).

3.3 Previous Reviews Referenced

1. Memo to File Regarding MAO inhibition studies for TR-701 submitted to DAARTS for IND 106307 on 5/23/2014.
2. IND 77872 Pharmacology/Toxicology 30-Day Safety Review by Dr. Maria Rivera, Ph.D. submitted to DARRTS on 10/24/2008.
3. IND 106307 Pharmacology/Toxicology 30-Day Safety Review by Dr. Maria Rivera, Ph.D. submitted to DARRTS on 9/22/2009.

4 Pharmacology

4.1 Primary Pharmacology

The primary pharmacology studies were reviewed by the Microbiology reviewer.

4.2 Secondary Pharmacology

Serotonin syndrome and monoamine oxidase (MAO) inhibition have been reported in association with clinical administration of linezolid. *In vitro* studies with TR-701 and TR-700 indicated that TR-700, but not TR-701, is a weak inhibitor of MAO-A and MAO-B with IC_{50} values comparable to linezolid (Study Nos.: SPH-07-0701-040, SPH-07-0701-015A, SPH-08-0701-011A, PHA-12-0701-030, and SPH-12-0701-061). However, in a mouse head-twitch experiment (PHA-12-0701-031) and a tyramine-challenge experiments in rats (SPH-07-0701-016; SPH-08-0701-008), linezolid doses comparable to the human therapeutic dose produced positive results (increased head twitch or increased mean arterial pressure), but TR-701 doses associated with plasma TR-700 C_{max} and AUC values greatly exceeding the equivalent clinical therapeutic exposure values did not. These data indicate that in nonclinical experiments TR-701 and TR-700 are less potent than linezolid in stimulating *in vivo* responses mediated by MAO inhibition. Taken as a whole the nonclinical study data suggests TR-701 does not pose an urgent concern for MAO inhibition at the clinical exposures expected with the therapeutic dose. These findings are reviewed in the Memo to File by James Wild Ph.D., submitted to DARRTS for IND 106307 on 5/23/2014.

4.3 Safety Pharmacology

TR-701 and/or TR-700 were examined in an extensive panel of neurological, cardiovascular, pulmonary, renal and gastrointestinal safety pharmacology studies.

Neurological Safety Pharmacology

1. **Effect of TR-701 on General Behavior in Rats** (Study No.: SPH-07-0701-018).

   This study was previously reviewed for IND 77872 (30-day safety review; 10/24/2008) by Dr. Maria Rivera. Sprague-Dawley rats (4/sex/dose) treated with single oral doses of TR-701 (10, 30, and 100 mg/kg) or vehicle did not exhibit any TR-701-related changes in any evaluated parameter. The parameters evaluated were locomotor activity, tail elevation, tremors, convulsion, abdominal tone, catalepsy, righting reflex, traction (grip strength), exophalmos, salivation, pinna reflex, piloerection, lacrimation, respiratory rate, eyelid, skin color, diarrhea, startle reflex, and death measured for up to 24 hours after dosing.

2. **Effects of TR-701 on Spontaneous Locomotor Activity in Mice** (Study No.: SPH-07-0701-019).

   This study was reviewed for IND 77872 (30-day safety review; 10/24/2008) by Dr. Maria Rivera. Male SPF ICR mice (8/dose) administered a single oral dose of 10, 30, and 100 mg/kg TR-701 or vehicle exhibited significantly reduced spontaneous locomotor activity 30 minutes after dosing with 30 and 100 mg/kg TR-701. Compared to baseline, the mean locomotor activity at 30 and 100 mg/kg decreased 16.7 counts/5 minutes and 30.6 counts/5 minutes 30 minutes after dosing respectively whereas activity decreased 0.2 counts/5 minutes in the vehicle control group and 25.6 counts/5 minute in the positive control group receiving 10 mg/kg diazepam.

3. **Effect of TR-701 on Hexobarbital-Induced Sleep Time in Mice** (Study No.: SPH-07-0701-068).

   This study was reviewed for IND 77872 (30-day safety review; 10/24/2008) by Dr. Maria Rivera. Male SPF ICR mice (8/dose) were administered single oral doses of 10, 30, and 100 mg/kg TR-701 or vehicle followed 30 minutes later by intraperioneal hexobarbital (70 mg/kg). The high-dose of TR-701 produced a significant increase in hexobarbital-induced sleep time (51.5 minutes) compared to vehicle control animals (41.8 minutes). The positive control, 6 mg/kg chlorpromazine significantly increased hexobarbital-induced sleep time to 72.6 minutes.

4. **Effect of TR-701 on Strychnine-Induced Convulsions in Mice** (Study No.: SPH-07-0701-072)

   This study was reviewed for IND 77872 (30-day safety review; 10/24/2008) by Dr. Maria Rivera. Male SPF ICR mice (8/dose) were administered single oral doses of 10, 30, and 100 mg/kg TR-701 or vehicle followed 30 minutes later by intraperioneal strychnine (1 mg/kg) to induce convulsions. None of the doses of TR-701 changed the clonic convulsion response or mortality compared to vehicle, but the positive control agent, 20 mg/kg diazepam, completely blocked strychnine-induced convulsions and mortality.

5. **Effect of TR-701 on Analgesia in Mice Exposed to Acetic Acid** (Study No.: SPH-07-0701-069).

   This study was reviewed for IND 77872 (30-day safety review; 10/24/2008) by Dr. Maria Rivera. Male SPF ICR mice (8/group) were treated with a single oral dose of 10, 30, and 100 mg/kg TR-701 or vehicle followed by injection of acetic acid to induce a writhing-pain response. None of the TR-701 doses affected the writhing response compared to the control treatment. In contrast, a positive control agent, 2 mg/kg morphine significantly decreased the writhing response.

6. **Effect of TR-701 on Analgesia in Mice Evaluated by the Hot Plate Test** (Study No.: SPH-07-0701-070).

   This study was reviewed for IND 77872 (30-day safety review; 10/24/2008) by Dr. Maria Rivera. Male SPF ICR mice (8/group) were treated with a single oral dose of 10, 30, and 100 mg/kg TR-701 or vehicle and effects on pain threshold were assessed by the hot-plate test. None of the TR-701 doses affected the licking response to hot-plate exposure compared to the control treatment. In contrast, a positive control agent, 12 mg/kg morphine, significantly increased the time lapse before a licking response occurred.

7. **Effect of TR-701 on Pentylenetetrazole-Induced Convulsions in Mice** (Study No.: SPH-07-0701-071).

   This study was reviewed for IND 77872 (30-day safety review; 10/24/2008) by Dr. Maria Rivera. Male SPF ICR mice (8/dose) were treated with a single oral dose of 10, 30, or 100 mg/kg TR-701 or vehicle followed 30 minutes later by a single intraperitoneal dose of pentylenetetrazole (100 mg/kg) to induce clonic convulsions. None of the doses of TR-701 was observed to change the clonic

convulsion response or mortality rate compared to the vehicle control group. In contrast, a positive control agent (10 mg/kg diazepam) completely blocked pentylenetetrazole-induced convulsions and mortality.

8. **Effect of TR-701 on Electric-Shock Induced Convulsions in Mice** (Study No.: SPH-07-071-073).

   This study was reviewed for IND 77872 (30-day safety review; 10/24/2008) by Dr. Maria Rivera. Male SPF ICR mice were administered a single-oral dose of 10, 30, and 100 mg/kg TR-701 or vehicle followed 30 minutes later by an electric shock to induce tonic convulsion. None of the doses of TR-701 changed the tonic convulsion response or mortality compared to vehicle, but the positive control agent, 30 mg/kg diazepam, completely blocked convulsions and mortality.

9. **Effect of TR-701 on Body Temperature in Mice** (Study No.: SPH-07-0701-074).

   This study was reviewed for IND 77872 (30-day safety review; 10/24/2008) by Dr. Maria Rivera. Male SPF ICR mice (8/dose) were administered single oral doses of 10, 30, and 100 mg/kg TR-701, or vehicle followed by measurements of body temperature for up to 24 hours after dosing. All of the TR-701 doses produced a small but significant decrease in body temperature 60 to 120 minutes after dosing. However, the decreased values were still within normal range, and not considered to be toxicologically relevant.

10. **Assessing the Effects of TR-701 on the Autonomic Nervous System Using Isolated Guinea Pig Ileum** (Study No.: SPH-07-0701-079).

    This study was reviewed for IND 77872 (30-day safety review; 10/24/2008) by Dr. Maria Rivera. In an organ bath preparation, a longitudinal muscle preparation from guinea-pig ileum was exposed to TR-701 concentrations of 0.1, 1, or 10 μM in the presence of agents known to stimulate contraction (0.5 μM acetylcholine; 2 μM histamine; or 2 mM barium chloride). TR-701 did not block the contractile responses at any of the tested concentrations.

Cardiovascular Safety Pharmacology

1. **Effect of TR-701 on Blood Pressure, Heart Rate, and ECG in Dogs Using a Radiotelemetry System** (Study No.: SPH-07-0701-017).

   This study was reviewed for IND 77872 (30-day safety review; 10/24/2008) by Dr. Maria Rivera. Four nonanesthetized Beagle dogs received sequential single oral doses of vehicle or increasing concentrations of TR-701 (20, 60, and 200 mg/kg) with a 2-week washout period between doses. Body temperature and cardiovascular parameters including blood pressure, heart rate and ECG intervals (PR, QRS, QT, RR, and QTc (corrected using Bazett's formula) were monitored for up to 24 hours after dosing. None of the measured parameters was affected by any of the TR-701 doses.

2. **Effect of TR-701 on hERG Channel Tail Currents** (Study No.: SPH-07-0701-083).

This study was reviewed for IND 77872 (30-day safety review; 10/24/2008) by Dr. Maria Rivera. In a typical preparation of hERG channel expressed in human embryonic kidney cells, 1 and 10 μM concentrations of TR-701 had no significant effects on hERG channel K+ current amplitude compared to pretreatment values.

3. **The Effect of TR-700 on hERG Channel Tail Current** (Study No.: SPH-07-0701-067).

   This study was reviewed for IND 77872 (30-day safety review; 10/24/2008) by Dr. Maria Rivera. In a typical preparation of hERG channel expressed in human embryonic kidney cells, the maximum soluble concentration of TR-700 (20.25 μM) had no effect on hERG channel K+ current amplitude compared to pretreatment values. In contrast, a positive control agent, 50 nM terfenadine, reduced hERG channel current amplitude by 72% compared to pretreatment values.

4. **Effect of TR-701 on Cardiac Function of Isolated Rat Heart** (Study No.: SPH-07-0701-080).

   In a Langendorff preparation, TR-701 in a concentration range of 0.1 to 10 μM had no effects on measured parameters in isolated perfused rat hearts (n = 6/dose). The measured parameters included: left ventricular peak systolic pressure), left ventricular end diastolic pressure, left ventricular developing pressure, heart rate, double product, and coronary flow rate.

5. **Effect of TR-700 on Cardiac Function of Isolated Rat Heart** (Study No.: SPH-07-0701-081).

   In a Langendorff preparation, TR-700 in a concentration range of 0.1 to 10 μM had no effects on measured parameters in isolated perfused rat hearts (n = 6/dose). The measured parameters included: left ventricular peak systolic pressure), left ventricular end diastolic pressure, left ventricular developing pressure, heart rate, double product, and coronary flow rate.

Pulmonary Function Safety Pharmacology

1. **Effect of TR-701 on Respiration Rate and Volume in Rats** (Study No.: SPH-07-0701-075).

   Male Sprague-Dawley rats (8/group) were administered single oral doses of 10, 30, and 100 mg/kg TR-701 or vehicle and respiratory rate, tidal volume, and minute volume were measured for up to 24 hours after dosing. TR-701 did not alter any of the parameters relative to the control group.

Gastrointestinal Safety Pharmacology

1. **Effect of TR-701 on Gastrointestinal Transport in Mice** (Study No.: SPH-07-0701-076).

Male SPF ICR mice (8/dose) were treated with a single oral dose of 10, 30, and 100 mg/kg TR-701 or vehicle followed by oral administration of a 10 ml/kg charcoal mixture 30 minutes after administration. After an additional 30 minutes, animals were sacrificed and intestines isolated. Two measurements were obtained: total intestine length and the migration distance of the charcoal meal through the intestine measured from the pylorus. The transport ratio was defined as the ratio of the charcoal meal distance divided by the total intestinal length multiplied by 100. TR-701 did not affect intestinal transport compared to the control group. In contrast a positive control agent, 200 mg/kg l-hyoscyamine produced a significantly lower transport ratio of 17.6% compared to a ratio of 54.2% for the control group.

2. **Effect of TR-701 on Gastric Secretion in Rats** (Study No.: SPH-07-0701-077).

   Following fasting overnight, anesthetized male Sprague-Dawley rats (5/group) were treated with single doses of 10, 30, and 100 mg/kg TR-701, 1 mg/kg atropine as a positive control, or vehicle introduced directly into the duodenum. Animals were maintained for a further 5 hours without food or water, then sacrificed, their stomachs were removed, and gastric juices were collected by centrifugation. The volume of recovered gastric juice, gastric juice pH, and gastric juice total acidity were determined. The high dose of TR-701 significantly reduced mean gastric volume by approximately 40% compared to control animals. Also mean total acidity was lowered in high-dose animals by approximately 48% compared to control animals but the reduction was not statistically significant. Stomach juice pH was not changed compared to the control group. The lower doses of TR-701 did not affect any of the measured parameters. The positive control agent, atropine produced significant reductions in all three parameters with reductions of approximately 52% and 65% for gastric volume and total acidity, and an increased pH of approximately 39% compared to control values.

Renal Safety Pharmacology

1. **Effect of TR-701 on Renal Function in Rats** (Study No.: SPH-07-0701-078). Male Sprague-Dawley rats (8/dose) hydrated with water and saline were subsequently administered single oral doses of 10, 30, and 100 mg/kg TR-701, a positive control, 15 mg/kg furosemide, or vehicle followed by urine collection for the next 5 hours. Urine was analyzed for volume, electrolyte content, pH, total protein, specific gravity, and bilirubin. The high dose of TR-701 (100 mg/kg) produced significant reductions in urinary sodium and chloride concentrations of approximately 30% and 35% respectively compared to the vehicle control group. Urinary sodium and chloride were also reduced by approximately 20% and 32% respectively in the rats administered 30 mg/kg TR-701 compared to the control group, but the changes were not statistically significant. Urine volume was increased but not significantly in the 30 and 100 mg/kg groups by approximately 76% and 73% respectively compared to the control group. Treatment with the positive control agent, furosemide, significantly increased urine volume by

approximately 195% and reduced urine sodium and chloride by approximately 42% and 37% respectively compared to the control group.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Methods of Analysis

Validated analytical methods were developed to measure TR-701 and TR-700 in plasma from mice, rats, and dogs and TR-700 enantiomer quantities in rat plasma. The validated analysis techniques employed liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). For the different plasma sample types, the lower limit of quantification (LLOQ) for TR-700 measurements ranged from 2.5 ng/ml to 100 ng/ml and the LLOQ for TR-701 was 10 ng/ml. Additional non-validated analysis techniques (LC-MS/MS and HPLC-UV) were developed for detection of TR-701, TR-700 and other TR-701 metabolites in samples from plasma, bile, urine, feces, and tissues in mouse, rat and/or dog.

Absorption

Study Title: Pharmacokinetics of TR-701 and TR-700 Following Intravenous or Oral Administration to Mice (Study No.: PDM-07-0701-023).

Methods

In this non-GLP Study (three different sub-studies; RN-pk-001, RN-pk-010, and RN-pk-011) performed by Dong-A Pharmaceutical Co. Ltd in Korea, the pharmacokinetics of TR-701 and TR-700 were evaluated in male and female ICR mice after single oral or intravenous administrations (10 mg/kg IV and oral TR-700 for RN-pk-001; 10 mg/kg IV and oral TR-701 for RN-pk-010; and an extended range of oral TR-701 doses for RN-pk-011). Blood samples were collected for pharmacokinetic analysis at the time points shown below in Table 9.

Table 9: Collection Time Points for the Three Sub-Studies Included in Study No.: PDM-07-0701-023. (Sponsor's Table)

Treated with:	Study RN-pk-001 [a]	Study RN-pk-010 [a,b]	Study RN-pk-011 [c]
TR-700	0 min (control) 1 min (IV only); 5, 15, 30 min 1, 2, 4, 6, 8, 16, 24 h		
TR-701		0 min (control) 1 min (IV only); 5, 15, 30 min 1, 2, 4, 6, 8, 16, 24 h	0 min (control) 0.5, 1, 2, 4, 6, 8, 16, 24 h

a. Compound administered orally and intravenously; $n = 5$ mice/time point.
b. Plasma was acidified with 10 µL of 1 M HCl prior to storage and extraction/analysis.
c. Compound administered orally; $n = 3$ mice/group/time point.

Results

RN-pk-001: As shown in Table 10, After IV and oral administration of TR-700, $t_{1/2}$ values for TR-700 administered by either route were a little over 3 hours. The steady state volume of distribution was 512 ml/kg indicating distribution beyond the vascular compartment. Bioavailability by the oral route was 48.5% with a delayed t_{max} occurring at 2 hours.

Table 10: Pharmacokinetic Parameters for TR-700 after Administration of 10 mg/kg TR-700 in Male Mice. (Sponsor's Table)

PK parameters	IV	PO
Half-life (hr)	3.49	3.12
T_{max} (hr)		2
C_{max} (ug/mL)		4.62
$AUC_{0\text{-}24\,h}$ (ug hr/mL)	102.3	49.80
AUC_{inf} (ug hr/mL)	103.4	50.15
CL (mL/min/kg)	1.612	
Vdss (L/kg)	0.512	
F (%)		48.5

Data source: Study RN-pk-001

RN-pk-010: TR-701 was undetectable in plasma 15 minutes after IV administration and undetectable at all collection time-points following oral administration in both genders indicating rapid metabolism or elimination. As shown in Table 11, plasma levels of TR-700 were persistent with a $t_{1/2}$ of about 3 hours for both genders with both routes of administration. Also t_{max} values occurred sooner and bioavailability for TR-700 was higher following oral administration of TR-701 compared to when TR-700 was orally administered highlighting the advantages of the prodrug (TR-701). TR-700 C_{max} and AUC values were similar for both genders and for both routes of administration.

RN-pk-011: As shown in Table 12, plasma AUC values for TR-700 were roughly linear with TR-701 dose over an extended dosage range.

Table 11: Pharmacokinetic Parameters for TR-700 After Administration of 10 mg/kg TR-701 in Male and Female Mice. (Sponsor's Table)

	Intravenous		Oral	
	Male	Female	Male	Female
Mean BW (g)	30	25	30	25
$T_{1/2}$ (hr)	3.42	3.05	3.82	2.83
AUC (ug hr/mL)	53.6	41.9	49.8	37.5
T_{max} (hr)	0.0167	0.0167	0.5	0.5
C_{max} (ug/mL)	8.81	9.51	8.37	6.99
CL (mL/min/kg)	3.11	3.98		
Vd_{ss} (L/kg)	0.918	0.949		
AUC_{oral}/AUC_{IV} ratio (%)			92.8	89.4

Data source: Study RN-pk-010

Table 12: Pharmacokinetic Parameters of TR-700 in Mice After Oral Administration of 1.33-341 mg/kg TR-701. (Sponsor's Table)

	1.33 mg/kg	2.67 mg/kg	5.33 mg/kg	10.7 mg/kg	21.3 mg/kg	85.3 mg/kg	341 mg/kg
$T_{1/2}$ (hr)	6.04	5.50	6.22	5.57	5.21	7.57	
T_{max} (hr)	0.5	0.5	0.5	0.5	0.5	1	2
C_{max} (ug/mL)	0.73	1.07	2.19	4.61	9.37	44.49	71.15
$AUC_{0\text{-}24\ h}$ (ug hr/mL)	6.5	11.8	21.7	41.5	95.6	425	1331
AUC (ug hr/mL)	6.90	12.30	23.17	43.38	98.98	471	
CL/F (mL/min/kg)	2.42	2.71	2.88	3.07	2.69	2.26	
Vss/F (L/kg)	1.26	1.29	1.55	1.48	1.22	1.48	

Data source: Study RN-pk-011

Study Title: Pharmacokinetics of TR-701 and TR-700 after Single Intravenous and Oral Administration of TR-701 to Beagle Dogs (Study No.: PDM-07-0701-025).

Methods

In this non-GLP study conducted (b) (4) fasted male Beagle dogs (3/group) were administered oral TR-701 at doses of 10 or 30 mg/kg or the same doses of intravenous TR-701. Blood samples were collected for pharmacokinetic analysis at 0, 1, 5, 15, and 30 minutes and at 1, 2, 4, 6, and 10 hours after administration. Urine was collected for 0 to 6 hours and 6-24 hours after administration. Plasma and urine concentrations of TR-701 and TR-700 were analyzed using a LC/MS/MS method.

Results

As summarized in Table 13, TR-701 was rapidly eliminated with a $t_{1/2}$ of 0.05 hours for intravenous doses. The plasma TR-700 $t_{1/2}$ ranged from approximately 0.5 to 1 hour with the highest values occurring for the higher dose (30 mg/kg). Plasma C_{max} and AUC values increased in a roughly dose-proportional manner. Bioavailability of TR-701 was 63 and 76% for the 10 and 30 mg/kg doses respectively. Only very small amounts (generally less than 0.1% of the total) of TR-700 were excreted in urine through 24 hours following administration by either route (data not shown).

Table 13: Mean Pharmacokinetic Parameters of TR-701 and TR-700 Upon Single IV or PO Administration of TR-701 to Dogs. (Sponsor's Table)

Mean PK Parameters	Intravenous		Oral	
	10 mg/kg	30 mg/kg	10 mg/kg	30 mg/kg
Body weight (kg)	10.5	10.2	10.3	10.4
TR-701				
$t_{1/2}$ (hr)	0.05	0.05	not calculated	
AUC (µg hr/mL)	10.0	17.2		
CL (mL/min/kg)	17.0	29.1		
Vdss (L/kg)	0.031	0.077		
TR-700				
$t_{1/2}$ (hr)	0.58	0.90	0.64	0.94
AUC (µg hr/mL)	4.42	18.59	2.78	14.18
t_{max} (hr)	0.08	0.19	1.67	0.83
C_{max} (µg/mL)	5.37	17.43	1.38	5.85
CL/F (mL/min/kg)	39.2	26.9	not calculated	
Vd_{ss}/F (L/kg)	1.66	1.93		

Study Title: Pharmacokinetics of TR-701 and TR-700 Following Intravenous or Oral Administration in Rats (Study No.: PDM-07-0701-027).

Methods

In this non-GLP study (composed of 3 sub-studies: RN-pk-006, RN-pk-008, and RN-pk-015) performed (b) (4) male and female Sprague Dawley rats were administered TR-701 or TR-700 by intravenous or oral administration.

In sub-study RN-pk-006, TR-700 was administered to male and female rats in single intravenous doses of 10 mg/kg (n =9/group), and single oral gavage doses of 10 mg/kg TR-700 were administered to 8 male rats and 10 females.

In sub-study RN-pk-008, male and rats were administered single intravenous doses of 5 or 20 mg/kg TR-701 (n = 5-7/group) or single oral gavage doses of 20 mg/kg TR-701 (n = 8 males and 7 females).

In sub-study RN-pk-015, TR-701 was administered to male rats via single intravenous injections at doses of 5, 10, or 20 mg/kg (n = 7, 7, or 9/group respectively). In another experiment, TR-701 was administered by oral gavage in single oral doses of 20, 50, or 100 mg/kg (n = 11, 9, and 9 respectively).

For all the studies except where noted after IV dosing, blood was collected at 0, 1, 5, 15, 30, and 45 minutes (no 45-minute collection for sub-study RN-pk-006), and at 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 hours. After oral dosing, blood samples were collected at 0, 15, and 30 minutes and at 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 hours. For sub-study RN=pk-015, blood samples were also collected at 16 and 24 hours after dosing. For Study No.: RN-pk-006, urine was collected for a 24 hour period and the entire GI tract including contents was removed and TR-700 was extracted. Plasma, urine, and/or GI track TR-701 and TR-700 were quantified in rat plasma using a HPLC with UV detection method. For study RN-pk-008, urine was collected over a 24 hour period.

Results

Study No.: RN-pk-006

Females weighed significantly less than males by about 33%. After IV or oral administration of TR-700 the plasma AUC0-∞ in females was significantly greater (2.6 to 2.8 fold) and the $t_{1/2}$ longer (1.9 fold) than in males (Table 14). Plasma clearance of TR-700 was significantly lower (-65%) in females compared to males, and the steady-state volume of distribution was significantly higher (+25%) in females compared to males. After oral dosing, bioavailability was similar in both genders (29% males, 27% females), but C_{max} was significantly higher by 1.6 fold compared to the C_{max} value in males (Table 15).

Females excreted a slightly greater percent of the TR-700 dose in urine and feces in the first 24 hours after dosing compared to males.

Table 14: TR-700 PK Parameters for Male and Female Rats After a Single 10 mg/kg IV dose of TR-700 in Study # RN-pk-006. (Sponsor's Table)

	Male (N = 9)	Female (N = 9)
BW (g)	319 ± 11.7	228 ± 10.9***
$T_{1/2}$ (hr)	2.15 ± 0.330	3.98 ± 0.595***
AUC (ug hr/mL)	53.1 ± 10.8	149 ± 31.0***
CL (mL/min/kg)	3.15 ± 0.638	1.13 ± 0.290***
Vdss (L/kg)	0.274 ± 0.049	0.343 ± 0.107*
CL_R (mL/min/kg)	0.019 ± 0.026	0.012 ± 0.006
CL_{NR} (mL/min/kg)	3.13 ± 0.619	1.11 ± 0.286***
$Xu_{0\text{-}24\ hr}$ (% of dose)	0.801 ± 0.512	1.13 ± 0.264
$GI_{0\text{-}24\ hr}$ (% of dose)	0.356 ± 0.182	0.511 ± 0.260

*** $p<0.001$; * $p<0.05$
Data source: Study RN-pk-006

Table 15: TR-700 PK Parameters for Male and Female Rats After a Single 10 mg/kg Oral Dose of TR-700 in Study # RN-pk-006. (Sponsor's Table)

	Male (N = 8)	Female (N = 10)
BW (g)	328 ± 11.6	206 ± 7.25***
$T_{1/2}$ (hr)	3.59 ± 1.77	6.75 ± 2.17**
AUC (ug hr/mL)	15.8 ± 6.35	40.8 ± 19.4**
T_{max} (hr)	5.00 ± 2.78	5.10 ± 1.79
C_{max} (ug/mL)	1.87 ± 0.61	3.04 ± 1.68
CL_R (mL/min/kg)	0.021 ± 0.030	0.021 ± 0.067
$Xu_{0\text{-}24\ hr}$ (% of dose)	0.337 ± 0.368	1.00 ± 0.853
$GI_{0\text{-}24\ hr}$ (% of dose)	2.07 ± 1.57	6.88 ± 4.47*
F	29.8	27.4

*** P<0.001. **P<0.01. *P<0.05.
Data source: Study RN-pk-006

Study RN-pk-008

Pharmacokinetics for both genders were similar for TR-701 after IV (5 and 20 mg/kg) or oral (20 mg/kg) administration of both doses of TR-701, but plasma AUC values for TR-700 in females were significantly greater by approximately 3-fold compared to males. Also the mean female $t_{1/2}$ values for TR-700 were significantly longer by greater than 2-fold compared to males (Table 16 and Table 17). Bioavailability for oral TR-701 was measured as a low 8% for both genders but these values did not factor in the amount of TR-701 rapidly converted to TR-700.

Table 16: PK Parameters for TR-701 (DA-7218) and TR-700 (DA-7157) After Single IV Doses of 5 mg/kg and 20mg/kg TR-701 to Male and Female Rats. (Sponsor's Table)

Parameter	5 mg/kg		20 mg/kg	
	Male (N = 5)	Female (N = 5)	Male (N = 6)	Female (N = 7)
Body weight (g)	377 ± 9.75	237 ± 11.5[a]	379 ± 17.4	246 ± 17.7[a]
DA-7218				
AUC (ug min/ml)	204 ± 54.4	164 ± 73.3	570 ± 81.5	567 ± 192
Terminal half-life (min)	19.8 ± 11.5	23.6 ± 5.11	83.8 ± 21.8	74.1 ± 55.7
MRT (min)	7.55 ± 1.50	6.71 ± 1.13	22.8 ± 6.42	35.3 ± 13.3
CL (mi/min/kg)	49.0 ± 16.1	61.0 ± 33.7	35.1 ± 4.66	30.3 ± 19.7
V_{ss} (ml/kg)	367 ± 103	402 ± 244	740 ± 246	862 ± 636
DA-7157				
AUC (ug·min/ml)	760 ± 248	2,030 ± 740[b]	3,560 ± 561	12,100 ± 4,200[b]
Terminal half-life (min)	73.9 ± 13.5	183 ± 34.7[a]	96.8 ± 13.6	247 ± 48.3[b]
C_{max} (ug/ml)	16.0 ± 4.10	14.6 ± 5.61	42.4 ± 14.4	58.1 ± 9.61[c]
T_{max} (min)	1.00 ± 0	1.80 ± 1.79	3.67 ± 2.07	5.00 ± 6.83

[a] $P < 0.001$ compared to male.

[b] $P < 0.01$ compared to male.

[c] $P < 0.05$ compared to male.

Data source: Study RN-pk-008

Table 17: PK Parameters for TR-701 (DA-7218) and TR-700 (DA-7157) After a Single Oral Dose of 20mg/kg TR-701 to Male and Female Rats. (Sponsor's Table)

Parameter	Male (N = 8)	Female (N = 7)
Body weight (g)	273 ± 13.9	221 ± 9.88[a]
DA-7218		
AUC (ug min/ml)	48.3 ± 20.6	44.2 ± 7.82
Terminal half-life (min)	126 ± 26.2	118 ± 17.4
C_{max} (ug/ml)	0.250 ± 0.0959	0.273 ± 0.0457
T_{max} (min)	54.4 ± 17.8	53.6 ± 21.0
F (%)	8.47	7.80
DA-7157		
AUC (ug min/ml)	2,710 ± 663	8,620 ± 1,530[a]
Terminal half-life (min)	219 ± 41.1	420 ± 72.5[a]
C_{max} (ug/ml)	14.6 ± 3.45	14.6 ± 0.873
T_{max} (min)	30.0 ± 11.3	57.9 ± 28.0[b]

[a] $P < 0.001$ compared to male.

[b] $P < 0.05$ compared to male.

Data source: Study RN-pk-008

Study No.: RN-pk-015

After IV dosing of TR-701, plasma AUC values for TR-701 were approximately proportional to dose, but clearance and volume of distribution were largely dose independent (Table 18). After IV dosing TR-701 was rapidly converted to TR-700, and based on $t_{1/2}$ values for TR-701 and TR-700, clearance of TR-700 was much slower than for TR-701. The TR-700 C_{max} and AUC values increased in an approximately dose-proportional manner.

After oral dosing, TR-701 was rapidly absorbed from the GI track (Table 19), TR-701 was detected in plasma at the 15 minute sampling time. TR-701 rapidly converted to TR-700 which was also detected in plasma at the 15 minute sampling time and the TR-700 T_{max} (0.43-0.75 hours) occurred before the TR-701 T_{max} (0.73-2 hours) in plasma. TR-701 and TR-700 plasma AUC values increased in an approximately dose-proportional manner. After oral dosing, plasma AUCs for TR-700 were 65 to 106-fold higher than for TR-701 and much higher than after IV dosing suggesting substantial first pass metabolism. The bioavailability for TR-700 ranged from 72.5 to 87.0%, much higher than after oral administration of TR-700 (oral bioavailability of 27-29%) in Study No.: RN-pk-006.

Table 18: PK Parameters for TR-701 (DA-7218) and TR-700 (DA-7157) after a Single IV Dose of TR-701 at 5, 10, or 20 mg/kg to Male Rats. (Sponsor's Table)

PK parameters	5 mg/kg (N = 7)	10 mg/kg (N = 7)	20 mg/kg (N = 9)
Body weight (g)	307.9 ± 7.0	301.4 ± 16.8	273.9 ± 11.7
DA-7218			
$T_{1/2}$ (hr)	0.230 ± 0.060	0.375 ± 0.118	0.908 ± 0.117
AUC (ug hr/mL)	5.22 ± 1.26	11.00 ± 2.52	21.74 ± 4.32
CL (mL/min/kg)	15.96 ± 3.38	15.14 ± 3.40	15.33 ± 3.26
Vdss (L/kg)	0.085 ± 0.014	0.073 ± 0.040	0.117 ± 0.044
DA-7157			
$T_{1/2}$ (hr)	1.44 ± 0.30	1.86 ± 0.32	1.92 ± 0.49
AUC (ug hr/mL)	15.09 ± 1.77	29.62 ± 5.57	67.83 ± 19.05
C_{max} (ug/mL)	12.48 ± 1.16	20.80 ± 1.41	45.42 ± 8.95
T_{max} (hr)	0.20 ± 0.08	0.13 ± 0.08	0.18 ± 0.21

Data source: Study RN-pk-015

Table 19: PK Parameters for TR-701 (DA-7218) and TR-700 (DA-7157) After a Single Oral Dose of TR-701 at 20, 50, or 100 mg/kg to Male Rats. (Sponsor's Table)

PK parameters	20 mg/kg (N = 11)	50 mg/kg (N = 9)	100 mg/kg (N = 9)
Body weight (g)	294.1 ± 38.6	276.1 ± 4.2	276.7 ± 22.6
DA-7218			
$T_{1/2}$ (hr)	1.38 ± 0.48	2.73 ± 1.49	2.91 ± 1.78
AUC (ug hr/mL)	0.75 ± 0.26	1.33 ± 0.64	2.83 ± 1.13
C_{max} (ug/mL)	0.23 ± 0.10	0.29 ± 0.11	0.43 ± 0.12
T_{max} (hr)	0.73 ± 0.39	1.36 ± 0.42	2.00 ± 1.09
DA-7157			
$T_{1/2}$ (hr)	2.76 ± 0.84	5.35 ± 2.98	6.52 ± 2.44
AUC (ug hr/mL)	49.2 ± 14.1	140.0 ± 50.3	294.8 ± 109.1
C_{max} (ug/mL)	14.7 ± 4.7	21.3 ± 9.0	34.6 ± 10.1
T_{max} (hr)	0.43 ± 0.23	0.75 ± 0.40	0.75 ± 0.40

Data source: Study RN-pk-015

Study Title: A Single-dose Oral (Gavage) Pharmacokinetic Study of TR-701 FA in Long Evans Rats (Study No.: PDM-11-0701-021).

Methods

In this non-GLP study, the pharmacokinetic profile of TR-701 FA was evaluated in pigmented Long Evans rats following a single oral (gavage) administration. Animals (6/sex/group) were administered vehicle or 10, 30, or 100 mg/kg (males); 5, 15, and 50 mg/kg TR-701 FA on Day 0. Blood samples were collected for pharmacokinetic analysis from 3 rats/sex/group/timepoint just prior to administration and approximately 1, 2, 4, 8, and 24 hours after dosing. TR-701 and TR-700 plasma concentrations were determined using a validated LC-MS/MS procedure.

Results

TR-701 rapidly decreased in systemic circulation and plasma concentrations were not determined. As shown in Table 20, the active moiety of TR-701, TR-700 increased as dose increased with AUC increasing in a more than dose-proportional manner, and C_{max} increasing in a less than dose-proportional manner in males. In females, C_{max} also increased in a less than dose-proportional manner and AUC increased in a roughly dose-proportional manner. Despite the differences in doses, the resulting plasma C_{max} and AUC values for males and females were similar as were T_{max} values. Plasma $t_{1/2}$ and MRT values tended to be slightly greater in females compared to males and to increase with dose in both genders.

Table 20: TR-700 PK Parameters Following a Single Oral Dose of TR-701 FA in Long Evan's Rats. (Sponsor's Table)

Dose	AUC_{last} (ng•h/mL)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	MRT (h)
Males					
10 mg/kg	24,656	6179	1	3.3	3.1
30 mg/kg	133,841	15,660	1	2.1	5.5
100 mg/kg	500,243	35,267	8	2.8	6.6
Females					
5 mg/kg	61,277	7244	1	4.3	5.7
15 mg/kg	218,823	21,460	1	3.5	6.0
50 mg/kg	581,190	40,163	4	5.1	6.8

Abbreviations: AUC=area under the concentration-time curve; C_{max}=maximum plasma concentration; h=hour; MRT=mean residence time; $t_{1/2}$=half-life; T_{max}=time to maximum plasma concentration.

Distribution

Study Title: TR-700 Levels in Plasma, Bone Marrow, and Liver Tissues of Mice Following Once-daily Oral Administration of TR-701 for Three Days (Study No.: PDM-07-0701-026).

Methods

Two groups of male mice (18/group) were dosed with three daily oral doses of 500 or 2000 mg/kg/day. Blood, bone marrow, and liver homogenates were obtained from 3 animals/timepoint at 0, 1, 2, 4, and 24 hours after the first administration, 24 hours after the second administration (48 hour time-point), and 24 hours after the Day 3 administration (72 hour time-point). Samples were analyzed for TR-701 and TR-700 with a LC/MS/MS method.

Results

In all three tissue matrices (blood, bone marrow, and liver homogenates) C_{max} levels of TR-700 at both dose levels occurred at four hours. In all three matrices, and at both doses, TR-700 concentrations increased in a less than dose-dependent manner. Plasma and tissues levels plateaued between 48 and 72 hours for the 500 mg/kg/day dose, but increased between 24 and 72 hours suggesting tissue accumulation with repeated dosing with the high dose. Tissue to plasma concentrations were relatively constant suggesting tissue-specific accumulation was not pronounced.

Table 21: Mean Tissue Concentration of TR-700 after Three Oral Daily Doses of TR-701. (Sponsor's Table)

Time (hours)	500 mg/kg TR-701			2000 mg/kg TR-701		
	Plasma[a]	Liver	Bone Marrow	Plasma	Liver	Bone Marrow
0	ND	ND	ND	ND	ND	ND
1	45.8	109	28.1	66.3	196	56.8
2	51.1	146	37.7	81.7	233	77.2
4	66.4	209	37.7	82.6	260	82.0
24	40.7	113	21.0	26.9	73	15.8
48	21.8	63.6	14.8	57.0	190	37.8
72	29.8	65.4	15.2	82.8	198	59.0

[a]Concentrations are listed in µg/mL

Study Title: Tissue Distribution and Excretion Balance in Male and Female Beagle Dogs Following Intravenous and Oral Administration of [^{14}C]-TR-701 (Study No.: PDM-08-0701-020A).

Methods

This GLP-compliant study including a quality assurance report was performed (b) (4) beginning in October 2008. Male and Female Beagle dogs (5/sex/group) were administered 25 mg/kg [^{14}C]-TR-701 by oral gavage or 10 mg/kg [^{14}C]-TR-701 by IV bolus. Following administration, radioactivity in plasma and select tissues was evaluated in one euthanized dog of each gender at 2, 4, 8, 24, and 72 hours after dosing. Additional blood samples were collected at 10, 30, and 60 minutes after dosing from animals scheduled for the 24 hour post-dose euthanasia and blood, urine, and feces were collected periodically from the animals scheduled for the 72 hour post-dose euthanasia. Radioactivity was quantified using liquid scintillation counting. Selected plasma, feces, and urine samples were analyzed by HPLC with radiometric detection to profile and quantify radiolabelled metabolites.

Results

Oral Bioavailability: Following oral administration, oral bioavailability for radioactivity was calculated to be 63% for males and 49% for females.

Tissue Distribution Following a Single Oral Dose: Following a single oral dose, the T_{max} for [^{14}C]-TR-701-derived radioactivity in most tissues was approximately 2 hours after dosing. The tissues with the most radioactivity at the T_{max} were liver and small intestine for both genders. Total radioactivity exposure based on AUC was greatest in the large intestine, liver, small intestine, kidney and eye solids for both genders (Table 22 and Table 23). These tissues also had the greatest tissue to plasma AUC ratios ranging from approximately 3.4 for eye solids in males to 21 for large intestine in males. The tissues with the lowest exposures included: brain, eye vitreous, bone, and fat. For both genders, in most tissues, radioactivity was below the lower limit of quantification (LLOQ) by 24 hours after dosing. Select tissue including brain, eye vitreous, skin and fat and

ovaries and uterus for females accumulated the least amount of radioactivity and levels were below the LLOQ at 2-8 hours after dosing. Tissue $t_{1/2}$ values where calculable, ranged from 1.34 hours (muscle) to 28 hours (submaxillary gland) in males and 1.12 hours (mesenteric lymph node) to 13 hours (kidney) in females.

Table 22: Pharmacokinetic Parameters of [^{14}C]-TR-701-Derived Radioactivity in Plasma and Tissues of Male Dogs Following a Single Oral Dose of [^{14}C]-TR-701. (Sponsor's Table)

Tissue	C_{max} (µg/g)	T_{max} (hr)	$T_{½}$ (hr)	r^2	AUC_{last} (µg·hr/g)	$AUC_{0-\infty}$ (µg·hr/g)	Tissue: Plasma*
Plasma	3.30	2	1.33	1.00	10.62	10.89	1.00
Adrenal Glands	9.52	2	1.50	0.95	23.69	24.83	2.23
Bone	0.32	2	NC	NC	NC	NC	NC
Brain	1.20	2	NC	NC	2.41	NC	0.23
Epididymis	7.13	2	1.64	1.00	21.94	23.25	2.07
Eye (solids)	7.51	2	**6.01**	0.88	35.90	**66.92**	3.38
Eye (vitreous)	0.73	2	NC	NC	1.87	NC	0.18
Fat	1.95	2	NC	NC	NC	NC	NC
Heart	5.76	2	1.50	0.96	14.70	15.40	1.38
Kidney	17.02	2	**12.19**	0.60	54.67	**56.56**	5.15
Large Intestine	17.50	2	**19.62**	1.00	224.99	**400.37**	21.18
Liver	32.36	2	4.33	0.94	116.91	119.20	11.00
Lung	6.85	2	1.53	0.97	17.93	18.85	1.69
Mesenteric Lymph Nodes	4.50	2	1.65	0.96	12.02	12.79	1.13
Muscle	4.65	2	1.34	0.98	11.46	11.82	1.08
Pancreas	7.68	2	1.49	1.00	24.64	25.68	2.32
Prostate	5.17	2	1.39	0.97	18.15	18.73	1.71
Small Intestine	29.51	2	**3.16**	0.88	101.43	**102.23**	9.55
Skin	3.31	2	NC	NC	8.57	NC	0.81
Spleen	4.88	2	1.90	0.92	13.35	14.66	1.26
Stomach	9.10	2	1.43	1.00	26.53	27.55	2.50
Submaxillary Glands	6.03	2	**28.26**	0.72	21.82	**27.84**	2.05
Testes	7.84	2	2.05	0.98	24.90	27.75	2.34
Thymus	6.26	2	1.62	0.92	15.75	16.73	1.48
Thyroid	4.57	2	1.68	0.96	12.33	13.17	1.16

Bold values are an approximation.
*Ratio of AUC_{last} values.
NC=Not Calculable.

Table 23: Pharmacokinetic Parameters of [^{14}C]-TR-701-Derived Radioactivity in Plasma and Tissues of Female Dogs Following a Single Oral Dose of [^{14}C]-TR-701.
(Sponsor's Table)

Tissue	C_{max} (µg/g)	T_{max} (hr)	$T_{½}$ (hr)	r^2	AUC_{last} (µg·hr/g)	$AUC_{0-\infty}$ (µg·hr/g)	Tissue: Plasma*
Plasma	2.59	2	NC	NC	7.83	NC	1.00
Adrenal Glands	5.46	2	1.22	0.95	19.50	19.87	2.49
Bone	0.28	2	NC	NC	0.70	NC	0.09
Brain	0.66	2	NC	NC	1.73	NC	0.22
Eye (solids)	7.22	2	**3.45**	0.98	34.98	**46.13**	4.47
Eye (vitreous)	0.00	NC	NC	NC	0.00	NC	0.00
Fat	1.48	2	NC	NC	NC	NC	NC
Heart	4.03	2	1.15	0.94	14.39	14.60	1.84
Kidney	12.72	2	**13.34**	0.50	59.07	**62.34**	7.55
Large Intestine	6.17	8	NC	NC	94.82	NC	12.12
Liver	25.66	2	**4.21**	0.80	109.12	**112.46**	13.94
Lung	5.42	2	1.25	0.96	18.99	19.38	2.43
Mesenteric Lymph Nodes	3.75	2	1.12	0.94	13.20	13.38	1.69
Muscle	2.99	2	1.20	0.94	10.77	10.96	1.38
Ovaries	3.63	2	NC	NC	9.35	NC	1.20
Pancreas	5.42	2	1.23	0.97	18.39	18.76	2.35
Small Intestine	18.62	2	**3.60**	0.86	85.92	**87.22**	10.98
Skin	2.73	2	NC	NC	7.22	NC	0.92
Spleen	3.92	2	1.27	0.96	13.64	13.95	1.74
Stomach	6.19	2	1.72	0.99	22.20	23.64	2.84
Submaxillary Glands	4.42	2	1.34	0.97	15.42	15.85	1.97
Thymus	4.10	2	1.20	0.95	14.35	14.61	1.83
Thyroid	3.00	2	1.42	0.96	10.89	11.26	1.39
Uterus	3.05	2	NC	NC	7.80	NC	1.00

Bold values are an approximation.
*Ratio of AUC_{last} values.
NC=Not Calculable.

Tissue Distribution Following a Single IV Dose

Tissue distribution following IV administration was similar to that following oral exposure Total exposure based on AUC was greatest in the liver, large intestine, small intestine, eye solids, and kidney of male and female dogs (Table 24 and Table 25). High levels of [^{14}C]-TR-701 equivalents were also detected in the testes, epididymis and spleen of males and the adrenal glands and lung of females. Tissues with the highest tissue:plasma AUC ratios included the kidney (12), liver (11), small intestine (10), eye solids (5.7), and large intestine (5.4) in males and the liver (15), eye (solids) (9.5), small intestine (9.0) and kidney (5.8) in females. The lowest exposure was found in eye vitreous, brain, bone, skin, and fat (tissue:plasma exposure ratios of essentially 0 to 0.27).

Table 24: Pharmacokinetic Parameters of [^{14}C]-TR-701-Derived Radioactivity in Plasma and Tissues of Male Dogs Following a Single IV Dose of [^{14}C]-TR-701.
(Sponsor's Table)

Tissue	C_{max} (µg/g)	C_0	T_{max} (hr)	$T_{½}$ (hr)	r^2	AUC_{last} (µg·hr/g)	$AUC_{0-\infty}$ (µg·hr/g)	Tissue: Plasma*
Plasma	NA	5.77	0.167	**1.45**	0.46	6.80	**9.04**	1.00
Adrenal Glands	2.13	NA	2	1.22	0.95	10.61	10.75	1.56
Bone	0.11	NA	2	NC	NC	0.48	NC	0.07
Brain	0.38	NA	2	NC	NC	1.68	NC	0.25
Epididymis	3.27	NA	2	1.55	0.97	16.93	17.48	2.49
Eye (solids)	6.24	NA	2	**3.75**	0.97	38.90	**50.49**	5.72
Eye (vitreous)	0.00	NC	NC	NC	NC	0.00	NC	0.00
Fat	0.00	NC	NC	NC	NC	0.00	NC	0.00
Heart	1.53	NA	2	NC	NC	6.66	NC	0.98
Kidney	10.31	NA	8	NC	NC	80.00	NC	11.77
Large Intestine	5.46	NA	4	**5.55**	0.87	36.46	**39.03**	5.36
Liver	10.65	NA	2	**24.73**	0.86	73.51	**77.92**	10.82
Lung	2.55	NA	2	1.34	0.99	12.98	13.22	1.91
Mesenteric Lymph Nodes	1.36	NA	2	NC	NC	5.74	NC	0.84
Muscle	1.32	NA	2	NC	NC	5.75	NC	0.85
Pancreas	1.87	NA	2	1.19	0.98	9.33	**9.44**	1.37
Prostate	1.47	NA	2	1.41	0.99	7.54	7.70	1.11
Small Intestine	11.59	NA	2	3.82	0.97	69.91	70.42	10.28
Skin	1.76	NA	2	NC	NC	NC	NC	NC
Spleen	2.56	NA	2	**29.69**	0.84	16.91	**18.10**	2.49
Stomach	1.65	NA	2	2.58	1.00	9.28	10.49	1.36
Submaxillary Glands	1.68	NA	2	1.24	0.98	8.47	8.58	1.25
Testes	5.42	NA	2	1.44	1.00	28.11	28.76	4.13
Thymus	1.56	NA	2	1.27	0.98	7.83	**7.94**	1.15
Thyroid	1.28	NA	2	1.43	0.98	6.56	**6.71**	0.96

Bold values are an approximation.
*Ratio of AUC_{last} values.
NC=Not Calculable.
NA=Not Applicable.

Table 25: Pharmacokinetic Parameters of [^{14}C]-TR-701-Derived Radioactivity in Plasma and Tissues of Female Dogs Following a Single IV Dose of [^{14}C]-TR-701.
(Sponsor's Table)

Tissue	C_{max} (ng/g)	C_0	T_{max} (hr)	$T_{½}$ (hr)	r^2	AUC_{last} (ng·hr/g)	$AUC_{0-\infty}$ (ng·hr/g)	Tissue: Plasma*
Plasma	NA	8.20	0.167	**1.31**	0.33	6.41	**8.53**	1.00
Adrenal Glands	2.85	NA	2	NC	NC	16.24	NC	2.53
Bone	0.15	NA	2	NC	NC	NC	NC	NC
Brain	0.31	NA	2	NC	NC	1.75	NC	0.27
Eye (solids)	5.59	NA	2	**10.00**	0.03	60.75	**93.50**	9.48
Eye (vitreous)	0.33	NA	2	NC	NC	NC	NC	NC
Fat	0.00	NC	NC	NC	NC	0.00	NC	0.00
Heart	2.00	NA	2	NC	NC	13.33	NC	2.08
Kidney	5.73	NA	2	**3.89**	0.78	36.98	**37.31**	5.77
Large Intestine	2.66	NA	2	6.67	0.92	23.75	26.24	3.71
Liver	12.91	NA	2	**24.84**	0.89	93.44	**97.19**	14.59
Lung	2.32	NA	2	**1.78**	0.87	15.30	**15.78**	2.39
Mesenteric Lymph Nodes	1.43	NA	2	1.19	0.99	8.51	8.59	1.33
Muscle	1.40	NA	2	NC	NC	8.64	NC	1.35
Ovaries	2.16	NA	2	NC	NC	11.17	NC	1.74
Pancreas	2.33	NA	2	1.28	0.98	14.39	14.55	2.25
Small Intestine	9.47	NA	2	4.01	0.97	57.74	58.30	9.01
Skin	1.37	NA	2	NC	NC	NC	NC	NC
Spleen	2.03	NA	2	7.09	0.92	13.99	14.63	2.18
Stomach	2.04	NA	2	**2.59**	0.71	13.30	**14.56**	2.08
Submaxillary Glands	1.96	NA	2	1.25	0.98	11.98	12.10	1.87
Thymus	2.06	NA	2	1.18	1.00	12.04	12.14	1.88
Thyroid	1.46	NA	2	**35.50**	0.91	11.60	**12.69**	1.81
Uterus	2.79	NA	4	NC	NC	6.90	NC	1.08

Bold values are an approximation.
*Ratio of AUC_{last} values.
NC=Not Calculable.
NA=Not Applicable.

Elimination in Urine and Feces

The percent of radioactive dose eliminated in urine and feces is shown in Table 26. Approximately 90% of the radioactivity was excreted in feces with most of the remainder excreted in urine regardless of gender or route of administration. Approximately 70% of the excreted [^{14}C]-TR-701-derived radioactivity was recovered in the first 24 hours after dosing regardless of dose route.

Table 26: Percent of Radioactivity in Urine and Feces of Dogs Following a Single Oral or IV Dose of [^{14}C]-TR-701. (Sponsor's Table)

Oral Dosing

Hours Post-Dosing	Males (% of Dose)		Females (% of Dose)	
	Urine*	Feces	Urine*	Feces
0 - 6	0.00	NA	5.71	NA
6 - 12	9.92	40.29	1.22	58.30
12 - 24	0.47	32.71	0.34	23.69
24 - 48	0.36	16.29	0.40	2.17
48 - 72	0.18	1.41	0.39	0.11
Total	10.94	90.70	8.06	84.27
Cage Wash	0.11		0.35	
Sum by Sex	**102**		**92.7**	
Average Total Excretion	**97.2**			

IV Dosing

Hours Post-Dosing	Males (% of Dose)		Females (% of Dose)	
	Urine*	Feces	Urine*	Feces
0 - 6	8.83	NA	7.60	NA
6 - 12	0.48	56.25	1.65	68.87
12 - 24	0.18	5.28	0.70	28.92
24 - 48	0.29	17.01	0.31	3.88
48 - 72	0.26	2.82	0.16	0.58
Total	10.05	81.35	10.43	102
Cage Wash	0.11		0.04	
Sum by Sex	**91.5**		**113**	
Average Total Excretion	**102**			

*Includes cage rinse.
NA=Not Applicable.

Metabolite Identification in Dog Urine, Feces and Plasma

Qualitative assessment of HPLC retention times indicated that no TR-701 parent compound was present in plasma, urine, or feces. The detected metabolites are identified in Table 27, and their distribution to urine, feces and plasma is summarized in Table 28. Only the active metabolite, TR-700, was detected in plasma. Metabolities in urine included primarily N-desmethyl TR-700 with lesser contributions of carboxy-TR-700 and desmethyl TR-700 sulfate, hydroxyl TR-700, TR-700, and an unknown metabolite. In feces, the primary metabolite was TR-700 sulfate (77-95% in feces) with lesser contributions from TR-700 and N-desmethyl TR-700.

Table 27: TR-701 Metabolites in Dog

Metabolite Number	Description
M1	N-desmethyl TR-700
M2	Carboxy-TR-700
M3	Hydroxy TR-700
M4	TR-700 sulfate
M5	TR-700
M6	Unknown
M7	N-desmethyl TR-700 sulfate

Table 28: Relative Amounts of Measured Radioactivity from TR-701 Metabolites in Plasma, Urine, and Feces. (Sponsor's Table)

Sample	Percent of Total Radioactivity(a)							
	Not Retained	M6 unknown	M7 demethyl sulfate	M1 demethyl	M2 carboxylic acid	M3 hydroxy	M4 sulfate	M5 TR-700
Female urine 6 hr oral 674011MA1-21-6	1.1	1.6	2.5	55.5	1.5	*< 1%	37.4	*< 1%
Male urine 12 hr oral 674011MA1-21-7	*< 1%	*1.0		39.6	*1.1		57.6	*< 1%
Female urine 12 hr oral 674011MA1-21-8	*3.1		*2.2	58.3			36.5	
Male feces 12 hr oral 674011MA1-25-5	7.2			*2.3			87.9	*2.6
Female feces 12 hr oral 674011MA1-25-6	10.7			3.1	*< 1%		68.9	16.8
Male plasma 10 min IV 674011MA1-22-3								*100.0
Male urine 6 hr IV 674011MA1-21-9	*< 1%		*1.8	65.6	*2.6		27.7	*1.5
Female urine 6 hr IV 674011MA1-21-10				56.5	*< 1%		41.8	*1.5
Male feces 12 hr IV 674011MA1-25-7	14.0			*2.0			78.5	*5.5
Female feces 12 hr IV 674011MA1-25-8	12.9			*1.1			84.9	*1.1

* indicates that the radioactive peak height had a S/N ratio that was less than 3.

Study Title: Tissue Distribution Using Quantitative Whole Body Autoradiography and Excretion Balance in Male and Female Rats Following Intravenous and Oral Administration of [^{14}C]-TR-701 (Study No.: PDM-08-0701-021A).

Methods

This GLP-compliant study including a quality assurance report was conducted (b) (4) between Sept., 2008 and January, 2013. Male and female albino Sprague-Dawley and pigmented Long Evans rats were administered single oral

(25 mg/kg) or IV (10 mg/kg) doses of [^{14}C]-TR-701. The distribution of radioactivity into select tissues was evaluated using quantitative whole body radiography. In addition, radioactivity in whole blood, plasma, and selected tissues were evaluated in a single male and female rat from each strain with liquid scintillation counting used to determine radioactivity. Urine and fecal samples were collected separately from SD rats beginning 24 hours after dosing and every 24 hours through 168 hours after dosing.

Results

Distribution studies with [^{14}C]-TR-701 with both IV and oral administration in rats and dogs indicated that radioactivity distributed to many tissues. In Sprague-Dawley rats, the organs with the highest concentrations in both genders following oral administration included liver, plasma, adrenal gland, brown fat, small intestine, and stomach. The tissues with the lowest concentrations included eye and brain. The high concentrations in small intestine following IV administration suggest biliary excretion. Reproductive organs in males (prostate, testis) contained moderate concentrations of radioactivity while female reproductive organs (uterus, ovary) contained 4-6 fold higher concentrations of radioactivity. In almost all tissues, radioactive levels decreased to below the lowest level of quantification by 72 hours after administration (Table 29 and Table 30).

While eye concentrations were very low, uveal tract tissue within the eye contained moderate concentrations of radioactivity. Concentrations were approximately 3-20 fold increased in pigmented Long Evans rats compared to albino Sprague-Dawley rats and the half-life was greatly increased by 10 fold to approximately 40 hours (Table 31 and Table 32).

Tissue distribution following IV administration (data not shown) was similar to that resulting from oral administration. However, large intestine radioactivity concentrations were much higher following oral administration. Following oral administration of 25 mg/kg [^{14}C]-TR-701, systemic exposure in terms of dose normalized AUC_{last} (plasma and whole blood), was roughly equivalent to the systemic exposure following IV administration of 10 mg/kg [^{14}C]-TR-701 in male and female Sprague-Dawley and Long Evans rats.

Table 29: Pharmacokinetics Parameters of [^{14}C]-TR-701-Derived Radioactivity in Plasma and Tissues of Male Sprague-Dawley Rats Following a Single Oral Dose of [^{14}C]-TR-701. (Sponsor's Table)

Tissue	C_{max} (ng equiv./g)	T_{max} (hr)	$T_{½}$ (hr)	r^2	$AUC_{0\text{-last}}$ (ng equiv-hr/g)	$AUC_{0\text{-}\infty}$ (ng equiv-hr/g)	Tissue: Plasma*
Plasma	17805	1	3.3	0.922	119258	119860	1.0
Blood	11674	1	3.4	0.936	79896	80419	0.7
Adrenal gland	13111	1	3.1	0.981	104899	105593	0.9
Bone	3267	2	**3.0**	0.774	11868	**14851**	0.1
Bone marrow	6139	1	**13.7**	0.600	37486	**114756**	0.3
Brain	956	1	**13.0**	0.211	4954	**14337**	0.0
Eye	346	8	**76.8**	0.902	16476	**36844**	0.1
Fat	9187	1	**5.0**	0.316	21339	**36618**	0.2
Fat - brown	12754	1	**13.3**	0.622	70688	**222031**	0.6
Harderian gland	8430	1	**10.1**	0.622	49959	**118016**	0.4
Heart	8929	2	**7.3**	0.976	56132	**109749**	0.5
Kidney	15223	1	3.0	0.996	107227	107789	0.9
Kidney - cortex	14032	1	3.0	0.997	113128	113803	0.9
Kidney - medulla	11382	2	**5.1**	0.952	68453	**106773**	0.6
Large intestine	52095	8	NC	NC	355136	NC	3.0
Liver	27612	4	3.1	0.999	219213	220658	1.8
Lung	9835	1	**8.6**	0.754	48001	**112470**	0.4
Lymph node	8845	2	**5.6**	0.981	52143	**86542**	0.4
Muscle	5554	1	**16.1**	0.703	34754	**123942**	0.3
Pancreas	8871	1	**7.3**	0.759	48605	**91650**	0.4
Pituitary gland	7700	4	NC	NC	40100	NC	0.3
Prostate	5884	1	**11.4**	0.904	35156	**95453**	0.3
Salivary gland	7046	1	**9.0**	0.710	42648	**91835**	0.4
Skin	8819	1	**8.4**	0.343	34052	**86343**	0.3
Small intestine	16806	2	3.0	0.993	121079	121740	1.0
Spleen	9411	1	**6.7**	0.678	43612	**79486**	0.4
Stomach	15839	24	NC	NC	240432	NC	2.0
Testis	4186	1	**25.4**	0.997	21954	**113498**	0.2
Thymus	6894	4	NC	NC	42241	NC	0.4
Thyroid gland	8236	4	NC	NC	43336	NC	0.4
Urinary bladder	5624	2	4.3	0.975	62375	63963	0.5
Uveal Tract	6321	1	**3.2**	0.712	19074	**25470**	0.2

NC = Not calculated due to insufficient data.
* =Tissue:plasma ratio based on $AUC_{0\text{-last}}$
Values in **bold** are an approximation.

Table 30: Pharmacokinetics Parameters of [^{14}C]-TR-701-Derived Radioactivity in Plasma and Tissues of Female Sprague-Dawley Rats Following a Single Oral Dose of [^{14}C]-TR-701. (Sponsor's Table)

Tissue	C_{max} (ng equiv./g)	T_{max} (hr)	$T_{½}$ (hr)	r^2	$AUC_{0\text{-last}}$ (ng equiv·hr/g)	$AUC_{0\text{-}\infty}$ (ng equiv·hr/g)	Tissue: Plasma*
Plasma	43548	4	3.7	0.999	343753	349643	1.0
Blood	25780	4	3.8	0.999	204799	208587	0.6
Adrenal gland	25958	4	3.7	0.997	225935	229318	0.7
Bone	2127	2	5.9	0.970	19239	20511	0.1
Bone marrow	11997	2	3.7	0.996	108714	110366	0.3
Brain	1612	4	NC	NC	8428	NC	0.0
Eye	990	8	**88.5**	0.956	49389	**67876**	0.1
Fat	15410	4	3.4	1.000	101151	102331	0.3
Fat - brown	27110	4	3.8	1.000	201356	204954	0.6
Harderian gland	19117	4	3.7	1.000	143655	145936	0.4
Heart	18781	4	3.8	1.000	154143	156836	0.4
Kidney	21498	4	4.0	0.999	187710	191699	0.5
Kidney - cortex	21842	4	4.0	0.999	192770	196910	0.6
Kidney - medulla	20187	4	3.9	1.000	167764	171002	0.5
Large intestine	27419	8	NC	NC	260457	NC	0.8
Liver	40057	4	4.1	0.999	361246	369755	1.1
Lung	21311	4	3.7	0.999	173597	176553	0.5
Lymph node	15523	4	4.0	1.000	131389	134355	0.4
Muscle	11607	4	3.8	0.999	97487	99168	0.3
Ovaries	21002	4	3.9	1.000	169512	172795	0.5
Pancreas	15740	4	3.8	0.999	134528	136816	0.4
Pituitary gland	18292	4	3.9	1.000	139957	142872	0.4
Salivary gland	15489	4	3.9	1.000	122702	125059	0.4
Skin	24689	4	3.3	0.997	148159	149716	0.4
Small intestine	29647	4	5.3	0.988	329624	348147	1.0
Spleen	13574	4	4.0	1.000	115750	118246	0.3
Stomach	43627	2	7.8	0.990	211141	236862	0.6
Thymus	13687	4	3.8	0.999	113828	115935	0.3
Thyroid gland	16299	4	3.3	1.000	120256	121450	0.3
Urinary bladder	15160	4	4.9	1.000	138471	144690	0.4
Uterus	26054	4	3.6	0.999	200601	203463	0.6
Uveal Tract	10445	4	3.6	0.985	60542	61530	0.2

NC = Not calculated due to insufficient data.

* =Tissue:plasma ratio based on $AUC_{0\text{-last}}$

Values in **bold** are an approximation.

Table 31: Pharmacokinetics Parameters of [^{14}C]-TR-701-Derived Radioactivity in Plasma and Tissues of Male Long-Evans Rats Following a Single Oral Dose of [^{14}C]-TR-701. (Sponsor's Table)

Tissue	C_{max} (ng equiv./g)	T_{max} (hr)	$T_{½}$ (hr)	r^2	$AUC_{0\text{-last}}$ (ng equiv·hr/g)	$AUC_{0\text{-}\infty}$ (ng equiv·hr/g)	Tissue: Plasma*
Plasma	21869	4.0	2.8	0.999	162621	163343	1.0
Blood	13039	4.0	2.9	0.999	99688	100211	0.6
Eye	478	8.0	NC	NC	6856	NC	0.0
Fat	3457	2.0	**7.1**	0.506	15374	**33061**	0.1
Skin	6938	4.0	NC	NC	44588	NC	0.3
Uveal Tract	165938	8.0	44	0.986	3773492	3990934	23

NC = Not calculated due to insufficient data.

* =Tissue:plasma ratio based on $AUC_{0\text{-last}}$

Values in **bold** are an approximation.

Table 32: Pharmacokinetics Parameters of [^{14}C]-TR-701-Derived Radioactivity in Plasma and Tissues of Female Long-Evans Rats Following a Single Oral Dose of [^{14}C]-TR-701. (Sponsor's Table)

Tissue	C_{max} (ng equiv./g)	T_{max} (hr)	$T_{½}$ (hr)	r^2	$AUC_{0\text{-last}}$ (ng equiv·hr/g)	$AUC_{0\text{-}\infty}$ (ng equiv·hr/g)	Tissue: Plasma*
Plasma	30848	4.0	7.8	0.909	314804	315501	1.0
Blood	18805	4.0	3.7	0.993	182755	185549	0.6
Eye	1287	8	**30**	0.837	33843	**44222**	0.1
Fat	10872	2	17	0.997	85230	93378	0.3
Skin	12792	2	4.5	0.998	114850	118451	0.4
Uveal Tract	148786	8	**37**	0.836	4321973	**4614069**	14

NC = Not calculated due to insufficient data.

* =Tissue:plasma ratio based on $AUC_{0\text{-last}}$

Values in **bold** are an approximation.

Concentration in Plasma and Whole Blood Following a Single IV Dose

The dose normalized $AUC_{0\text{-last}}$ for plasma (Table 33) and blood (Table 34) was similar for Sprague-Dawley and Long Evans rats with females from both species having higher values than males. Plasma and blood half-life values were similar for both genders and both species. The blood:plasma $AUC_{0\text{-last}}$ ratio was consistently 0.6 indicating ^{14}C-TR-701 derived radioactivity did not partition well into red blood cells.

Table 33: Plasma Pharmacokinetics Following a Single IV Dose of ^{14}C-TR-701. (Sponsor's Table)

	SD		LE	
	M	F	M	F
C_0 (ng equiv./g)	34783	33887	46685	19374
$T_{1/2}$ (h)	4.1	4.0	3.3	3.3
$AUC_{0\text{-last}}$ (µg equiv•hr/g)	42166	101634	63895	99102
Dose Normalized $AUC_{0\text{-last}}$ (µg equiv•hr•kg/[g•mg])*	4634	11169	7021	10890

Table 34: Blood Pharmacokinetics Following a Single Oral Dose of ^{14}C-TR-701. (Sponsor's Table)

	SD		LE	
	M	F	M	F
C_{max} (ng equiv./g)	11674	25780	13039	18805
$T_{1/2}$ (h)	3.4	3.8	2.9	3.7
$AUC_{0\text{-last}}$ (µg equiv•hr/g)	79896	204799	99688	182755
Dose Normalized $AUC_{0\text{-last}}$ (µg equiv•hr•kg/[g•mg])*	2755	7062	3438	6302

Elimination in Urine and Feces

Fecal excretion was the primary route of elimination in rats treated with ^{14}C-TR-701 regardless of the route of administration. Approximately 87-89% and 83-86% of the dose was excreted in feces for males and females respectively. Males appeared to

eliminate the radioactivity more quickly with 80% elimination in the first 24 hours compared to 48 hours needed for females. The calculated cumulative excretion is summarized in Table 35.

Table 35: Percent of Dose of Radioactivity in Urine and Feces of Spraue-Dawley Rats Following a Single Oral or IV Dose of [^{14}C]-TR-701. (Sponsor's Table)

Oral Dosing

Hours Post-Dosing	Males (Mean %±SD)				Females (Mean %±SD)			
	Urine		Feces		Urine		Feces	
0 - 6	4.77	(2.30)	NS	NS	4.80	(0.58)	NS	NS
6 - 12	3.18	(0.97)	26.69	(11.56)	3.16	(0.64)	2.37	(2.45)
12 - 24	0.54	(0.25)	53.24	(10.20)	1.83	(0.49)	58.58	(4.47)
24 - 48	0.09	(0.03)	7.16	(3.08)	0.45	(0.13)	18.86	(2.18)
48 - 72	0.06	(0.05)	1.73	(0.55)	0.11	(0.06)	2.91	(1.13)
72- 96	0.02	(0.00)	0.54	(0.36)	0.05	(0.04)	0.40	(0.21)
96 - 120	0.01	(0.00)	0.06	(0.03)	0.04	(0.04)	0.05	(0.01)
120 - 144	0.01	(0.00)	0.02	(0.00)	0.03	(0.03)	0.03	(0.02)
144 - 168	0.00	(0.00)	0.01	(0.00)	0.02	(0.02)	0.02	(0.01)
Cage Wash	0.01	(0.01)	NS	NS	0.17	(0.20)	NS	NS
Total	8.69		89.44		10.67		83.22	
Sum by Sex	**98.1**				**93.9**			
Average Total Excretion:	**96.0**							

IV Dosing

Hours Post-Dosing	Males (Mean %±SD)				Females (Mean %±SD)			
	Urine		Feces		Urine		Feces	
0 - 6	8.42	(1.86)	NS	NS	5.78	(1.34)	NS	NS
6 - 12	2.45	(0.95)	44.25	(35.65)	2.55	(0.97)	6.49	(5.62)
12 - 24	0.28	(0.07)	40.04	(22.94)	1.16	(0.40)	65.44	(5.31)
24 - 48	0.08	(0.01)	2.87	(1.97)	0.32	(0.14)	11.82	(2.45)
48 - 72	0.03	(0.01)	0.46	(0.48)	0.05	(0.02)	1.87	(0.96)
72- 96	0.01	(0.00)	0.12	(0.10)	0.01	(0.01)	0.27	(0.15)
96 - 120	0.01	(0.00)	0.03	(0.00)	0.01	(0.00)	0.05	(0.01)
120 - 144	0.01	(0.00)	0.02	(0.01)	0.01	(0.00)	0.02	(0.00)
144 - 168	0.01	(0.00)	0.01	(0.00)	0.01	(0.00)	0.02	(0.00)
Cage Wash	0.02	(0.01)	NS	NS	0.01	(0.01)	NS	NS
Total	11.31		87.79		9.92		85.98	
Sum by Sex	**99.1**				**95.9**			
Average Total Excretion:	**97.5**							

NS=No Sample collected.

Metabolite Identification in Plasma, Urine, and Feces

Seven TR-701 metabolites were identified in the plasma, urine, and feces of Sprague-Dawley rats (Table 36).

Table 36: TR-701 Metabolites in Sprague-Dawley Rats

Metabolite Number	Description
M1	N-demethyl TR-700
M2	Carboxy-TR-700
M3	Hydroxy TR-700
M4	TR-700 sulfate
M5	TR-700
M6	Unknown
M7	N-demethyl TR-700 sulfate

The majority (>90%) of radioactivity in plasma was determined to be TR-700 in both males and females (Table 37). Also in both males and females, the number of metabolites in urine was the same, but males had more M7 and less M1 in urine compared to females. In feces, males and females had the same metabolites, but greater amounts of M4 were detected in the feces from males compared to females.

Table 37: Relative Amounts of Measured Radioactivity from TR-701 Metabolites Excluding Unretained Activity. (Sponsor's Table)

Sample	Percent of Total Radioactivity (b)						
	M6 unknown	M7 demethyl sulfate	M1 demethyl	M2 carboxylic acid	M3 hydroxy	M4 sulfate	M5 TR-700
Male plasma 4 hr oral 674012MA1-27-17							100.0
Female plasma 2 hr oral 674012MA1-27-16							100.0
Male urine 6 hr oral 674012MA1-21-9	2.4	24.5	*<1	7.8	*<1	62.3	2.3
Female urine 6 hr oral 674012MA1-21-11	3.3	*<1	11.8	9.2	*1.0	67.8	6.0
Male feces 24 hr oral 674012MA1-38-5				*1.4		96.7	*1.9
Female feces 24 hr oral 674012MA1-38-6 (c)						90.0	*8.7
Male plasma 2 hr IV 674012MA1-27-21							100.0
Female plasma 2 hr IV 674012MA1-27-22							100.0
Male urine 6 hr IV 674012MA1-21-13	1.5	21.3	1.6	7.5	<1	65.0	2.6
Female urine 6 hr IV 674012MA1-21-15	3.3	*<1	14.0	8.3	*<1	65.0	8.3
Male feces 24 hr IV 674012MA1-38-2				*1.9		97.6	*<1
Female feces 24 hr IV 674012MA1-38-3						87.1	12.9

* Indicates that the radioactive peak height had a S/N ratio that was less than 3.

(a) No TR-701 was identified in any of the samples analyzed.

(b) The relative amounts of radioactivity for individual components are calculated as a percentage of the sum of all of the integrated peaks.

(c) This sample also contained a small amount of unretained radioactivity corresponding to approximately 1.3% of the total measured radioactivity.

Study Title: Protein Binding of TR-700 in Heparinized Mouse, Rat, Dog, and Human Plasma (Study No.: PDM-13-070-080).

Methods
In this non-GLP study performed at Dong-A Protein Pharmaceuticals, Co., Ltd. in Korea, binding of TR-700 (1 and 10 μg/ml) in mouse, rat, dog, and human heparinized plasma was evaluated using ultracentrifugation.

Results
A high percentage of TR-700 bound plasma proteins in all species independent of TR-700 concentration. The rank order of binding was rat (97.5%) > mouse (85.1%) > human (80.4%) > dog (74.3%).

Study Title: Protein Binding of TR-701 and TR-700 in EDTA-Treated Mouse, Rat, Dog, and Human Plasma (Study No.: PDM-13-070-081).

Methods
In a non-GLP study conducted at Dong-A Pharmaceuticals, Co., Ltd. in Korea, protein binding of TR-701 and TR-700 (concentrations of 0.1, 1, 3, 10 and 50 μg/ml each) in mouse, rat, dog, and human EDTA-treated plasma was evaluated using ultracentrifugation. TR-701 was stable in EDTA-treated plasma from all species and not significantly metabolized to TR-700.

Results
A high percentage of both compounds were bound to plasma proteins in all test species independent of concentration over the range tested. The rank order of TR-701 plasma protein binding was rat (97.2%) > human (86.6%) > dog (85.1%), > mouse (74.8%). For TR-700 the rank order of plasma protein binding was rat (97.7%) > mouse (92.6%) > human (84.6%), > dog (78.0%).

Study Title: Study of the Cellular Uptake and Subcellular Distribution of the Oxazolidinone Tedizolid in Murine J774 Macrophages: Lack of Association with Mitochondria (Study No.: PDM-13-0701-082).

Methods
In this non-GLP study, TR-700 at concentrations of 20 μg/ml was incubated with murine J774 macrophages for two hours followed by cell collection, homogenization and subcellular separation through sucrose gradient centrifugation. Localization of TR-700 was assessed through differential centrifugation and isopycnic centrifugation. TR-700 was extracted from cell fractions by phase partition and protein precipitation in chloroform:methanol (8:4) and TR-700 was quantified after extraction by LC/MS.

Results
Most of the added TR-700 was associated after centrifugation with the high-speed supernatant which also contained the largest part of lactate dehydrogenase (LDHase), but not with enzymes associated with mitochondria (cyt-oxidase) or lysozomes (NABgase).

Metabolism

The primary metabolites for dogs and rats were described in Study Nos. PDM-08-0701-020A and PDM-08-0701-021A (reviewed above).

Study Title: *In Vitro* Metabolic Stability of TR-701 and TR-700 (Study No.: PDM-0701-07-028).

Methods
In several sub-studies, the *in vitro* metabolic stability of TR-701 and TR-700 was examined when incubated with different buffers and stabilizing solutions (Study No.: RN-pk-016), liver microsomes from rat, dog, monkey, and human (Study No.: RN-pk-007, RN-pk-020, and RN-pk-021), liver S9 fraction, liver homogenate, gastric juice bile and urine from rat (Study No.: RN-pk-016), plasma from mouse, rat, dog, and human (Study No.: RN-pk-034), or blood from rat, dog, and human (Study No.: RN-pk-035 and RN-pk-037).

Results
Study No. RN-pk-007: TR-701 was determined to be stable in PBS (pH 7.4) and in liver microsomes derived from mice, rats, and humans, but unstable in rat liver S9 fraction with 49% and 62% of TR-701 remaining after a 2 hour incubation with and without NADPH.
Study No. RN-pk-016: Both TR-701 and TR-700 were stable for up to 48 hours in solutions with pH 2 to 11, but both were unstable and largely destroyed in pH 12 buffer solution. Both compounds were stable in gastric juices, urine, and liver microsomes of rats. TR-701 was very unstable in rat plasma, blood, bile, and liver homogenates at 37°C, but it was stable for 18 hours at room temperature in deproteinized plasma and in rat plasma at -70°C. TR-700 was stable in rat plasma at a concentration of 1 μg/ml, rat blood at 1 and 10 μg/ml; rat bile at 1 μg/ml; and liver homogenate at 0.1 and 10 μg/ml.
Study RN-pk-020 and RN-pk-021: TR-701 and TR-700 were largely stable in rat, dog, monkey, and human liver microsomes after incubation for up to 120 minutes.
Study RN-pk-034: TR-701 (2 μg/ml) diminished rapidly and converted to TR-700 at 37°C in mouse, rat, dog and human plasma with $t_{1/2}$ values on the order of 30 minutes for all test species.
Study RN-pk-035 and RN-pk-037: EDTA prevented the conversion of TR-701 to TR-700 in rat, dog, and human blood samples at room temperature (approximately 3% TR-700 formed) and to a slightly greater degree at 4°C (approximately 0.6% TR-700 formed). TR-701 was greater than 50% converted to TR-700 in heparin-treated blood samples from humans at room temperature, but only 17.4% converted at 4°C.

Study Title: Effect of Twice-Daily Oral Dosing of TR-701 for 14 Days on Rat Liver Microsomal Enzyme Activity (Study No.: PDM-07-0701-031).

Methods
Male Sprague-Dawley rats received BID oral doses of vehicle (distilled water; n = 4) or 20 mg/kg TR-701 (n = 5) for two weeks (Days 0-13). On Day 14, animals were sacrificed and approximately 2 grams of liver was excised from each rat followed by liver homogenization. Liver extracts were assessed for specific CYP-450 isozyme activity including activity of CYP1A, CYP2B, CYP3A, and CYP2E.

Results

The enzymatic activities of CYP1A, CYP3A, and CYP2E in liver homogenate extracts prepared from the TR-701-treated animals were similar to those occurring in liver extracts from vehicle-control animals. CYP2B activity was higher in liver extracts for TR-701-treated animals (106.8 ± 2.6 pmol/mg protein/min.) compared to control extracts (95.1 ± 7.9 pmol/mg protein/min.).

Study Title: Metabolic Stability of TR-701 and Formation of TR-700 Using a Rat Liver S9 Fraction (Study No.: PDM-09-0701-010).

Methods
This non-GLP study was conducted (b) (4) beginning in 2007 and ending in 2009. TR-701 (50 μg/ml) was incubated with an S9 metabolic activation system produced from a phenobarbital/5,6-benzoflavone-induced liver from a male Sprague-Dawley rat. Incubations were conducted in duplicate at 37°C for 30, 60, 120 and 240 minutes.

Results
TR-701 at a concentration of 50 μg/ml was readily metabolized by induced-liver S9 fraction. Approximately 50% of TR-701 was metabolized after 4-hour incubation with a $t_{1/2}$ calculated to be 3.9 hours. The metabolism was dependent on S9 activation, but occurred in the absence of an NADPH-generation system indicating that NADPH-dependent oxidative systems (like CYP-450 enzymes) were not required for the metabolism of TR-701. The formation of TR-700 paralleled the disappearance of TR-701 and essentially all the TR-701 was metabolized to TR-700. Metabolism of TR-701 was abolished with a combination of acetic acid and a phosphatase inhibitor cocktail.

Study Title: Assessment of Systemic Exposure to TR-700 Enantiomers After Single-Dose Oral Administration of TR-701 FA to Sprague Dawley Rats (Study No.: PDM-13-0701-083).

Methods
This non-GLP study was conducted (b) (4) in 2013. The pharmacokinetic profiles of the (R)- and (S)-enantiomers of TR-700 were evaluated in Sprague-Dawley rats (6 male and 6 female) after administration of a single oral dose of TR-701 FA (a pure [R]-enantiomer). Dose levels were 30 mg/kg for males and 10 mg/kg for females. Blood samples for PK analysis were collected from the jugular vein at approximately 1, 2, 3, 6, 12, and 24 hours following dose administration. Blood was collected from 3 rats/gender at each time-point.

Results
Following oral administration of TR-701 FA, only the [R]-TR-700 enantiomer was detected in plasma. Plasma concentrations of the [S]-TR-700 enantiomer were below the limit of quantification (<50 ng/ml) at all sampling time points in both genders.

Excretion
The primary excretion pathways (feces and urine) for dogs and rats were described in Study Nos. PDM-08-0701-020A and PDM-08-0701-021A (reviewed above). In addition, biliary excretion was examined in the Study No.: PDM-07-0701-030 below.

1. **Measuring Biliary Excretion of TR-700 After a Single Intravenous Administration of TR-701 to Rats** (Study No.: PDM-07-0701-030).

Methods
In this Non-GLP study, TR-701 was administered in an IV bolus 0f 13.3 mg/kg to 3 male Sprague-Dawley rats and the extent of biliary excretion of TR-700 was assessed. The bile ducts of rats were cannulated and bile samples were obtained between 0 and 1 hours, 1-2 hours, 2-3 hours, 4-7 hours, and 7-24 hours after administration. TR-700 was quantified using UV-HPLC with a detection limit of 0.1 μg/ml.

Results
TR-700 was detected in the bile of all 3 animals between 1 and 3 hours after administration with negligible levels after 4 hours. The mean amount of TR-700 in bile collected 0-4 hours after dosing was low (1.76 ± 0.93% of total dose).

6 General Toxicology

6.1 Single-Dose Toxicity

Table 38: Summary of Single-Dose Toxicology Studies

Study Type/number	TR-701 doses	N/sex/dose	Results	NOAEL
Single oral dose study in Mice/ TOX-07-0701-007.	0, 500, 1000, and 2000 mg/kg	5/sex/dose	Rough fur, loss of fur, ptosis, and decreased locomotor activity at ≥ 1000 mg/kg	500 mg/kg
Single oral dose study in rats/ TOX-07-0701-008.	0, 500, 1000, and 2000 mg/kg	5/sex/dose	At mid- and highest doses: Two high-dose female deaths, rough fur, loss of fur, decreased locomotor activity, weakening, diarrhea, body weight decrease cecal di./'lation, splenic atrophy, thymic atrophy, reddish spots on stomach. At all doses: marked cecal blood vessels, decreased body weight	No NOAEL identified
Single IV dose study of TR-701 in Mice/ TOX-07-0701-010	0, 62, 125, and 250 mg/kg	5/sex/dose	In the high-dose group, 2 male and 1 female deaths, decreased locomotor activity and dyspnea (high dose males, mid-dose females), dilation of cecum in mid-dose males, and high-dose females, decreased/suppressed body weight gain at all doses.	No NOAEL identified
Single IV dose toxicity of TR-701 in Rats/ TOX-07-0701-010	0, 62, 125, and 250 mg/kg	5/sex/dose	In the high-dose group, 3 male and 3 female deaths, dyspnea in all high-dose animals, decreased body weight in mid-dose males and high-dose females, dilation of cecum in all males and females at ≥ 125 mg/kg, marked blood vessels in cecum in females in 125 mg/kg group.	NOAEL of 62 mg/kg

6.2 Repeat-Dose Toxicity

Study title: 4-Week Repeated Oral Dose Toxicity Study of DA-7218 in Rats

Study no.:	TOX-07-0701-014A
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 9, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TR-701 (DA-7218), Lot # Oxa-004, purity of 97.91%

Key Study Findings

- Gender differences were observed in toxicity in association with greater systemic exposure in females.
- The high-dose (100 mg/kg/day) produced substantial mortality in females with a total of 13 animals found dead or euthanized in extremis on Days 9 or 10.
- The target organs for toxicity in females were blood cells (reduced WBC, reticulocyte and neutrophil percentages) mesenteric lymph nodes (atrophy), spleen (atrophy), thymus (atrophy, reduced weights), bone marrow (atrophy), liver (increased ALT and ALP, hepatocellular hypertrophy), and GI tract (enlarged cecums, stomach erosion, duodenal inflammation, erosion).
- High-dose males demonstrated decreased RBCs, increased BUN, enlarged cecums, and reduced thymus and liver weights; however, no histopathology was noted in males.
- The NOAEL was estimated to be 30 mg/kg/day for males and 10 mg/kg/day for females.

Methods

Doses:	0, 10, 30, and 100 mg/kg/day
Frequency of dosing:	Once per day
Route of administration:	Oral gavage
Dose volume:	10 ml/kg
Formulation/Vehicle:	Sterile distilled water
Species/Strain:	Sprague-Dawley rats
Number/Sex/Group:	10/sex/group for the Main Study as shown in Table 39.
Age:	6-weeks at study initiation
Weight:	At study initiation; Males: 73.9 to 120.4g; Females: 117.5 to 148.4 g.
Satellite groups:	Toxicokinetic animals: 11/sex/group. Recovery animals: 5/sex/group for the Vehicle Control Group and the high-dose group (100 mg/kg/day)
Unique study design:	Male and female rats were administered TR-701 by oral gavage for 28 days. Main Study animals were euthanized after 28 days of dosing (Day 29), and Recovery Animals were euthanized four weeks later.

Deviation from study protocol: Deviations from the study protocol were noted, but none was considered to have altered the results or integrity of the study.

Table 39: Study Design for Study No.: TOX-07-0701-014A. (Sponsor's Table)

Group		Sex	No. of animals	Animal ID		Dosing Volume (mL/kg)	Dose level (mg/kg)
				main	recovery		
Main Study	Vehicle Control	M	15	1 - 10	11 - 15	10	0
		F	15	89 - 98	99 - 103	10	0
	T1	M	10	16 - 25		10	10
		F	10	104 - 113		10	10
	T2	M	15	26 - 35	36 - 40	10	30
		F	15	114 - 123	24 - 128	10	30
	T3	M	15	41 - 50	51 - 55	10	100
		F	15	129 - 138[a)]	13 9-143 [a,b)]	10	100
TK	T1	M	11	51 - 66		10	10
		F	11	144 - 154		10	10
	T2	M	11	67 - 77		10	30
		F	11	155 - 165		10	30
	T3	M	11	78 - 88		10	100
		F	11	166 – 176[b)]		10	100

[a)] Ten main T3 females (No. 129 - No. 138) and one recovery T3 female (No. 142) were found dead or sacrificed moribund on Day 10.

[b)] Six TK T3 females (Nos. 166, 167, 172, 174, 175, and 176) and four T3 females assigned to recovery group (Nos. 139, 140, 141, and 143) were used for plasma concentration analysis on Day 10.

Observations and Results

Table 40: Observation Table for Study No.: TOX-07-0701-014A

Parameter	Schedule
Mortality and Clinical Signs	Mortality was assessed BID for the duration of the study. Clinical signs were assessed daily for 7 days before randomization and BID during the Main and Recovery Studies.
Body Weights	Individual body weights were measured approximately once per week beginning before the start of dosing and throughout the dosing period including on the scheduled necropsy day.
Food Consumption	Individual food consumption (g/animal/day) was recorded approximately weekly during the pretest period and throughout the study.
Hematology and Coagulation Clinical Chemistry Urinalysis	Blood samples for hematology and clinical chemistry analysis were collected from all animals on the scheduled Main Study and Recovery necropsy days. Animals were fasted overnight prior to blood collection. Urine samples were collected from 5 animals/sex for all

	groups except high-dose females during the last week of the Main Study, and for all groups except high-dose females during the Recovery period.
Toxicokinetics	Blood samples were collected from each toxicokinetic animal (10, 30, and 100 mg/kg/day TR-701) prior to dosing, and at 0.25, 0.5, 1, 2, 4, 6, 10 and 24 hours after dosing on Days 1, 10 (100 mg/kg/day females only) and 28. Plasma concentrations of TR-700 were determined using a LC/MS/MS technique.
Ophthalmic Examinations	Ophthalmologic examinations were conducted on all animals prior to randomization, in the last weeks of the treatment period for all animals in the vehicle control males and females, mid-dose females, and high-dose males and in vehicle control males and females, mid-dose males and females and high-dose males during recovery period.
Necropsy	All surviving Main Study animals were euthanized and necropsied on Day 29. Recovery animals were euthanized and necropsied four weeks later.

Mortality

Eleven high-dose females from the Main Study and Recovery animals and two high-dose females from the toxicokinetic animals were found dead or euthanized in extremis on Days 9 or 10.

Clinical Signs

Treatment-related clinical signs in the high-dose females included abdominal distention, weakening, emaciation, decrease of locomotor activity, rough fur, loss of fur, and ptosis. In high-dose males 3 animals with loss of fur, 1 animal with rough fur, 1 animal with eye discharge, and 3 animals with abdominal distention were noted. Isolated animals of both genders in lower dose groups demonstrated loss of fur.

Body Weights

High-dose females demonstrated a significant decrease in body weight (-26%) compared to control animals on Day 9.

Feed Consumption

In male animals, significant reductions in food intake were noted on Days 2 (mid- and high-dose animals) and 16 (high-dose only). In females significantly lower food intake was noted on Days 2 (low- and mid-dose groups), Day 9 (all TR-701 treatment groups) and Day 22 (mid-dose group only)

Ophthalmoscopy

Ocular examinations included: binocular indirect ophthalmoscopy after pupil dilation.

No treatment-related ophthalmology findings were observed.

ECG: not performed

Hematology

In high-dose males from the Main Study, significantly decreased RBCs (-8%) and neutrophil percentage (-29%) and increases in mean corpuscular volume (MCV; 7%) and mean corpuscular hemoglobin (MCH; -8%) were observed. In the low- and mid-dose groups, a significant increase in lymphocyte percentage (mid-dose group only), and decreases in neutrophil (mid-dose group only; -50%) and monocyte percentages (low- and mid-dose groups only) were detected.

In females from the Main Study, high-dose females were not examined for hematology due to substantial mortality. Mid-dose females demonstrated significantly decreased WBC (-23%), reticulocyte (-45%) and neutrophil (-53%) percentages and increases in MCV (+5%), MCH (+4.6%) and lymphocyte percentage (+6.6%). Low-dose females demonstrated significantly decreased neutrophil percentage (-37%), and increased lymphocyte percentage (+5%).

Following the recovery period, significant increases in mean corpuscular hemoglobin concentration (MCHC) were observed in the mid- and high-dose males.

Clinical Chemistry

In high-dose males in the Main Study, significantly increased BUN plasma levels were noted. In mid-dose females (no measurements for high-dose females), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were significantly decreased and serum glucose, albumin/globulin ratio (also in low-dose females), and phospholipid were observed.

In Recovery animals, significantly increased albumin/globulin ratio (mid- and high-dose males), significantly decreased creatinine and sodium (mid-dose females), and increased inorganic phosphorus and potassium (mid-dose females) were noted.

Urinalysis

Low urine pH (7.3 compared to 8.5 for control males) was noted in high-dose males in the Main Study. In Recovery animals, significantly increased urine volume was noted in mid-dose males.

Gross Pathology

In males and females, enlarged cecums were observed in a majority of all TR-701 groups with only a 20% incidence in the low-dose males. This effect was considered to be secondary to antibiotic effects on GI microflora. In addition small spleen was noted in 3/11, and small thymus in 5/11 of the moribund high-dose females. Also in moribund females, enlarged (7/11) and thin walled (4/11) intestine were observed. At recovery, enlarged spleen was observed in 2 males at 100 mg/kg/day, but no gross pathology findings were observed in Recovery mid-dose females.

Organ Weights

Absolute organ weights were measured and relative organ weights (organ to terminal body weight ratios) were calculated. Organ weights for animals found dead or euthanized in extremis were not measured.

In Main Study animals, absolute and relative heart and thymus weights and relative liver weights were significantly reduced by more than 10% in high-dose males. In mid-dose females, the absolute and relative weights for thymus were significantly decreased as well as absolute weights for heart and lung and relative weights for salivary glands. In low-dose but not mid-dose females relative heart weights were significantly reduced by 10%.

At Recovery, male organ weights were similar to control, but in mid-dose females had increased relative weights for spleen (+15%), kidneys (+10%), and adrenal glands (+23%).

Histopathology

Adequate Battery

Yes. The histopathology findings are shown in Table 126.

Peer Review

No

Histological Findings

All of the histopathology findings were observed in mid- and high-dose (found dead and euthanized in extremis) females as shown below in Table 41 below. Bone marrow atrophy was noted in a majority of mid-dose females and all the high-dose females. In addition, liver hepatocellular hypertrophy, thymus atrophy, spleen atrophy, stomach erosion, mucosal inflammation/atrophy, and mesenteric lymph node atrophy were noted in high-dose females.

Table 41: Female Histopathology Findings for Study No.: TOX-07-0701-014A.
(Sponsor's Table)

Organ	Dose (mg/kg/day)			
	0	10	30	100
No. of examinations	10	10	10	0 (11)[a)]
Liver				
Hepatocellular hypertrophy	0	-	0	0 (4)
Thymus				
Atrophy	0	0	0	0 (11)
Spleen				
Atrophy	0	0	0	0 (8)
Stomach				
Erosion	0	-	0	0 (3)
Duodenum				
Mucosal inflammation/atrophy	0	0	0	0 (11)
Ulcer	0	0	0	0 (1)
Erosion	0	0	0	0 (7)
Cecum				
Atrophy	0	-	0	0 (5)
Mesenteric lymph node				
Atrophy	0	-	0	0 (8)
Femur				
Bone marrow atrophy	0	0	5 *	0 (11)
Sternum				
Bone marrow atrophy	0	0	6 *	0 (11)

* Significantly different from control using Fisher's exact two tailed test ($P<0.05$).

a) The number in the parenthesis represents numbers of animals found dead and sacrificed moribund.

Special Evaluation

Immunology Evaluation

Spleen cells were collected and Natural Killer cell activity and lymphocyte population analysis (flow cytometry) were examined in all animals euthanized at the scheduled necropsy. NK cell activity was not decreased in any TR-701 animals. In high-dose males in the Main study, B cells and a non-B cell/T cell subpopulation were significantly decreased, but the same changes were not noted in mid-dose females or in any Recovery animals.

Bone marrow cellularity was examined in all animals euthanized at the scheduled necropsy. Bone Marrow cells in fixed left femurs were counted (total hematopoietic cells) in five adjacent fields on a slide. Bone marrow cellularity was markedly decreased in mid- and high-dose females, but not in males.

Toxicokinetics

The plasma C_{max} and AUC values for TR-700 increased with TR-701 dose and mean systemic exposures were generally higher in females compared to males (Table 42 and Table 43). AUC values decreased with repeated-dosing with male AUC values decreased 1.8 fold for the 100 mg/kg/day group on Day 1 compared to Day 28 and similar exposure for the 10 and 30 mg/kg/day groups on both days. In females, plasma AUC values were 5.7, 1.4, and 2.9-fold higher on Day 1 compared to Day 28 for the 10 and 30 mg/kg/day groups and compared to Day 10 for the 100 mg/kg/day group respectively.

Table 42: Toxicokinetic Parameters for Plasma TR-700 on Day 1 in Study No.: TOX-07-0701-014A. (Sponsor's Table)

STUDY NO.: G04185		Male			Female		
Group	Dose (mg/kg)	C_{max} (ng/ml)	T_{max} (hr)	AUC_{0-24hr} (ng*hr/ml)	C_{max} (ng/ml)	T_{max} (hr)	AUC_{0-24hr} (ng*hr/ml)
T1	10	5679.0 ± 1167.9[a)]	0.67 ± 0.29	29138.7 ± 8884.3	27382.3 ± 18238.8	4.67 ± 4.62	309565.7 ± 242921.1
T2	30	19000.3 ± 5050.5	2.67 ± 1.15	99288.3 ± 43497.2	29370.7 ± 5864.0	2.67 ± 1.15	320551.7 ± 38867.5
T3	100	72478.7 ± 3156.5	4.67 ± 1.15	740321.2 ± 232756.9	72798.0 ± 8760.2	6.00 ± 0.00	828472.7 ± 160232.3

[a)] Each value represents the mean± S.D., n=3

Table 43: Toxicokinetic Parameters for Plasma TR-700 on Day 28 in Study No.: Study No.: TOX-07-0701-014A. (Sponsor's Table)

STUDY NO.: G04185		Male			Female		
Group	Dose (mg/kg)	C_{max} (ng/ml)	T_{max} (hr)	AUC_{0-24hr} (ng*hr/ml)	C_{max} (ng/ml)	T_{max} (hr)	AUC_{0-24hr} (ng*hr/ml)
T1	10	6622.3 ± 1070.2[a)]	0.83 ± 0.29	23330.5 ± 5604.5	10458.0 ± 406.5	0.42 ± 0.14	54141.3 ± 8435.3
T2	30	23598.0 ± 6171.5	1.17 ± 0.76	81485.6 ± 18116.0	24106.0 ± 4223.2	2.25 ± 3.25	219644.4 ± 58938.6
T3[b)]	100	55130.0 ± 2365.8	3.33 ± 1.15	402853.2 ± 110731.9	33128.3 ± 9058.9	2.33 ± 1.53	289954.5 ± 45485.5

[a)] Ea ch value represents the mean±S.D., n=3

[b)] In case of female rat, blood samples were taken on day 10.

Dosing Solution Analysis

In a stability test, preparations of TR-701 at concentrations of 1, 3, 10, 30, and 100 mg/kg were shown to be stable when refrigerated for 7 days. The mean actual concentrations of TR-701 dosing solutions were shown to be with ± 10% of the nominal concentrations when assessed on the first day and in the last week of dosing.

Study title: Repeated Dose 4-Week Oral Toxicity Study of DA-7218 in Beagle Dogs

Study no.:	TOX-07-0701-013A
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 30, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TR-701 (DA-7218), Lot # OXA-005, purity of 97.35%

Key Study Findings

- Dose-dependent vomiting and salivation occurred in all TR-701 treatment groups during the dosing period.
- No other adverse findings in body weight, food consumption, clinical pathology or histopathology was observed.
- Unlike in rats, toxicokinetic values were similar for both genders.
- Exclusive of vomiting, the NOAEL was considered to be the high-dose of 400 mg/kg/day.

Methods

Doses:	0, 100, 200, 400 mg/kg/day
Frequency of dosing:	Once per day
Route of administration:	Oral
Dose volume:	In capsule
Formulation/Vehicle:	Gelatin capsule, size 12
Species/Strain:	Beagle dogs
Number/Sex/Group:	3/sex/group
Age:	6 months of age
Weight:	At start of dosing, Males: 7.46 to 9.78 kg, Females: 6.12 to 9.46 kg.
Satellite groups:	Recovery animals: 2/sex/group for the vehicle control and high-dose groups only.
Unique study design:	Each animal received on capsule daily filled with an amount of TR-701 calculated from the animal's most recent body weight measurement. On Day 29, Main Study animals were euthanized and necropsied. Recovery animals were euthanized after an additional 1-month with no dosing.
Deviation from study protocol:	Deviations in the study protocol were noted.

However, none was considered to have altered the results or compromised the integrity of the study.

Observations and Results

Table 44: Observation Schedule for Study No.: TOX-07-0701-13A

Parameter	Schedule
Mortality and Clinical Signs	QD for the duration of the study.
Body Weights	Individual body weights were measured approximately once per week beginning before the start of dosing and throughout the dosing period including on the scheduled necropsy day, and during the Recovery period
Food Consumption	Individual food consumption (g/animal/day) was recorded approximately weekly during the pretest period and throughout the study.
Hematology and Coagulation Clinical Chemistry Urinalysis	Blood samples for hematology and clinical chemistry analysis were collected from all animals once pretreatment and on the scheduled Main Study and Recovery necropsy days. Animals were fasted 16 hours prior to blood collection. Urine samples including 24 hour urine volume were collected from all animals once during pretreatment and once during the Main and Recovery studies.
Electrocardiograms (ECG)	ECG (6-lead measurements) recordings were obtained from all animals once during pre-treatment and on Days 22 of both the Main and Recovery Studies.
Toxicokinetics	Blood samples were collected from each animal prior to dosing, and at 0.5, 1, 2, 3, 4, 6, 8, 10 and 24 hours after dosing on Days 1 and 28. Plasma concentrations of TR-700 were determined using a validated LC/MS/MS technique.
Ophthalmic Examinations	Ophthalmologic examinations were conducted once during pretreatment and on Days 21 of both the Main and Recovery Studies.
Necropsy	All surviving Main Study animals were euthanized and necropsied on Day 29. Recovery animals were euthanized and necropsied four weeks later.

Mortality

No unscheduled deaths were noted.

Clinical Signs

Dose-dependent vomiting and salivation were observed in both genders in all the TR-701 groups during the Main Study. Vomiting occurred with dose-dependent frequency (almost every dosing day for high-dose males and females). Other findings without dose-dependency included soft and/or mucous stools.

Body Weights

No TR-701-related changes in body weight occurred for any dose throughout the entire study.

Feed Consumption

Food consumption was also unaffected by TR-701 administration.

Ophthalmoscopy

No TR-701-related ophthalmoscopy findings were noted.

ECG

No TR-701-related changes in any ECG parameters were noted.

Hematology

On Day 20, significant reductions in the percent of basophils and large unstained cells were noted for low- and high-dose males and in low-dose females. Due to the lack of dose-dependency, these effects were not considered to be TR-701-related. On the 20th day of Recovery, RBCs were significantly increased from the male control values by 10% in high-dose males, but not females. No other changes in hematology or coagulation parameters were noted during dosing or following Recovery.

Clinical Chemistry

On Day 20, significant increases in serum calcium were observed in mid- and high-dose males, but values still fell within normal physiological ranges. Significantly increased blood urea nitrogen (BUN) observed only in low-dose females, was not considered to be related to TR-701 administration because of a lack of dose-dependency.

Urinalysis

Occult blood, unrelated to treatment was observed in low-dose males prior to the start of dosing.

Gross Pathology

A fibrotic scar on the spleen was noted for one low- and one mid-dose male. Also one high-dose male demonstrated duodenal congestion. These findings were not considered to be related to TR-701 administration due to a lack of dose-dependency and/or occurrence in only one gender. No gross pathology was noted in females during dosing or in either gender during Recovery.

Organ Weights

The weighed organs are listed in Table 126.

Significant increases in mean absolute brain weights of the low- and high-dose males and adrenal glands of mid-dose females were noted. At recovery, mean absolute adrenal gland weights were significantly increased for high-dose males. Due to a lack of histopathology correlates, these organ weight changes were not considered toxicologically relevant.

Histopathology

Adequate Battery

Yes, the examined tissues are listed in Table 126

Peer Review

Yes

Histological Findings

No histopathology findings were noted by either the study pathologist or the peer review pathologist.

Toxicokinetics

Plasma TR-700 concentrations were determined using a previously validated LC/MS/MS method. Toxicokinetic values were variable between genders with males generally demonstrating higher values (Table 45). Also in some instances, toxicokinetic values decreased with higher doses. For both males and females, C_{max} and AUC values tended to increase on Day 28 compared to Day 1, although there were exceptions. These effects may have been influenced by disrupted absorption due to vomiting.

Table 45: Summary of TR-700 Toxicokinetic Parameters on Days 1 and 28 Following Oral Administration of TR-701 to Male and Female Dogs. (Sponsor's Table)

Sex	Dose (mg/kg/day)	Mean C_{max} (ng/mL)		Mean AUC_t (ng·h/mL)	
		Day 1	Day 28	Day 1	Day 28
Male	100	3118	16986	10821	39166
	200	20476	25598	56555	71263
	400	12709	40617	31725	201802
Female	100	5575	9124	11763	23328
	200	36604	7167	116279	18049
	400	31995	20422	81843	66062

Dosing Solution Analysis

Description of tablet analysis was not included in the study report.

Study title: A 3-Month Oral (Gavage) Toxicity and Toxicokinetics Study of TR-701 FA in Sprague-Dawley Rats with a 28-Day Recovery Period

Study no.:	Tox-11-0701-027
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 14, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TR-701 FA, Batch No.: 02090118, purity of 97.3%

Key Study Findings

- Four high-dose males and one high-dose female died early or were euthanized early due to TR-701-related toxicity including lesions in the bone marrow, thymus, small and/or large intestine, stomach, and/or liver.
- At the early termination in Week 5, high-dose male and female body weights were reduced 27.1% and 15.7% respectively compared to the control group. After the recovery period, partial weight gain occurred for high-dose animals compared to controls.
- Hematology changes including significantly lower platelets and reticulocytes in high-dose males correlated with bone marrow atrophy and demonstrated reversal during the recovery period.
- Serum chemistry changes including increased liver enzymes (ALT, AST, SDH) in high-dose males correlated with hepatocellular degeneration and atrophy. Liver enzyme levels returned to normal after the recovery period.
- Histopathology mainly occurring in early death animals included bone marrow hypocellularity, small and large intestine degeneration and atrophy, hepatic degeneration and atrophy, and in early death males, seminiferous tubule degeneration and testicular hemorrhage. These histopathologies occurred at a lower incidence and severity in high-dose animals sacrificed early in Week 5. In general histopathology was reduced or reversed following the recovery period.
- The NOAEL values were considered to be the mid-doses, 30 mg/kg/day and 10 mg/kg/day for males and females respectively.

Methods

Doses:	0, 10, 30, and 100 mg/kg/day for males (Groups 1-4 respectively) and 0, 3, 10, and 30 mg/kg/day for females (Groups 1-4 respectively). Males and females received differential high doses that previously were shown to produce similar exposure levels in Study No.: TOX-07-0701-014.
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	10 ml/kg
Formulation/Vehicle:	25 mM disodium hydrogen phosphate
Species/Strain:	Sprague Dawley rats (Crl:CD[SD])
Number/Sex/Group:	15/sex/group for Groups 1 and 4 and 10/sex/group for Groups 2 and 3. For Groups 1 and 4, 10 animals were used for the Main Study and the remaining 5 animals were Recovery animals.
Age:	Approximately 7-weeks old at the start of dosing
Weight:	Males: 208 to 265 g; Females: 143 to 191 g
Satellite groups:	Toxicokinetics animals included 3 animals/sex/group for Group 1A and 9 animals/sex/group for Groups 2A, 3A, and 4A treated up to 37 days (Group 4A) or 91

	consecutive days (Groups 1A-3A).
Unique study design:	Group 4/4A males were dosed for 35 days (with a 2-day dosing holiday on Days 27 and 28) and Group 4/4A females were dosed for 37 consecutive days. All animals in Groups 1/1A-3/3A were dosed for 91 consecutive days. Group 4 Recovery animals were euthanized on Day 65, 28 days after the Main Study Sacrifice. Group 1 Recovery animals were euthanized on Day 120, 29 days after the Main Study necropsy.
Deviation from study protocol:	Multiple deviations from the study protocol occurred, most notably an early necropsy date for high-dose animals. However, none was considered to have compromised the results or the integrity of the study.

Table 46: Study Design for the 3-Month Rat Toxicology Study with Oral TR-701 FA. (Sponsor's Table)

Group Number	Treatment	Dosage Level (mg/kg/day)	Dose Volume (mL/kg)	Number of Males [a]
1	Vehicle	0	10	15
2	TR-701 FA	10	10	10
3	TR-701 FA	30	10	10
4	TR-701 FA	100/60 [b]	10	15

Group Number	Treatment	Dosage Level (mg/kg/day)	Dose Volume (mL/kg)	Number of Females [a]
1	Vehicle	0	10	15
2	TR-701 FA	3	10	10
3	TR-701 FA	10	10	10
4	TR-701 FA	30	10	15

[a] = For Group 4, all except 5 animals/sex of the surviving animals were euthanized on study day 37 (study week 5) following 35 (males) or 37 (females) days of dose administration; the remaining 5 animals/sex were euthanized following a 28-day nondosing (recovery) period on study day 65 (study week 9). For Groups 1-3, 10 animals/sex/group were euthanized following a minimum of 91 days of dose administration; the remaining 5 animals/sex in Group 1 were euthanized following a 29-day nondosing (recovery) period. Evaluations conducted and data for these control toxicology group animals collected during the recovery period are reported separately in Appendix O, and are not discussed further in this report.

[b] = Based on mortality and observations of toxicity, males in Group 4 were placed on a 2-day dosing holiday (study days 27 and 28) after which the 100 mg/kg/day dosage level was lowered to 60 mg/kg/day (study days 29-36).

Observations and Results

Table 47: Observation Schedule for the 3-Month Rat Study with Oral TR-701 FA

Parameter	Schedule
Mortality	BID for the duration of the study.
Clinical Signs	Daily for 7 days before randomization and BID (prior to dosing and 1-2 hours following dosing) for the duration of the study. Recovery animals were examined daily
Body Weights	Individual body weights were approximately once per week beginning before the start of dosing and throughout the dosing period.
Food Consumption	Individual food consumption (g/animal/day) was recorded approximately weekly during the pretest period and throughout the study and weekly averages were reported for the corresponding body weight interval
Hematology and Coagulation Clinical Chemistry Urinalysis	Blood and urine samples were collected from all animals prior to the initiation of dose administration and on the scheduled Main Study and Recovery necropsy days. In addition blood and urine samples were collected from all animals in Groups 1-3 during Week 6. Animals were fasted overnight prior to blood collection.
Toxicokinetics	Blood samples were collected from all animals prior to dosing, and at 1, 2 (Study Day 0 only), 3, 6, 12, and 24 hours after dosing on Days 0 and 90. Blood samples were collected from control group animals at approximately 2 hours after dose administration on Days 0 and 90. Blood was collected from 3 animals/sex/group per time point for Groups 1A-4A on Study Day 0 and for Groups 1A-3A on Study Day 90. All surviving animals in Group 4A were euthanized without blood collection on Day 37.
Ophthalmic Examinations	Ocular examinations were conducted on all animals prior to randomization, near the end of the treatment period for all animals in Groups 1-3, and near the end of the recovery period for all remaining animals in Group 4.
Necropsy	Main Study Group 4 males and females were euthanized and necropsied on Day 36 and Day 38 respectively (Week 5). All Main Study animals in Groups 1-3 were euthanized and necropsied on Day 92 (Week 13). Group 4 Recovery animals were euthanized and necropsied on Day 65 (Week 9), and Group 1 Recovery animals were euthanized and necropsied on Day 120 (Week 17).

Mortality

Four high-dose males and one high-dose female in the Main Study died early or were euthanized in extremis as well as one high-dose toxicokinetic animal. These deaths were considered to be TR-701-related and the causes of death were determined to be

lesions in the bone marrow, thymus, small and/or large intestine, stomach and/or liver. All but one male animal that was found dead on Day 17 died within two days of the scheduled necropsies on Day 36 for males and Day 38 for females.

Clinical Signs

No clinical signs were observed in the low- and mid-dose groups for both males and females. In Main-Study high-dose males and females, particularly those found dead or euthanized in extremis, clinical signs included: red material around nose, mouth, and/or forelimbs, yellow/brown material on the urogenital/anogenital area, thin appearance, soft feces, small feces, decreased defecation, pale extremities, body cool to the touch, dermal atonia, and prostration. In addition, the high-dose female that was euthanized in extremis exhibited labored respiration, decreased respiration, prostration, and dilated pupils. Among the recovery animals, only one male demonstrated clinical signs of red material around the nose and mouth, yellow material in the urogenital area, thinness, and pale extremites, but most of the Recovery females demonstrated red material around the nose and mouth.

Body Weights

Markedly lower mean body weight gain and/or mean body weight loss was noted in high-dose males and females compared to the control group. Weight loss was more severe in males and noted throughout the dosing period and less severe in females beginning in Week 3. At the early termination in Week 5, high-dose male and female body weights were reduced 27.1% and 15.7% respectively compared to the control group. A lesser weight reduction was noted in mid-dose animals with mean body weights 6.1 and 5.8% reduced for mid-dose males and females respectively compared to the control group at the end of Week 12.

During the recovery period, weight recovery occurred and at the end of recovery, body weights for high-dose males and females were 10.6% and 5.3% reduced respectively compared to the control group.

Feed Consumption

High-dose males and to a lesser extent, high-dose females demonstrated significantly lower food consumption for the duration of the truncated dosing period. At the end of dosing in Week 5, food consumption was 27.6 and 19.0% lower in high-dose males and females respectively compared to the control group. The low- and mid-dose groups were not similarly affected.

Ophthalmoscopy

Ocular examinations were conducted using an indirect ophthalmoscope and slit-lamp biomicroscope preceded by pupillary dilation with a mydriatic agent.

No ophthalmic lesions considered to be related to TR-701 administration were observed.

ECG: Not performed

Hematology

In Week 5, significantly higher mean neutrophil counts, significantly lower platelet counts and absolute reticulocyte counts were noted in high-dose males. The neutrophil counts were largely due to a high value in one male. The platelet and absolute reticulocyte counts correlated with bone marrow atrophy in two males. Significantly lower RBCs in Week 5 in high-dose females were largely due to a low value in one female, and significantly lower mean RBC counts in mid-dose females in Week 6 was due to very low values in two females.

In Weeks 9 and 13, all of the changes at Week 5 were no longer significantly different than control values.

Clinical Chemistry

Significant changes for the high-dose animals sacrificed in Study Week 5 (necropsy for surviving high-dose animals) could not be determined due to the lack of concurrent sacrifice for control animals. High-dose males and females had lower albumin and total protein values (compared to Week 6 control animals) which were consistent with intestinal histopathology. Also in high-dose males several liver enzymes alanine aminotransferase (ALT; more than 3-fold elevated compared to Week 6 control animals), aspartate aminotransferase (AST; more than 2-fold elevated compared to Week 6 control animals), sorbitol dehydrogenase (SDH; more than 2-fold elevated compared to Week 6 control animals) were significantly elevated which corresponded with hepatocellular degeneration and atrophy in two high-dose males. Higher cholesterol and GGT in high-dose males were suggestive of liver cholestasis. In both high-dose males and females, significantly elevated serum calcium, phosphorus, and potassium values and lower chloride values were noted suggesting renal insufficiency and/or acidosis. Urea nitrogen was slightly elevated in males and females which is consistent with minimal dehydration. As there was not histopathology correlating with renal insufficiency, diarrhea was considered to be a possible contributing factor to acidosis and minimal dehydration. Other findings in high-dose males and females included lower alkaline phosphatase (ALP) and triglycerides (possibly related to lower food intake) and higher serum glucose values.

In Week 9 (the Recovery necropsy for high-dose animals), the serum albumin and total protein levels males and females and liver enzymes in males returned to values more similar to those of control animals suggesting a trend toward recovery. This was also true for urea nitrogen levels. However, serum calcium was still elevated and only slight corrections occurred for the phosphorus, potassium and chloride levels. Also ALP, triglyceride, and serum glucose values did not correct.

In mid-dose animals in Week 6, many of the serum chemistry changes noted for high-dose animals also occurred but the changes were of smaller magnitude. Total protein and albumin values were minimally decreased in mid-dose males as was ALP. Blood urea nitrogen in mid-dose females was minimally elevated. Mean ALT and AST were 1.25-fold and 1.15-fold elevated in mid-dose males compared to control males. All of these changes were considered to be TR-701-related, but due to the small magnitude of changes, and lack of histopathology correlates, the changes were not considered toxicologically relevant.

In Week 13 (the necropsy date for low- and mid-dose animals), ALP values in mid-dose males were reduced compared to Week 6 values, and lowered compared to control males. ALT and AST values in mid-dose males and blood urea nitrogen levels in mid-dose females were not different compared to control values. Mean total protein was significantly lower in mid-dose males, but individual values all fell within the range of concurrent control values.

Urinalysis

The following urinalysis parameters were assessed: specific gravity, pH, urobilinogen, total volume, color, clarity, protein, glucose, ketones, bilirubin, occult blood, leukocytes, nitrites, sediment microscopy.

No TR-701-related changes in urine parameters were observed.

Gross Pathology

Gross Pathology examinations included external surface examination, examination of all orifices, and internal examination of the cranial, thoracic, abdominal and pelvic cavities including all viscera.

Gross pathology in the early death animals of both genders included: small thymuses and/or spleens, distended stomachs, and dark red stomach contents. The early death males also exhibited dark red contents of the urinary bladder, small prostates and seminal vesicles, and firm and/or dark red testes.

Much of the same gross pathology was evident in the surviving high-dose animals that survived until euthanasia in Week 5. However, in conjunction with the Week 9 sacrifice of the Recovery animals, no TR-701-associated gross pathology was reported. Also no gross pathology was observed at the Week 13 necropsy in low- and mid-dose animals.

Organ Weights

Organ weights were not collected in Weeks 5 (Main Study sacrifice) or 9 (Recovery Study sacrifice) for high-dose animals. For the Week 13 sacrifice, organ weights for the low- and mid-dose groups were determined. Paired organs were weighed together. Absolute organ weights were recorded and organ to final body weight and organ to brain weight ratios were calculated. Thyroid/parathyroid was weighed after fixation.

Absolute thymus weights were significantly reduced by approximately 21% for both mid-dose males and females, and thymus weights relative to brain weights were reduced by similar amounts. The thymus weight reduction was associated with lymphoid necrosis in the thymus and may have been due to stress.

Reviewer Comment: *Because organ weights were not collected for high-dose animals, dose-dependent trends in organ weight changes not assessable.*

Histopathology

Adequate Battery

Yes. See

Peer Review

No

Histological Findings

Multiple-organ histopathology was observed in the early death high-dose animals and at a lower incidence and severity in all high-dose animals at the Week 5 necropsy as summarized below. In association with the Week 13 necropsy, no TR-701-related histopathology was noted in the low- and mid-dose males and females.
Bone Marrow: Mild to severe hypocellularity (myeloid and erythroid cells), mild to severe megakaryocyte degeneration, and moderate to severe hemorrhage of sternal and femoral bone marrow was observed in all early death animals.
GI Tract: Degenerative and atrophic changes in the small and large intestine were observed. Histopathology included: villous atrophy, villous enterocyte degeneration, single cell necrosis of crypt or gland epithelium, gland necrosis and ulceration and/or edema. Changes occurred in the duodenum, jejunum and/or ileum. In addition single cell necrosis of gland epithelium was noted in the cecum and/or colon with submucosal edema in the single early death female and gland necrosis was observed in the rectum of 4 early death rats with severe ulceration also occurring in the rectum one early death male. Also mild hemorrhage and/or mild erosion of glandular mucosa was observed in the stomach of 3 of the 5 early death animals.
Liver: In all early death animals hepatic histopathology included: minimal to moderate degeneration of centrilobular hepatocytes and minimal to mild diffuse atrophy of hepatocytes. Degeneration consisted of vacuolation and/or pyknosis of centilobular hepatocytes, and atrophy was characterized by small hepatocytes with condensed eosinophilic cytoplasm.
Kidney: Mild proteinosis (hyaline droplets) of the distal tubule was noted in one high-dose male and a moderated degree of the same histopathology and moderate tubular hyaline droplets were observed in another high-dose male.
Reproductive Tract: In all four early-death male rats, minimal to moderate seminiferous tubule degeneration occurred with more marked involvement of the later stage tubules. Also the testes of 3/4 of the rats demonstrated severe hemorrhage and in one male the hemorrhage was associated with suppurative inflammation. Other male reproductive organs were affected with minimal to severe cell debris in the epididymides and mild atrophy and/or minimal to severe decreased secretions in the prostate and seminal vesicles and in the coagulating gland of one male. In the early death female, mild follicular involution of the ovaries and severe mucosal atrophy of the vagina and cervix was noted.
Other histopathology: Multiple lymphatic and glandular changes were considered to be possibly associated with stress secondary to TR-701 primary toxicity. These included: degenerative changes including lymphoid depletion and necrosis in the thymus (mild to severe), lymph nodes (minimal to severe), and spleen (minimal to severe). Adrenal changes included mild hemorrhage and/or single cell necrosis in 3 of 5 early death rats, congestion and atrophy in the zona reticularis in the early death female, hypertrophy of

the zona fasciculata in 3 of 4 early death males, and congestion and vacuolation of the zona fasciculata in one early death male.

Multiple lesions were observed that were considered to be related to reduced food intake and low body weights in the early death animals. These included: mucosal atrophy in the stomach and intestine, acinar gland atrophy in the exorbital lacrimal glands, salivary glands, and Harderian glands, atrophy of the pituitary pars distalis, decreased zymogen granules in the pancreas, squamous epithelial atrophy of the esophagus, skin, nonglandular stomach, and tongue, skeletal muscle atrophy and fat atrophy in the mammary gland.
Histopathology in surviving high-dose animals: As noted above the high dose animals that survived until the Main Study sacrifice in Week 5 exhibited much of the same primary and secondary histopathology as the early death animals but at a lower incidence and severity including the male reproductive organ effects. One surviving male had severe hemorrhage and moderate suppurative inflammation of the testes. Two surviving males and one female demonstrated the broad spectrum of TR-701 histopathology occurring in the early death animals.
Histopathology in Recovery Study animals: One high-dose male euthanized in Week 9 after recovery demonstrated multifocal mild atrophy and vacuolation of seminiferous epithelium with moderate epithelial hyperplasia, mild interstitial edema, and mild hypospermia in the epidydmides. Another recovery male also demonstrated minimal focal seminiferous tubule atrophy in one testes.

Special Evaluation

None

Toxicokinetics

All of the rats administered TR-701 FA were systemically exposed to both TR-701 and TR-700, but the TR-701 exposure was much less (1-3%) than for TR-700 based on AUC measurements (Table 48 and Table 49). In general, C_{max} measurements increased in a less than dose-proportional manner for both genders and AUC increased in an approximately dose-proportional manner for both genders.

Exposure to TR-700 was higher on Day 90 versus Day 0 indicating plasma accumulation. T_{max} for both TR-700 and TR-701 ranged from 1 to 6 hours with the higher T_{max} values associated with the higher doses of TR-701. Half-life values for both TR-701 and TR-700 ranged from approximately 3 to 5 hours for both genders.

Table 48: TR-701 FA Toxicokinetic Parameters in Rats. (Sponsor's Table)

Gender:	Male			Female		
TR-701 FA Dosage (mg/kg/day):	10	30	100	3	10	30
Parameter (Unit)			**Study Day 0**			
AUC_{last} (ng•h/mL)	1020	2290	8020	287	1660	3190
DN AUC_{last}	102	76.3	80.2	95.8	166	106
C_{max} (ng/mL)	173	320	733	74.8	245	368
DN C_{max}	17.3	10.7	7.33	24.9	24.5	12.3
T_{max} (h)	1	3	6	1	1	3
$T_{1/2}$ (h)	NR	3.2	3.5	NR	3.3	4.9
Parameter (Unit)			**Study Day 90**			
AUC_{last} (ng•h/mL)	866	2540	NA	497	2350	NA
DN AUC_{last}	86.6	84.6	NA	166	235	NA
C_{max} (ng/mL)	170	345	NA	77.5	235	NA
DN C_{max}	17.0	11.5	NA	25.8	23.5	NA
T_{max} (h)	1	1	NA	1	6	NA
$T_{1/2}$ (h)	NR	4.0	NA	3.6	3.4	NA
Accumulation Ratio*	0.847	1.11	NA	1.73	1.41	NA

DN = Dose-normalized; units for DN AUC_{last} and C_{max} are (ng•h/mL)/(mg/kg) and (ng/mL)/(mg/kg), respectively. NA = Not applicable. NR = Not reportable (failed to meet acceptance criteria).
*Ratios calculated using AUC_{last}.

Table 49: TR-700 Toxicokinetic Parameters in Rats. (Sponsor's Table)

Gender:	Male			Female		
TR-701 FA Dosage (mg/kg/day):	10	30	100	3	10	30
Parameter (Unit)			**Study Day 0**			
AUC_{last} (ng•h/mL)	30700	99600	337000	19200	84900	159000
DN AUC_{last}	3070	3320	3370	6400	8490	5310
C_{max} (ng/mL)	5580	12200	31600	3030	10900	19000
DN C_{max}	558	405	316	1010	1090	634
T_{max} (h)	1	3	6	1	1	3
$T_{1/2}$ (h)	NR	2.2	3.5	2.8	3.2	4.7
TR-700/TR-701 FA Ratio	36.5	52.9	51.1	81.3	62.0	60.7
Parameter (Unit)			**Study Day 90**			
AUC_{last} (ng•h/mL)	44000	161000	NA	32100	124000	NA
DN AUC_{last}	4400	5350	NA	10700	12400	NA
C_{max} (ng/mL)	8010	19300	NA	4460	12900	NA
DN C_{max}	801	642	NA	1490	1290	NA
T_{max} (h)	1	1	NA	1	1	NA
$T_{1/2}$ (h)	2.5	2.9	NA	3.7	3.6	NA
TR-700/TR-701 FA Ratio	61.9	77.0	NA	78.5	64.1	NA
Accumulation Ratio*	1.43	1.61	NA	1.67	1.46	NA

DN = Dose-normalized; units for DN AUC_{last} and C_{max} are (ng•h/mL)/(mg/kg) and (ng/mL)/(mg/kg), respectively. NA = Not applicable. NR = Not reportable (failed to meet acceptance criteria).
*Ratios calculated using AUC_{last}.

Dosing Solution Analysis

The analyzed dosing formulations were homogeneous and contained 94.9 to 102% of the nominal concentrations of each TR-701 dosing solution. TR-701 was not detected in the vehicle control dosing solution.

Study title: 3-month Dog Study of TR-701 FA Toxicity and Toxicokinetics TR-701

Study no.:	TOX-11-0701-026
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 3 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TR-701 FA, Lot No.: 02120030, purity of 99.6%

Key Study Findings

- No TR-701-related deaths occurred. The primary clinical signs were abnormal feces and emesis occurring 1-2 hours after dosing for all TR-701 groups.
- Other than emesis, no other toxicity clearly related to TR-701 administration was observed.
- The NOAEL value for both males and females was considered to be 400 mg/kg/day.

Methods

Doses:	0, 100, 200, and 400 mg/kg/day for Groups 1, 2, 3, and 4 respectively
Frequency of dosing:	daily
Route of administration:	Oral gavage
Dose volume:	5 ml/kg
Formulation/Vehicle:	250 mM disodium hydrogen phosphate buffered solution, pH 7.5
Species/Strain:	Beagle dogs
Number/Sex/Group:	6 animals/sex/group (Groups 1 and 4) and 4 animals/sex/group for Groups 2 and 3.
Age:	Approximately 5-6 months old for both genders at receipt.
Weight:	Males: 5.9 to 7.9 kg for males and 4.9 to 7.7 kg for females at the initiation of dosing
Satellite groups:	none
Unique study design:	Male and female Beagle dogs were orally administered vehicle or TR-701FA daily for 91 days. Recovery animals were evaluated following a recovery period of 28 days
Deviation from study protocol:	Multiple deviations from the study protocol were noted, but none was considered to have altered

the results or integrity of the study.

Observations and Results

Table 50: Observation Schedule for the 3-Month Dog Study with Oral TR-701 FA

Parameter	Schedule
Mortality	BID for the duration of the study.
Clinical Signs	Daily for 7 days before randomization and BID (prior to dosing and 1-2 hours following dosing) for the duration of the study. Recovery animals were examined daily
Body Weights	Individual body weights were obtained generally twice per week beginning before the start of dosing and throughout the dosing period.
Food Consumption	Individual food consumption (g/animal/day) was recorded daily during the pretest period and throughout the study and weekly averages were reported for the corresponding body weight interval
Hematology and Coagulation Clinical Chemistry Urinalysis	Blood and urine samples were collected from all animals prior to the initiation of dose administration and on the scheduled necropsy days. Animals were fasted overnight prior to blood collection.
Toxicokinetics	Blood samples were collected from all animals prior to dosing, and at 1, 2, 4, 8, and 24 hours after dosing on Days 0, 27, 60, and 88.
Ophthalmic Examinations	Ocular examinations were conducted on all animals prior to randomization, near the end of the treatment period, and near the end of the recovery period.
Electrocardiograms	Multilead (I, II, III, aVR, aVL, aVF, and V2) ECGs were recorded for all animals prior to the initiation of dose administration and during Study Week 12 (1-2 hours following dose administration).
Necropsy	Day 92 for Main Study animals and Day for Recovery animals.

Mortality

No mortalities or early sacrifices were considered to be related to TR-701 administration. One male in the low dose TR-701 group was euthanized following dose administration on Study Day 27 and another mid-dose male was euthanized also after dose administration on Day 48. Neither animal had been displaying signs of toxicity before dosing, and following euthanasia, gross examination of the lungs for both animals revealed lung pathology including partially collapsed lungs, dark red discolorations, and foamy contents in the trachea. This pathology suggests aspiration accidents occurred in conjunction with oral gavage.

Clinical Signs

TR-701-related clinical signs were noted in males and females in all the TR-701 groups in a dose-related manner. These clinical signs consisted of abnormal feces and emesis which generally occurred 1-2 hours after dosing.

More serious clinical signs, dose-dependent for severity were noted for the TR-701 mid- and high-dose males and females. These included diarrhea, watery diarrhea, and red mucoid feces (high-dose only) excessive salivation, clear and frothy material around the mouth, emesis containing food emesis and feces containing white and yellow material, and clear material around the ventral neck, forelimbs, and ears.

Body Weights

Body weights were unaffected by TR-701 FA administration. Significantly higher mean cumulative body weight gains were sporadically noted for high-dose females but this was not considered TR-701-related because of a lack a dose-response trend and a lack of correlating changes in food consumption.

Feed Consumption

Food consumption was not affected by TR-701 administration. Although low-dose females consumed significantly more food in some weeks, and mid-dose females consumed significantly less food in other weeks, the changes were not considered TR-701 related due to lack of dose and time-response trends and inconsistent changes in the direction of greater or lesser food consumption.

Ophthalmoscopy

No ophthalmic changes were noted in any group.

ECG

The following ECG parameters were measured or calculated: heart rate, PR, RR, QRS, QT, and QTcV (Van de Water's correction) by a veterinary cardiologist.

No changes in any of the ECG parameters noted above were noted.

Hematology

The mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values were slightly (≤ +7%) but significantly higher in mid- and high-dose males and high-dose females (+8% for both parameters) following dosing but not after recovery. The relative but not absolute number of basophils was reduced 50% compared to control animals in high-dose males after dosing but not after recovery. Also large unstained cells were lower compared to control animals in all low-, mid- and high-dose males (-33 to 66%) after dosing, but not after recovery. These changes were not considered toxicologically significant because of slight magnitude of change, changes in only one gender, and/or a lack of concurrence with absolute values and because all of the changes reversed during recovery. Other than these changes no other significant hematological or coagulation value changes were noted.

Clinical Chemistry

Following dosing, significantly lower mean serum globulin levels were observed in high-dose males (-12.5%) and females (-12.5%). A corresponding higher A/G ratio (+19%) occurred in high-dose males following dosing. These changes were of small magnitude, and reversed during the recovery period and were not considered toxicologically relevant.

Urinalysis

The urinalysis parameters listed in Table 125 were measured.

No TR-701-related changes in urinalysis parameters were noted.

Gross Pathology

No TR-701-related gross pathology findings were noted.

Organ Weights

The organs shown in Table 126 were weighed following necropsy. Paired organs were weighed together. Absolute weights and relative weights (organ to final body weight, organ to brain weight) were determined.

Male Reproductive Organs: Testes and Prostate weight were reduced in all of the TR-701 treatment groups, but not in a dose-dependent manner which suggests a lack of relationship to TR-701 administration. Testes weights were significantly lower in the low- and mid-dose males, but not in the high-dose males (Table 51).
Ovaries and Uterus: Decreased absolute and relative ovary and uterus weights occurred in a TR-701 dose-dependent manner except for uterus absolute and relative weights where high-dose values were greater than the mid-dose values. Absolute ovary weights in high-dose females were decreased approximately 33% in mid-dose females and 50% in high-dose females. However, none of the changes were statistically significant relative to control values (Table 52). The Sponsor attributed the changes to females in different stages of the estrus cycle.
Spleen: Spleen weights in females were reduced in the TR-701 treatment groups, but not in a dose-dependent manner and not significantly except relative to body weight and relative to brain weight (mid-dose group only).
Thyroid/Parathyroid: The combined weights of the thyroid/parathyroid were lighter in high-dose males compared to the control group. The absolute thyroid/parathyroid weight and the weights relative to body weight, and relative to brain weight were 25.3%, 33.3%, and 28.3% reduced compared to the control group respectively, but only the ratio to body weight change was significant. In females, thyroid/parthyroid weights were not significantly changed compared to the control group.

No TR-701-related changes in organ weights were noted for Recovery animals.

Table 51: Male Organ Weights

Organ	Weights and Weight Ratios[a]	Control	TR-701 Doses (mg/kg/day)		
			100	200	400
Spleen	Absolute	117.04	129.01	121.63	109.11
	Ratio to body weight	1.465	1.452	1.538	1.263

	Ratio to brain weight	159.745	174.417	158.482	142.904
Thyroid/Parathyroid	Absolute	0.7325	0.5786	0.6834	0.5470
	Ratio to body weight	0.009	0.007	0.009	0.006*
	Ratio to brain weight	1.000	0.782	0.891	0.717
Testes	Absolute	12.67	10.08	10.26	12.01
	Ratio to body weight	0.157	0.113**	0.130*	0.139
	Ratio to brain weight	17.240	13.585*	13.368*	15.691
Weights are expressed in mg. Ratios are to 100g body or brain weight.					

Table 52: Female Organ Weights

Organ	Weight ratios	Control	TR-701 Doses (mg/kg/day)		
			100	200	400
Spleen	Absolute	126.95	102.00	88.49	100.87
	Ratio to body weight	1.795	1.283*	1.222*	1.322*
	Ratio to brain weight	185.183	141.572	130.342*	149.340
Thyroid/Parathyroid	Absolute	0.6138	0.6376	0.6583	0.7176
	Ratio to body weight	0.009	0.008	0.009	0.009
	Ratio to brain weight	0.899	0.877	0.972	1.072
Ovaries	Absolute	1.43	1.2324	0.9575	0.7185
	Ratio to body weight	0.021	0.015	0.013	0.009
	Ratio to brain weight	2.100	1.747	1.383	1.055
Uterus	Absolute	14.28	11.20	7.76	9.39
	Ratio to body weight	0.207	0.141	0.102	0.115
	Ratio to brain weight	20.949	15.588	11.065	13.667
Weights are expressed in mg. Ratios are to 100g body or brain weight					

Histopathology

Adequate Battery: Yes, the tissues and organs that were examined for histopathology are noted in Table 126.

Peer Review: No

Histological Findings

No TR-701-related histological findings were reported. One confounding factor in terms of evaluating reproductive organ histopathology was the variable sexual maturity of the dogs. Mononuclear cell infiltrate was observed in the liver of animals from all groups including control animals. Mononuclear cell infiltrate was noted in the brains of one high-dose female and one low-dose male. Also axonal spheroids were observed in the cochlear nucleus of the brain of one 200 mg/kg/day group female and two high-dose females. However since the cochlear nucleus was not consistently observable in brain sections this finding was not considered to be clearly TR-701 related.

Toxicokinetics

Oral administration of TR-701 FA to male and female dogs resulted in systemic exposure to both TR-701 and TR-700, but TR-701 was rapidly converted to TR-700.

The ratio of TR-700 AUC to TR-701 AUC where measureable was on the order of 100 to 1000. T_{max} values for both compounds were typically 1-2 hours and the estimated plasma half-life values for both compounds were on the order of 1-3 hours.

Due to emesis, TR-700 C_{max} and AUC values were variable (Table 53). However, TR-700 C_{max} and AUC values tended to increase proportionally or less than proportionally with TR-701 dose. TR-700 plasma exposures were generally higher for females but gender differences were generally < 2 fold.

TR-700 tended to accumulate in plasma with repeated dosing. Mean accumulation ratios for AUC in males ranged from 0.85 to 3.2 and from 1.2 to 5.4 in females with a trend toward increasing accumulation with longer duration of dosing.

Table 53: TR-700 Toxicokinetic Parmameters. (Sponsor's Table)

Gender:	Male			Female		
TR-701 FA Dosage (mg/kg/day):	100	200	400	100	200	400
Parameter (Unit)	**Study Day 0**					
AUC_{last} (ng•hr/mL)	28,800†[3]	31,200	68,800	25,600	46,700	52,300
DN AUC_{last}	288†[3]	156	172	256	233	131
C_{max} (ng/mL)	14,600†[3]	16,100	24,900	11,200	17,600	19,600
DN C_{max}	146†[3]	80.7	62.2	112	88.2	49.0
T_{max} (h)	1.0†[3]	1.0	1.2	1.3	1.3	1.5
$T_{1/2}$ (h)	1.0†[3]	0.87†[3]	1.1†[3]	1.1†[3]	1.2†[3]	1.4†[4]
TR-700/TR-701 FA Ratio	NA	NA	525†[3]	NA	974†[1]	679†[4]
Parameter (Unit)	**Study Day 27**					
AUC_{last} (ng•hr/mL)	39,700†[3]	72,600	58,500	34,700	60,500	141,000
DN AUC_{last}	397†[3]	363	146	347	303	353
C_{max} (ng/mL)	19,500†[3]	30,200	20,500	16,700	22,900	31,100
DN C_{max}	195†[3]	151	51.3	167	114	77.8
T_{max} (h)	1.7†[3]	1.3	1.2	1.0	1.5	2.7
$T_{1/2}$ (h)	0.72†[3]	2.0†[3]	1.6†[5]	1.0†[3]	1.1†[2]	2.3†[5]
TR-700/TR-701 FA Ratio	98†[1]	469†[2]	902†[5]	766†[1]	774†[2]	594
Accumulation Ratio	2.3†[2]	2.9	0.85	1.3	1.2	2.9
Parameter (Unit)	**Study Day 60**					
AUC_{last} (ng•hr/mL)	32,200†[3]	40,700†[3]	151,000	40,000	48,200	245,000
DN AUC_{last}	322†[3]	204†[3]	377	400	241	613
C_{max} (ng/mL)	14,100†[3]	14,700†[3]	45,900	18,400	16,100	53,900
DN C_{max}	141†[3]	73.4†[3]	115	184	80.5	135
T_{max} (h)	1.3†[3]	2.0†[3]	1.5	1.0	1.8	2.5
$T_{1/2}$ (h)	1.1†[1]	1.1†[1]	1.2†[5]	1.2†[3]	1.6†[3]	2.1
TR-700/TR-701 FA Ratio	NA	446†[1]	729†[5]	727†[1]	421†[1]	524
Accumulation Ratio	2.4†[2]	1.9†[3]	2.5	1.4	1.2	4.8
Parameter (Unit)	**Study Day 88**					
AUC_{last} (ng•hr/mL)	42,400†[3]	66,900†[3]	141,000	53,700	99,300	229,000
DN AUC_{last}	424†[3]	335†[3]	354	537	497	572
C_{max} (ng/mL)	24,600†[3]	24,300†[3]	36,900	24,000	26,500	47,700
DN C_{max}	246†[3]	121†[3]	92.1	240	133	119
T_{max} (h)	1.3†[3]	1.3†[3]	2.2	1.3	3.3	2.8
$T_{1/2}$ (h)	0.97†[2]	1.1†[3]	1.5	1.0†[3]	1.0†[1]	1.7†[5]
TR-700/TR-701 FA Ratio	695†[1]	654†[3]	8056†[4]	855†[3]	1020†[3]	863
Accumulation Ratio	3.0†[2]	3.2†[3]	2.7	2.1	2.9	5.4

N = 4 at 100 and 200 mg/kg/day and N = 6 at 400 mg/kg/day, except where indicated as †[n]
DN = Dose-normalized; units for DN AUC_{last} and C_{max} are (ng•hr/mL)/(mg/kg) and (ng/mL)/(mg/kg), respectively.
NA = Not applicable

Dosing Solution Analysis

The dosing solutions were found to contain 100 to 109% of the nominal concentrations of TR-701, and the dosing solutions were stable following 10 days of room temperature, refrigerated (4°C)and frozen (-70°C) storage. TR-701 was not detected in the vehicle formulation.

Study title: A 1-, 3-, 6-, and 9-month Oral (Gavage) Neurotoxicity Study of TR-701 in Long Evans Rats with a 3-Month Recovery Period

Study no.:	TOX-11-0701-028
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 16, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TR-701 FA; Lot # 02120030, purity of 99.6%

Key Study Findings

- Body weights were reduced in high-dose males (30 mg/kg/day) and females (10 mg/kg/day) with greater reductions occurring with the duration of dosing.
- No gross pathology or histopathology was noted for a battery of examined neural tissues, the eyes or skeletal muscle. No TR-701-related ophthalmic lesions were observed.
- Functional neural assessments including Functional Observational Battery assessments, and assessments of locomotor activity were negative when measured in Weeks 3, 12, 25 and 38.

Methods

Doses:	0, 7.5, 15 and 30 mg/kg/day for males and 0, 2.5, 5, and 10 mg/kg/day for females
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	10 ml/kg
Formulation/Vehicle:	25 mM disodium hydrogen phosphate, pH 7.5
Species/Strain:	Long Evans rats (Crl:LE) rats
Number/Sex/Group:	Group 2: 36/sex/group; all other groups 60/sex/group.
Age:	9-weeks old at initiation of dosing
Weight:	At randomization, Main and Recovery Animals: Males: 220-323g, Females: 143-215g; Toxicokinetic animals: Males: 229-301g; Females: 160-207g.
Satellite groups:	Toxicokinetic Animals: Group 1A: 5/sex/group; all other groups 9/sex/group.
Unique study design:	Animals in the toxicology groups (Groups 1-4)

	were dosed once daily by oral gavage for a minimum of 28, 91, 182, or 273 days. Up to 12 animals/sex/group were euthanized following a minimum of 28 (Groups 1, 3, and 4), 91 (all groups), 182 (all groups), and 273 (Groups 1, 3, and 4) days of dose administration; the remaining animals (Recovery animals; up to 12 animals/sex/group for all groups) were euthanized following a minimum of 182 days of dose administration and a 3-month non-dosing (Recovery) period. Toxicokinetic animals (Groups 1A-4A) were dosed for 273 days.
Deviation from study protocol:	Multiple deviations from the study protocol were noted, but the deviations were not considered to have altered the results or the integrity of the study.

Table 54: Study Design for Main Study Animals for Study No.: TOX-11-0701-028. (Sponsor's Table)

Group Number	Treatment	Dosage Level (mg/kg/day)	Dose Volume (mL/kg)	Number of Males[a]
1	Vehicle	0	10	60
2	TR-701 FA	7.5	10	36
3	TR-701 FA	15	10	60
4	TR-701 FA	30	10	60

Group Number	Treatment	Dosage Level (mg/kg/day)	Dose Volume (mL/kg)	Number of Females[a]
1	Vehicle	0	10	60
2	TR-701 FA	2.5	10	36
3	TR-701 FA	5	10	60
4	TR-701 FA	10	10	60

[a] = Up to 12 animals/sex/group were euthanized following a minimum of 28 (Groups 1, 3, and 4), 91 (all groups), 182 (all groups), and 273 (Groups 1, 3, and 4) days of dose administration; the remaining animals (up to 12 animals/sex/group for all groups) were euthanized following a minimum of 182 days of dose administration and a 3-month nondosing (recovery) period.

Table 55: Study Design for Toxicokinetic Animals for Study No.: TOX-11-0701-028. (Sponsor's Table)

Group Number	Treatment	Dosage Level (mg/kg/day)	Dose Volume (mL/kg)	Number of Males[a]
1A	Vehicle	0	10	5
2A	TR-701 FA	7.5	10	9
3A	TR-701 FA	15	10	9
4A	TR-701 FA	30	10	9

Group Number	Treatment	Dosage Level (mg/kg/day)	Dose Volume (mL/kg)	Number of Females[a]
1A	Vehicle	0	10	5
2A	TR-701 FA	2.5	10	9
3A	TR-701 FA	5	10	9
4A	TR-701 FA	10	10	9

[a] = All surviving animals were euthanized following 273 days of dose administration

Observations and Results

Table 56: Study Design for the Rat Neurological Study

Parameter	Schedule
Mortality and morbundity	BID for the duration of the study.
Clinical Signs	Twice daily (prior to dosing and 1-2 hours following dosing) for the duration of the study. Recovery animals were examined daily.
Body Weights	Individual body weights were obtained weekly beginning before the start of dosing and throughout the dosing period and on the day prior to the scheduled necropsy.
Food Consumption	Individual food consumption (g/animal/day) was recorded at least once weekly during the pretest period and throughout the study.
Functional Observational Battery (FOB)	FOB assessments were recorded for the animals selected for the Recovery Study (up to 12 animals/sex/group) prior to the initiation of the dose administration and during Weeks 3, 12, and 25. In addition, FOB assessments were recorded during Week 38 for the 9-month necropsy (up to 12 animals/sex/ group in Groups 1, 3, and 4).
Locomotor Activity	Locomotor activity was assessed for the same animals that were assessed for FOB
Toxicokinetics	Blood samples were collected from all animals prior to dosing, and at 1, 2, 4, 8, and 24 hours after dosing on Days 0 and during Weeks 3, 12, 25, and 38. Also blood was collected from vehicle control animals (Group 1A)

	approximately 2 hours after dosing. Blood was collected from 3 animals/sex/group for all groups at all time points.
Ophthalmic Examinations	Ocular examinations were conducted on all animals prior to randomization, near the end of each of the 1-, 3-, 6-, and 9-month treatment periods.
Necropsy	Necropsies were scheduled at the end of the 1-, 3-, 6-, and 9-month treatment periods as well as at the end of recovery for Recovery animals.

Mortality

A total of 11 animals were found dead or euthanized in extremis including 7 males (5 found dead and 2 euthanized in extremis) and four females (1 found dead and three euthanized in extremis). The deaths occurred sporadically, not occur in a clear dose-dependent manner and some of the deaths appeared to be related to gavage error.

Clinical Signs

TR-701-related clinical signs, considered non-adverse, were limited to clear material around the mouth of high-dose males and females.

Body Weights

Slightly lower body weight gains were noted for the high-dose males and females. At the 1, 3, 6, and 9 month necropsies body weights for the high-dose males were reduced 1.1, 3.9, 5.7, and 6.7% respectively compared to the vehicle controls. Body weights for the high-dose females were reduced 1.8, 0.9, 3.3, and 5.8% respectively for the same necropsies. Following recovery body weights for high-dose males remained depressed by 5.6% while the high-dose female body weights had returned to control levels.

Feed Consumption

No clear changes in food consumption were considered related to TR-701 administration.

Ophthalmoscopy

No TR-701-related ophthalmic lesions were observed.

ECG: Not performed

Hematology: Not performed

Clinical Chemistry: Not performed

Urinalysis: Not performed

Gross Pathology

A complete gross pathology assessment (external and internal examination) was performed on animals that were found dead or euthanized in extremis, but these animals were not subjected to neuropathological examination. Surviving animals that were euthanized at each scheduled necropsy were assessed for brain and spinal cord

gross pathology. Any observable gross changes, abnormal coloration, or lesions of the brain and spinal cord were recorded in addition to brain weight and dimensions.

No TR-701-related gross pathology was noted in the brain or spinal cord at any of the necropsy time-points in the Main Study (1-, 3-, 6- and 9-month) or Recovery Study.

Organ Weights

Brain weights were recorded for surviving animals euthanized at each scheduled necropsy.

Brain weights were not significantly changed with TR-701 administration at any of the necropsy time-points.

Histopathology

Adequate Battery

The battery of tissues examined for histopathology were limited predominantly to neural tissues in 10 randomly selected rats/gender/group in the control and high-dose groups from the 1-, 3-, and 6-month necropsies but not the 9-month necropsy. The examined tissues included: brain (olfactory bulbs, cerebral cortex, hippocampus, basal ganglia, thalamus, hypothalamus, midbrain, cerebellum, pons and medulla oblagata, optic tract including optic chiasm), spinal cord (at cervical swellings C3-C7 and at lumbar swellings T13-L4), trigeminal ganglia/nerves, lumbar dorsal root ganglia at T13-L4, lumbar dorsal root fibers at T13-L4, lumbar ventral root fibers at T13-L4, cervical dorsal root ganglia at C3-C7, cervical dorsal root fibers at C3-C7, cervical ventral root fibers at C3-C7, cervical spinal nerve, lumbar spinal nerve, sciatic nerves (mid-thigh region), sciatic nerves (sciatic notch), sural nerves, tibial nerves, peroneal nerves, optic nerves (retrobulbar and intracranial), eyes with optic nerve, and skeletal muscle gastronemius.

Peer Review

No

Histological Findings

No histopathology in the examined tissues was attributed to TR-701 administration.

Special Evaluation

Functional Observational Battery: Animals were assessed for FOB parameters including Home Cage Observations, Handling Observations, Open Field Observations, Sensory Observations, Neuromuscular Observations, and Physiological Observations.

Home Cage Observations: Home cage observations were not affected by TR-701 administration in the Study Weeks 3, 12, 25, and 38 evaluations.

Handling Observations: handling observations were not clearly affected by TR-701 in the Study Week 3, 12, 25, and 38 evaluations. Significantly fewer males in the high-dose group (6/10) exhibited no resistance upon handling in the Week 38 observation compared to the control group where 12/12 males exhibited slight resistance upon handling. Of the remaining four high-dose males, three exhibited slight resistance upon

handling and one was tense or rigid upon handling. However at other observation times, for instance Week 12, a higher proportion (5/12) of control males exhibited slight resistance to being handled suggesting variation in handling observations could be unrelated to TR-701 administration. Also in Week 38 all of the high-dose females exhibited no resistance to handling.

Open Field Observations: Open field observations appeared to be unaffected by TR-701 administration in the Week 3, 12, 25, and 38 observations.

Sensory Observations: Sensory observations appeared to be unaffected by TR-701 administration in the Week 3, 12, 25, and 38 observations.

Neuromuscular Observations: Neuromuscular observations appeared to be unaffected by TR-701 administration in the Week 3, 12, 25, and 38 observations.

Physiological Observations: Physiological observations appeared to be unaffected by TR-701 administration in the Week 3, 12, 25, and 38 observations.

Locomotor Activity: Locomoter activity was assessed for the animals selected for the recovery period (up to 12 animals/sex/group) prior to the initiation of dose administration and during Weeks 3, 12, and 25. In addition, locomotor activity was recorded for the animals selected for the 9-month necropsy (up to 12 animals/sex/group in Groups 1, 3, and 4) during Week 38. Data for ambulatory and total locomotor activity was tabulated. Total locomotor activity was defined as a combination of fine locomotor skills including grooming and ambulatory locomotor activity. Within-session repeated measures analysis of variance were conducted across the subintervals of each test session for total and ambulatory counts and for overall interval means (representing the entire 60-minute session activity) during each test session.

While some significant changes in locomotor activity occurred in TR-701 treatment groups, the changes were not dose-dependent and generally occurred sporadically in different test periods. Also, no meaningful changes in the pattern of habituation occurred in any of the TR-701-treatment groups

Toxicokinetics

The toxicokinetic parameters for TR-700 in male and female rats are shown in Table 57 below. Both plasma C_{max} and AUC measurements increased in a roughly dose-proportional manner. Exposure values (C_{max} and AUC) were somewhat higher in females compared to males despite the lower dosage range administered to females. A notable observation in both genders is that TR-700 continued to accumulate with longer durations of exposure, with gradual increases in C_{max} and AUC values observed at each sampling interval. The plasma AUC values following 273 days of administration of TR-701 were approximately 3-fold and 2-fold increased in males and females respectively compared to AUC values after the first dose.

Table 57: Toxicokinetic Parameters for TR-700 in Male and Female Rats Following Oral Administration of TR-701 FA. (Sponsor's Table)

Sex	Males			Females		
TR-701 FA Dosage (mg/kg/day):	**7.5**	**15**	**30**	**2.5**	**5**	**10**
Parameter (Units)			Study Day 0			
AUC_{last} (µg·h/mL)	16.9	32.4	69.9	24.9	55.7	95.6
SE AUC_{last} (µg·h/mL)	1.66	2.04	6.31	0.874	2.34	2.86
DN AUC_{last}	2.25	2.16	2.33	9.96	11.1	9.56
AUC_{inf} (µg·h/mL)	16.9	32.5	70.0	25.3	56.9	98.0
C_{max} (µg/mL)	3.67	5.17	10.4	3.60	8.48	14.7
DN C_{max}	0.489	0.345	0.346	1.44	1.70	1.47
T_{max} (h)	2	2	1	1	2	2
$T_{1/2}$ (h)	2.4	2.6	2.4	4.0	4.2	4.4
			Study Day 21			
AUC_{last} (µg·h/mL)	20.9	45.8	85.2	26.1	52.0	97.8
SE AUC_{last} (µg·h/mL)	0.900	4.16	7.70	1.29	1.08	5.72
DN AUC_{last}	2.79	3.05	2.84	10.4	10.4	9.78
C_{max} (µg/mL)	4.92	8.31	19.4	4.89	8.99	14.7
DN C_{max}	0.656	0.554	0.647	1.96	1.80	1.47
T_{max} (h)	1	1	1	1	1	1
$T_{1/2}$ (h)	2.8	3.1	2.8	4.2	4.4	4.9
Accumulation Ratio	1.2	1.4	1.2	1.0	0.93	1.0
			Study Day 84			
AUC_{last} (µg·h/mL)	27.2	81.8	145	34.3	82.6	147
SE AUC_{last} (µg·h/mL)	1.35	4.45	9.98	2.65	3.52	11.1
DN AUC_{last}	3.63	5.45	4.83	13.7	16.5	14.7
C_{max} (µg/mL)	7.08	15.0	27.6	4.91	12.8	22.1
DN C_{max}	0.944	0.999	0.921	1.96	2.56	2.21
T_{max} (h)	1	1	1	2	1	1
$T_{1/2}$ (h)	3.5	3.6	2.9	4.5	5.1	4.5
Accumulation Ratio	1.6	2.5	2.1	1.4	1.5	1.5

Table 57 continued.

Sex	Males			Females		
TR-701 FA Dosage (mg/kg/day):	7.5	15	30	2.5	5	10
Parameter (Units)			Study Day 175			
AUC_{last} (µg·h/mL)	34.1	82.7	204	45.8	97.1	174
SE AUC_{last} (µg·h/mL)	3.56	5.44	33.3	2.74	6.97	10.2
DN AUC_{last}	4.55	5.51	6.80	18.3	19.4	17.4
C_{max} (µg/mL)	7.90	17.3	31.2	6.00	11.7	21.5
DN C_{max}	1.05	1.15	1.04	2.40	2.34	2.15
T_{max} (h)	1	1	2	1	2	1
$T_{1/2}$ (h)	2.8	2.7	3.9	4.5	5.3	5.0
Accumulation Ratio	2.0	2.6	2.9	1.8	1.7	1.8
			Study Day 272			
AUC_{last} (µg·h/mL)	41.9	101	222	51.7	97.2	189
SE AUC_{last} (µg·h/mL)	6.33	9.14	27.3	3.42	11.1	7.43
DN AUC_{last}	5.59	6.73	7.40	20.7	19.4	18.9
C_{max} (µg/mL)	8.73	21.0	29.1	6.36	12.0	23.6
DN C_{max}	1.16	1.40	0.970	2.54	2.39	2.36
T_{max} (h)	1	1	2	1	2	2
$T_{1/2}$ (h)	2.9	3.2	3.5	4.4	5.5	5.3
Accumulation Ratio	2.5	3.1	3.2	2.1	1.7	2.0

DN = Dose-normalized; units for DN AUC_{last} and C_{max} are (µg·h/mL)/(mg/kg) and (µg/mL)/(mg/kg), respectively. SE = Standard error. Accumulation ratios calculated using AUC_{last}.

Dosing Solution Analysis

Samples for concentration analysis were collected from each of the dosing formulations including the control group during study weeks 0, 12, 25, and 38. Samples were analyzed for TR-701 content using a validated HPLC method.

The analyzed dosing formulations for TR-701 FA were found to contain 98.3% to 105% of the nominal concentration of TR-701 FA which was within the target range.

Study title: A 28-Day Intravenous (Injection) Toxicity Study of TR-701 With a 28-Day Recovery Period in Sprague Dawley Rats

Study no.: TOX-08-0701-009
Study report location: Electronic transmission
Conducting laboratory and location: (b) (4)
Date of study initiation: May 5, 2008
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: TR-701, Lot No.: CMLW-304/07-TR3, Purity of 90% including 3% moisture and (b) (4) impurities*.

*A later batch analysis reported by the Applicant for TR-701, Lot No.: CMLW-304/07-TR3 indicates that substance impurities included (b) (4) and this percentage

was used to calculate the impurity NOAEL in the present study and qualify the commercial acceptance level (b) (4)

Key Study Findings

- No TR-701-related deaths were observed. Clinical signs included: soft and/or mucoid feces primarily in high-dose males and females, and labored and shallow respiration, hyporeactivity, pale extremities, and muscle rigidity in several high-dose males.
- Body weights for high-dose males were reduced by 8% compared to controls at the end of dosing.
- Serum potassium, total protein, and globulin were reduced and the albumin/globulin ratio was increased in a dose-dependent manner in mid- and high-dose males and females. These serum chemistry changes may have occurred in association with soft and mucoid feces and demonstrated total or partial reversal in recovery animals.
- No TR-701-related gross pathology or histopathology findings were reported.
- The NOAEL values in this study were considered to be the mid-doses, 30 and 15 mg/kg/day for males and females respectively.

Methods

Doses:	0, 10, 30, and 90 mg/kg/day for males and 5, 15, and 45 mg/kg/day for females.
Frequency of dosing:	Once daily
Route of administration:	Intravenous bolus injection via lateral caudal tail vein.
Dose volume:	10 ml/kg
Formulation/Vehicle:	Sterile water for injection
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	Main Study: 10/sex/group; Recovery Study: 5/sex/group for Groups 1 and 4 only.
Age:	Approximately 7 weeks old at the initiation of dose administration
Weight:	Main and Recovery Study Males ranged from 213 to 270 g and females ranged from 141 to 179 g. Toxicokinetic males ranged in individual body weight from 224 to 259 g and females from 126 to 180 g.
Satellite groups:	Toxicokinetic animals: 3/sex/group for the vehicle control group (Group 1A) and 9/sex/group for all the TR-701 treatment groups (Groups 2A-4A).
Unique study design:	See Table 58. Male and female rats received intravenous bolus injections of TR-701 for 28 days followed by a 28-day recovery period.
Deviation from study protocol:	Deviations from the study protocol were noted but none was considered to have altered the

results or limited the integrity of the study.

Table 58: Study Design for Study No.: TOX-08-0701-009. (Sponsor's Table)

Toxicology Groups ((b) (4)-674005M and (b) (4)-674005F)

Group Number	Test Article	Dosage Level (mg/kg/day)	Dose Volume (mL/kg)	Number of Animals [a] Males	Females
1	Vehicle	0	10	15	15
2	TR-701	10 (M), 5 (F)	10	10	10
3	TR-701	30 (M), 15 (F)	10	10	10
4	TR-701	90 (M), 45 (F)	10	15	15

Toxicokinetic Groups ((b) (4)-674005A, 674005B, 674005T and 674005K) [b]

Group Number	Test Article	Dosage Level (mg/kg/day)	Dose Volume (mL/kg)	Number of Animals [c] Males	Females
1A	0	0	10	3	3
2A	TR-701	10 (M), 5 (F)	10	9	9
3A	TR-701	30 (M), 15 (F)	10	9	9
4A	TR-701	90 (M), 45 (F)	10	9	9

[a] = For toxicology animals, up to 10 animals/sex/group were euthanized at the primary necropsy. The remaining animals (≤5 animals/sex/group) were euthanized at the recovery necropsy.

[b] = Toxicokinetic animals (Groups 1A-4A) were dosed and received dose formulations prepared in the same manner as the toxicology (Groups 1-4) animals until euthanasia following the final blood collection on study day 23.

[c] = An additional 6/animals/sex/group (Groups 2A-4A) received a single dose on 19 June 2008, and were euthanized following the final blood collection at 30 minutes post-infusion completion.

Observations and Results

Table 59: Observation Schedule for Study No.: TOX-08-0701-009

Parameter	Schedule
Mortality and morbundity	BID for the duration of the study.
Clinical Signs	Twice daily (prior to dosing and 1-2 hours following dosing) with an additional examination at approximately 5 minutes after dosing beginning on Day 1. Recovery animals were examined daily. In addition, detailed physical examinations were conducted on all animals weekly, beginning 12-days prior to dosing, at randomization and on the scheduled termination days.
Body Weights	Individual body weights were obtained at least weekly beginning before the start of dosing and twice weekly throughout the dosing period, and once weekly during

	recovery.
Food Consumption	Individual food consumption (g/animal/day) was recorded at least once weekly during the pretest period and throughout the dosing period, and during the Recovery period.
Hematology and Coagulation Serum Chemistry Urinalysis	Blood and urine samples were collected from all animals just prior to the scheduled necropsies. Animals were fasted overnight prior to collection.
Toxicokinetics	Blood samples were collected from all animals in Groups 2A-4A prior to dosing, and at 1, 2, 4, 8, and 24 hours after dosing on Days 0 and 22. In addition, on Day 22, blood was also collected at approximately 5, 15, and 30 minutes after dosing. Also blood was collected from vehicle control animals (Group 1A) approximately 2 hours after dosing. Blood was collected from 3 animals /sex/group for all groups at all time-points.
Ophthalmic Examinations	Ocular examinations were conducted on all animals prior to randomization, during the last week of dosing, and during the last week of the recovery period.
Necropsy	Necropsies were scheduled at the end of the 28-day treatment period as well as 28 days later for Recovery animals.

Mortality

One female in the low- and mid-dose TR-701 groups and two females in the high-dose group were found dead at the 5-minute post-dosing observation on Days 11 (low-dose), 24 (mid-dose), 5 and 19 (high-dose). Also one male was found dead on Day 0. Each of these deaths was not considered to be related to TR-701 administration due to proximity to the dosing time, a lack of dosing for the male and/or a lack of clinical signs for the low- and mid-dose animals. One low dose female was euthanized in extremis on Day 19 due to a procedure-related injury.

Clinical Signs

TR-701-related clinical signs included soft and/or mucoid feces, yellow and/or brown material on the uro/anogenital area(s) and clear material around the mouth. Findings were noted after several days of administration 5-minutes post-dosing, 1-2 hours post-dosing, and at the time of dosing on the following day in males and females in the mid- and high-dose groups with much greater incidence in the high-dose group. Also more severe clinical signs including labored and shallow respiration, increased respiration rate, hypoactivity, pale extremities, impaired equilibrium, and prostate body were noted in several high-dose males primarily on Days 0 and 1, but not after Day 4. Near the end of the dosing period, muscular rigidity was noted in 4 high-dose males.

Body Weights

TR-701 related reductions in body weight were noted in mid-dose and high-dose males. In mid-dose males, body weights were reduced during Days 3-6, but comparable to control for the rest of the dosing period. High-dose males demonstrated significantly

reduced body weights compared to the control group on Days 3-6 and lower weights throughout the dosing period including 8% lower than control on Day 27

Mean body weight gain was lower for high-dose males was similar to that of the control group during the recovery period, but the mean body weight remained significantly lower.

Feed Consumption

Lower mean food consumption was noted for the mid- and high-dose group males from Study Days 0 to 13 and the differences compared to the control group were generally significant for the high-dose males. Food consumption was similar for all TR-701 treatment groups from Days 14-27. TR-701 did not experience a reduction in food consumption.

Despite the similar food consumption for high-dose males compared to controls from Days 14-27, food consumption for Recovery high-dose males remained slightly but significantly reduced compared to controls during the Recovery period (Days 28 – 35).

Ophthalmoscopy

All ocular examinations were conducted using an indirect ophthalmoscope and slit lamp biomicroscope following pupillary dilation.

No ophthalmic changes indicative of TR-701-induced toxicity were noted.

ECG: Not performed

Hematology

The hematology parameters shown in Table 123 were assessed.

No hematology parameters were greatly or significantly altered in a dose-dependent manner.

Mean red cell numbers were slightly lower, and mean corpuscular volume was slightly higher in high-dose males and females in Week 4, but not to a significant degree. Also platelet numbers were lower in 50% of high-dose males in Week 4, but individual and group mean values were within the historical reference range. APTT was reduced in the mid- and high-dose males and high-dose females, but not significantly so. All of the hematology and coagulation value changes were not considered toxicologically relevant because of the small magnitude of change and all of the value changes showed evidence of reversal in Recovery animals.

Clinical Chemistry

The serum chemistry parameters shown in Table 124 were assessed.

Significantly lower mean potassium levels occurred in a dose-related manner in mid- and high-dose males and females during Study Week 4 compared to controls. After 4-weeks recovery, potassium levels for high-dose males were similar to control males but

remained lower than controls for the high-dose females. The lower potassium levels may have been related to increased gastrointestinal loss associated with soft and mucoid feces, but did not correlate in individual animals with higher urine volume noted for high-dose females. Also possibly related to gastrointestinal loss were lower mean total protein, globulin, and higher albumin/globulin ratio that occurred in a dose-dependent manner in mid- and high-dose males and females. Lower mean albumin levels also occurred in mid- and high-dose females. These effects were totally or partially reversed in recovery animals.

Urinalysis

The urinalysis parameters shown in Table 125 were assessed.

All urinalysis parameters were not changed by TR-701 administration except for a significantly higher mean total volume in high-dose females during Week 4 only. This change may have been influenced by water contamination of the urine sample in one outlier female.

Gross Pathology

No TR-701-related gross-pathology findings were noted.

Organ Weights

The organs listed in Table 126 were weighed.

Several organ weight changes were noted in male and female rats, but most of the changes were consistent with stress effects and/or the TR-701-related depression in weight gain. In high-dose males, significantly lower absolute and relative heart weights (relative to brain weight), and lower liver weight relative to brain weight were observed. Significantly increased absolute and relative (to body and brain weights) adrenal weights were increased in high-dose males. Also though not statistically significant relative to control values, absolute liver weights, liver weights relative to body weights were lower in high dose males and absolute and relative (to brain and body weights) thymus weights were noted.

In high-dose females, significantly lower absolute and relative (to brain and body weights) thymus weights were noted and a trend toward higher absolute and relative (to body and brain weights) adrenal weights.

At the Recovery sacrifice, when high-dose males had significantly less body weight than controls, high-dose males demonstrated significantly reduced absolute and relative (relative to brain weight) adrenal weights, higher relative brain weights (relative to body weight), and higher relative (relative to body weight) thryroid/parathyroid weight.

Histopathology

Adequate Battery

Yes, the panel of tissues listed in Table 126 was examined for histopathology

Peer Review

No

Histological Findings

No histopathology considered related to TR-701 administration was noted.

Toxicokinetics

Plasma TR-701 rapidly converted to TR-700 as evidenced by much lower TR-701 plasma concentrations compared to TR-700 plasma concentrations (Table 60).

Table 60: Summary of Toxicokinetic Parameters for TR-701 and TR-700 in Study No.: TOX-08-0701-009. (Sponsor's Table)

Sex	Study Day	Group (mg/kg/day)	C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24} (µg·h/mL)
		TR-700			
Male	0	10	15.00	0.0833	26.60 [a]
		30	55.10	0.250	117.0
		90	111.0	0.250	505.0
	22	10	16.80	0.250	25.90
		30	52.70	0.250	127.0
		90	121.0	0.250	491.0
Female	0	5	7.510	0.500	38.60
		15	25.80	0.250	101.0
		45	67.20	0.0833	370.0
	22	5	7.510	0.500	33.40
		15	21.00	0.250	85.10
		45	66.90	0.250	281.0
		TR-701			
Male	0	10	27.80	0.0833	8.830 [a]
		30	59.40	0.0833	18.80
		90	151.0	0.0833	56.90
	22	10	29.60	0.0833	8.600 [a]
		30	65.60	0.0833	22.00
		90	148.0	0.0833	50.70
Female	0	5	13.20	0.0833	5.230 [a]
		15	26.40	0.0833	10.60
		45	53.10	0.0833	25.20
	22	5	12.00	0.0833	4.920
		15	22.20	0.0833	11.00
		45	44.20	0.0833	21.80

a = Value is AUC_{all} instead of AUC_{0-24h}

Plasma AUC and C_{max} values for TR-700 increased in a roughly dose-proportional manner in females for both the Day 0 and Day 22 sampling days. The plasma C_{max} and AUC values for males increased in a dose-linear manner, but with more variability regarding dose proportionality. Plasma C_{max} increased in a dose-proportional manner

between the low- and mid-doses, and less than dose proportional manner between the mid- and high-dose. Plasma AUC values increased in a less than dose proportional manner for all doses. Neither TR-700 nor TR-701 appeared to accumulate in either gender with repeated dosing.

Dosing Solution Analysis

The analyzed dosing formulations were found to always contain 90 to 110% of the nominal concentrations.

Study title: A 14-Day (Twice Daily) Intravenous 30-Minute Infusion Toxicity Study of TR-701 in Beagle Dogs (Revised Final Report)

Study no.:	TOX-08-0701-019
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 8, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TR-701, Lot # CMLW-304/07-TR3 purity of 90%; lot # OXA-004, purity of 97.5%; and Lot # DUG-AG-124, purity of 99.6%

Key Study Findings

- The primary clinical signs were severe injection-site reactions primarily in high-dose (200 mg/kg/day) males and females, with lesser incidence in mid-dose animals. Emesis occurred in individual animals in all TR-701 treatment groups with incidence and severity increasing with dose.
- Because of severe injection-site reactions, all high-dose (200 mg/kg/day) males and females were euthanized early on Day 8 or 9.
- Body weights for high-dose males and females were approximately 10% lower than for control animals.
- In mid-dose animals hematology changes including increased WBC, absolute neutrophils, and monocytes may have been secondary to the injection-site reactions.
- Thymic atrophy which was demonstrated in a dose-dependent manner in all groups and much of the TR-701-related gross pathology and histopathology in the low- and mid-dose groups may have been related to stress secondary to the injection-site reactions. Bone marrow hypocellularity occurred in 2/3 high-dose females euthanized early.
- Because of injection-site reactions, no NOAEL was determined. Exclusive of injection-site reactions, the NOAEL was considered to be the low dose, 50 mg/kg/day, in both genders.

Methods

Doses:	0, 25, 50, and 100 mg/kg/dose
Frequency of dosing:	Twice per day
Route of administration:	IV infusion over approximately 30 minutes
Dose volume:	1 ml/kg
Formulation/Vehicle:	0.9% NaCl
Species/Strain:	Beagle dogs
Number/Sex/Group:	3/sex/group
Age:	6-7 months of age at dosing initiation
Weight:	Males: 8.5 to 10.8 kg; females: 6.3 to 7.9 kg
Satellite groups:	none
Unique study design:	See Table 61.
Deviation from study protocol:	Study deviations were noted, but none was considered to have altered the study results or the integrity of the study.

Table 61: Study Design for the 14-Day IV Toxicology Study in Dogs

Group	Treatment	N/sex/group
1	Vehicle	3
2	TR-701: 25 mg/kg/dose; 50 mg/kg/day	3
3	TR-701: 50 mg/kg/dose; 100 mg/kg/day	3
4*	TR-701: 100 mg/kg/dose; 200 mg/kg/day	3

*Following 8 or 9 days of dosing, Group 4 animals were euthanized due to severe clinical signs.

Observations and Results

Table 62: Observation Schedule for the 14-Day IV Toxicology Study in Dogs

Parameter	Schedule
Mortality and morbundity	BID for the duration of the study.
Clinical Signs	Prior to dosing and approximately 1 hour following dosing for both daily doses. In addition, detailed physical examinations were conducted on all animals weekly, beginning once during acclimation prior to randomization, at randomization, weekly during the dosing period and prior to the scheduled necropsies.
Body Weights	Individual body weights were obtained before the start of dosing and twice weekly throughout the dosing period, and one day before the scheduled necropsy.
Food Consumption	Individual food consumption (g/animal/day) was recorded at least once weekly during the pretest period and throughout the dosing period. The weekly and daily averages were reported as g/animal/day.

Hematology and Coagulation Serum Chemistry Urinalysis	Blood and urine samples were collected from all animals just prior to initiation of dosing and at the scheduled necropsies for Groups 1-3. Clinical pathology was not evaluated for animals in Group 4. Animals were fasted overnight prior to collection.
Toxicokinetics	Blood samples were collected from all animals prior to dosing and at approximately 15, 30 (immediately following infusion), 40, and 50 minutes, and 1, 2, 4, 6, 8, and 12 hours (prior to administration of the second dose) following the start of infusion of all groups on study days 0, 7 (Group 4 females), 8 (Group 4 males), and 13 (Groups 1-3).
Ophthalmic Examinations	Ocular examinations were conducted on all animals prior to randomization and near the end of the dosing period with the exception of Group 4 animals.
ECG	ECGs were recorded for all animals prior to randomization and during study Week 1 (Groups 1-3). During the treatment period ECGs were recorded approximately 5 to 15 minutes post-infusion completion of the first daily dose.
Necropsy	Necropsies were scheduled at the end of the 14-day treatment period. Group 4 animals were euthanized following 8-9 days of dosing due to morbidity.

Mortality

Males and females in the high-dose group (100 mg/kg/dose; 200 mg/kg/day) were euthanized early (study Days 9 and 8 respectively) due to significant weight loss, low food consumption, adverse clinical signs, and local irritation at injection sites resulting in swollen/impaired hindlimbs.

Clinical Signs

Individual male and female dogs in the low- (25 mg/kg/dose; 50 mg/kg/day) and mid-dose (50 mg/kg/dose; 100 mg/kg/day) groups experienced emesis and/or excess salivation. High-dose animals demonstrated emesis, excess salivation, and frothy material around the mouth. These signs occurred in males more than in females and were present during dosing and up to 1-2 hours after dosing.

Local injection-site reactions occurred in a few low- and mid-dose animals but were most severe and prevalent in high-dose animals beginning as early as the second day of dosing. The effects consisted of swollen hindlimbs and/or forelimbs resulting in impaired use of limbs.

Body Weights

Mean body weights were lower and/or weight gains were lower for high-dose males and females. In the second week of dosing before early euthanasia mean body weights were 8.2% and 12.5% lower in males and females respectively compared to control

animals. Weight loss relative to control animals did not occur for low- and mid-dose animals.

Feed Consumption

Significantly lower mean food consumption was observed for high-dose males and females at different intervals during the dosing period beginning as early as the first day of dosing. Affected animals were supplemented with Alpo (wet food supplement), which increased food consumption to values similar to still slightly lower than control values. Significantly lower food consumption was also noted for mid-dose males and females during the Day 7 to Day 8 interval. Subsequent supplementation with Alpo improved food consumption to values approximating control values.

Ophthalmoscopy

All ocular examinations were conducted using an indirect ophthalmoscope and slit lamp biomicroscope preceded by pupillary dilation with an appropriate mydriatic agent.

No ophthalmic toxicity was noted for the TR-701 treatment groups.

ECG

Multilead (I, II, II, aVR, aVL, aVF, and V2) electrocardiograms (ECGs) were recorded. Measurements of heart rate and ECG intervals (PR, QRS and QT) were performed. QTc was determined using Van der Water's correction.

No TR-701-related changes in any of the ECG parameters were observed.

Hematology

The measured hematology and coagulation parameters are shown in Table 123.

On Day 14, hematology effects were noted in the low- and mid-dose animals compared to pretest values and control group values. These effects may have been secondary to the TR-701-related severe local irritation. In mid-dose animals higher counts reaching statistical significance in males were noted for WBC counts (+101% in males; +82% in females), mean absolute neutrophils (+151% in males; +131% in females), and monocytes (+142% in males; +113% in females). Although statistical significance was not achieved, TR-701-related lower mean RBC counts (-14% in males and -17% females), hemoglobin (-13% males and -17% females), hematocrit (-13% males and -16% females), and absolute basophils (-50% males and females) and reticulocytes (-49% males; -51% females) were observed in mid-dose animals and occurred in a dose-dependent manner with changes of lesser magnitude in low-dose animals.

Clinical Chemistry

The measured clinical chemistry parameters are shown in Table 124.

Compared to control values, significantly lower serum albumin in mid-dose males, lower A/G ratio, higher globulin and cholesterol in low- and mid-dose males, and higher cholesterol and triglyceride levels in mid-dose females were noted. The injection-site inflammation may have influenced some of these changes such as the lower albumin.

Urinalysis

The measured urinalysis parameters are shown in Table 125.

None of the urinalysis parameters were altered by treatment with TR-701.

Gross Pathology

Necropsy examinations included examination of the external surface, all orifices, and the cranial, thoracic, abdominal and pelvic cavities including viscera.

Reportedly, the only gross-pathology findings were associated with the pronounced injection-site reactions. The gross-pathology findings increased with dose with only minor findings associated with vehicle injections. Injection-site findings and their relative incidence are shown in Table 63 below.

Table 63: Incidence of Gross Pathology Findings in Association with Injection Sites. (Sponsor's Table)

	Males				Females			
Dosage (mg/kg/dose):	**0**	**25**	**50**	**100**	**0**	**25**	**50**	**100**
Injection Site [a]: no. dogs (no. injection sites)	**3 (7)**	**3 (12)**	**3 (17)**	**3 (12)**	**3 (7)**	**3 (12)**	**3 (15)**	**3 (12)**
Discoloration dark red [b]	3 (6)	3 (11)	3 (16)	2 (8)	1 (1)	3 (11)	3 (12)	3 (10)
Edema [b]	0 (0)	2 (5)	2 (9)	3 (11)	0 (0)	2 (5)	0 (0)	2 (5)
Swollen [b]	0 (0)	2 (6)	3 (13)	3 (10)	0 (0)	2 (4)	3 (7)	3 (7)
Open sore [b]	0 (0)	1 (1)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Thickened [b]	0 (0)	2 (5)	2 (5)	0 (0)	0 (0)	0 (0)	2 (6)	0 (0)
Dark red areas [b]	1 (1)	0 (0)	0 (0)	1 (2)	2 (5)	1 (1)	0 (0)	2 (2)
Scabbing [b]	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	2 (3)	0 (0)

[a] Total number of dogs examined (total number injection sites examined, all dogs in that group)
[b] Total number dogs affected (total number of injection sites affected)

Organ Weights

The organs that were assessed for weight are listed in Table 126.

Organs were collected from high-dose animals following their early euthanasia dates on Days 8 and 9, while organ weights for the low- and mid-dose animals were determined on Day 14. Given the disparity in the euthanasia times, the Sponsor chose not to statistically compare the organ weights from high-dose animals to those of control animals and comparisons between the high-dose animals and other groups were restricted to qualitative comparisons. TR-701-related organ weight changes were limited to reduced thymus weights with significant reductions for mid-dose males (Table 64). This finding is consistent with a stress response. Other significant organ weight changes (lower relative adrenal gland and lung weights in females; lower absolute heart, lung and higher kidney weights in males) noted in mid-dose animals were not consistent with qualitative comparisons to the high-dose animals, and therefore not considered related to TR-701 administration.

Table 64: TR-701-Related Organ Weight Changes for the 14-Day IV Toxicology Study in Dogs. (Sponsor's Table)

Parameter	Direction and magnitude of change	Dosage level (mg/kg/dose)	Sex
Final Body Weight	↓ 10%	100	Male
	↓ 11%	100	Female
Thymus			
Absolute	↓ 52*, 46%	50, 100	Male
Relative to body weight	↓ 50**, 40%	50, 100	Male
Relative to brain weight	↓ 51*, 45%	50, 100	Male
Thymus			
Absolute	↓ 39, 55, 59%	25, 50, 100	Female
Relative to body weight	↓ 36, 55, 56%	25, 50, 100	Female
Relative to brain weight	↓ 42, 57, 60%	25, 50, 100	Female

* = Significantly different from the control group at 0.05 using Dunnett's test
** = Significantly different from the control group at 0.01 using Dunnett's test

Histopathology

Adequate Battery

Yes. The organs and tissues prepared for histopathology are listed in Table 126.

Peer Review

No

Histological Findings

Consistent with the gross pathology findings, much of the TR-701-related histopathology was associated with the injection-site reactions as shown in Table 65. More injection sites were required for the animals receiving TR-701 because debilitating injection-site reactions often necessitated the use of new injection sites. Consequently more injection sites were available for evaluation in the TR-701 groups compared to the control group. Although injection-site histopathology occurred with vehicle administration, the incidence and severity of the injection site histopathology was increased with TR-701, although not in a dose-dependent manner.

Other TR-701-related histopathology included thymic atrophy, lymphoid depletion of Peyer's patches, ulceration of the pylorus, mucosal atrophy and karyomegaly of the esophagus, duodenum and jejunum, inflammation of the duodenum and bone marrow hypercellularity or hypocellularity (Table 66). Of these findings, thymic atrophy correlated with lower absolute and relative thymus weights in all TR-701-treated females and the mid- and high-dose males. Peyer's Patch lymphoid depletion occurred in a dose-dependent manner with the greatest incidence and severity in high-dose males and females. Bone-marrow hypocellularity was observed in 2/3 high-dose females, but not in any other TR-701-treated animals. The histopathology in the GI tract (ulcers, atrophy and/or karyomegaly of the pyloric stomach, esophageal mucosa, and

duodenal and jujunal mucosa) was observed in only a single high-dose female. Bone marrow hypercellularity was noted in multiple male and female animals in all of the TR-701 treatment groups, but it is unclear if this finding was a direct effect of TR-701 or a consequence of injection-site inflammation.

Table 65: Incidence of Histopathology at Injection Sites. (Sponsor's Table)

	Males				Females			
Dosage (mg/kg/dose):	**0**	**25**	**50**	**100**	**0**	**25**	**50**	**100**
Injection Site [a]: no. dogs (no. injection sites)	**3 (7)**	**3 (12)**	**3 (17)**	**3 (12)**	**3 (7)**	**3 (12)**	**3 (15)**	**3 (12)**
Edema [b, c]	0 (0)	3 (12)	3 (16)	3 (11)	0 (0)	3 (12)	3 (15)	3 (9)
severe	-	3	3	3	-	3	3	3
Inflammation, necro-suppurative [b, c]	0 (0)	3 (12)	3 (17)	3 (11)	1 (1)	3 (12)	3 (14)	3 (10)
mild	-	0	0	0	1	0	0	0
moderate	-	1	2	2	0	1	0	3
severe	-	2	1	1	0	2	3	0
Ulceration/necrosis [b, c]	0 (0)	1 (1)	3 (6)	0 (0)	0 (0)	0 (0)	2 (3)	3 (3)
moderate	-	1	2	-	-	-	0	2
severe	-	0	1	-	-	-	2	1
Vasculitis [b, c]	3 (3)	3 (12)	3 (16)	3 (11)	2 (3)	3 (12)	3 (15)	3 (11)
minimal	2	0	0	0	2	0	0	0
mild	1	0	0	0	0	0	0	0
severe	0	3	3	3	0	3	3	3
Thrombosis [b, c]	0 (0)	3 (10)	3 (15)	3 (11)	0 (0)	3 (12)	3 (15)	3 (10)
moderate	-	2	2	2	-	1	0	1
severe	-	1	1	1	-	2	3	2
Hemorrhage [b, c]	3 (5)	3 (11)	3 (15)	3 (12)	2 (3)	3 (11)	3 (14)	3 (12)
minimal	1	0	0	0	1	0	0	0
mild	2	0	0	0	1	3	0	1
moderate	0	2	2	3	0	0	2	1
severe	0	1	1	0	0	0	1	1

[a] - Total number of dogs examined (total number of injection sites examined, all dogs in that group)
[b] - Total number of dogs affected (total number of injection sites affected)
[c] - Most severe grade for any injection site in the animal presented in the table
- No noteworthy findings

Table 66: Incidence of TR-701-Related Histopathology Not Directly Associated with Injection-Site Reactions. (Sponsor's Table)

	Males				Females			
Dosage (mg/kg/dose):	**0**	**25**	**50**	**100**	**0**	**25**	**50**	**100**
Thymus [a]:	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Atrophy	3	3	3	3	1	2	3	3
minimal	3	2	0	0	1	2	2	1
moderate	0	1	2	1	0	0	1	0
severe	0	0	1	2	0	0	0	2

Table 66 continued

Dosage (mg/kg/dose):	Males 0	25	50	100	Females 0	25	50	100
Duodenum [a]	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Karyomegaly	0	0	0	0	0	0	0	1
mild	-	-	-	-	-	-	-	1
Inflammation, acute	0	0	0	0	0	0	0	1
minimal	-	-	-	-	-	-	-	1
Jejunum [a]	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Karyomegaly	0	0	0	0	0	0	0	1
minimal	-	-	-	-	-	-	-	1
Peyer's patch [a]	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Depletion, lymphoid	0	0	1	2	0	0	1	2
minimal	-	-	1	1	-	-	0	0
mild	-	-	0	0	-	-	0	2
moderate	-	-	0	1	-	-	1	0
Bone Marrow, Sternum [a]	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Hypercellular	0	0	1	0	0	0	0	0
mild	-	-	1	-	-	-	-	-
Hypocellular	0	0	0	0	0	0	0	2
minimal	-	-	-	-	-	-	-	1
moderate	-	-	-	-	-	-	-	1
Bone Marrow, Femur [a]	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Hypocellular	0	0	0	0	0	0	0	2
minimal	-	-	-	-	-	-	-	1
moderate	-	-	-	-	-	-	-	1
Hypercellular	0	1	1	2	0	1	1	0
minimal	-	1	1	2	-	1	1	-
Stomach, pylorus [a]	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Ulcer	0	0	0	0	0	0	0	1
minimal	-	-	-	-	-	-	-	1
Esophagus [a]	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Atrophy, mucosal	0	0	0	0	0	0	0	1
mild	-	-	-	-	-	-	-	1
Karyomegaly	0	0	0	0	0	0	0	1
mild	-	-	-	-	-	-	-	1

[a] - Number of animals examined
- No noteworthy findings

Special Evaluation

None

Toxicokinetics

TR-701 was rapidly converted to TR-700 in plasma. Plasma C_{max} and AUC values for TR-700 increased in a roughly dose-proportional manner. Plasma TR-700 concentration

measurements were somewhat variable, but pronounced gender differences did not occur, and in both genders TR-700 did not greatly accumulate with repeated dosing.

Table 67: Toxicokinetic Parameters for TR-700 and TR-701 Following IV Administration of TR-701 in Dogs. (Sponsor's Table)

Gender	Dose (mg/kg/dose)	T_{max} (hr)		C_{max} (µg/mL)		AUC_{0-12h} (µg•hr/mL)	
		Day 0	Day 13 [a]	Day 0	Day 13 [a]	Day 0	Day 13 [a]
TR-700							
Males	25	0.50	0.50	16.9	21.0	22.6	29.2
	50	0.50	0.67	35.2	30.8	49.9	38.5
	100	0.50	0.33	74.1	32.5	156	59.2
Females	25	0.42	0.50	9.82	16.1	12.9	19.9
	50	0.50	0.50	28.7	37.2	43.2	49.1
	100	0.67	0.67	48.5	62.5	119	152
Combined	25	0.46	0.50	13.4	18.5	17.7	24.5
	50	0.50	0.58	32.0	34.0	46.5	43.8
	100	0.58	0.50	61.3	47.5	138	106
TR-701							
Males	25	0.42	0.33	15.4	11.1	6.65	4.84
	50	0.33	0.47	33.4	17.4	13.3	6.90
	100	0.25	0.50	68.6	19.4	30.7	8.76
Females	25	0.33	0.33	13.6	11.9	4.87	4.49
	50	0.33	0.33	33.4	11.0	12.8	4.74
	100	0.42	0.50	72.2	39.5	30.1	17.1
Combined	25	0.37	0.33	14.5	11.5	5.76	4.66
	50	0.33	0.40	33.4	14.2	13.0	5.82
	100	0.33	0.50	70.4	29.5	30.4	12.9

a. Study day 13 for the 25 and 50 mg/kg/dose groups; study day 8 and 7 for the 100 mg/kg/dose group males and females, respectively.
$AUC_{0-12h} = AUC_{Tau}$

Dosing Solution Analysis

The analyzed dosing formulations were found to contain (b) (4) TR-701, (b) (4) (b) (4) of the target concentrations.

7 Genetic Toxicology

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

Study title: TR-701 Bacterial Reverse Mutation Assay

Study no.:	TOX-07-0701-001
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 21, 2005
GLP compliance:	Yes ((b) (4) FDA)
QA statement:	Yes
Drug, lot #, and % purity:	DA-7218 (TR-701), Lot No.: Oxa-003, purity of 99.07%

Key Study Findings

TR-701 was considered to be negative for mutagenesis in the Ames assay for all strains tested.

Methods

Strains:	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, and *Escherichia coli* WP2 uvrA
Concentrations in definitive study:	Definitive concentrations for each strain are shown in Table 68.
Basis of concentration selection:	TR-701 concentrations for the definitive study were based on the results of a range-finding test which included a high dose of 5000 μg/plate and made note of cytotoxic effects. The lowest dose causing at least 50% cytotoxicity in individual strains was chosen as the high-dose in the definitive study for each strain.
Negative control:	Sterile distilled water
Positive control:	Sodium azide (SA) for TA100 and TA1535 without S9 ; 2-nitrofluorene (2-NF) for TA98 without S9; 4-nitroquinoline-1-oxide (4NQO) for WP2 uvrA; 9-aminoacridine (9-AA) for TA1537; 2-aminoanthracene (2-AA) for TA1535 and WP2 uvrA with S9; Benzo(a)pyrene (BP) for TA98, TA100, and TA1537 with and without S9.
Formulation/Vehicle:	Sterile distilled water
Incubation & sampling time:	48 hours incubation at 37°C followed by sampling.

Table 68: Ames Assay TR-701 Test Concentrations for the Definitive Assay

Test Strain	Concentrations Tested With and Without S9 (μg/plate)
TA100	0, 9.8, 19.6, 39.1, 78.2, 156.3, 312.5, and 625
TA1535	0, 19.6, 39.1, 78.2, 156.3, 312.5, and 625
TA98	0, 39.1, 78.2, 156.3, 312.5, 625, 1250, and 2500
TA1537	0, 9.8, 19.6, 39.1, 78.2, 156.3, and 312.5
WP2 uvrA	0, 156.3, 312.5, 625, 1250, 2500, and 5000

Study Validity

The assay was considered valid because:

1. The number of spontaneous revertants in negative control plates fell within the normal range.
2. The positive control chemicals induced significant increases in the number of colonies.

Results

Positive test results were considered to have occurred if a concentration-related increase over the range tested and/or a reproducible increase in the number of revertant colonies per plate occurred at one or more concentrations with or without metabolic activation. A cytotoxic effect was considered to have occurred if the number of colonies fell significantly below negative control levels or a lack of background lawn was observed.

Dose-dependent cytotoxicity occurred at 312.5, 625, and 1250 μg per plate for the *Salmonella* strains, but no cytotoxicity was observed at any test concentration for WP2 uvrA in the presence of S9 activation. In all strains tested, no increase in the number of revertant colonies occurred compared to the negative control at any concentration of TR-701 either in the presence or absence of S9 metabolic activation. In contrast, the positive control agents induced marked increases in the number of revertant colonies compared to the vehicle control group (Table 69).

Table 69: Mean Revertants/Plate for the Definitive Ames Assay with TR-701.
(Sponsor's Table)

G04197

Test strain	Chemical Treated	Dose (μg/plate)	Revertant colonies/plate (Mean)[Factor][a] Without S-9 mix		With S-9 mix	
TA100	Test Item	0	116 ± 6		126 ± 6	
		9.8	114 ± 12	[1.0]	No treatment	
		19.6	111 ± 12	[1.0]	126 ± 9	[1.0]
		39.1	112 ± 6	[1.0]	111 ± 3	[0.9]
		78.2	104 ± 3	[0.9]	126 ± 7	[1.0]
		156.3	81 ± 4	[0.7]	112 ± 7	[0.9]
		312.5	44 ± 5	[0.4] *	74 ± 12	[0.6]
		625	No treatment		45 ± 3	[0.4] *
TA1535	Test Item	0	12 ± 2		12 ± 3	
		19.6	9 ± 2	[0.8]	9 ± 3	[0.8]
		39.1	10 ± 3	[0.8]	10 ± 2	[0.8]
		78.2	11 ± 2	[0.9]	7 ± 2	[0.6]
		156.3	9 ± 1	[0.8]	7 ± 2	[0.6]
		312.5	7 ± 1	[0.6]	6 ± 2	[0.5] *
		625	4 ± 1	[0.3] *	6 ± 2	[0.5] *
TA98	Test Item	0	16 ± 2		27 ± 2	
		39.1	19 ± 3	[1.2]	No treatment	
		78.2	18 ± 3	[1.1]	29 ± 2	[1.1]
		156.3	20 ± 2	[1.3]	25 ± 2	[0.9]
		312.5	17 ± 1	[1.1]	23 ± 2	[0.9]
		625	12 ± 5	[0.8]	18 ± 1	[0.7]
		1250	8 ± 3	[0.5] *	13 ± 2	[0.5] *
		2500	No treatment		8 ± 2	[0.3] *
TA1537	Test Item	0	10 ± 2		20 ± 7	
		9.8	14 ± 4	[1.4]	21 ± 2	[1.1]
		19.6	10 ± 3	[1.0]	20 ± 7	[1.0]
		39.1	10 ± 2	[1.0]	20 ± 2	[1.0]
		78.2	9 ± 2	[0.9]	15 ± 6	[0.8]
		156.3	6 ± 0	[0.6]	12 ± 3	[0.6]
		312.5	5 ± 3	[0.5] *	9 ± 5	[0.5] *
E. coli WP2 *uvrA*	Test Item	0	9 ± 2		12 ± 3	
		156.3	8 ± 3	[0.9]	8 ± 1	[0.7]
		312.5	8 ± 3	[0.9]	11 ± 2	[0.9]
		625	9 ± 3	[1.0]	10 ± 3	[0.8]
		1250	7 ± 3	[0.8]	9 ± 1	[0.8]
		2500	7 ± 3	[0.8]	9 ± 1	[0.8]
		5000	5 ± 1	[0.6]	11 ± 3	[0.9]
Positive controls						
TA100	SA	0.5	432 ± 22	[3.7]		
TA1535	SA	0.5	398 ± 16	[33.2]		
TA98	2-NF	2	279 ± 12	[17.4]		
TA1537	9-AA	50	238 ± 39	[23.8]		
WP2 *uvrA*	4NQO	0.5	120 ± 11	[13.3]		
TA100	BP	2			768 ± 80	[6.1]
TA1535	2-AA	2	11 ± 2	[0.9]	271 ± 10	[22.6]
TA98	BP	2	17 ± 3	[1.1]	627 ± 7	[23.2]
TA1537	BP	2			233 ± 12	[11.7]
WP2 *uvrA*	2-AA	4			224 ± 12	[18.7]

[a] No. of revertant colonies of treated plate/No. of revertant colonies of vehicle control plate
SA, Sodium azide; 2-NF, 2-Nitrofluorene; 9-AA, 9-Aminoacridine
4NQO, 4-Nitroquinoline-1-oxide; 2-AA, 2-Aminoanthracene; BP, Benzo(a)pyrene
Test item: DA-7218
* Growth inhibition (decrease in No. of revertant colonies and/or formation of micro-colonies)

Study title: TR-700 Bacterial Reverse Mutation Assay

Study no.:	TOX-07-0701-005A
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 16, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	DA-7157 (TR-700), Lot No.: PJS4-38, purity of 99.24%

Key Study Findings

TR-700 was considered to be negative for mutagenesis in the Ames assay for all strains tested.

Methods

Strains:	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, and *Escherichia coli* WP2 uvrA
Concentrations in definitive study:	The definitive study concentrations of TR-700 are shown in Table 70.
Basis of concentration selection:	TR-700 concentrations for the definitive study were based on the results of a range-finding test which included a high dose of 5000 μg/plate and made note of cytotoxic effects. The lowest dose causing at least 50% cytotoxicity in individual strains was chosen as the high-dose in the definitive study for each strain.
Negative control:	Vehicle: Dimethylsulfoxide (DMSO)
Positive control:	Sodium azide (SA) for TA100 and TA1535 without S9 ; 2-nitrofluorene (2-NF) for TA98 without S9; 4-nitroquinoline-1-oxide (4NQO) for WP2 uvrA; 9-aminoacridine (9-AA) for TA1537; 2-aminoanthracene (2-AA) for TA1535 and WP2 uvrA with S9; Benzo(a)pyrene (BP) for TA98, TA100, and TA1537 with and without S9.
Formulation/Vehicle:	Dimethylsulfoxide (DMSO)
Incubation & sampling time:	48 hour incubation at 37°C followed by sampling.

Table 70: Concentrations of TR-100 tested for Each Strain in the Definitive Study

Test Strain	Concentrations Tested With and Without S9 (μg/plate)
TA100	0, 0.313, 0.625, 1.25, 2.5, 5, 10, and 20
TA1535	0, 0.625, 1.25, 2.5, 5, 10, 20, and 40
TA98	0, 0.625, 1.25, 2.5, 5, 10, 20, and 40

TA1537	0, 0.625, 1.25, 2.5, 5, 10, 20, and 40
WP2 uvrA	0, 1.25, 2.5, 5, 10, 20, 40, and 80

Study Validity

The assay was considered valid because:

1. The number of spontaneous revertants in negative control plates fell within the normal range.
2. The positive control chemicals induced significant increases in the number of colonies.

Results

Cytotoxicity (50-70%) occurred at the highest concentrations of TR-700 for all strains except WP2 uvrA which experienced 30 and 40% cytotoxicity respectively in the absence and presence of S9 activation at the highest TR-700 concentration of 80 μg/plate. In the previous range-finding assay, TR-700 concentrations of 40 and 200 μg/plate respectively caused 20% and 100% cytotoxicity. None of the tested concentrations of TR-700 caused increased numbers of revertant colonies with or without S9 activation. In contrast, the positive control agents all substantially increased the number of revertant colonies compared to the negative control (Table 71).

Table 71: Mean Revertants/Plate for the Definitive Ames Assay with TR-701.
(Sponsor's Table)

G05065

Tester strain	Chemical Treated	Dose (μg/plate)	Revertant colonies/plate (Mean)[Factor][a] Without S-9 mix		With S-9 mix	
TA100	Test Item	0	107 ± 5		124 ± 16	
		0.313	111 ± 5	[1.0]	121 ± 22	[1.0]
		0.625	113 ± 10	[1.1]	122 ± 17	[1.0]
		1.25	102 ± 1	[1.0]	106 ± 5	[0.9]
		2.5	101 ± 2	[0.9]	119 ± 3	[1.0]
		5	95 ± 5	[0.9]	104 ± 2	[0.8]
		10	90 ± 18	[0.8] *	93 ± 12	[0.8]
		20	46 ± 7	[0.4] *	62 ± 10	[0.5] *
TA1535	Test Item	0	15 ± 1		14 ± 3	
		0.625	10 ± 1	[0.7]	15 ± 1	[1.1]
		1.25	12 ± 1	[0.8]	11 ± 2	[0.8]
		2.5	14 ± 2	[0.9]	11 ± 1	[0.8]
		5	13 ± 2	[0.9]	15 ± 2	[1.1]
		10	12 ± 2	[0.8]	13 ± 4	[0.9]
		20	12 ± 1	[0.8]	13 ± 3	[0.9]
		40	4 ± 4	[0.3] *	4 ± 2	[0.3] *
TA98	Test Item	0	16 ± 5		33 ± 5	
		0.625	21 ± 6	[1.3]	32 ± 4	[1.0]
		1.25	17 ± 1	[1.1]	33 ± 3	[1.0]
		2.5	17 ± 3	[1.1]	32 ± 5	[1.0]
		5	19 ± 2	[1.2]	29 ± 1	[0.9]
		10	20 ± 1	[1.3]	31 ± 1	[0.9]
		20	18 ± 2	[1.1]	20 ± 3	[0.6]
		40	8 ± 2	[0.5] *	15 ± 4	[0.5] *
TA1537	Test Item	0	17 ± 2		18 ± 2	
		0.625	22 ± 1	[1.3]	15 ± 2	[0.8]
		1.25	22 ± 2	[1.3]	18 ± 1	[1.0]
		2.5	17 ± 2	[1.0]	14 ± 3	[0.8]
		5	21 ± 3	[1.2]	16 ± 1	[0.9]
		10	20 ± 2	[1.2]	18 ± 2	[1.0]
		20	9 ± 2	[0.5] *	14 ± 4	[0.8]
		40	5 ± 1	[0.3] *	10 ± 2	[0.6]
E. coli WP2 *uvrA*	Test Item	0	12 ± 2		19 ± 2	
		1.25	15 ± 1	[1.3]	14 ± 2	[0.7]
		2.5	12 ± 1	[1.0]	13 ± 2	[0.7]
		5	9 ± 1	[0.8]	17 ± 3	[0.9]
		10	13 ± 2	[1.1]	14 ± 2	[0.7]
		20	12 ± 3	[1.0]	14 ± 3	[0.7]
		40	11 ± 2	[0.9]	13 ± 4	[0.7]
		80	8 ± 3	[0.7]	11 ± 3	[0.6]
Positive controls						
TA100	SA	0.5	487 ± 15	[4.6]		
TA1535	SA	0.5	407 ± 14	[27.1]		
TA98	2-NF	2	315 ± 17	[19.7]		
TA1537	9-AA	50	209 ± 11	[12.3]		
WP2 *uvrA*	4NQO	0.5	187 ± 6	[15.6]		
TA100	BP	2			806 ± 12	[6.5]
TA1535	2-AA	2	21 ± 2	[1.4]	373 ± 11	[26.6]
TA98	BP	2	20 ± 3	[1.3]	387 ± 26	[11.7]
TA1537	BP	2			259 ± 36	[14.4]
WP2 *uvrA*	2-AA	4			284 ± 12	[14.9]

[a] No. of revertant colonies of treated plate/No. of revertant colonies of vehicle control plate
SA, Sodium azide; 2-NF, 2-Nitrofluorene; 9-AA, 9-Aminoacridine
4NQO, 4-Nitroquinoline-1-oxide; 2-AA, 2-Aminoanthracene; BP, Benzo(a)pyrene
* Growth inhibition (decrease in No. of revertant colonies and/or formation of micro-colonies)
Test item: DA-7157

7.2 *In Vitro* Assays in Mammalian Cells

Study title: TR-701 In Vitro Mammalian Chromosome Aberration Test

Study no.: TOX-07-0701-021
Study report location: Electronic transmission

Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	February 28, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TR-701 (DA-7218), Lot No.: OXA-003, purity of 99.07%

Key Study Findings

TR-701 did not produce significant increases in the number of aberrant metaphases under any of the tested incubation conditions.

Methods

Cell line:	Chinese hamster lung cells
Concentrations in definitive study:	For the 6 hour incubation minus S9: 0, 39.1, 78.1, 156.3, 312.5, and 625 μg/ml; for the 6 hour plus S9: 0, 78.1, 156.3, 312.5, and 625 μg/ml; for the 24 hour incubation minus S9: 0, 19.5, 39.1, 78.1, 156.3 μg/ml.
Basis of concentration selection:	The high concentrations used in the definitive study were based on precipitation and cytotoxicity data obtained in a preliminary range-finding assay.
Negative control:	Sterile distilled water
Positive control:	Cyclophosphamide (CPA), ethylmethanesulfonate (EMS),
Formulation/Vehicle:	Sterile distilled water
Incubation & sampling time:	Cells were incubated with TR-701 for 6 and 24 hours in the absence of S9 activation and 6 hours in the presence of S9 activation. The 6 hour incubations were washed free of TR-701 then the cells were left in culture until after the addition of colchicine. Approximately 22 hours after the start of treatment, 1 μM colchicine was added to each culture for two hours after which the cells were harvested.

Study Validity

The following validation criteria were met:

1. The number of aberrant metaphases in the negative and positive control groups was within historical ranges.

Results

Results were considered to be positive for induction of chromosomal aberrations if a statistically significant, concentration related increase or clear, reproducible, and

statistically significant increase in the number of cells with chromosome aberrations occurred at any concentration level.

In the 6-hour treatment in the absence of S9 activation as well as plus S9 activation, only small insignificant increases in the numbers of metaphases with structural aberrations were observed with TR-701 treatment at any concentration and a dose-dependent effect was not observed (Table 72). Similarly only insignificant increases in structural aberrations were noted after 24 hours of treatment. After 24 hours, the highest elevation in structural aberrations occurred with the highest concentration of TR-701 (156.3 μg/ml) which produced 6.5 aberrations/100 metaphases compared to 1.5/100 metaphases for the negative control group, but this increase was not significant when analyzed by χ^2 analysis. Also for all the 6- and 24-hour incubations, the frequency metaphases with numerical aberrations (polyploidy plus endoreduplication) was not significantly increased over control levels.

For all three incubations conditions (6 hours ± S9 activation, 24 hours without S9 activation), the positive control agents produced significant increases in the mean number of structurally aberrant metaphases, but did not stimulate increased frequencies of numerical aberrations.

Table 72: Mean Number of Structural and Numerical Aberrant Metaphases Following Treatment with TR-701. (Sponsor's Table)

Study No. G04198						
Nominal conc. of test item (μg/mL)	S9 mix	Treatment Times [b] (hours)	Mean Aberrant Metaphases	Mean Total Aberrations	Mean of PP + ER	Relative Cell Counts (%)
6 h treatment (+S9)						
0	+	6-18	0.5/0.5 [c]	0.5/0.5	0.0+ 0.0	100
39.1	+	6-18		Not Counted		93
78.1	+	6-18	3.0/2.5	4.0/3.5	1.0 + 0.0	83
156.3 &	+	6-18	2.0/2.0	2.0/2.0	1.5 + 0.0	81
312.5 #	+	6-18	3.5/3.5	6.0/6.0	2.0 + 0.0	77
625 #	+	6-18	4.0/4.0	4.5/4.5	2.0 + 0.0	75
CPA 6	+	6-18	23.0/22.0**[d]	36.5/35.5	1.0 + 0.0	78
6 h treatment (-S9)						
0	–	6-18	0.5/0.5	0.5/0.5	0.5 + 0.0	100
78.1 &	–	6-18	2.0/2.0	2.0/2.0	2.5 + 0.0	102
156.3 #	–	6-18	2.0/2.0	4.0/4.0	1.0 + 0.0	98
312.5 #	–	6-18	1.5/1.5	1.5/1.5	2.5 + 0.0	97
625 #	–	6-18	2.0/2.0	2.0/2.0	2.5 + 0.0	87
EMS 800	–	6-18	23.5/23.0**	34.5/33.0	1.0 + 0.0	75
24 h treatment (-S9)						
0	–	24-0	1.0/1.0	1.5/1.5	1.0 + 0.0	100
19.5	–	24-0	2.0/2.0	2.0/2.0	1.0 + 0.0	89
39.1	–	24-0	2.5/2.5	4.5/4.5	2.0 + 0.0	83
78.1 &	–	24-0	2.0/2.0	2.0/2.0	2.0 + 0.0	75
156.3 &	–	24-0	5.0/5.0	6.5/6.5	3.0 + 0.0	65
EMS 600	–	24-0	33.0/33.0**	44.0/43.5	1.0 + 0.0	62

** Significantly different from the control at $P<0.01$.

Visible precipitation observed when treated and at the end of treatment.
& Visible precipitation observed only when treated.

Test item : DA-7218

[a] See Appendix 1, 2 & 3 for individual data
[b] Treatment time-recovery time
[c] Gaps included/excluded, means of duplicate cultures
100 metaphases were examined per culture.
[d] The negative and positive control groups: Fisher's exact test

Abbreviation:
PP : Polyploid
ER : Endoreduplication
CPA : Cyclophosphamide · H_2O
EMS : Ethylmethanesulfonate

Study title: TR-700 In Vitro Chromosome Aberration Test

Study no.:	TOX-07-0701-086
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 16, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TR-700 (DA-7157), lot # PJS4-38, purity of 99.24%

Key Study Findings

TR-700 significantly induced structural chromosomal aberrations in a dose-dependent manner in 6 hour incubations with and without S9 activation at soluble concentrations not associated with prohibitive cytotoxicity.

Methods

Cell line:	Chinese Hamster Lung (CHL) cells
Concentrations in definitive study:	See Table 73 below.
Basis of concentration selection:	The concentrations used in the definitive study were based on the cytotoxicity and precipitation results of a range-finding study.
Negative control:	Dimethylsulfoxide (DMSO)
Positive control:	Without S9: ethylmethanesulfonate (EMS); With S9: Cyclophosphamide $\cdot H_20$
Formulation/Vehicle:	DMSO
Incubation & sampling time:	See Table 73 below. Approximately 22 hours after the start of treatment, 1 μM colchicine was added to each culture and incubations were continued for 2 more hours before cell harvest.

Table 73: Study Design for the Chromosomal Aberration Assay with TR-700.
(Sponsor's Table)

Treatment Groups	S-9 Mix	Concentration (μg/mL)	Treatment time - Recovery time (hrs.)
SERIES I (6+S)			
Vehicle control	+	0	6 – 18
Test item	+	50	6 – 18
Test item	+	100	6 – 18
Test item	+	200	6 – 18
Test item	+	400	6 – 18
Positive control	+	CPA 6	6 – 18
SERIES II (6-S)			
Vehicle control	–	0	6 – 18
Test item	–	12.5	6 – 18
Test item	–	25	6 – 18
Test item	–	50	6 – 18
Test item	–	100	6 – 18
Positive control	–	EMS 800	6 – 18
SERIES III (24-S)			
Vehicle control	–	0	24 – 0
Test item	–	12.5	24 – 0
Test item	–	25	24 – 0
Test item	–	50	24 – 0
Test item	–	100	24 – 0
Positive control	–	EMS 600	24 – 0

Test item : DA-7157 Vehicle control : DMSO
CPA : Cyclophosphamide·H_2O EMS : Ethylmethanesulfonate
S : S-9 mix

Study Validity

The following criteria for a valid study were satisfied in this study.

1. The number of aberrant metaphases in the negative and both positive control groups was within historical ranges.

Results

Results were considered to be positive for induction of chromosomal aberrations if a statistically significant, concentration related increase or clear, reproducible, and statistically significant increase in the number of cells with chromosome aberrations occurred at any concentration level.

In the 6-hour treatment in the presence of S9 activation, a concentration-dependent increase in the mean number of metaphases with structural aberrations including gaps occurred with values of 0.5, 4.0, 7.0, 33.0 and 37.5 for TR-700 concentrations of 0, 50, 100, 200, and 400 μg/ml respectively. The increase in aberrant metaphases was significant for all TR-700 concentrations. In addition, significantly increased numbers of metaphases with numerical aberrations (polyploidy and/or endoreduplication) were noted for the highest two TR-700 concentrations (200 and 400 μg/ml). Precipitation

occurred only at the two highest concentrations and cytotoxicity above 50% was observed only with the highest TR-700 concentration. In the positive control incubation with CPA, the mean number of structurally aberrant metaphases, but not the mean number of numerically aberrant metaphases was significantly increased relative to the vehicle control incubations.

Similarly, in the 6-hour treatment in the absence of S9 activation, a concentration-dependent increase in the mean number of metaphases with structural aberrations including gaps occurred with values of 1.0, 3.5, 5.0, 7.0 and 17.5 for TR-700 concentrations of 0, 12.5, 25, 50, and 100 μg/ml respectively. The increases in metaphases with structural aberrations were significantly increased for TR-700 concentrations ≥ 25 μg/ml. However, unlike the results with S9-activation, no significant changes in the number of metaphases with numerical aberrations occurred with any of the TR-700 concentrations. Precipitation and cytotoxicity above 50% occurred only with the highest TR-700 concentration of 100 μg/ml. In the positive control incubation with EMS, the mean number of structurally aberrant metaphases, but not the mean number of numerically aberrant metaphases was significantly increased relative to the vehicle control incubations.

In contrast, in the 24-hour treatment without S9 activation, the mean number of metaphases with structural aberrations and/or numerical aberrations were not increased with any of the TR-700 concentrations (12.5, 25, 50, and 100 μg/ml) compared to the vehicle control incubations. In the positive control incubation with EMS, the mean number of structurally aberrant metaphases, but not the mean number of numerically aberrant metaphases was significantly increased relative to the vehicle control incubations.

Table 74: Chromosome Aberration Assay Results for TR-700. (Sponsor's Table)

Study No. G05066

Nominal conc. of test item (μg/mL)	S9 mix	Treatment Times [b] (hours)	Mean Aberrant Metaphases	Mean Total Aberrations	Mean of PP + ER	Relative Cell Counts (%)
6 h treatment (+S9)						
0	+	6-18	0.5/0.5 [c]	0.5/0.5	0.5 + 0.0	100
50	+	6-18	4.5/4.0*[d]	5.5/5.0	2.5 + 0.0	96
100	+	6-18	7.0/7.0**	10.0/10.0	2.5 + 0.0	80
200 &	+	6-18	33.0/33.0**	42.0/42.0	5.0 + 0.0*[d]	52
400 #	+	6-18	37.5/37.5**	46.5/46.5	7.0 + 0.0**	44
CPA 6	+	6-18	24.5/24.5**[e]	40.0/40.0	1.0 + 0.0	64
6 h treatment (-S9)						
0	–	6-18	1.0/1.0	1.5/1.5	1.0 + 0.0	100
12.5	–	6-18	3.5/3.5	5.5/5.5	4.0 + 0.0	84
25	–	6-18	5.0/5.0*[d]	7.5/7.5	3.5 + 0.0	80
50	–	6-18	7.0/7.0**	10.5/10.5	3.5 + 0.0	73
100 #	–	6-18	17.5/17.5**	25.0/25.0	5.0 + 0.0	43
EMS 800	–	6-18	29.0/28.0**[e]	43.0/42.0	0.0 + 0.0	69
24 h treatment (-S9)						
0	–	24-0	1.5/1.5	1.5/1.5	0.5 + 0.0	100
12.5	–	24-0	2.5/2.5	2.5/2.5	1.5 + 0.0	89
25	–	24-0	3.0/2.5	4.0/3.5	1.5 + 0.0	85
50	–	24-0	4.5/4.5	6.0/6.0	2.0 + 0.0	81
100 #	–	24-0	3.5/3.5	5.5/5.5	2.0 + 0.0	59
EMS 600	–	24-0	39.0/38.5**[e]	56.5/56.0	1.0 + 0.0	67

* Significantly different from the control at $P<0.05$.
** Significantly different from the control at $P<0.01$.

Visible precipitation observed when treated and at the end of treatment.
& Visible precipitation observed only when treated.

Test item : DA-7157

[a] See Appendix 1, 2 & 3 for individual data
[b] Treatment time-recovery time
[c] Gaps included/excluded, means of duplicate cultures
100 metaphases were examined per culture.
[d] The negative and test-item treated groups: χ^2-test and Fisher's exact test
[e] The negative and positive control groups: Fisher's exact test

Abbreviation:
PP : Polyploid
ER : Endoreduplication
CPA : Cyclophosphamide · H_2O
EMS : Ethylmethanesulfonate

Study title: TR-700 In Vitro Mouse Lymphoma Cell Mutation Assay

Study no.:	TOX-07-0701-089
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 8, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TR-700 (DA-7157), Lot # PJS4-45, purity of 99.28%

Key Study Findings

An increased mutation frequency compared to the vehicle control group was induced by a high-TR-700 concentration of 112.3 μg/ml after 3 hours of incubation without S9 activation, but only in the presence of precipitate.

Methods

Cell line:	L5178Y Mouse Lymphoma cells
Concentrations in definitive study:	See Table 75.
Basis of concentration selection:	The definitive concentrations of TR-700 were based on the cytotoxicity and precipitation results from a preliminary study.
Negative control:	Dimethylsulfoxide (DMSO)
Positive control:	In absence of S9: methyl methanesulphonate (MMS); in the presence of S9: 3-methylcholanthrene (3MC)
Formulation/Vehicle:	Dimethylsulfoxide (DMSO)
Incubation & sampling time:	In the Main Mutation Test #1 cells were incubated with TR-700 with and without S9 activation for 3 hours. In the Main Mutation Test #2, cells were incubated with TR-700 without S9 activation for 24 hours.

Table 75: Concentrations of TR-700 Used in the Main Mutation Tests. (Sponsor's Table)

Mutation tests:	-S9 mix	Test 1 (3 hours)	3.51, 7.02, 14.04, 28.08, 56.15, 112.3 μg/mL
	+S9 mix	Test 1 (3 hours)	7.02, 14.04, 28.08, 56.15, 112.3, 224.6 μg/mL
	-S9 mix	Test 2 (24 hours)	1.75, 3.51, 7.02, 14.04, 28.08, 56.15 μg/mL

Study Validity

1. The highest concentration tested allowed the maximum exposure of 5000 μg/ml or 10 μM for freely soluble compounds, or the limit of toxicity (80-90% cytotoxicity; 10-20% relative total growth) or the limit of solubility.
2. For a toxic substance, a least 4 analyzable concentrations were used ideally spanning the toxicity range of 100 to 10% relative total growth.
3. For the vehicle control group the mean mutant frequency (MF) was between 50 to 170 x 10^6, the mean cloning efficiency was between 65-120% and the mean suspension growth was between 8 and 32 on Day 2 followed by 3 hour treatments. Also after obvious outliers were excluded, at least 2 vehicle control cultures remained.
4. Positive control groups showed an absolute increase in mean total MF of at least 300 x 10^6 above the mean vehicle control MF with at least 40% small colony mutants. Also the mean relative total growth for positive control cultures was greater than 10%. In addition there was not excessive heterogeneity between replicate cultures and there was an absence of confounding technical problems such as contamination, excessive numbers of outliers, or excessive toxicity.

Results

Main Mutation Test #1 without S9 activation: Relative total growth of 30 to 123% of the solvent control was obtained. Precipitate was visibly observed at TR-700 concentrations of 56.15 and 112.3 μg/ml. At the high-dose of 112.3 μg/ml, the induced mutant frequency (266 x 10^6) exceeded the mutant frequency of the vehicle control (96 x 10^6) by more than the Global Evaluation Factor (GEF), but this result took place in the presence of precipitate. The positive control agent, MMS, induced an acceptable increase in mutation frequency (743 x 10^6) and an acceptable increase in the number of small colony mutants (90%) compared to the vehicle control results (65%).

Main Mutation Test #1 with S9 activation: Relative total growth of 50 to 127% of the solvent control was obtained for the TR-700 groups. Precipitate was visibly observed at TR-700 concentrations of ≤ 112.3 μg/ml. None of the TR-700 groups induced mutant frequencies above the GEF. The positive control agent, 3-MC, induced an acceptable increase in mutation frequency (901 x 10^6) compared to 143 x 10^6 for the vehicle control and an acceptable increase in the number of small colony mutants (80%) compared to the vehicle control results (61%).

Main Mutation Test #2 without S9 activation: Relative total growth of 20 to 97% of the solvent control was obtained for the TR-700 groups. Precipitate was visibly observed at the high concentration of TR-700 (56.15 μg/ml). None of the TR-700 groups induced mutant frequencies above the GEF. The positive control agent, MMS, induced an acceptable increase in mutation frequency (1798 x 10^6) compared to 107 x 10^6 for the vehicle control and an acceptable increase in the number of small colony mutants (93%) compared to the vehicle control results (60%).

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

Study title: TR-701 Mammalian Erythrocyte Micronucleus Test in Male Mice

Study no: TOX-07-0701-084

Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 8, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	DA-7218 (TR-701), Batch No: Oxa-003, purity of 99.07%

Key Study Findings

TR-701 at doses as high as 2000 mg/kg/day administered daily for three days did not induce micronuclei in bone marrow cells in male mice compared to the vehicle control group.

Methods

Doses in definitive study:	0, 500, 1000, and 2000 mg/kg/day
Frequency of dosing:	Once per day for 3 days
Route of administration:	Oral gavage; IP for the positive control.
Dose volume:	20 ml/kg
Formulation/Vehicle:	Sterile distilled water
Species/Strain:	ICR mice
Number/Sex/Group:	Males only; 6/group
Satellite groups:	none
Basis of dose selection:	Dose-range finding study indicated tolerability to 2000 mg/kg/day (limit dose for ≥ 2 weeks according to OECD guideline)
Negative control:	Sterile distilled water
Positive control:	Cyclophosphamide (CPA)

Table 76: Study Design for Male Mouse Micronucleus Study with TR-701
(Sponsor's Table)

Treatment Groups	Animals per dose	Animal Number	Dose level (mg/kg)	Total Number of Administrations	Route
Vehicle control [a)]	6	1 - 6	0	3	P.O.
T1	6	7 - 12	500	3	P.O.
T2	6	13 - 18	1000	3	P.O.
T3	6	19 - 24	2000	3	P.O.
Positive control (CPA) [b)]	6	25 - 30	70 [b)]	1	I.P.

[a)] Vehicle control: sterile distilled water
[b)] Cyclophosphamide·H_2O (Positive control, mg/10 ml/kg b.w.); once at 3rd day

Study Validity

The following study validation criteria were met in this study.

1. The incidence of MCPCEs in the negative and positive control groups should fall within the historical control range.
2. All of the PCE/(PCE/NCE) ratios should be greater than 0.1

Results

The mean frequencies of micronucleated polychromatic erythrocytes (MNPCEs) per 2000 polychromatic erythrocytes (PCEs) were 1.50, 0.83, 2.17, and 1.80 for the vehicle, 500, 1000, and 2000 mg/kg/day groups respectively with no statistically significant difference between groups. MNPCEs were significantly increased compared to the vehicle control group for the positive control group with 87.83 MNPCEs/2000 PCEs.

The ratio of PCEs to PCE/normochromatic erythrocytes (NCE) was significantly reduced compared to the vehicle control group in all the treatment groups with values of 0.61, 0.15, 0.14, and 0.12 for the vehicle control, 500, 1000, and 2000 mg/kg/day groups respectively. This measurement indicative of cytotoxicity was not reduced in the positive control group (0.49).

Study title: TR-701 Mammalian Erythrocyte Micronucleus Test in Female Mice.

Study no:	TOX-07-0701-085
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 6, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	DA-7218 (TR-701); Lot # OXA-005, purity of 97.35%

Key Study Findings

TR-701, administered orally for three days at doses as high as 2000 mg/kg/day to female mice did not induce an increased frequency of micronuclei in bone marrow cells.

Methods

Doses in definitive study:	0, 500, 1000, 2000 mg/kg/day
Frequency of dosing:	Once per day for three days
Route of administration:	Oral gavage (positive control was administered once intraperitoneally)
Dose volume:	20 ml/kg
Formulation/Vehicle:	Sterile distilled water
Species/Strain:	ICR mice
Number/Sex/Group:	Females only: 6/group
Satellite groups:	None
Basis of dose selection:	Based on preliminary tolerability findings from a range-finding study.
Negative control:	Sterile distilled water
Positive control:	Cyclophosphamide (CPA)

Table 77: Study Design for Female Mouse Micronucleus Study with TR-701.
(Sponsor's Table)

Treatment Groups	Animals per dose	Animal Number	Dose level (mg/kg) [c]	Total Number of Administrations	Route
Vehicle control [a]	6	1 - 6	0	3	P.O.
T1	6	7 - 12	500	3	P.O.
T2	6	13 - 18	1000	3	P.O.
T3	6	19 - 24	2000	3	P.O.
CPA [b]	6	25 - 30	70	1	I.P.

[a] Vehicle control: Sterile distilled water

[b] Cyclophosphamide·H_2O (Positive control, mg/10 ml/kg b.w.): once at 3rd day

[c] mg/20 ml/kg b.w, Doses are expressed without considering (b) (4) impurity in the test item.

Study Validity

The following study validation criteria were satisfied in this study.

1. The incidence of MNPCEs in the negative and positive control groups should fall within the historical control range.
2. All of the PCE/(PCE/NCE) ratios should be greater than 0.1

Results

The mean frequencies of micronucleated polychromatic erythrocytes (MNPCEs) per 2000 polychromatic erythrocytes (PCEs) were 1.67, 1.50, 2.17 and 0.80 respectively for the vehicle control, and 500, 1000, and 2000 mg/kg/day TR-701 groups. All of the values were similar and not significantly different for these groups. The ratio of PCE to PCE + normochromatic erythrocytes (NCE), indicative of cytotoxicity was significantly reduced for the TR-701 treatment groups with values of 0.14, 0.14, and 0.12 for the 500, 1000, and 2000 mg/kg/day groups respectively compared to 0.59 for the vehicle control group. The positive control group had a significantly increased frequency of MNPCEs (83.00) compared to the vehicle control group, but no decrease in the PCE/PCE +NCE ratio (0.47).

Study title: TR-700 Mammalian Erythrocyte Micronucleus Test in Male Mice

Study no:	TOX-07-0701-022
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 24, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TR-700, Lot # PJS4-38, purity of 99.24%

Key Study Findings

TR-700 at doses as high as 2000 mg/kg/day administered daily for three days did not induce micronuclei in bone marrow cells in male mice compared to the vehicle control group

Methods

Doses in definitive study:	0, 500, 1000, and 2000 mg/kg/day
Frequency of dosing:	Once per day for three days for TR-700
Route of administration:	Oral for TR-700; IP for positive control
Dose volume:	20 ml/kg
Formulation/Vehicle:	1% hydroxyPropylMethyl cellulose
Species/Strain:	CrljBgi: CD1 (ICR) mice
Number/Sex/Group:	6 males/group
Satellite groups:	none
Basis of dose selection:	Based on a previous range-finding study where a high dose of 2000 mg/kg/day was shown to be well tolerated
Negative control:	1% hydroxyPropylMethyl cellulose
Positive control:	70 mg/kg Cyclophosphamide (CPA)

Table 78: Study Design for Male Micronucleus Study with TR-700. (Sponsor's Table)

Treatment Groups	Animals per dose	Animal Number	Dose level [b)]	Total Number of Administrations	Route
Vehicle control [a)]	6	1 - 6	0	3	P.O.
Low (T1)	6	7 - 12	500	3	P.O.
Middle (T2)	6	13 - 18	1000	3	P.O.
High (T3)	6	19 - 24	2000	3	P.O.
Positive control [c)]	6	25 - 30	CPA 70	1	I.P.

[a)] Vehicle control: 1% HPMC
[b)] mg/20 ml/kg b.w.
[c)] Cyclophosphamide·H_2O (Positive control, mg/10 ml/kg b.w.); once at 3rd day

Study Validity

The following study validation criteria were satisfied in this study.

1. The incidence of MNPCEs in the negative and positive control groups should fall within the historical control range.
2. All of the PCE/(PCE+NCE) ratios should be greater than 0.1

Results

The mean frequencies of micronucleated polychromatic erythrocytes (MNPCEs) per 2000 polychromatic erythrocytes (PCEs) were 1.17, 1.17, 0.50 and 1.17 for the vehicle control, and 500, 1000, and 2000 mg/kg/day groups respectively with no significant differences between groups. The ratios of PCE/PCE+NCE, a measure of cytotoxicity

were 0.58, 0.25, 0.22, and 0.21 for the same respective groups indicating TR-700 concentration-related cytotoxicity. The mean frequency in the cyclophosphamide group (87.50) was significantly greater than the vehicle control group, but the cyclophosphamide treatment did not produce significant toxicity with a PCE/PCE+NCE ratio of 0.45.

Study title: TR-700 Mammalian Erythrocyte Micronucleus Test in Female Mice

Study no:	TOX-07-0701-087
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 5, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TR-700, Lot # PJS4-45, purity of 99.46%

Key Study Findings

TR-700 at doses as high as 2000 mg/kg/day administered daily for three days did not induce micronuclei in bone marrow cells in female mice compared to the vehicle control group.

Methods

Doses in definitive study:	0, 500, 1000, and 2000 mg/kg/day
Frequency of dosing:	Once per day for three days for TR-700 and one day for cyclophosphamide
Route of administration:	Oral for TR-700 and IP for cyclophosphamide
Dose volume:	20 mg/kg
Formulation/Vehicle:	HydroxyPropylMethyl cellulose
Species/Strain:	(SPF) CrljBgi: CD1(ICR) mice
Number/Sex/Group:	6 females/group
Satellite groups:	none
Basis of dose selection:	In a preliminary range-finding study, the high dose of 2000 mg/kg was shown to be tolerated
Negative control:	HydroxyPropylMethyl cellulose
Positive control:	70 mg/kg Cyclophosphamide $\cdot H_2O$

Table 79: Study Design for the Female Micronucleus Assay with TR-700.
(Sponsor's Table)

Treatment Groups	Animals per dose	Animal Number	Dose level (mg/kg)	Total Number of Administrations	Route
Vehicle control [a)]	6	1 - 6	0	3	P.O.
Low (T1)	6	7 - 12	500	3	P.O.
Middle (T2)	6	13 - 18	1000	3	P.O.
High (T3)	6	19 - 24	2000	3	P.O.
CPA [b)]	6	25 - 30	70	1	I.P.

[a)] Vehicle control: 1% HPMC
[b)] Cyclophosphamide·H_2O (Positive control, mg/10 ml/kg b.w.); once at 3rd day

Study Validity

The following study validation criteria were satisfied in this study.

1. The incidence of MNPCEs in the negative and positive control groups should fall within the historical control range.
2. All of the PCE/(PCE + NCE) ratios should be greater than 0.1

Results

The mean frequencies of micronucleated polychromatic erythrocytes (MNPCEs) per 2000 polychromatic erythrocytes (PCEs) were 2.83, 1.67, 0.83 and 2.67 for the vehicle control, and 500, 1000, 2000 mg/kg/day groups respectively with no significant differences between groups. The ratios of PCE/PCE+NCE, a measure of cytotoxicity were 0.59, 0.38, 0.26, and 0.23 for the same respective groups indicating TR-700-related cytotoxicity. The mean frequency in the cyclophosphamide group (87.83) was significantly greater than the vehicle control value, but the cyclophosphamide treatment did not produce significant cytotoxicity with a PCE/PCE+NCE ratio of 0.47 (not significantly different from the vehicle control value).

7.4 Other Genetic Toxicity Studies

Study title: TR-701 In Vivo Unscheduled DNA Synthesis Test in Rats

Study no:	TOX-07-0701-090
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 7, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TR-701 (DA-7218), Lot # OXA-007, purity of 98.28%

Key Study Findings

Oral TR-701 did not significantly increase DNA synthesis in male Sprague-Dawley rats.

Methods

Doses in definitive study:	0, 600 and 2000 mg/kg/day for TR-701
Frequency of dosing:	Twice in one day; the second dose administered 14 hours after the first dose.
Route of administration:	Oral gavage
Dose volume:	10 ml/kg
Formulation/Vehicle:	Purified water
Species/Strain:	Male Sprague-Dawley rats
Number/Sex/Group:	4 males/group
Satellite groups:	none
Basis of dose selection:	In a preliminary test, TR-701 doses of 1000 and 2000 mg/kg/day did not elicit major clinical signs or lethality.
Negative control:	Purified water
Positive control:	75 mg/kg 2-Acetylaminofluorene (2-AAF)

Study Validity

The following validation criteria were satisfied for this study.

1. The slide preparations should contain sufficient cells of normal morphology to permit meaningful assessment of unscheduled DNA synthesis.
2. Vehicle-control responses should fall within the historic control range.
3. A positive response would be indicated by a significant and reproducible increase in the net nuclear grain count outside the historical control range.
4. A clear negative response is indicated by a mean net nuclear grain count that is not significantly greater than the vehicle control.

Results

TR-701 at doses of 600 and 2000 mg/kg/day did not stimulate unscheduled DNA synthesis in rat hepatocytes. In contrast, the positive-control agent, 75 mg/kg 2-AAF, significantly increased the net nuclear grain count indicating a significant increase in DNA synthesis (Table 80).

Table 80: Study Design for the Unscheduled DNA Synthesis Study with TR-701.
(Sponsor's Table)

Treatment	Dose level (mg/kg/day)	Animal number	No cells scored	Mean (gross) nuclear grain count		Mean cytoplasmic grain count		Mean net nuclear grain count		% cells in repair
				Mean	SD	Mean	SD	Mean	SD	
Vehicle	-	201	100	5.88	4.22	10.04	4.53	-4.16	4.71	1
		202	100	4.26	2.82	7.59	3.95	-3.33	2.93	0
		203	100	3.62	2.20	7.91	4.74	-4.29	4.17	0
		204	100	4.45	2.52	6.86	2.63	-2.41	2.94	3
DA-7218	600	211	100	3.74	2.58	6.23	3.57	-2.49	2.97	2
		212	100	4.69	3.02	7.96	3.66	-3.27	3.95	1
		213	100	4.93	2.79	9.12	4.45	-4.19	4.02	1
		214	100	3.42	2.06	7.18	3.93	-3.76	3.98	0
DA-7218	2000	221	100	3.10	2.28	5.74	3.52	-2.64	3.09	0
		222	100	3.64	2.82	6.87	3.71	-3.23	3.24	2
		224	100	5.48	3.61	10.91	7.99	-5.43	6.56	0
		225	100	6.79	6.37	11.78	8.82	-4.99	5.39	2
2-acetylaminofluorene (2-AAF)	75	232	100	18.96	11.25	10.82	6.26	8.14	7.73	62
		233	100	27.43	21.68	17.34	10.81	10.09	14.96	70

Vehicle: Purified water
SD: Standard deviation

Statistical significance:
** $p<0.01$
Otherwise $p>0.01$

8 Carcinogenicity

Carcinogenicity studies were not performed or required based on the limited duration of clinical administration planned for TR-701.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: A study of the Effects of TR-701 Free Acid on Fertility and Early Embryonic Development in Rats.

Study no.:	TOX-08-0701-026
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 24, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TR-701FA, Lot No.: 5133-D-R1-01-16-01, purity of 98.5%.

Key Study Findings

- Parameters of reproductive performance including mating index, fertility index, copulation index (males only), mean estrous cycle length (females only) or mean pre-coital interval (females only) were minimally affected by daily oral doses of TR-701 ≤ 50 mg/kg/day for males or ≤ 15 mg/kg/day for females compared to the vehicle control group.

- Mean testicular sperm numbers, sperm production rate, sperm motility, and morphology for male rats administered 50 mg/kg/day TR-701 (high-dose) were not significantly different compared to the control group values.
- In the high-dose male rats, epididymal sperm numbers and epididymal weights (significant only for the L. cauda epididymis) were reduced compared to the concurrent control values. However, because male fertility parameters were not significantly altered, these findings were not considered toxicologically significant.
- No TR-701-related gross pathology or histopathology findings were noted for males or females.
- None of the intrauterine parameters were significantly altered in gravid female rats by TR-701 doses ≤ 15 mg/kg/day compared to control values.
- The NOAEL values for males and females were considered to be the high doses of 50 and 15 mg/kg/day respectively.

Methods

Doses:	0, 5, 15, 50 mg/kg/day for males and 0, 2.5, 5. 15 mg/kg/day for females
Frequency of dosing:	Once daily
Dose volume:	10 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	25 mM disodium hydrogen basic, pH 7.5
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	25/sex/group
Satellite groups:	none
Study design:	Males were dosed from Study Days 0-58 (a minimum of 28 days prior to pairing through 1 day before euthanasia for a total of 58-59 doses. Females were dosed from Days 14 to 57, a minimum of 14 days prior to pairing through Gestation Day (GD) 7 for a total of 22-35 doses. Females with no evidence of mating were dosed daily through the day prior to euthanasia for a total of 44 doses.
Deviation from study protocol:	Deviations in the study protocol occurred, but none of the deviations was considered to have altered the results or integrity of the study.

Table 81: Study Design for the Male and Female Fertility Study in Rats.

Group No.	TR-701 FA Dosage Level (mg/kg/day)		Number/Group	
	Males	Females	Males	Females
Group 1: Vehicle	0	0	25	25
Group 2: low dose	5	2.5	25	25
Group 3: mid dose	15	5	25	25
Group 4: high dose	50	15	25	25

Observations and Results

Table 82: Observation Schedule for Male and Female Fertility Study in Rats.

Mortality and Clinical signs	Twice daily and approximately 2 hours following dose administration.
Body Weights	Individual body weights were recorded twice weekly throughout the study and prior to the scheduled euthanasia.
Food Consumption	Twice weekly
Estrous Cycle	Vaginal lavages were performed daily and slides were evaluated microscopically to determne the stage of estrus of each female for 10 days prior to TR-701 administration and continuing until evidence of copulation was observed or until termination of the mating period for females.
Necropsy	Males were euthanized for necropsy on GD 15 for the females. Surviving females were euthanized for necropsy on GD 15.

Mortality

One low-dose male (5 mg/kg/day) and one high-dose male (50 mg/kg/day) was euthanized in extremis on Study Days 25 and 53 respectively due to excessive weight loss.

All females in all the TR-701 treatment groups survived until the scheduled necropsies.

Clinical Signs

Clinical signs for the males euthanized in extremis included: red material around nose and excreta-related findings (small feces, decreased defecation) beginning 4 days before euthanasia. Other clinical signs in males included salivation around the time of dosing.

No TR-701-related clinical signs were reported for females.

Body Weight

Males: Other than for the two males that died in extremis, no TR-701-related changes in mean body weight or body weight gains were noted during the treatment period.

Females: Body weight gains in the TR-701 treatment groups were significantly lower than the control group values during Study Days 17-21. As a consequence, mean body weight gain in the high-dose group but not the low- and mid-dose groups was significantly lower by 33% compared to the control group for the premating period as a whole (Study Days 14-27). However, mean body weights were not lower for the high-dose group or the other TR-701 treatment groups compared to the control group for the pre-mating treatment period as a whole.

During Gestation Days (GDs) 0-7, TR-701 at all the administered doses did not significantly affect mean body weight gain or mean body weights compared to the control group.

Feed Consumption

Males: Significantly reduced mean food consumption was noted for males in the mid- and high-dose groups during study Days 0-3 and at other sporadic intervals, but a consistent pattern of TR-701-related change in food consumption was not observed.

Females: During the premating period (Study Days 14-27), mean food consumption was significantly reduced on Study Days 14-17 for the low-dose group, Study Days 14-21 for the mid-dose group, and Study Days 14-24 for the high-dose group. During the remainder of the premating treatment period, Study Days 24-27, mean food consumption for the control and treatment groups was similar.

During the gestation treatment period (GDs 0-7), mean food consumption was significantly reduced for the high-dose group females (15 mg/kg/day) compared to the control group for the g/animal/day values, but not for the g/kg/day values. After cessation of dosing (GDs 8-15), food consumption significantly increased relative to the control group. Food consumption for the low- and mid-dose groups during the gestation-treatment period was either higher than or the same as the food consumption for the control group. Greater food consumption compared to the control group was noted for both the low- and mid-dose groups following cessation of dosing (GDs 8-15).

Toxicokinetics: not performed

Dosing Solution Analysis

Quadruplicate samples for concentration analysis were collected from each dosing formulation prepared during Weeks 1, 2, 5, and 8 of the study. Samples were stored frozen until analysis.

The actual concentrations of the dosing solutions were within 10% of the nominal concentrations.

Necropsy

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

Males: Male mating index, fertility index and copulation index were calculated. Spermatid counts, sperm counts, sperm motility, sperm morphology were assessed. In males, the right testes and epididymis from control and high-dose males and all males euthanized in extremis were prepared for histology and acrosome morphology was assessed.

No significant TR-701-related effects on male reproductive performance were observed at any dose level (Table 83). High-dose TR-701 administration tended to reduce the fertility and copulation indexes, but the high-dose values were not significantly lower than concurrent control values and fell within the historical control range.

Table 83: Male Fertility Parameters. (Sponsor's Table)

Parameter	Dosage Level (mg/kg/day)				WIL HC[a] Mean (Range)
	0	5	15	50	
Male Mating Index (%)	96.0	91.7	96.0	96.0	97.0 (86.7-100.0)
Male Fertility Index (%)	96.0	87.5	92.0	84.0	91.2 (73.3-100.0)
Male Copulation Index (%)	100.0	95.5	95.8	87.5	94.5 (78.3-100.0)

[a] = (b) (4) historical control data

Mean testicular sperm numbers, sperm production rate, motility, and morphology for the high-dose group were not significantly different than for the vehicle control group. However, mean epididymal sperm numbers in the 50 mg/kg/day group (285.1 million/g) were lower but not statistically lower than the concurrent control group (328.0 million/g) and the minimum mean historical value (324.0 million/g). The reduced epididymal sperm numbers correlated with significantly lower mean caudal epididymal weights. While the reduction of epididymal sperm numbers and weights appear to be caused by administration of the high dose of TR-701, no correlative effect was noted for male fertility suggesting the epididymal effects were of limited toxicological significance.

Histopathology in the epididymis was not considered to be associated with TR-701 administration. Minimal to mild mononuclear infiltrate (lymphocytes and macrophages) was noted at a similar (not significantly different) incidence and severity in the control (17/25; 16 minimal, 1 mild), and high-dose groups (19/24; 14 minimal, 5 mild) in the scheduled necropsy. Also the epididymis in one control male and in one high-dose male had focal areas of granulomatous inflammation consistent with a sperm granuloma.

Females: In addition to the assessments shown below, each animal underwent internal and external gross pathology examination and the weights of select organs (brain, ovaries, and pituitary gland) were recorded. Females with evidence of mating (copulatory plugs, presence of sperm in vaginal lavages) were euthanized on GD 15. Females without evidence of mating and males were also euthanized on GD 15. Upon necropsy, the uterus and ovaries were exposed and opened, the number of corpora lutea in each ovary was counted as well as the number and location of all embryos, early resorptions, and the total number of implantation sites. Viability of the embryos was determined.

No significant TR-701-related effects on female reproductive performance were observed at any dosage level (Table 84). As was the case for the male reproductive performance parameters, high-dose TR-701 (15 mg/kg/day for females) tended to reduce specific parameters (female fertility index, female conception index, mean pre-coital interval), but the high-dose values were not significantly lower than concurrent control values and fell within the historical control range.

Table 84: Female Reproductive Performance. (Sponsor's Table)

Parameter	Dosage Level (mg/kg/day)				WIL HC[a] Mean (Range)
	0	2.5	5	15	
Female Mating Index (%)	100.0	96.0	100.0	96.0	98.4 (86.7-100.0)
Female Fertility Index (%)	96.0	88.0	96.0	84.0	93.3 (73.3-100.0)
Female Conception Index (%)	96.0	91.7	96.0	87.5	95.0 (78.3-100.0)
Mean Estrous Cycle Length (days)	4.2	4.8	4.2	4.1	4.4 (3.6-5.8)
Mean Pre-Coital Interval (days)	3.1	3.4	3.4	2.8	3.0 (1.8-4.8)

[a] = (b) (4) historical control data

At necropsy, no TR-701-related gross pathology or histopathology findings or changes in organ weights (brain, ovaries, pituitary gland) were observed at any dose. The control, low-, mid- and high-dose groups had 1, 2, 1, and 3 nongravid females respectively.

None of the intrauterine survival parameters for any of the TR-701 dosage groups were significantly different compared to the control group (Table 85).

Table 85: Summary of Embryonic Data at the Scheduled Necropsy

	Group 1	Group 2	Group 3	Group 4
Number of Gravid Females	24	22	24	20 (21)*
Pre-implantation Loss	8.1 ± 2.16	3.5 ± 0.81	7.5 ± 2.22	11.4 ± 2.76
Post-implantation Loss	8.8 ± 2.51	4.7 ± 1.20	4.6 ± 1.25	2.7 ± 1.01
Number of Corpora Lutea	17.1 ± 0.46	16.3 ± 0.34	16.4 ± 0.43	16.9 ± 0.66
Number of Implantation Sites	15.7 ± 0.47	15.7 ± 0.29	15.1 ± 0.39	14.8 ± 0.31
Viable Fetuses	91.2 ± 2.51	95.3 ± 1.20	95.4 ± 1.25	97.3 ± 1.01
Dead fetuses	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Early Resorptions	8.8 ± 2.51	4.7 ± 1.20	4.6 ± 1.25	2.7 ± 1.01
Late Resorptions	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Values are expressed as the mean percent/litter ± Standard Error of the Mean. * Includes one female that had no evidence of mating but delivered.				

9.2 Embryonic Fetal Development

Study title: A Study of the Effects of TR-701 on Embryo/Fetal Development in Rats

Study no.:	TOX-08-0701-004
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 20, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TR-701, Lot No.: CMLW-304/07-TR3

Key Study Findings

- No TR-701-related maternal mortality or clinical signs were noted, but dose-related reductions in maternal food consumption and mean body weight gains were observed throughout the treatment period for all the TR-701 doses.

- Mean fetal weights in the mid- (5 mg/kg/day) and high-dose (15 mg/kg/day) groups were significantly reduced compared to control animals. Other cesarean section data including number of gravid females, implantation loss, number of corpora lutea, number of viable fetuses, fetal sex ratios, and number of resorptions were not changed.
- Malformations were not increased relative to the control group, but specific skeletal variations were increased in the high-dose group.
- The TR-701 NOAEL for maternal and fetal toxicity was considered to be 2.5 mg/kg/day which corresponded to plasma C_{max} and AUC_{0-24h} values of 3.56 μg/ml and 30.2 μg x hr/ml on GD17.

Methods

Doses:	0, 2.5, 5, and 15 mg/kg
Frequency of dosing:	Once daily during Gestation Days 6-17
Dose volume:	10 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Sterile water for injection
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	25 females/group
Satellite groups:	Toxicokinetic animals (8 females/group) and Supplemental Toxicokinetic animals (4 females/group)
Study design:	TR-701 or vehicle was administered by oral gavage once daily from gestation day (GD) 6-17 to bred female rats. On GD 20, surviving females were euthanized and necropsied.
Deviation from study protocol:	Study protocol deviations were noted, but none was considered to have substantially altered the results or integrity of the study.

Observations and Results

Table 86: Observation Schedule for the Rat Embryo-Fetal Study

Mortality and Clinical Signs	All rats were observed twice daily throughout the study for morbidity and mortality. Animals were also observed for signs of toxicity approximately 2 hours following dose administration.
Body Weights	Individual maternal body weights were recorded on GDs 0, 6-18, and 20 for the embryo/fetal development phase and on GDs 0 and 6-17 for the toxicokinetic phases (regular and supplemental phases). Net body weights exclusive of gravid uterine weight were calculated for GD 20.
Food Consumption	Individual food consumption was recorded daily on GDs 0, 6-18, and 20.

Toxicokinetic and supplemental toxicokinetic phases	Blood samples (≈ 1 ml) were collected from toxicokinetic phase females on gestation days 6 and 17 at approximately 0(predose), 1, 2, 4, 8, and 24 hours after dose administration. Blood samples were also collected from the supplemental toxicokinetic animals at 15 and 30 minutes after dose administration.
Necropsy	Animals underwent necropsy (Laparohysterectomy) on GD 20.

Mortality

All animals survived to Gestation Day 20.

Clinical Signs

No TR-701 associated clinical signs were noted.

Body Weight

Dose-related reductions in mean maternal body weight gains were noted in the 2.5, 5, and 15 mg/kg/day groups during Gestation Days 6-9, 9-12, and 12-18 and for the overall treatment period (Gestation Days 6-18). As a result of reduced weight gain, mean maternal body weights during gestation were as much as 4.6%, 6.3% and 10.0% reduced for the 2.5, 5, and 15 mg/kg/day groups respectively compared to the control group. The differences from the control group were generally significant. Following cessation of dosing, mean maternal body weights gains remained slightly suppressed relative to the control group, but not significantly so.

Dose-related significant reductions in mean gravid uterine weights due to reduced mean fetal weights were also noted for the 5 and 15 mg/kg/day groups.

Feed Consumption

Maternal food consumption was significantly reduced throughout the treatment period for all the TR-701 dose groups except food consumption for the lowest dose group (2.5 mg/kg/day) was not different than those of the vehicle control group from Gestation Days 12-18.

Toxicokinetics

Exposures to TR-701 were minimal indicating rapid metabolism to TR-700 (Table 87). Plasma C_{max} and AUC for TR-700 increased in a roughly dose-proportional manner. Plasma C_{max}, but not $AUC_{0\text{-}24h}$ values increased on GD 17 relative to GD 6, and T_{max} values changed from 2 hours to 1 hour (Table 88).

Table 87: Toxicokinetic Parameters for TR-701 after Oral Administration of TR-701 in Rats. (Sponsor's Table)

Test Period	Dose (mg/kg)	T_{max} (h)	C_{max} (µg/mL)	AUC_{Tau} (h• µg/mL)
Gestation Day 6	2.5	1.00	0.0792	0.801
	5	4.00	0.142	1.600
	15	4.00	0.397	4.77
Gestation Day 17	2.5	1.00	0.0806	0.677
	5	1.00	0.127	1.15
	15	1.00	0.368	3.52

Note: AUC_{Tau}=$AUC_{0\text{-}24\ h}$

Table 88: Toxicokinetic Parameters for TR-700 after Oral Administration of TR-701 to Rats. (Sponsor's Table)

Test Period	Dose (mg/kg)	T_{max} (h)	C_{max} (µg/mL)	AUC_{Tau} (h• µg/mL)
Gestation Day 6	2.5	2.00	2.81	27.3
	5	2.00	4.85	59.1
	15	2.00	12.3	156
Gestation Day 17	2.5	1.00	3.56	30.2
	5	1.00	6.46	54.4
	15	1.00	17.1	152

Note: AUC_{Tau}=$AUC_{0\text{-}24\ h}$

Dosing Solution Analysis

Dosing formulations were prepared approximately weekly as single formulations for each dosage level, divided into aliquots and stored refrigerated. Stability analysis was not performed as stability had previously been established. Quadruplicate samples for each concentration were collected from the middle of each dosing formulation including the control from the first and last weeks of dose administration for the embryo/fetal development and toxicokinetic phases and the last week of the dose administration for the supplemental toxicokinetic phase.

All of actual concentrations of the TR-701 dosing solutions fell within 10% of the nominal concentrations at all of the testing time-points. TR-701 was not detected in the vehicle formulation administered to the control group.

Necropsy

No TR-701-related gross pathology findings were observed.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Mean fetal weights in the 5 and 15 mg/kg/day groups were significantly lower (7.9 and 23.7% respectively) than the control group (Table 89). This pattern was consistent across genders for both groups.

Table 89: Summary of Fetal Data at the Scheduled Necropsy

	Group 1	Group 2	Group 3	Group 4

Fetal Weight in Grams	3.8 ± 0.27	3.7 ± 0.22	3.5 ± 0.26**	2.9 ± 0.33**
Number of Gravid Females	25	25	25	24
Pre-implantation Loss	25 (1.0)	24 (1.4)	22 (0.9)	21 (0.9)
Post-implantation Loss	22 (0.9)	23 (0.9)	16 (0.6)	26 (1.1)
Number of Corpora Lutea	410 (16.4)	408 (16.3)	393 (15.7)	389 16.2)
Number of Implantation Sites	385 (15.4)	374 (15.0)	371 (14.8)	368 (15.3)
Viable Fetuses	363 (14.5)	351 (14.0)	355 (14.2)	342 (14.3)
Dead fetuses	0	0	0	0
Fetal Sex Ratios (M/F)	49.1/	49.1	53.1	48.0
Early Resorptions	21 (0.8)	23 (0.9)	16 (0.6)	26 (1.1)
Late Resorptions	1	0	0	0
Values are expressed as the total value and in parentheses, the mean number/litter Significantly lower than the control value; *****: p ≤ 0.05; ******: p ≤ 0.01.				

Offspring (Malformations, Variations, etc.)

The number of visceral and skeletal malformations for each group is shown below in Table 90. The incidence of malformations was considered to be similar in all groups with 3/2/2, 1/1/1, 0/0/0 and 7/4/4 malformations/individual animals/litter in the control (Group 1), 2.5 (Group 2), 5 (Group 3), and 15 (Group 4) mg/kg/day groups respectively. The incidences for individual malformations in all the TR-701 dosage groups were not significantly different than the incidences for the control group and/or fell within the historical control range and were not considered related to TR-701 administration. Malformations included: ectrodactlyly (Group 4), brachydactyly (Group 4), microphthalmia and/or anophthalmia (Groups 1, 2, and 4) localized limb edema (Group 4), transposition of the great vessels (Group 4), coarctation of the arotic arch (Group 4), vertebral anomaly (Group 1), bent limb bone (Group 1), and small limb bone (Group 4).

Table 90: Summary of External, Visceral, and Skeletal Malformations in Fetal Rats

Groups	Number of fetuses (litters) examined	Number of Malformations (litters)			
		External	Visceral	Skeletal	Total
Group 1	363 (25)	1(1)	0	2 (2)	3 (2)
Group 2	351 (25)	1(1)	0	0	1 (1)
Group 3	355 (25)	0	0	0	0
Group 4	342 (24)	4(3)	2 (2)	1(1)	7 (3)

No external variations were noted for any of the groups. The visceral malformations that were noted occurred at equal frequency (pale spleen) or less frequency (renal papilla(e) not developed and/or distended ureter) in the TR-701 groups compared to the control

group or only in a single animal in the TR-701 high-dose group (hemorrhagic ring around iris, major blood vessel variation). None of the visceral variations were considered TR-701 related.

Mean litter proportions of skeletal developmental variations in the low- and mid-dose groups were generally similar to the control group and within historical control ranges. Several developmental variations were significantly increased in the TR-701 high-dose group compared to the control group and/or fell outside the historical control range as shown in Table 91. The increased skeletal variations (mean litter proportions) included: ossified pubis, unossified sternebra(e) numbers 5 and/or 6, unossified sternebra(e) numbers 1, 2, 3, and/or 4, and increased mean litter proportions of reduced ossification of vertebral arches and skull, and the presence of 14th rudimentary and full rib(s) and 27 presacral vertebrae. Also lower mean litter proportions of cervical centrum number 1 ossified were noted for the TR-701 high-dose group. These variations were considered related to high-dose TR-701 administration and correlated with the reduced mean fetal weights in this group.

Table 91: Mean Litter Proportions of Fetal Skeletal Developmental Variations. (Sponsor's Table)

Dose (mg/kg/day):	0	15	Historical Control mean (range)
Cervical centrum no. 1 ossified	19.6	7.8^{+}	20.1 (6.6-35.8)
Reduced ossification of the vertebral arches	0.3	5.6	0.1 (0.0-1.1)
14th rudimentary rib(s)	8.3	23.0	6.7 (1.4-15.1)
Sternebra(e) nos. 5 and/or 6 unossified	2.9	23.8^{+}	7.1 (0.2-23.1)
Sternebra(e) nos. 1, 2, 3 and/or 4 unossified	0.5	8.1	0.2 (0.0-1.3)
Pubis unossified	0.2	3.4	0.1 (0.0-2.3)
Reduced ossification of the skull	0.3	1.0	0.1 (0.0-1.0)
27 presacral vertebrae	0.0	6.4	0.1 (0.0-1.8)
14th full rib(s)	0.0	1.4	0.1 (0.0-0.9)

$^{+}$ = Significantly different from control group at 0.05.

Study title: An Oral (Gavage) Study of the Effects of TR-701 FA on Embryo-Fetal Development in Mice.

Study no.:	TOX-09-0701-019, (b) (4)-674019
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 27, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TR-701FA, Lot No.: 02090118

Key Study Findings

- TR-701 did not cause an increase in maternal death or weight loss.

- Mean Fetal weights for both genders were significantly reduced by high-dose (25 mg/kg/day) TR-701.
- Increased numbers of malformations occurred in fetuses born to high-dose dams (12 fetuses in 6 litters) compared to control dams (1 fetus in 1 litter). However, the percent of fetuses/litter (mean ± SD) with malformations (5.8 ± 11.38% for the high-dose group and 0.0 ± 0.00% for the control group) were not significantly different.
- All but one of the malformations in the high-dose group was a costal cartilage anomaly (11 fetuses in 5 litters). The mean litter proportions for this malformation was not significantly higher than for the control group, but since it did not occur at all in the concurrent controls and occurs only a very low incidence in historical controls, it is considered to be TR-701-related.
- The NOAEL for maternal toxicity was considered to be the high dose of 25 mg/kg/day which corresponded to plasma TR-700 $AUC_{0\text{-}24h}$ values of 93.8 and 95.7 µg·h/mL on Gestation Days 6 and 15 respectively.
- The NOAEL for embryo/fetal development was considered to be 5 mg/kg/day which corresponded to plasma TR-700 $AUC_{0\text{-}24h}$ values of 20.4 and 17.6 µg·h/mL on Gestation Days 6 and 15 respectively.

Methods

Doses:	0, 1, 5, and 25 mg/kg/day
Frequency of dosing:	Once daily during Gestation Days (GD) 6-15
Dose volume:	10 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	25 mM disodium hydrogen phosphate
Species/Strain:	Crl:CD1(ICR) mice
Number/Sex/Group:	25 females/group
Satellite groups:	48 females/group for toxicokinetics
Study design:	See Table 92 below.
Deviation from study protocol:	Several deviations from the study protocol were noted, but none of the deviations was considered to have affected the integrity or the results of the study.

Table 92: Study Design for the Embryo-Fetal Study in Mice. (Sponsor's Table)

Group Number	Treatment	Dosage Level (mg/kg/day)	Dosage Volume (mL/kg)	Number of Females	
				Main	TK
1	Vehicle	0	10	25	48
2	TR-701FA	1	10	25	48
3	TR-701FA	5	10	25	48
4	TR-701FA	25	10	25	48

Main = Embryo-fetal development phase
TK = Toxicokinetic phase

Observations and Results

Table 93: Observation Schedule

Clinical Observations and Mortality	All mice were observed twice daily, once in the morning and once in the afternoon for morbidity and mortality throughout the study. Animals were also observed for signs of toxicity approximately 2 hours following dose administration.
Body Weights	Individual maternal body weights were recorded daily on GDs 0 and 6-18 for the embryo/fetal development phase and GDs 0 and 6-16 for the toxicokinetic phase. Net body weight exclusive of the gravid uterine weight was recorded on GD 18.
Food Consumption	Individual food consumption was recorded according to the same schedule as for body weights.
Necropsy	Surviving dams were necropsies on Day 18
Toxicokinetics	Blood samples were collected on GD 6 and GD 15, at predose, 0.5, 1, 2, 8, and 24 hours after dose administration. Samples were collected via the retro-orbital sinus and processed to plasma.

Mortality

Three females were euthanized in extremis, one in the control group on GD 13, one in the low-dose group (1 mg/kg/day) on GD 9, and one in the high-dose group on GD 11. In addition four animals were found dead, one control animal on GD 14, and three in the high-dose group (GDs 14, 15, and 16). Most of the deaths were attributed to intubation errors based on observed esophageal and/or skeletal muscle perforations or dark red contents in the thoracic cavity at necropsy.

Clinical Signs

No TR-701-related clinical signs were noted.

Body Weight

Mean maternal body weights, body weight gains, and mean gravid uterine weights were not significantly affected by TR-701 administration.

Feed Consumption

Mean maternal food consumption was not significantly affected by TR-701 administration when considered as grams food/animal/day and g/kg body weight/day.

Toxicokinetics

The C_{max} and AUC values for plasma TR-700 increased in a roughly dose-proportional manner (Table 94). Mean values for these two parameters did not increase between GD6 and GD15 indicating TR-700 did not accumulate in plasma with repeated dosing.

Table 94: Select Toxicokinetic Parameters for Plasma TR-700 Following Oral Administration of TR-701 on GD 6-15 in Pregnant Mice. (Sponsor's Table)

Dosage Level (mg/kg/day)	C_{max} (µg/mL)	T_{max} (h)	AUC_{last} (µg·h/mL)	AUC_{0-24h} (µg·h/mL)
	Gestation Day 6			
0	0.00	NC	0.00	NC
1	0.559	1.00	2.69	3.75
5	2.86	0.500	20.4	20.4
25	13.2	0.500	93.8	93.8
	Gestation Day 15			
0	0.00	NC	0.00	NC
1	0.601	1.00	2.99	3.94
5	2.99	0.500	17.6	17.6
25	10.4	2.00	95.7	95.7

NC: Not Calculated

Dosing Solution Analysis

Duplicate samples were collected for concentration analysis from the middle stratum of the first and last dosing formulations prepared at each concentration and also for the vehicle control.

All of the actual concentrations of the dosing solutions were within 10% of the nominal concentrations. The vehicle control solutions did not contain detectable TR-701.

Necropsy

A gross necropsy was performed on females that died or were euthanized in extremis as well as scheduled laparohysterectomies for surviving dams. The thoracic, abdominal, and pelvic cavities were examined. The uterus and ovaries were excised and the number and location of corpora lutea, fetuses, early and late resorptions, and implantation sites were recorded.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

The number of gravid females in the high-dose group was lower than for the control group, but this was a reflection of the greater number of dams found dead or euthanized in the high-dose group (Table 95). The percent males and females were similar for all groups. Mean fetal weights were significantly reduced approximately 10% in the high-dose group (1.25 g) compared to the control group (1.37 g) and both genders were significantly affected. No dead fetuses were found in any of the groups and the percent of viable fetuses was similar for all groups. As shown in Table 96, the high-dose group experienced increased early, late, and total resorptions. However these differences were not significant and fell within the historical control values, and thus do not clearly indicate a TR-701-related toxicological effect. The other parameters were not significantly different from control for the high-dose group, and all of the parameters were unchanged by treatment with the low- and mid-dose administrations of TR-701.

Table 95: Summary of Fetal Data in the Mouse Embryo-Fetal Study

Group	Gravid Dams	Fetal Weights (g)			Litter size	Percent Male	Percent Female	Viable Fetuses	Dead Fetuses
		Male	Female	Combined					
1	23	1.39	1.35	1.37	22	53.1	46.9	269 (91.5)	0
2	22	1.44	1.35	1.40	22	53.6	46.4	246 (93.5)	0
3	25	1.36	1.32	1.35	25	51.8	48.2	285 (92.6)	0
4	19	1.29*	1.21**	1.25*	19	55.3	44.7	203 (87.7)	0
Significantly lower than the control value; *: $p \leq 0.05$; **: $p \leq 0.01$.									

Table 96: Summary of Fetal Resorptions, Implantation Loss, Implantation Sites and Corpora Lutea in the Mouse Embryo-Fetal Study

Group	Resorptions			Implantation Loss		Implantation Sites	Corpora Lutea
	Early	Late	Total	Pre	Post		
1	18 (8.1)	1 (0.4)	19 (8.5)	16 (4.9)	19 (8.5)	288 (12.5)	304 (13.2)
2	18 (6.1)	1 (0.4)	19 (6.5)	27 (10.1)	19 (6.5)	265 (12.0)	292 (13.3)
3	16 (6.5)	3 (0.8)	19 (7.4)	22 (7.8)	19 (7.4)	304 (12.2)	326 (13.0)
4	24 (10.7)	4 (1.6)	28 (12.3)	16 (6.0)	28 (12.3)	231 (12.2)	247 (13.0)
Data expressed as total numbers/group with percent/litter in parentheses.							

Offspring (Malformations, Variations, etc.)

Increased numbers of malformations occurred in the high-dose group compared to the control group. The numbers of fetuses (litters) with malformations for the control, low-, mid-, and high-dose groups were 1(1), 5(4), 3(2) and 12(6) respectively (Table 97).

External malformations were noted, but none was significantly increased compared to the control group, none was increased in a TR-701 dose-related manner, and/or did not exceed historical control levels. Therefore none of the external or visceral malformations was considered TR-701 related.

A TR-701 skeletal malformation, a costal cartilage fusion anomaly, was noted in 11 fetuses in 5 litters in the high-dose group. This finding was not observed in the concurrent control or in the mid-dose TR-701 group, but did occur in 2 fetuses in 2 litters in the low-dose group. The mean litter proportion of costal cartilage anomaly in the high-dose group (mean $\pm$ SD; 5.4 $\pm$ 11.45% per litter) was not significantly different than control proportions (0.0 $\pm$ 0.00% per liter). However, the proportion of costal cartilage anomaly in the high-dose group exceeded the maximum mean value (0.7% per litter) in the historical control range suggesting that the anomaly was TR-701-related. This particular malformation has also been reported in mice treated with linezolid.

Table 97: Absolute Numbers of Malformations in the Mouse Embryo-Fetal Study
(Sponsor's Table)

	FETUSES				LITTERS			
DOSE GROUP:	1	2	3	4	1	2	3	4
NUMBER EXAMINED EXTERNALLY	269	246	285	203	22	22	25	19
OMPHALOCELE	1	1	0	0	1	1	0	0
SPINA BIFIDA	0	2	0	0	0	1	0	0
CARPAL AND/OR TARSAL FLEXURE	0	0	2	0	0	0	1	0
CLEFT PALATE	0	0	1	1	0	0	1	1
NUMBER EXAMINED VISCERALLY	269	246	285	203	22	22	25	19
NUMBER WITH FINDINGS	0	0	0	0	0	0	0	0
NUMBER EXAMINED SKELETALLY	269	246	285	203	22	22	25	19
COSTAL CARTILAGE ANOMALY	0	2	0	11	0	2	0	5
STERNEBRAE FUSED	0	0	0	1	0	0	0	1
TOTAL NUMBER WITH MALFORMATIONS								
EXTERNAL :	1	3	3	1	1	2	2	1
SOFT TISSUE :	0	0	0	0	0	0	0	0
SKELETAL :	0	2	0	11	0	2	0	5
COMBINED :	1	5	3	12	1	4	2	6

1- 0 MG/KG/DAY 2- 1 MG/KG/DAY 3- 5 MG/KG/DAY 4- 25 MG/KG/DAY

Table 98: Litter Proportions of Malformations in the Mouse Embryo-Fetal Study

Malformations		Group 1	Group 2	Group 3	Group 4
External	Mean	0.4	1.1	0.9	0.4
	SD	1.94	3.90	3.38	1.53
	SEM	0.41	0.83	0.68	0.35
Visceral	Mean	0.0	0.0	0.0	0.0
	SD	0.00	0.00	0.00	0.00
	SEM	0.00	0.00	0.00	0.00
Skeletal	Mean	0.0	0.9	0.0	5.4
	SD	0.00	2.89	0.00	11.45
	SEM	0.00	0.62	0.00	2.63
Total	Mean	0.4	2.0	0.9	5.8
	SD	1.94	4.63	3.38	11.38
	SEM	0.41	0.99	0.68	2.61
Data expressed as the percent per litter.					

The total percent of fetuses per litter with variations were 57.8, 61.6, 60.9, and 67.3 for Groups 1, 2, 3, and 4 respectively with most of the variations comprised of skeletal variations (Table 99). Visceral variations occurred in 0.8, 0.5 and 2.9% of fetuses per litter in the 1, 5, and 25 mg/kg/day groups respectively compared to none in control group fetuses. However, none of the visceral variations was considered to be TR-701-related because none of the variations occurred in more than one fetus or litter, and the mean litter proportions were not significantly greater than for the control group. Substantially more skeletal variations occurred for all the groups including the control group and slightly more as a whole occurred in the TR-701 high-dose group. However, none of the individual skeletal variations were considered to be TR-701 related because they occurred in single fetuses or litters, at a similar incidence in the control group, did not occur in a dose-related manner, and/or the mean litter proportion was within the historical range.

Table 99: Total Variations in the Mouse Embryo-Fetal Study (Sponsor's Table)

	FETUSES				LITTERS			
DOSE GROUP:	1	2	3	4	1	2	3	4
NUMBER EXAMINED EXTERNALLY	269	246	285	203	22	22	25	19
NUMBER WITH FINDINGS	0	0	0	0	0	0	0	0
NUMBER EXAMINED VISCERALLY	269	246	285	203	22	22	25	19
SPLEEN- PALE	0	2	2	1	0	1	1	1
ACCESSORY SPLEEN(S)	0	0	0	1	0	0	0	1
LIVER- PALE	0	0	0	2	0	0	0	1
NUMBER EXAMINED SKELETALLY	269	246	285	203	22	22	25	19
7TH CERVICAL RIB(S)	54	46	47	15	14	13	17	8
14TH RUDIMENTARY RIB(S)	54	50	55	47	16	18	21	18
ACCESSORY SKULL BONE(S)	15	24	15	27	8	10	8	10
14TH FULL RIB(S)	47	44	66	59	15	13	13	14
27 PRESACRAL VERTEBRAE	1	0	0	9	1	0	0	3
7TH STERNEBRA	8	12	11	11	5	8	7	4
STERNEBRA(E) MALALIGNED(SLIGHT OR MODERATE)	9	6	8	7	6	6	7	3
STERNEBRA(E) #5 AND/OR #6 UNOSSIFIED	0	0	0	1	0	0	0	1
25 PRESACRAL VERTEBRAE	3	10	6	0	2	3	4	0
STERNEBRAE WITH THREAD-LIKE ATTACHMENT	1	0	0	0	1	0	0	0
EXTRA SITE OF OSSIFICATION ANTERIOR TO CERVICAL ARCH #2	1	0	0	1	1	0	0	1
REDUCED OSSIFICATION OF THE RIB(S)	0	0	0	2	0	0	0	1
VERTEBRAL CENTRA UNOSSIFIED	1	0	0	0	1	0	0	0

1- 0 MG/KG/DAY 2- 1 MG/KG/DAY 3- 5 MG/KG/DAY 4- 25 MG/KG/DAY

Study title: A Dose Range-Finding Study of the Effects of TR-701 on Embryo/Fetal Development in Rabbits.

Study no.:	TOX-08-0701-012
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 22, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TR-701, Lot No.: CMLW-304/07-TR3, purity of 90% (3% moisture, (b) (4) impurities)

Key Study Findings

- In pregnant rabbits orally administered 0, 1, 2.5, and 5 mg/kg/day, TR-701-related maternal toxicity manifest as mortality, morbidity and/or abortions and dose-dependent weight loss accompanied by corresponding reductions in food intake.
- Mean fetal weights in the 2.5 and 5 mg/kg/day were 23.5% and 19.8% lower than mean fetal weights in the control group with corresponding reductions in mean gravid uterine weights in both groups.
- NO TR-701-related developmental malformations or variations were noted.
- The NOAEL for maternal and fetal developmental toxicity was considered to be 1 mg/kg/day which corresponded with a plasma $AUC_{0\text{-}24h}$ value of 1.09 μg x hr/ml on Gestation Day 20.

Methods

Doses:	0, 1 (low), 2.5 (mid), or 5 (high) mg/kg/day
Frequency of dosing:	Once per day
Dose volume:	10 ml/kg

Route of administration:	Oral gavage
Formulation/Vehicle:	Distilled water
Species/Strain:	New Zealand White rabbits
Number/Sex/Group:	8 females/group
Satellite groups:	4 females/group for toxicokinetic analysis
Study design:	In the Main Study, gravid females time mated on Gestation Day (GD) 1 were dosed once daily on GDs 7-20. Necropsies were performed on animals that died, were euthanized in extremis or aborted. Surviving Main Study, dams were euthanized and necropsied on Day 29.
Deviation from study protocol:	Several deviations from the study protocol were noted, but none of the deviations was considered to have altered the results of the study or compromised the integrity of the study.

Observations and Results

Table 100: Observation Schedule for the Rabbit Embryo-Fetal Study.

Clinical Observations and Mortality	All rabbits were observed twice daily for morbundity and mortality throughout the study. Animals were also observed approximately 2 hours after dose administration.
Body Weights	Individual body weights were recorded once per day on GDs 0, 4, 7-21, 24, and 29 for animals assigned to the embryo/fetal development phase and on GDs 0, 4, and 7-20 for toxicokinetic animals. On GD 29 the animals were euthanized, gravid uterine weight was collected and net maternal body weight exclusive of the weight of the uterus and contents was calculated.
Food Consumption	Individual food consumption was recorded in GDs 4 through 29.
Necropsy	All surviving Main Study animals were euthanized on GD 29. The thoracic, abdominal and pelvic cavities of each dam were opened and the contents examined.
Toxicokinetics	Blood samples were collected on GD 7 and GD 20, at predose, 15 minutes, 30 minutes, and 1, 2, 4, 8 and 24 hours after dose administration.

Mortality

Two females each in the 2.5 and 5 mg/kg/day groups were found dead or euthanized in extremis due to severe weight loss. All other females survived to the scheduled necropsy on GD 29.

Clinical Signs

In the low- (1 mg/kg/day), mid- (2.5 mg/kg/day) and high-dose (5 mg/kg/day) groups, 1, 2, and 2 females respectively aborted during the course of the study. These animals

were euthanized. Red material on the urogenital area was noted in 5 of the early termination animals, four of whom aborted. Decreased defecation occurred in a dose-dependent manner.

Body Weight

Significant dose-related body weight losses were noted during the treatment period (GD 7-10, GD 10-13, GD 13-21, and GD 7-21) in the mid- and high-dose females. During the dosing period from GD 7 to 21, the mid-dose group lost an average of 250 grams body weight relative to the mean starting weight and the high-dose group lost 293 grams. In contrast the control group gained an average of 274 grams body weight.

Feed Consumption

Significantly reduced maternal food consumption occurred in the mid- and high-dose groups throughout the treatment period with corresponding weight loss. Following cessation of treatment, food consumption for the mid- and high-dose groups was significantly lower for GDs 21-24, but similar to the control group on GDs 24-29.

Toxicokinetics

Plasma C_{max} and AUC values increased in a greater than dose-proportional manner. Plasma AUC values were similar on Day 7 and Day 20, but plasma C_{max} values were lower on Day 20 compared to Day 7.

Table 101: Toxicokinetic Parameters for the Rabbit Embryo-Fetal Study.
(Sponsor's Table)

Test Period	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	$AUC_{0\text{-}24h}$ (h·ng/mL)	AUC_{last} (h·ng/mL)
Gestation Day 7	1	137	4.00	1200	1110
	2.5	451	3.50	4330	4330
	5	922	3.25	8750	8750
Gestation Day 20	1	87.5	2.75	1090	1090
	2.5	222	6.50	4000	4000
	5	610	8.00	9980	9980

Dosing Solution Analysis

Quadruplicate samples were collected in the first and last week of administration from all the dosing formulations including the vehicle for concentration analysis.

All of the actual concentrations of the dosing formulations were within 10% of the nominal concentrations. No TR-701 was detected in the vehicle formulations.

Necropsy

A gross necropsy was performed on female rabbits that died or were euthanized before the scheduled necropsy. In addition scheduled laparohysterectomies were performed by researchers blinded to the treatment group on Day 29 for all surviving female rabbits. The contents of the thoracic, abdominal, and pelvic cavities were examined. The uterus

and ovaries were exposed and excised. The number of corpora lutea in each ovary, and number and location of fetuses, early and late resorptions and total implantation sites in each uterus were recorded.

No remarkable gross pathology findings were observed in the rabbits euthanized early or at the scheduled necropsy that were considered to be related to TR-701 administration. One mid-dose and two high-dose females were determined to be non-gravid.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Based on the laparohysterectomy parameters from the surviving females only, mean gravid uterine weights were lower in the mid- and high-dose females compared to the control group with a significant reduction in the mid-dose females. In agreement with this finding, mean fetal weights in the mid- and high-dose groups were 23.5% and 19.8% lower than the control group respectively, but the differences were not statistically significant. Mean litter proportions of early and late resorptions were increased in the mid- and high-dose groups 7.4% and 10.7% per litter respectively compared to 2.6% in the control group, but again the differences did not reach statistical significance. In agreement with these findings, slightly but not significantly lower litter proportions of viable fetuses were noted in the mid- (92.6%) and high-dose (89.3%) groups compared to the control group (97.4%). Late resorptions took place mainly in 1 mid-dose dam and 2 high-dose dams. Mean numbers of corpora lutea, implantation sites, and the mean litter proportions of implantation loss were similar across all groups.

Table 102: Summary of Fetal Data at Scheduled Necropsy for the Rabbit Embryo-Fetal Study

Fetal Parameter	Calculations	Group 1 (Vehicle)	Group 2 (1 mg/kg/day)	Group 3 (2.5 mg/kg/day)	Group 3 (5 mg/kg/day)
Viable Fetuses	(Total) mean	(75) 9.4	(66) 9.4	(24) 8.0	(26) 8.7
	SD	1.30	1.99	1.00	1.53
Dead Fetuses	Total	0	0	0	0
Early Resorptions	(Total) mean	0 (0.0)	(2) 0.3	(0) 0.0	(1) 0.3
	SD	0.00	0.49	0.00	0.58
Late Resorptions	(Total) mean	(2) 0.3	(2) 0.3	(2) 0.7	(2) 0.7
	SD	0.46	0.76	1.15	0.58
Post-Implantation Loss	(Total) mean	(2) 0.3	(4) 0.6	(2) 0.7	(3) 1.0
	SD	0.46	0.79	1.15	1.00
Implantation Sites	(Total) mean	(77) 9.6	(70) 10.0	(26) 8.7	(29) 9.7
	SD	1.19	2.58	0.33	0.58
Corpora Lutea	(Total) mean	(80) 10.0	74 (10.6)	(28) 9.3	(30) 10.0
	SD	1.31	2.44	1.53	0.00
Pre-Implantation Loss	(Total) mean	(3) 0.4	(4) 0.6	(2) 0.7	(1) 0.3
	SD	0.74	1.13	1.15	0.58
Fetal Weights in Grams	mean	37.9	37.9	29.0	30.4
	SD	4.80	4.76	6.21	6.23
Number of Gravid Females	Total	8	7	3	3
Fetal weights compared using Dunnett's test.					

Offspring (Malformations, Variations, etc.)

The number of fetuses (litters) available for morphological variation were 75(8), 66(7), 24(3), and 26(3) in the control, 1, 2.5, and 5 mg/kg/day groups. One malformation, bilateral carpal flexure was noted in one high-dose fetus. No external developmental variations were observed in any of the groups.

9.3 Prenatal and Postnatal Development

Study title: An Oral (Gavage) Study of the Effects of TR-701 FA on Pre- and Postnatal Development Including Maternal Function in Rats

Study no.:	TOX-11-0701-025
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 2, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TR-701 FA, Lot # 02090118, 97.3% purity.

Key Study Findings

- Oral administration of TR-701 FA at doses of ≤ 3.75 to pregnant F_0 dams did not produce clinical signs, adversely affect survival, alter body weight, alter gestation length or the process of parturition, produce F_0 gross pathology or alter the mean number of F_1 pups born, live litter size, percentage of males at birth, postnatal F_1 survival, or F_1 birth weights.
- Oral administration of TR-701 FA to pregnant F_0 rats resulted in TR-700 exposure to fetuses in utero and the presence of TR-700 in milk.
- Developmental landmarks in the F_1 generation were unaffected by F_0 maternal exposure to TR-701 at all dosage levels. No TR-701-related effects on behavior, including startle response on PND 20 and 60, locomotor activity on PND 21 and 61 and learning and memory assessment on PND 22 and 62 were observed. Also no TR-701-related effects were noted in the F_1 generation on survival, clinical signs, mean body weights, or weight gain, gestation, process of parturition, lactation (females), reproductive endpoints (pre-coital intervals, estrous cycle length, mating, fertility, copulation/conception indices).
- No TR-701-related effects on F_2 pup live litter size, percentage of males per litter, postnatal survival, pup body weights, or pup external or internal condition were noted.

Methods

Doses:	0, 1.25, 2.5, and 3.75 mg/kg/day
Frequency of dosing:	daily
Dose volume:	10 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	25 mM disodium hydrogen phosphate, pH 7.5
Species/Strain:	Crl: CD(SD) rats

Number/Sex/Group:	24 females/group in the pre-postnatal development phase, 3 females/group in the placental transfer phase, and 4 females/group in the lacteal transfer phase.
Satellite groups:	none
Study design:	Three groups of bred female Crl:CD(SD) rats were administered TR-701 FA once daily by oral gavage from gestation day (GD) 6 through lactation day (LD) 20 (pre- and postnatal development phase; 36-38 doses total), GD 6 to GD 20 (15 doses total; placental transfer phase) or from GD 6 to postnatal day (PND) 10 (26 to 28 doses total; lacteal transfer phase). Females that failed to deliver were dosed through postmating Day 24 for a total of 19 doses. F_0 Females were allowed to deliver naturally and rear their offspring to PND 10 (lacteal transfer phase) or weaning (PND 21; pre- and postnatal development phase).
Deviation from study protocol:	Study protocol deviations were noted; however, none was considered to have altered the results or integrity of the study.

Observations and Results

F_0 Dams

Survival:	All F_0 maternal animals in the control, 1.25, 2.5, and 3.75 mg/kg/day group survived to the scheduled necropsy.
Clinical signs:	No TR-701-related clinical signs were noted during daily examinations and at observations 2 hours after dose administration.
Body weight:	No TR-701-related changes in body weight were noted during gestation and lactation for any dose level.
Feed consumption:	Lower food consumption was noted in the high-dose (3.75 mg/kg/day) group throughout gestation, but lower dose groups were unaffected. However, corresponding effects on body weight were not noted. Food consumption during lactation was unaffected.
Uterine content:	No TR-701-related effects were noted for the mean number of implantation sites, mean number of F_1 pups born, live litter size, or percentage of males at birth.
Necropsy observation:	No TR-701-related necropsy findings were noted.
Toxicokinetics:	See Table 103 below. Plasma concentrations collected two hours after dosing increased in less

than a dose-proportional manner on GD 20 and a roughly dose-proportional manner on LD 10. The fetus/dam plasma ratio on GD 20 ranged from 0.241 to 0.368. On LD 20 about equal concentrations of TR-700 were found in maternal milk and plasma respectively.

Dosing Solution Analysis: The actual concentrations for the dosing solutions were within 98-102% of the nominal concentrations. No TR-701 was detected in the vehicle formulation.

Other: Gestation length and parturition were not affected by administration of any of the doses of TR-701 relative to the control group. Also at the LD 21 necropsy, no TR-701 effects on the number of former implantation sites or number of unaccounted for sites were noted.

Table 103: Toxicokinetic Measurements in Maternal and Fetal Plasma and Maternal Milk in the Pre- Postnatal Study in Rats. (Sponsor's Table)

Dosage (mg/kg/day):	1.25 mg/kg/day	2.5 mg/kg/day	3.75 mg/kg/day
Study Day	Mean ± SD (ng/mL)	Mean ± SD (ng/mL)	Mean ± SD (ng/mL)
GD 20			
Dam's Plasma	1723 ± 312	3402 ± 400	4198 ± 2777
Fetal Plasma	442 ± 122	816 ± 137	1075 ± 230
Fetus/Dam Ratio	0.266 ± 0.103	0.241 ±0.0396	0.368 ± 0.279
LD 10			
Plasma	1285 ± 374	2661 ± 209	4093 ± 527
Milk	1306 ± 181	3547 ± 757	5191 ± 671
Milk/Plasma Ratio	1.09 ± 0.375	1.34 ± 0.310	1.29 ± 0.247

N=3 for dams and fetuses (placental transfer phase, GD 20); N = 4 dams (lacteal transfer phase, LD 10).

F_1 Generation (PND 0-21)

Survival: Survival was similar for all groups from PND 4 to 21. Pups(litters) that were found dead or euthanized in extremis between birth and PND 21 were 15(9), 12(8), 17(10), and 22(13) in the control, 1.25, 2.5, and 3.75 mg/kg/day groups respectively. In addition, 1(1), 4(4), 7(3), and 10(7) in the same respective groups were missing and presumed cannibalized. The higher incidence in the 2.5 and 3.75 mg/kg/day groups were due to 1 litter in each group with a higher percentage of dead/missing/ euthanized pups (7 and 8 pups in one litter each in the 2.5 and 3.75 mg/kg/day groups respectively). However, the remaining litters in each group were not similarly affected,

and thus the differences were not clearly related to TR-701 administration.

Clinical signs: The general physical condition of all F_1 pups in the 1.25 and 2.5 mg/kg/day groups was unaffected by maternal administration. In the high-dose group (3.75 mg/kg/day), 12 pups were observed with pale body and 11 pups had small stature compared to 2 and 1 pups respectively with pale body and small stature in the control group. The majority of affected high-dose animals also had lower body weights compared to the control group, and 7 affected high-dose animals were subsequently found dead, euthanized in extremis or missing. However, 4 of the 7 pups were in the same litter. Because of the higher incidence in one litter and because the mean values for body weights, body weight gains, and postnatal survival in the high-dose group did not differ compared to the control group, the increased incidence of high-dose pups with pale body and/or small stature is of uncertain relatedness to TR-701 administration. Also the findings do not appear to be toxicologically relevant because the mean body weights, body weight gains, and postnatal survival in the high-dose group for the period of PND 0-21 did not differ compared to the control group.

Body weight: Mean male and female pup body weight gains in the high-dose group, and mean female pup body weight gain in the 2.5 mg/kg/day group was significantly lower than for the control pups during PND 4-7 only. However, at PND 21 weaning, mean body weights in these groups were comparable to the control group.

Feed consumption: Not assessed

Physical development: Balanopreputial Separation: TR-701 did not affect the mean age of balanopreputial separation (about 45 days for all groups including the control group) or significantly alter the mean body weights at the age of attainment.
Vaginal Patency: TR-701 did not affect the mean ages of attainment of vaginal patency (approximately 32 days for all groups) or the mean body weights at the age of attainment (control group: 113.6g; 1.25 mg/kg/day: 115.0g; 2.5 mg/kg/day: 116.2g; 3.75 mg/kg/day: 113.6g.

Neurological assessment: Auditory Startle Response: Select F_1 animals were assessed on PND 20 (weaning) and PND 60 (sexual maturity). For both dates, all measurement values were similar for the TR-701 treatment groups and the control group.
Locomotor Activity: Locomotor activity (total activity and ambulatory activity counts) in F_1 animals were largely

unaffected by maternal TR-701 administration as assessed on PND 21 and 61. One exception, high-dose F_1 females exhibited significantly higher (+21%) mean cumulative ambulatory counts compared to the concurrent control. However, males were not similarly affected and the value for high-dose females was similar to the historical control value suggesting the effect was not TR-701 related.

Biel Maze Swimming Trials: With a few exceptions, swimming ability, times to criterion, mean time to escape and number of errors committed during various phases of evaluation on PND 22 or PND 62 were similar for F_1 pups born to the F_0 control and TR-701 treatment groups. Low-dose female pups on PND 22 demonstrated a significantly shorter mean time to escape. Also in the PND 22 learning trials, high-dose males demonstrated significantly longer mean escape times and higher mean numbers of errors compared to controls, but not historical control values. Similarly compared to control females, mid- and high-dose females demonstrated significantly higher escape times and higher mean numbers of errors for the overall path B learning trials and/or the path A memory trial (high-dose females; escape times only) on PND 62. However, all of the differences were limited to one gender at each evaluation time, and a single age of testing, and a single path for specific tests suggesting the differences were not related to maternal TR-701 administration.

Reproduction: The number of litters, mean number of F_1 pups born, live litter size, percentage of males/litter at birth, and postnatal survival were all unaffected by maternal TR-701 administration at any dose.

Other: Necropsy of the pups found dead or euthanized in extremis did not reveal external or internal gross pathology related to TR-701 administration. This was also the case for the scheduled necropsies of the pups on PND 21

F_1 Generation Postweaning Period (PND 21-114)

Survival: F1 offspring that were found dead during the postweaning period included one low-dose male on PND 105 and one high-dose male on PND 114. No clinical signs were associated with these deaths and in the absence of a dose-response, the deaths were considered incidental. All other animals survived until the scheduled necropsy.

Clinical signs: No clinical signs considered related to maternal treatment with TR-701 were observed in F_1 offspring during the post weaning period during detailed weekly physical

examinations.

Body weight: Only minor transient changes in weekly body weights, body weight gains, and cumulative body weight gains were noted. Absolute mean body weights were not affected, and the effects were not considered related to maternal TR-701 administration. Similarly, mean F_1 maternal body weights and body weight gains during gestation or lactation were unaffected by TR-701 administration to F_0 dams.

Reproductive Performance: As shown in Table 104, none of the measured male or female reproductive performance parameters was altered by maternal F_0 dosing with TR-701.

Gestation Length and Parturition: The process of parturition and mean F_1 gestation lengths for the TR-701-exposed groups were comparable to the control group.

Gross Pathology: Necropsy of the two F_1 males found dead during the postweaning period did not reveal external or internal gross pathology related to TR-701 administration. This was also the case for the F_1 females scheduled for necropsy on LD 4, post-mating Day 25 or post-cohabitation Day 25 and for the scheduled necropsy of F_1 males allowed to breed.

Reproduction: The number of litters, mean number of F_2 pups born, live litter size, percentage of males/litter at birth, and postnatal survival were all unaffected by F_0 maternal TR-701 administration at any dose.

Other: At the LD 4 necropsy, a TR-701-effect on the number of former implantation sites and the number of unaccounted-for sites in the uterus was not observed in F_1 dams. Significantly fewer mean implantation sites were observed for F_1 dams in the mid-dose group (13.8 sites/litter) compared to the concurrent control value (16.3 sites/litter) but the effect was not dose-responsive and a similar effect did not occur in low-dose or high-dose F_1 dams (14.9 sites/litter for both groups).

Table 104: Male and Female Reproductive Performance for F_1 Pups. (Sponsor's Table)

	Dosage Level (mg/kg/day)				WIL HC[a]
Parameter	0	1.25	2.5	3.75	Mean (Range)
Male Mating Index (%)	91.3	95.8	95.7	95.8	95.8 (84.0-100.0)
Female Mating Index (%)	95.7	100.0	100.0	100.0	98.2 (92.0-100.0)
Male Fertility Index (%)	87.0	91.7	91.3	91.7	90.0 (60.0-100.0)
Female Fertility Index (%)	87.0	95.8	95.7	95.8	92.9 (60.0-100.0)
Male Copulation Index (%)	95.2	95.7	95.5	95.7	93.2 (71.4-100.0)
Female Conception Index (%)	90.9	95.8	95.7	95.8	92.9 (65.2-100.0)
Estrous Cycle Length (days)	4.3	4.3	4.2	4.5	4.3 (4.0-5.0)
Pre-Coital Interval (days)	2.7	3.0	3.5	2.7	3.3 (2.4-4.8)

[a] = (b) (4) historical control data

F_2 Generation

Survival:	Maternal F_0 administration of TR-701 did not affect postnatal F_2 pup survival up to PND 4 relative to control F_2 pups. The numbers of F_2 pups (litters) found dead between birth and PND 4 were 13(5), 13(7), 5(3) and 18(7) for the control, low-, mid- and high-dose TR-701 maternal-dose groups respectively. The numbers of pups(litters) found missing (presumed to be cannibalized) were 7(3), 7(5), 10(3) and 0(0) for the same respective groups.
Body weight:	Mean F_2 pup body weights and body weight changes were not affected up to PND 4 by F_0 maternal exposure to TR-701 at any dose.
External and Internal evaluation:	The general physical condition of the F_2 pups was unaffected by F_0 maternal exposure to TR-701. Also among the pups found dead no gross pathology attributable to F_0 exposure to TR-701 was observed.
Male/Female ratio:	Maternal F_0 administration of TR-701 did not affect the percentage of F_2 males per litter at birth.
Other:	Maternal F_0 administration of TR-701 did not affect the mean number of F_2 pups born or live litter size. These two measurements were significantly reduced in the mid-dose F_2 group (13.0 pups per litter and live litter size of 12.9 pups per litter) compared to the concurrent control (15.5 and 15.4 per litter respectively), but similar differences did not occur for the high-dose group (14.0 and 13.6 respectively). The lower values for the mid-dose pups correlated with significantly lower mean number of F_1 dam implantations for this group, but the values were within the historical control range.

10 Special Toxicology Studies

Immunotoxicology

Study title: 4-Week Oral Immunotoxicity Study of TR-701 FA in Rats

Study no.:	TOX-12-0701-060
Study report location:	Electronic transmission
Conducting laboratory and location:	Bayer Pharma AG, Toxicology, 42096 Wuppertal, Germany
Date of study initiation:	August 14, 2012
GLP compliance:	German Principles of GLP
QA statement:	Yes
Drug, lot #, and % purity:	TR-701 FA, Lot 02120030, purity of 99.6%

Key Study Findings

- Total spleen cell counts were significantly reduced by 40% in high-dose (100 mg/kg/day) males and nonsignificantly by 20% in high-dose (30 mg/kg/day) females.
- High-dose males had significantly decreased T cells (-17%), B cells (-25%) and double positive T cells (CD4+/CD8+; -40%) in spleen cell populations. Spleen T cells were reduced 13% in high-dose females.
- Mean IgG titers were significantly decreased in the mid- and high-dose males by 61% in both groups and non-significantly in the low-dose males (-43%). IgG titers were also significantly decreased in high-dose females by 76%.
- Following pre-treatment with sheep red blood cells, IgM and IgG-mediated plaque formation was significantly reduced in high-dose females and in a dose-dependent manner in in male animals. The total number of plaque forming spleen cells were substantially reduced in both high-dose males (-76%) and females (-80%) compared to control groups with statistically significant reductions in males only.

Methods

Doses:	Males: 0, 10, 30 and 100 mg/kg; Females: 0, 3, 10, or 30 mg/kg
Frequency of dosing:	daily
Route of administration:	oral
Dose volume:	10 ml/kg
Formulation/Vehicle:	25 mM disodium hydrogen phosphate buffer, pH 7.5
Species/Strain:	Sprague-Dawley Rats
Number/Sex/Group:	8/sex/group
Age:	5-weeks at delivery
Weight:	At start of study: Males: 196-216 g; Females: 137 to 168 g.
Satellite groups:	None
Unique study design:	See Table 105. TR-701 FA or vehicle was administered by oral gavage to Sprague-Dawley rats (8/sex/group) daily for a period of 29 days for males and 30 days for females
Deviation from study protocol:	Deviations from the study protocol were noted; however, the deviations were not considered to have altered the results or integrity of the study.

Table 105: Study Design for the Rat Immunotoxicity Study

Group No.	N/group	Sex	Compound	Dose (mg/kg)
1	8	Male	Vehicle	0
2	8	Male	TR-701 FA	10
3	8	Male	TR-701 FA	30
4	8	Male	TR-701 FA	100
5	8	Male	CPA	80
6	8	Female	Vehicle	0

7	8	Female	TR-701 FA	3
8	8	Female	TR-701 FA	10
9	8	Female	TR-701 FA	30
10	8	Female	CPA	80
CPA = the positive control, cyclophosphamide. Animals in Groups 5 and 10 were treated once intravenously with 80 mg/kg cyclophosphamide on Day 24 (males, Group 5) or Day 25 (females, Group 10).				

Observations and Results

Table 106: Observation Schedule for the Rat Immunotoxicity Study

Clinical Observations and Mortality	Rats were inspected twice daily inspections for mortality and morbidity. Detailed clinical examinations were conducted weekly.
Body Weights	Individual body weights were measured daily for animals in Groups 1-4 and 6-8
Food Consumption	weekly
Water Intake	weekly
Sheep Erythrocyte Immunization	Animals were immunized with sheep erythrocytes 5 days before necropsy.
Immunotoxicity Evaluations	Performed with spleen and blood samples obtained after necropsy
Necropsy	Surviving animals
Toxicokinetics	Not performed

Mortality

All animals survived until the scheduled sacrifice.

Clinical Signs

Detailed clinical examinations included assessments of changes in skin, fur, eyes, mucus membranes, occurrence of secretions and excretions, autonomic activity, changes in gait, posture, and response to handling, clonic/tonic movements, and bizarre behavior.

One male in Group 5 (high-dose, 100 mg/kg/day TR-701 FA) exhibited paleness and piloerection on Days 24 and 29 and increased squatting behavior on Day 29.

Body Weights

Body weights of high-dose males were significantly decreased compared to the vehicle control group for Days 5-10. However, the body weight decreases were strongly influenced by weight loss for a single male that also displayed clinical signs.

Feed Consumption

Mean food consumption was not significantly changed for any of the TR-701 FA-treatment groups compared to the vehicle control group.

Water Intake

Water intake increased in a TR-701 FA dose-dependent manner throughout the 4-week treatment period. In the high-dose group, mean water intake per day over the treatment period was approximately 46% higher in males and 27% higher in females versus respective control values.

Immunotoxicity Evaluations

Spleen Cell Counts: Total spleen cell counts for viable cells were determined using Trypan Blue exclusion and manual cell counting for individual animals at necropsy. Total spleen cell counts were significantly reduced by approximately 40% in high-dose males compared to control males. Spleen cell counts were also reduced to a non-significant degree by approximately 20% in high-dose females.

Flow Cytometric Analysis: Subpopulations of spleen cells were stained with surface markers for specific immune-cell types and evaluated with flow cytometry. High-dose males demonstrated significant decreases in T cells (CD3+; -17%), B cells (CD45 RA+, PanB+; -25%), and double-positive T cells (CD4+/CD8+; -40%). High-dose females demonstrated similar significant decreases in CD3+ T cells (-13%) only. NK cells (CD161+), CD4+ T cells and CD8+ T cells were not significantly reduced in high-dose animals for either gender. No immune cell decreases were apparent in lower-dose groups for either gender.

Antibody Titers: Serum antibodies (IgG, IgM, and IgA) titers were determined following necropsy using a sandwich ELISA technique. At the end of dosing, mean IgA antibody titers in males were significantly decreased at all TR-701 dose levels but not in a dose-dependent fashion (11-19%). Mean IgG titers were significantly decreased in the mid- and high-dose males by 61% in both groups and non-significantly in the low-dose males (-43%). IgG titers were also significantly decreased in high-dose females by 76%. IgM was not significantly reduced by TR-701 in either gender.

Plaque Forming Cell Assay (PFCA): Animals were intravenously injected with sheep red blood cells 5 days before necropsy. At necropsy, spleen cells from each animal were incubated with guinea pig complement, and the number of plaque-forming cells was enumerated. At the end of dosing, dose-dependent decreases in IgM and IgG-mediated plaque formation was noted in male animals. IgM-mediated plaque formation/10^6 spleen cells was reduced only in high-dose males (-76%), while IgG-mediated plaque formation was reduced in both the mid- (-54%) and high-dose (-83%) males. IgM- and IgG-mediated plaque formation were both significantly reduced in high-dose females with reductions of 80% and 85% respectively compared to the control group. The total number of plaque forming spleen cells were substantially reduced in both high-dose males (-76%) and females (-80%) compared to control groups with statistically significant reductions in males only.

Organ Weights

Absolute weights of brain, spleen, and thymus were determined.

Absolute and relative thymus weights were significantly decreased in high-dose males by 30 and 25% respectively. In high-dose females, absolute and relative thymus

weights were decreased by 22 and 19% respectively. Absolute spleen weights were significantly reduced in high-dose males (-19%), but not females. Brain weights were not affected by any TR-701 dose in either gender.

Toxicokinetics: Not performed

Dosing Solution Analysis

Stability analysis indicted that TR-701 solutions in a concentration range from 0.1 mg/ml to 175 mg/ml were stable for at least 10 days at room temperature. Actual concentrations of TR-701 dosage solutions determined during the first and last preparation verified that TR-701 content was within (b) (4) of the target concentrations.

Local Toxicity

Study Title: Acute Perivascular, Intramuscular, and Subcutaneous Irritation Study of TR-701 FA in Rabbits

Study no.:	TOX-12-0701-058
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Oct. 11, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TR-701 FA for injection, Lot No.: P42601A, purity of 98.9%

Key Study Findings

One day and four days after TR-701 FA administrations in single doses by perivascular (PV), intramuscular (IM), and subcutaneous (SC) injections, slightly more severe signs of local irritation including hemorrhage, focal muscle degeneration with histocyte infiltration were observed compared to vehicle injections.

Methods

Each of 6 New Zealand White rabbits (4 males and 2 females) was administered single doses of vehicle and TR-701 FA by perivascular (PV), intramuscular (IM), and subcutaneous (SC) injections.

PV Injections: TR-701 (200 mg) was administered by single bolus injection (0.3 ml) into the perivascular space around the marginal ear vein of the right ear and vehicle was injected into the left ear in the same manner of the same animals.

IM Injections: TR-701 (200 mg) was administered by single IM injection (0.5 ml) at a site in the M. vastus lateralis muscle of the right leg of each animal, and vehicle was injected in the same manner into the M. vastus lateralis in the left leg of the same animals.

SC Injections: TR-701 (200 mg) was administered by single SC injection (1.0 ml) at a shave site located to the right of the midline of the back of each animal and vehicle was injected in the same manner at a site to the left of the midline of the back of the same animals.

Following 24 hours of observation, 3 animals were euthanized and the remaining 3 were euthanized after a 96 hour nondosing period. Surviving rabbits were observed for mortality twice daily, received body weight measurements and detailed physical examinations on the day of dosing and on the day of each animal's scheduled necropsy. Irritation observations were performed for all animals at approximately 1 and 24 hours after dosing with additional observations at approximately 48, 72, and 96 hours after dosing for the animals remaining after the 24-hour necropsy. All injection sites were examined macroscopically and microscopically on the day of necropsy.

Observations and Results

Mortality

All animals survived to the scheduled necropsy.

Clinical Signs

No TR-701-related clinical signs were noted.

Injection Site Irritation Observations (at 1, 24, 48, 72 and 96 hours after dosing)

PV sites: Irritation findings were observed mostly at the PV sites including slight erythema or moderate erythema and swelling with some discoloration of the marginal ear vein and surrounding tissue. The incidence of these findings were slightly increased at the TR-701 injection sites (17 observations in 6 animals) compared to vehicle sites (8 observations in 4 animals), but without clear correspondence with macroscopic and histopathology findings.
SC sites: slight swelling was observed at 2 TR-701 injection sites and 1 control site.
IM sites: No irritation was reported for IM injection sites.

Macroscopic Examination

None of the gross necropsy observations were considered to be related to administration of TR-701.

Histopathology

At the necropsy occurring 24 hours after dosing, subacute inflammation and hemorrhage were noted in the dermis and/or subcutis in the perivascular, intramuscular, and subcutaneous injection sites with similar incidence in the vehicle and TR-701 injection sites. Focal muscle degeneration with hystiocyte and heterophil infiltrate was noted in the panniculus of the IM and SC injection sites with a slightly higher incidence in the TR-701 injection sites.

At the necropsy occurring 96 hours after dosing, subacute inflammation was noted in the dermis and/or subcutis of the PV, IM, and SC injection sites with a slightly higher incidence in the TR-701 injected IM sites. Hemorrhage was noted at PV and IM sites. Focal muscle degeneration with histiocytic infiltration was noted in the panniculus in the IM and SC sites with a slightly higher incidence in the SC TR-701 injection sites.

Studies Intended to Qualify TR-701 Drug Substance Impurities

General Toxicology Studies

Study title: A 14-Day Intravenous (Bolus) Injection Impurity Safety Qualification Study in Sprague Dawley Rats

Study no.:	TOX-13-0701-075
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 11, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) lot # DUG-AY-2(1), 98.5% purity; (b) (4) lot # AJL-N-86(4), 98% purity; (b) (4) lot # WZH-U-119(2), 95.6%

Key Study Findings

The TR-701 drug substance impurities, (b) (4) administered at doses of 0.25 mg/kg/day for 14 days did not produce any signs of toxicity for any of the measured parameters. (b) (4)

Methods

Doses:	0.25 mg/kg/day (b) (4)
Frequency of dosing:	Once per day
Route of administration:	Intravenous bolus via tail vein
Dose volume:	5 ml/kg
Formulation/Vehicle:	25 mM disodium hydrogen phosphate, pH 7.5
Species/Strain:	Sprague-Dawley rats
Number/Sex/Group:	10/sex/group
Age:	Approximately 8 weeks old at dose initiation
Weight:	At randomization: 207 to 262 g for males and 161 to 205 g for females
Satellite groups:	None
Unique study design:	See Table 107
Deviation from study protocol:	Deviations were noted but none was considered to have altered the results or integrity of the study.

Table 107: Study Design for Study No.: TOX-13-0701-075. (Sponsor's Table)

Group Number	Treatment	Dosage Level (mg/kg/day)	Dose Volume (mL/kg)	Number of Animals [a] Males	Females
1	Control	0	5	10	10
2	(b) (4)	0.25	5	10	10
3		0.25	5	10	10
4		0.25	5	10	10

[a] = All surviving animals/sex/group were euthanized following 14 days of dose administration.

Observations and Results

Table 108: Observation Schedule for Study No.: TOX-13-0701-075

Parameter	Schedule
Mortality and morbundity	BID for the duration of the study.
Clinical Signs	Twice daily (immediately following dosing and 1-2 hours following dosing). In addition, detailed physical examinations were conducted on all animals weekly, beginning prior to dosing and on the scheduled termination days.
Body Weights	Individual body weights were obtained at weekly beginning before the start of dosing and the day prior to necropsy
Food Consumption	Individual food consumption (g/animal/day) was recorded at least once weekly during the pretest period and throughout the dosing period.
Hematology and Coagulation Serum Chemistry Urinalysis	Blood and urine samples were collected from all animals just prior to the scheduled necropsies. Animals were fasted overnight prior to collection.
Necropsy	Necropsies were scheduled at the end of the 14-day treatment period.

Mortality

One female receiving (b) (4) was found dead on Day 7. No clinical, gross or microscopic findings indicated the cause of death and the death was not attributed to administration of (b) (4)

Clinical Signs

No test article-related clinical signs were noted.

Body Weights

Body weights were not affected by administration of any of the test articles.

Feed Consumption

Food consumption was not affected by administration of any of the test articles.

Hematology

A standard panel of hematology parameters was examined for this study (the same as for Study No.: TOX-07-0701-014A).

None of the hematology and coagulation parameters was significantly altered by any of the test articles relative to the vehicle control group.

Clinical Chemistry

A standard panel of serum chemistry parameters was examined for this study (the same as for Study No.: TOX-07-0701-014A).

None of the serum chemistry parameters was significantly altered by any of the test articles relative to the vehicle control group.

Urinalysis

The following urinalysis parameters were examined: specific gravity, pH, urobilinogen, total volume, color, clarity, protein, glucose, ketones, bilirubin, occult blood, leukocytes, and nitrites. Also urine sediment was examined microscopically.

None of the examined urinalysis parameters was significantly altered by administration of any of the test articles.

Gross Pathology

No test article-related gross pathology was noted in this study compared to the vehicle control group.

Organ Weights

The same panel of organs listed in Table 126 for Study No.: TOX-07-0701-014A was weighed for this study.

No test article-related changes in organ weight were noted in this study compared to the vehicle control group.

Histopathology

Adequate Battery

Yes, the list of organs noted in Table 126 for Study No.: TOX-07-0701-014A was also examined for histopathology in this study.

Peer Review

No

Histological Findings

No test-article-related histopathology was observed.

Dosing Solution Analysis

Duplicate samples for the vehicle and test article dosing formulations were collected from each sample which were prepared approximately weekly for each dose level.

The analyzed formulations were found to contain 100% to 107% of the nominal concentrations.

Study title: A 14-Day Intravenous (Bolus) Toxicity Study [redacted] in Female Sprague Dawley Rats

Study no.:	TOX-10-0701-001
Study report location:	Electronic transmission
Conducting laboratory and location:	[redacted]
Date of study initiation:	January 11, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	[redacted] Batch No.: DKH-B-47(3), purity of 97.2%

Key Study Findings

The TR-701 impurity, [redacted] administered QD intravenously for 14 days did not produce any toxicity. [redacted]

Methods

Doses:	0, 0.5, 2.0 mg/kg/day
Frequency of dosing:	Once per day for 14 days
Route of administration:	Intravenous bolus through the tail vein
Dose volume:	5 ml/kg
Formulation/Vehicle:	25 mM disodium hydrogen phosphate in sterile water
Species/Strain:	Crl:CD Sprague-Dawley rats
Number/Sex/Group:	10 females per group
Age:	Approximately 8 weeks old at dose initiation
Weight:	At randomization: 171-215 grams
Satellite groups:	None
Unique study design:	Table 109
Deviation from study protocol:	Study protocol deviations occurred but none was considered to have altered the results or integrity of the study.

Table 109: Study Design for Study No.: TOX-10-0701-001. (Sponsor's Table)

Group Number	Treatment	Dosage Level (mg/kg/day)	Dose Volume (mL/kg)	Number of Animals [a] Females
1	Vehicle[b]	0	5	10
2	(b) (4)	0.5	5	10
3		2	5	10

[a] = All surviving animals were euthanized following 14 consecutive days of dose administration.

[b] = 25 mM disodium hydrogen phosphate anhydrous (Na_2PO_4, food grade) in sterile water.

Observations and Results

Parameter	Schedule
Mortality and morbundity	BID for the duration of the study.
Clinical Signs	Twice daily (immediately following dosing and 1-2 hours following dosing). In addition, detailed physical examinations were conducted on all animals weekly, beginning prior to dosing and on the scheduled termination days.
Body Weights	Individual body weights were obtained weekly beginning before the start of dosing and the day prior to necropsy
Food Consumption	Individual food consumption (g/animal/day) was recorded at least once weekly during the pretest period and throughout the dosing period.
Hematology and Coagulation Serum Chemistry Urinalysis	Blood and urine samples were collected from all animals just prior to the scheduled necropsies. Animals were fasted overnight prior to collection.
Necropsy	Necropsies were scheduled at the end of the 14-day treatment period.

Mortality

No deaths attributed to (b) (4) occurred. One control female died on Study Day 5.

Clinical Signs

No (b) (4)-related clinical signs were observed.

Body Weights

No (b) (4)-related changes in body weights were observed.

Feed Consumption

Food consumption was not altered by (b) (4) administration.

Hematology

The same hematology and coagulation parameters that were assessed for Study No.: TOX-07-0701-014A as noted in Table 123 were assessed for the present study.

No (b) (4)-related changes in any of the hematology or coagulation parameters were observed.

Clinical Chemistry

The same panel of clinical chemistry parameters that was assessed in Study No.: TOX-07-0701-014A as listed in Table 124 was assessed for this study.

No (b) (4)-related changes in any of the serum chemistry parameters were observed. Mean GGT levels were decreased by 67% in the low-dose (b) (4) group compared the vehicle control group, but no dose-dependent effect was noted.

Urinalysis

The following urinalysis parameters were assessed: specific gravity, pH, urobilinogen, total volume, color, clarity, protein, glucose, ketones, bilirubin, occult blood, leukocytes, and nitrites. Also urine sediment was examined microscopically.

None of the urinalysis parameters were altered by (b) (4) administration compared to the vehicle control group.

Gross Pathology

No (b) (4)-related gross pathology was observed.

Organ Weights

The same list of organs listed in Table 126 for Study No.: TOX-07-0701-014A was weighed for this study.

No (b) (4)-related changes in organ weight were noted in this study compared to the vehicle control group.

Histopathology

Adequate Battery

Yes, the panel of tissues noted in Table 126 for Study No.: TOX-07-0701-014A was also examined for this study.

Peer Review

No

Histological Findings

Histopathology associated with the tail vein injection sites were noted in all the groups including the vehicle control group. (b) (4) administration did not increase the incidence or severity of the injection site histopathology, and in general (b) (4)-related histopathology was not observed.

Dosing Solution Analysis

Duplicate samples for the vehicle and [redacted] dosing formulations were collected from each sample which were prepared approximately weekly for each dose level.

The dosing formulations were found to contain 94.2 to 99.7% of the nominal doses.

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

Study title: Intermediate 6 Reference Standard [redacted] *Salmonella-E coli*/Mammalian Microsome Reverse Mutation Assay

Study no.:	TOX-11-0701-015
Study report location:	Electronic transmission
Conducting laboratory and location:	[redacted]
Date of study initiation:	March 8, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	[redacted] Lot # DUG-AS-176(1), 99.4% purity

Key Study Findings

The TR-701 drug substance impurity, [redacted] at concentrations ≥ 25 μg/plate, increased the mean number of revertant colonies/plate 3.1 to 5.8 fold compared to vehicle control plates in strain TA100 with metabolic activation. [redacted] was considered positive for mutagenicity in the Ames test.

Methods

Strains:	*Salmonella typhimurium* strains: TA1537, TA98, TA100, and TA1535 and one *Escherichia coli* strain: WP2 *uvrA*
Concentrations in definitive study:	25, 50, 100, 250, 500, 1000, and 2500 μg/plate.
Basis of concentration selection:	Preliminary experiments noted precipitation at ≥ 2500 μg/plate, and cytotoxicity at ≥ 500 μg/plate for TA1537 without metabolic activation and ≥ 1000 μg/plate for TA1537 with metabolic activation.
Negative control:	Dimethyl sulfoxide (DMSO)
Positive control:	See Table 110
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Plates were incubated at 37°C for 2 days followed by counting of revertant colonies.

Table 110: Positive Controls for Study No.: TOX-11-0701-015

Strain	Positive Control
Without S9 activation	
TA1537	ICR-191 acridine
TA98	2-nitrofluorene

TA100	Sodium azide
TA1535	Sodium azide
WP2 *uvrA*	4-nitroquinoline-N-oxide
With S9 activation	
TA1537	2-aminoanthracene
TA98	
TA100	
TA1535	
WP2 *uvrA*	

Study Validity

All of the vehicle and positive control plates produced revertant counts that fell within the historical ranges.

Results

Results for mutagenicity were considered positive if the increase in revertants/plate increased with increasing concentration with the increases of at least 2-fold higher than the vehicle control background frequency for strains with high background frequencies (TA100), and three times for those with low spontaneous background frequencies (TA1537, TA98, TA1535, WP2 *uvrA*). The results of the current study (Table 111) indicate that all of the positive control agents with and without S9 activation increased revertant colonies/plate at least three times higher than the vehicle control group. For the impurity (b) (4) 3.1 to 5.8 fold increases in the mean number of revertant colonies/plate were observed in strain TA100 with metabolic activation at (b) (4) concentrations ≥ 25 μg/plate. In all other strains with and without S9 activation and in TA100 without S9 activation, (b) (4) was negative for mutagenicity. Precipitates were observed with 2500 μg/plate (b) (4) but cytotoxicity did not occur for any strain under any conditions in the confirmatory assay.

Table 111: Summary of the Ames Assay Results for [(b) (4)] (Sponsor's Table)

REVERTANT COLONIES PER PLATE—Mean (SD)[a]

Treatment Group	µg/plate	TA1537		TA98		TA100		TA1535		WP2*uvrA*	
		WITHOUT ACTIVATION									
DMSO	50 µL	5	(4)	14	(3)	86	(11)	6	(2)	47	(9)
ICR	0.5	179	(39)								
2NF	2.5			519	(84)						
SA	1.0					604	(91)	529	(41)		
NQNO	2.0									932	(22)
Intermediate 6 Reference Standard	25	3	(3)	12	(1)	102	(28)	9	(4)	60	(10)
	50	3	(2)	18	(2)	96	(26)	6	(3)	56	(2)
	100	4	(3)	14	(2)	107	(13)	12	(4)	57	(9)
	250	3	(1)	14	(2)	120	(15)	7	(3)	45	(9)
	500	3	(2)	15	(2)	107	(8)	10	(2)	49	(5)
	1000	3	(3)	14	(2)	119	(6)	5	(3)	43	(3)
	2500 [b]	2	(1)	10	(3)	121	(15)	8	(3)	46	(11)
		WITH ACTIVATION									
DMSO	50 µL	3	(2)	16	(4)	95	(4)	6	(2)	49	(9)
2AA	2.5	77	(15)	1200	(41)	1626	(140)	193	(24)		
2AA	10.0									381	(31)
Intermediate 6 Reference Standard	25	5	(3)	17	(3)	294	(27)	12	(4)	57	(6)
	50	4	(4)	17	(1)	340	(26)	8	(1)	77	(4)
	100	3	(1)	15	(2)	390	(20)	7	(3)	87	(5)
	250	5	(4)	21	(8)	554	(18)	7	(4)	98	(17)
	500	3	(3)	20	(4)	417	(23)	10	(1)	85	(4)
	1000	7	(2)	12	(1)	354	(18)	5	(3)	84	(12)
	2500[b]	5	(4)	14	(3)	348	(53)	5	(4)	70	(1)

2AA: 2-Aminoanthracene
2NF: 2-Nitrofluorene
ICR: ICR-191 Acridine
DMSO: Dimethylsulfoxide
NQNO: 4-nitroquinoline-N-oxide
SA: Sodium azide
SD: standard deviation
[a] Calculated from triplicate plates
[b] Precipitates present

Study title: [(b) (4)] Mammalian Microsome Reverse Mutation Assay

Study no.: TOX-12-0701-035
Study report location: Electronic transmission
Conducting laboratory and location: [(b) (4)]
Date of study initiation: March 11, 2009
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: [(b) (4)] Lot # DKH-A-193(2), purity of 97%.

Key Study Findings

The TR-701 impurity, [(b) (4)] at concentrations including those causing precipitation, did not increase the mean number of revertant colonies/plate for any of the tester strains with and without S9 activation. Under the conditions of this assay, [(b) (4)] was negative for mutagenicity.

Methods

Strains:	Salmonella strains: TA1537, TA98, TA100, TA1535 and Escherichia coli strain: WP2 *uvrA*
Concentrations in definitive study:	25, 50, 100, and 250 μg/plate.
Basis of concentration selection:	In a preliminary study, concentrations up to 5000 μg/plate were examined. Precipitation occurred at concentrations ≥250 μg/plate. Cytotoxicity occurred at ≥100 μg/plate in TA98 without S9 activation and in TA1537, TA100, TA1535 with and without S9 activation, and at ≥250 μg/plate in TA98 with S9 activation.
Negative control:	Dimethyl sulfoxide (DMSO)
Positive control:	See Table 112
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Plates were incubated at 37°C for 2 days

Table 112: Positive Control Agents Used in Study No.: TOX-12-0701-035

Strain	Positive Control
Without S9 activation	
TA1537	ICR-191 acridine
TA98	2-nitrofluorene
TA100	Sodium azide
TA1535	Sodium azide
WP2 *uvrA*	4-nitroquinoline-N-oxide
With S9 activation	
TA1537	2-aminoanthracene
TA98	
TA100	
TA1535	
WP2 *uvrA*	

Study Validity

All of the vehicle and positive control plates produced revertant counts that fell within the historical ranges.

Results

Results for mutagenicity were considered positive if the increase in revertants/plate increased with increasing concentration with the increases at least 2-fold higher than the vehicle control background frequency for strains that have high background frequencies (TA100), and three times for those with low spontaneous background frequencies (TA1537, TA98, TA1535, WP2 *uvrA*). The results for the definitive study indicated that none of the tested (b) (4) concentrations increased the mean number of revertant colonies/plate for any of the tester strains with and without S9 activation (Table 113).

As in the initial assay, precipitates were observed at 250 μg/plate in all strains with and without S9 activation. In the definitive assay, cytotoxicity was observed at ≥50 μg/plate in TA1537 with S9 activation; at ≥50 μg/plate in TA100 both with and without S9 activation; at ≥100 μg/plate in TA1537 without S9 activation, and at 100 μg/plate in TA98 and TA1535 with and without S9 activation.

Table 113: Summary Mutagenicity Results [(b) (4)] in an Ames Assay. (Sponsor's Table)

REVERTANT COLONIES PER PLATE—Mean (SEM)

Treatment Group	μg/plate	TA1537		TA98		TA100		TA1535		WP2*uvrA*	
WITHOUT ACTIVATION											
DMSO	50 μL	5	(1)	8	(0)	70	(3)	9	(1)	21	(2)
ICR	0.5	328	(10)								
2NF	2.5			545	(43)						
SA	1.0					265	(12)	324	(15)		
NQNO	2.0									1408	(78)
[(b) (4)]	2.5	4	(2)	12	(2)	69	(4)	6	(2)	28	(4)
	5	6	(1)	7	(1)	66	(5)	5	(2)	23	(5)
	10	5	(0)	8	(1)	60	(3)	9	(4)	27	(2)
	25	4	(1)	7	(2)	42	(5)	4	(2)	23	(3)
	50	6	(3)	7	(1)	9	(3)	5	(2)	17	(2)
	100	0	(0)	0	(0)	0	(0)	0	(0)	19	(5)
	250 [a]	0	(0)	0	(0)	0	(0)	0	(0)	19	(2)
WITH ACTIVATION											
DMSO	50 μL	5	(1)	14	(1)	55	(3)	8	(3)	28	(4)
2AA	2.5	218	(10)	1645	(87)	638	(86)	158	(4)		
2AA	10.0									298	(13)
[(b) (4)]	2.5	6	(1)	11	(1)	56	(1)	5	(1)	23	(4)
	5	6	(1)	17	(3)	46	(1)	6	(1)	26	(3)
	10	4	(0)	13	(1)	53	(11)	8	(3)	20	(3)
	25	7	(2)	8	(2)	43	(1)	6	(1)	20	(2)
	50	0	(0)	9	(0)	20	(3)	6	(1)	21	(5)
	100	0	(0)	3	(1)	1	(0)	0	(0)	19	(1)
	250 [a]	0	(0)	0	(0)	2	(1)	0	(0)	12	(3)

DMSO – Dimethylsulfoxide; 2AA – 2-Aminoanthracene; 2NF – 2-Nitrofluorene; ICR – ICR-191 Acridine; NQNO – 4-nitroquinoline-N-oxide; SA – Sodium azide; SEM= standard error of the mean
a: Precipitates present.

Study title: [(b) (4)] Salmonella-Ecoli Mammalian Microsome Reverse Mutation Assay

Study no.: TOX-12-0701-036
Study report location: Electronic transmission
Conducting laboratory and location: [(b) (4)]
Date of study initiation: March 10, 2009
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: [(b) (4)] Lot # DKH-A-195(2), 99% purity.

Key Study Findings

None of the tested (b) (4) concentrations increased the mean number of revertant colonies/plate for any of the tester strains with and without S9 activation.

Methods

Strains:	*Salmonella typhimurium* strains: TA1537, TA98, TA100, and TA1535 and one *Escherichia coli* strain: WP2 *uvrA*
Concentrations in definitive study:	100, 250, 500, 1000, and 2500 μg/plate
Basis of concentration selection:	In a preliminary study precipitates were observed at ≥ 2500 μg/plate with all strains with and without S9 activation. Cytotoxicity was observed at ≥ 2500 μg/plate in TA100 both with and without metabolic activation.
Negative control:	Dimethyl sulfoxide (DMSO)
Positive control:	See Table 114
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Plates were incubated at 37°C for 2 days followed by counting of revertant colonies.

Table 114: Positive Controls for Study No.: TOX-11-0701-036

Strain	Positive Control
Without S9 activation	
TA1537	ICR-191 acridine
TA98	2-nitrofluorene
TA100	Sodium azide
TA1535	Sodium azide
WP2 *uvrA*	4-nitroquinoline-N-oxide
With S9 activation	
TA1537	2-aminoanthracene
TA98	
TA100	
TA1535	
WP2 *uvrA*	

Study Validity

All of the vehicle and positive control plates produced revertant counts that fell within the historical ranges.

Results

Results for mutagenicity were considered positive if the increase in revertants/plate increased with increasing concentration with the increases at least 2-fold higher than the vehicle control background frequency for strains with high background frequencies (TA100), and three times for those with low spontaneous background frequencies (TA1537, TA98, TA1535, WP2 *uvrA*). The results for the definitive study indicated that

none of the tested [(b) (4)] concentrations increased the mean number of revertant colonies/plate for any of the tester strains with and without S9 activation.

As in the initial assay, precipitates were observed at 2500 µg/plate in all strains with and without S9 activation. In the definitive assay, cytotoxicity was observed at ≥ 1000 µg/plate in TA100 with and without metabolic activation.

Table 115: Summary Mutagenicity Results for the Definitive Ames Assay [(b) (4)] (Sponsor's Table)

REVERTANT COLONIES PER PLATE—Mean (SEM)

Treatment Group	µg/plate	TA1537		TA98		TA100		TA1535		WP2*uvrA*	
WITHOUT ACTIVATION											
DMSO	50 µL	7	(1)	16	(4)	73	(8)	8	(0)	34	(3)
ICR	0.5	389	(19)								
2NF	2.5			563	(21)						
SA	1.0					375	(5)	469	(28)		
NQNO	2.0									1416	(23)
[(b) (4)]	50	5	(2)	12	(2)	69	(11)	9	(1)	45	(3)
	100	8	(2)	12	(4)	69	(5)	10	(1)	32	(3)
	250	5	(2)	12	(3)	57	(2)	9	(3)	32	(3)
	500	7	(1)	16	(4)	66[c]	(6)	3	(1)	35	(3)
	1000	9	(2)	13	(3)	--[b]	--[b]	5	(1)	31	(8)
	2500[a]	6	(1)	6	(1)	--[b]	--[b]	4	(1)	20	(3)
WITH ACTIVATION											
DMSO	50 µL	6	(1)	15	(1)	57	(3)	13	(3)	34	(4)
2AA	2.5	312	(34)	1853	(78)	430	(15)	214	(11)		
2AA	10.0									414	(17)
[(b) (4)]	50	6	(1)	20	(1)	64	(7)	10	(3)	34	(2)
	100	6	(1)	14	(4)	58	(3)	10	(1)	38	(4)
	250	5	(0)	13	(1)	54	(10)	6	(1)	31	(5)
	500	4	(1)	14	(3)	47[c]	(2)	6	(1)	36	(7)
	1000	6	(1)	21	(2)	--[b]	--[b]	3	(0)	22	(0)
	2500[a]	6	(0)	10	(2)	--[b]	--[b]	4	(0)	18	(2)

DMSO – Dimethylsulfoxide; 2AA – 2-Aminoanthracene; 2NF – 2-Nitrofluorene; ICR – ICR-191 Acridine; NQNO – 4-nitroquinoline-N-oxide; SA – Sodium azide; SEM= standard error of the mean
a: Precipitates present. b: Reduced background lawn, plates not counted. c: Slightly reduced background lawn.

Study title: Bacterial Reverse Mutation Assay [(b) (4)]

Study no.: TOX-13-0701-078
Study report location: Electronic transmission
Conducting laboratory and location: [(b) (4)]
Date of study initiation: February 26, 2013
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: [(b) (4)] Lot # 33130044, purity of 99.7%

Key Study Findings

The TR-701 substance impurity, [redacted] in the presence of S9 activation increased the revertant colony counts in TA100 compared to the treatment with the vehicle control. Under the conditions of this study, [redacted] was considered positive for mutagenicity.

Methods

Strains:	*Salmonella typhimurium* strains: TA1537, TA98, TA100, and TA1535 and one *Escherichia coli* strain: WP2 *uvrA*
Concentrations in definitive study:	0, 33, 67, 100, 333, 667, 1000, 3333, and 5000 μg/plate for TA100 with S9 activation and 0, 50, 150, 500, 1500, and 5000 μg/plate for all other strains with and without S9 activation and TA100 without S9 activation.
Basis of concentration selection:	In a preliminary study, precipitate was observed at 333 μg/plate but no cytotoxicity was observed for any strains under any conditions at doses of ≤ 5000 μg/plate.
Negative control:	Dimethyl sulfoxide (DMSO)
Positive control:	See Table 116
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Plates were incubated at 37°C for 2 days

Study Validity

The following study validity criteria were met:

1. All the tester strains must demonstrate the expected mutations.
2. All tester strains must be plated at culture titers ≥ 0.3 x 10^9 cells/ml.
3. A minimum of 3 non-toxic concentration levels are required to evaluate an assay.
4. Vehicle control cultures for each tester strain must demonstrate the characteristic mean number of spontaneous revertants.
5. Positive control agents must increase the number of revertants by at least 3-fold above the respective vehicle control.

Table 116: Study Design and the Positive Controls for Study No.: TOX-13-0701-078. (Sponsor's Table)

Strain	S9 Activation	Positive Control	Concentration (μg/plate)
TA98, TA1535 and TA1537	Rat	2-aminoanthracene (Sigma Aldrich Chemical Co., Inc.) Lot No. STBB1901V Exp. Date 13-Oct-2014 CAS No. 613-13-8 Purity 97.5%	1.0
TA100			2.0
WP2 *uvr*A			15
TA98	None	2-nitrofluorene (Sigma Aldrich Chemical Co., Inc.) Lot No. S43858 Exp. Date 31-Jan-2014 CAS No. 607-57-8 Purity 97.9%	1.0
TA100, TA1535		sodium azide (Alfa Aesar) Lot No. A23U048 Exp. Date 21-Nov-2013 CAS No. 26628-22-8 Purity 100.0%	1.0
TA1537		9-aminoacridine (Sigma Aldrich Chemical Co., Inc.) Lot No. 07620TD Exp. Date 30-Nov-2013 CAS No. 52417-22-8 Purity 99.9%	75
WP2 *uvr*A		methyl methanesulfonate (Sigma Aldrich Chemical Co., Inc.) Lot No. MKBH9900V Exp. Date 13-Mar-2015 CAS No. 66-27-3 Purity 99.9%	1,000

Results

A result was considered positive if the test article caused a dose-related increase in the mean revertants/plate of at least on tester strain over a minimum of two increasing concentrations of test article. Positive results required at least a 3-fold increase in mean revertants over the vehicle control for tester strains TA1535 and TA1537 and a 2-fold increase for tester strains TA98, TA100, WP2 *uvrA*. (b) (4) produced a positive mutagenic response in TA100 with S9 activation. The mean revertants/plate following treatment with (b) (4) ranged from 2.0 to 3.2 fold in excess of the vehicle control values (Table 117). No other positive mutagenic responses were observed for (b) (4) treatment of any of the other tester strains with and without S9 activation or for TA100 without S9 activation (data not shown). In contrast all of the positive control agents produced revertants/plate values at least 3 fold above the concurrent vehicle control values in all tester strains under all conditions. Precipitates were observed at concentrations ≥ 150 μg/plate and no cytotoxicity was observed.

Table 117: Mutagenicity Results (b) (4) with S9 activation in TA100.
(Sponsor's Table)

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA100	(b) (4)	5000 µg	*228*	*10*	*2.2*	238^{M} 6 IP, 219^{M} 6 IP, 227^{M} 6 IP
		3333 µg	*210*	*19*	*2.0*	210^{M} 6 NP, 191^{M} 6 NP, 229^{M} 6 NP
		1000 µg	*251*	*31*	*2.4*	278^{M} 6 NP, 259^{M} 6 NP, 217^{M} 6 NP
		667 µg	*311*	*31*	*3.0*	297^{M} 6 NP, 346^{M} 6 NP, 289^{M} 6 NP
		333 µg	*324*	*33*	*3.1*	356^{M} 1 NP, 291^{M} 1 NP, 325^{M} 1 NP
		100 µg	*333*	*24*	*3.2*	312^{M}, 327^{M}, 359^{M}
		67 µg	*317*	*58*	*3.0*	383^{M}, 278^{M}, 289^{M}
		33 µg	*236*	*15*	*2.3*	253^{M}, 231^{M}, 224^{M}
	DMSO	100 µL	*104*	*11*		92^{M}, 109^{M}, 112^{M}
TA98	**2AA**	1.0 µg	*455*	*41*	*19.0*	441^{A}, 501^{A}, 423^{A}
TA100	**2AA**	2.0 µg	*481*	*87*	*4.6*	386^{A}, 500^{A}, 556^{A}
TA1535	**2AA**	1.0 µg	*62*	*9*	*5.6*	51^{A}, 68^{A}, 66^{A}
TA1537	**2AA**	1.0 µg	*25*	*2*	*3.1*	26^{A}, 27^{A}, 23^{A}
WP2*uvr*A	**2AA**	15 µg	*212*	*33*	*9.2*	222^{A}, 238^{A}, 175^{A}

Key to Positive Controls

2AA 2-aminoanthracene

Key to Automatic & Manual Count Flags

^{M}: Manual count ^{A}: Automatic count

11 Integrated Summary and Safety Evaluation

TR-701, and for some assays also TR-700, was tested in neural system, cardiovascular, respiratory, renal and gastrointestinal GI safety pharmacology studies. TR-701 altered some neural system, renal and GI parameters, but generally only at high doses and with results that were not greatly impactful in terms of toxicity.

Neural system safety pharmacology studies indicated that at doses of 10, 30, and 100 mg/kg, TR-701 produced no adverse effects in general behavior in rats, and hexobarbital-induced sleep, electroshock-induced convulsions, pentylenetetrazol- and strychnine-induced siezures, motor coordination, pain perception in the hot-plate and acetic acid writhing test, and rectal temperature in mice. Autonomic nervous system contractile and relaxant responses in isolated guinea pig ileum were not altered by 0.1 to 10 µM concentrations of TR-701 or TR-700. Hexobarbital-induced sleep time was significantly increased with an oral dose of 100 mg/kg TR-701 and spontaneous locomotor activity in mice was significantly reduced with oral administration of 30 and 100 mg/kg TR-701 compared to vehicle control mice.

Serotonin syndrome and monoamine oxidase (MAO) inhibition have been reported for linezolid. *In vitro* studies with TR-701 and TR-700 indicated that TR-700, but not TR-701, was a weak inhibitor of MAO-A and MAO-B with IC_{50} values comparable to linezolid. However, in a mouse head-twitch experiment and a tyramine-challenge experiment in rats, linezolid doses comparable to the human therapeutic dose produced positive results (increased head twitch or increased mean arterial pressure), but TR-701 doses associated with plasma TR-700 C_{max} and AUC values greatly exceeding the equivalent clinical therapeutic exposure values did not. These nonclinical data indicate that TR-701 and TR-700 are less potent than linezolid in stimulating *in vivo* responses mediated by MAO inhibition (Table 118). As is the apparent case for mitochondrial protein synthesis inhibition and other drug class-related toxicities, the animal study data suggests TR-701 does not pose an urgent concern for MAO inhibition at the clinical exposures expected with the therapeutic dose.

Table 118: Safety Margins Associated with the NOAEL doses of TR-701 and Active Doses of Linezolid *In Vivo* in Mice and Rats.

Study Type	NOAEL Dose for TR-701 or Acitve Dose for Linezolid	HED[a]	Associated Plasma Concentrations	Safety Margin Based on BSA	Safety Margin Based on C_{max}[b,c]
TR-701					
Tyramine-induced pressor effects in rats	150 mg/kg	24 mg/kg	57.6 μg/ml (C_{max})	7.2	19.2
Serotonin-induced head twitch in mice	300 mg/kg	24 mg/kg	60171 ng/ml	7.2	20.1
Linezolid					
Tyramine-induced pressor effects in rats	50 mg/kg	8 mg/kg	Not measured	0.4	----
Serotonin-induced head twitch in mice	50 mg/kg	4 mg/kg	26609 ng/ml	0.2	1.26

a Human equivalent doses (HED) for mice and rats were based on whole body surface area comparisons and conversion factors of 0.08 and 0.16 in mice and rats respectively.

b The therapeutic dose of TR-700 FA is 200 mg/day which for an average 60 kg human = 3.33 mg/kg/day. The steady-state C_{max} plasma level of TR-700 associated with IV doses of the 200 mg/day TR-701 is 3.0 μg/ml.

c The therapeutic dose of linezolid (Zyvox®) is 1200 mg/day which for an average 60 kg human = 20 mg/kg/day. The C_{max} plasma level of linezolid associated with IV therapeutic doses of linezolid is 21.2 μg/ml.

TR-701 produced no significant effects in cardiovascular and respiratory safety pharmacology studies. TR-701 (1-10 μM) and TR-700 (1-20 μM) did not alter hERG channel K+ currents. TR-701 and TR-700 also did not alter cardiac function in isolated rat heart at concentrations of 0.1-10 μM. *In vivo* TR-701 did not significantly change blood pressure, heart rate, or ECG intervals in conscious male dogs at doses of 20, 60 and 200 mg/kg, or alter respiratory rate, tidal volume, or minute volume in male rats at doses of 10, 30 and 100 mg/kg.

In a renal safety pharmacology study in rats, a high-oral dose of TR-701 (100 mg/kg) significantly increased urinary sodium and chloride concentrations compared to control rats and non-significantly increased urine volume. A similar trend of lesser non-significant effects occurred with a lower dose of 30 mg/kg TR-701. GI transport was not altered in mice by oral TR-701 (10, 30, and 100 mg/kg), but mean gastric volume was significantly reduced by 39% and mean total gastric acidity was reduced 48% (not statistically significant) while gastric pH remained unchanged.

Single-dose pharmacokinetics of TR-701 and TR-700 were evaluated in mice, rats (Sprague-Dawley and Long Evans) and dogs after oral and IV doses of TR-701. The toxicokinetics of TR-700 and also often TR-701 was evaluated after multiple IV and oral doses of TR-701 in conjunction with toxicology studies. Distribution studies included mass balance studies in rats and dogs and *in vitro* protein binding assessments in plasma from mice, rats, dogs and humans. The *in vitro* metabolic stability of TR-701 and TR-700 in hepatic microsomes (rat, dog, monkey and human), rat liver homogenates, and rat liver cytosolic fractions were evaluated. Also, the effects of TR-700 on inhibition or induction of major human CYP-450s was evaluated.

Regarding absorption and excretion, TR-701 whether administered intravenously or orally was rapidly and extensively metabolized to TR-700 by GI and serum phosphatases. The plasma $t_{1/2}$ of TR-700 ranged from 2-8 hours in rodents and was generally < 1 hour in dogs following IV or oral TR-701 administration. The bioavailability of TR-700 after oral administration of TR-701 was > 60% in mice, rats and dogs. The steady state volume of distribution for TR-700 but not TR-701 in all species was much greater than vascular compartment volumes suggesting widespread distribution. The primary route of excretion was in feces (80% in rats, 90% in dogs) with less excretion in urine (20% in rats, 10% in dogs). Although fecal excretion is often accompanied by biliary excretion, only minimal biliary excretion was observed in rats. TR-700 plasma exposure following oral dosing was similar between genders for mice and dogs, but female exposure was roughly twice as high as male exposure in rats necessitating different dose ranges in toxicology studies. TR-700 accumulated in plasma only moderately with repeated-dosing. After 90 days of oral dosing in conjunction with the 3-month toxicology study in rats, plasma AUC values were less than 2-fold higher than plasma AUC values from the first day of dosing in both males and females. Similar magnitudes of accumulation or an absence of accumulation was noted for both IV and oral dosing in other toxicology studies in both rats and dogs.

Distribution studies with [^{14}C]-TR-701 with both IV and oral administration in rats and dogs indicated that TR-700 distributed to many tissues. In Sprague Dawley rats, the organs with the highest concentrations in both genders included liver, plasma, adrenal gland, brown fat, small intestine, and stomach. The tissues with the lowest concentrations included eye and brain. Reproductive organs in males (prostate, testis) contained moderate concentrations of radioactivity while females reproductive organs (uterus, ovary) contained 4-6 fold higher concentrations of radioactivity. Large intestine concentrations were much higher following oral administration. In almost all tissues, radioactive levels decreased to below the lowest level of quantification by 72 hours after administration. While eye concentrations were very low, uveal tract within the eye contained moderate concentrations of radioactivity. Concentrations were approximately 3-20 fold increased in pigmented Long Evans rats compared to albino Sprague-Dawley rats, and the half-life was greatly increased by 10 fold to approximately 40 hours suggesting melanin binding. Distribution patterns were similar in dogs except relatively less radioactivity accumulated in female reproductive organs compared to rats. Protein binding of TR-701 and TR-700 was similarly high ranging from a low value of approximately 75% in dog plasma and approximately 98% in rat plasma with human plasma protein binding in the range of 80-85%.

Metabolite identification (summarized in Table 119) indicated similar metabolic profiles for rats, dogs and humans. The only large percentage metabolite for all three species was TR-700 (tedizolid). All of the human plasma metabolites in urine were represented in both rats and dogs. Analysis of TR-701 stability under different conditions indicated that metabolism to TR-700 was not mediated by liver microsomes from mice, rats, monkeys, and humans, gastric juice, urine, or deproteinized plasma. TR-701 metabolism in blood was blocked by EDTA, but not heparin. Metabolism occurred in liver S9 fraction with and without NADPH, in plasma from many species, and in blood, bile, and liver homogenates suggesting metabolism by a soluble phosphatase. When TR-701 was orally administered to rats twice daily for 14 days, liver CYP2B activity, but not the activity of CYP1A, CYP3A, and CYP2E was slightly higher than in control animals.

Table 119: Metabolite Profiles in Rats, Dogs, and Humans. (Sponsor's Table)

Metabolite	Rat	Dog	Human
Tedizolid	P U F	P U F	P U F
Tedizolid sulfate	U F	U F	U F
N-Desmethyl tedizolid	U	U F	U
N-Desmethyl tedizolid sulfate	U	U	-
Hydroxy tedizolid	U	-	-
Carboxy tedizolid	U F	U	U F

Abbreviations: - =less than 1% of total radioactive dose; F=Feces; P=Plasma; U=Urine
Note: Only metabolites present at >1.0% of total radioactive dose (in any sex or route of administration) are listed.

The primary toxicities associated with oral administration of TR-701 FA to rats targeted the gastrointestinal tract, blood cells, thymus, spleen, and bone marrow, and the effects were dose and duration dependent. In 1- and 3-month oral toxicology studies in rats, hematopoietic effects included mild to moderate reductions in the number of circulating red blood cells, white blood cells, and platelets and decreased splenic B cells in conjunction with hypocellularity in bone marrow, splenic and thymic atrophy and lymphoid necrosis in the thymus. GI effects at tolerated doses were characterized by decreased food intake, reduced body weight gain and soft mucoid stool. These effects also occurred with IV dosing in rats, but as with oral TR-701, the hematopoietic and GI effects showed evidence of reversibility and generally occurred at multiples of the TR-700 therapeutic exposure in humans. At higher doses associated with pronounced morbidity and mortality in a 3-month oral-dose study in rats, other toxicities were noted including hepatocellular centrolobular degeneration and atrophy accompanied by hepatic enzyme elevations (ALT, AST, and GGT), renal tubular degeneration associated with hyaline droplets, and degeneration and atrophy of male and female reproductive organs including testes, male accessory glands, ovaries, uterus, vagina and cervix. These changes also showed evidence of reversibility. Other effects that occurred with high doses were consistent with stress or reduced food intake including thymus degeneration, changes in Harderian, lacrimal, salivary glands, and pituitary and necrosis and hemorrhage in the adrenal cortex.

The reduction of WBCs and splenic B cells by TR-701 in 1- and 3-month oral toxicology studies in rats are consistent with immunotoxicity. In a separate immunotoxicity study at the highest oral dose of 100 mg/kg/day for 28 days, TR-701 was shown to significantly reduce cell counts, T cells, and B cells in the spleen of male rats. Also, plasma IgG titer was significantly reduced in males administered 30 and 100 mg/kg/day TR-701 and in females administered the high-female dose of 30 mg/kg/day. IgM and IgG-induced plaque formation was significantly reduced for male rats receiving 100 mg/kg/day for IgM and 30 and 100 mg/kg/day for IgG. In females, the high-dose of 30 mg/kg/day was associated with significantly reduced IgG-mediated plaque formation and a trend toward reduction of plaque formation mediated by IgM. In other repeated-dose studies the 30 mg/kg/day oral doses of TR-701 were associated with plasma TR-700 AUC exposures of approximately 100 and 200 µg·h/mL in male and female rats respectively. The results indicate that at doses associated with TR-700 exposures 4-8 times the human therapeutic exposure, TR-701 is immunosuppressive, but as evidenced by the results of the recovery study in the 3-month oral toxicology study, the immunotoxicity appears to be reversible.

The potential for peripheral and optic neuropathy previously associated with nonclinical administration and prolonged clinical administration of linezolid, was evaluated in a 9-month neurotoxicity study for oral TR-701 administered daily to pigmented rats. The results of this study indicated that TR-701 at doses ≤ 30 mg/kg/day in males and ≤ 10 mg/kg/day in females corresponding to plasma TR-700 exposures approximately 7-8 times the clinical plasma exposures did not change functional observational battery reactions or locomotor activity or produce peripheral nerve or ocular histopathology in rats. However, in a 1-month IV toxicology study in rats (Study No.: TOX-08-0701-009),

a subset of dose-dependent clinical signs occurring only in male rats, including labored and shallow breathing, hyporeactivity impaired equilibirium, prostate body, and muscular rigidity were consistent with neurotoxicity. These effects were transient and not accompanied by neural histopathology. Also the TR-700 plasma C_{max} and AUC values associated with these effects were approximately 30- and 17-fold higher than the respective plasma C_{max} and AUC values associated with the clinical dose. These data suggest TR-701 poses a low potential for neurotoxicity in the clinical setting.

Table 120: Summary of NOAEL Values, LOAEL Values, and Exposure Margins Associated with the Immunotoxicity and Neurotoxicity Studies.

Study	Subcategories (TR-701 Doses, mg/kg/day)	NOAEL or LOAEL (mg/kg/day)	TR-700 $AUC_{0\text{-}24h}$ (µg·h/mL)	Exposure Margin
Oral Immunotoxicology Study in Rats [a]	Male (0, 10, 30, 100)	NOAEL = 10	23.3*	0.91
		LOAEL = 30	81.4*	3.2
	Female (0, 3, 10, 30)	NOAEL = 10	54.1*	2.1
		LOAEL = 30	219.6*	8.6
9-month Oral Neurotoxicity Study in Pigmented Rats [b]	Male (0, 7.5, 15, 30)	NOAEL = 30 (high dose)	222	8.7
	Female (0, 2.5, 5, 10)	NOAEL = 10 (high dose)	189	7.4

The mean tedizolid plasma steady state $AUC_{0\text{-}24h}$ associated with multiple doses of the clinical daily dose (200 mg/kg/day) has been determined to be 25.6 (µg·h/mL).

[a] *Toxicokinetics not performed with study.*

[b] *Toxicokinetics performed with study.*

**Plasma $AUC_{0\text{-}24h}$ value derived from the same dose in the 1-month oral toxicology study in rats.*

The primary toxicity of orally administered TR-701 in dogs in a study of 3-months duration was dose-dependent emesis with an absence of other significant toxicity. The high-dose and NOAEL in the 3-month oral toxicology study in Beagle dogs was 400 mg/kg/day for both genders corresponding to maximal AUC plasma exposure values of 141 and 229 µg·h/mL for TR-700 in males and females respectively (approximately 6 and 9 times greater than the plasma TR-700 exposure associated with the 200 mg/day clinical dose). Intravenous administration of TR-701 in dogs in a 14-day BID administration study also produced emesis, weight loss and GI tract histopathology, and additionally, severe injection-site reactions with marked inflammation resulting in swollen and impaired hindlimbs and forelimbs. In a single high-dose female receiving 200 mg/kg/day TR-701, bone marrow hypocellularity, inflammation of the duodenum, atrophy of the esophageal mucosa, karyomegaly of the mucosa of the esophagus, duodenum, and jejunum, and ulcers of the pyloric stomach was observed. The NOAEL for this study (50 mg/kg/day) provided TR-700 plasma exposures roughly equal to plasma exposures for the clinical dose.

The injection site reactions occurred predominantly in dogs among test species. Rats did not demonstrate injection site reactions in the 1-month IV toxicology study.

Additionally rabbits did not experience injection site irritation for perivascular, intramuscular, or subcutaneous injections of TR-701.

Table 121: Summary of NOAEL Values, LOAEL Values, and Exposure Margins Associated with the TR-701 General Toxicology Studies in Rats and Dogs.

Study	Subcategories (TR-701 Doses, mg/kg/day)	NOAEL or LOAEL (mg/kg/day)	TR-700 $AUC_{0\text{-}24h}$ (µg·h/mL)	Exposure Margin
1-month oral-toxicology study in rats	Male (0, 10, 30, 100)	NOAEL = 30	81.4	3.2
		LOAEL = 100	402.8	15.7
	Female (0, 10, 30, 100)	NOAEL = 10	54.1	2.1
		LOAEL = 30	219.6	8.6
1-month oral-toxicology study in dogs	Male (0, 100, 200, 400)	NOAEL = 400 (highest dose)	201.8	7.9
	Female (0, 100, 200, 400)	NOAEL = 400 (highest dose)	66.0	2.6
3-month oral-toxicology study in rats	Male (0, 10, 30, 100/60)	NOAEL = 30	161 (Day 90)	6.3
		LOAEL = 100	337 (Day 0)	13.2
	Female (0, 3, 10, 30)	NOAEL = 10	124 (Day 90)	4.8
		LOAEL = 30	159 (Day 0)	6.2
3-month oral-toxicology study in dogs	Male (0, 100, 200, 400)	NOAEL = 400 (highest dose)	141 (Day)	5.5
	Female (0, 10, 30, 100)	NOAEL = 400 (highest dose)	229 (Day)	8.9
1-month IV-toxicology study in rats	Male (0, 10, 30, 90)	NOAEL = 30	127	5.0
		LOAEL = 90	491	19.2
	Female (0, 5, 15, 45)	NOAEL = 15	85.1	3.3
		LOAEL = 45	281	11.0
14-Day IV-toxicology study in dogs	Male (0, 50, 100, 200)	No NOAEL with regard to injection site reactions (ISR)		
		Exclusive of ISR: 50 mg/kg/day	29.2	1.1
	Female (0, 50, 100, 200)	No NOAEL with regard to injection site reactions (ISR)		
		Exclusive of ISR: 50 mg/kg/day	19.9	0.8
The mean tedizolid plasma steady state $AUC_{0\text{-}24h}$ associated with multiple oral doses of the clinical daily dose (200 mg/kg/day) has been determined to be 25.6 (µg·h/mL).				

Inhibition of mitochondrial protein synthesis could account for the hematopoietic effects most prominently associated with high doses of TR-701 in repeated-dose toxicology studies in rats and to a lesser extent in dogs. In *in vitro* experiments using mitochondria isolated from rat heart, TR-700 was 20-25 fold more potent than linezolid in inhibiting mitochondrial protein synthesis. However, in another experiment, TR-700 did not distribute into mitochondrial subcellular compartments in isolated macrophages concentrating instead more in phagolysosomes and cytosolic fractions. This data and the data from the repeated-oral dose toxicology studies suggests TR-701 has the potential to inhibit mitochondrial protein synthesis *in vivo* but at doses associated with high TR-700 exposures substantially exceeding the exposures expected with the recommended duration of clinical treatment at the therapeutic dose.

In genetic toxicology assays, TR-701 and TR-700 were found to be negative for mutagenicity in bacterial mutagenicity assays, and TR-701 was found to be negative for

clastogenesis in a mammalian cell chromosome aberration assay. In a chromosome aberration assay with TR-700, statistically significant increases in chromosome aberration frequency occurred after 6-hours incubation both plus and minus S9 activation at concentrations not associated with precipitation or >50% cytotoxicity. However, 24 hour incubations minus S9 activation with the same TR-700 concentration range that produced positive results in the 6-hour incubation minus S9 activation did not cause significant increases in chromosome aberration frequency. Also TR-700 was shown to induce a small but statistically significant (compared to vehicle control) increase in mutant frequency at the highest test concentration (112 μg/ml) in a mouse lymphoma cell thymidine kinase study conducted *in vitro*, but the effective concentration was associated with precipitation. *In vivo* mouse bone marrow-micronucleus studies with oral administration of TR-701 and TR-700 were negative for clastogenesis, and in an *in vivo* rat liver unscheduled DNA synthesis assay, oral TR-701 was shown to be negative for DNA damage. The weight of evidence from these studies as a whole indicates that neither TR-701 nor TR-700 present substantial genotoxic risk in humans.

In the 1- and 3-month oral-toxicology studies in rats, atrophy of male and female reproductive organs occurred with high doses of TR-701 associated with morbidity and mortality. These results suggest TR-701 may have the potential to adversely impact fertility. In a male and female fertility study, TR-701 doses as high as 50 mg/kg/day did not alter reproductive performance, sperm production rate, sperm motility, sperm morphology, or produce reproductive organ histopathology in male rats. Slight reductions in mean epididymal sperm numbers (13%) and decreased absolute and relative (to brain weight) epididymal weights (<10%) were observed with the high dose, but since the reproductive performance and other indices were not affected, this result was not considered to be toxicologically relevant. In females, doses of TR-701 as high as 15 mg/kg/day did not adversely affect female reproductive performance, absolute or relative weights of female reproductive organs, or intrauterine survival of embryos. The NOAEL values for male and female reproductive toxicity were considered to be 50 and 15 mg/kg/day respectively. In other toxicology studies, male and female doses of 30 and 10 mg/kg/day produced similar exposures of 130 and 104 μg·h/mL respectively which are on the order of 4-5 times greater than plasma exposures expected for the 200 mg/day clinical dose. These results suggest that TR-701 has limited potential to adversely affect fertility at clinical dose levels.

In embryo-fetal studies, oral TR-701 FA produced fetal toxicity in mice, rats, and rabbits. In mice, oral administration of the highest dose of 25 mg/kg/day resulted in lower mean fetal weights, and an increase in costal cartilage anomalies in fetuses. The plasma TR-700 AUC exposure associated with the NOAEL of 5 mg/kg/day for fetal toxicity was approximately 20 μg·h/mL or about equal to the AUC exposure (25.6 μg·h/mL) associated with the expected human oral therapeutic dose. In rats, maternal body weight gains and mean food consumption were reduced for all the oral TR-701 doses (2.5, 5, and 15 mg/kg/day), and lower mean fetal weights occurred with the mid- and high-TR-701 doses. Fetal developmental delay occurred with the high-dose as indicated by increased litter proportions of fetal skeletal variations indicative of delayed ossification. Other rib and skeletal variations not indicative of delayed fetal development

occurred at litter proportions above the historical control values but were not significantly increased relative to the concurrent vehicle control group. The NOAEL for both maternal and fetal toxicity was considered to be the low dose of 2.5 μg/kg/day which was associated with a plasma TR-700 AUC of 30.5 μg·h/mL or about equal to the AUC exposure associated with the clinical dose. In a rabbit embryo-fetal study, maternal GI toxicity limited TR-701 doses to ≤ 5 mg/kg/day. Mean fetal weights were reduced by maternal doses of 2.5 and 5 mg/kg/day, but TR-701-related developmental malformations or variations were not observed. The NOAEL in rabbits of 1 mg/kg/day for maternal and fetal toxicity corresponded to a plasma TR-700 $AUC_{0\text{-}24h}$ value of 1.09 μg x hr/ml or approximately 25-fold less than the expected clinical exposure. The TR-701-related maternal and fetal toxicity that occurred in animal studies suggests that TR-701 should be administered to pregnant women only when the potential benefits strongly outweigh potential risks.

In a pre-postnatal study with oral TR-701 doses of 1.25, 2.5, 3.75 mg/kg/day in rats, no adverse effects on offspring growth, maturation, or measures of behavior or reproductive function were observed. In this study, the high dose was chosen based on the 4.6% maternal weight loss produced by a dose of 2.5 mg/kg/day in the rat embryo-fetal study. The high dose (3.75 mg/kg/day) for the pre- postnatal study is estimated to have provided plasma TR-700 exposures approximately equal to that produced by a 200 mg/day clinical dose. Also in this study, TR-700 exposure to fetuses was confirmed. TR-700 was detected in maternal milk at concentrations approximating maternal plasma levels, and mean plasma fetal concentrations were approximately 24-37% of the maternal plasma concentration 2 hours after dosing.

Table 122: Summary of NOAEL Values and Exposure Margins Associated with the TR-701 Developmental and Reproductive Toxicology Studies.

Study	Subcategories	NOAEL (mg/kg/day)	$AUC_{0\text{-}24h}$ (μg·h/mL)	Exposure Margin
Rat Fertility Study[a]	Male	50	130*	5.08
	Female	15	104*	4.06
Rat Embryo-Fetal Study[b]	Maternal Toxicity	2.5	30.2	1.18
	Fetal Toxicity	2.5		
Mouse Embryo-Fetal Study[b]	Maternal Toxicity	25	95.7	3.74
	Fetal Toxicity	5	17.6	0.69
Rabbit Embryo-Fetal Study[b]	Maternal Toxicity	1.0	1.09	0.04
	Fetal Toxicity	1.0		
Rat Pre-Post Natal Study[a]	F_0, F_1, F_2 generations	3.75	30.2*	1.18

The mean tedizolid plasma steady state $AUC_{0\text{-}24h}$ associated with multiple doses of the clinical daily dose (200 mg/kg/day) has been determined to be 25.6 (μg·h/mL).

[a] Toxicokinetics not performed with study.

[b] Toxicokinetics performed with study.

***** Plasma AUC_{0-24h} value derived from a similar dose in a repeated-dose toxicology study in the same species.

Taken as a whole, the nonclinical toxicology data for TR-701 and TR-700 suggests relative safety for clinical administration of the clinical therapeutic dose of 200 mg/day for up to 14 days. TR-701 was immunotoxic in animals studies at high doses suggesting immune cells should be monitored in patients. TR-701 has also shown a potential to produce toxicities associated with mitochondrial protein synthesis inhibition and MAO inhibition, as well as effects consistent with transient neural impairment, but not at exposures expected to occur at the clinical therapeutic dose. The weight of evidence suggests TR-701 and TR-700 are not genotoxic. While TR-701 should not impair male or female fertility at therapeutic doses, it caused maternal and fetal toxicity in rodent embryo-fetal studies suggesting it should be restricted for administration to pregnant women.

12 Appendix/Attachments

Table 123: Hematology and Coagulation Parameters

Study No.	TOX-07-0701-014A	TOX-07-0701-013A	TOX-11-0701-027	TOX-11-0701-026	TOX-08-0701-009	TOX-08-0701-019
Species	**Rat**	**Dog**	**Rat**	**Dog**	**Rat**	**Dog**
Hemoglobin concentration	X	X	X	X	X	X
Hemoglobin distribution width	X	X	X	X	X	X
Hematocrit	X	X	X	X	X	X
Erythrocyte count	X	X	X	X	X	X
Platelet count	X	X	X	X	X	X
Plateletcrit / thrombocrit						
Mean platelet volume						
Mean corpuscular volume	X	X	X	X	X	X
Mean corpuscular hemoglobin		X		X		X
Mean corpuscular hemoglobin concentration		X		X		X
Red cell distribution width	X	X	X	X	X	X
Total leukocyte count	X	X	X	X	X	X
Reticulocyte count (absolute and relative)	X	X	X	X	X	X
Reticulocyte hemoglobin content						
Differential leukocyte count (Absolute and relative neutrophil, lymphocyte, monocyte, eosinophil, basophil counts)	X	X	X	X	X	X
Blood smear for cell morphology (if necessary for interpretation)						

Red cell morphology	X	X	X	X	X	X
Activated partial thromboplastin time (APTT)	X	X	X	X	X	X
Prothrombin time (PT)	X	X	X	X	X	X

Table 124: Clinical Chemistry Parameters

Study No.	**TOX-07-0701-014A**	**TOX-07-0701-013A**	**TOX-11-0701-027**	**TOX-11-0701-026**	**TOX-08-0701-009**	**TOX-08-0701-019**
Species	**Rat**	**Dog**	**Rat**	**Dog**	**Rat**	**Dog**
Aspartate aminotransferase	X	X	X	X	X	X
Alanine aminotransferase	X	X	X	X	X	X
Alkaline phosphatase	X	X	X	X	X	X
Blood urea nitrogen	X	X	X	X	X	X
Urea						
Creatinine	X	X	X	X	X	X
Creatinine kinase						
Glucose	X	X	X	X	X	X
Cholesterol	X	X	X	X	X	X
Triglycerides	X	X	X	X	X	X
Total protein	X	X	X	X	X	X
Albumin	X	X	X	X	X	X
Total bilirubin	X	X	X	X	X	X
Sodium	X	X	X	X	X	X
Sorbitol dehydrogenase	X	X	X	X	X	X
Potassium	X	X	X	X	X	X
Chloride	X	X	X	X	X	X
Calcium	X	X	X	X	X	X
Inorganic phosphorus or phosphate	X	X	X	X	X	X
Gamma-glutamyl transferase	X	X	X	X	X	X
Glutamate dehydrogenase						
Globulin	X	X	X	X	X	X
Albumin/globulin ratio	X	X	X	X	X	X
Hemolysis, Lipemia, Icterus		X		X		X

Table 125: Urinalysis Parameters

Study No.	**TOX-07-0701-014A**	**TOX-07-0701-013A**	**TOX-11-0701-027**	**TOX-11-0701-026**	**TOX-08-0701-009**	**TOX-08-0701-019**
Species	**Rat**	**Dog**	**Rat**	**Dog**	**Rat**	**Dog**
Specific gravity	X	X	X	X	X	X
pH	X	X	X	X	X	X
Urobilinogen	X	X	X	X	X	X
Total volume	X	X	X	X	X	X
Color	X	X	X	X	X	X
Clarity	X	X	X	X	X	X
Protein	X	X	X	X	X	X
Glucose	X	X	X	X	X	X

Ketones	X	X	X	X	X	X
Bilirubin	X	X	X	X	X	X
Occult blood	X	X	X	X	X	X
Leukocytes	X	X	X	X	X	X
Nitrites	X	X	X	X	X	X
Microscopy of sediment	X	X	X	X	X	X

Table 126: Histopathology and Organ Weight Inventory

Study #	TOX-07-0701-014A	TOX-07-0701-013A	TOX-11-0701-027	TOX-11-0701-026	TOX-08-0701-009	TOX-08-0701-019
Species	**Rat**	**Dog**	**Rat**	**Dog**	**Rat**	**Dog**
Adrenals	X, *	X, *	X, *	X, *	X, *	X, *
Aorta	X	X	X	X	X	X
Bone Marrow smear	X	X	X	X	X	X
Bone (sternum, and/or femur and/or rib)	X	X	X	X	X	X
Brain	X, *	X, *	X, *	X, *	X, *	X, *
Bronchi, main stem						
Cecum	X	X	X	X	X	X
Cervix	X	X	X	X	X	X
Colon	X	X	X	X	X	X
Conjunctiva						
Duodenum	X	X	X	X	X	X
Epididymis	X, *	X, *	X, *	X, *	X, *	X, *
Esophagus	X	X	X	X	X	X
Eye	X	X	X	X	X	X
External ear						
Fallopian tube						
Gall bladder		X		X		X
Gross lesions	X	X	X	X	X	X
Harderian gland	X		X		X	
Heart	X, *	X, *	X, *	X, *	X, *	X, *
Hypophysis						
Ileum	X	X	X	X	X	X
Infusion site						
Jejunum	X	X	X	X	X	X
Joint, tibiofemoral						
Kidneys	X, *	X, *	X, *	X, *	X, *	X, *
Lachrimal gland	X		X		X	
Larynx						
Liver	X, *	X, *	X, *	X, *	X, *	X, *
Lungs	X	X	X	X	X	X
Lymph nodes, inguinal						
Lymph nodes, axillary	X	X	X	X	X	X

Lymph nodes, mediastinal						
Lymph nodes mandibular	X	X	X	X	X	X
Lymph nodes, mesenteric,	X	X	X	X	X	X
Mammary Gland						
Muscle (biceps, femoris)						
Nasal cavity						
Nasal turbinates						
Optic nerves	X		X		X	
Ovaries	X, *	X, *	X, *	X, *	X, *	X, *
Oviduct	*	X	*	X	*	X
Pancreas	X	X	X	X	X	X
Parathyroids	X, *	X, *	X, *	X, *	X, *	X, *
Peripheral nerve		X		X		X
Peyer's patches	X	X	X	X	X	X
Pharynx						
Pituitary	X, *	X, *	X, *	X, *	X, *	X, *
Prostate	X	X, *	X	X, *	X	X, *
Rectum	X	X	X	X	X	X
Salivary gland	X	X	X	X	X	X
Sciatic nerve	X		X		X	
Seminal vesicles	X		X		X	
Skeletal muscle	X	X	X	X	X	X
Skin	X	X	X	X	X	X
Spinal cord	X	X	X	X	X	X
Spleen	X, *	X, *	X, *	X, *	X, *	X, *
Sternum						
Stomach	X	X	X	X	X	X
Testes	X, *	X, *	X, *	X, *	X, *	X, *
Thymus	X, *	X, *	X, *	X, *	X, *	X, *
Thyroid	X, *	X, *	X, *	X, *	X, *	X, *
Tongue	X		X		X	
Tonsils						
Trachea	X	X	X	X	X	X
Ureter						
Urinary bladder	X	X	X	X	X	X
Uterus	X, *	X, *	X, *	X, *	X, *	X, *
Vagina	X	X	X	X	X	X
Vertebra, Lumbar						
Zymbal gland						
X = tissue collected for histopathology; * weighed organ						

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/s/

JAMES S WILD
05/28/2014

WENDELYN J SCHMIDT
05/28/2014
I concur with Dr. Wild's assessment of the adequacy and scope of the non-clinical studies as well as his interpretation of the data.

Comments on NDAs 205-435 and 205436 tedizolid phosphate

From A. Jacobs, AD

Date: 5/23/14

1. I concur that there are no pharm-tox approval issues and that the pregnancy category should be a C.

2. I have conveyed other comments to the reviewer and they will be addressed as appropriate.

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/s/

ABIGAIL C JACOBS
05/27/2014