CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-505

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology/Toxicology Review

From: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO

NDA: 22-505
Agency receipt date: May 29, 2009
Drug: Egrifta (tesamorelin acetate), synthetic analog of human growth hormone releasing hormone
Sponsor: Theratechnologies
Indication: to induce and maintain a reduction of excess visceral abdominal fat (VAT) in HIV-infected patients with lipodystrophy

Reviewing Division: Division of Metabolism and Endocrinology Products

Background:
The pharm/tox reviewer and supervisor conclude that the nonclinical data support approval of tesamorelin for the indication listed above. Initially, the reviewer recommended that the applicant conduct an additional embryofetal toxicity study as a postmarketing requirement. Hydrocephaly was observed in a rat pre/postnatal study, and reduced ossification of the skull was observed in a rat embryofetal study.

Rat pre/postnatal study:
Four F1 pups from two litters had hydrocephaly in a group in which rat dams received 1.2 mg tesamorelin/kg from gestation day 6 to lactation day 21. Hydrocephaly was noted either at post-weaning necropsy or as a clinical sign beginning at lactation day 11. The maternal AUC at this dose was approximately 1.6 to 2.7 fold the AUC in humans at the maximum recommended dose.

Rat fertility and teratogenicity study:
A significant increase in reduced ossification of the interparietal bone of the skull was noted in all tesamorelin treated groups (0.1, 0.3 and 0.6 mg/kg). Dams were treated with drug in this study from 14 days prior to mating until day 17 of gestation. The doses produced AUCs in the rat dams that were <0.2 fold to approximately equal the AUC in humans at the maximum recommended clinical dose.

The findings of hydrocephaly and reduced skull ossification are potentially concerning for pregnant women particularly given the lack of margin between the exposures associated with these findings and the human exposures.

The objective of the proposed postmarketing study was to obtain a better understanding of the dose response relationship for these findings and to determine the vulnerable period of exposure in the developing fetus or offspring.

Subsequent to the initial recommendation of the pharm/tox reviewer, the Maternal Health Team completed a consult in which they recommended that tesamorelin be contraindicated in pregnant women if approved. The pharm/tox reviewer, therefore, reconsidered the recommendation for a postmarketing study and concluded that if the drug will be contraindicated in pregnant women, an additional embryofetal study is not needed as a postmarketing requirement.

Carcinogenicity:
Two year carcinogenicity studies were not required or conducted for this application. The chronic toxicity study in rats included staining for proliferating cell nuclear antigen and did not reveal any drug-related increase in cell proliferation. Additional information from the literature did not suggest a particular concern for carcinogenicity.

Conclusions:
I agree with the division pharm/tox conclusion that this application can be approved from a pharm/tox perspective. If this product were considered appropriate for use in pregnant women, then an additional study or studies may be warranted to better delineate the dose- and time-related occurrence of hydrocephaly in rat pups. This information could be useful in informing pregnant women of the potential for risk to the fetus or nursing child. If the product is contraindicated in pregnant women, then I agree that additional developmental and reproductive studies are not needed at this time.

The division has proposed that the established pharmacologic class for this product be growth hormone releasing factor (GRF) analog. This is a new established pharmacologic class, and it is scientifically valid and clinically meaningful.

Although the data from the chronic toxicity studies with tesamorelin did not indicate preneoplasia, a negative carcinogenicity conclusion is not usually made based on these data, so this information is not usually included in the carcinogenicity section of labeling.

Description of animal toxicology data in the labeling beyond that for developmental and reproductive toxicity does not appear to be necessary.

I have discussed these potential labeling changes with the pharm/tox reviewer and supervisor.

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/s/

PAUL C BROWN
11/04/2010

Memorandum

PHARMACOLOGY/TOXICOLOGY MEMO TO FILE

Date: 23 August 2010
NDA# 22-505
Sponsor: Theratechnologies
Drug: Egrifta^{TM}
Reviewer: Lauren Murphree Mihalcik, Ph.D.
Re: Addendum to Review Regarding Pregnancy Category

In the original pharmacology/toxicology review submitted on 1 March 2010 of NDA 22-505 (Egrifta^{TM} for reduction of visceral adipose tissue (VAT) in patients with HIV-related lipodystrophy), the reviewer recommended a Pregnancy Category C for this indication. This recommendation was based on the finding of hydrocephaly in rats in the peri- and postnatal reproductive toxicity studies and the uncertainty surrounding the finding of incomplete ossification in the skull in the embryofetal development studies. These findings are considered adverse and drug-related by the reviewer and would have supported either a designation of Category C or X, depending on how the review team viewed the clinical utility of Egrifta use during pregnancy.

After consultations with the clinical reviewer and Maternal Health Team, the reviewer considers a Category X designation appropriate. The characteristics that distinguish a Category C drug/indication from a Category X drug/indication related to the clinical benefit of using the drug during pregnancy, not solely to the strength of the nonclinical evidence of harm. The Maternal Health Team considers there to be no clinical benefit to reducing VAT during pregnancy (a time during which VAT usually increases). With the concurrence of the primary clinical reviewer and the clinical review team, assigning this drug and indication to Pregnancy Category X is consistent with 21 CFR 201.57.

Because this drug will be contraindicated in pregnant women, the recommended embryofetal development study described in the review will not be a post-marketing requirement. Any new indications for this drug will be subject to a new assessment of risk and benefit to pregnant women and may require additional nonclinical studies.

Pharmacology/Toxicology concurs with the Pregnancy Category X designation.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22505	ORIG-1	THERATECHNOLOGIES INC	Egrifta

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/s/

LAUREN M Mihalcik
08/27/2010

TODD M BOURCIER
08/27/2010
I concur with Dr. Mihalcik's assessment

Memorandum

PHARMACOLOGY/TOXICOLOGY MEMO TO FILE

Date: 27 July 2010

NDA# 22505

Sponsor: Theratechnologies

Drug: EGRIFTA® (tesamoreline acetate)

Reviewer: Lauren Murphree Mihalcik, Ph.D.

Background

After review of the reproductive toxicity data for Egrifta, the reviewer was concerned about a possible signal for hydrocephaly observed in four fetuses (from two litters) in the Segment 3 study in rats at a dose of 1.2 mg/kg (~2X MRHD, AUC basis). In the Segment 2 study, the high dose of 0.6 mg/kg (~1X MRHD, as well as lower doses) caused an increase in delayed ossification in the skull relative to controls. This effect was not dose-dependent, but it occurred in the context of advancing ossification parameters in other parts of the body, suggesting a specific effect in the skull. To clarify whether the observation in the Segment 2 study was real and premonitory to hydrocephaly, the Division informed the sponsor that we planned to request a repeat Segment 2 study using a dose of ≥ 1.2 mg/kg. The results would allow us to at least know if the window of risk was pre- or post-natal and would allow pregnant women to make informed decisions about taking Egrifta. At the time, Pharm/Tox was considering a Pregnancy Category C designation. The Maternal Health Team has subsequently recommended a Pregnancy Category X designation, citing lack of clinical benefit of Egrifta during pregnancy. ***Since Egrifta will be contraindicated in pregnancy, a further exploration of teratogenic effects is not necessary, and this PMR will be cancelled***. The protocol synopsis is described and evaluated below for future reference, should a new indication be approved for use in pregnant women.

Sponsor's Synopsis of Proposed Protocol

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Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22505	ORIG-1	THERATECHNOLOGIES INC	Egrifta

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/s/

LAUREN M Mihalcik
08/27/2010

TODD M BOURCIER
08/27/2010

Memorandum

Pharmacology/Toxicology
Center for Drug Evaluation and Research
Division of Metabolic & Endocrine Products

NDA SECONDARY REVIEW

Date:	10 March 2010
NDA #	22-505
Sponsor:	Theratechnologies
Drug:	Egrifta (tesamorelin acetate injection) human GHRH analogue
Primary Reviewer:	Lauren Murphree Mihalcik, Ph.D.
Secondary Reviewer:	Todd Bourcier, Ph.D.

Theratechnologies is seeking marketing approval for tesamorelin acetate, proposed trade name Egrifta, as a treatment 'to induce and maintain a reduction of excess (b) (4) abdominal fat (b) (4) in HIV-infected patients with lipodystrophy'. Tesamorelin acetate is a 44 amino acid synthetic analogue of human growth hormone releasing hormone (hGHRH). The peptide's N-terminus is modified with a hexenoyl moiety to reduce enzymatic cleavage of the peptide, thereby prolonging its plasma half-life and pharmacodynamic activity. Tesamorelin is intended to simulate the endogenous pulsatile release of growth hormone and its effector insulin-like growth hormone-1 (IGF1). This approach presumably would limit adverse effects of excess growth hormone, such as insulin resistance, while maintaining sufficient efficacy for the desired effect of lipolysis and fat redistribution in the patient population.

If approved, tesamorelin would be the first full-length hGHRH drug product marketed, offering a new treatment modality for the HIV lipodystrophy indication. To date, growth hormone has been investigated as a potential therapy in this patient population, with mixed results.

Dr. Lauren Murphree Mihalcik, the primary nonclinical reviewer, concludes that the pharmacology and toxicology data support approval of tesamorelin. She recommends a post-marketing requirement to conduct an additional embryofetal development study in rats administered higher doses in order to clarify adverse findings observed in the post-natal development study. *I concur with Dr. Murphree Mihalcik's assessment.*

Our recommendation for approval is based on a toxicity profile in rats and dogs that is consistent with the profile of excess growth hormone/IGF1 activity. Rats exhibited gains in organ and body weight, liver (lipid) vacuolation, and elevations in glucose and cholesterol. Dogs, in which exposure was much higher, exhibited signs of acromegaly, lipid alterations, and frank insulin resistance. It is interesting to note that a presumed advantage of tesamorelin is the potential avoidance of growth hormone excess. While tesamorelin may prove to confer a 'wider'

therapeutic window compared to growth hormone, the animal studies clearly demonstrate that tesamorelin treatment does not eliminate the potential for growth hormone excess and its adverse sequelae. The adverse findings in the general toxicology studies are reasonably explained as being secondary to elevated levels of growth hormone and IGF1. Therefore, clinical toxicities of tesamorelin are predicted to be similar to those known for approved growth hormone products and in those with acromegaly.

Dr. Murphree Mihalcik recommends a nonclinical post-marketing requirement to conduct an additional embyrofetal toxicology study in rats administered higher doses of tesamorelin, to address a hydrocephaly signal detected in the peri/post-natal rat study. I agree with her recommendation. Given the proposed indication, it is expected that relatively few women of child-bearing potential would be exposed to tesamorelin, even fewer so while pregnant, but such exposure cannot be completely excluded. Moreover, exposure may become broader than anticipated should off-label use of tesamorelin resemble such practices as with growth hormone products. Unlike growth hormone products, tesamorelin is a modified version of the endogenous GHRH peptide, and should be viewed as toxicologically distinct from growth hormone until demonstrated otherwise (as done in the general toxicology studies). Therefore, I believe it is justified to fully characterize the potential for adverse effects of tesamorelin on embryofetal development, with relevant results communicated in the label.

At issue is the finding of hydrocephaly in some rat pups born to dams administered 1.2mg/kg tesamorelin during the period of organogenesis through lactation. This dose is only ~2-fold higher than the proposed clinical dose, and did not cause maternal toxicity other than an expected slight increase in body weight. When administered to dams only during the period of organogenesis, when the risk of teratogenicity is highest, hydrocephaly was not observed but fetuses presented with reduced ossification of interparietal bones of the skull. This is notable because fetuses had higher (not lower) body weight and reduced ossification was not observed at other skeletal sites. Unfortunately, this study tested a dose of 0.6mg/kg instead of the 1.2mg/kg dose that was associated with hydrocephaly in the post-natal period, so a link between the findings in these two studies is suggestive but not conclusive. Because of the difference in doses, this set of studies is unable to discern whether tesamorelin imparted hydrocephaly during the peri/post-natal period, during the period of organogenesis, or both. This has clinical implications regarding labeling of risk: for example, hydrocephaly induced only in the post-natal period via lactation presents a different risk than hydrocephaly induced during organogenesis, and would be conveyed differently in the product label. Of note, reproductive toxicology studies conducted with growth hormone or IGF-1 did not identify skeletal malformations as seen with tesamorelin. A post-marketing study on embryofetal development in rats administered at least 1.2mg/kg tesamorelin during the period of organogenesis will address this concern.

Nonclinical labeling issues to be resolved prior to final regulatory action include refining the post-marketing requirement and revising language in the Pregnancy section.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22505	ORIG-1	THERATECHNOLOGIES INC	Egrifta

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/s/

TODD M BOURCIER
03/11/2010
Supervisor secondary memo, pharm/tox

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-505**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**29 May 2009**
PRODUCT:	**Egrifta (tesamorelin acetate injection)**
INTENDED CLINICAL POPULATION:	**HIV-infected patients with lipodystrophy**
SPONSOR:	**Theratechnologies (Agent: Kendle International)**
DOCUMENTS REVIEWED:	**eCTD Submissions**
REVIEW DIVISION:	**Division of Metabolism and Endocrinology Products (HFD-510)**
PHARM/TOX REVIEWER:	**Lauren Murphree Mihalcik, Ph.D.**
PHARM/TOX SUPERVISOR:	**Todd Bourcier, Ph.D.**
DIVISION DIRECTOR:	**Mary Parks, M.D.**
PROJECT MANAGER:	**Jennifer Johnson**

Date of review submission to Division File System (DFS): 1 March 2010

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability
Pharmacology/Toxicology recommends approval of NDA 22-505 (Egrifta®).

B. Recommendation for nonclinical studies
The Division recommends that an additional reproductive toxicity study be performed in the rat to more thoroughly characterize the risk of hydrocephaly in offspring of treated dams. Specifically the sponsor should perform an embryofetal development study using ≥1.2 mg/kg to assess the risk of hydrocephaly in the developing fetus at the dose in which it was observed in the peri- and post-natal study. Higher doses would be preferred as the 1.2 mg/kg dose produced exposures that were only 1.6X MRHD (AUC basis) in pregnant rats. Given the wide range of exposures seen in the target population (0.2-3.0 ng.h/mL, mean 1.12 ng.h/mL), the sponsor should characterize the risk that at least covers this range of clinical exposures.

C. Recommendations on labeling

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II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Pharmacology
TH9507 is an N-terminally modified version of human growth hormone releasing hormone (GHRH or GRF) that is resistant to cleavage and inactivation by DPP-4, thus prolonging its half-life *in vivo*. Its potency for binding the GHRH receptor *in vitro* is similar to that of the unmodified protein (0.069 nM vs. 0.083 nM). The sponsor has demonstrated robust growth hormone (GH) stimulation in both *in vivo* and *in vitro* models, including transfected mammalian cells, porcine pituitary cells, and Landrace-Yorkshire pigs. No studies directly related to the indication (reduction of (b) (4) adipose tissue in HIV-positive patients) were submitted, but the published literature contains examples of GH as an effective, if problematic, treatment for lipodystrophy. In the safety pharmacology studies, the only drug-related effect was an increase in blood pressure and heart rate in dogs given a very high dose (50 mg/kg).

Pharmacokinetics
TH9507 rapidly absorbed after subcutaneous dosing in rats and dogs (T_{max} values of 5 min and 35-45 min, respectively). Bioavailability was not measurable in rats due to rapid clearance after i.v. injection and was measured to be 6-21% in dogs. After s.c. injection in the rat, radiolabeled protein distributes primarily to excretory organs, including bladder, kidney, liver, and intestine, suggesting both urinary and fecal routes of elimination. Metabolism studies in plasma samples of various species show degradation of the peptide into smaller peptide fragment, as would be expected. This degradation occurred rapidly in rats, but the peptide was more stable in human and dog plasma, consistent with longer half-lives in these species. The half-life of TH9507 in human plasma was ~15 times longer than that of unmodified GHRH.

General Toxicology
Single dose studies were conducted in rats, mice, and dogs. Minimum lethal doses of ~100 and 200 mg/kg were established for mice and rats, respectively (240X and 975X MRHD, BSA basis). No single lethal dose was established for dogs at up to 100 mg/kg i.v. (1600X MRHD, BSA basis).

Repeat dose studies were conducted in CD-1 mice (up to 13 weeks), Sprague-Dawley rats (up to 26 weeks), and beagle dogs (up to 52 weeks). Injection site reactions in the mice precluded longer-term administration of doses adequate to provide sufficient margins to human exposures. Effects in rats and dogs were generally consistent with the expected effects of long-term increases in GH levels.

After six months of dosing, rats given 1.5X to 26X MRHD (AUC basis) weighed 5-28% more than controls, had 5-16% increases in glucose, and had increases in cholesterol and decreases in red blood cells (males only). All doses caused lipid vacuolation in the liver with necrosis in 1/15 animals per sex at the high dose (considered adverse). There was no evidence of increased proliferative potential of spleen cells in a mitogenicity assay, and PCNA staining in multiple tissues did not reveal a treatment-

related effect. This data was provided to show a lack of carcinogenic potential in chronically dosed rats. The NOAEL for this study was 0.6 mg/kg, or 15X MRHD (AUC basis).

In the 52-week study in dogs, there were large increases in exposure over time (~100X over Day 1 levels), due in part to development of anti-drug antibodies which appeared to prolong the half-life of the drug and increase pharmacodynamic activity as measured by IGF-1 concentrations. Animals at all doses exhibited signs of canine acromegaly, including increased size of body (↑18-59%), paws, abdomen, and skin folds, leading to secondary adverse effects such as foot and leg lesions. Two females developed a severe diabetes-like syndrome, one of which was euthanized due to very poor condition. Animals at all doses had increases in cholesterol (2-7X), LDL (14-85X), HDL (↑50-100%), and triglycerides (2-33X). There were dose-dependent decreases in red blood cells (up to 25%), with increases in platelets and decreases in APTT. Microscopic effects that were attributed to pharmacodynamic effects included hypertrophy in numerous tissues, vacuolation in the adrenal, and diffuse pituitary hyperplasia. Effects secondary to the metabolic effects noted above included alterations in the gallbladder and pancreas, hydropic degeneration of the liver, and vacuolar degeneration in the kidney. The reviewer considers all the effects to be secondary, tertiary, or compensatory effects to the expected pharmacodynamic effects of the drug. Although no NOAEL was identified, the very high exposures (averaging 1.5-42X MRHD at the beginning of the study and 280X-6400X MRHD at the end) did not cause any effects that would not be best explained by increased GH activity in these animals.

Special Toxicology
The sponsor provided 28-day rat studies and appropriate genetic toxicity studies to qualify the impurities reaching relevant threshold levels [(b) (4)].

Genetic Toxicology
TH9507 was not mutagenic or clastogenic in two *in vitro* assays (the Ames test and mammalian chromosome aberration assay) and one *in vivo* assay (murine micronucleus induction). Additional *in vitro* studies performed to qualify impurities (b) (4) were also negative.

Reproductive Toxicology
Reproductive toxicity studies were conducted in rats and rabbits. There was evidence of changes to skull formation in offspring of both species, particularly in rats. Rats given 0.2X-1X MRHD (AUC basis) had pups with increased incidence of reduced ossification of the interparietal bone when other areas of ossification were more progressed consistent with higher fetal weights. Most notably, animals given 1.6X MRHD in the peri- and post-natal study produced pups with hydrocephaly (four pups from two litters). In rabbits given ~300X MRHD, there was one occurrence of hydrocephaly (within the historical control range) and an increase in fetal rates of incomplete ossification in the bones of the skull (not statistically significant). There were no adverse effects in dams in these studies, but slight increases in weight gain were evidence of pharmacodynamic activity.

B. Nonclinical safety issues relevant to clinical use

1. Chronic dosing with TH9507 caused slight elevations (↑6%) in plasma glucose in rats given 1.6X MRHD, increasing in males to ↑12% at 26X MRHD (AUC basis). A severe diabetes-like syndrome developed in dogs exposed to very high drug levels. Since hyperglycemia and insulin resistance has been associated with GH treatment in HIV patients with lipodystrophy, there is a risk for similar effects with TH9507. Clinical trials showed trends towards increased plasma glucose and HOMA-R scores for insulin resistance that were sometimes statistically significant.
2. Increases in lipids were seen in both rats and dogs and appeared to precede most of the adverse effects associated with metabolic changes; however, changes in lipid parameters were not observed in clinical subjects.
3. Evidence from the reproductive toxicity studies suggests that there is a risk of hydrocephaly or altered intracranial pressure in the offspring of animals given doses that provide exposures that

are 1-2X MRHD (AUC basis). Given the known clinical risk of intracranial hypertension associated with GH treatment (see, e.g., approved labeling for Genotropin®, Humatrope®, and Norditropin®), there appears to be a risk for hydrocephaly and/or intracranial hypertension in the offspring of patients taking TH9507 during pregnancy or while breastfeeding.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-505
Review number: 1
Sequence number/date/type of submission: SN000 / 29 May 2009 / Original NDA
Information to sponsor: Yes ( ) No (x)
Sponsor and/or agent: Theratechnologies (Agent: Kendle Int'l)
Manufacturer for drug substance: (b) (4)
Reviewer name: Lauren Murphree Mihalcik, Ph.D.
Division name: DMEP
HFD #: 510
Review completion date: January 22, 2010

Drug:

Trade name: Egrifta
Generic name: Tesamorelin acetate injection
Code name: TH9507
Chemical name: N-(trans-3-hexenoyl)-human growth hormone releasing factor (1-44) acetate
CAS registry number: 218949-48-5
Molecular formula/molecular weight: $C_{221}H_{366}N_{72}O_{67}S$ / 5132.7

Structure: trans-CH_3-CH_2-CH=CH-CH_2-CO-Tyr-Ala-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys-Val-Leu-Gly-Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Met-Ser-Arg-Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala-Arg-Ala-Arg-Leu-$CONH_2$.

Relevant INDs/NDAs/DMFs: IND 61226 (Theratechnologies)

Drug class: Hypothalamic hormones, modified human growth hormone releasing factor (GHRH or GRF)

Intended clinical population: HIV-infected patients with lipodystrophy

Clinical formulation: Lyophilized tesamorelin acetate (1 mg) and 55 mg mannitol per vial (with 10% overfill), as shown in the sponsor's table below, packaged with sterile water for injection. Two vials are used per dose.

Component	Quantity (mg/vial)	Function	% Of the total unit weight*
N-[trans-3-Hexenoyl]-Human Growth Hormone-Releasing Factor (1-44) Acetate, In-house	1.1**	Active Pharmaceutical Ingredient	0.1
Mannitol USP	55 mg	(b) (4)	
Water for injection USP***	--------	Solvent	--------
(b) (4)	--------	(b) (4)	--------
	--------		--------
	--------		--------

* Based on a theoretical unit filling weight of 1.1 g

** This weight is corrected for peptide content

(b) (4)

Route of administration: Subcutaneous injection

Maximum Recommended Human Dose: 2 mg per day (2 x 1 mg injection given at the same time), providing an average AUC in the target population of 1.12 ng.h/mL and a C_{max} of 2.3 ng/mL, based on the results of the PK analysis in study CTR-1015 as shown in the sponsor's table below.

Table 16: Summary of pharmacokinetic results following administration of tesamorelin 2 mg s.c. injection

Parameters		Day 1 (n = 17)					Day 14 (n = 15)				
		Mean	SD (±)	CV (%)	Geo. Mean	Geo. Mean CV(%)	Mean	SD (±)	CV (%)	Geo. Mean	Geo. Mean CV(%)
AUC_{0-t}	(pg·h/mL)	1149.55	1008.70	87.75	852.80	91.87	1117.23	953.40	85.34	794.68	108.59
AUC_{0-inf}	(pg·h/mL)	1255.40	1104.49	87.98	933.35	90.94	1312.60	1124.19	85.65	940.40	104.73
$AUC_{t/inf}$	(%)	91.44	3.62	3.96	-	-	84.73	6.39	7.54	-	-
C_{max}	(pg/mL)	3106.4	1375.3	44.27	2822.3	48.89	2333.3	1185.0	50.78	2013.2	66.52
T_{max}	(h)	0.162	0.060	37.23	-	-	0.157	0.042	26.61	-	-
T_{max}**	(h)	0.150	0.000	-	-	-	0.150	0.025	-	-	-
K_{el}	(h^{-1})	4.3214	2.7194	62.93	-	-	2.5071	1.9692	78.54	-	-
$T_{½ el}$	(h)	0.31	0.32	104.79	-	-	0.63	0.61	96.54	-	-
Cl/F	(L/(h·kg))	38.71	26.85	69.38	-	-	40.97	31.15	76.04	-	-
V_d/F	(L/kg)	10.48	6.10	58.25	-	-	20.19	9.87	48.90	-	-

** Median and interquartile ranges are also presented.

"-" = Not applicable.

Disclaimer: Some Tables and Figures from the electronic NDA submission have been copied for use in this review.

Studies reviewed within this submission

Primary Pharmacodynamics	Assessment of TH9507 as a prodrug in porcine pituitary cells GH release and IGF-1 production in Landrace x Yorkshire barrow pigs
Safety Pharmacology	*In vitro* hERG Cardiovascular effects in the beagle dog CNS effects in the Sprague Dawley rat Respiratory effects in the Sprague Dawley rat
Pharmacokinetics	**Absorption** Pharmacokinetics in rats and dogs (s.c. and i.v. dosing) Bridging RIA-LC/MS/MS studies in rats and dogs
	Distribution Tissue distribution in the rat
	Metabolism Stability in human plasma Biodegradants in rat, dog, and human plasma Induction/inhibition of drug-metabolizing enzymes in the rat
General Toxicology	Single dose studies in CD-1 mice, SD rats, and beagle dogs Repeat dose studies: CD-1 mice: 13 weeks (s.c.) SD rats: 2 weeks (i.v.), 4 weeks (i.v.), 13 weeks (s.c.), and 26 weeks (s.c.) Beagle dogs: 2 weeks (i.v.), 4 weeks (i.v.), 16 weeks (s.c.), and 52 weeks (s.c.)
Genetic Toxicology	Ames Test (*Salmonella typhimurium and Escherichia coli)* Chromosome Aberration Assay in Chinese Hamster Ovary cells Erythrocyte Micronucleus Assay in ICR Mice
Reproductive and Developmental Toxicology	Fertility and teratology study in the rat (male and female) Rabbit embryofetal development (two dose-range finding and two pivotal) Rat pre- and post-natal development study
Special Toxicology Studies	Local tolerance in rabbits Immunotoxicity study in rats Impurity qualification studies: 28-day study in the rat with heat-stressed batch Genetic toxicity studies with impurities (b) (4): Ames Test (*Salmonella typhimurium and Escherichia coli)* Chromosome Aberration Assay in Chinese Hamster Ovary cells

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

TH9507 is an N-terminally modified version of human growth hormone releasing hormone (GHRH or GRF) that is resistant to cleavage and inactivation by DPP-4. Its potency for binding the GHRH receptor *in vitro* is similar to that of the unmodified protein (0.069 nM vs. 0.083 nM). The sponsor has demonstrated robust growth hormone (GH) stimulation in both *in vivo* and *in vitro* models, including transfected mammalian cells, porcine pituitary cells, and Landrace-Yorkshire pigs. No studies directly related to the indication (reduction of (b) (4) adipose tissue in HIV-positive patients) were submitted, but the published literature contains examples of GH as an effective, if problematic, treatment for lipodystrophy. In the safety pharmacology studies, the only drug-related effect was an increase in blood pressure and heart rate in dogs given a very high dose (50 mg/kg).

2.6.2.2 Primary pharmacodynamics

Mechanism of action:
General Rationale for TH9507 treatment of lipodystrophy: HIV-infected patients on antiretroviral therapy often develop a lipodystrophy characterized by excess visceral adipose tissue (VAT) accumulation and loss of subcutaneous fat coupled with dyslipidemia and insulin resistance. Increased VAT in these patients is associated with elevated cardiovascular risk. HIV lipodystrophy is also associated with lowered growth hormone (GH) levels (basal and pulse amplitude). Since GH is known to be lipolytic, restoring physiological levels and patterns of GH may be beneficial. Previous clinical studies using GH itself to treat these patients resulted in fluid retention and hyperglycemia in a significant number of subjects. Using TH9507 (a long-acting GRF) could provide a more physiological stimulation of GH by inducing a pulsatile release of the hormone.

TH9507 binds to the human GRF receptor in vitro: In an assay using recombinant hGRF-R expressed in BHK cells, TH9507 displaced labeled ligand at the receptor with an IC_{50} of 0.069 nM, compared to an IC_{50} of 0.083 nM for unmodified hGRF. The sponsor did not provide data on selectivity of TH9507 for the hGRF-R versus other receptors.

TH9507 elicits GH release in vitro: Since the primary mechanism of action of TH9507 is to stimulate GH release in the pituitary (via GRF-R), pharmacodynamic activity was measured by evaluating GH release from cultured (non-transformed) pituitary cells isolated from a Landrace-Yorkshire pig. As shown below (Figure 1), both human GRF and TH9507 caused an increase in GH release into the media of cultured cells after a four-hour incubation. This effect was not blocked by the addition of protease-containing fetal calf serum, which did blunt the effect of low-concentration hGRF (Figure 2). The addition of the hexenyl group on TH9507 was incorporated to reduce susceptibility to proteases and prolong its half-life. The sponsor also uses this data to support their contention that TH9507 is not a pro-drug that requires cleavage by a protease for activity.

Figure 1. GH Release from Porcine Pituitary Cells

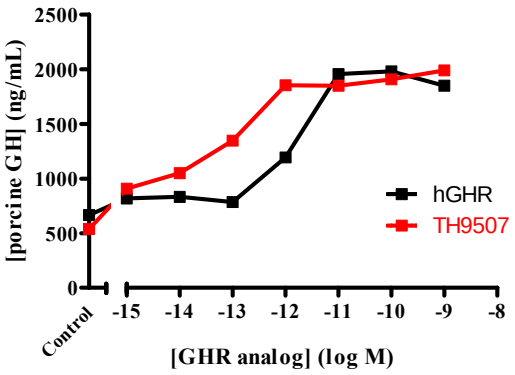
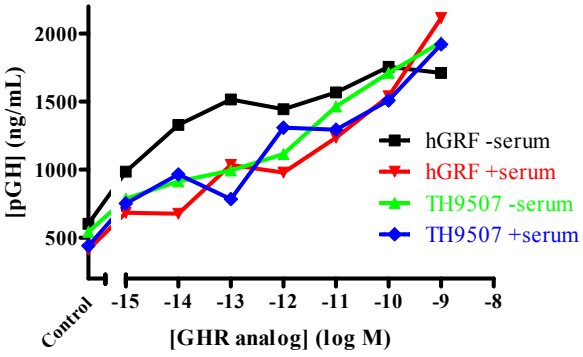


Figure 2. Effect of Serum on GH Release from Porcine Pituitary Cells



TH9507 elicits GH release in vivo: *In vivo* efficacy of TH9507 was demonstrated by measuring GH or IGF-1 release in Landrace-Yorkshire pigs. TH9507 was more potent than hGRF in eliciting porcine GH (pGH) release as shown in Figure 3. Pulsatile release of pGH was demonstrated after s.c. dosing (see Figure 4 below) or i.v. dosing (data not shown). Dosing TH9507 in a vehicle of 2.5% mannitol did not appear to affect its ability to stimulate GH release, although a direct comparison to TH9507 in saline was not performed.

Figure 3. GH AUC after a single s.c. dose in pigs

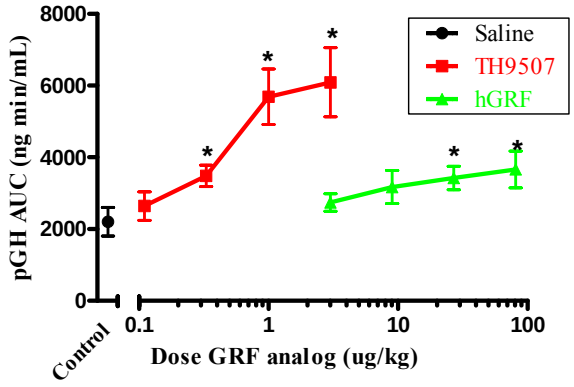
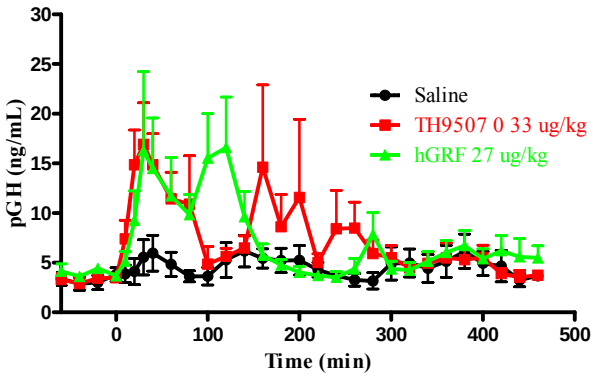
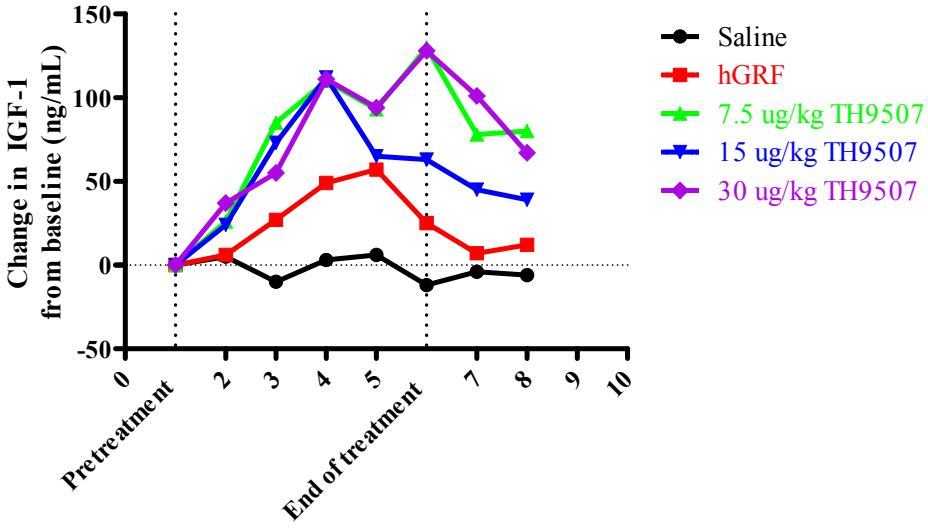


Figure 4. Pulsitile release of GH after a single s.c. dose in pigs



With repeated dosing of hGRF or TH9507 twice daily for 5 days, IGF-1 levels increased above baseline and saline controls, as shown in Figure 5 below. The changes in IGF-1 were not dose-dependent.

Figure 5. IGF-1 production with twice daily treatment in pigs



Drug activity related to proposed indication: The sponsor did not provide any nonclinical studies designed to demonstrate efficacy in treating lipodystrophy or related disorders.

2.6.2.3 Secondary pharmacodynamics

No secondary pharmacodynamics studies were included in the application.

2.6.2.4 Safety pharmacology

Neurological effects: *NOEL ≥ 50 mg/kg (rats)*

Effects on the CNS were evaluated in male Sprague Dawley rats using functional observational battery (FOB), motor activity, grip strength, and body temperature assessments performed at 15, 120, and 1320 minutes after dosing with 0, 0.6, 6, or 50 mg/kg. There were no treatment-related effects at any dose.

Cardiovascular effects: *NOEL 6 mg/kg (dogs)*

hERG activity: There was no statistically significant inhibition of hERG current when concentrations up to 800 ng/mL of TH9507 were applied to stably transfected Chinese Hamster Ovary (CHO) expressing this potassium channel.

Cardiovascular Telemetry Study: Four conscious male Beagle dogs previously implanted with telemetry transmitters were given 0, 0.6, 6, or 50 mg/kg (s.c.) in 1 mL/kg 5% mannitol in a Latin square design with at least two days between doses. In the 2-3 hours after dosing, animals given 50 mg/kg (800X MRHD, BSA basis) had a slight elevation in blood pressure (systolic, diastolic, and mean) and heart rate. Data for early time points are shown in Figures 6 and 7 below, with some error bars removed for clarity. There were no apparent effects on ECG parameters (PR, QRS, QT, and QTc) at any dose.

Figure 6. Mean Blood Pressure at Early Time Points

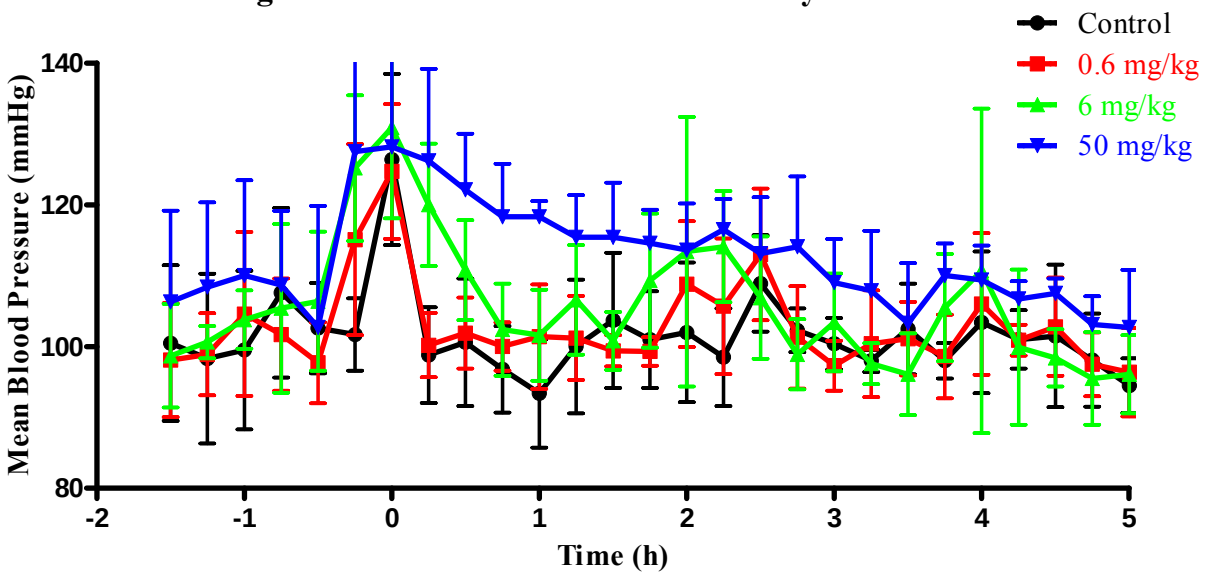
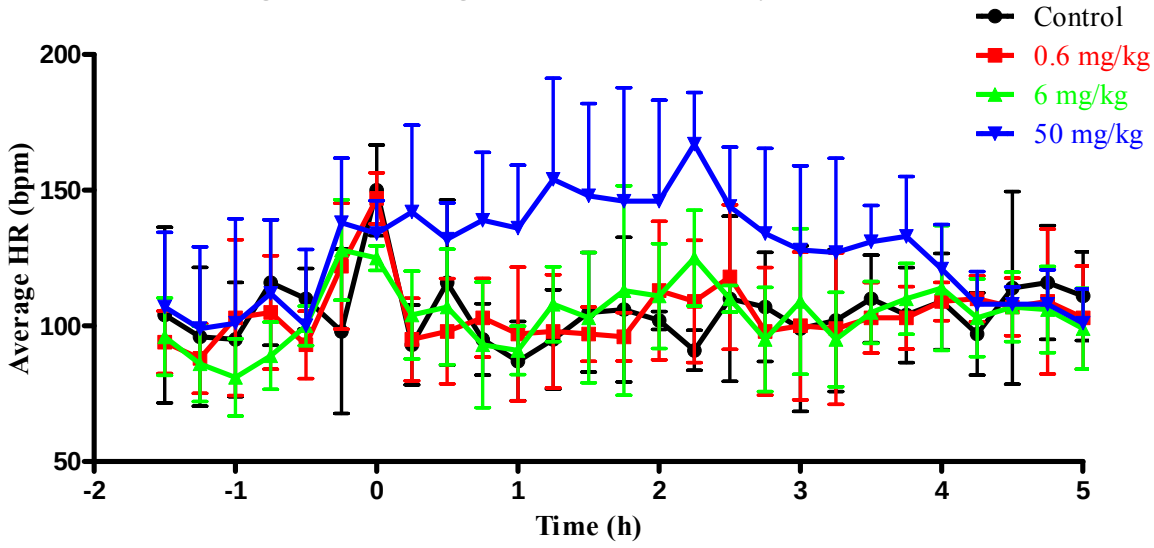


Figure 7. Average Heart Rate at Early Time Points



Pulmonary effects: *NOEL ≥ 50 mg/kg (rats)*

Respiratory function (including respiratory rate, tidal volume, and derived minute ventilation) was assessed in conscious Sprague Dawley rats placed in "head out" plethysmographs. There were no treatment-related effects observed at 15, 120, or 1320 minutes after doses of 0.6, 6, or 50 mg/kg.

Renal effects: The sponsor did not submit any specific assessments of renal function.

Gastrointestinal effects: The sponsor did not submit any specific assessments of gastrointestinal effects.

Abuse liability: The sponsor did not submit any studies to assess the abuse liability of TH9507.

2.6.2.5 Pharmacodynamic drug interactions

No pharmacodynamic drug interaction studies were performed.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

TH9507 rapidly absorbed after subcutaneous dosing in rats and dogs (T_{max} values of 5 min and 35-45 min, respectively). Bioavailability was not measurable in rats due to rapid clearance after i.v. injection and was measured to be 6-21% in dogs. After s.c. injection in the rat, radiolabeled protein distributes primarily to excretory organs, including bladder, kidney, liver, and intestine, which suggests both urinary and fecal routes of elimination. Metabolism studies in plasma samples of various species show degradation of the peptide into smaller peptide fragment, as would be expected. This degradation occurred rapidly in rats, but the peptide was more stable in human and dog plasma, consistent with longer half-lives in these species. The half-life of TH9507 in human plasma was ~15 times longer than that of unmodified GHRH.

2.6.4.2 Methods of Analysis

When development of TH9507 began, the sponsor used a commercially available radioimmunoassay (RIA) against hGRF to measure TH9507 in the plasma of nonclinical species. During later regulatory validation, it became clear that this assay could both underestimate TH9507 concentrations due to matrix effects and overestimate total exposure since it also recognized C-terminal truncated fragments of TH9507. The replacement assay, using LC/MS/MS, is more specific for intact TH9507 and was used for analysis of the pivotal chronic toxicity studies. Due to limitations in the LC/MS/MS assay, especially at low concentrations, an ELISA assay was developed for rats and rabbits and used in reproductive toxicity studies (rat pre- and post-natal study (PCL-277) and rabbit embryofetal development study (PCL-276)). Parameters for each assay are described in the sponsor's table at right.

Table 2: Summary of methods used to detect TH9507

Method		Selectivity	Quantification Limits	Accuracy (Intra-assay)[a]	Accuracy (Inter-assay)[a]
RIA					
Rat plasma (Heparin)	E-PCL-068	TH9507, hGRF and C-terminal truncated TH9507 and hGRF peptides	0.030-30 ng/mL	≤ 77% (≤ 73.6% at LLOQ)	N/A
Dog plasma (Heparin)	E-PCL-067	TH9507, hGRF and C-terminal truncated TH9507 and hGRF peptides	0.030-30 ng/mL	≤ 61% (≤ 57.9% at LLOQ)	≤ 45.9% (≤ 22.3% at LLOQ)
LC/MS/MS					
Rat serum	E-PCL-107	TH9507	1-500 ng/mL	≤11.2% (≤11.8% at LLOQ)	≤9.7% (≤4.4% at LLOQ)
Dog serum	E-PCL-110	TH9507	5-1000 ng/mL	≤-11.7% (≤16% at LLOQ)	N/A
ELISA					
Rat serum	E-PCL-277	TH9507	0.625-12.5 ng/mL	≤20% (≤20.6% at LLOQ)	≤14.1% (≤11% at LLOQ)
Rabbit plasma	E-PCL-276	TH9507	5-100 ng/mL	≤6% (≤5.9% at LLOQ)	≤15.5% (≤5.1% at LLOQ)

N/A = not available
[a] % Theoretical concentration

2.6.4.3 Absorption

TH9507 was rapidly absorbed following subcutaneous injection in both rats and dogs. In rats, T_{max} was approximately 5 minutes post dose. Absorption was somewhat slower in dogs, with T_{max} values generally ranging from 35-45 minutes. Bioavailability could not be measured in the rat due to rapid elimination of the drug from the plasma following i.v. dosing. In dogs, bioavailability was estimated to be 6-21% after a single dose, but these studies were based on RIA measurements of the AUC which, as described above, may not reliable. For both species, bioavailability appears to be low, and the uncertainty regarding the precise value is not important to the assessment of safety.

Toxicokinetic parameters for rats and dogs from the repeat dose studies are shown in the tables below. For comparison, the average clinical C_{max} and $AUC_{0\text{-}24}$ are 2.3 ng/mL and 1.12 ng.h/mL.

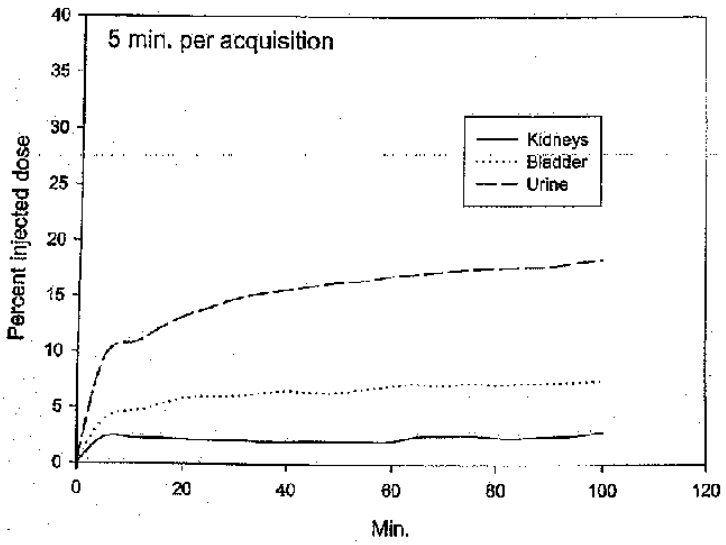
Rat Toxicokinetic Parameters Following Repeated Dosing							
Dose	Parameter	16 week s.c.		28-day i.v.		52-week s.c.	
		Male	Female	Male	Female	Male	Female
0.1	T_{max} (min)	14.2	14.2	5	5	5	30
	C_{max} (ng/mL)	11.6	5.55	46.5	33.2	4.91	3.44
	$AUC_{0\text{-}t,last}$ (ng.h/mL)	632	47	ND	ND	72.5	106
0.3	T_{max} (min)	5	5	5	5	-	-
	C_{max} (ng/mL)	18.7	14.5	158	108	-	-
	$AUC_{0\text{-}t,last}$ (ng.h/mL)	989	188	ND	ND	-	-
0.6	T_{max} (min)	5	14.2	5	5	5	5
	C_{max} (ng/mL)	33.2	28.5	263.4	256.7	50	58.5
	$AUC_{0\text{-}t,last}$ (ng.h/mL)	1917	1418	ND	ND	1859	1069
1.2	T_{max} (min)	-	-	-	-	5	5
	C_{max} (ng/mL)	-	-	-	-	72.5	58.3
	$AUC_{0\text{-}t,last}$ (ng.h/mL)	-	-	-	-	1859	1676

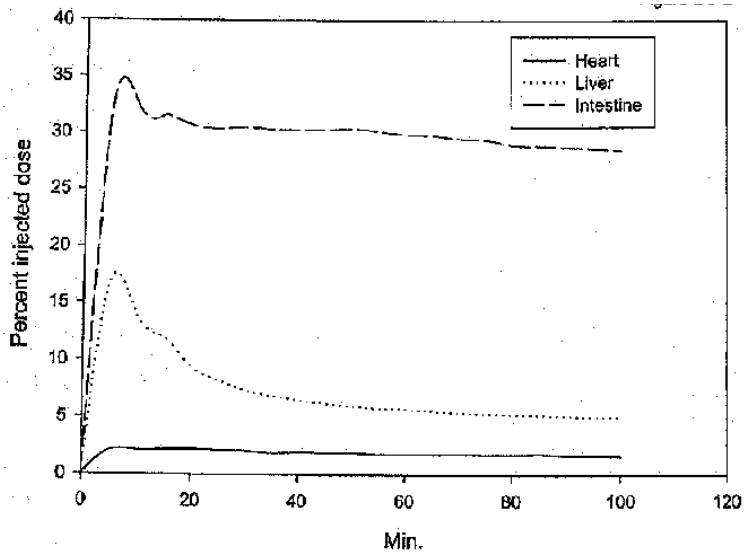
Dog Toxicokinetic Parameters Following Repeated Dosing							
Dose	Parameter	16 week s.c.		28-day i.v.		52-week s.c.	
		Male	Female	Male	Female	Male	Female
0.1	T_{max} (min)	38	45	0	0	26	34
	C_{max} (ng/mL)	22	54	736	593	110	210
	$AUC_{0\text{-}t,last}$ (ng.h/mL)	114	198	144	128	115	255
	$T_{1/2}$ (min)	159	137	19	25	34	63
0.3	T_{max} (min)	34	60	10	0	-	-
	C_{max} (ng/mL)	92	77	1015	1043	-	-
	$AUC_{0\text{-}t,last}$ (ng.h/mL)	301	287	445	458	-	-
	$T_{1/2}$ (min)	103	151	48	43	-	-
0.6	T_{max} (min)	43	35	0	0	34	45
	C_{max} (ng/mL)	191	266	2250	2423	551	618
	$AUC_{0\text{-}t,last}$ (ng.h/mL)	771	966	1008	879	552	675
	$T_{1/2}$ (min)	173	130	45	40	26	84
1.2	T_{max} (min)	-	-	-	-	30	45
	C_{max} (ng/mL)	-	-	-	-	794	1192
	$AUC_{0\text{-}t,last}$ (ng.h/mL)	-	-	-	-	837	1461
	$T_{1/2}$ (min)	-	-	-	-	58	23

2.6.4.4 Distribution

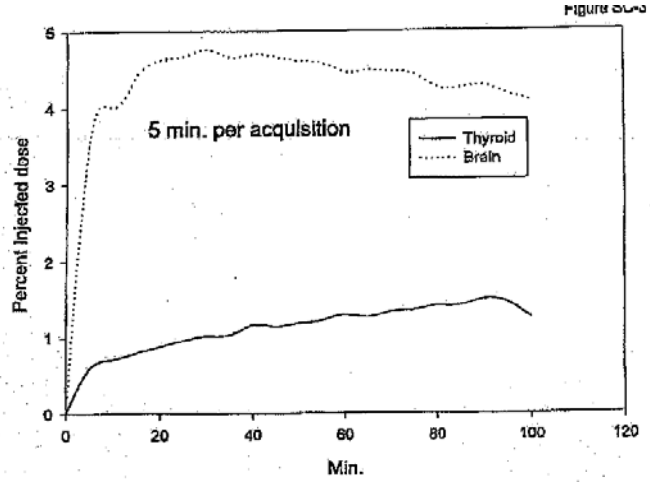
The sponsor attempted to assess the distribution of TH9507 by following the radioactivity in Sprague-Dawley rats given [^{123}I]-labeled TH9507. The iodine label should bind transiently to the two tyrosine residues in the peptide, allowing for a short window of opportunity to assess distribution *in vivo*. Rats were dosed intravenously with 15.5 µg/kg of the labeled peptide and euthanized at 2.5 hours post dose (with scintographic images obtained up to 100 minutes post dose). These animals were compared with those given [^{123}I]NaI and an iodinated reference peptide. A second study used subcutaneous dosing and compared the radioactivity distribution to that of iodinated hGRF and truncated versions (1-29) of TH9507 and hGRF.

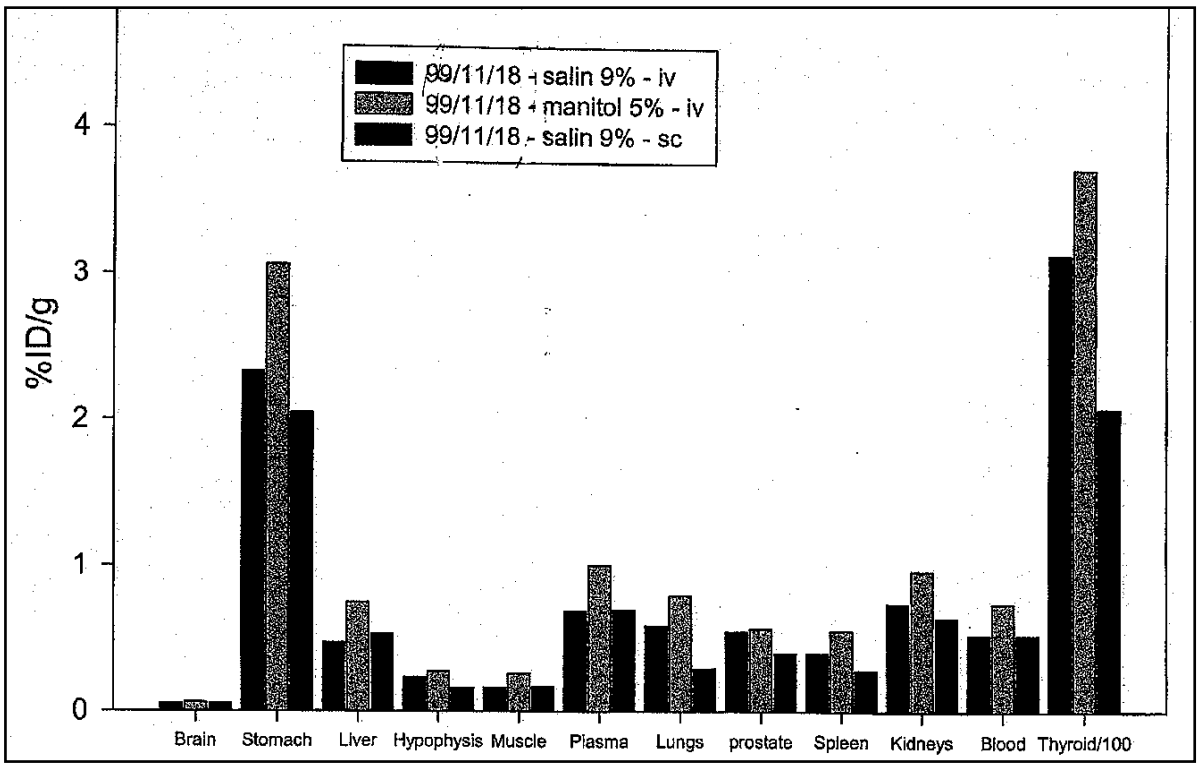
After i.v. dosing, the majority of the dosed radioactivity was found in the excretory tissues, including kidney, bladder, liver, and intestine (see the sponsor's figures below). This was consistent with the observations made after s.c. dosing. There was very little radioactivity seen in the brain by either route, suggesting low permeability across the blood-brain barrier. With the exception of the lung, distribution of TH9507 and hGRF were similar after either i.v. or s.c. injection. Levels of radioactivity in the lung were significantly higher after i.v. TH9507 compared to the unmodified peptide (but not after s.c. injection).





Radioactivity measured by whole-body scintillation in the brain after i.v. dosing showed levels of ~4.5% of the injected dose, as shown in the sponsor's graph at right; however, tissue levels measured after i.v. or s.c. injection showed measured radioactivity to be very low (0.06% of injected dose per gram compared to 0.8% for blood). The sponsor's graph below shows distribution measured after i.v. injection using saline or mannitol as the vehicle and after s.c. injection in saline.





2.6.4.5 Metabolism

Per ICH S6, traditional metabolism studies are generally not needed for peptide drugs. The sponsor has included two studies of the degradation of TH9507 in plasma samples. In study PCL-034, TH9507 was incubated in human, rat, and dog plasma for up to 5 hours. As shown in the sponsor's Figure 7 below, TH9507 was fairly stable in human and dog plasma but was rapidly degraded in rat plasma, consistent with differences observed *in vivo*. Human GRF, in contrast, was rapidly degraded in human plasma (presumably by DPP-4) to hGRF(1-2) and hGRF(3-44), as shown in the sponsor's Figure 8. TH9507 comprised ~83% of drug-related material after 5 hours of incubation, with 9 peptides comprising the remainder. One degradant comprising >5% of the drug-related material was a human-specific degradant. Dog and rat plasma contained 15 and 31 identified peptide fragments, respectively.

Table 2. Number of putative TH9507 biodegradants following incubation in human, dog, or rat plasma

Peptide	Plasma Matrix	Number of Identified Peptide Biodegradants				
		Total	Biodegradants > 5%		Biodegradants < 5%	
			Matrix-Specific	Common	Matrix-Specific	Common
hGRF	Human	14	2*	0	8	4
TH9507	Human	10	1*	0	3	2*+ 4
TH9507	Dog	15	2*	1	6* + 1	2* + 3
TH9507	Rat	31	1*	2* + 2	11 *+ 13	2

*Contain the hexenoyl group on the N-terminal tyrosine of TH9507.

A second study measuring the *in vitro* half-life of hGRF and TH9507 in human plasma (PCL-342) measured values of 33 minutes for hGRF and ~8 hours for TH9507 using a concentration of 200 μg/mL.

Figure 7: Degradation/metabolism of TH9507 in human, rat, and dog plasma

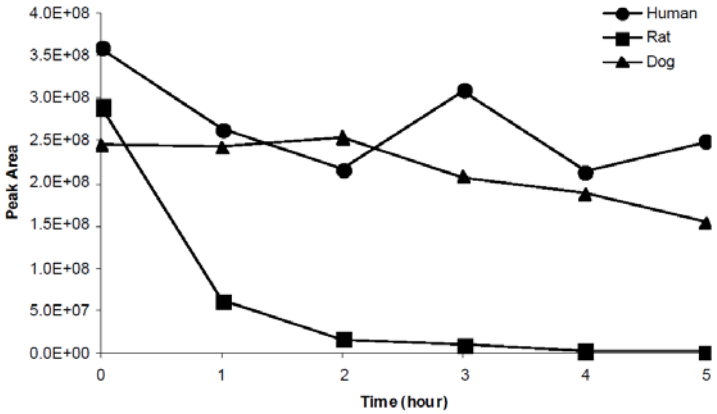
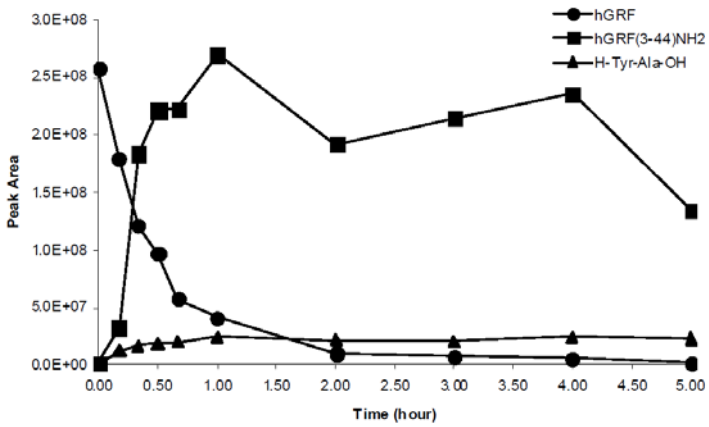


Figure 8: Degradation/metabolism of hGRF in human plasma



In a 28-dat rat study using s.c. dosing, liver samples were taken from male and female rats to assess Cytochrome P450 activities. The only statistically significant effect was a reduction in CYP3A1/2 activity in male animals, as shown in the sponsor's table below (testosterone 6β-hydroxylase activity (TESH)). This enzyme is known to be sensitive to GH levels in male rats, suggesting that this is related to the pharmacodynamic activity of the drug.

Dose (mg/kg)	Sex	Cytochrome b_5 (nmol/mg protein)	Cytochrome P_{450} (nmol/mg protein)	CZXH Activity (nmol/min/mg protein)	TESH Activity (nmol/min/mg protein)	EROD Activity (nmol/min/mg protein)	PROD Activity (pmol/min/mg protein)	UGTase Activity (nmol/min/mg protein)
0	M	0.183 ± 0.034	0.847 ± 0.181	1.883 ± 0.190	4.329 ± 1.228	0.257 ± 0.029	13.01 ± 3.23	9.075 ± 2.205
	F	0.207 ± 0.054	0.935 ± 0.155	1.890 ± 0.148	1.658 ± 0.171	0.387 ± 0.084	6.868 ± 1.421	4.609 ± 0.716
0.1	M	0.217 ± 0.064	0.682 ± 0.128	1.623 ± 0.381	2.571 ± 0.594*	0.205 ± 0.035	8.134 ± 1.877	8.423 ± 1.024
	F	0.188 ± 0.037	0.754 ± 0.290	2.095 ± 0.273	1.077 ± 0.634	0.393 ± 0.066	7.086 ± 1.392	5.556 ± 1.300
0.6	M	0.195 ± 0.027	0.737 ± 0.086	1.848 ± 0.453	1.364 ± 0.402**	0.318 ± 0.038	9.102 ± 1.304	10.47 ± 1.71
	F	0.173 ± 0.064	0.845 ± 0.151	1.844 ± 0.224	1.716 ± 0.402	0.349 ± 0.040	5.890 ± 1.257	4.650 ± 0.727
1.2	M	0.258 ± 0.035	0.715 ± 0.101	1.341 ± 0.153	1.692 ± 0.468**	0.276 ± 0.083	9.903 ± 1.783	7.922 ± 0.582
	F	0.132 ± 0.079	0.766 ± 0.336	1.647 ± 0.457	1.222 ± 0.558	0.334 ± 0.026	4.668 ± 0.943	5.828 ± 2.389

2.6.4.6 Excretion

Although no dedicated excretion studies were performed, the distribution study described above showed probable drug distribution to the excretory organs (kidney, bladder, liver, and intestine), suggesting elimination by both urine and feces.

2.6.4.7 Pharmacokinetic drug interactions

No nonclinical drug interaction studies were submitted with the application.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: Pivotal toxicity studies, conducted in rats and dogs, achieved exposures to TH9507 far in excess of those expected in clinical practice. Rats were treated for six months with up to 25X MRHD (AUC basis), and exposures in dogs reached >1000X MRHD in the 52-week study. TH9507 caused dose-dependent increases in body weight gain in all species, consistent with the known effects of increasing growth hormone levels. Other findings were also consistent with known effects of GH in these species (e.g., slight anemia, increased lipids, and hypertrophy in various tissues) or secondary to these effects, especially to metabolic effects. Increased lipids resulted in lipid vacuolation in the liver in rats and dogs. Effects in dogs were particularly severe given the very high exposure levels. Two female dogs developed a severe diabetes-like syndrome, requiring humane sacrifice of one animal. The dogs in the chronic study developed symptoms of canine acromegaly, consistent with observations of dogs given growth hormone.

Genetic toxicology: There was no evidence of genetic toxicity in the Ames test, *in vitro* mammalian chromosome aberration assay, or *in vivo* micronucleus assay in ICR mice at doses up to 100 mg/kg. Additional *in vitro* studies performed to qualify impurities (b) (4) were also negative.

Carcinogenicity: Two year carcinogenicity studies in rodents were not required for this compound.

Reproductive toxicology: Reproductive toxicity studies were conducted in rats and rabbits. There was evidence of changes to skull formation in both species, particularly in rats. Rats given 0.2X-1X MRHD (AUC basis) had pups with increased incidence of reduced ossification of the interparietal bone when other areas of ossification were more progressed consistent with higher fetal weights. Most notably, animals given 1.6X MRHD in the peri- and post-natal study produced pups with hydrocephaly (four pups from two litters). In rabbits given ~300X MRHD, there was one occurrence of hydrocephaly (within the historical control range) and an increase in fetal rates of incomplete ossification in the bones of the skull (not statistically significant).

Special toxicology: The sponsor provided 28-day rat studies and appropriate genetic toxicity studies to qualify the impurities reaching relevant threshold levels [(b) (4)].

2.6.6.2 Single-dose toxicity

Rodents: Single dose toxicity studies were performed in mice and rats using 100 and 200 mg/kg (i.v.) with no control groups. Treatment-related mortality was seen at ≥ 100 mg/kg in mice and 200 mg/kg in rats. Deaths generally occurred within one hour of dosing following clinical signs including decreased activity, tremor, convulsive spasms, and gasping respiration. Other findings considered treatment-related were evidence of gastric bleeding (two mice given 100 mg/kg) and fluid accumulation in the lung (one rat given 200 mg/kg). Many animals of both species had dark discoloration of the tail (often persisting for the 14 days of observation). Without control groups for comparison, these effects on the tail can not be definitively ascribed to injection trauma or drug-related effects.

Dogs: The sponsor submitted two single dose toxicity studies in dogs. In the first study, a dose of 100 mg/kg given to a single animal caused significant adverse clinical signs which persisted for approximately six hours (including redness of the mouth and eyes, prone position, incontinence, diarrhea and vomitus, each positive for occult blood. A dose of 50 mg/kg (i.v.) given to another animal produced similar but slightly milder clinical signs. Both animals had a persistent reduction in food intake leading

to a 0.6 kg weight loss. In the second study, doses of 25 and 5 mg/kg (i.v.) both again caused redness of the mouth, eyes, and ears, effects that correlated with increased histamine in plasma, decreased activity, prone position, foamy vomitus, soft stool, and pressing against the cage. At 25 mg/kg, animals had increases in ALT, ALP, and cholesterol with decreased weight, but these effects were not observed at 5 mg/kg.

SINGLE DOSE TOXICITY STUDIES			
Species	**NOAEL or MLD[§]**	**MRHD Multiple [§§]**	**Findings**
CD-1 Mice GLP Study #EPCL-287 100, 200 mg/kg (i.v.)	MLD ~100 mg/kg	240X	Mortality on D1 in 10% of LD and 80% of HD animals within 20 minutes. Dark discoloration of the caudal tail in both groups; evidence of gastric bleeding in two LD animals.
Rat GLP Study #EPCL-288 100, 200 mg/kg (i.v.)	MLD 200 mg/kg	975X	Mortality in 70% of HD animals within 12 minutes. Decreased activity and dark discoloration of the tail at both doses; HD animals that died showed convulsive spasms, no activity, and gasping spasmatic respiration.
Dog Non-GLP Study #EPCL-301 50, 100 mg/kg (i.v.)	No Lethal Dose	> 1600X	Clinical signs lasting 1h (LD) or 3h (HD): Redness of the mouth and eyes, prone position, incontinence, diarrhea and vomitus, both positive for occult blood; Reduced body weight
Dog Non-GLP Study #EPCL-302 5, 25 mg/kg (i.v.)	No Lethal Dose	>400X	Both doses: Redness of the mouth, eyes, and ears correlated with ↑histamine in plasma Decreased activity, prone position, foamy vomitus, soft stool, and pressing against the cage. HD: ↑ALT, ↑ALP, ↑Chol. on D3; ↓weight.
§ Minimal Lethal Dose §§ BSA basis, assumes 60 kg subject			

2.6.6.3 Repeat-dose toxicity

General toxicity studies of 2 and 4 weeks duration were conducted in Sprague-Dawley rats and beagle dogs by intravenous administration. These species were also used for the pivotal subchronic (13- or 16-week) and chronic studies by the clinical administration route (26 and 52 weeks duration, respectively). An additional 13-week study was performed in CD-1 mice using s.c. dosing. Important findings from the repeat dose studies are discussed below and shown in the following table, and a detailed review of each chronic study is provided later in this section. In all nonclinical species, the effects noted in the repeat-dose toxicity studies were generally attributable to increases in pharmacodynamic activity (i.e., release of growth hormone).

Mortality
Effects of TH9507 on mortality was limited to the 52-week dog study, in which one of two MD females developing a severe diabetes-like syndrome was euthanized for humane reasons. This animal had exposure to TH9507 that was >4000X MRHD (AUC basis). There were no treatment-related effects on

mortality in the repeat-dose studies in mice or rats at doses up to 1.2 mg/kg (25X MRHD, AUC basis, in rats).

Body Weight
All species displayed a dose-dependent increase in body weight gain that was typically greater in magnitude in females by two- to three-fold. The effect increased in magnitude with dosing duration. This correlated with increased food consumption in rats but not in dogs. Increased weight gain is an expected effect of growth hormone, so this and the sequelae of weight gain are attributed to pharmacodynamic activity of TH9507. The weight gain in dogs was particularly robust, leading to body weight that were 18-59% higher than controls at the termination of the 52-week study. The large increase in body weight in the dogs was accompanied by other clinical signs consistent with canine acromegaly, including skin folds, enlarged paws, and increased abdomen depth (all consistent with increased GH levels) in the 52-week study, but these effects were not seen in the shorter-term studies.

Hematology
TH9507 caused a dose-dependent decrease in red blood cell parameters. Mean RBC counts decreased by as much as 7% in rats and 25% in dogs. This anemia appeared to be regenerative, with some associated increase in reticulocytes. Platelets also increased slightly across species, leading to decreases in APTT (up to ↓10%).

Metabolic Changes
Increases in lipids were seen after 12 weeks of dosing in male rats given ≥ 0.6 mg/kg and in dogs given ≥ 0.1 mg/kg following 4 weeks (i.v.) or 16 weeks (s.c.) of dosing. Increases in cholesterol were generally smaller in rats (~30%) compared to dogs (>100%) in the subchronic studies. In dogs, triglycerides generally increased along with cholesterol, and the changes in cholesterol were primarily due to increases in LDL (14X-85X control in the 52-week dog study). This increase in lipids correlated with vacuolation in the livers of both species, which stained positive for lipids (using Oil Red O) in rats and was hypothesized (but not confirmed) to be glycogen in the dogs. The vacuolation in the rats was associated with bile duct hyperplasia (13 weeks) and minimal necrosis (26 weeks) in the high dose groups.

Injection Site
Minimal to moderate inflammation was seen at all doses in the subchronic rat study, with the addition of hemorrhage observed with chronic dosing. In dogs, congestion, inflammation and vasculitis were observed at ≥ 0.3 mg/kg in the subchronic study. When the dogs were exposed to higher levels of drug in the chronic study, dermal hypertrophy and diffuse necrosis were seen at all doses (only in high-exposure LD animals).

Additional effects related to pharmacodynamic activity
Pituitary hyperplasia was observed in rats (HD) and dogs (all doses), consistent with continued stimulation of the GHRH receptor in that tissue. Other effects attributed to PD effects in the dog included vacuolation of the adrenal, increased plasma proteins, and hypertrophy in the adrenal, digestive organs, femur, skin, and bladder.

RODENT PIVOTAL TOXICOLOGY STUDIES			
SPECIES/ STUDY DOSES/AUC	**NOAEL**	**MRHD MULTIPLE***	**FINDINGS**
CD-1 Mouse 13 weeks (s.c.) 0, 0.3, 0.6, 1.2 mg/kg	0.3 mg/kg (local)	No TK	*Body weight gain* increased by 9-18% in males and 22-44% in females *Platelets* increased slightly and dose-dependently *Calcium and phosphorus* increased dose-dependently *Injection site toxicity*: minimal to mild inflammation at ≥ 0.6 mg/kg; muscular degeneration/regeneration at all doses
Rat 2 weeks (i.v.) 0, 12.5, 25, 50, 100 µg/kg	0.1 mg/kg	No TK	*No drug-related effects* in this dose-range finding study with n=3/sex/group.
4 weeks (i.v.) 0, 0.1, 0.3, 0.6 mg/kg C_{max}: 40, 133, 260 ng/mL[#]	0.1 mg/kg	17X C_{max} basis	*Body weight gain* increased by 16-36% in males and 82-114% in females (correlated with ↑ food consumption) *RBC* was decreased 5% in HDF *Liver toxicity*: Total bilirubin increased 11-46% and organ weights increased by up to 24% *Kidney toxicity:* UNa increased 50-100% with minimal tubular basophilia in HD *Lung toxicity:* Perivascular inflammation at ≥ 0.3 mg/kg. Minimal congestion/hemorrhage at all doses
13 weeks (s.c.) 0, 0.1, 0.3, 0.6 mg/kg D1: 2.9, 5.0, 8.4 ng h/mL[#] D92: compromised by ab	0.3 mg/kg	4X	*No effects* on mortality, clinical signs, ophthalmoscopy *Body weight gain* increased by 8-18% in males 42-81% in females (correlated with ↑ food consumption) *Liver toxicity*: Vacuolation (lipid) increased in HD (microvascular) with bile duct hyperplasia in 2/20 HD *Injection site toxicity*: minimal to mild inflammation was seen at all doses *Reproductive toxicity:* inflammation in the prostate or congestion in the ovary were seen in 3/sex at HD
26 weeks (s.c.) 0, 0.1, 0.6, 1.2 mg/kg 1.8, 17, and 29 ng.h/mL	0.6 mg/kg	15X	*No effects* on mortality, clinical signs, ophthalmoscopy, urinalysis *Final body weight* was greater by 6-18% in males 18-28% in females (correlated with ↑ food consumption) *RBC* decreased 7% in HDM *Glucose* increased 5-16% 5 ≥ 0.1 mg/kg and *cholesterol* increased 25-50% at ≥ 0.6 mg/kg (M) *Liver toxicity*: Vacuolation (lipid) and iron-rich pigment in Kupffer cells increased at all doses with minimal necrosis in 2/30 HD, ↑50% bilirubin in HDM *Injection site toxicity*: minimal to moderate inflammation and hemorrhage was seen at ≥ 0.1 mg/kg *Reproductive effects:* females given ≥ 0.6 mg/kg were more likely to be in diestrus at study termination

*Based on average clinical AUC of 1.12 ng h/mL following 2 mg dose

[#] RIA based measurement

NON-RODENT PIVOTAL TOXICOLOGY STUDIES			
SPECIES/ STUDY DOSES/AUC	**NOAEL**	**MRHD MULTIPLE***	**FINDINGS**
Beagle dog 2 weeks (i.v.) 0, 12.5, 25, 50, 100 µg/kg	0.1 mg/kg	No TK	*No drug-related effects* in this dose-range finding study.
4 weeks (i.v.) 0, 0.1, 0.3, 0.6 mg/kg 136, 450, 980 ng h/mL[#]	0.3 mg/kg	400X	*No effects* on mortality, clinical signs, ophthalmoscopy, ECG, urinalysis, organ weights, or gross pathology *Body weight gain* increased by 100% in males and 133-367% in females *Platelets* increased by 40% in HDM *Lipids and glucose* increased in all dose groups *Testes toxicity:* Tubular atrophy was seen in 1/3 and 2/3 MD and HD males *Lung toxicity:* histiocytosis in HDF, minimal hemorrhage in LD and MD males (2/3)
16 weeks (s.c.) 0, 0.1, 0.3, 0.6 mg/kg D1: 22, 33, 104 ng h/mL[#] W16: compromised by ab	< 0.1 mg/kg	20X	*No effects* on mortality, clinical signs, ophthalmoscopy, ECG, urinalysis *Body weight gain* increased by ~150% (M) and ~300(F) *Anemia* (mild, regenerative) at ≥ 0.1 mg/kg (↓~20% RBC, Hb, Hct) with slight increases in platelets at ≥ 0.3 mg/kg. *Lipids* (cholesterol and TG) increased >100% at all doses, and glucose increased in HDM by ~15% Protein levels increased ~5-10% and phosphorus increased by 20-40% in all dose groups. *Liver toxicity:* Increased weight (up to 50%), vacuolation at all doses *Adrenal effects:* vacuolation at HD *Kidney effects:* minimal tubular basophilia (all doses), mononuclear infiltrate *Injection site effects:* congestion, inflammation, and vasculitis at ≥ 0.3mg/kg
52 weeks (s.c.) 0, 0.1, 0.6, 1.2 mg/kg M:318,1545, 3776 ng h/mL F:2138,3944,7205 ng h/mL	<0.1 mg/kg	M: <284X F: <1909X	*Drug exposure* increased dramatically during the study, correlating with development of anti-drug antibodies *Mortality:* One of two MDF with a diabetes-like syndrome was euthanized. *Clinical signs* consistent with acromegaly were observed at all doses *Body weights* were 18-59% higher than controls at termination *Anemia* (up to ↓25% in RBC) was dose-dependent; platelets increased ↑80-120% with ↓10% APTT at ≥ 0.6 mg/kg *Lipid* changes include increased cholesterol (2-7X), LDL (14-85X), HDL (↑50-100%), and triglycerides (3-22X). *PD Effects:* Hypertrophy in adrenal, esophagus, femur, GI tract, skin, and bladder; vacuolation in the adrenal; diffuse pituitary hyperplasia *2° to metabolic changes:* alterations in the gallbladder and pancreas, hydropic degeneration of the liver, and vacuolar degeneration in the kidney

*Based on average clinical AUC of 1.12 ng.h/mL following 2 mg dose

[#] RIA based measurement

A 26-WEEK SUBCUTANEOUS TOXICITY STUDY OF TH9507 IN RATS (E-PCL-105)

Key study findings:

- SD rats given 0.1, 0.6, or 1.2 mg/kg/d for 26 weeks had exposures at the end of the study (sexes combined) of 1.8, 17, and 29 ng.h/mL, respectively, or 1.6X, 15X, and 26X MRHD (AUC basis). Exposures tended to increase with repeated dosing.
- There was only minimal development of anti-drug antibodies (2 animals).
- There were no treatment-related clinical signs or mortality, but body weight gain increased dose-dependently in males (↑10-33%) and with a plateau at ≥ 0.6 mg/kg in females (↑34-57%). Final body weights were 6-28% higher in drug-treated groups.
- High dose male animals had a slight, dose-dependent decrease in red blood cells (↓7%) and increase in reticulocytes (↑31%).
- Glucose and cholesterol (HDL and LDL) increased slightly in a dose-dependent manner. Additionally, high dose males (but not females) had total bilirubin levels which were 50% higher than controls.
- Increases in organ weights (heart, spleen, ovary, and kidney (F)) were generally attributable to increased body weights. A 10% increase in liver weight in the high dose females was statistically significant after correction for body weight.
- Dose-dependent increases in periportal vacuolation (lipid) and iron-rich pigments in the Kupffer cells were observed in the liver. Vacuolation occurred at all doses. Periportal necrosis was observed in one animal per sex at the high dose.
- Female animals receiving ≥ 0.6 mg/kg were more likely to be in diestrus at the end of the study.
- There were no treatment-related effects on the proliferative potential of spleen cells in the mitogenicity assay. PCNA staining also did not appear to vary with drug treatment.
- Liver samples from male animals had a dose-dependent decrease in the ability to metabolize testosterone *ex vivo* (i.e., reduced CYP3A1/2 activity).

Reviewer Comments: Effects observed in TH9507 could generally be attributed to increased GH exposure. In the liver, minimal necrosis occurred in two high dose animals with minimal or slight periportal lipid vacuolation. Alterations in lipid and carbohydrate metabolism in the liver are expected results of prolonged exposure to GH, but the reviewer considers the necrosis seen at the high dose (although low in incidence) to be adverse.

SD Rats, 26 weeks	NOAEL	MRHD multiple (1.1ng.h/mL)
Liver: Periportal necrosis	0.6 mg/kg	15X

Study: E-PCL-105
Volume and page: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: 2 April 2004
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: TH9507, FHEXGRF0201, > 98.5%

Methods	
Doses	0, 0.1, 0.3, 0.6, 1.2 mg/kg once per day for 26 weeks
Species/source	Sprague-Dawley CD (Crl:CD (SD)BR IGS) rats / (b) (4)
Age / Weight	~8 weeks/ 276-348 g (M) and 187-229 g (F)
n/sex/group (main study)	15/sex/group
TK groups	9/sex/group for TK and 4/sex/group for CYP450 study
Recovery groups	none
Route, formulation, dose volume	Subcutaneous injection in 1 mL/kg 5% mannitol in sterile water for injection

Observations and Times	
Mortality checks	Twice daily
Clinical Findings	Weekly (main study animals only)
Body weights	Weekly
Food consumption	Weekly (main study animals only)
Ophthalmoscopy	Pretreatment, W12, W26 (main study animals only)
Hematology	Main study animals: W12 and W26 RBC, WBC, and coagulation parameters
Clinical chemistry	Main study animals: W12 and W26 Lipids, proteins, glucose, electrolytes, LFTs, and kidney parameters
Urinalysis	Main study animals: W12 and W26, sixteen hour samples
Other clinical evaluation	Anti-TH9507 antibody titer: Day 1 (predose),W4, W13, W26 GH: D1 (predose), W4, W13, W26
Toxicokinetics	On Day 1 samples were taken at 5, 15, and 30 minutes post dose. During W13 and W26, samples were taken at 0, 5, 15, 30, 60, and 120 minutes post dose and measured using LC/MS/MS
P450 Evaluation	Satellite animals only at Week 4: 3g of liver tissue was assayed for activity of CYP1A1/2, CYP2B1/2, CYP2E1, CYP3A1/2, and UDPGT
Gross pathology	Main study animals at sacrifice
Organ weights	Main study animals at necropsy: adrenal glands, brain, heart, kidneys, liver, ovaries/testes, pituitary, prostate, spleen, thymus, thyroid and parathyroid, uterus
PCNA	Proliferating Cell Nuclear Antigen (PCNA) was evaluated qualitatively using immunohistochemical staining in the adrenals, cecum, colon, ileum, injection sites, jejunum, liver, pituitary, stomach and testes
Mitogenicity	Spleen cells were taken from 10 main study animals/sex/group and incubated with 0, 2.5, 5, or 10 µg/mL Concanavalin A. Proliferative capacity was measured by incorporation of tritiated thymidine into the DNA.
Histopathology	Performed at necropsy for main study animals (control and HD): adrenal, aorta, bone and marrow (femur), brain, cecum, colon, duodenum, epididymides, esophagus, eyes, heart, ileum, injection sites, jejunum, kidneys, liver (sample of 2 lobes), lungs + bronchi, lymph nodes (mandibular and mesenteric), mammary gland (inguinal) optic nerves, ovaries, pancreas, pituitary, prostate, rectum, salivary gland (mandibular), sciatic nerve, skeletal muscle, skin + subcutis (inguinal), spinal cord (cervical), spleen, sternum + marrow, stomach, testes, thymus, thyroid and parathyroids, tongue, trachea, urinary bladder, uterus, vagina, gross lesions. LD and MD animals: liver and gross lesions Oil Red O was used in the liver to identify lipid accumulation; Hall's, Perl's, and Schmorl's stains were performed to identify pigment deposits in liver Kupffer cells.
	Adequate Battery: yes (x), no ()
	Peer review: yes (X), no ()

Results:

Dose Formulations: The drug formulations used in the study were within the ±10% acceptance limits of the nominal concentration.

Mortality: Two deaths occurred during the study, both of which were considered unrelated to treatment by the sponsor. A LDM was euthanized on D78 following signs including "labored breathing, a severely swollen muzzle, and a severe skin lesion with red staining of the fur around the mouth." This animal had a fractured nasal bone at necropsy, which was considered accidental. One MDM from the toxicokinetics satellite group was found dead on D168 with no previous signs of ill-health.

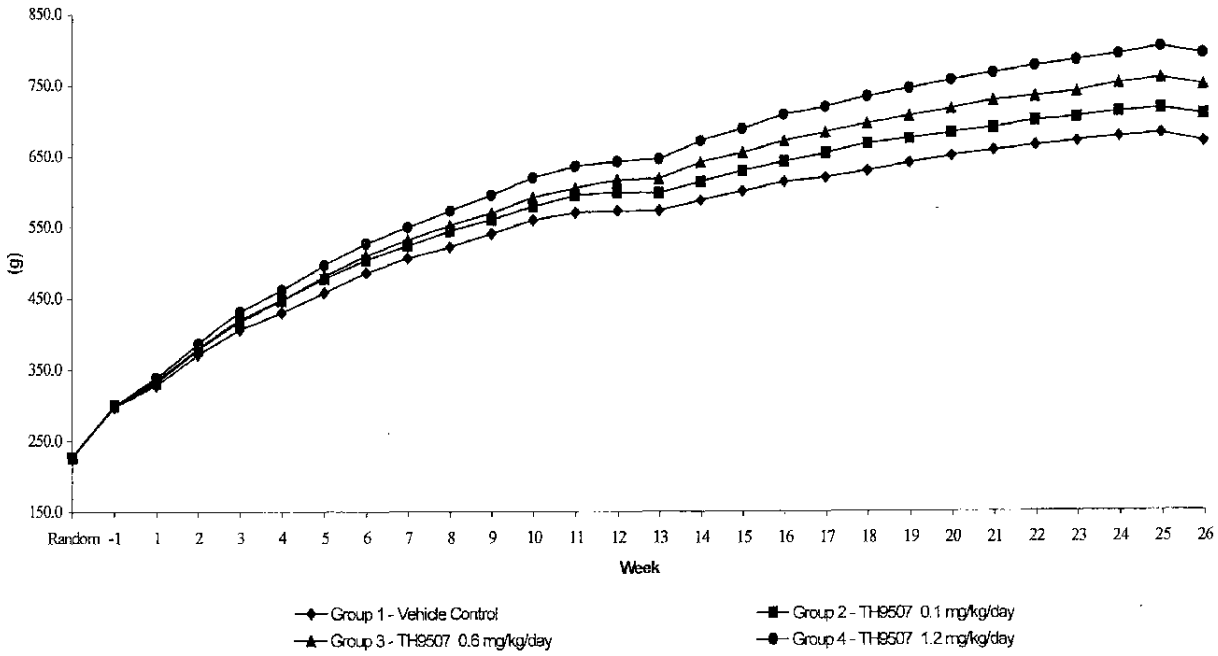
Clinical signs: There were no treatment-related clinical signs. Various signs observed throughout the study were those commonly seen in these animals and did not increase with dose.

Body weights: Treated animals at all doses gained more weight than controls over the study, as shown in the sponsor's figures below. Weight gain was 10-33% higher in treated males and 34-49% higher in treated females relative to control animals, leading to body weights that were up to 28% greater than untreated animals.

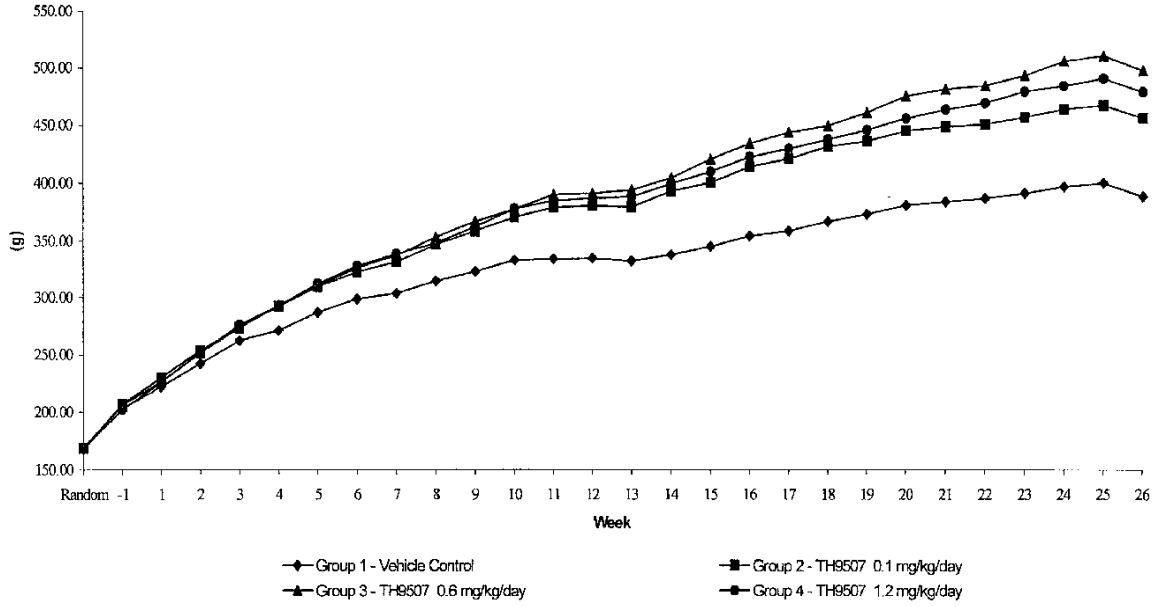
Body Weight					
Sex	Dose, mg/kg	BW gain (g) over study	% Increment	Final BW (g)	BW % control
Males	0	374	0%	672	100%
	0.1	411	↑10%	711	106%
	0.6	453*	↑21%	753	112%
	1.2	496*	↑33%	796*	118%
Females	0	187	0%	389	100%
	0.1	250*	↑34%	458*	118%
	0.6	293*	↑57%	499*	128%
	1.2	278*	↑49%	480*	123%

* $p<0.05$

MALE BODY WEIGHT



FEMALE BODY WEIGHT



Food consumption: Food consumption increased dose-dependently, with statistically significant increases in MD and HD animals (↑10-20%).

Ophthalmoscopy: There were no treatment-related effects.

Hematology: Female animals in drug-treated groups had 22-37% increases in total white blood cells compared to control animals, largely due to increases in lymphocyte numbers (↑33-50%), but this effect was not observed in males. High dose male animals had a slight, dose-dependent decrease in red blood

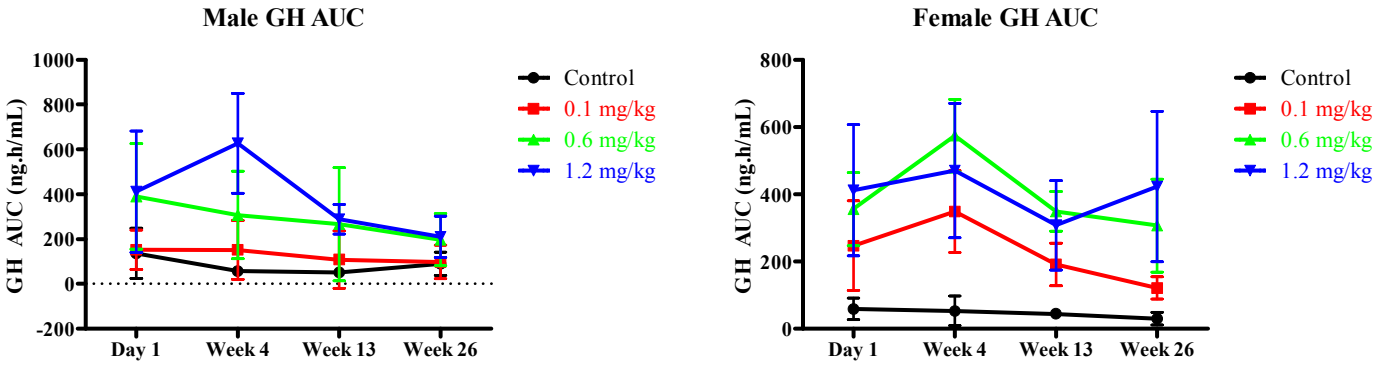
cells (↓7%) and increase in reticulocytes (↑31%), but no difference in total hemoglobin or hematocrit due to increases in MCV and MCH. Male animals receiving ≥ 0.6 mg/kg had slightly decreased clotting time (↓9-13% APTT) without significant changes to other clotting parameters.

Clinical chemistry: By the end of the dosing period, treated animals had slight but dose dependent increases in glucose and cholesterol (including HDL and LDL). These effects were statistically significant and larger in magnitude in male animals, as shown in the table below. Additionally, high dose males (but not females) had total bilirubin levels which were 50% higher than controls.

CLINICAL CHEMISTRY, WEEK 52								
Dose, mg/kg	Glucose		Cholesterol (mM)		HDL (mg/mL)		LDL (mg/mL)	
	Male	Female	Male	Female	Male	Female	Male	Female
0	111.1	107.1	96.7	101.7	76.2	72.5	9.1	5.4
0.1	117.1	115.5	104.6	88.9	81.9	63.1	8.2	5.1
0.6	124.0*	115.3	119.5*	89.0	94.5*	70.1	10.1	5.0
1.2	129.3**	118.0	145.7***	111.9	115.3***	84.5	17.1***	8.3

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Growth Hormone: The growth hormone AUC values measured throughout the study are shown below. Animals in drug-treated groups generally had higher values than controls, although there was significant overlap in control and low-dose males. For comparison, GH AUC values measured after dosing TH9507 to healthy clinical subjects in early studies were approximately 30-50 ng.h/mL.



Urinalysis: There were no treatment-related effects seen in the urinalysis.

Gross pathology: Drug-treated groups had an increase in the incidence of dark areas around the injection site, consistent with changes observed microscopically. Additionally, mid and high dose animals had an increase in the incidence of "enlarged spleen", consistent with an increase in observed absolute organ weights.

Organ weights: Increases in the absolute weights of heart and spleen were noted in males and females, as shown in the tables below. These effects were not evident when the organ weights were corrected for the increase in body weight in the treated animals. Females also had increases in liver, kidney, and ovary weights. While these effects also appear to have been influenced by higher body weights, the effects on the liver in HDF and on the ovary in MDF were still significantly higher (by 10 and 32%, respectively) after correcting for body weight.

ORGAN WEIGHTS, MALES

Dose mg/kg	Heart		Spleen	
	gram	%BW	gram	% BW
0	1.83	0.28	0.98	0.15
0.1	1.86	0.28	0.95	0.14
0.6	2.01* ↑10%	0.28	1.10	0.15
1.2	2.10** ↑14%	0.28	1.22* ↑23%	0.16

* $p < 0.05$; ** $p < 0.01$ (sponsor's calculations)

ORGAN WEIGHTS, FEMALES

Dose, mg/kg	Heart		Liver		Kidney		Ovary		Spleen	
	gram	%BW	gram	% BW	gram	%BW	mg	%BW.10^3	gram	% BW
0	1.22	0.33	8.08	2.17	2.19	0.59	70	19	0.59	0.16
0.1	1.32	0.31	9.64** ↑15%	2.23	2.32	0.55	90	20	0.71* ↑20%	0.16
0.6	1.40* ↑16%	0.30	10.39*** ↑25%	2.21	2.45* ↑11%	0.53*	118*** ↑67%	25* ↑32%	0.83*** ↑42%	0.18
1.2	1.42** ↑16%	0.31	10.91*** ↑35%	2.39* ↑10%	2.56*** ↑16%	0.56	93* ↑32%	20	0.84*** ↑42%	0.18

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (sponsor's calculations)

Histopathology: Finding which occurred more often in treated animals are shown in the table below. The sponsor identifies effects at the injection site and liver as being related to treatment. In the injection site, inflammation and hemorrhage were observed at all doses (results from a representative dorsal injection site are shown below). Myofiber degeneration was observed in one high dose male, which may be the result of mechanical injury.

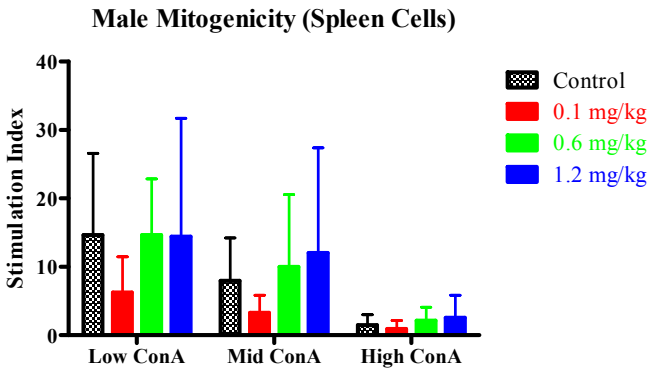
In the liver, there were dose dependent increases in periportal vacuolation and iron-rich pigments in the Kupffer cells. The vacuolation was identified by Oil Red O staining as lipid. Periportal necrosis was seen in the liver of one high dose animal of each sex and is considered treatment-related by the reviewer but not by the sponsor.

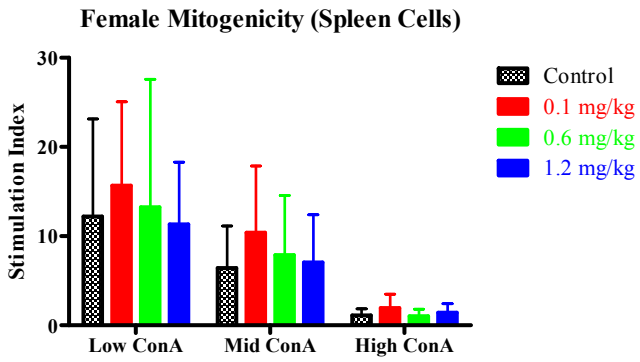
Other findings that were observed more often in treated animals include cysts and hyperplasia in the pituitary, extramedullary hematopoiesis in the portal triad of the liver, and hemorrhage in the lung.

Evaluation of the female reproductive system showed that animals receiving ≥ 0.6 mg/kg were more likely to be in diestrus compared to control animals with a concomitant decrease in the number in metaestrus.

HISTOPATHOLOGY									
Tissue	Finding	Dose	MALES				FEMALES			
			0	0.1	0.6	1.2	0	0.1	0.6	1.2
		n	15	15	15	15	15	15	15	15
Dorsal injection site	Inflammation (minimal to slight)		0	2	3	3	0	2	2	3
	Hemorrhage (minimal to moderate)		0	2	4	3	0	2	2	2
	Myofiber degeneration		0	0	0	1	0	0	0	0
Liver	Extramedullary hematopoiesis (portal triad)		0	0	0	2	0	0	0	0
	Pigment deposits (hemosiderin) in Kupffer cells		0	0	3	7	0	1	1	1
	Periportal vacuolation (lipid)		0	1	5	11	0	4	2	5
	Periportal necrosis		0	0	0	1	0	0	0	1
	Adenoma, hepatocellular		0	0	1	0	0	0	0	0
	Eosinophilic cell focus		0	0	0	2	0	0	0	0
Lung	Hemorrhage		1	2	-	4	0	-	-	1
	Fibrosis, pleura		0	-	-	1	0	-	-	0
Pituitary	Cyst		0	-	-	1	0	-	-	2
	Hyperplasia, pars distalis		0	-	-	2	1	-	-	0
Estrous cycle	Proestrus						3	4	2	3
	Estrus						4	2	4	2
	Metaestrus						7	6	2	3
	Diestrus						1	3	7	7

Mitogenicity assay: The proliferative potential of spleen cells from these animals was assessed by treating culture cells ex vivo with Concanavalin A and measuring the incorporation of tritiated thymidine into DNA. None of the samples from drug-treated animals had significantly greater stimulation of thymidine incorporation relative to control animals.





Hepatic Enzyme Activity: Liver samples from four satellite animals per sex per group were analyzed for activity of specific liver enzymes (CYP1A1/2, CYP2B1/2, CYP2B1, CYP3A1/2, UDPGT). The only statistically significant changes were dose-dependent decreases in CYP3A1/2 content (measured as a decrease in the hydroxylation of testosterone) in male animals. This effect was not observed in females. There also appeared to be a trend towards decreases in CYP2B1/2 activity in both sexes, but the effect was not statistically significant.

Male Liver Enzyme Activity				
Parameter	Control	0.1 mg/kg	0.6 mg/kg	1.2 mg/kg
Total b5 content	0.183 ± 0.034	0.217 ± 0.064	0.195 ± 0.027	0.258 ± 0.035
Total CYP450 content	0.847 ± 0.181	0.682 ± 0.128	0.737 ± 0.086	0.715 ± 0.101
CYP3A1/2	4.329 ± 1.228	2.571 ± 0.594 *	1.364 ± 0.402 **	1.692 ± 0.468 **
CYP2B1/2	13.01 ± 3.23	8.134 ± 1.877	9.102 ± 1.304	9.903 ± 1.783
Female Liver Enzyme Activity				
Parameter	Control	0.1 mg/kg	0.6 mg/kg	1.2 mg/kg
Total b5 content	0.207 ± 0.054	0.188 ± 0.037	0.173 ± 0.064	0.132 ± 0.079
Total CYP450 content	0.935 ± 0.155	0.754 ± 0.290	0.845 ± 0.151	0.766 ± 0.336
CYP3A1/2	1.658 ± 0.171	1.077 ±0.634	1.716 ± 0.402	1.222 ± 0.558
CYP2B1/2	6.868 ± 1.421	7.086 ± 1.392	5.890 ± 1.257	4.668 ± 0.943

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

PCNA Assay: PCNA staining in adrenals, cecum, colon, ileum, injection sites, jejunum, liver, pituitary, stomach and testes (the only tissues examined) did not appear to vary with drug treatment. The pathologist's report states that "upon qualitative assessment, none of the tissues in the treated groups examined demonstrated a higher proliferative rate (greater nuclear staining) than the control group."

Anti-drug antibodies: Antibodies against TH9507 were measured using a validated ELISA. Samples were considered positive for anti-TH9507 antibodies when the mean absorbance value was higher than the negative cut off value and was reduced by > 50% by immunodepletion. Three samples had absorbance values above the cut off value, and only two samples met the secondary criteria for a positive response (from a MDM on D85 and a HDF on D22). These data suggest that the immunologic response to the drug was minimal in this study.

Toxicokinetics: Plasma drug concentrations were measured using a validated LC/MS/MS assay with a LLOQ of 1 ng/mL. The calculated TK parameters are shown in the sponsor's table below. After s.c. injection, plasma drug concentrations increased rapidly with the C_{max} generally occurring within five minutes. Drug clearance occurred with a $t_{1/2}$ of 6-40 minutes. C_{max} and AUC increased with dose, but the effect was not strictly proportional.

Text Table 4 Summary of Selected Toxicokinetic Parameters for TH9507

		DOSE LEVEL (mg/kg/day)					
		0.1		0.6		1.2	
Parameter	Occasion	M	F	M	F	M	F
C_{max} (ng/mL)	Day 1	3.65	3.49	14.7	8.77	17.9	17.5
	Week 13	7.53	2.56	30.3	23.0	74.8	35.1
	Week 26	4.91	3.71	50.0	58.5	72.5	58.3
$AUC_{(0\text{-}30\ min)}$ (ng•min/mL)	Day 1	51.4	26.2	237	114	366	200
	Week 13	118	55.0	583	420	1507	688
	Week 26	62.5	91.4	688	1001	1242	1139
$AUC_{(0\text{-}120min)}$ (ng•min/mL)	Day 1	ND	ND	ND	ND	ND	ND
	Week 13	140	72.6	814	531	2401	1457
	Week 26	72.5	143	913	1069	1859	1676

ND: Not determined

For comparison to clinical exposures, the C_{max} and AUC values are shown as multiples of average human exposure at 2 mg QD in the table below. The low dose provided an AUC which is similar to expected clinical exposure with repeated dosing. The mid- and high-dose groups had exposures of ~15X and ~25X MRHD (AUC basis).

Multiples of Human Exposure							
Parameter	Occasion	0.1 mg/kg		0.6 mg/kg		1.2 mg/kg	
		M	F	M	F	M	F
Cmax (ng/mL)	Day 1	1.6	1.5	6.4	3.8	7.8	7.6
	Week 13	3.3	1.1	13.2	10.0	32.5	15.3
	Week 26	2.1	1.6	21.7	25.4	31.5	25.3
AUC^ (ng.h/mL)	Day 1	0.8	0.4	3.5	1.7	5.4	3.0
	Week 13	2.1	1.1	12.1	7.9	35.7	21.7
	Week 26	1.1	2.1	13.6	15.9	27.7	24.9

^AUC is for 0-30 minutes for Day 1 and 0-120 minutes for later time points

A ONE YEAR SUBCUTANEOUS TOXICITY STUDY IN DOGS WITH TH9507 (2875)

Key study findings:

- Dogs given 0.1, 0.6, and 1.2 mg/kg/d for 1 year had exposures to TH9507 that increased significantly with repeated dosing. $AUC_{0\text{-}90}$ values varied widely (especially dependent on anti-drug antibody status). Average exposures at W41 were 284X, 1379X, and 3371X (males) and 1909X, 3522X, and 6433X MRHD (females).
- Two MD females developed a severe diabetes-like syndrome, and one was euthanized during W48. Other animals had dose-dependent increases in insulin but normal glucose levels.
- Increased size of body, paws, abdomen, and skin folds, indicative of acromegaly, were dose-dependent, along with secondary foot and leg lesions.
- Body weight gains were 50-200% higher in treated animals, leading to 18-59% higher body weights.
- There were dose-dependent decreases in RBC parameters (↓25% at the HD), with increases in platelet numbers (↑80-120%) and a 10% decrease in APTT at ≥ 0.6 mg/kg.
- Treated animals had increased cholesterol (2-7X), LDL (14-85X), HDL (↑50-100%), and triglycerides (3-22X).
- Changes in electrolyte handling were seen as lower serum levels of chloride and sodium and elevated potassium and phosphorus, along with 50-100% increases in urine volume.
- IGF-1 (the primary PD marker) increased in treated animals, but the effect was not always dose dependent (again somewhat variable by antibody status).
- Significant increases in the weights of the adrenal, liver, kidney, and pituitary were observed at all doses with a decrease in spleen weight (↓~80%).
- Microscopic observations attributable to pharmacodynamic effects: Hypertrophy in adrenal, esophagus, femur, GI tract, skin, and bladder; vacuolation in the adrenal; diffuse pituitary hyperplasia
- Microscopic observations likely 2º to metabolic changes*:* alterations in the gallbladder and pancreas, hydropic degeneration of the liver, and vacuolar degeneration in the kidney

Dog, 52 weeks	NOAEL	MRHD multiple (µg.h/mL)
Acromegaly and metabolic disturbances	<0.1 mg/kg	<284X (M) <1909X (F)

Reviewer Comments: This study used doses that provided exposures far in excess of those experienced by clinical subjects or to be used by patients. (Low dose $AUC_{0\text{-}90}$ values were 100X (M) or 230X (F) on average, with the low outliers at 10X). Exposures to drug and IGF-1 were notably much higher in

animals with anti-TH9507 antibodies. Effects seen in this study appeared to be secondary or tertiary to pharmacodynamic effects of the drug and were generally consistent with the known effects of increased growth hormone levels in dogs (e.g., acromegaly and diabetes-like symptoms).

Given the very high and variable exposures in this study, it is worth considering the drug-related effects in the low-exposure animals. The male and female animal with the lowest exposures (#2202 and 2504) had 10X and 20X MRHD (AUC basis). They gained ~50% more weight over the study than did controls. The female had severe fissures in the footpads at the end of the study. Effects on RBC parameters, while milder, were evident in both animals. Both animals had notable increases in total cholesterol (particularly LDL) and triglycerides with little effect on insulin or glucose levels. The male had minimal hypertrophy in the stomach, minimal diffuse hyperplasia in the pituitary, and minimal fibrous material in the gallbladder. The female had minimal hypertrophy in the adrenal, minimal hydropic degeneration in the liver, and minimal tubular dilation. These results suggest that increased lipid levels (and increased GH activity) precede the main adverse findings. In clinical studies in lipodystrophy patients, triglycerides and non-HDL cholesterol actually decreased with prolonged TH9507 treatment.

Study: 2875
Volume and page: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: 1 March 2004
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: TH9507, FHEXBRF002A, >98.5% (W1-7)
TH9507, FHEXGRF002B, >98.5% (W8-38, 48-52)
TH9507, FHEXGRF0201, > 98.5% (W39-48)

Methods	
Doses	0, 0.1, 0.6, 1.2 mg/kg once per day
Species/source	Beagle dogs /
Age / Weight	~6 months/ 7.3-8.6 kg (M) and 5.4-7.4 kg(F)
n/sex/group (main study)	4/sex/group
TK groups	6/sex/group for TK and immunologic evaluation
Recovery groups	none
Route, formulation, dose volume	Subcutaneous injection in 1 mL/kg 5% mannitol in sterile water for injection

Observations and Times	
Mortality checks	Twice daily
Clinical Findings	Daily
Body weights	Weekly
Food consumption	Observed daily; Only 400g of chow was offered each day (for a 2h period).
Ophthalmoscopy	Pretreatment, W13, W26
EKG	Predose and 5-15 minutes post dose on D1 and in W13 and W52
Hematology	Pretreatment, W13, 26, 39, and 52
Clinical chemistry	Pretreatment, W13, 26, 39, and 52
Urinalysis	Pretreatment, W13, 26, 39, and 52: sixteen hour samples in main study animals.
Other clinical evaluation	Anti-TH9507 antibody titer and IGF-1 levels: Day 1 (predose) and Weeks 4, 13, 26, 39, and 52
Toxicokinetics	On Day 1 and in W13, 26, 41, and 52, samples were taken at 0, 5, 15, 30,

	60, and 120 minutes post dose and at 4, 8, and 24 hours post dose and measured using LC/MS/MS.
P450 Evaluation	Satellite animals only at Week 4: 3g of liver tissue was assayed for activity of CYP1A1/2, CYP2B1/2, CYP2Ea, CYP3A1/2, and UDPGT
Gross pathology	Main study animals at sacrifice
Organ weights	Main study animals at necropsy: adrenal glands, brain, heart, kidneys, liver, lungs + bronchi, ovaries/testes, pituitary, prostate, spleen, thymus, thyroid and parathyroid, uterus
Histopathology	Performed at necropsy for main study animals: adrenal, aorta, bone and marrow (femur), brain, cecum, colon, duodenum, epididymides, esophagus, eyes, heart, ileum, injection sites, jejunum, kidneys, liver (sample of 2 lobes), lungs + bronchi, lymph nodes (mandibular and mesenteric), mammary gland (inguinal) optic nerves, ovaries, pancreas, pituitary, prostate, rectum, salivary gland (mandibular), sciatic nerve, skeletal muscle, skin + subcutis (inguinal), spinal cord (cervical), spleen, sternum + marrow, stomach, testes, thymus, thyroid and parathyroids, tongue, trachea, urinary bladder, uterus, vagina, gross lesions
	Adequate Battery: yes (x), no ()
	Peer review: yes (X), no ()

Results:

Mortality: One MD female (3602B) was euthanized in W48 due to a suspected case of diabetes. While this animal did not have any marked clinical signs, it lost 3.6 kg between weeks 41 and 48 (↓27% of BW) and other clinical investigations revealed polyuria, glucosuria (5.5 mM), ketonuria (>7.8 mM), marked hyperglycemia (32.3 mM), and low serum insulin (4.6 uIU/mL). An additional female in this group (which was not euthanized) had similar characteristics (2.6 kg body weight loss W46-51, polyuria, glucosuria, hyperglycemia, and hypoinsulinemia).

Clinical signs: As shown in the sponsor's table below, there were dose-dependent increases in the incidence and severity of clinical signs associated with acromegaly, including increased size of body, paws, and abdomen, and skin folds. The sponsor also notes a treatment-related increase in the incidence of foot and leg lesions, which was thought to be secondary to increased body weight.

GROUP 1: CONTROL **GROUP 3: TH9507 (0.6 mg/kg/day)**
GROUP 2: TH9507 (0.1 mg/kg/day) **GROUP 4: TH9507 (1.2 mg/kg/day)**

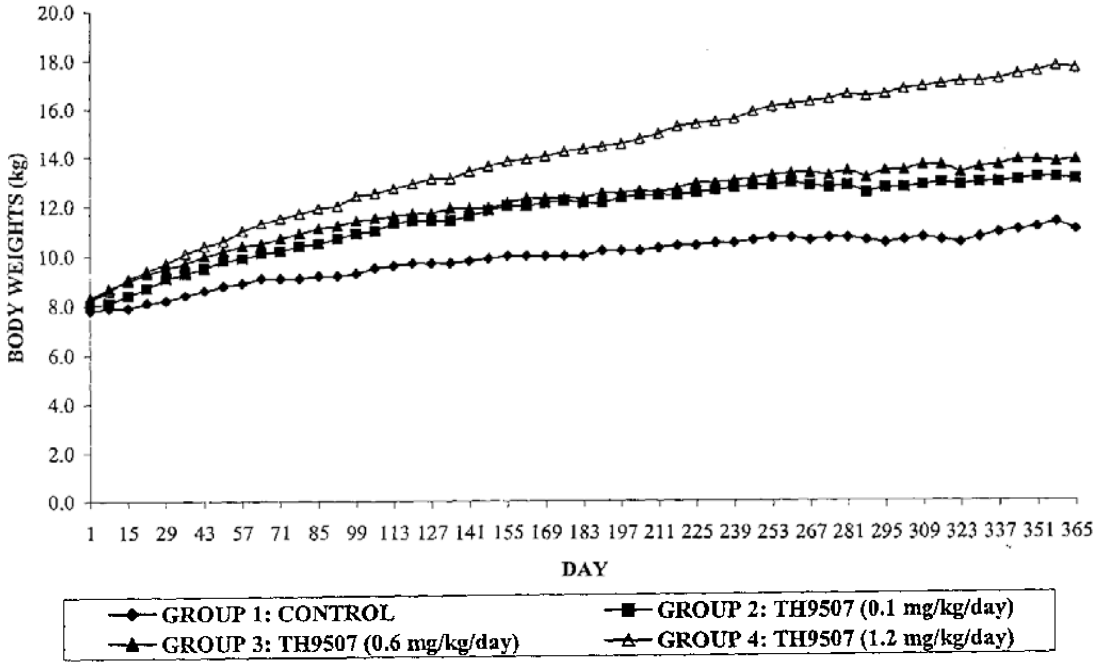
Sex		Male				Female			
Group		1	2	3	4	1	2	3	4
No. of Animals Examined		4	4	4	4	4	4	3	4
Increased body size:	normal	4	0	0	0	4	1	0	0
	slight	0	1	0	0	0	2	2	2
	moderate	0	3	4	0	0	1	1	0
	severe	0	0	0	3	0	0	0	2
	severe/marked	0	0	0	1	0	0	0	0
Skin folds (head):	normal	4	3	1	0	4	2	1	0
	slight	0	1	3	1	0	2	0	2
	moderate	0	0	0	1	0	0	2	0
	severe	0	0	0	1	0	0	0	1
	severe/marked	0	0	0	1	0	0	0	1
Skin folds (limbs/paws):	normal	4	4	2	0	4	2	1	2
	slight	0	0	1	1	0	2	1	0
	moderate	0	0	1	1	0	0	1	0
	severe	0	0	0	2	0	0	0	1
	severe/marked	0	0	0	0	0	0	0	1
Skin folds (flanks/tail):	normal	4	3	3	0	4	4	3	3
	slight	0	1	1	4	0	0	0	0
	moderate	0	0	0	0	0	0	0	1
	severe	0	0	0	0	0	0	0	0
	severe/marked	0	0	0	0	0	0	0	0
Enlarged paws:	normal	4	0	0	0	4	0	0	0
	slight	0	4	1	0	0	3	0	1
	moderate	0	0	3	0	0	1	3	1
	severe	0	0	0	4	0	0	0	1
	severe/marked	0	0	0	0	0	0	0	1
Increased abdomen depth:	normal	4	3	1	0	4	1	1	1
	slight	0	1	2	1	0	2	0	1
	moderate	0	0	1	3	0	1	2	1
	severe	0	0	0	0	0	0	0	1
	severe/marked	0	0	0	0	0	0	0	0

Body weights: Treated animals at all doses gained more weight than controls over the study (↑56-204%), leading to final body weights that were 18 to 59% higher than controls (see the sponsor's figure below). These effects were statistically significant in males and occasionally statistically significant in females (due to greater variability). These effects are consistent with the clinical observations described above.

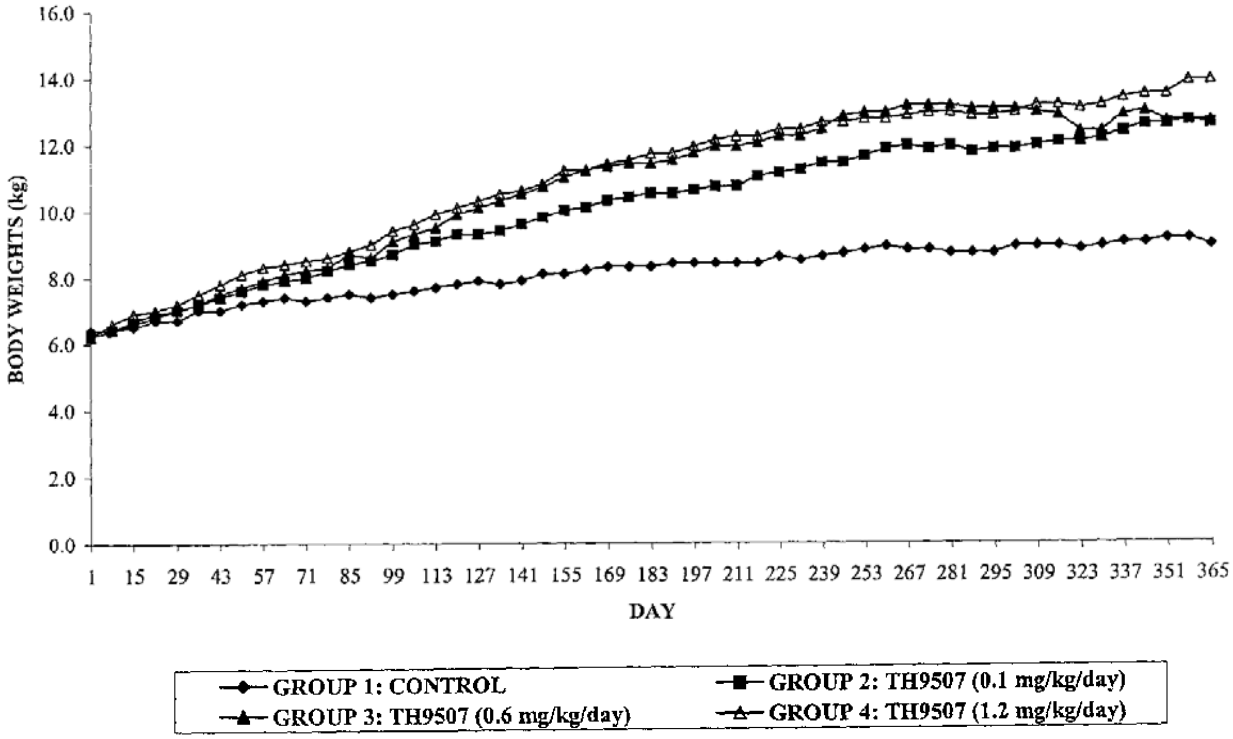
Body Weight					
Sex	Dose, mg/kg	BW gain (kg) over study	% Increment	Final BW (kg)	BW % control
Males	0	3.2 ± 0.26	-	11.0 ± 0.77	100%
	0.1	5.0 ± 0.28	↑56%	13.0 ± 0.21	118%
	0.6	5.5 ± 2.10	↑71%	13.8 ± 2.16*	125%*
	1.2	9.3 ± 1.39	↑190%	17.5 ± 1.53*	159%*
Females	0	2.5 ± 1.08	-	8.9 ± 1.14	100%
	0.1	6.4 ± 2.42	↑156%	12.5 ± 2.5	140%
	0.6	6.2 ± 2.45	↑145%	12.6 ± 3.04	142%
	1.2	7.6 ± 3.46	↑204%	13.8 ± 3.54	155%

* $p<0.05$

MALE BODY WEIGHT



FEMALE BODY WEIGHT



Food consumption: Food consumption was significantly increased in both males and females. The daily allotment of food was limited to 400 g per animal, and treated groups frequently consumed their entire portion, while control animals rarely did. In the final week, food consumption was ~30% higher than controls in treated males and ~20% higher in treated females.

Ophthalmoscopy: There were no treatment-related effects at W13 or W52.

EKG: There were no treatment-related effects.

Hematology: Decreases in RBC, hemoglobin, and hematocrit were noted by W13 at all doses. This effect became statistically significant at W13 for females and W52 for males. Low dose females appeared to adapt somewhat to this effect, as their RBC parameters were not statistically different from controls after W26. There were no statistically significant compensatory differences in reticulocyte number (but the fraction of reticulocytes was increased 40-60% in females receiving ≥ 0.6 mg/kg at W52).

RBC PARAMETERS, MALES

Dose, mg/kg	RBC (10^{12}/L)		Hgb(g/L)		Hct (g/L)	
	Pre	W52	Pre	W52	Pre	W52
0	6.49	6.95	141	160	0.45	0.48
0.1	6.21	5.54*	134	130*	0.42	0.39*
0.6	6.52	5.29*	141	125*	0.45	0.37*
1.2	6.19	5.12*	142	126*	0.45	0.39*

RBC PARAMETERS, FEMALES

Dose, mg/kg	RBC (10^{12}/L)		Hgb(g/L)		Hct (g/L)	
	Pre	W52	Pre	W52	Pre	W52
0	6.85	6.99	154	164	0.49	0.49
0.1	6.43	6.30	143	149	0.45	0.44
0.6	6.25	5.42*	142	130*	0.44	0.39*
1.2	6.32	5.28*	141	125*	0.45	0.37*

Clotting parameters: Increased platelets in treated animals (↑54-117%) at W52 correlated with decreases in APTT, which were statistically significant at ≥ 0.6 mg/kg as shown in the tables below.

CLOTTING PARAMETERS, MALES

Dose, mg/kg	Platelets (10^9/L)		APTT (sec)		PT (sec)	
	Pre	W52	Pre	W52	Pre	W52
0	267	274	9.7	8.9	6.3	6.5
0.1	354	529* ↑93%	10.2	8.5	6.4	6.3
0.6	275	502* ↑83%	9.8	8.0* ↓10%	6.4	6.3
1.2	259	511* ↑86%	9.5	8.0* ↓10%	6.7	6.6

CLOTTING PARAMETERS, FEMALES

Dose, mg/kg	Platelets (10^9/L)		APTT (sec)		PT (sec)	
	Pre	W52	Pre	W52	Pre	W52
0	287	310	9.5	8.9	6.5	6.5
0.1	310	478 ↑54%	10.1	8.2	6.7	6.5
0.6	236	673* ↑117%	9.9	8.0* ↓10%	6.4	6.3
1.2	232	581* ↑87%	10.2	8.1* ↓9%	6.5	6.5

Clinical chemistry: Clinical chemistry changes of note are shown in the tables below. Changes in lipid markers, including increases in cholesterol and triglycerides, was evident beginning W13 and tended to increase with longer dosing. The data for lipids at the end of the study is shown below. Treated animals had cholesterol values that were 2-7X controls. LDL levels were 14-85X controls while HDL levels only increased by 50-100%. Triglyceride levels were also elevated, especially at the high dose.

LIPIDS, WEEK 52								
Dose, mg/kg	Cholesterol (mM)		LDL (mg/mL)		HDL (mg/mL)		TG (mM)	
	Male	Female	Male	Female	Male	Female	Male	Female
0	3.83	4.83	5	7	126	158	0.35	0.42
0.1	10.80	11.03*	125	98	236*	267	1.48	1.46
0.6	12.19	12.73*	166	162	255*	287	2.82	1.23
1.2	28.83*	15.18*	429*	213*	275*	299*	7.93*	1.32*

* $p < 0.05$

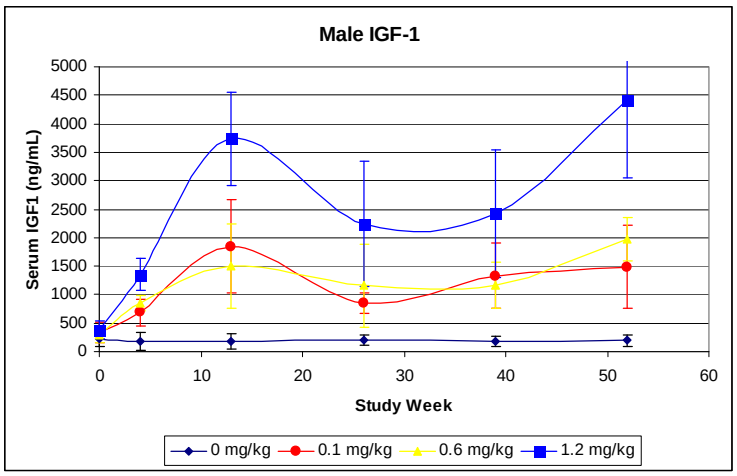
There was also clear evidence of disturbance of electrolyte homeostasis by the end of the study. Generally, chloride and sodium levels were lower in treated animals in a dose-dependent manner, while potassium and phosphorus were elevated. These effects are consistent with decreased aldosterone levels.

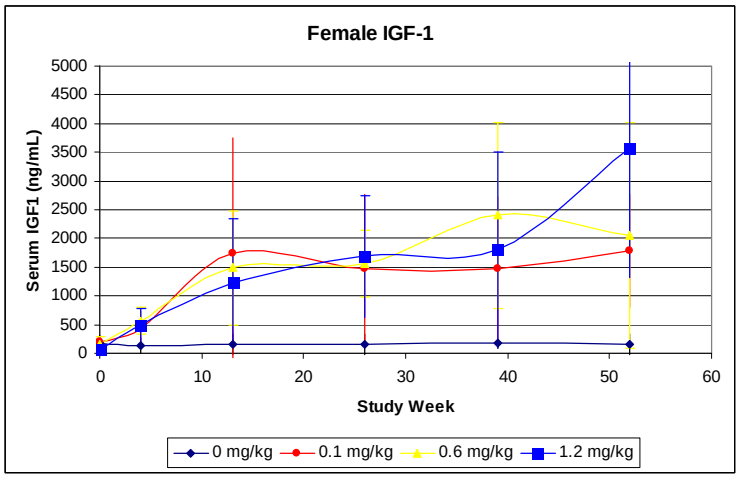
ELECTROLYTES, WEEK 52								
Dose, mg/kg	Chloride (mM)		Potassium (mM)		Sodium (mM)		Phosphorous (mM)	
	Male	Female	Male	Female	Male	Female	Male	Female
0	118	117	4.4	4.5	154	154	1.19	1.25
0.1	113	112*	5.0*	4.8	152	153	1.72*	1.70*
0.6	111*	113	5.3*	5.2	150	152	2.08*	1.69*
1.2	108*	110*	5.2*	5.3	148	149	1.85*	1.78*

Marked increases in fasted serum insulin were seen in 4/8, 5/7, and 8/8 animals in the low, mid, and high dose groups, respectively, at W52, consistent with development of insulin resistance. CRP and globulin levels were higher in treated females compared to controls, but these effects were not observed in male animals.

OTHER CLINICAL CHEMISTRY, WEEK 52						
Dose, mg/kg	CRP (mg/L)		Globulin		Insulin (μIU/mL)	
	Male	Female	Male	Female	Male	Female
0	0.06	0.02	30	23	3.6 ± 2	5 ± 5
0.1	0.03	0.03	32	29*	11 ± 13	29 ± 18
0.6	0.02	0.05	31	30*	27 ± 21	45 ± 48
1.2	0.02	0.07*	33	32*	114 ± 55*	101 ± 104

IGF-1 levels were markedly increased in treated animals, as shown in the graphs below. The effect was not always dose dependent (especially in females).





Urinalysis: Dose-dependent increases in urine volume (↑100-150%) were noted in animals receiving ≥ 0.6 mg/kg beginning in W39 (females) and by W52 (males). Glucosuria was noted in 2/4 MDF and 1/4 HDM.

Gross pathology: Some findings that occurred more often in treated animals are shown in the table below. Enlargement of the adrenal gland and pituitary appeared to be drug-related, as did thickening in a number of organs.

GROSS PATHOLOGY

Tissue	Finding	Dose	MALES				FEMALES			
		n	0 4	0.1 4	0.6 4	1.2 4	0 4	0.1 4	0.6 4	1.2 4
Adrenal	Enlargement		0	2	3	1	0	3	3	4
Brain	Depressed area		0	0	1	1	0	0	1	1
Bone Marrow	Gelatinous material		0	0	0	0	0	0	1	0
Digestive Contents	Dark Fluid		0	0	0	0	0	0	1	1
	Opaque fluid		0	0	0	0	0	0	0	1
Esophagus	Thickening		0	1	1	1	0	0	1	1
Femur & marrow	Thickening		0	0	2	2	0	0	0	2
Gallbladder	Dark material		0	0	1	3	0	0	2	2
	Dilatation		0	1	1	1	0	0	1	2
Lymph node	Dark discoloration		1	1	2	2	0	3	2	4
Lung	Dark area		1	1	3	1	0	2	2	2
Pituitary	Enlargement		0	2	4	4	0	1	4	4
	Pale discoloration		0	2	4	2	0	2	3	4
	Soft		0	1	4	3	0	3	3	4
Spleen	Small		0	0	2	2	0	0	2	2
Skin & subcutis	Thickening		0	0	1	3	0	1	1	2
	Scab		0	0	0	0	0	0	1	2
	Wound		0	0	0	1	0	0	1	1
Stomach	Thickening		0	4	3	3	0	3	3	3
	Dark focus		0	2	0	2	1	2	2	2
Urinary bladder	Thickening		0	0	2	2	0	1	1	1

Organ weights: Consistent with the observations of enlargement at necropsy, the weights of the adrenal and pituitary were increased in treated animals of both sexes. Liver weights also increased. Spleen weights decreased, consistent with the findings of "small" at gross necropsy. Relevant data is shown below as the absolute organ weight or percent body weight (controls) or percent change from control (treated animals).

ORGAN WEIGHTS, MALES										
Dose	Adrenal		Liver		Pituitary		Spleen		Kidney	
mg/kg	gram	%BW*10^3	gram	% BW	mg	%BW*10^3	gram	% BW	gram	% BW
0	1.29	11.9	322	2.93	71.4	0.7	180	1.68	65	0.59
0.1	86%	55%*	79%*	52%*	215%	143%	-69%*	-74%	23%	5%
0.6	150%*	96%*	118%*	74%*	834%*	600%*	-78%*	-82%*	40%	10%
1.2	87%	18%	162%*	67%*	908%*	471%*	-69%*	-81%*	46%	-8%

* $p < 0.05$; Values for drug-treated groups are percent change from control

ORGAN WEIGHTS, FEMALES										
Dose,	Adrenal		Liver		Pituitary		Spleen		Kidney	
mg/kg	gram	%BW*10^3	gram	% BW	mg	%BW*10^3	gram	% BW	gram	% BW
0	1.25	14.2	237	2.70	72	0.9	123	1.39	41	0.46
0.1	138%	31%	127%	64%*	264%	111%	-44%	-57%*	78%*	28%
0.6	107%*	47%	156%	80%*	679%*	389%*	-69%*	-80%*	93%*	39%
1.2	129%*	56%	187%*	195%*	1033%*	500%*	-66%*	-78%*	88%*	22%

* $p < 0.05$; Values for drug-treated groups are percent change from control

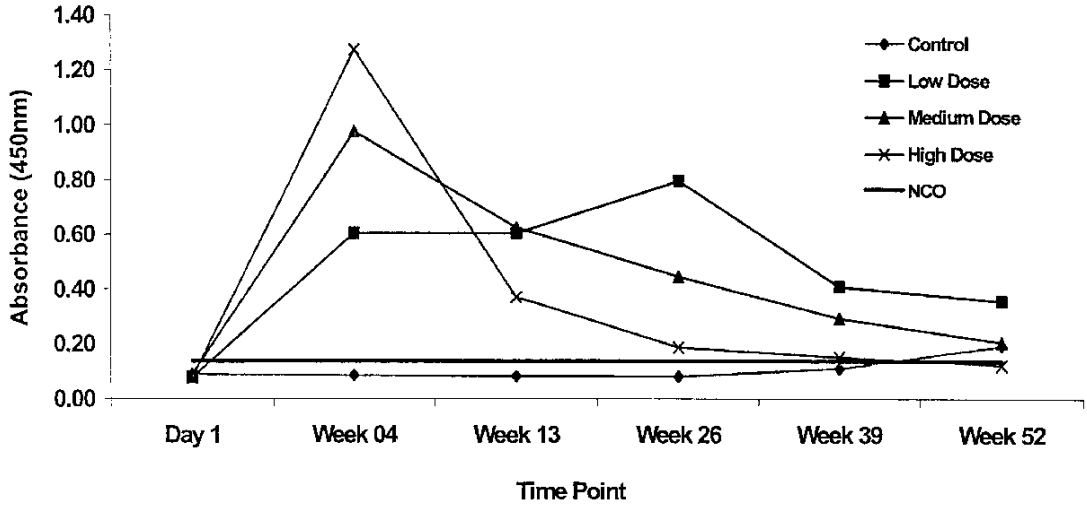
Histopathology: Most of the changes observed microscopically could be attributed to pharmacodynamic activity of the drug, including hypertrophy in numerous tissues (e.g., adrenal, esophagus, femur, GI tract, and bladder). Other changes appeared to be secondary to metabolic changes in these animals, including alterations in the gallbladder and pancreas, hydropic degeneration of the liver, and vacuolar degeneration in the kidney. The findings shown in the table below occurred more often in treated animals than in controls.

HISTOPATHOLOGY										
Tissue	Finding	Dose	MALES				FEMALES			
			0	0.1	0.6	1.2	0	0.1	0.6	1.2
		n	4	4	4	4	4	4	4	4
Adrenal Gland	Degeneration, vacuolar	Minimal	0	0	0	1	0	1	2	3
		Mild		1	1	1	0	0	0	1
	Hypertrophy	Minimal	0	1	0	1	0	2	2	1
		Mild		0	3	0	0	2	1	2
Esophagus	Hypertrophy	Minimal	0	1	0	2	0	0	0	1
		Mild		0	0	0			1	0
		Moderate		0	1	0			0	0
Eye	Mucoid degeneration	Moderate	0	0	0	0	0	0	1	0
	Cataract	Minimal	0	0	1	0	0	0	0	0
		Moderate			0				1	
		Severe			0				1	
Femur & marrow	Hypertrophy	Minimal	0	0	1	1	0	0	0	1
		Mild			0	0				1
		Moderate			0	1				0
Gallbladder	Degeneration, vacuolar	Minimal	0	1	1	1	0	1	1	0
		Mild		0	0	2		1	2	0
		Moderate		0	1	0		0	0	1
	Fibrous material, basophilic	Minimal	0	1	2	1	0	1	1	0
		Mild		2	1	1		2	2	1
		Moderate		0	1	1		0	1	0
		Severe		0	0	1		0	0	1
Heart	Mucoid degeneration	Moderate	0	0	0	0	0	0	1	0
	Calcification, metastatic	Minimal	0	0	0	1	0	0	0	0
Kidney	Tubular dilatation	Minimal	0	1	0	3	0	1	1	3
		Mild		1	1	0		0	0	0
	Degeneration, vacuolar, of collection ducts	Minimal	0	0	0	0	0	1	3	2
		Mild				1		0	0	2
	Basophilia, tubular	Minimal	1	1	1	0	0	1	1	3
		Mild	0	0	2	1		0	1	0
		Moderate	0	1	0	0		0	0	0
	Cell infiltrate, mononuclear	Minimal	0	1	3	1	0	1	1	0
		Mild		1	0	1		0	0	1
Liver	Degeneration	Minimal	0	0	0	0	0	1	0	1
		Mild		2	3	0		3	1	0
		Moderate		0	0	2		0	1	3
		Severe		0	1	2		0	1	0
Lung	Macrophages, alveolar	Minimal	0	0	0	0	0	0	0	1
		Mild			1	1				1
Pancreas	Degeneration, microcystic, of exocrine ducts	Minimal	0	0	1	2	0	0	2	2
		Mild			1	1		1	1	0
	Degeneration, vacuolar, epithelial	Minimal	0	0	0	1	0	0	0	2
		Mild				1			0	0
		Moderate				0			2	0
Pituitary	Hyperplasia, diffuse	Minimal	0	1	0	0	0	1	0	0
		Mild		1	1	1		1	2	0
		Moderate		1	2	2		1	2	2
		Severe		0	1	1		0	0	2

Duodenum	Hypertrophy	Minimal Mild	0	0	2 0	0 1	0	0 1	0	1 0
Spleen	Decreased blood volume	Minimal Mild	0	0	0 2	0 2	0	0	0 3	1 2
Sternum & marrow	Hypocellular marrow	Minimal Mild	0	0	0	1 0	0	0	0 1	0
Skin & subcutis	Hypertrophy, dermal	Minimal Mild Moderate	0	0	1 1 0	2 2 0	0	1 1 0	1 1 0	0 1 2
	Pododermatitis, ulcerative	Moderate Severe	0	0	0	0 1	0	0	1 0	1 0
	Calcinosis circumscripta	Moderate	0	0	0	0	0	0	0	1
Stomach	Calcification	Minimal Mild	0	0	0	0	0	0	1 1	0
	Hypertrophy	Minimal Mild Moderate	0	2 1 1	0 2 1	1 2 0	0	1 2 0	0 2 1	0 1 2
Thymus	Atrophy	Minimal Mild Moderate Severe	1 0 0 0	2 0 1 0	0 1 2 0	2 1 1 0	1 0 0 0	0 1 0 0	0 1 1 2	1 1 1 0
Thyroid	Increased C-cell complexes	Minimal Mild	0	0	0 1	0 1	0	0	1 0	0 1
Urinary bladder	Hypertrophy	Minimal Mild Moderate	0	0	0 2 0	1 3 0	0	0 1 0	0	0 0 1
Injection site L4	Necrosis, diffuse	Minimal Mild	0	1 0	0	1 1	0	0	1 1	2 1
	Hypertrophy, dermal	Minimal Mild Moderate	0	0	2 0 0	3 1 0	0	1 0 0	2 0 0	0 0 2
	Cell infiltrate, mononuclear	Minimal Mild Moderate	0	1 1 0	0 2 1	2 1 0	0 0 1	2 1 0	0 3 1	0 3 1
Injection site R4	Necrosis, diffuse	Mild	0	0	1	1	0	0	0	0
	Hypertrophy, dermal	Minimal Mild Moderate	0	0	2 0 0	2 2 0	0	1 0 0	1 1 0	0 0 2

Anti-drug antibodies: Antibodies to TH9507 were detected in two control animals, four animals given 0.1 mg/kg, and seven animals each in the mid- and high dose groups. In animals receiving ≥ 0.6 mg/kg, the titers tended to peak at week 4 and decrease thereafter. The sponsor does not offer an explanation for the appearance of anti-TH9507 antibodies in the control animals but calls the results, as well as those of a high dose animal, atypical.

Figure 2 **Mean Absorbance Results for Anti-TH9507 IgG Antibodies in Screening Assay for Control, Low (0.1 mg/kg/day), Medium (0.6 mg/kg/day), and High (1.2 mg/kg/day) TH9507 Dosing Levels (8 Dogs per Group)**



Toxicokinetics: The sponsor's table below shows TK parameters from D1 and Week 52. Note that exposures are measured as $AUC_{0\text{-}90min}$ in ng.min/mL rather than ng.h/mL. There was large variability in C_{max} and AUC values, but both parameters increased drastically with repeated dosing in all dose groups.

Parameter	0.1 mg/kg/day		0.6 mg/kg/day		1.2 mg/kg/day	
	Males	Females	Males	Females	Males	Females
Day 1						
C_{max} (ng/mL)	3.03±0.83	6.31±2.73	56.6±26.2	46.8±20.3	71.7±21.0	59.3±6.5
T_{max} (min)	20.0±12.2	41.3±33.3	22.5±8.7	30.0±21.2	18.8±7.5	22.5±8.7
$t_{1/2}$ (min)	ND[a]	ND[a]	22.3±7.2	14.2[b]	22.6±9.2	14.8±2.0
$AUC_{(0\text{-}90min)}$ (ng·min/mL)	98.0±29.0	160±72	2152± 1114	1781± 942	2802± 1134	2285± 386
$AUC_{(0\text{-}\infty)}$ (ng·min/mL)	ND[a]	ND[a]	2043[b]	1303[b]	3209± 1718	2356± 431
Week 52						
C_{max} (ng/mL)	110±149	210±256	551±312	618±480	794±374	1192±588
T_{max} (min)	26.3±7.5	33.8±18.9	33.8±18.9	45.0±17.3	30.0±0.0	45.0±17.3
$t_{1/2}$ (min)	34.1[b]	62.9±29.7	25.8[b]	83.5[b]	57.5±25.1	22.8[b]
$AUC_{(0\text{-}90min)}$ (ng·min/mL)	6873± 10182	15289± 20103	33108± 21962	40520± 29402	50192± 27456	87654± 50405

Data were also collected during Weeks 13, 26, and 41 but are not reported in this table.
a Not determined
b Standard deviation not calculated due to n<3.

Changing the units to the more commonly used ng.h/mL gives the mean exposure parameters described in the table below. TK samples were only taken over the first 90 minutes post dose on most occasions. During Week 41, more comprehensive analysis was done, covering a full 24 hours post dose. Since $AUC_{0\text{-}90}$ values were similar between W41 and W52, the more comprehensive $AUC_{0\text{-}24h}$ values from W41 will be used for the assessment of safety. During W41, mean exposures were ≥ 284X MRHD, and C_{max} values were ≥ 50X MRHD. There was quite a bit of variability in TK parameters (as shown in the sponsor's table above with large SD values). For example, at the low dose, the range in W41 TK values was 10X MRHD in an antibody negative LDM to >7000X for one antibody positive LDF.

Toxicokinetic Parameters							
Parameter	Occasion	0.1 mg/kg		0.6 mg/kg		1.2 mg/kg	
		M	F	M	F	M	F
C_{max} (ng/mL)	Day 1	3	6.3	56.6	46.8	72	59
	Week 41	119	220	607	745	1181	1362
	Week 52	110	210	551	618	794	1192
$AUC_{0\text{-}90\ min}$ (ng.h/mL)	Day 1	1.6	2.7	36	30	47	38
	Week 41	128	222	643	876	1,325	1,434
	Week 52	115	255	552	675	837	1,461
$AUC_{0\text{-}24h}$ (ng.h/mL)	Week 41	318	2,138	1,545	3,944	3,776	7,205
Multiples of Human Exposure							
C_{max} (multiple of 2.3 ng/mL)	Day 1	1	3	25	20	31	26
	Week 41	52	96	264	324	513	592
	Week 52	48	91	240	269	345	518
$AUC_{0\text{-}90\ min}$ (multiple of 1.12 ng.h/mL)	Day 1	1.5	2.4	32	27	42	34
	Week 41	114	198	574	782	1,183	1,280
	Week 52	102	228	493	603	747	1,304
$AUC_{0\text{-}24h}$ (multiple of 1.12 ng.h/mL)	Week 41	284	1,909	1,379	3,522	3,371	6,433

As shown in the sponsor's table below, the large increases in IGF-1 and drug exposure observed over time appear to be somewhat dependent on the development of anti-drug antibodies; however, drug accumulation of 5- to 18-fold versus Day 1 occurred in animals without detectably anti-drug antibodies.

IgG Positive								
Dose (mg/kg/day)	**IGF-1 (ng/mL)**				**TH9507 $AUC_{(0\text{-tlast})}$ ng·min/mL**			
		Week 1	**Week 52**	**Fold increase**		**Week 1**	**Week 52**	**Fold increase**
0.1	Mean n=4	213	1811	8.5	Mean n=4	135	20529	152
	SD	112	959		SD	80	17791	
0.6	Mean n=6	240	1274	5.3	Mean n=6	2295	52837	23
	SD	139	1057		SD	899	24021	
1.2	Mean n=6	277	4593	17	Mean n=6	2343	89525	38
	SD	238	2144		SD	307	48302	
IgG Negative								
Dose (mg/kg/day)	**IGF-1 (ng/mL)**				**TH9507 $AUC_{(0\text{-tlast})}$ ng·min/mL**			
		Week 1	**Week 52**	**Fold increase**		**Week 1**	**Week 52**	**Fold increase**
0.1	**Mean n=4**	317	1465	**4.6**	**Mean n=4**	123	1634	**13**
	SD	194	730		**SD**	46.5	696	
0.6	**Mean n=6**	205	2061	**10**	**Mean n=6**	1769	27200	**15**
	SD	167	1420		**SD**	1064	21000	
1.2	**Mean n=6**	189	3379	**18**	**Mean n=6**	2744	48321	**18**
	SD	169	1324		**SD**	1189	27662	

2.6.6.4 Genetic toxicology

BACTERIAL MUTATION ASSAY (AMES TEST) [TH9507]

Key findings: TH9507 was not mutagenic in the Ames test at concentrations up to 5000 µg/plate.

Study: E-PCL-295
Volume and page: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: 27 October 2000
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: TH9507, THEXGRF0001, 99.3%

Methods

Strains/species/cell line: *Salmonella typhimurium* histidine auxotrophs TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2 *uvr*A in the absence and presence of Aroclor 1254-induced rat liver S9.

Doses used in definitive study: 0, 33, 100, 333, 1000, 3333, and 5000 µg/plate

Basis of dose selection: Doses were chosen following initial assays of solubility (up to 500 mg/mL in water) and cytotoxicity (up to 5000 µg/plate). There were no conditions causing precipitate or appreciable toxicity, so the limit dose of 5000 µg/plate was selected as the high dose.

Negative controls: Vehicle (distilled water)

Positive controls: Positive controls are shown in the table below.

Strain	S9	Positive control	Concentration (µg/plate)
All *salmonella strains*	+	2-aminoanthracene	1.0
WP2 *uvr*A	+	2-aminoanthracene	10
TA98	-	2-nitrofluorene	1.0
TA100, TA1535	-	sodium azide	1.0
TA1537	-	9-aminoacridine	75
WP2 *uvr*A	-	Methyl methanesulfonate	1000

Incubation and sampling times: Bacterial strains were exposed to TH9507 and relevant controls using the plate incorporation technique. Plates were incubated 48-72 hours at 37ºC prior to colony counting.

Results

Study validity: Criteria for a valid assay included confirmation of strain integrity, vehicle controls within a specified range, positive controls with at least a 3X increase over vehicle control, and a minimum of three non-toxic doses for evaluation. The study met these criteria. S9 batches used had been appropriately characterized for their ability to metabolize both 2-aminoanthracene and 7,12-dimethylbenz(a)anthracene, and the positive controls used in the study were appropriate.

Study outcome:

Preliminary Plate Incorporation: In the initial toxicity-mutation assay, doses from 2.5 to 5000 µg/plate were evaluated for toxicity and mutations. There was no evidence of cytotoxicity in strains exposed to any dose of TH9507 (all plates scored as "normal"). The number of revertants was not significantly increased under any assay condition as shown in the table below.

Initial Toxicity-Mutation Assay Results

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2*uvrA*
- S9					
Vehicle	14 ± 1	97 ± 13	13 ± 2	6 ± 0	12 ± 1
2.5	17 ± 7	113 ± 4	12 ± 1	4 ± 1	11 ± 1
7.5	15 ± 1	82 ± --	13 ± 4	-- ± --	10 ± 3
25	14 ± 1	107 ± 3	9 ± 1	3 ± 1	12 ± 3
75	17 ± 5	99 ± 8	15 ± 1	5 ± 2	13 ± 1
200	20 ± 8	89 ± 1	8 ± 0	4 ± 3	12 ± 1
600	19 ± 1	90 ± 2	10 ± 1	8 ± 1	9 ± 1
1800	14 ± 1	57 ± 1	10 ± 3	9 ± 4	12 ± 3
5000	4 ± 1	25 ± 1	15 ± 5	6 ± 1	8 ± 4
Positive	209 ± 8	365 ± 5	232 ± 31	557 ± 190	87 ± 6
+S9					
Vehicle	16 ± 2	114 ± 16	11 ± 2	6 ± 1	18 ± 4
2.5	21 ± 2	103 ± 28	12 ± 3	6 ± 7	16 ± 6
7.5	28 ± 4	104 ± 8	7 ± 1	5 ± 2	11 ± 4
25	17 ± 4	107 ± 11	9 ± 3	4 ± 1	15 ± 5
75	21 ± 10	118 ± 32	10 ± 1	4 ± 2	16 ± 6
200	27 ± 4	112 ± 4	11 ± 3	6 ± 1	11 ± 4
600	26 ± 0	105 ± 6	16 ± 6	7 ± 2	17 ± 9
1800	18 ± 4	88 ± 8	14 ± 2	12 ± 1	10 ± 1
5000	23 ± --	46 ± 2	12 ± 3	9 ± --	6 ± 1
Positive	1025 ± 76	1863 ± 226	139 ± 15	115 ± 63	350 ± 14

Main Plate Incorporation: In the definitive assay, results for cytotoxicity and mutagenicity were similar to the initial assay. All plates were scored as "normal" for the condition of the background lawn, and no assay condition caused a significant increase in revertants. Positive controls were all at least three times greater than vehicle controls. The results confirm that TH9507 was not mutagenic in this assay.

Confirmatory Mutation Assay Results

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2*uvrA*
- S9					
Vehicle	18 ± 2	110 ± 3	11 ± 3	8 ± 2	13 ± 2
33	16 ± 3	103 ± 6	8 ± 3	7 ± 1	12 ± 3
100	15 ± 2	105 ± 17	8 ± 2	5 ± 2	15 ± 5
333	15 ± 2	106 ± 9	9 ± 2	2 ± 1	13 ± 3
1000	13 ± 2	82 ± 10	10 ± 2	4 ± 1	13 ± 1
3333	10 ± 1	52 ± 20	8 ± 2	5 ± 2	11 ± 1
5000	9 ± 3	37 ± 5	9 ± 1	5 ± 2	11 ± 2
Positive	240 ± 6	578 ± 55	269 ± 75	472 ± 46	115 ± 17
+S9					
Vehicle	18 ± 2	98 ± 11	12 ± 1	5 ± 3	16 ± 3
33	15 ± 3	97 ± 9	10 ± 1	7 ± 1	12 ± 3
100	17 ± 2	99 ± 13	10 ± 2	5 ± 1	12 ± 2
333	20 ± 0	99 ± 13	9 ± 2	6 ± 0	11 ± 2
1000	15 ± 2	105 ± 15	10 ± 2	5 ± 1	12 ± 1
3333	12 ± 2	63 ± 17	9 ± 2	5 ± 2	11 ± 4
5000	12 ± 1	41 ± 10	8 ± 3	6 ± 2	8 ± 3
Positive	604 ± 60	638 ± 12	63 ± 8	57 ± 13	74 ± 9

IN VITRO MAMMALIAN CHROMOSOME ABERRATION TEST [TH9507]

Key findings: TH9507 did not cause structural or numerical chromosome aberrations in CHO cells.

Study:	E-PCL-296
Volume and page:	eCTD
Conducting laboratory and location:	(b) (4)
Date of study initiation:	24 October 2000
GLP compliance:	Yes
QA report:	Yes
Drug, lot #, and % purity:	TH9507, THEXGRF0001, 99.3%

Methods

Strains/species/cell line: Chinese hamster ovary (CHO-K_1) cells ((b) (4)); passage number ≤ 20.

Doses used in definitive study:

4 hrs with S9:	125, 250, 500, 1000, 1200, 1400, 1600, 1800, 2000 µg/mL
4 hrs without S9:	125, 250, 500, 1000, 1200, 1400, 1600, 1800, 2000 µg/mL
24 hrs without S9:	125, 250, 500, 1000, 1200, 1400, 1600, 1800, 2000 µg/mL

Basis of dose selection: Doses were selected based on the results of a preliminary toxicity assay simulating the exposure conditions of the definitive chromosome aberration assay. In this initial experiment, CHO cells were exposed to 9 concentrations of TH9507 (0.5-5000 µg/mL), as shown in the sponsor's tables below. Precipitation of (presumed) test article was seen at 5000 µg/mL (-S9) and ≥ 150 µg/mL (+S9). Cell growth inhibition was 100% for all conditions with 5000 µg/mL of TH9507, so this dose was excluded from further testing.

Preliminary Toxicity Test

4 hours (-S9)

Treatment	Cell Count ($x10^6$)	Cell Viability (%)	Viable Cells/ Flask ($x10^6$)	Cell Growth Index (%)	Cell Growth Inhibition (%)
Water	1.71	97%	1.66	100%	
TH9507 0.5 ug/mL	1.87	98%	1.83	110%	-10%
1.5 ug/mL	1.31	97%	1.27	77%	23%
5 ug/mL	1.53	97%	1.48	90%	10%
15 ug/mL	1.76	97%	1.71	103%	-3%
50 ug/mL	1.43	100%	1.43	86%	14%
150 ug/mL	1.08	96%	1.04	63%	37%
500 ug/mL	1.35	98%	1.33	80%	20%
1500 ug/mL	0.95	96%	0.91	55%	45%
5000 ug/mL	0.69	0%	0.00	0%	100%

4 hours (+S9)

Treatment	Cell Count ($x10^6$)	Cell Viability (%)	Viable Cells/ Flask ($x10^6$)	Cell Growth Index (%)	Cell Growth Inhibition (%)
Water	1.67	97%	1.62	100%	
TH9507 0.5 ug/mL	1.63	97%	1.58	98%	2%
1.5 ug/mL	1.23	96%	1.18	73%	27%
5 ug/mL	1.71	98%	1.67	104%	-4%
15 ug/mL	1.50	96%	1.44	89%	11%
50 ug/mL	1.48	98%	1.43	89%	11%
150 ug/mL	1.27	97%	1.24	76%	24%
500 ug/mL	1.39	97%	1.35	84%	16%
1500 ug/mL	0.82	95%	0.80	50%	50%
5000 ug/mL	0.51	0%	0.00	0%	100%

20 hours (-S9)

Treatment	Cell Count ($x10^6$)	Cell Viability (%)	Viable Cells/ Flask ($x10^6$)	Cell Growth Index (%)	Cell Growth Inhibition (%)
Water	1.42	98%	1.39	100%	
TH9507 0.5 ug/mL	1.42	100%	1.42	102%	-2%
1.5 ug/mL	1.47	98%	1.44	103%	-3%
5 ug/mL	1.34	99%	1.32	95%	5%
15 ug/mL	1.28	100%	1.28	92%	8%
50 ug/mL	1.15	100%	1.15	83%	17%
150 ug/mL	0.81	98%	0.80	57%	43%
500 ug/mL	0.87	98%	0.85	62%	38%
1500 ug/mL	0.42	97%	0.41	30%	70%
5000 ug/mL	0.25	0%	0.00	0%	100%

Negative controls: Vehicle (sterile water)

Positive controls: Mitomycin C (MMC) and cyclophosphmide (CP)

Incubation and sampling times: The cells were incubated for 4 hours (±S9) or 20 hours (-S9) in a cell culture incubator at 37ºC. For the 4-hour treatment, the culture media was replaced with drug-free media after 4 hours. All preparations received Colcemid solution (final concentration 0.1 µg/mL) for the final two hours of incubation.

Results

Study validity: Adequate criteria for a valid test were outlined in the protocol and met by the study. S9 batches used had been appropriately characterized for their ability to metabolize both 2-aminoanthracene and 7,12-dimethylbenz(a)anthracene, and the positive controls used in the study were appropriate.

Study outcome: Samples with at least a 50% inhibition of cell growth (mitotic index) were chosen as the highest dose for analysis. As shown in the sponsor's table below, there was no condition under which TH9507 caused a significant increase in aberrations. The positive controls have the expected results.

SUMMARY

Treatment	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored	Aberrations Per Cell (Mean +/- SD)	Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
Water	-	4	11.0	200	0.030 ±0.171	1.0	3.0
TH9507							
500 ug/mL	-	4	9.3	200	0.035 ±0.210	2.5	3.0
1000 ug/mL	-	4	7.9	200	0.045 ±0.252	1.5	3.5
1400 ug/mL	-	4	4.5	200	0.060 ±0.238	1.0	6.0
MMC, 0.2 ug/mL	-	4	6.5	100	0.760 ±1.577	1.5	40.0**
Water	+	4	10.2	200	0.005 ±0.071	4.0	0.5
TH9507							
500 ug/mL	+	4	9.5	200	0.010 ±0.100	3.5	1.0
1000 ug/mL	+	4	9.4	200	0.005 ±0.071	4.5	0.5
1400 ug/mL	+	4	4.3	200	0.015 ±0.122	4.0	1.5
CP 10 ug/mL	+	4	2.8	200	0.155 ±0.460	4.0	13.0**
Water	-	20	7.9	200	0.025 ±0.157	0.5	2.5
TH9507							
125 ug/mL	-	20	7.3	200	0.045 ±0.231	1.5	4.0
250 ug/mL	-	20	6.2	200	0.025 ±0.157	1.0	2.5
500 ug/mL	-	20	3.6	200	0.030 ±0.171	1.0	3.0
MMC, 0.1 ug/mL	-	20	5.8	100	1.410 ±2.297	1.0	54.0**

MAMMALIAN ERYTHROCYTE MICRONUCLEUS TEST [TH9507]

Key findings: TH9507 did not cause an increase in micronucleated PCEs in ICR mice at doses up to 100 mg/kg.

Study: E-PCL-297
Volume and page: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: 26 October 2000
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: TH9507, THEXGRF0001, 99.3%

Methods	
Doses	Pilot Toxicity Study: 1, 10, 100, 1000, 2000 mg/kg Toxicity Study: 200, 400, 600, 800 mg/kg Micronucleus Study: 0, 25, 50, 100 mg/kg

Species/source	ICR mice, 6-8 weeks old, from (b) (4)
Number/sex/group	Pilot Toxicity Study: 5/sex (high dose), 2 males/group for lower doses Toxicity Study: 5/sex/group Micronucleus Study: 5/sex/group
Route, formulation, dose volume	i.p. injection in water in 20 mL/kg
Study design	In the pilot and main toxicity studies, animals were given a single i.p. dose and observed for 3 days for clinical signs and body weights. In the micronucleus assay, animals were given a single dose and euthanized after 24 hours (all doses) or 48 hours (control and HD animals only). Cyclophosphamide (50 mg/kg) was used as a positive control.

Results:

Pilot toxicity study: All animals given ≥ 1000 mg/kg died after dosing. Lethargy and ~6% weight loss were seen at ≥ 10 mg/kg while piloerection was observed at ≥ 100 mg/kg. Animals given 1 mg/kg had no treatment-related clinical signs.

Main toxicity study: As shown in the sponsor's table below, mortality was observed at all doses. Only the 200 mg/kg group had surviving animals (females).

Treatment	Clinical Observation	Number of Animals With Clinical Signs/Total Number of Animals Dosed		Number of Animals Died/Total Number of Animals Dosed	
		Males	Females	Males	Females
TH9507 200 mg/kg	Lethargy Piloerection Prostration	5/5 5/5 2/5	5/5 5/5 1/5	5/5	1/5
TH9507 400 mg/kg	Lethargy Piloerection	2/5 2/5	2/5 2/5	5/5	5/5
TH9507 600 mg/kg	Lethargy Piloerection	1/5 1/5	0/5 0/5	5/5	5/5
TH9507 800 mg/kg	Lethargy Piloerection	0/5 0/5	1/5 1/5	5/5	5/5

Micronucleus test: At the lower doses used in the micronucleus test, there was no mortality. Clinical signs of lethargy and piloerection were seen at all doses.

Treatment	Clinical Observation	Number of Animals With Clinical Signs/Total Number of Animals Dosed		Number of Animals Died/Total Number of Animals Dosed	
		Males	Females	Males	Females
Distilled water 20 mL/kg	*	*/10	*/10	0/10	0/10
TH9507 25 mg/kg	Lethargy Piloerection	5/5 5/5	5/5 5/5	0/5	0/5
TH9507 50 mg/kg	Lethargy Piloerection	5/5 5/5	5/5 5/5	0/5	0/5
TH9507 100 mg/kg	Lethargy Piloerection	15/15 15/15	15/15 15/15	0/15	0/15
CP 50 mg/kg	*	*/5	*/5	0/5	0/5

*= no clinical signs observed, all dosed animals appeared normal

Evaluation of the erythrocytes showed a dose-dependent decrease in the PCE to total erythrocyte ratio, suggesting an effect on the bone marrow, but no effect on the incidence of micronucleated PCEs, as shown in the sponsor's table below.

Treatment	Sex	Time (hr)	Number of Mice	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Micronucleated Polychromatic Erythrocytes: Number per 1000 PCEs (Mean +/- SD)	Micronucleated Polychromatic Erythrocytes: Number per PCEs Scored[1]
Water							
20 mL/kg	M	24	5	0.493 ± 0.06	---	0.3 ± 0.27	3 / 10000
	F	24	5	0.548 ± 0.02	---	0.5 ± 0.00	5 / 10000
TH9507							
25 mg/kg	M	24	5	0.470 ± 0.05	-5	0.3 ± 0.27	3 / 10000
	F	24	5	0.503 ± 0.06	-8	0.1 ± 0.22	1 / 10000
50 mg/kg	M	24	5	0.418 ± 0.08	-15	0.4 ± 0.22	4 / 10000
	F	24	5	0.453 ± 0.06	-17	0.4 ± 0.22	4 / 10000
100 mg/kg	M	24	5	0.377 ± 0.06	-24	0.2 ± 0.27	2 / 10000
	F	24	5	0.448 ± 0.06	-18	0.3 ± 0.27	3 / 10000
CP,							
50 mg/kg	M	24	5	0.333 ± 0.02	-32	28.3 ± 6.51	*283 / 10000
	F	24	5	0.353 ± 0.03	-36	26.7 ± 2.89	*267 / 10000
Water							
20 mL/kg	M	48	5	0.529 ± 0.06	---	0.2 ± 0.27	2 / 10000
	F	48	5	0.513 ± 0.06	---	0.3 ± 0.27	3 / 10000
TH9507							
100 mg/kg	M	48	5	0.389 ± 0.06	-26	0.2 ± 0.27	2 / 10000
	F	48	5	0.429 ± 0.04	-16	0.3 ± 0.27	3 / 10000

[1]*, p≤0.05 (Kastenbaum-Bowman Tables)

BACTERIAL MUTATION ASSAY (AMES TEST) [TH05111]

Key findings: (b) (4) was not mutagenic in the Ames test at concentrations up to 5000 µg/plate.

Study: E-PCL-368
Volume and page: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: 24 December 2008
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: (b) (4), BB-08-017, 95.44%

Methods

Strains/species/cell line: *Salmonella typhimurium* histidine auxotrophs TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2 *uvr*A in the absence and presence of Aroclor 1254-induced rat liver S9.

Doses used in definitive study: 0, 50, 100, 150, 500, 1500, and 5000 µg/plate

Basis of dose selection: Doses were chosen following initial assays of solubility (up to 50 mg/mL in water) and cytotoxicity (up to 5000 µg/plate). Non-interfering precipitate was seen at ≥ 1500 µg/plate and a > 50% reduction in revertant counts were seen at 5000 µg/plate under some conditions, so the limit dose of 5000 µg/plate was selected as the high dose.

Negative controls: Vehicle (distilled water)

Positive controls: Positive controls are shown in the table below.

Strain	S9	Positive control	Concentration (µg/plate)
TA98, TA1535, TA1537	+	2-aminoanthracene	1.0
TA100	+	2-aminoanthracene	2.0
WP2 *uvr*A	+	2-aminoanthracene	10
TA98	-	2-nitrofluorene	1.0
TA100, TA1535	-	sodium azide	1.0
TA1537	-	9-aminoacridine	75
WP2 *uvr*A	-	Methyl methanesulfonate	1000

Incubation and sampling times: Bacterial strains were exposed to (b) (4) and relevant controls using the plate incorporation technique. Plates were incubated 48-72 hours at 37ºC prior to colony counting.

Results

Study validity: Criteria for a valid assay included confirmation of strain integrity, vehicle controls within a specified range, positive controls with at least a 3X increase over vehicle control, and a minimum of three non-toxic doses for evaluation. The study met these criteria. S9 batches used had been appropriately characterized for their ability to metabolize at least two pro-mutagens, and the positive controls used in the study were appropriate. There was some deviation in measured vs. nominal (b) (4) concentrations that were outside the acceptance limits, but these deviations did not significantly affect the study outcome (see below).

Study outcome:

Drug formulation: In both the initial and confirmatory assays, some dosing formulations had lower measured concentrations than allowed under the protocol acceptance criteria (see the sponsor's table below); however, the high doses were within acceptance limits, thus meeting the recommendations of the relevant guidances to use 5000 µg/plate.

Phase (preparation date)	Nominal concentration (mg/mL)	Measured concentration (mg/mL)	Percent of nominal	Mean measured concentration (mg/mL)	Mean percent of nominal
Initial Toxicity-Mutation Assay (30 Dec 2008)	0	<LLOQ	-	< LLOQ	-
		<LLOQ	-		
	0.050	0.0331	66.2[A]	0.0297	59.4[A]
		0.0263	52.6[A]		
	0.15	0.135	90.2	0.127	84.8[A]
		0.119	79.4[A]		
	0.50	0.422	84.4[A]	0.420	83.9[A]
		0.417	83.4[A]		
	1.5	1.19	79.2[A]	1.21	80.4[A]
		1.22	81.5[A]		
	5.0	4.20	84.0[A]	4.22	84.5[A]
		4.25	85.0		
	15	12.4	82.8[A]	12.6	84.1[A]
		12.8	85.3		
	50	47.7	95.4	46.3	92.7
		45.0	90.0		

LLOQ = lower limit of quantitation (0.0210 mg/mL)
A = outside acceptance criteria

Phase (preparation date)	Nominal concentration (mg/mL)	Measured concentration (mg/mL)	Percent of nominal	Mean measured concentration (mg/mL)	Mean percent of nominal
Confirmatory Mutagenicity Assay (14 Jan 2009)	0	<LLOQ	-	< LLOQ	-
		<LLOQ	-		
	0.50	0.433	86.5	0.446	89.1[A]
		0.459	91.7		
	1.5	1.36	91.0	1.37	91.3
		1.37	91.6		
	5.0	4.50	90.0	4.48	89.7
		4.47	89.4		
	15	14.2	94.4	13.9	93.0
		13.7	91.5		
	50	51.0	102	49.1	98.2
		47.2	94.4		

LLOQ = lower limit of quantitation (0.0210 mg/mL)
A = outside acceptance criteria

Preliminary Plate Incorporation: In the initial toxicity-mutation assay, doses from 1.5 to 5000 µg/plate were evaluated for toxicity and mutations. There was no evidence decrease in the background lawn in

strains exposed to any dose of (b) (4), but non-interfering precipitate was seen at ≥ 1500 µg/plate. The number of revertants was not significantly increased under any assay condition, as shown in the table sponsor's below. Some evidence of cytotoxicity (decreased revertants) was observed at the highest dose for TA100 (-S9) and WP2*uvr*A (+S9).

Bacterial Mutation Assay
Summary of Results - Initial Toxicity-Mutation Assay
Table 21

Test Article Id : (b) (4), Lot No.:BB-08-017
Study Number : AC22RZ.503.(b)
Experiment No : B1

Average Revertants Per Plate ± Standard Deviation

Activation Condition : None

Dose (µg per plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	12 ± 1	138 ± 14	13 ± 1	4 ± 2	29 ± 10
1.5	16 ± 1	135 ± 1	11 ± 0	5 ± 0	37 ± 8
5.0	15 ± 6	133 ± 15	9 ± 4	7 ± 1	32 ± 2
15	12 ± 4	126 ± 15	10 ± 1	9 ± 2	28 ± 0
50	16 ± 4	117 ± 18	18 ± 3	5 ± 0	37 ± 8
150	8 ±	139 ± 3	10 ± 4	5 ± 1	32 ± 4
500	13 ± 2	117 ± 6	17 ± 6	6 ± 1	30 ± 8
1500	16 ± 4	100 ± 19	11 ± 4	4 ± 1	31 ± 2
5000	19 ± 7	68 ± 18	10 ± 3	4 ± 1	21 ± 6
Positive	277 ± 27	623 ± 15	464 ± 0	1502 ± 71	440 ± 28

Activation Condition : Rat Liver S9

Dose (µg per plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	19 ± 1	115 ± 14	16 ± 3	9 ± 1	36 ± 10
1.5	20 ± 2	117 ± 13	12 ± 1	11 ± 3	28 ± 4
5.0	22 ± 1	118 ± 6	17 ± 3	6 ± 1	42 ± 21
15	24 ± 8	95 ± 6	13 ± 0	7 ± 3	34 ± 5
50	20 ± 4	126 ± 7	13 ± 4	5 ± 2	32 ± 10
150	27 ± 1	122 ± 8	11 ± 1	5 ± 3	41 ± 3
500	14 ± 3	105 ± 16	15 ± 1	4 ± 1	26 ± 14
1500	17 ± 1	91 ± 2	10 ± 2	8 ± 1	36 ± 5
5000	22 ± 8	63 ± 6	10 ± 1	7 ± 2	17 ± 1
Positive	755 ± 74	1695 ± 80	117 ± 13	83 ± 11	551 ± 59

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot = 100 µL

Main Plate Incorporation: In the definitive assay, results for cytotoxicity and mutagenicity were similar to the initial assay (precipitation at ≥ 1500 µg/plate and reduction in revertants at 5000 µg/plate under some conditions). All plates were scored as "normal" for the condition of the background lawn, and no assay condition caused a significant increase in revertants. Positive controls were all at least three times greater than vehicle controls. The results confirm that (b) (4) was not mutagenic in this assay.

Bacterial Mutation Assay
Summary of Results - Confirmatory Mutagenicity Assay
Table 22

Test Article Id : (b) (4), Lot No.:BB-08-017
Study Number : AC22RZ.503 (b) (4)
Experiment No : B2

Average Revertants Per Plate ± Standard Deviation

Activation Condition : None

Dose (µg per plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	12 ± 2	143 ± 4	10 ± 3	6 ± 4	31 ± 6
50	16 ± 10	139 ± 5	8 ± 3	7 ± 4	41 ± 6
150	16 ± 2	115 ± 21	16 ± 5	7 ± 2	30 ± 2
500	15 ± 3	119 ± 14	11 ± 2	9 ± 0	37 ± 4
1500	16 ± 4	77 ± 13	11 ± 4	7 ± 2	27 ± 4
5000	14 ± 1	50 ± 10	7 ± 2	8 ± 2	11 ± 3
Positive	187 ± 26	506 ± 101	302 ± 28	2044 ± 234	386 ± 45

Activation Condition : Rat Liver S9

Dose (µg per plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	26 ± 1	161 ± 7	13 ± 3	7 ± 3	39 ± 14
50	26 ± 4	166 ± 6	13 ± 1	5 ± 1	44 ± 16
150	20 ± 2	157 ± 3	12 ± 2	8 ± 2	43 ± 7
500	33 ± 6	156 ± 15	15 ± 10	10 ± 4	41 ± 5
1500	18 ± 7	133 ± 9	12 ± 3	6 ± 2	33 ± 9
5000	26 ± 2	71 ± 9	5 ± 1	7 ± 3	15 ± 6
Positive	931 ± 64	1112 ± 223	107 ± 16	72 ± 11	452 ± 18

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot = 100 µL

IN VITRO MAMMALIAN CHROMOSOME ABERRATION TEST [TH05111]

Key findings: (b) (4) did not cause structural or numerical chromosome aberrations in CHO cells.

Study: E-PCL-369
Volume and page: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: 16 December 2008
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: (b) (4), BB-08-017, 95.44%

Methods

Strains/species/cell line: Chinese hamster ovary (CHO-K_1) cells ((b) (4) ; passage number ≤ 20. Metabolic activation was achieved using the S9 fraction of Aroclor 1254-induced rat liver.

Doses used in definitive study:

Treatment Condition	Treatment Time	Recovery Time	Dose levels (µg/mL)
Non-activated	4 hr	16 hr	125, 250, 500, 750, 1000, 1250
	20 hr	0 hr	125, 250, 500, 750, 1000, 1250
S9-activated	4 hr	16 hr	125, 250, 500, 1000, 1250, 1500, 2000, 2500

Basis of dose selection: Doses were selected based on the results of a preliminary toxicity assay simulating the exposure conditions of the definitive chromosome aberration assay. In this initial experiment, CHO cells were exposed to 9 concentrations of TH9507 (0.5-5000 µg/mL), as shown in the sponsor's tables below. Precipitation of (presumed) test article was seen at ≥ 1500 µg/mL at the beginning of the incubation for all conditions, but the threshold for precipitate decreased to 500 µg/mL for the 4h (+S9) condition at the end of the incubation period. Cell growth inhibition was >100% for all conditions with 5000 µg/mL of (b) (4), so this dose was excluded from further testing. Doses of 1250 µg/mL (-S9) and 2500 µg/mL (+S9) were chosen as the high doses, with the difference due to variable sensitivity at 1500 µg/mL.

Preliminary Toxicity Test

4 hours (-S9)

Treatment µg/mL	Cell Count (x10^6)	Cell Viability (%)	Viable Cells/ Flask (x10^6)	Cell Growth Index (%)	Cell Growth Inhibition (%)
Baseline A	0.84	99%	0.83		
Baseline B	0.77	99%	0.76		
Baseline Average			0.80		
Water	1.90	99%	1.88		
(b) (4) Lot No.: BB-08-017					
0.5	1.85	100%	1.85	98	2
1.5	2.02	98%	1.98	109	-9
5	1.86	98%	1.82	94	6
15	1.91	99%	1.89	101	-1
50	1.73	99%	1.71	85	15
150	1.69	98%	1.66	80	20
500	1.65	97%	1.60	74	26
1500	0.89	97%	0.87	7	93
5000	0.42	*	0.00	-74	174

4 hours (+S9)

Treatment µg/mL	Cell Count (x10^6)	Cell Viability (%)	Viable Cells/ Flask (x10^6)	Cell Growth Index (%)	Cell Growth Inhibition (%)
Baseline A	0.84	99%	0.83		
Baseline B	0.77	99%	0.76		
Baseline Average			0.80		
Water	1.54	100%	1.54		
(b) (4) Lot No.: BB-08-017					
0.5	1.70	98%	1.67	117	-17
1.5	1.67	98%	1.64	113	-13
5	1.68	99%	1.66	116	-16
15	1.53	100%	1.53	99	1
50	1.57	98%	1.54	100	0
150	1.65	97%	1.60	108	-8
500	1.48	97%	1.44	86	14
1500	1.22	98%	1.20	54	46
5000	0.29	*	0.00	-107	207

20 hours (-S9)

Treatment µg/mL	Cell Count ($x10^6$)	Cell Viability (%)	Viable Cells/ Flask ($x10^6$)	Cell Growth Index (%)	Cell Growth Inhibition (%)
Baseline A	0.84	99%	0.83		
Baseline B	0.77	99%	0.76		
Baseline Average			0.80		
Water	2.07	99%	2.05		
(b) (4) Lot No.: BB-08-017					
0.5	2.06	100%	2.06	101	-1
1.5	2.04	99%	2.02	97	3
5	2.09	97%	2.03	98	2
15	2.01	99%	1.99	95	5
50	1.91	98%	1.87	86	14
150	1.75	99%	1.73	74	26
500	1.76	98%	1.73	74	26
1500	0.49	94%	0.46	-27	127
5000	0.04	*	0.00	-63	163

Negative controls: Vehicle (sterile water)

Positive controls: Mitomycin C (MMC) and cyclophosphmide (CP)

Incubation and sampling times: The cells were incubated for 4 hours (±S9) or 20 hours (-S9) in a cell culture incubator at 37ºC. For the 4-hour treatment, the culture media was replaced with drug-free media after 4 hours. All preparations received Colcemid solution (final concentration 0.1 µg/mL) for the final two hours of incubation.

Results

Study validity: Adequate criteria for a valid test were outlined in the protocol and met by the study. S9 batches used had been appropriately characterized for their ability to metabolize both 2-aminoanthracene and 7,12-dimethylbenz(a)anthracene, and the positive controls used in the study were appropriate. Dose formulation analysis was adequate.

Study outcome: Samples with at least a 50% inhibition of cell growth (mitotic index) were chosen as the highest dose for analysis. In the assay with a 4 hour exposure in the absence of S9, the 1250 µg/mL (top) dose produced 60% cell growth inhibition. The 20 hour exposure (-S9) showed >50% inhibition at ≥ 500 µg/mL, so 500 µg/mL was chosen as the high dose for analysis. With a 4 hour exposure in the presence of S9, the highest dose (2500 µg/mL) inhibited cell growth by only 18%. As shown in the sponsor's table below, there was no condition under which (b) (4) caused a significant increase in aberrations. The positive controls had the expected results.

SUMMARY (INITIAL ASSAY)

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored Numerical	Cells Scored Structural	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
Water	-S9	4	8.3	200	200	0.005	±0.071	0.0	0.5
(b) (4) Lot No.: BB-08-017									
750	-S9	4	8.3	200	200	0.005	±0.071	0.0	0.5
1000	-S9	4	7.4	200	200	0.000	±0.000	0.5	0.0
1250	-S9	4	5.9	200	200	0.000	±0.000	0.0	0.0
MMC, 0.2	-S9	4	5.3	200	100	0.440	±1.038	0.0	19.0**
Water	+S9	4	9.0	200	200	0.005	±0.071	0.0	0.5
(b) (4) Lot No.: BB-08-017									
500	+S9	4	8.8	200	200	0.000	±0.000	1.5	0.0
1500	+S9	4	7.2	200	200	0.005	±0.071	0.0	0.5
2500	+S9	4	6.6	200	200	0.010	±0.141	0.5	0.5
CP, 10	+S9	4	1.9	200	50	0.800	±1.088	0.0	42.0**
Water	-S9	20	7.9	200	200	0.005	±0.071	0.0	0.5
(b) (4) Lot No.: BB-08-017									
125	-S9	20	7.4	200	200	0.000	±0.000	0.5	0.0
250	-S9	20	7.0	200	200	0.005	±0.071	0.5	0.5
500	-S9	20	7.4	200	200	0.005	±0.071	0.0	0.5
MMC, 0.1	-S9	20	5.3	200	100	0.350	±0.809	0.0	18.0**

Treatment: Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, p≤0.05; **, p≤0.01; using Fisher's Exact test.

Because of the lack of significant toxicity in the 4h (+S9) flasks, this condition was repeated using higher doses (3500 and 5000 µg/mL). The 5000 µg/mL flasks examined for aberrations had 4 and 1 chromatid breaks (compared to 0 and 1 in control flasks and 13 and 8 in the positive control flasks), but this apparent increase was not statistically significant.

4-HOUR TREATMENT, 16-HOUR RECOVERY PERIOD (REPEAT ASSAY)

Treatment µg/mL	Flask	Cell Count Averages ($x10^6$)	Cell Viability (%)	Mean Cells per Flask ($x10^6$)	Cell Growth Index (%)	Cell Growth Inhibition (%)	Mitotic Index (%)	Mitotic Inhibition (%)
Baseline	A	0.94	100%					
	B	0.95	98%	0.93				
Water	A	2.07	98%					
	B	1.89	97%	1.93	100		8.3	
(b) (4) Lot No.: BB-08-017								
3500	A	0.49	83%					
	B	0.51	85%	0.42	-51	151	7.5	10
5000	A	0.44	66%					
	B	0.43	65%	0.28	-65	165	5.1	39
CP, 10	A	0.91	99%					
	B	0.89	97%	0.88	-5	105	3.2	61
CP, 20	A	0.92	96%					
	B	0.98	98%	0.92	-1	101	N/A	N/A

SUMMARY (REPEAT ASSAY)

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations	
				Numerical	Structural			Numerical (%)	Structural (%)
Water	+S9	4	8.3	200	200	0.005	±0.071	0.5	0.5
(b) (4) Lot No.: BB-08-017									
5000	+S9	4	5.1	200	200	0.025	±0.291	0.0	1.0
CP, 10	+S9	4	3.2	200	100	0.440	±1.048	0.0	20.0**

BACTERIAL MUTATION ASSAY (AMES TEST) [TH07117]

Key findings: (b) (4) was not mutagenic in the Ames test at concentrations up to 5000 µg/plate.

Study: E-PCL-370
Volume and page: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: 24 December 2008
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: (b) (4), BB-08-023, 93.65%

Methods

Strains/species/cell line: *Salmonella typhimurium* histidine auxotrophs TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2 *uvr*A in the absence and presence of Aroclor 1254-induced rat liver S9.

Doses used in definitive study: 0, 50, 100, 150, 500, 1500, and 5000 µg/plate

Basis of dose selection: Doses were chosen following initial assays of solubility (up to 50 mg/mL in water) and cytotoxicity (up to 5000 µg/plate). Non-interfering precipitate was seen at ≥ 1500 µg/plate with interfering precipitate at 5000 µg/plate (all conditions). There was no obvious toxic effect to the background lawn, but a > 50% reduction in revertant counts were seen at 1500 or 5000 µg/plate under some conditions, so the limit dose of 5000 µg/plate was selected as the high dose.

Negative controls: Vehicle (distilled water)

Positive controls: Positive controls are shown in the table below.

Strain	S9	Positive control	Concentration (µg/plate)
TA98, TA1535, TA1537	+	2-aminoanthracene	1.0
TA100	+	2-aminoanthracene	2.0
WP2 *uvr*A	+	2-aminoanthracene	10
TA98	-	2-nitrofluorene	1.0
TA100, TA1535	-	sodium azide	1.0
TA1537	-	9-aminoacridine	75
WP2 *uvr*A	-	Methyl methanesulfonate	1000

Incubation and sampling times: Bacterial strains were exposed to (b) (4) and relevant controls using the plate incorporation technique. Plates were incubated 48-72 hours at 37ºC prior to colony counting.

Results

Study validity: Criteria for a valid assay included confirmation of strain integrity, vehicle controls within a specified range, positive controls with at least a 3X increase over vehicle control, and a minimum of three non-toxic doses for evaluation. The study met these criteria. S9 batches used had been appropriately characterized for their ability to metabolize at least two pro-mutagens, and the positive controls used in the study were appropriate. There was some deviation in measured vs. nominal (b) (4) concentrations that were outside the acceptance limits, but these deviations did not significantly affect the study outcome (see below).

Study outcome:

Drug formulation: In both the initial and confirmatory assays, some dosing formulations had lower measured concentrations than allowed under the protocol acceptance criteria (see the sponsor's table below); however, the high doses were within acceptance limits, thus meeting the recommendations of the relevant guidances to use 5000 µg/plate.

Phase (preparation date)	Nominal concentration (mg/mL)	Measured concentration (mg/mL)	Percent of nominal	Mean measured concentration (mg/mL)	Mean percent of nominal
Initial Toxicity-Mutation Assay (31 Dec 2008)	0	<LLOQ[A]	-	< LLOQ[A]	-
		<LLOQ[A]	-		
	0.050	0.0446	89.2	0.0449	89.8
		0.0452	90.4		
	0.15	0.122	81.4[B]	0.121	80.8[B]
		0.120	80.2[B]		
	0.50	0.417	83.3[B]	0.416	83.2[B]
		0.416	83.1[B]		
	1.5	1.30	86.5	1.30	86.8[B]
		1.31	87.1		
	5.0	4.34	86.8	4.37	87.5[B]
		4.40	88.1		
	15	13.3	88.5	13.3	88.4[B]
		13.2	88.2		
	50	46.0	92.0	45.9	91.9
		45.9	91.7		

LLOQ = lower limit of quantitation (0.0210 mg/mL)
A = a peak was detected at the retention time of the test article in addition to several peaks not at the retention time of the test article. The peak at the retention time of the test article is estimated to be approximately 1.00 µg/mL, however it is < LLOQ and below the lowest concentration standard
B = outside acceptance criteria

Phase (preparation date)	Nominal concentration (mg/mL)	Measured concentration (mg/mL)	Percent of nominal	Mean measured concentration (mg/mL)	Mean percent of nominal
Confirmatory Mutagenicity Assay (14 Jan 2009)	0	<LLOQ[C]	-	< LLOQ[C]	-
		<LLOQ[C]	-		
	0.15	0.122	81.3[B]	0.123	82.1[B]
		0.124	82.9[B]		
	0.50	0.485	97.0	0.476	95.2
		0.466	93.3		
	1.5	1.49	99.3	1.48	98.6
		1.47	97.9		
	5.0	4.72	94.5	4.72	94.4
		4.72	94.3		
	15	16.3	109	16.0	107
		15.8	105		
	50	51.2	102	51.2	102
		51.2	103		

LLOQ = lower limit of quantitation (0.0210 mg/mL)
A = a peak was detected at the retention time of the test article in addition to several peaks not at the retention time of the test article. The peak at the retention time of the test article is estimated to be approximately 1.00 µg/mL, however it is < LLOQ and below the lowest concentration standard
B = outside acceptance criteria
C = several peaks not at the retention time of the test article were detected

Preliminary Plate Incorporation: In the initial toxicity-mutation assay, doses from 1.5 to 5000 µg/plate were evaluated for toxicity and mutations. There was no evidence decrease in the background lawn in strains exposed to any dose of (b) (4) Non-interfering precipitate was seen at ≥ 1500 µg/plate with interfering precipitate at 5000 µg/plate (all conditions). There was no obvious toxic effect to the background lawn, but a > 50% reduction in revertant counts were seen at 1500 or 5000 µg/plate under some conditions (TA100(±S9) and WP2*uvr*A (-S9)). The number of revertants was not significantly

increased under any assay condition, as shown in the table sponsor's below. Some evidence of cytotoxicity (decreased revertants) was observed at the highest dose for TA100 (-S9) and WP2*uvr*A (+S9).

Bacterial Mutation Assay
Summary of Results - Initial Toxicity-Mutation Assay
Table 21

Test Article Id : (b) (4), Lot No.: BB-08-023
Study Number : AC22SA.503 (b) (4)
Experiment No : B1

Average Revertants Per Plate ± Standard Deviation

Activation Condition : None

Dose (µg per plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	12 ± 2	109 ± 16	13 ± 6	3 ± 1	38 ± 5
1.5	13 ± 3	124 ± 1	11 ± 0	3 ± 1	41 ± 3
5.0	10 ± 1	118 ± 8	10 ± 1	4 ± 0	41 ± 4
15	10 ± 1	116 ± 17	9 ± 3	5 ± 3	47 ± 6
50	11 ± 1	115 ± 9	13 ± 3	4 ± 3	38 ± 6
150	11 ± 2	100 ± 1	12 ± 1	4 ± 1	36 ± 1
500	13 ± 3	95 ± 11	15 ± 2	6 ± 1	31 ± 1
1500	8 ± 4	53 ± 11	7 ± 6	6 ± 1	39 ± 1
5000	8 ± 2	26 ± 4	8 ± 1	3 ± 3	15 ± 4
Positive	129 ± 13	446 ± 3	390 ± 11	1395 ± 298	428 ± 18

Activation Condition : Rat Liver S9

Dose (µg per plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	13 ± 4	96 ± 20	7 ± 1	7 ± 2	38 ± 5
1.5	19 ± 1	119 ± 14	13 ± 1	5 ± 5	30 ± 1
5.0	16 ± 6	115 ± 18	8 ± 4	4 ± 1	39 ± 7
15	17 ± 4	125 ± 13	11 ± 1	7 ± 1	38 ± 8
50	15 ± 2	117 ± 3	7 ± 1	3 ± 3	30 ± 3
150	17 ± 4	102 ± 5	10 ± 3	4 ± 0	43 ± 11
500	13 ± 0	81 ± 20	11 ± 0	4 ± 3	42 ± 6
1500	17 ± 2	57 ± 5	9 ± 1	4 ± 2	24 ± 7
5000	12 ± 1	32 ± 6	9 ± 3	6 ± 2	22 ± 7
Positive	600 ± 82	1140 ± 59	90 ± 1	73 ± 6	481 ± 37

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot = 100 µL

Main Plate Incorporation: In the definitive assay, patterns of precipitant formation were somewhat different from the initial assay. Non-interfering precipitate was seen in the absence of S9 at 5000 µg/plate for all tester strains. In the presence of S9, non-interfering precipitate was seen beginning at 500 µg/plate (TA1537) or 1500 µg/plate (all other strains) with interfering precipitate at 5000 µg/plate. There was no evidence of toxicity to the background lawn, and no assay condition caused a significant increase in revertants. Positive controls were all at least three times greater than vehicle controls. The results confirm that (b) (4) was not mutagenic in this assay.

Bacterial Mutation Assay
Summary of Results - Confirmatory Mutagenicity Assay
Table 22

Test Article Id : (b) (4) Lot No.: BB-08-023
Study Number : AC22SA.503. (b)
Experiment No : B2

Average Revertants Per Plate ± Standard Deviation

Activation Condition : None												
Dose (µg per plate)	TA98		TA100		TA1535		TA1537		WP2 uvrA			
Vehicle	14	± 3	130	± 4	14	± 5	5	± 4	33	± 5		
15	18	± 2	135	± 15	13	± 2	6	± 5	35	± 5		
50	16	± 5	118	± 17	13	± 2	7	± 1	42	± 12		
150	14	± 5	151	± 5	13	± 4	11	± 4	35	± 5		
500	11	± 5	94	± 10	11	± 1	8	± 3	30	± 7		
1500	10	± 2	89	± 9	9	± 2	5	± 3	28	± 2		
5000	13	± 2	38	± 9	10	± 4	5	± 2	11	± 2		
Positive	217	± 14	569	± 33	440	± 17	1863	± 253	282	± 10		

Activation Condition : Rat Liver S9												
Dose (µg per plate)	TA98		TA100		TA1535		TA1537		WP2 uvrA			
Vehicle	27	± 3	139	± 10	18	± 3	7	± 3	41	± 6		
15	24	± 3	126	± 18	13	± 3	6	± 2	46	± 2		
50	29	± 7	124	± 21	15	± 3	7	± 2	38	± 8		
150	26	± 5	122	± 4	15	± 2	6	± 0	39	± 4		
500	22	± 3	113	± 5	12	± 2	6	± 2	41	± 6		
1500	24	± 6	86	± 19	14	± 3	10	± 1	30	± 4		
5000	20	± 2	62	± 15	12	± 2	7	± 1	22	± 6		
Positive	621	± 93	1052	± 26	79	± 14	62	± 9	458	± 33		

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot = 100 µL

IN VITRO MAMMALIAN CHROMOSOME ABERRATION TEST [TH07117]

Key findings: (b) (4) did not cause structural or numerical chromosome aberrations in CHO cells.

Study: E-PCL-371
Volume and page: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: 15 December 2008
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: (b) (4), BB-08-023, 93.65%

Methods

Strains/species/cell line: Chinese hamster ovary (CHO-K_1) cells ((b) (4) ; passage number ≤ 20. Metabolic activation was achieved using the S9 fraction of Aroclor 1254-induced rat liver.

Doses used in definitive study:

Treatment Condition	Treatment Time	Recovery Time	Dose levels (µg/mL)
Non-activated	4 hr	16 hr	125, 250, 500, 750, 1000, 1250, 1500, 2000
	20 hr	0 hr	62.5, 125, 250, 400, 500, 750, 1000
S9-activated	4 hr	16 hr	625, 1250, 2500, 5000

Basis of dose selection: Doses were selected based on the results of a preliminary toxicity assay simulating the exposure conditions of the definitive chromosome aberration assay. In this initial experiment, CHO cells were exposed to 9 concentrations of TH9507 (0.5-5000 µg/mL), as shown in the sponsor's tables below. Precipitation of (presumed) test article was seen at ≥ 500 µg/mL for all conditions. Cell growth inhibition was >50% was observed at ≥ 1500 µg/mL (4h –S9) and at ≥ 500 µg/mL (20h –S9). In the presence of S9 there was no significant inhibition of cell growth (25% at 5000 µg/mL).

Preliminary Toxicity Test

4 hours (-S9)

Treatment µg/mL	Cell Count ($x10^6$)	Cell Viability (%)	Viable Cells/ Flask ($x10^6$)	Cell Growth Index (%)	Cell Growth Inhibition (%)
Baseline A	0.98	99%	0.97		
Baseline B	1.14	99%	1.13		
Baseline Average			1.05		
Water	3.06	99%	3.03		
(b) (4) Lot No.: BB-08-023					
0.5	2.81	98%	2.76	86	14
1.5	3.37	95%	3.21	109	-9
5	2.95	96%	2.83	90	10
15	2.93	96%	2.81	89	11
50	2.73	95%	2.59	78	22
150	2.99	95%	2.84	90	10
500	2.35	99%	2.33	65	35
1500	1.70	95%	1.61	29	71
5000	1.13	93%	1.05	0	100

4 hours (+S9)

Treatment µg/mL	Cell Count ($x10^6$)	Cell Viability (%)	Viable Cells/ Flask ($x10^6$)	Cell Growth Index (%)	Cell Growth Inhibition (%)
Baseline A	0.98	99%	0.97		
Baseline B	1.14	99%	1.13		
Baseline Average			1.05		
Water	2.17	97%	2.11		
(b) (4) Lot No.: BB-08-023					
0.5	2.25	98%	2.21	110	-10
1.5	2.36	99%	2.34	122	-22
5	2.54	100%	2.54	140	-40
15	2.38	99%	2.36	124	-24
50	2.59	99%	2.57	143	-43
150	2.73	98%	2.68	154	-54
500	2.62	98%	2.57	143	-43
1500	1.92	93%	1.79	70	30
5000	1.90	97%	1.84	75	25

20 hours (-S9)

Treatment µg/mL	Cell Count ($x10^6$)	Cell Viability (%)	Viable Cells/ Flask ($x10^6$)	Cell Growth Index (%)	Cell Growth Inhibition (%)
Baseline A	0.98	99%	0.97		
Baseline B	1.14	99%	1.13		
Baseline Average			1.05		
Water	2.56	99%	2.53		
(b) (4) Lot No.: BB-08-023					
0.5	2.56	98%	2.51	99	1
1.5	2.51	93%	2.33	87	13
5	2.36	94%	2.22	79	21
15	2.44	96%	2.35	88	12
50	2.43	96%	2.33	87	13
150	2.51	95%	2.38	90	10
500	1.83	95%	1.74	47	53
1500	0.97	97%	0.94	-8	108
5000	0.59	96%	0.56	-33	133

Negative controls: Vehicle (sterile water)

Positive controls: Mitomycin C (MMC) and cyclophosphmide (CP)

Incubation and sampling times: The cells were incubated for 4 hours (±S9) or 20 hours (-S9) in a cell culture incubator at 37ºC. For the 4-hour treatment, the culture media was replaced with drug-free media after 4 hours. All preparations received Colcemid solution (final concentration 0.1 µg/mL) for the final two hours of incubation.

Results

Study validity: Adequate criteria for a valid test were outlined in the protocol and met by the study. S9 batches used had been appropriately characterized for their ability to metabolize both 2-aminoanthracene and 7,12-dimethylbenz(a)anthracene, and the positive controls used in the study were appropriate. Dose formulation analysis was adequate.

Study outcome: Samples with at least a 50% inhibition of cell growth (mitotic index) were chosen as the highest dose for analysis. In the assay with a 4 hour exposure in the absence of S9, the 1000 µg/mL (top) dose produced 65% cell growth inhibition. The 20 hour exposure (-S9) showed no inhibition of cell growth at any concentration tested, but the mitotic index was reduced by 59% at the highest dose (750 µg/mL). With a 4 hour exposure in the presence of S9, the highest dose (1250 µg/mL) inhibited cell growth by 70%. The condition lacked a sufficient number of lower dose groups, so a repeat assay was performed using 313 µg/mL (see below). As shown in the sponsor's table below, there was no condition under which (b) (4) caused a significant increase in aberrations. The positive controls had the expected results.

TABLE 10

SUMMARY (INITIAL ASSAY)

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored Numerical	Cells Scored Structural	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
Water	-S9	4	11.2	200	200	0.015	±0.158	0.5	1.0
(b) (4) Lot no.: BB-08-023									
250	-S9	4	10.8	200	200	0.005	±0.071	0.5	0.5
500	-S9	4	9.5	200	200	0.000	±0.000	0.0	0.0
1000	-S9	4	6.5	200	200	0.000	±0.000	0.0	0.0
MMC, 0.2	-S9	4	7.2	200	100	0.490	±1.020	1.0	21.0**
Water	+S9	4	13.2	200	200	0.000	±0.000	1.0	0.0
(b) (4) Lot no.: BB-08-023									
625	+S9	4	10.7	200	200	0.000	±0.000	1.0	0.0
1250	+S9	4	6.2	200	200	0.005	±0.071	0.5	0.5
CP, 10	+S9	4	2.6	200	100	0.540	±1.123	0.0	21.0**
Water	-S9	20	10.9	200	200	0.000	±0.000	0.5	0.0
(b) (4) Lot no.: BB-08-023									
125	-S9	20	9.3	200	200	0.010	±0.100	0.0	1.0
400	-S9	20	8.2	200	200	0.005	±0.071	0.5	0.5
750	-S9	20	4.5	200	200	0.005	±0.071	1.0	0.5
MMC, 0.1	-S9	20	4.5	200	100	0.500	±1.000	0.0	23.0**

TABLE 11

SUMMARY (REPEAT ASSAY)

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored Numerical	Cells Scored Structural	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
Water	+S9	4	8.3	200	200	0.005	±0.071	0.5	0.5
(b) (4) Lot no.: BB-08-023									
313	+S9	4	10.8	200	200	0.000	±0.000	0.5	0.0
CP, 10	+S9	4	3.2	200	100	0.440	±1.048	0.0	20.0**

2.6.6.5 Carcinogenicity

Dedicated two year carcinogenicity studies in rodents were not required for this application. This decision was communicated to the sponsor in December 2005 and was partly based on review of the mitogenesis and PCNA data from the chronic toxicology study in rats.

1. Per ICH S6 (R1, Addendum), *in vivo* carcinogenicity studies are not generally required for growth factors, and while TH9507 is not itself a growth factor, it's primary mechanism of action is through stimulation of GH release. Published carcinogenicity studies of GH in mice and rats showed a lack of neoplastic effects in these assays.
2. TH9507 was neither mutagenic nor clastogenic in the genetic toxicity assay battery.
3. Chronic studies of TH9507 in rats and dogs did not reveal a propensity of the drug to stimulate neoplastic processes. Specifically, cell proliferation assays quantified by PCNA staining of several tissues did not reveal any drug-related effect in the rats, and hyperplasia in dog tissues was limited to those caused by pharmacodynamic activity (i.e., diffuse pituitary hyperplasia).
4. Excess cancer risk has not been conclusively demonstrated in humans with acromegaly, a disease associated with long-standing GH excess, although epidemiological data suggest a statistical association between acromegaly and increased incidence of colon cancer[1].
5. Carcinogenicity studies have not been conducted for any of the approved recombinant human growth hormone products.

2.6.6.6 Reproductive and developmental toxicology

SUBCUTANEOUS FERTILITY AND TERATOLOGY STUDY OF TH9507 IN THE RAT

Key study findings:

- Body weight gains increased in treated animals, consistent with increases in food consumption.
- There were no effects on estrous cycle parameters, mating, or fertility indices.
- There was a trend towards an increase in the number of corpora lutea and higher pre-implantation loss in drug treated animals, but the values were within the historical control range.
- There were no effects on sperm or histopathology of the testes.
- Dams given 0.6 mg/kg had higher fetal weights (↑10%).
- All treated groups had increase in the fetal incidence of reduced ossification of the interparietal bone, but decreases in the number of animals with reduced ossification of the pubic bone and decreases of poorly developed sternebrae 1-4.

Reviewer Comments: None of the effects observed were considered precisely adverse on its own; however, when the reduced ossification of the interparietal bone in the skull is considered in the context of 1) increased fetal body weights, 2) fewer animals with reduced ossification in other areas, and 3) hydrocephaly in the pre- and post-natal study, this finding stands out as a potential precursor to the hydrocephaly. Reduced ossification in skeletal structures are usually associated with lower fetal body weights, related to delays in development, while higher body weights tend to correlate with reductions in the finding. In this study, findings in the pubic bone and sternebrae were generally consistent with this paradigm, but all groups had increases in reduced ossification in the skull (interparietal bone). This finding occurred at the high end of the historical control for fetal incidence (19%) and was not dose-dependent. Historical litter incidence was not reported.

[1] Jenkins et al, Clin. Endocrinol., 2006; 64, 115-121.

SD Rats Effect	NOAEL	Multiple of MRHD (1.1 ng.h/mL AUC)
Males No adverse effects	0.6 mg/kg	1X
Females No adverse effects	0.6 mg/kg	1X
Fetuses Reduced ossification of interparietal bone	<0.1 mg/kg	<0.2X

Study: EPCL-021 ((b) (4) 98082)
Volume and page: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: 16 December 2002
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: TH9507, LotFHEXGRF0002A, peptide content of 99.4%, peptide purity of 99.2%

Methods	
Doses	0, 0.1, 0.3, 0.6 mg/kg QD
Dosing Schedule	The males were treated for 28 days prior to mating, during mating until necropsy. Females were given the drug 14 days prior to mating, during mating and up to day 17 of gestation and sacrificed on day 20.
Species/source	Sprague Dawley Rats, Crl:CD(SD) IGS / (b) (4)
Number/sex/group (main study)	22/sex/group
Route, formulation, dose volume	s.c. injection in the scapular region in 1 mL/kg in 5% mannitol
Toxicokinetic groups	4 females/group in drug-treated groups only; samples were collected but not analyzed.
IN-LIFE OBSERVATIONS	**FREQUENCY**
Mortality & cageside observations	Twice daily
Clinical examination	Detailed examinations weekly
Body weight	Males: twice weekly Females: twice weekly premating and every 3 days thereafter
Food consumption	Males: twice weekly Females: twice weekly premating and every 3 days thereafter Not measured during cohabitation
Estrous cycle determination	10 days prior to mating and during mating until positive for sperm
Breeding procedure	Following the premating drug treatment period, animals were cohabitated (1:1) until there was evidence of mating (≤ 21d)
Non-mated females	Non-mated females were euthanized and subject to necropsy and uterine exam.
POST-MORTEM EVALUATIONS	
Macroscopic	Gross pathology performed for all animals

Organ weights	As appropriate: epididymides, ovaries, prostate, seminal vesicles, testes
Uterine & Ovarian exams	The gravid uterus was weighed and the contents examined (including placenta). The number and position of fetuses was recorded also with evidence of resorption. Non-pregnant animals were evaluated for evidence of implantation sites.
Sperm analysis	Sperm motility, morphology, and count were evaluated and the testes were examined histopathologically
Fetal observation	Each fetus was weighed, identified for sex, examined externally, and euthanized. Evaluation of skeletal or visceral abnormalities were performed for ~50% of fetuses each

Results

Mortality: There were two early deaths, but neither appeared to be related to treatment. One control female was found dead on D1 with a clot in its cranial cavity. A MDF designated as a TK animal was euthanized on gestation day seven (GD7) in poor condition ("increased respiratory rate, ectopic pupils, and severe, uncoordinated, non-sustained spinning") after toxicokinetic blood sampling. The sponsor attributes this death to complications of either the blood sampling or a fall earlier in the day.

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

Body weight: Treatment with TH9507 caused dose-dependent increases in weight gain in both males and females. In females, the effect plateaued at ≥ 0.3 mg/kg, as shown in the tables below.

Male Body Weight				
Study Time	Dose, mg/kg	BW gain (g)	% Increment	BW % control
Premating/Pairing/ Post-mating	0	144	-	100%
	0.1	163	↑13%	103%
	0.3	173*	↑20%	105%
	0.6	175*	↑22%	106%

* $p < 0.05$

Female Body Weight				
Study Time	Dose, mg/kg	BW gain (g)	% Increment	BW % control
Pre-mating, Day 1 to GD0	0	23.5	-	100%
	0.1	34.3***	↑45%	105%*
	0.3	36.6***	↑55%	107%***
	0.6	36.3***	↑55%	106%**
Gestation, GD0 to GD20	0	148.1	-	100%
	0.1	153.7	↑4%	103%
	0.3	162.5*	↑10%	107%**
	0.6	161.9*	↑9%	107%**

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Food consumption: Food consumption was increased overall in treated animals in a generally dose-dependent manner (although MD females consumed more than HD females during gestation).

Males: During the pre-mating period, treated males given 0.1, 0.3, and 0.6 mg/kg consumed 101%, 103%, and 105% of controls. Food conversion efficiency (food consumed per gram body weight gained) decreased from 11.6 g/g in controls to ~10.4 g/g in treated groups.

Females, pre-mating period: During the 14 days prior to mating, treated females given 0.1, 0.3, and 0.6 mg/kg consumed 106%, 110%, and 109% of controls. Food conversion efficiency decreased from 12.9 g/g in controls to ~9 g/g in treated groups.

Females, gestational period: During gestation, total food consumption in low, mid, and high dose groups was 102%, 110%, and 108% of control values. Food conversion efficiency was ~3.8 across all treatment groups.

Toxicokinetics: Samples were collected but not analyzed.

Necropsy:
Macroscopic observations: Thickening of the stomach was noted in one MD and one HD male. Other findings appeared to be unrelated to treatment.
Organ weights: There were no treatment related differences in organ weights.

Estrous Cycle Effects: There were no treatment-related effects on estrous cycle parameters.

Fertility parameters: The mating and fertility indices were not affected by the drug treatment as shown in the sponsor's table below.

	NO. PLACED FOR MATING MALES	FEMALES	NO. MATING	MEAN (S.D.) DAY TO MATING	NO. FEMALES PREGNANT	MATING INDEX (%)	FERTILITY INDEX (%)	CONCEPTION RATE (%)
GROUP 1 VEHICLE CONTROL	22	22	22	3.4 (2.60)	19	100.0	86.4	86.4
GROUP 2 TH-9507 0.1 MG/KG/DAY	22	22	22	3.5 (1.50)	21	100.0	95.5	95.5
GROUP 3 TH-9507 0.3 MG/KG/DAY	22	22	22	3.6 (1.50)	22	100.0	100.0	100.0
GROUP 4 TH-9507 0.6 MG/KG/DAY	22	22	22	4.1 (2.86)	22	100.0	100.0	100.0

Uterine & Ovarian Examination: There was trend towards an increase in the number of corpora lutea and higher pre-implantation loss for all drug treated animals. Gravid uterine weight was slightly higher at the two HDs; however, the sponsor claims that all these findings are within the historical control range and not toxicologically significant. The maximum difference in uterine weights (~6.7 g) does not account for the large differences in maternal body weights (↑15-31g).

UTERINE PARAMETERS								
Parameter	**Control**		**0.1 mg/kg**		**0.3 mg/kg**		**0.6 mg/kg**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Corpora lutea	18.1	2.23	18.8	2.61	19.5	1.99	20.0	2.19
Implantation Sites	17.2	1.92	15.4	2.44	17.4	1.53	17.5	1.40
Male Fetuses	8.1	2.22	6.9	1.79	7.9	2.21	8.5	2.42
Female Fetuses	8.2	2.27	8.5	2.40	8.6	1.65	8.0	2.31
Sex Ratio (%M)	49.6	13.4	45.2	10.3	47.3	10.8	51.5	12.9
Live Fetuses	16.3	1.59	15.4	2.69	16.5	1.92	16.5	1.68
Dead Fetuses	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Early Resorptions	0.8	0.96	1.0	1.07	0.9	1.19	0.9	1.27
Middle Resorptions	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Late Resorptions	0.1	0.23	0.0	0.0	0.0	0.21	0.0	0.0
All Resorptions	0.9	0.99	1.0	1.07	1.0	1.21	0.9	1.27
Pre-implantation loss (%)	4.9	6.06	12.3**	8.94	10.5*	7.56	12.0**	6.83
Post-implantation loss (%)	5.0	5.33	6.0	7.17	5.5	7.12	5.1	7.32
Gravid uterus weight (g)	87.8	9.77	85.8	15.15	91.8	8.78	94.5	9.83

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

Sperm Evaluation: The percent motile sperm, spermatozoa count, sperm morphology were all unaffected as shown below. Histopathology of the right testis and spermatogenic cycle assessment showed few abnormalities, which are considered incidental.

		CAUDA EPIDIDYMIS WEIGHT (G)	SPERMATOZOA PER GRAM (MILLIONS)	MOTILITY (%)
GROUP 1 - VEHICLE CONTROL	MEAN	.309	779.66	72.1
	SD	.0279	110.363	10.48
	N	22	21	22
GROUP 2 - TH-9507	MEAN	.308	815.04	69.1
0.1 MG/KG/DAY	SD	.0385	120.860	11.64
	N	22	22	22
GROUP 3 - TH-9507	MEAN	.301	796.64	69.5
0.3 MG/KG/DAY	SD	.0335	156.787	11.28
	N	22	22	21
GROUP 4 - TH-9507	MEAN	.324	805.12	73.4
0.6 MG/KG/DAY	SD	.0312	95.523	9.39
	N	22	22	22

Fetal Evaluation*:* Offspring of high dose dams had significantly higher body weights on average (↑9-10%) compared to controls.

FETAL BODY WEIGHT								
Gender	**Control**		**0.1 mg/kg**		**0.3 mg/kg**		**0.6 mg/kg**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Male	3.51	0.27	3.65	0.24	3.62	0.24	3.85***	0.27
Female	3.31	0.24	3.45	0.25	3.43	0.22	3.60***	0.26
Total	3.41	0.25	3.54	0.24	3.53	0.22	3.73***	0.26

*** $p < 0.001$

The drug did not produce any significant increases in major malformations, but minor fetal external and visceral anomalies were higher at LD and MD in litters and fetuses, as shown in the sponsor's table below:

	GROUP 1 VEHICLE CONTROL		GROUP 2 TH-9507 0.1 MG/KG/DAY		GROUP 3 TH-9507 0.3 MG/KG/DAY		GROUP 4 TH-9507 0.6 MG/KG/DAY	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
EXTERNAL (EXT)	19	309	21	324	22	362	22	364
VISCERAL (VIS)	19	154	21	163	22	182	22	182
SKELETAL (SKE)	19	156	21	161	22	178	22	171
TECHNIQUE OF WILSON (WT)	19	154	21	163	22	182	22	182
MAJOR MALFORMATIONS (CONT'D)	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A
LIMBS								
Digit(s) shortened (SKE)	0	0	1	1	0	0	0	0
MINOR EXTERNAL AND VISCERAL ANOMALIES (TOTAL)	1	1	4	4	5	5	1	2
HEART								
Innominate artery absent (VIS)	0	0	1	1	0	0	0	0
URETER(S)								
Dilated (VIS)	1	1	3	3	5	5	1	2

L/E = Litters examined
F/E = Fetuses examined
L/A = Litters affected
F/A = Fetuses affected

All doses produced reduced ossification of interparietal bones as measured by fetal or litter incidence, which is usually associated with reduced fetal weights rather than the increased weights observed in this study. These were just within the historical control range for fetuses (0-19%) and were not considered significant by the sponsor. There was decreased finding of reduced ossification of the pubic bones at LD and HD.

	GROUP 1 VEHICLE CONTROL		GROUP 2 TH-9507 0.1 MG/KG/DAY		GROUP 3 TH-9507 0.3 MG/KG/DAY		GROUP 4 TH-9507 0.6 MG/KG/DAY	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
EXTERNAL (EXT)	19	309	21	324	22	362	22	364
VISCERAL (VIS)	19	154	21	163	22	182	22	182
SKELETAL (SKE)	19	156	21	161	22	178	22	171
TECHNIQUE OF WILSON (WT)	19	154	21	163	22	182	22	182
	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A
MINOR SKELETAL ANOMALIES (TOTAL)	15	51	19	57	20	65	21	53
SKULL								
Frontal bone(s): Reduced ossification	1	2	0	0	2	2	0	0
Hyoid bone: Reduced ossification	9	18	11	20	12	21	11	21
Hyoid bone: Irregular ossification	0	0	0	0	0	0	1	1
Parietal bone(s): Reduced ossification	2	2	2	2	3	3	1	1
Interparietal bone: Reduced ossification	6	9	15	30 ***	14	30 **	13	28 **
Interparietal bone: Irregular ossification	1	2	0	0	1	1	0	0
Supraoccipital bone: Reduced ossification	2	4	6	6	5	9	6	6
Supraoccipital bone: Irregular ossification	0	0	0	0	1	1	0	0
RIBS								
Reduced	1	1	0	0	2	5	1	1
Ossification center(s) on 7th cervical vertebra	2	2	1	1	1	1	1	1
Rib(s) on 7th cervical vertebra	1	3	0	0	0	0	0	0
Rudimentary 14th rib(s)	1	1	3	4	2	2	2	2
Rudimentary 14th rib(s) with contralateral ossification center	0	0	1	1	2	2	1	2
Extra 14th rib with contralateral rudimentary rib	1	1	0	0	0	0	0	0
PELVIC GIRDLE								
Pubic bone(s): Absent	1	1	0	0	2	2	0	0
Pubic bone(s): Reduced ossification	8	13	4	4 *	6	9	3	3 **
Ischial bone(s): Reduced ossification	1	1	0	0	2	2	1	1
LIMBS								
Femur: Misshaped	0	0	1	1	0	0	0	0
Femur/Tibia/Fibula: Reduced ossification	0	0	0	0	1	1	0	0

L/E = Litters examined
F/E = Fetuses examined
L/A = Litters affected
F/A = Fetuses affected

Significantly different from control group (group 1) value: * - P<=0.05 ** - P<=0.01 *** - P<=0.001 (Fisher's)

The percentage of fetuses with common changes to sternebrae 1 to 4 was significantly reduced at 0.6 mg/kg/day as well as the percentage of fetuses with sternebra 5 and xiphisternum variants was lower at a HD. The sponsor states that these were probably due to increased ossification due to higher fetal weights at a HD.

	GROUP 1 VEHICLE CONTROL	GROUP 2 TH-9507 0.1 MG/KG/DAY	GROUP 3 TH-9507 0.3 MG/KG/DAY	GROUP 4 TH-9507 0.6 MG/KG/DAY
	AFFECTED FETUSES/LITTERS MEAN % (S.D.)	AFFECTED FETUSES/LITTERS MEAN % (S.D.)	AFFECTED FETUSES/LITTERS MEAN % (S.D.)	AFFECTED FETUSES/LITTERS MEAN % (S.D.)
COMMON SKELETAL VARIANTS				
Thoracic centrum variants (absent/reduced/ irregular/semi-bipartite/bipartite)	27.2 (19.98)	32.7 (26.14)	30.4 (25.68)	38.7 (26.68)
Sternebrae 1 to 4 (absent/reduced/irregular/ semi-bipartite/bipartite)	7.2 (8.01)	4.7 (9.85)	6.4 (13.74)	1.0 b (3.27)
Sternebra 5 and Xiphisternum (absent/reduced/ irregular/semi-bipartite/bipartite)	87.8 (19.32)	79.0 (24.77)	80.7 (23.40)	74.1 (27.35)

Significantly different from control group (group 1) value: a - P<=0.05 b - P<=0.01 c - P<=0.001 (Wilcoxon)

A SUBCUTANEOUS TERATOLOGY STUDY OF TH9507 IN THE RABBIT [LOW DOSES]

Key study findings:
Dams: Dams given 0.1, 0.3, or 0.6 mg/kg had dose-dependent increases in weight gain (17-28%), with no adverse clinical effects. There were no treatment-related effects on uterine parameters.

Fetuses: Offspring of HD dams weighed 6% (F) or 9% (M) less than controls.

Reviewer comments: During development of TH9507, this study was considered inadequate by the division due to a lack of obvious maternal toxicity at the high dose. The doses chosen were based on a dose range finding study (E-PCL-019) that used doses from 0.1 or 0.6 mg/kg (n=5/group). No adverse effects were noted at any dose in the DRF study. The reviewer notes that two fetuses (from two litters) had reduced ossification in the parietal bone (skull).

Study: E-PCL-020
Volume and page: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: 13 December 2001
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: TH9507, FHEXGRF0002A & FHEXGRF0001, 90.4% & 87.7% peptide, 99.2% & 99.3% peptide purity

METHODS	
Doses	Dosed with 0, 0.1, 0.3 or 0.6 mg/kg from GD7-19
Species/source	Time mated female New Zealand White rabbits / (b) (4)
Number/sex/group	22 females per group
Route, formulation, dose volume	s.c. injection in 1 mL/kg 5% mannitol
Toxicokinetic groups	Four animals per group (satellite)
Basis of dose selection	A dose-range study (E-PCL-019) showed no maternal or fetal toxicity at doses of 0.1, 0.2, 0.4, or 0.6 mg/kg.

IN-LIFE OBSERVATIONS	FREQUENCY
Cageside observations & clinical exams	Twice daily with detailed examinations on GD 5, 7, 10, 13, 16, 20, 23, and 26
Body weight & food consumption	Body weights: GD 5, 7, 10, 13, 16, 20, 23, and 26 Food consumption: daily
TK analysis	Samples taken from TK animals on GD7 and GD19 at 0, 15, 30, and 60 minutes post dose.
POST-MORTEM EVALUATIONS	
Maternal necropsy	Animals were euthanized on GD29. All main study animals were subject to necropsy
Ovarian/Uterine examinations	"The reproductive tract was dissected out, the ovaries removed and the corpora lutea counted. The gravid uterus was weighed, the uterine contents (including the placentas) were examined and the number and position of live and dead fetuses and early, middle and late resorptions and/or empty implantation sites were recorded. The uterus of any animal judged to be non-pregnant was stained with 10% (v/v) aqueous ammonium sulfide solution and examined for implantation sites."
Placental examination	At necropsy
Teratologic (Fetal) examination	Each fetus was weighed, sexed, and given a detailed external examination. The head was examined by coronal section between the frontal and parietal bones. Visceral and skeletal anomalies were noted.

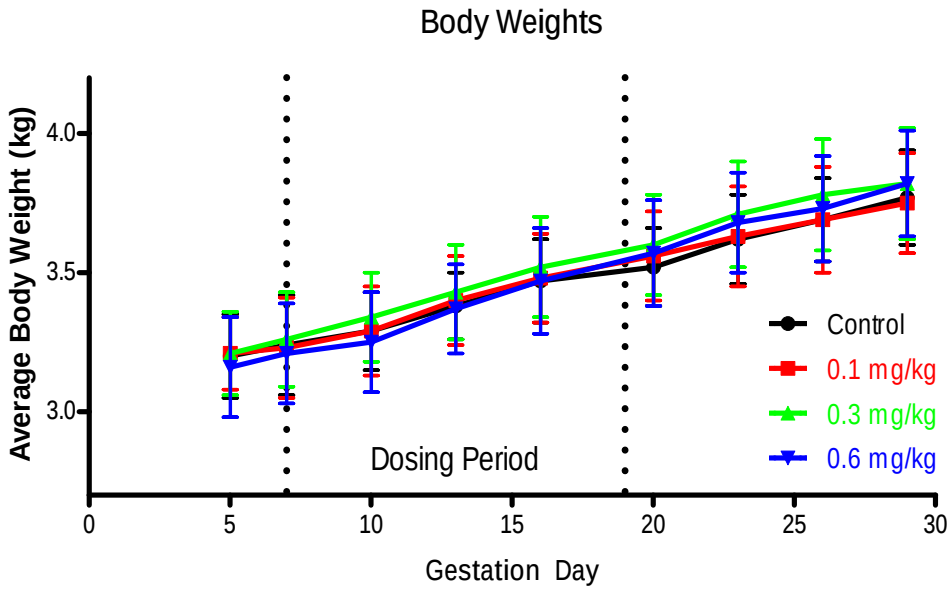
Results

Mortality: There were no unscheduled deaths.

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

Body weight: Body weight gains increased slightly with drug treatment (17-28%) during the dosing period), but the effect was not statistically significant.

Body Weight				
Study Time	Dose, mg/kg	BW gain (kg)	% Increment	BW % control
Dosing Period (GD7-19)	0	0.28	-	100%
	0.1	0.33	↑17%	101%
	0.3	0.34	↑21%	102%
	0.6	0.36	↑28%	101%
Entire Study (GD7-29)	0	0.53	-	100%
	0.1	0.52	↓2%	99%
	0.3	0.56	↑5%	101%
	0.6	0.61	↑15%	101%



Food consumption: There were no significant differences in food consumption.

Toxicokinetics: Toxicokinetic parameters were not evaluated.

Maternal necropsy: Dark areas near the injection site increased in incidence with dose (from 3 in control group to 8 in the high dose group).

C-section data: As shown in the sponsor's tables below, there were no significant differences in uterine/ovarian parameters, fetal numbers, or resorptions.

Parameter	Control		0.1 mg/kg		0.3 mg/kg		0.6 mg/kg	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Corpora Lutea	9.2	1.6	8.5	1.99	9.2	1.19	9.6	2.13
Implantation sites	8.3	1. 72	7.7	1. 99	8.3	1.17	8.7	2.51
Male Fetuses	3.8	1. 71	3.5	1. 99	3.9	1.48	4.4	1.71
Female Fetuses	4.3	1.67	3.9	1.66	3.9	1.19	4.1	1.55
Sex Ratio (%males)	46.9	22.2	44.7	23.19	49.3	15.25	49.8	17.81
Live Fetuses	8.1	1.8	7.5	1	7.8	1.37	8.5	2.5
Dead Fetuses	0	0	0	0	0	0	0	0
Early Resorptions	0.1	0.29	0.1	0.35	0.4	0.58	0.1	0.29
Middle Resorptions	0	0	0	0.21	0	0	0	0
Late Resorptions	0.1	0.29	0	0.21	0.1	0.35	0.1	0.29
All Resorptions	0.2	0.39	0.2	0.43	0.5	0.74	0.2	0.39
Empty Implantation Sites	0	0	0	0	0	0	0	0
Pre-implantation loss (%)	10	12.56	10.4	17.17	8.9	13.81	11.3	15.37
Post-implantation loss (%)	2.6	5.89	2.7	5.13	6.1	9.2	2	4.47
Gravid uterus weight (g)	536.6	97.87	483.9	109.95	512.5	75.85	521.5	131.53

Fetal Body Weight: Fetal body weights were slightly lower (↓9%) in HD male fetuses ($p < 0.05$) and HD female fetuses (↓6%, $p > 0.05$). The sponsor did not consider this to be toxicologically significant since the average weights were within the historical control range.

Parameter	Control		0.1 mg/kg		0.3 mg/kg		0.6 mg/kg	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Male Fetuses	48	4.78	45.9	4.14	46.5	3.64	43.7**	3.60
Female Fetuses	46.3	3.75	45.0	6.00	45.6	3.88	43.3	5.50
Total	47.6	3.99	46.1	5.21	46.2	3.47	43.7**	4.62

Offspring (malformations, variations, etc.):

Major malformations: Major malformations occurred in 2, 2, and 1 fetus in the low, mid, and high dose groups, respectively, but were not observed in the offspring of control animals.

Major malformations		
Dose	Fetus	Finding
0.1 mg/kg	2505/4	Dilatation of the ascending aorta Stenosis of pulmonary truncus and ductus arteriosus
	2505/1	Membrane-covered herniation of abdominal musculature at the umbilicus
0.3 mg/kg	3502/9	Indentation encircling cervical region (possibly due to umbilical cord) Exencephaly, unilateral anotia, brachygnathia, micrognathia Cleft palate, lack of turbinate formation, lack of forelimbs, midline cleft with cranial major organ displacement
	3509/3	Fused and pelvic kidneys
0.6 mg/kg	4511/3	Intraventricular septal defect with stenosis of the aortic arch

Variations: Some variations occurred more often in TH9507-treated animals, but there did not appear to be a toxicologically significant effect. The fetal incidence of "additional sutures in the frontal/parietal bones" increased in MD animals by a statistically significant amount, but HD offspring were similar to controls. Reduced ossification of the parietal bone (skull) was noted in two fetuses from two litters.

Variations (# litters/# fetuses)				
	Control n=155 F n=21 L	0.1 mg/kg n=164 F N=22 L	0.3 mg/kg n=172 fetus n=22 litters	0.6 mg/kg n=187 fetus n=22 litters
All visceral variations	**3/3**	**5/6**	**7/7**	**3/3**
Retrocaval ureters	1/1	4/4	2/2	2/2
All skeletal variations	**20/44**	**17/38**	**16/34**	**20/47**
Hyoid bone: irregular ossification	4/4	5/5	6/6	6/10
Parietal bone: reduced ossification	0/0	0/0	0/0	2/2
Additional sutures in frontal/parietal	3/3	1/1	5/10*	4/4
Additional sutures in nasal bones	0/0	1/1	2/2	3/3

A SUBCUTANEOUS TERATOLOGY STUDY OF TH9507 IN THE RABBIT [HIGH DOSES]

Key study findings:
Dams: Dams given 2 mg/kg were exposed to > 300X MRHD (C_{max} or AUC basis). There were no significant treatment-related differences in clinical signs or uterine parameters.

Fetuses: There were no significant differences in fetal weights. Two fetuses from TH9507-treated dams had major malformations, including hydrocephaly (in a single animal), but the incidence was within the historical control range. The finding of incomplete or irregular ossification of bones in the skull was increased in the treated group, but the effect was not statistically significant. The rate of 13th rib increased by ~50% in offspring of treated dams.

Reviewer comments: This study was requested by the division to attempt to elicit a minimal toxic reaction in this species since doses up to 0.6 mg/kg did not cause adverse effects in previous studies. The sponsor has complied with the request and verified exposure at a high multiple of MRHD. The results of this study would generally be considered unconcerning except for the fact that hydrocephaly was also noted in the Segment II-III rat study. The reviewer considers the hydrocephaly noted here to be possibly related to treatment with TH9507, but since the incidence is well within the historical control range for hydrocephaly, a definitive attribution cannot be made with any certainty. Additionally, offspring of dams given 2 mg/kg had higher fetal rates of incomplete ossification in the bones of the skull. Although the effect was not statistically significant, it is consistent with effects seen in the rat embryofetal development study. The NOAEL for this study is 2 mg/kg, with the cautions noted above.

The reviewer notes that this study is a repeat of study E-PCL-226, in which a low pregnancy rate (~50% in both groups) produced an inadequate study. Since no fetal examination was performed in E-PCL-226, it is not reviewed herein. Other results are generally consistent with the current study.

Study: E-PCL-269
Volume and page: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: 21 December 2007
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: TH9507, FHEXGRF0501, 91% peptide, 99.6% peptide purity

METHODS	
Doses:	Dosed with 0 or 2 mg/kg from GD7-19
Species/source	Time mated female New Zealand White rabbits / (b) (4)
Number/sex/group:	21 females per group
Route, formulation, dose volume	s.c. injection in 1 mL/kg 5% mannitol
Toxicokinetic groups	Four animals per group (satellite)

IN-LIFE OBSERVATIONS	FREQUENCY
Cageside observations & clinical exams	Twice daily with detailed examinations on GD 5, 7, 10, 13, 16, 20, 23, and 26
Body weight & food consumption	Body weights: GD 5, 7, 10, 13, 16, 20, 23, and 26 Food consumption: daily
TK analysis	Samples taken from TK animals on GD7 and GD19 at 0, 5, 15, 30, and 60 minutes post dose.

POST-MORTEM EVALUATIONS	
Maternal necropsy	Animals were euthanized on GD29. All main study animals were subject to necropsy
Ovarian/Uterine examinations	"The reproductive tract was dissected out, the ovaries removed and the corpora lutea counted. The gravid uterus was weighed, the uterine contents (including the placentas) were examined and the number and position of live and dead fetuses and early, middle and late resorptions and/or empty implantation sites were recorded. The uterus of any animal judged to be non-pregnant was stained with 10% (v/v) aqueous ammonium sulfide solution and examined for implantation sites."
Placental examination	Not performed.
Teratologic (Fetal) examination	Each fetus was weighed, sexed, and given a detailed external examination. The head was examined by coronal section between the frontal and parietal bones. Visceral and skeletal anomalies were noted.

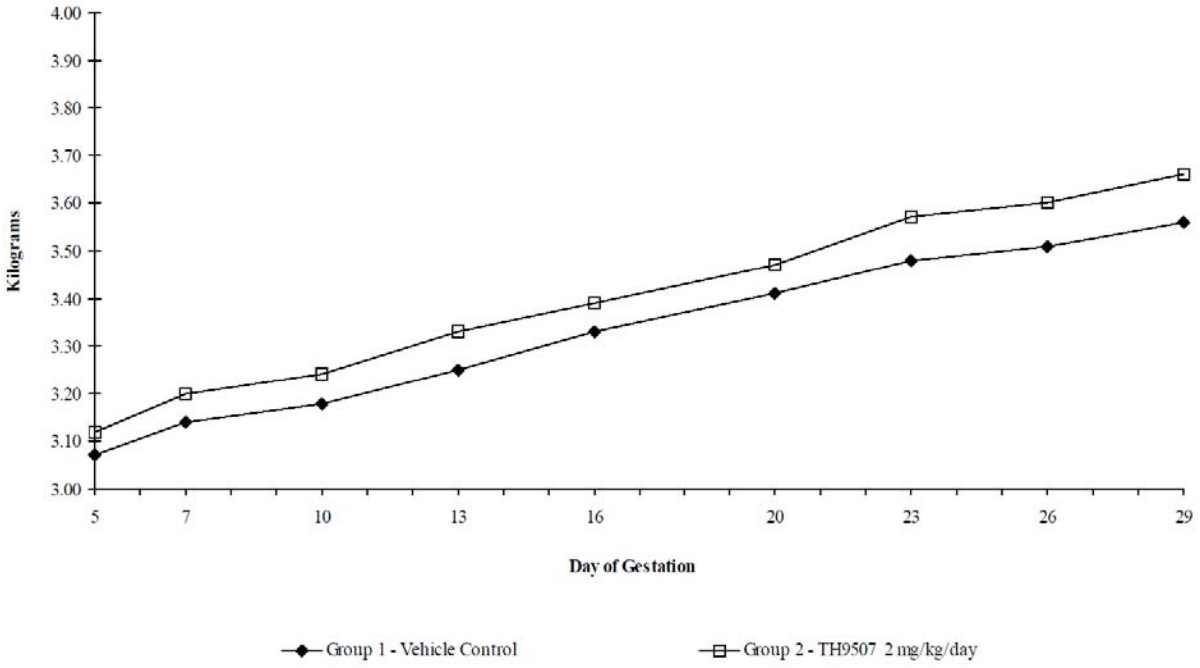
Results

Mortality: There were no unscheduled deaths.

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

Body weight: Body weight gains were similar in control and TH9507 treated animals during the dosing period, but treated animals gained 10% more weight over the entire study due to a 25% increase in weight gain during GD20-29.

Body Weight				
Study Time	Dose, mg/kg	BW gain (kg)	% Increment	BW % control
Dosing Period (GD7-19)	0	0.27	-	100%
	2	0.27	0%	102%
Entire Study (GD7-29)	0	0.42	-	100%
	2	0.46	↑10%	103%



Food consumption: There were no significant differences in food consumption.

Toxicokinetics: Toxicokinetic parameters for GD7 and GD19 are shown in the sponsor's tables and figure below. There was not an appreciable difference in C_{max} or AUC between the two timepoints. C_{max} values are ~300X MRHD. AUC values are not directly comparable to clinical values since they were measured over only 60 minutes; however, the 1 hour exposure values are ~500X daily exposures for clinical subjects given 2 mg.

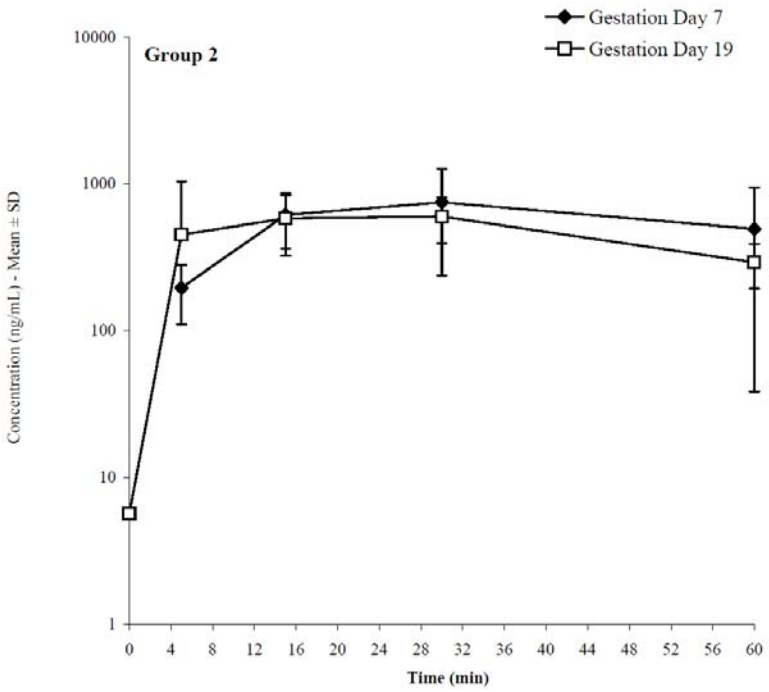
Gestation Day 7

Group No.	Dose Level (mg/kg/day)	Animal No.	Tmax (min)	C_{max} (ng/mL)	$AUC_{(0\text{-tlast})}$ (ng•min/mL)	C_{max}/ Dose	$AUC_{(0\text{-tlast})}$/ Dose
2	2	2523	30	725	29439	363	14720
		2524	30	1484	63260	742	31630
		2525	30	464	21441	232	10721
		2526	5	302	9248	151	4624
		2527	30	956	43971	478	21986
		Mean [a]	30	786	33472	393	16736
		SD		463	20889	232	10444

Gestation Day 19

Group No.	Dose Level (mg/kg/day)	Animal No.	Tmax (min)	C_{max} (ng/mL)	$AUC_{(0\text{-tlast})}$ (ng•min/mL)	C_{max}/ Dose	$AUC_{(0\text{-tlast})}$/ Dose
2	2	2523	15	770	33476	385	16738
		2524	15	714	31559	357	15779
		2525	5	1488	38990	744	19495
		2526	30	239	10461	119	5230
		2527	30	693	28429	346	14215
		Mean [a]	15	781	28583	390	14291
		SD		449	10834	224	5417

[a] Median value reported for Tmax.



C-section data: As shown in the sponsor's tables below, there were no significant differences in uterine parameters, fetal numbers, or resorptions.

Group	Summary Information	Total Number of Corpora Lutea	Total Implantation Sites	Male Fetuses	Female Fetuses	Sex Ratio (% Males)
1	Mean	8.5	7.7	3.8	3.6	49.72
	SD	1.3	1.9	1.4	1.6	19.07
	N	21	21	21	21	21
2	Mean	8.2	7.7	3.6	4.0	47.65
	SD	1.7	1.6	1.4	1.5	18.38
	N	21	21	21	21	21

Group	Summary Information	Live Fetuses	Dead Fetuses	Early Resorptions	Middle Resorptions	Late Resorptions
1	Mean	7.4	0.0	0.1	0.0	0.2
	SD	2.0	0.0	0.5	0.0	0.4
	N	21	21	21	21	21
2	Mean	7.5	0.0	0.0	0.1	0.0 a
	SD	1.5	0.0	0.2	0.3	0.0
	N	21	21	21	21	21

Group	Summary Information	Sum of Resorptions	Number of Empty Implantation Sites	Pre-implantation Loss (%)	Post Implantation Loss (%)	Gravid Uterus Weight (g)
1	Mean	0.3	0.0	8.63	4.27	440.2
	SD	0.6	0.0	19.85	7.69	107.6
	N	21	21	21	21	21
2	Mean	0.1	0.0	5.76	1.59	464.9
	SD	0.5	0.0	8.91	5.31	69.0
	N	21	21	21	21	21

Fetal Body Weight: Fetal body weights were similar in both groups.

Group	Summary Information	Males	Females	Total
1	Mean	44.13	43.28	43.90
	SD	4.62	6.78	5.50
	N	20	21	21
2	Mean	45.07	44.51	44.80
	SD	3.89	3.81	3.54
	N	20	21	21

Offspring:

Major malformations: Two fetuses from two different TH9507-treated dams had major malformations, as shown in the table below. Each of the findings was within the historical control range, but generally at the high end (due to the low incidence and generally standardized study designs). One finding of concern is hydrocephaly, which the reviewer notes was also seen in the Segment 3 rat study. The fetus with this finding also had incomplete ossification of the parietal, frontal, and interparietal bones of the skull.

Major malformations					
Fetus	Finding	Litter incidence	Fetal incidence	Historical control range (litter)	Historical control range (fetal)
2520/3	Hydrocephaly	4.8%	0.63%	0-7.7%	0-2.5%
	Diaphragmatic hernia	4.8%	0.63%	0-5%	0-0.67%
2512/8	Dilatation of ascending aorta	4.8%	0.63%	0-5.26%	0-0.64%
	Stenosis of pulmonary trunk	4.8%	0.63%	0-4.76%	0-0.63%
	Interventricular septal defect	4.8%	0.63%	0-6.72%	0-0.84%

Variations: There were a few variations that occurred more often in TH9507 treated animals and are shown below. A missing accessory lung lobe was noted in four fetuses from treated dams but not in any from controls. Incomplete ossification of several bones of the skull was noted at higher rates in treated fetuses, but the effect was not statistically significant. The presence of a 13th rib (bilateral or unilateral) was seen at a ~50% higher rate and the presence of delayed development of the sternebrae was lower in fetuses from treated dams. Thus, while ossification was relatively progressive in treated animals in the sternebrae and ribs, it was delayed in the skull.

Variations (# litters/# fetuses)		
	Control n=155 fetus n=21 litters	2mg/kg n=158 fetus n=21 litters
All visceral variations	**1/2**	**4/6**
Accessory lung lobe absent	0/0	2/4
Abnormal flexure of forepaw(s)	0/0	1/1
All skeletal variations	**19/96**	**21/103**
Incomplete ossification: parietal	2/2	5/6
Incomplete ossification: frontal	15/28	16/42
Incomplete ossification: interparietal	1/1	4/4
Irregular ossification: parietal	0/0	1/1
Common skeletal variants (%)		
13^{th} rib (total)	40.47%	61.89%*
Sternebrae (unossified/incomplete/ semibipartite/bipartite)	61.69	46.24

SUBCUTANEOUS PRE- AND POSTNATAL STUDY IN THE RAT

Key study findings:

F0 Generation: There were no adverse effects in the dams (up to 1.2 mg/kg/d), but treated animals gained more weight, especially during lactation.

F1 Generation
Four F1 pups from two HD litters had hydrocephaly, noted either at post-weaning necropsy (n=1) or as a clinical sign beginning LD11 (n=3). There were no other treatment-related effects on the F1 generation prior to mating. F1 animals from the HD group had more dead F2 pups (12 versus 5 in controls) but were otherwise not impaired in reproductive assessments.

F2 Generation
F2 pups from treated offspring gained more weight over LD0-4 than controls (5-12%).

Reviewer Comments: The reviewer considers the hydrocephaly observed in the F_1 pups to be related to treatment. This effect could either be due to 1) direct exposure to the drug in milk (although measurements of F_1 drug concentrations in the two hours after dosing showed F_1 plasma concentrations below the LLOQ, 0.6 ng/mL or ~25% MRHD C_{max}) or 2) exposure to anti-GHRH antibodies transferred from the dams during lactation. (Anti-GHRH antibodies were detected in two animals in the 26-week rat study and in all animals in the 13-week study using a compromised assay). The sponsor has not provided sufficient data to distinguish between these two possibilities and does not attempt to do so in their application. In either case, tesamorelin should not be taken by pregnant or breastfeeding women due to the risk of hydrocephaly in the developing fetus or infant. Please see section 2.6.6.9 for further discussion

SD Rats, Segment 3 Adverse Effect	NOAEL	Multiple of MRHD (1.1 ng.h/mL)
Dams No maternal toxicity	1.2 mg/kg	1.6X (Gestation) 2.7X (Lactation)
F1 Generation Hydrocephaly	0.6 mg/kg	1X (Gestation) 1.8X (Lactation)
F2 Generation No adverse effects	1.2 mg/kg	1.6X (Gestation) 2.7X (Lactation)

Study: EPCL-227
Volume and page: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: 15 May 2007
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: TH9507, lot FHEXGRF0501, peptide purity > 98.5%, peptide content 91.0%

Methods	
Doses	0, 0.1, 0.6, 1.2 mg/kg QD
Dosing Schedule	Animals were treated from GD6 to LD21
Species/source	Time-mated F Sprague Dawley Rats, Crl:CD(SD) IGS / (b) (4)
Number/sex/group (main study)	22/sex/group
Route, formulation, dose volume	s.c. injection in the scapular region in 1 mL/kg in 5% mannitol
Toxicokinetic groups	Samples collected from main study animals on GD6, GD17, and LD10 (F0) and 10 days after birth for F1.

IN-LIFE OBSERVATIONS	FREQUENCY
Mortality & cageside observations	Twice daily (adults)
Clinical examination	F_0: Detailed examinations every three days and daily GD20-parturition F_1: Examined on LD0 for malformations, sex, weight, and live status and daily for general condition until weaning. Detailed observations twice weekly as adults. F_2: Daily during lactation
Body weight	F_0:Approximately every three days F_1: At birth, LD4, 7, 14, and 21; Mated females weighed every three days F_2: At birth and LD4

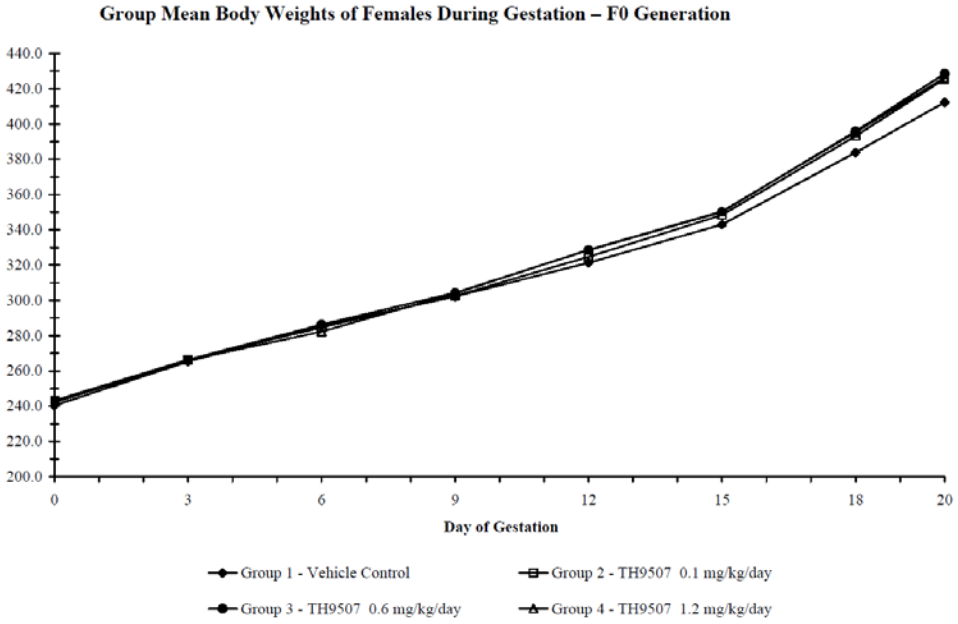
Food consumption	F_0:Approximately every three days
Culling	On LD4, F_1 litters were reduced to 8 pups per litter (4/sex where possible); Culled pups were subject to skeletal exam.
F_1 Breeding procedure	On LD21 (weaning), one male and one female per litter were selected for further breeding. At 85 days old, one male and one female cohabitated for 14 days, avoiding sibling matings.
F_1 Observations	Physical development, pupillary closure response, Motor activity in Figure 8 maze, auditory startle habituation, "E" water maze
F_2 Observations	General condition was evaluated during lactation
POST-MORTEM EVALUATIONS	
Termination	F_0 dams were euthanized on LD21; F_1 dams that failed to mate were euthanized 26d after the end of the mating period; F_1 generation dams that were pregnant were euthanized on LD4-6; mated F_1 males were euthanized 3 weeks after the end of the mating period. F1 animals not selected for mating were euthanized after weaning.
Macroscopic	Gross pathology performed for all F_0 and F_1 animals; F_2 animals were not examined if they were externally normal
Organ weights	Not measured
Uterine & Ovarian exams	Implantation site scars were counted when appropriate. Non-pregnant animals were evaluated for evidence of implantation sites.
Fetal observation	Culled F1 pups (D4) and non-mating F1 weanlings were prepared for skeletal exam, but it was not performed.

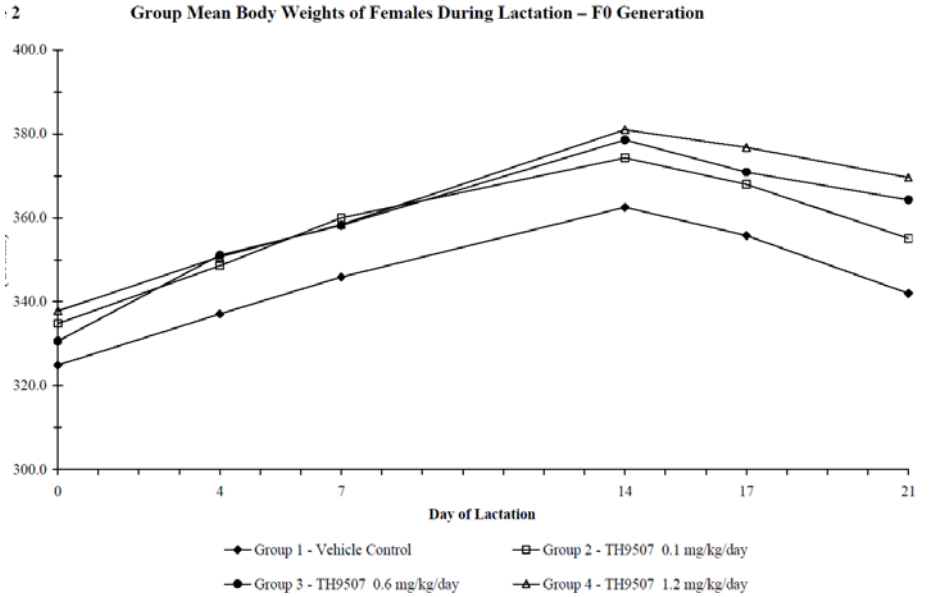
Results

F_0 Generation
Mortality: There were no unscheduled deaths in the F_0 generation.
Clinical Signs: Clinical signs noted in the F_0 animals (red or scabbed skin and staining and/or thinning of the fur) occurred in control and treated animals with similar frequency.
Body Weight: Over the entire study, F_0 dams given TH9507 had significantly greater weight gain than controls. This effect was most pronounced during lactation. The sponsor's figures below illustrate the effects.

Female Body Weight				
Study Time	Dose, mg/kg	BW gain (g)	% Increment	BW % control
Gestation GD0-GD20	0	171.9	-	100%
	0.1	182.3	↑6%	102%
	0.6	185.6	↑8%	103%
	1.2	184.1	↑7%	103%
Lactation LD0-LD21	0	17.1	-	100%
	0.1	20.3	↑19%	104%*
	0.6	33.7	↑97%	106%***
	1.2	31.8	↑86%	108%***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$





Food Consumption: Food consumption during gestation was similar across dose groups after correcting for body weight.

F_0 necropsy: Injection sites were more likely to be dark and/or swollen in drug-treated animals (generally at ≥ 0.6 mg/kg). Other sporadic findings did not appear to be related to treatment.

F_0 Reproductive Performance: There were no treatment-related effects on reproductive performance.

F0 Generation

Group 1 - Vehicle Control
Group 2 - TH9507 0.1 mg/kg/day
Group 3 - TH9507 0.6 mg/kg/day
Group 4 - TH9507 1.2 mg/kg/day

Group	No. of Mated Females	No. of Pregnant Females	Pregnancy Rate (%)	Gestation Index (%)	Dead Pups		Malformed Pups	
					Litters Affected	Pups Affected	Litters Affected	Pups Affected
1	24	23	95.8	100.0	5	11	0	0
2	24	24	100.0	100.0	2	2	0	0
3	24	23	95.8	100.0	3	5	1	1
4	24	24	100.0	100.0	2	3	0	0

Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Fisher's)

F_1 Weaning period

F_1 Viability: F1 pups from high dose dams had lower survival (not statistically significant) due to humane sacrifice of pups with clinical findings (see below).

F1 Generation - Pups
Day Post Partum

Group 1 - Vehicle Control
Group 2 - TH9507 0.1 mg/kg/day
Group 3 - TH9507 0.6 mg/kg/day
Group 4 - TH9507 1.2 mg/kg/day

Group	Summary Information	Day 4 Viability Index	Day 7 Survival Index	Day 14 Survival Index	Day 21 Lactation Index
1	Mean	99.67	100.0	100.0	100.0
	SD	1.61	0.0	0.0	0.0
	N	23	23	23	23
2	Mean	99.72	100.0	100.0	100.0
	SD	1.37	0.0	0.0	0.0
	N	24	24	24	24
3	Mean	99.01	100.0	100.0	100.0
	SD	2.62	0.0	0.0	0.0
	N	23	23	23	23
4	Mean	99.23	100.0	98.9	98.9
	SD	2.57	0.0	5.2	5.2
	N	23	23	23	23

Significantly different from control group (group 1) value: a - P < 0.05 b - P < 0.01 c - P < 0.001 (Wilcoxon)

Group mean litter size: There were no significant differences in average litter size.

F1 Generation - Pups
Day Post Partum

Group 1 - Vehicle Control
Group 2 - TH9507 0.1 mg/kg/day
Group 3 - TH9507 0.6 mg/kg/day
Group 4 - TH9507 1.2 mg/kg/day

Group	Summary Information	Day 0 Males	Day 0 Females	Day 0 Total	Day 4 (Pre-Cull) Males	Day 4 (Pre-Cull) Females	Day 4 (Pre-Cull) Total
1	Mean	5.9	6.1	12.0	5.9	6.0	12.0
	SD	2.6	2.6	3.3	2.6	2.6	3.3
	N	23	23	23	23	23	23
2	Mean	6.3	6.8	13.2	6.3	6.8	13.1
	SD	2.2	2.1	2.0	2.2	2.1	2.0
	N	24	24	24	24	24	24
3	Mean	7.4	6.5	13.9	7.3	6.5	13.8
	SD	2.1	1.9	2.1	2.2	1.9	2.1
	N	23	23	23	23	23	23
4	Mean	6.6	6.6	13.1	6.5	6.5	13.0
	SD	2.4	2.1	2.2	2.4	2.1	2.3
	N	23	23	23	23	23	23

F_1 Body Weight: There were no treatment-related differences in fetal body weights at birth or during lactation.

Body Weight of Live F_1 Newborns				
	Control n=23	0.1 mg/kg n=23	0.6 mg/kg n=22	1.2 mg/kg n=22
Males (g)	7.12	7.20	7.11	7.11
Females (g)	6.77	6.80	6.77	6.75

MALES: Body Weight to LD21				
Study Time	Dose, mg/kg	BW gain (g)	% Decrement	BW % control
LD0 to LD21	0	51.36	-	100%
	0.1	51.09	0%	99%
	0.6	52.05	↑1%	101%
	1.2	51.47	0%	100%

FEMALES: Body Weight to LD21				
Study Time	Dose, mg/kg	BW gain (g)	% Decrement	BW % control
LD0 to LD21	0	49.24	-	100%
	0.1	49.69	↑1%	100%
	0.6	50.19	↑2%	101%
	1.2	48.97	↓1%	99%

F_1 physical Exam: Three pups from the same HD litter (1 male and 2 females), exhibited swelling of the cranial region indicative of hydrocephaly beginning D11 post partum. Other signs in these animals

included thinness, cold to touch, signs of dehydration, and uncoordination. No historical control data was provided for physical exam findings.

Physical Exam (Sexes Combined)				
	C	LD	MD	HD
Cold to touch	0	0	0	3
Dehydrated suspected	0	0	0	3
Swollen, firm cranium	0	0	0	3
Swollen cranium	0	0	0	1
Thin	0	0	0	4
Uncoordinated	0	0	0	2

F_1 External, Visceral, and Skeletal Examination, sacrificed at weaning: The male fetus with hydrocephaly was noted at necropsy to have dilatation of the ventricle and dark, clear fluid and in the brain, confirming the diagnosis. Four HD offspring (of 65) had dark area on the lung. Three females in the high dose offspring died between D8 and D21 post partum, each with dilated ventricle in the brain with pale, clear fluid in the brain or cranial cavity. Note that one of the three females with hydrocephaly was from a separate litter. The litter incidence exceeded the historical control range, but a fetal incidence historical control range was not provided. The reviewer concludes that this is a significant, drug-related effect.

NECROPSY FINDINGS (AT WEANING AND EARLY DECEDENTS)											
Tissue	Finding		MALES				FEMALES				
		Dose	0	0.1	0.6	1.2	0	0.1	0.6	1.2
		n	67	69	69	71	62	75	69	68
Brain	Dilatation of the ventricle		0	0	0	1	0	0	0	3+
	Fluid, dark, clear		0	0	0	1	0	0	0	0+
	Fluid, pale, clear		0	0	0	0	0	0	0	1+
Cranial Cavity	Fluid, pale, clear		0	0	0	0	0	0	0	2+
Lung	Area dark		1	1	0	1	0	0	1	4

+ Three female animals found dead or euthanized prior to weaning

DIAGNOSIS OF HYDROCEPHALY										
	MALES				FEMALES				Litter Incidence (1.2 mg/kg)	Historical control
Dose	0	0.1	0.6	1.2	0	0.1	0.6	1.2		
Fetuses Examined:	67	69	69	71	62	75	69	68		
Litters Examined:	23	24	23	23	23	24	23	23		
Animals diagnosed:	0	0	0	1	0	0	0	3	8.7%	0-4%
Litters with diagnosis	0	0	0	1	0	0	0	2		

F_1 Post-weaning Period

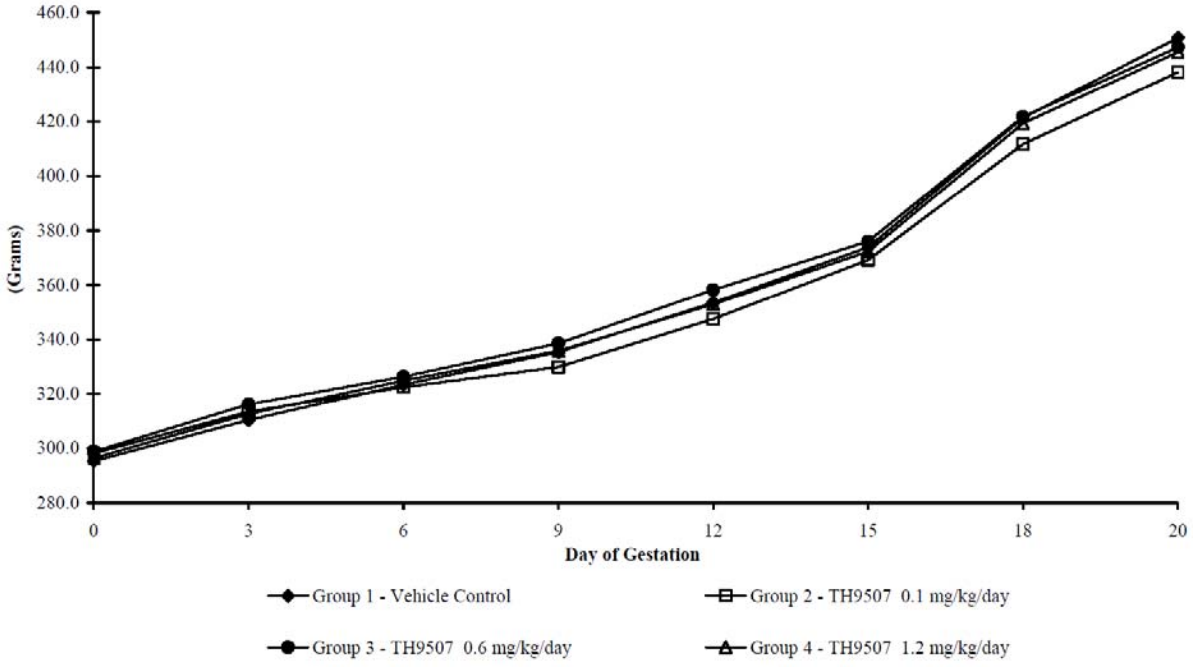
Post-weaning Viability: There were no deaths in TH9507-treated animals during this time.
Post-weaning physical exam: There were no treatment-related effects.
Post-weaning body weight: There were no treatment-related differences.

Post-weaning Body Weight				
Post weaning week	Control	0.1 mg/kg	0.6 mg/kg	1.2 mg/kg
Females Week 1	59.6 ± 4.3	59.8 ± 5.5	60.7 ± 5.5	59.2 ± 3.2
Week 8	290 ± 37	293 ± 32	287 ± 23	288 ± 27
GD20	450 ± 41	438 ± 39	447 ± 36	446 ± 40
LD4	355 ± 24	351 ± 26	359 ± 24	353 ± 31
Males Week 1	62.4 ± 4.7	61.3 ± 5.1	62.5 ± 5.0	62.3 ± 4.5
Week 13	646 ± 61	642 ± 67	630 ± 57	655 ± 68

Development post-weaning (n= 23-24 per group)
Visual function: There were no differences in pupillary closure
Behavioral performance: Activity counts were similar across groups assessed in Figure 8 mazes. There were no differences in startle habituation or in performance in a water maze.
Physical development: All groups had similar times to vaginal opening or preputial separation.

F_1 Mating to Sacrifice Period
Mortality: One control male was found dead on D114 post partum.
Physical Exam: There were no treatment-related effects.
Body weight: Body weights and weight gains did not show any treatment-related effects, as shown in the sponsor's figure below.

Figure 5 **Group Mean Body Weights of Females during Gestation F_1 Generation – Adults**



Reproductive Performance: There were no treatment-related effects on reproductive performance in either males or females, as shown in the sponsor's tables below; however, there were significantly more dead pups in the litters of the offspring of high dose animals (12 versus 5 in the control group). This resulted in a slightly lower number of live pups per litter and a 2-fold increase in the number of dead pups per litter, but the effect on the average was not statistically significant.

Table 33 Group Mean Parental Performance

F1 Generation

Group 1 - Vehicle Control
Group 2 - TH9507 0.1 mg/kg/day
Group 3 - TH9507 0.6 mg/kg/day
Group 4 - TH9507 1.2 mg/kg/day

Group	Number Placed for Mating: Males	Number Placed for Mating: Females	Number Mating	Mean (SD) Day to Mating	Number Females Pregnant	Mating Index (%)	Fertility Index (%)	Conception Rate (%)
1	23	23	21	2.5 1.5 (N = 19)	18	91.3	78.3	85.7
2	24	24	21	2.2 1.2 (N = 21)	21	87.5	87.5	100.0
3	23	23	22	3.6 2.9 (N = 22)	19	95.7	82.6	86.4
4	23	23	19	2.8 1.5 (N = 19)	18	82.6	78.3	94.7

Significantly different from control group (group 1) value: a - P ≤ 0.05 b - P ≤ 0.01 c - P ≤ 0.001 (Wilcoxon - day to mating only)
Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Fisher's)

F1 Generation

Group 1 - Vehicle Control
Group 2 - TH9507 0.1 mg/kg/day
Group 3 - TH9507 0.6 mg/kg/day
Group 4 - TH9507 1.2 mg/kg/day

Group	No. of Mated Females	No. of Pregnant Females	Pregnancy Rate (%)	Gestation Index (%)	Dead Pups: Litters Affected	Dead Pups: Pups Affected	Malformed Pups: Litters Affected	Malformed Pups: Pups Affected
1	21	18	85.7	88.9	4	5	1	1
2	21	21	100.0	100.0	2	3 **	0	0
3	22	19	86.4	100.0	5	6	0	0
4	19	18	94.7	100.0	8	12 *	0	0

Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Fisher's)

Group Mean Data
F1 Generation

Group 1 - Vehicle Control
Group 2 - TH9507 0.1 mg/kg/day
Group 3 - TH9507 0.6 mg/kg/day
Group 4 - TH9507 1.2 mg/kg/day

Group	Summary Information	Length of Gestation (Days)	Duration of Parturition (h)	Sex Ratio (%)	Number of Pups at Birth/Litters Live	Dead	Malformed	No. of Implant Scars	Live Birth Index (%)
1	Mean	21.9	3.32	51.60	15.1	0.3	0.06	16.7	91.32
	SD	0.3	1.68	13.66	3.0	0.6	0.25	3.0	7.07
	N	16	5	16	16	16	16	18	16
2	Mean	21.7	3.13	46.81	14.8	0.1	0.00	16.1	92.03
	SD	0.5	1.24	14.50	2.7	0.5	0.00	2.7	6.11
	N	21	5	21	21	21	21	21	21
3	Mean	21.9	1.87	44.45	13.7	0.3	0.00	15.5	89.66
	SD	0.5	0.83	15.37	3.4	0.6	0.00	4.1	6.79
	N	19	3	19	19	19	19	19	19
4	Mean	21.9	3.82	49.52	14.4	0.7	0.00	15.9	91.09
	SD	0.5	0.35	15.20	3.4	0.8	0.00	3.7	6.14
	N	18	6	18	18	18	18	18	18

Significantly different from control group (group 1) value: a - P ≤ 0.05 b - P ≤ 0.01 c - P ≤ 0.001 (Wilcoxon)

F_2 Generation

Clinical signs: There were no relevant clinical signs.

Viability: Although there were more dead pups at birth in the HD group, at D4 viability was slightly higher in treated groups (~98%) relative to controls (~92%).

F2 Generation - Pups
Day Post Partum

Group 1 - Vehicle Control
Group 2 - TH9507 0.1 mg/kg/day
Group 3 - TH9507 0.6 mg/kg/day
Group 4 - TH9507 1.2 mg/kg/day

Group	Summary Information	Day 4 Viability Index
1	Mean	92.18
	SD	24.84
	N	16
2	Mean	98.07
	SD	3.18
	N	21
3	Mean	98.29
	SD	2.95
	N	19
4	Mean	98.67
	SD	2.56
	N	18

Significantly different from control group (group 1) value: a - P ≤ 0.05 b - P ≤ 0.01 c - P ≤ 0.001 (Wilcoxon)

F2 Generation - Pups
Day Post Partum

Group 1 - Vehicle Control
Group 2 - TH9507 0.1 mg/kg/day
Group 3 - TH9507 0.6 mg/kg/day
Group 4 - TH9507 1.2 mg/kg/day

Group	Summary Information	Day 0 Males	Day 0 Females	Day 0 Total	Day 4 Males	Day 4 Females	Day 4 Total
1	Mean	7.6	7.5	15.1	6.8	7.0	13.8
	SD	2.0	2.6	3.0	2.4	3.2	4.7
	N	16	16	16	16	16	16
2	Mean	7.0	7.9	14.8	6.8	7.8	14.5
	SD	2.6	2.4	2.7	2.5	2.4	2.7
	N	21	21	21	21	21	21
3	Mean	6.1	7.7	13.7	5.9	7.5	13.5
	SD	2.4	2.7	3.4	2.3	2.7	3.3
	N	19	19	19	19	19	19
4	Mean	7.1	7.3	14.4	6.9	7.3	14.2
	SD	2.9	2.7	3.4	2.8	2.7	3.3
	N	18	18	18	18	18	18

Body weight: There were no statistically significant treatment-related effects on body weight, but weight gain appeared to be higher in the F2 animals of TH9507-treated groups.

F2 Weight Gain						
Sex	Dose, mg/kg	D0 (g)	D4 (g)	BW gain (g)	% Increment	BW % control
Males LD0 to LD4	0	6.89	11.00	4.11	0%	100%
	0.1	6.63	11.25	4.62	12%	102%
	0.6	7.03	11.41	4.38	7%	104%
	1.2	6.96	11.41	4.45	8%	104%
Females LD0 to LD4	0	6.44	10.52	4.08	0%	100%
	0.1	6.36	10.65	4.29	5%	101%
	0.6	6.6	11.00	4.40	8%	105%
	1.2	6.63	11.16	4.53	11%	106%

Gross Pathology: There were no treatment-related effects.

A SUBCUTANEOUS INJECTION PHARMACOKINETIC STUDY OF TH9507 IN THE NON-PREGNANT, PREGNANT, AND LACTATING RAT

Key study findings:

- Pregnant rats given 0.1, 0.6, or 1.2 mg/kg (s.c.) had exposures that were 0.2X, 1X, and 1.6X MRHD (AUC basis) on GD17; after delivery (LD10), exposures were 0.2X, 1.8X, and 2.7X MRHD, respectively.
- TH9507 was below the LLOQ (0.625 mg/kg or 0.3X MRHD) on LD10 in the pups.

Reviewer Comments: These exposure levels were used to assess the safety margins for the reproductive toxicity studies in the rat. Exposures were markedly lower than those seen after the first dose in the 26-week study (which used an LC/MS/MS method, as opposed to the ELISA method used here). Both methods appear to have been adequately validated.

Study: E-PCL-282
Volume and page: eCTD
Conducting laboratory and location: (b) (4)

Date of study initiation: 7 March 2008
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: TH9507 FHEXGRF0601, 89.7% peptide, 99.0% purity

Purpose: To measure plasma concentrations of TH9507 following a single dose in male and non-pregnant female rats and repeated doses to pregnant/lactating rats and concentration in mild in lactating rats.

Methods: Pregnant female SD rats (26/group) were given s.c. doses of TH9507 at 0.1, 0.6, and 1.2 mg/kg from GD6 to LD10. Maternal concentrations of TH9507 were measured at 0, 3, 6, 12, 20, and 60 minutes post dose on GD6, GD17, and LD10. Fetal plasma concentrations were measured at euthanasia 30, 60 and 120 minutes after the maternal dose on LD10. Non-pregnant females and males were assessed for exposure only after a single dose. Serum samples were measured using a validated ELISA method (LLOQ = 0.625 ng/mL).

Results: The TK parameters from this study are shown in the sponsor's table below. All TH9507 concentrations for the pups were below the LLOQ. The reviewer notes that the LLOQ for the assay used in this study is ~25% of the clinical C_{max}. Please also note that the table below shows exposure (AUC) values in ng.min/mL rather than the more standard ng.h/mL. For comparison, the MRHD provides exposure that is ~66 ng.min/mL, or between the exposures of groups 2 and 3. The levels of TH9507 in maternal milk were not measured. The high dose animals in this study had exposures that were ~2X MRHD.

Day 1 * (Pregnant Females)

Group No.	Dose Level (mg/kg/day)	C_{max} (ng/mL)	$AUC_{(0\text{-}tlast)}$ (ng•min/mL)	C_{max}/ Dose	$AUC_{(0\text{-}tlast)}$/ Dose
1	0.1	1.34	20.7	13.4	207
2	0.6	4.09	38.8	6.82	64.6
3	1.2	5.97	133	4.98	110

Day 12 * (Pregnant Females)

Group No.	Dose Level (mg/kg/day)	C_{max} (ng/mL)	$AUC_{(0\text{-}tlast)}$ (ng•min/mL)	C_{max}/ Dose	$AUC_{(0\text{-}tlast)}$/ Dose
1	0.1	1.19	11.2	11.9	112
2	0.6	4.73	55.0	7.89	91.7
3	1.2	2.57	110	2.14	92.1

Day 32 * (Post- Partum Females)

Group No.	Dose Level (mg/kg/day)	C_{max} (ng/mL)	$AUC_{(0\text{-}tlast)}$ (ng•min/mL)	C_{max}/ Dose	$AUC_{(0\text{-}tlast)}$/ Dose
1	0.1	1.14	18.6	11.4	186
2	0.6	4.21	119	7.01	198
3	1.2	6.03	176	5.03	147

Day 1 (Males)

Group No.	Dose Level (mg/kg/day)	C_{max} (ng/mL)	$AUC_{(0\text{-}tlast)}$ (ng•min/mL)	C_{max}/ Dose	$AUC_{(0\text{-}tlast)}$/ Dose
4	0.1	1.30	9.78	13.0	97.8
5	0.6	2.69	37.5	4.49	62.6
6	1.2	3.94	39.8	3.28	33.2

Day 1 (Non-Pregnant Females)

Group No.	Dose Level (mg/kg/day)	C_{max} (ng/mL)	$AUC_{(0\text{-}tlast)}$ (ng•min/mL)	C_{max}/ Dose	$AUC_{(0\text{-}tlast)}$/ Dose
4	0.1	0.85	4.99	8.50	49.9
5	0.6	3.75	24.3	6.24	40.5
6	1.2	5.85	45.5	4.88	37.9

* Nominal day of treatment corresponds to gestation day 6, day 17, post partum day 10 respectively.

REPRODUCTIVE TOXICITY SUMMARY			
STUDY	**NOAEL**	**MRHD MULTIPLE (2mg; 1.1 ng.h/mL)**	**FINDINGS**
Male Rat Fertility 0.1, 0.3, 0.6 mg/kg 0.2, n/a, 0.6 ng h/mL	**0.6 mg/kg**	**0.5X**	No adverse effects Exposures were measured in a separate study.
Female Rat Fertility & Early Embryonic Development 0.1, 0.3, 0.6 mg/kg 0.3, n/a, 1 ng.h/mL	**0.6 mg/kg**	**1X**	Dose-dependent increase in maternal weight gain All doses: increased reduced ossification of interparietal bone of the skull HD: ↑10% fetal weights
Rat Post-Natal Development 0.1, 0.6, 1.2 mg/kg GD17: 0.2, 0.9, 1.8 ng h/mL LD10: 0.3, 2, 3 ng.h/mL	**0.6 mg/kg**	**1X**	HD: hydrocephaly in 4 F_1 pups (2 litters) Fetal: < 0.625 ng/mL (25% C_{max} @ MRHD)
Rabbit Embryonic Development [low doses] 0.1, 0.3, 0.6 mg/kg No TK	**0.6 mg/kg**	**Est. 150X**	No adverse effects Dose-dependent increases in maternal weight gain (↑17-28%) MRHD multiple assumes linearity from 0 to 2 mg/kg
Rabbit Embryonic Development [high dose] 2 mg/kg 558 ng.h/mL	**2 mg/kg**	**500X**	Two fetuses from treated dams had major malformations, including hydrocephaly in one (within historical control range)

2.6.6.7 Local tolerance

SUBCUTANEOUS ADMINISTRATION IRRITATION STUDY OF HEX-HGRF USING RABBITS

Key study findings:

- An s.c. dose of 2 mg was not irritating to the skin of Japanese white rabbits.

Study: E-PCL-299
Volume and page: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: 19 November 2003
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: HEX-hGRF [TH9507], FHEXGRF0201, 90.5% peptide, 98.8% purity

Methods	
Doses	Vehicle, TH9507 (2 mg), and 5% acetic acid
Species/source	Japanese white rabbits (male) / (b) (4)
Age / Weight	13 weeks / 2.78-2.97 kg
n/sex/group (main study)	6 animals total
TK groups	None
Recovery groups	None
Route, formulation, dose volume	Single subcutaneous injection to three sites of 2 mL/site (positive and negative controls and test article).

Observations and Times	
Mortality checks	Once daily
Clinical Findings	Once daily
Body weights	D0 and D7
Gross pathology	Only the skin was examined
Histopathology	Only the skin was examined
	Adequate Battery: yes (x), no () Note: adequate for the aims of this study
	Peer review: yes (), no (x)

Results:

Clinical evaluations: There were no treatment-related effects on clinical signs or changes to the skin using TH9507. Acetic acid had the expected results.

Macroscopic examination: In the post mortem assessment, TH9507 was similar to controls for assessment of hyperemia, bleeding, swelling, and discoloration on D2 and D14.

Table 5-1: Macroscopic examination in male rabbits – Day 2

Test article	Animal No.	Hyperemia				Bleeding				Swelling				White discoloration				Brown discoloration				Total Score	Total area of changed site
		Score	L	W	S	Score	L	W	S	Score	L	W	S	Score	L	W	S	Score	L	W	S		
Physiological saline	1	0	-	-	-	2	5.1	2.4	12.2	0	-	-	-	0	-	-	-	0	-	-	-	2	12.2
	2	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	0	0.0
	3	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	0	0.0
	Mean	0.0				0.7				0.0				0.0				0.0				0.7*	4.07
1 mg/mL HEX-hGRF	1	1	2.2	0.8	1.8	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	1	1.8
	2	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	0	0.0
	3	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	0	0.0
	Mean	0.3				0.0				0.0				0.0				0.0				0.3*	0.60
5 vol% Acetic acid	1	0	-	-	-	1	2.2	0.3	0.7	3	9.5	7.2	68.4	0	-	-	-	2	5.1	4.0	20.4	6	89.5
	2	2	4.0	2.1	8.4	2	3.0	1.7	5.1	0	-	-	-	0	-	-	-	0	-	-	-	4	13.5
	3	2	4.3	3.8	16.3	0	-	-	-	2	4.5	3.0	13.5	0	-	-	-	0	-	-	-	4	29.8
	Mean	1.3				1.0				1.7				0.0				0.7				4.7*	44.27

Notes) L: Maximum length (cm) of the reactive site
W: Maximum width (cm) of the reactive site
S: L × W (cm^2)
-: Not measured
*: Total of the mean scores for each parameter

Table 5-2: Macroscopic examination in male rabbits – Day 14

Test article	Animal No.	Hyperemia				Bleeding				Swelling				White discoloration				Brown discoloration				Total Score	Total area of changed site
		Score	L	W	S	Score	L	W	S	Score	L	W	S	Score	L	W	S	Score	L	W	S		
Physiological saline	4	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	0	0.0
	5	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	0	0.0
	6	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	0	0.0
	Mean	0.0				0.0				0.0				0.0				0.0				0.0**	0.00
1 mg/mL HEX-hGRF	4	0				0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	0	0.0
	5	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	0	0.0
	6	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	0	0.0
	Mean	0.0				0.0				0.0				0.0				0.0				0.0**	0.00
5 vol% Acetic acid	4	0	-	-	-	1	0.6	0.7	0.4	0	-	-	-	0	-	-	-	0	-	-	-	1	0.4
	5	0	-	-	-	1	0.9 0.3	0.8 0.3	0.7* 0.1*	1	2.2	2.0	4.4	0	-	-	-	0	-	-	-	2	5.2
	6	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	1	1.3	1.2	1.6	1	1.6
	Mean	0.0				0.7				0.3				0.0				0.3				1.3**	2.40

Notes) L: Maximum length (cm) of the reactive site
W: Maximum width (cm) of the reactive site
S: L × W (cm^2)
-: Not measured
*: Two sites of bleeding were observed
**: Total of the mean scores for each parameter

Microscopic evaluations: Animals receiving TH9507 injections had lower total scores for irritation than the saline control on D2. TH9507 treated sites were similar to controls by the end of the study.

Table 6-1: Microscopic examination in male rabbits - Day 2

Test Article	Animal No.	Score						
		Cell infiltration	Bleeding	Edema	Fibrosis	Necrosis	Degeneration	Total Score
Physiological saline	1	2	3	0	0	2	1	8
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
	Mean	0.7	1.0	0.0	0.0	0.7	0.3	2.7*
1 mg/mL HEX-hGRF	1	0	1	0	0	0	0	1
	2	0	0	0	0	0	0	0
	3	1	1	0	0	0	0	2
	Mean	0.3	0.7	0.0	0.0	0.0	0.0	1.0*
5 vol% acetic acid	1	2	3	3	0	2	2	12
	2	1	2	0	0	0	0	3
	3	2	2	3	0	3	2	12
	Mean	1.7	2.3	2.0	0.0	1.7	1.3	9.0*

Notes) 0: No abnormal change, 1: Very slight, 2: Slight, 3: Moderate
*: Total of the mean scores for each parameter

Table 6-2: Microscopic examination in male rabbits - Day 14

Test Article	Animal No.	Score						
		Cell infiltration	Bleeding	Edema	Fibrosis	Necrosis	Degeneration	Total Score
Physiological saline	4	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0
	Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0*
1 mg/mL HEX-hGRF	4	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0
	Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0*
5 vol% acetic acid	4	1	2	2	2	1	1	9
	5	2	1	2	2	2	2	11
	6	2	1	1	2	2	2	10
	Mean	1.7	1.3	1.7	2.0	1.7	1.7	10.1*

Notes) 0: No abnormal change, 1: Very slight, 2: Slight, 3: Moderate
*: Total of the mean scores for each parameter

2.6.6.8 Special toxicology studies

28-DAY SUBCUTANEOUS INJECTION REPEAT DOSE TOXICITY STUDY OF TH9507 IN THE ALBINO RAT

Key study findings:

- Animals given nominal doses of 4 mg/kg of control or stressed TH9507 for 28 days had similar treatment-related effects on body weight gain, hematology (slight regenerative anemia), growth hormone levels, organ weights, and gross pathology.
- Toxicokinetics were similar between groups with parent exposures of 5.3-7.6 ng.h/mL (5X-7X MRHD, AUC basis) on D28.
- Microscopic findings were generally similar between control and stressed TH9507 treated animals, including periportal vacuolation in the liver and injection site reactions. The severity of inflammation and hemorrhage at the injection site was somewhat elevated in animals treated with the stressed TH9507.

Reviewer Comments: The goal of this study was to qualify impurities for the marketed product that exceeded the levels used in the previous nonclinical studies. Although the nominal dose in the study was 4 mg/kg, there were significant deviations from the nominal concentrations in weeks one and four. For the purposes of qualifying the impurities, the reviewer considers the lowest actual delivered dose to be appropriate for comparison. During week one only 80% of the nominal dose appeared to be delivered, or 3.2 mg/kg. For the two most abundant impurities in the final drug product, (b) (4) *and* (b) (4) *this resulted in delivered doses of* (b) (4) *respectively. The potential clinical exposures to these two impurities are* (b) (4) *, giving dosing multiples 8X and 3X MRHD (BSA basis). The reviewer considers this study adequate to qualify the higher levels of* (b) (4) *and* (b) (4) *in the Egrifta® formulation.*

Study: E-PCL-232
Volume and page: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: 28 February 2008
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: TH9507 (control), 7E530, 95.4%
TH9507 (stressed), 7E530 (treated), 90.3%

Methods							
Doses	Group Number / Identification	Dose Level (mg/kg/day)	Dose Volume (mL/kg/day)	Number of Animal: Main Study Males	Main Study Females	Toxicokinetic Study Males	Toxicokinetic Study Females
	1/ Vehicle control	0	4	10	10	3	3
	2/ TH9507 control	4	4	10	10	15	15
	3/ TH9507 stressed	4	4	10	10	15	15
Species/source	Sprague-Dawley CD (Crl:CD (SD)BR IGS) rats / (b) (4)						
Age / Weight	~6 weeks/ 176-211g (M) and 150-179 g (F)						
n/sex/group (main study)	See above						
TK groups	See above						
Recovery groups	none						

Route, formulation, dose volume	Subcutaneous injection in 4 mL/kg 5% mannitol in sterile water for injection

Observations and Times	
Mortality checks	Twice daily
Clinical Findings	Cageside observations twice daily with detailed observations weekly (main study animals only)
Body weights	Weekly
Food consumption	Weekly (main study animals only)
Ophthalmoscopy	Pretreatment and W4 (main study animals only)
Hematology	Main study animals at scheduled necropsy: Parameters for RBC, WBC, and coagulation
Clinical chemistry	Main study animals at necropsy: Parameters for lipids, proteins, glucose, electrolytes, LFTs, and kidney function
Urinalysis	Main study animals on D28 using sixteen hour samples (food and water withheld)
Other clinical evaluation	Anti-TH9507 antibody titer was measured on D1 and D29 by ELISA. GH was measured on D1 and D28 predose and at 5, 60 and 120 minutes post dose by RIA assay.
Toxicokinetics	On Days 1 and 28 samples were taken predose and at 5, 15, 30, 60, and 120 minutes post dose. Concentration of TH9507 was measured by validated ELISA. Impurities were not measured *in vivo*.
Gross pathology	Main study animals at sacrifice
Organ weights	Main study animals at necropsy: adrenal glands, brain, heart, kidneys, liver, ovaries/testes, pituitary, prostate, spleen, thymus, thyroid and parathyroid, uterus
Histopathology	Performed at necropsy for main study animals (all dose groups) adrenal, aorta, bone and marrow (sternum), brain, cecum, colon, duodenum, epididymides, esophagus, eyes, Harderian glands, heart, ileum, injection sites, jejunum, kidneys, lacrimal glands, liver (sample of 2 lobes), lungs, lymph nodes (mandibular, unilateral and mesenteric), mammary gland (inguinal), optic nerves, ovaries, pancreas, pituitary, prostate, rectum, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin (inguinal), spinal cord (cervical), spleen, stomach, testes, thymus, thyroid and parathyroids, tongue, trachea, urinary bladder, uterus, vagina, gross lesions.
	Adequate Battery: yes (x), no ()
	Peer review: yes (), no (x)

Results:

Dose Formulations: The drug formulations failed to meet acceptance criteria on numerous occasions, as shown in the sponsor's table below. During the first week, preparations of the control and stressed TH9507 (for groups 2 and 3) were ~15% and ~20% lower than their nominal values. In the final week, one preparation for Group 3 was 15% lower than planned. Other variations appeared to be due to errors in the analysis of the dosing formulations. Given that this is a pivotal study for impurity qualification, the reviewer will consider the actual dose given during the first week (80% of the nominal dose, averaging the actual and retention samples) to be the dose used for the study for the purpose of impurity qualification (80% of 4 mg/kg, or 3.2 mg/kg).

In Text Table 3: Summary of Dose Formulation Results Outside the Acceptance Criteria

Week (Preparation Date)	Group	Nominal Concentration (mg/mL)	Percent of Nominal	Mean Percent of Nominal
1 (12 Mar 2008)	2	1	86.5	87.0[A]
			87.5	
	3	1	82.0[A]	81.4[A]
			80.8[A]	
	2[B]	1	85.4	84.8[A]
			83.6[A]	
			85.4	
	3[B]	1	78.6[A]	79.0[A]
			79.4[A]	
			79.1[A]	
2 (19 Mar 2008)	3 (pre-filtration)[C]	1	87.0	87.3[A]
			87.5	
4 (02 Apr 2008)	3[D]	1	68.3[A]	76.3[A]
			84.2[A]	
4 (09 Apr 2008)	3[E]	1	84.3[A]	85.4[A]
			86.5	

A=out of acceptance criteria
B = analysis of retention samples in triplicate
C = post-filtration samples met acceptance criteria
D = results of analysis of retention samples in triplicate were within acceptance criteria. The inconsistency of these original results was considered likely due to a dilution error during dose formulation analysis.
E = retention samples not analyzed

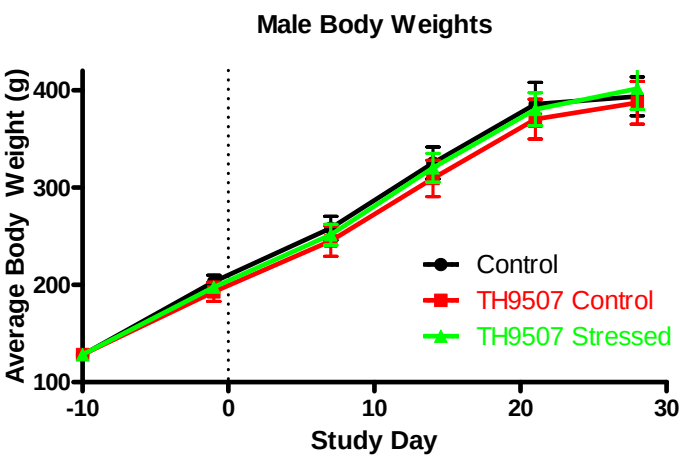
Mortality: Two animals died following blood collection on D28, one control female and one Group 2 TK female. These deaths were attributed to the blood collection procedure and were not considered treatment-related.

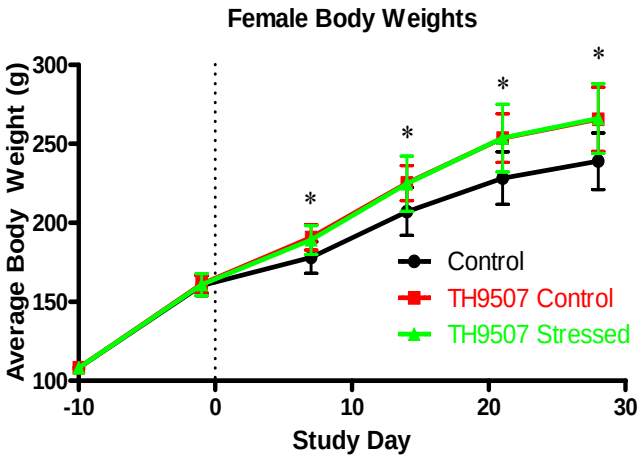
Clinical Signs: None of the abnormal findings appeared to be related to treatment.

Body Weights: Body weight gains were greater in treated animals, especially in females. The effects of the control and stressed TH9507 formulations appeared to be similar.

Body Weight					
Sex	Group	BW gain (g) over study	% Increment	Final BW (g)	BW % control
Males	1	190	-	394	100%
	2	194	↑2%	387	98%
	3	204	↑7%	401	102%
Females	1	79	-	239	100%
	2	104*	↑32%	266*	111%
	3	105*	↑33%	266*	111%

* $p<0.05$





Food Consumption: Females given both version of TH9507 consumed ~10% more food than controls. There were no significant differences in food consumption in the male animals.

Ophthalmology: The ophthalmologist's report states that there were no treatment-related findings.

Urinalysis: There were no treatment-related changes.

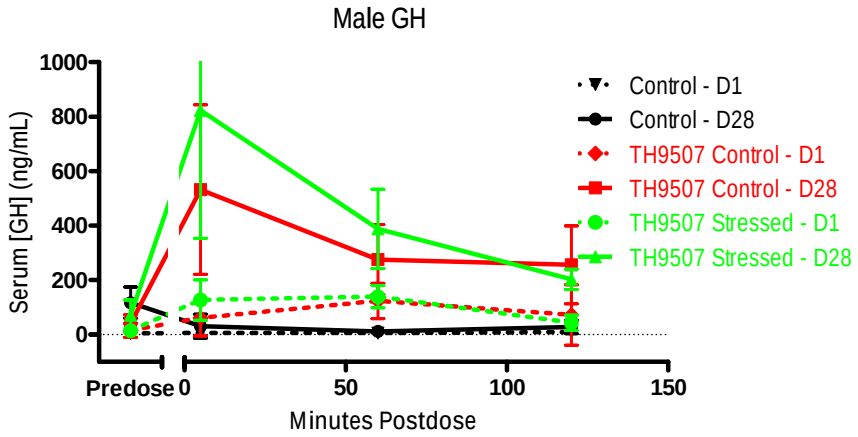
Hematology: Treated animals had slight decreases in RBC and increases in reticulocytes compared to control animals as shown below. The differences between the two TH9507 was not toxicologically significant.

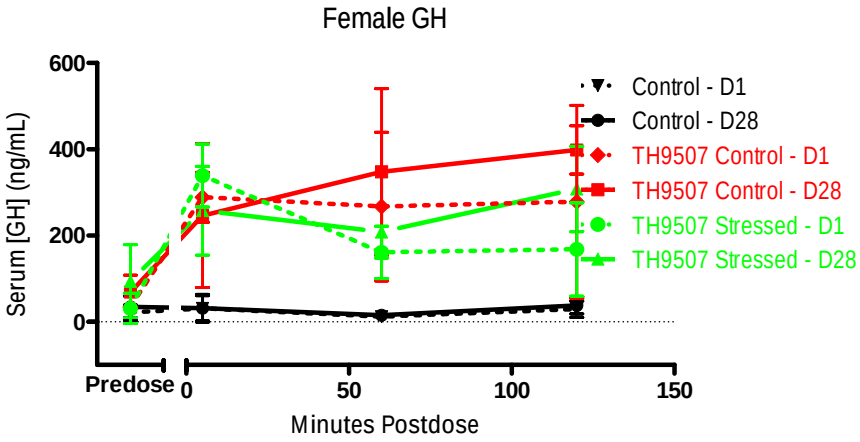
HEMATOLOGY				
Parameter	Males		Females	
	Unstressed	Stressed	Unstressed	Stressed
RBC	↓8.2%	↓8.9%	↓5.1%	↓5.7%
% Reticulocytes	↑24%*	↑39%**	↑31%	↑36%
# Reticulocytes	↑14%	↑25%*	↑24%	↑24%

Values represent the % change from control; * $p < 0.05$ vs. control; ** $p < 0.001$ vs. control

Clinical Chemistry: There were no meaningful changes in clinical chemistry parameters.

Growth Hormone: Dosing with either TH9507 formulation produced increases in serum growth hormone concentrations with no significant difference between the formulations. Males, unlike females, had an increase in GH over the 28-day dosing period, but this was consistent with higher predose levels in control animals on D28. Average GH concentration over 120 minutes post dose are shown in the graphs below for D1 (dotted lines) and D28 (solid lines).





Organ Weights: Significant changes in organ weights included increased adrenal weights in males (30-40%), increased liver weights in females (↑9-24%), and increased absolute heart and kidney weights in females (11-14%) that appear to be due to increased body weight. In all cases, the effects of the two TH9507 formulations were similar, as shown in the sponsor's table below.

In Text Table 5 Differences in Organ Weights* Compared to Vehicle Control Group

Gender		Male			Female		
Group		1	2	3	1	2	3
Body (g)		375	369	383	220	247	246
Adrenals							
	Absolute	—	**38**	**32**	—	10	9
	% body	—	**40**	**30**	—	-3	-3
Liver							
	Absolute	—	-1	1	—	**24**	**22**
	% body	—	1	-1	—	**10**	**9**
Heart							
	Absolute	—	-1	1	—	**14**	**12**
	% body	—	1	-1	—	1	0
Kidney							
	Absolute	—	-2	2	—	**11**	**11**
	% body	—	-1	0	—	-1	-1

* **Expressed as percent difference of group means.**
Based upon statistical analysis of group means, values highlighted in bold are significantly different from vehicle control group - P≤ 0.05; refer to data tables for actual significance levels and tests used.

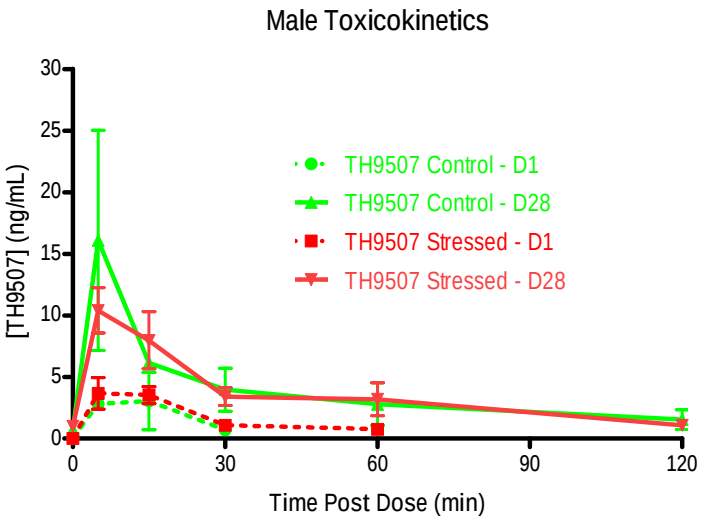
Gross Pathology: The only findings at necropsy which appeared to be related to treatment were darkened areas at the injection sites. As shown in the sponsor's table below, the incidence of "area dark" was similar for both TH9507 formulations.

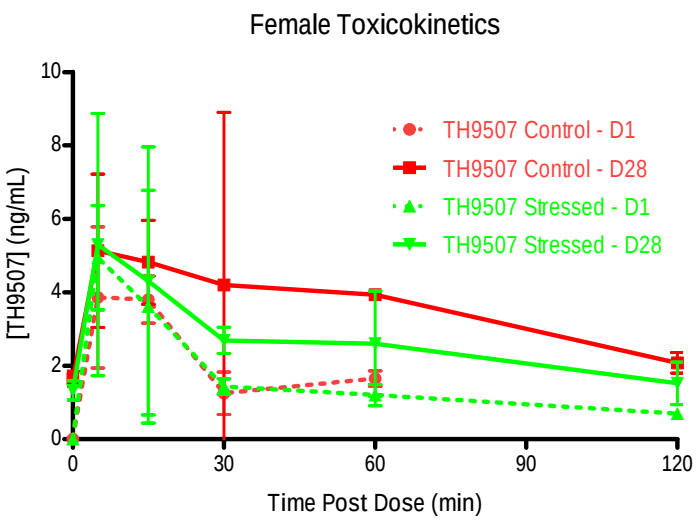
Tissue/Finding	Gender	Male			Female		
	Group	1	2	3	1	2	3
	Number of animals examined	10	10	10	10	10	10
Right dorsal thoracic injection site							
	Area dark	6	10	10	3	9	10
Left scapular injection site							
	Area dark	—	5	7	—	7	8
Interscapular injection site							
	Area dark	1	9	6	—	4	4
Right scapular injection site							
	Area dark	—	3	2	—	4	5

Histopathology: Findings that occurred more often in TH9507-treated animals are shown in the table below, including alterations at the injection site and vacuolation of periportal hepatocytes. Injection site reactions appeared to be somewhat more severe in animals treated with the stressed TH9507, with increases in "moderate" inflammation and hemorrhage. In the liver, microvesicular vacuolation occurred with similar incidence and severity in both TH9507-treated groups. Hemorrhage in the skeletal muscle was seen in treated female animals but not in males. This effect was considered incidental by the sponsor and the reviewer.

HISTOPATHOLOGY									
Tissue	Finding	Group	MALES 1	MALES 2	MALES 3	FEMALES 1	FEMALES 2	FEMALES 3
		n	10	10	10	10	10	10
Injection Site	Inflammation: subcutis	Minimal	3	5	3	3	2	0
		Slight	0	5	3	0	6	5
		Moderate	0	0	4	0	1	5
	Hemorrhage	Minimal	3	1	2	3	2	2
		Slight	1	8	4	0	5	8
		Moderate	0	1	4	0	0	0
	Necrosis	Minimal		0	1			3
		Slight	0	5	5	0	0	0
		Moderate		1	0			0
Liver	Periportal vacuolation	Minimal	0	3	6	0	2	4
		Slight		0	0		1	0
	Granulomatous inflammation		1	3	0	0	0	0
	Reactive sinusoidal lining cells		0	0	0	0	2	0
Skeletal muscle	Hemorrhage		0	0	0	0	1	2

Toxicokinetics: Average TH9507 concentration over the first 120 minutes post dose are shown in the graphs below for D1 (dotted lines) and D28 (solid lines). Exposures (measured as $AUC_{0\text{-tlast}}$) and C_{max} values were similar for both TH9507 formulations.





TOXICOKINETIC PARAMETERS					
Sex	Day	TH9507 Control		TH9507 Stressed	
		C_{max} (ng/mL)	$AUC_{0\text{-tlast}}$ (ng.h/mL)	C_{max} (ng/mL)	$AUC_{0\text{-tlast}}$ (ng.h/mL)
Male	1	3.0	1.02	3.7	1.75
	28	16	7.57	10	7.17
Female	1	3.9	2.15	4.9	3.15
	28	5.1	7.28	5.3	5.33

Anti-drug antibodies: Anti-TH9507 antibodies were measured using a validated ELISA method. Two samples were designated as potentially positive after the screening assay (a D1 sample from a control animal and a D29 sample from a Group 2 animal). In the confirmatory immunodepletion assay, an additional sample was also tested by mistake (sample TS-62 in the sponsor's table below). After immunodepletion, only the sample from the control animal was deemed positive (>50% depletion in signal). The reviewer does not consider this evidence of dosing error since the original screening value was very close to the negative cut-off. The lack of an immunogenic response in TH9507-treated animals is consistent with previous studies in the rat.

Sample ID	Animal ID	Time Point	Screening			Immunodepletion					
			Assay ID	NCO [c]	Mean A_{450nm} Value of Duplicate	Assay ID	Dilution Fold	Results (Mean A_{450nm} value of duplicate)		Difference (%) [a]	Results [b] (Positive or Negative)
								Non Immunodepleted	Immunodepleted		
HPC	-	-	-	-	-	PRO-29	-	1.3050 [f]	0.0905 [f]	-93.1	**Positive**
LPC	-	-	-	-	-		-	0.1885 [f]	0.0455 [f]	-75.9	**Positive**
Pool Rat Serum (1/10) (PRS)	-	-	-	-	-		10	0.0515	0.0530	2.9	Negative
TS-32	1507	Day 1	PRO-25	0.0792	0.081 [d]	PRO-29	10	0.1810	0.0470	-74.0	**Positive**
TS-51	2006	Day 29	PRO-25	0.0792	0.728	PRO-29	10	0.7820	0.7460	-4.6	Negative
TS-62	2502	Day 1	PRO-25	0.0792	0.069 [d]	PRO-29	10	0.0460	0.0440	-4.3	Negative
RATBREC.69 499F	-	-	PRO-19	0.0792	0.071/ 0.086/ 0.099 [e]	PRO-29	10	0.0760	0.0730 [f]	-3.9	Negative

a = % Difference = [(Mean A_{450nm} value of immunodepleted - Mean A_{450nm} value of Non immunodepleted)/Mean A_{450nm} value of Non immunodepleted] x 100
b = Positive if the % reduction (%Difference) > 50 %
c = NCO = Mean + 1.645 x SD
d = Flagged values due to % difference between duplicate > 25%
e = NCO screening result
f = Mean A_{450nm} value of 2 sample replicates (n=2)

EVALUATION OF THE EFFECT OF TH9507 ON THE PRIMARY ANTIBODY RESPONSE TO A T-CELL DEPENDENT IMMUNOGEN DURING A 28-DAY SUBCUTANEOUS INJECTION STUDY IN ALBINO RATS

Key study findings:

- Rats given 0.1, 0.6, or 1.2 mg/kg had mounted a similar antibody response to KLH injection as animals given vehicle control.

Reviewer Comments: There was no evidence of compromised immune response in this study.

Study: E-PCL-159
Volume and page: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: 3 December 2004
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: TH9507, FHEXGRF020185.5% peptide content, 98.5% peptide purity

Methods	
Doses	0, 0.1, 0.6, 1.2 mg/kg/day for 28 days
Species/source	Sprague-Dawley (Crl:CD (SD)BR IGS) rats / (b) (4)
Age / Weight	~7 weeks/ 219-237g (M) and 158-183 g (F)
n/sex/group (main study)	10/sex/group

TK groups	none
Study Design	Animals were immunized with Keyhole Limpet Hemocyanin (KLH) (300 µg in 500 µL sterile water, i.v.) on D22 at 3h post TH9507-dose.
Route, formulation, dose volume	Subcutaneous injection of drug in 1 mL/kg 5% mannitol in sterile water for injection, rotating injection sites daily.

Observations and Times	
Mortality checks	Twice daily
Clinical Findings	Cageside observations twice daily with detailed observations weekly
Body weights	Weekly
Food consumption	Weekly
Immunotoxicity Assessment	Blood samples were collected pre-dose on D22 and on D27, 28, and 29. Serum was analyzed for the presence of anti-KLH IgM antibodies.
Post-mortem	All animals were euthanized and discarded without further examination.

Results:

Mortality and clinical signs: There were no treatment-related deaths or clinical signs. One MD animal died following blood collection on D22, and that death was attributed to a collection error.

Body Weights: All treated female groups gained significantly more weight than controls (↑36-60%). HDM also gained more weight than controls (↑7%), but the effect was not statistically significant. Growth curves for each sex are shown in the sponsor's figures below.

Body Weight					
Sex	Dose, mg/kg	BW gain (g) over study	% Increment	Final BW (g)	BW % control
Males	0	171	-	399.8	100%
	0.1	174	↑2%	403.9	101%
	0.6	169	↓1%	397.3	99%
	1.2	183	↑7%	412.0	103%
Females	0	55.2	-	224.4	100%
	0.1	75.3*	↑36%	248.0**	111%
	0.6	83.3***	↑60%	255.8***	114%
	1.2	78.7**	↑43%	248.7**	111%

* $p < 0.05$; ** $p<0.01$; *** $p < 0.001$

Figure 1 Group Mean Body Weights - Males

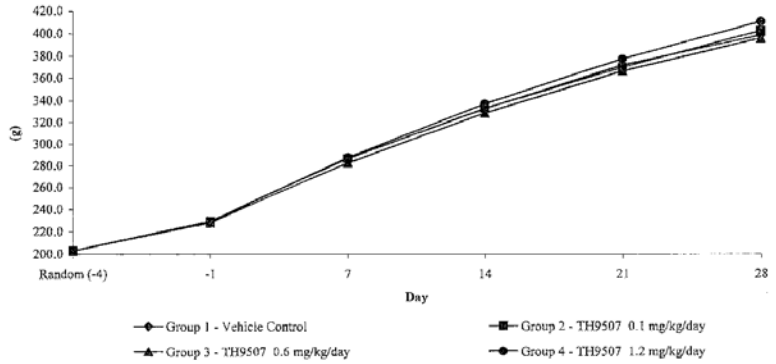
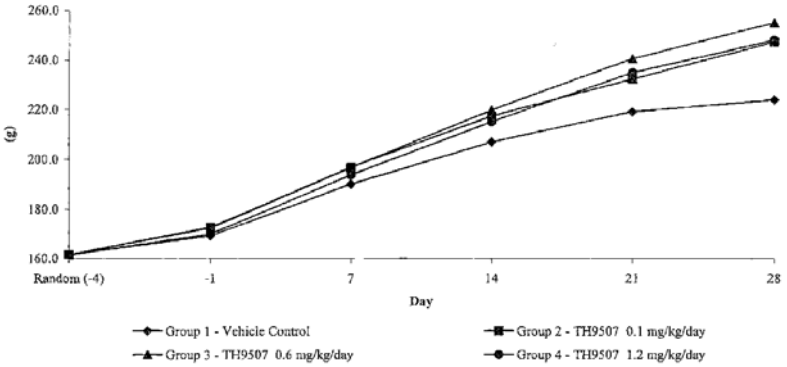
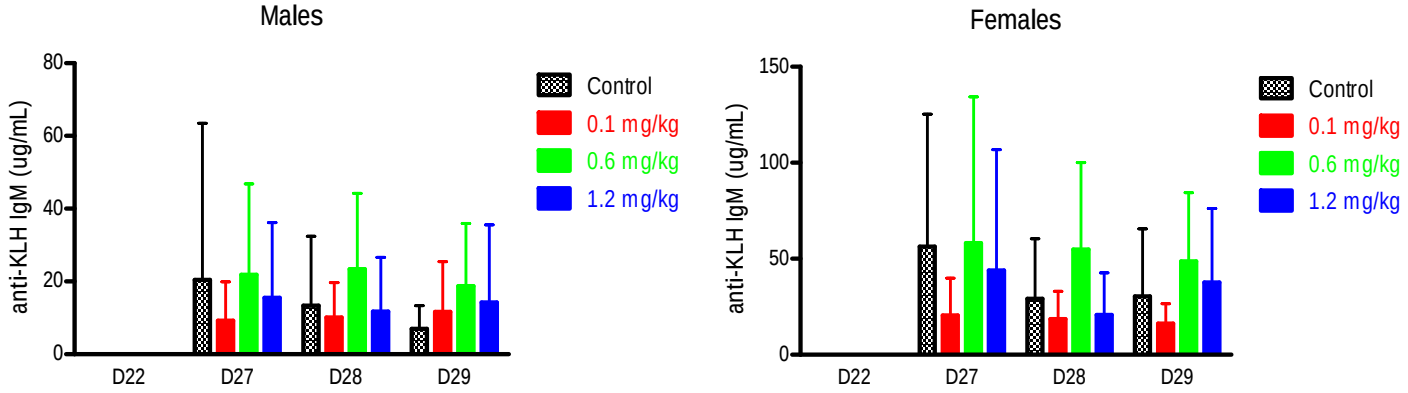


Figure 2 Group Mean Body Weights - Females



Immunotoxicity Assessment: As shown in the graphs below, there were no significant differences in antibody responses as measured on Day 27, 28, or 29. These results suggest that TH9507 is not overtly toxic to the immune system in these animals.



2.6.6.9 Discussion and Conclusions

The pivotal repeat dose studies in rats and dogs are discussed in detail in section 2.6.6.3. The NOAEL for the rat study was 0.6 mg/kg (15X MRHD, AUC basis) after 26 weeks of dosing. The NOAEL for the chronic dog study was <0.1 mg/kg (<284X/1909X MRHD for males and females, respectively). Note

that these multiples of clinical exposure differ from those presented by the sponsor, who used AUC values from the healthy volunteer study rather than from the study in HIV-positive patients.

In the reproductive toxicity assessment, hydrocephaly in the offspring of dams given 1.2 mg/kg during GD6 to LD21 was the most concerning finding. This, coupled with the decreased ossification in the skull during the fertility and embryonic development study which used lower doses, suggests that there is a real effect of treatment with TH9507 on the heads of developing rats. Three possible explanations (not necessarily mutually exclusive) for this effect emerge when examining the relevant data and literature: 1) exposure to anti-GHRH antibodies; 2) exposure *in utero* or upon lactation to TH9507; and 3) exposure to increased maternal GH or IGF-1 (or other downstream effector of GHRH) through the placenta or milk.

The antibody hypothesis (i.e., that hydrocephaly is caused by lowered GHRH activity) is supported by the following:

- Anti-GHRH antibodies were detected in two animals in the 26-week rat study and in all animals in the 13-week study (using a compromised assay).
- Hydrocephaly was not observed in the early embryofetal development study and was only observed after lactation (and transfer of maternal antibodies)
- Pediatric patients with hydrocephaly often also have low GH or GH resistance. Although alterations in GH may simply be secondary to physical pressure on relevant brain structures, there does not appear to be a clearly established causal relationship.[2]
- Mice overexpressing IGFBP1 (which modulates IGF-1 activity) had a high incidence of hydrocephaly.[3]
- TH9507 was not detected in the plasma of the F_1 animals. (Plasma concentrations were below the LLOQ, 0.6 ng/mL or ~25% MRHD C_{max}).

The tesamorelin exposure hypothesis is supported by additional data:

- Doses used in the EFD study were lower than those used in the current study (HD of 0.6 vs. 1.2 mg/kg), and the degree of placental drug transfer is unknown. The measurement of F_1 drug levels may have been insufficiently sensitive to detect low but efficacious drug levels.
- Both rats and rabbits showed reduced ossification in the skull during embryofetal development (but advanced progression of ossification in other areas), suggesting that there may have been increased intracranial pressure prior to the time when maternal antibodies would have been transferred.
- This reduced ossification occurred in a much greater proportion of dams than did anti-drug antibodies in the 26-week study.
- Intracranial hypertension is a known side effect of growth hormone administration (see e.g., Genotropin label).
- GHRH is secreted in the milk of lactating rats (as well as humans). In humans, the concentration of GHRH in milk exceeds that of plasma by several folds[4].
- Neonatal rat pituitary somatotrophs respond more robustly to GHRH than those from older animals[5], so a plasma concentration that would be sub-efficacious in an adult may be sufficient to cause GH release in the neonate and/or developing fetus. Concentrations as low as 0.001 nM (verses LLOQ of 0.1 nM) cause significant release of GH *ex vivo* for pituitary cells of 2 day old rats compared to older animals.

[2] Lopponen, et al., *Archives of Disease in Childhood,* 77:32-37 (1997).
[3] Doublier et al., *Growth Hormone and IGF Research*, 10:267-274 (2000).
[4] Werner et al., *Biochemical and Biophysical Research Communications*, 135:1084-1089 (1986).
[5] Szabo and Cuttler, *Endocrinology,* 118(1):69-73 (1986).

Exposure to downstream effectors (e.g., growth hormone, IGF-1) or compensatory hormones (e.g., somatostatin) could also play a role in the adverse effect. If a single component was responsible for both the delayed ossification in the skull and the hydrocephaly, it would mostly like be present during both gestation and lactation, although a priming effect of *in utero* exposure can not be excluded as a possibility. The literature suggests that growth hormone and IGF-1 do not cross the placental barrier, but may signal the placenta to increase fetal nutrition and regulate placental IGF-1 production, respectively. An effect downstream of growth-promoting peptides could also account for the lack on increased body weight in F_1 pups (which might be expected if animals were exposed to minimally efficacious levels of growth hormone).

The sponsor does not provide sufficient data or explanation to distinguish between these possibilities, complicating the assessment of safety for women of childbearing potential. The reviewer considers it likely that a combination of tesamorelin exposure (especially during lactation) and interactions of downstream effects (especially during gestation) to have the most scientific support, as described above. There have not been definitive studies to demonstrate whether GHRH crosses the placenta in rats, but low levels of transfer (~2%) were demonstrated for exenatide (39aa) in an ex vivo preparation[6], and oxytocin (9aa) appears to cross the placenta in a similar preparation by passive diffusion[7].

The reviewer concurs with the sponsor's recommendation that TH9507 be discontinued when breastfeeding or pregnant. We are recommending that TH9507 be assigned to Pregnancy Category C due to the hydrocephaly noted at clinically relevant exposures (2X MRHD).

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: The sponsor has provided an adequate set of toxicity studies to support approval of Egrifta®.

Unresolved toxicology issues: The sponsor should conduct a rat embryofetal development study in rats using higher doses, including a 1.2 mg/kg dose for comparison with the peri- and post-natal study and to cover the range of exposures seen clinically to clarify the risk of hydrocephaly in offspring of WOCBP. Specifically, this post-marketing requirement will clarify whether the risk of hydrocephaly is present from exposure to tesamorelin during the pre-natal or post-natal period, or both.

Recommendations: Pharmacology/Toxicology recommends approval of Egrifta®.

Suggested labeling: To be provided in a subsequent document.

[6] Hiles et al., *Human and Experimental Toxicology,* 22(12): 623-628 (2003).
[7] Malek et al., *Journal of Maternal and Fetal Medicine,* 5:245-255 (1996).

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22505	ORIG-1	THERATECHNOLOGIES INC	Egrifta

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

/s/

LAUREN M Mihalcik
03/01/2010

TODD M BOURCIER
03/02/2010
P/T recommends approval
Requests Seg 2 PMR to address hydrocephaly

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 22-505 **Applicant: Theratechnologies** **Stamp Date: 2 June 2009**

Drug Name: Egrifta **NDA/BLA Type: Standard**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	x		Fully in eCTD format
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	x		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	x		Not ideal, but legible.
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	x		Carcinogenicity studies were not required per communication with the Division, but the sponsor did a mitogenicity assay as part of the chronic rat study. Reproductive toxicity study in the rabbit was repeated, as requested by the Division.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	x		Degradants in the refrigerated formulation meeting the threshold for qualification were tested for genotoxicity and in a repeat dose (4 week) rat toxicity study.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	x		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	x		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	x		The sponsor has submitted studies to qualify the degradants in the final formulation and a requested repeat of the Seg 2 rabbit study.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?		X	(b) (4) [redacted] This will be a review issue. Impurities
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		Impurities/degradants identified as (b) (4) [redacted]
11	Has the applicant addressed any abuse potential issues in the submission?		x	Sponsor states the drug is "unlikely to be associated with drug abuse or dependence"
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?	n/a		n/a

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

This NDA appears to be fileable, but the nonclinical toxicology section of the label may be inadequate. Judging from previous reviews of the chronic toxicity studies, there were significant drug-related findings. In the rat lipid accumulation in the liver and increases in glucose were observed at all doses (not considered adverse by the sponsor). Exposures in the rat study were 10X, 100X, and 200X MRHD (AUC basis). In the dog, two of four females given 0.6 mg/kg (50X) developed diabetes and were euthanized. Surviving dogs were acromegalic due to GH excess with moderate to severe histopathology findings (e.g., degeneration of the liver, pancreas, and gallbladder, pituitary diffuse hyperplasia, stomach hypertrophy, and thymus atrophy). Most of these findings had no NOAEL. Exposures in dogs were 5X, 50X, and 138X MRHD (AUC basis) at the low, mid, and high dose, respectively. Although these effects occurred at doses higher than the expected clinical exposure, some mentioning of at least some of them may be warranted.

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

Pharm/tox has no comments at this time.

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

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

/s/

Lauren J Murphree
7/14/2009 12:03:44 PM
PHARMACOLOGIST

Todd Bourcier
7/14/2009 01:44:52 PM
PHARMACOLOGIST
Concur, OK to file